

## **Abstracts**

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## FACTORS INFLUENCING ON CLINICAL MANIFESTATION OF HCV IN GROUP OF CLEAN-UP WORKERS OF CHORNOBYL NPP ACCIDENT

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Immunological monitoring of 536 clean-up workers of Chornobyl NPP accident revealed significant prevalence of hepatitis C virus (HCV) infection (19.6%) compared with non-irradiated person (9.5%). Among 105 HCV carriers clinical signs of infection was found in 39 (37.41%) persons.

Factors that influenced on manifestation of HCV were male gender ( $r = 0,3553$ ,  $p < 0,0001$ ), serological signs of previous hepatitis B virus (HBV) infection ( $r = 0,2896$ ,  $p = 0,003$ ), antibodies against cytomegalovirus (CMV) and *T. gondii* in serum ( $r = 0,3418$ ,  $p < 0,0001$ ).

Logistic regression ( $P = 1/1+e^{-z}$ , where  $z = 1,5040 - 1,8405 \times \text{Mix}(1) - 2,6026 \times \text{Mix}(2) - 0,6567 \times \text{Mix}(3)$ ;  $\text{Mix}(1)$  – serological signs of one of above mentioned infections,  $\text{Mix}(2)$  – serological signs of two infections, and  $\text{Mix}(3)$  – serological signs of previous HBV, CMV and *T. gondii* infection) for male clean-up workers allowed to predict HCV manifestation in 72% of cases.

For patients with serological signs of two or three infections the most significant predictor for HCV manifestation was absolute content of CD4+ T helper cells ( $>1.0 \times 10^9/\text{L}$  or  $<1.0 \times 10^9/\text{L}$ ) ( $r = 0,5745$ ,  $p = 0,005$ ). These parameters allowed to predict reactivation of HCV in 28 out of 31 (90,33%) male HCV carriers.

These data suggest to additional negative influence of TORCH-infections and immunological reactivity on maintenance of HCV in latent form. In connection with our previously investigations, that revealed significant prevalence of CMV and *T. gondii* infections among the acute radiation syndrome convalescents, clean-up workers and inhabitants of contaminated territories, the persistence of TORCH-infections among irradiated persons may disturb their health state.

## EXPERIMENTAL AND CLINICAL BIOLOGY OF CARBON-ION THERAPY AT HIMAC; AN OVERVIEW

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Purpose Carbon-ion therapy using HIMAC synchrotron started at Year 1994, and treated more than 1,800 patients by Year 2004. Therapeutic outcome of this new modality is well recognized so that Japanese government approved our institute December 2003 to charge the treatment fee from patients. Biology of heavy ions in past has attracted only limited number of basic scientists, but is now becoming a general topics in all oncology areas. I here shortly report experimental results and also biological analysis of clinical data that have been obtained recently.

Materials and Methods 1. in vitro experiments: 10 cell lines of human melanoma (MM) and 11 cell lines of human squamous cell carcinoma (SCC) were cultured. These cells were exposed to either carbon ions of 50 keV/micrometer or reference 200 kVp X rays. Colony formation assay was carried out to obtain survival curves. 2. in vivo experiments: C3H/HeMsNrsf mice aged 12-18 weeks were used: males for the tumor study and females for the skin study. The tumor was a syngeneic NFSa fibrosarcoma, and its 16 through 18th generations were transplanted intramuscularly into the right hind legs of mice 7 days before the first irradiation. Hairs on the right hind leg of female mice were removed by applying a depilatory agent 7 to 8 days before the first irradiation. A total of 881 male mice for the tumor experiment and of 2,323 female mice for the skin reaction experiment were used with 5 mice for each irradiation dose point. All of the data collected from repeated experiments were combined. 3. Clinical data analysis: Local control rates of non-small cell lung cancer (NSCLC) by carbon-ion therapy were achieved by a treatment schedule of 18 fractions in 6 weeks with dose escalation. Local control rates for photon therapy that were obtained other institutions were used to compare with those for carbon-ion therapy.

Results and Discussion Survival parameters such as SF2 and D10 were plotted for X rays and carbon ions. No difference between the two cell lines was obtained except alpha/beta ratio, which distribution was wider for SCC than MM after X-ray irradiation. For carbon ions, the distribution was wider for MM than SCC. RBE values of carbon ions for tumor growth delay was higher than RBE values for early skin reaction after high LET carbon ions, implying therapeutic gain of carbon ions even for early reaction. Tumor control probabilities of NSCLC were analyzed by using alpha and beta values of a cultured human salivary gland tumor, HSG. We found that the slope of TCP for carbon ions was steeper than that for photons. This strongly support an concept that high LET radiation could overcome photon-resistant tumors that are heterogeneously included in spontaneous human tumors.

## 4D NUCLEAR ORGANISATION AND RADIATION INDUCED CHROMOSOME ABERRATIONS: QUANTITATIVE RELATIONSHIPS

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Principal theories on the mechanisms of chromosome aberrations (CA) formation are common in suggestion that chromatin lesions must be in contacts to form exchanges. They are different in suggestion whether these contacts are pre-existent or lesion proximity occurs after irradiation. To resolve this uncertainty, the study of how does structural organisation of interphase nucleus affect induction and interaction of chromosome lesions is necessary. Quantification of lesion contacts in space and time requires knowledge of chromosome states in the whole nucleus. Two models of large scale chromosome organisation are numerically studied by Monte Carlo experiments, as to random walk and globular chromosome models. The random walk model reveals highly dynamic behaviour of chromosomal loci in the nucleus, massive intermingling of chromatin and unrestricted movement of open broken ends. The globular model predicts that interphase chromosomes are restricted in movement and thus rejoining distance has to be small compared to the chromosome size. Unlike random walk model, the globular chromosome model is in agreement with all available data on chromosome structure *in situ* and dynamics *in vivo*. Basing on globular chromosome model, a variety of 4D structures for whole interphase nuclei of human lymphocytes as well as a pattern of intra- and inter-chromosomal contacts are obtained by Monte Carlo technique. On this basis the simple and complex intra- and inter-chromosomal aberrations induced by low and high LET radiation are calculated and compared with the full-colour and dual-arm labelling FISH data. Simulation results for complete and total interchanges agree well with data, except of complex interchanges which are underpredicted. To explain the underprediction, a new mechanism is introduced dealing with chromatin dynamic repositioning following irradiation as a part of rejoining process.

A direct comparison of CA simulation and data requires taking into account the fact that computer experiments are made for interphase nucleus and the CA detection (Giemsa, FISH) is performed in metaphase at single sampling time. The radiation induced mitotic delay or/and cell death may influence the frequency of aberrations in metaphase cells at a given sampling time. The exact incorporation of the cell cycle delay into chromosomal aberrations analysis requires knowledge of kinetics of cell progression through cell cycle stages. The frequencies of aberrations seen in first metaphase at any time with taking into account the mitotic delay are calculated using Monte Carlo technique. The predicted dose responses for simple and complex interchanges induced by low LET radiation are compared with the experimental CA data for human lymphocytes.

## RADIATION INFLUENCE OF THE NPP ON ENVIRONMENT

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The big number of biosystems sensitive to radioactive pollution of an environment is known. A problem of a choice of the most sensitive test-system is one of the most complicated problem in radioecology. The soil-vegetable top located around NPP is the first and basic depot of radioactive substance arriving on earth during emission from the NPP. This thin biosphere layer being most rich in life is known to be most sensitive to radiation affection of radioactive pollution. The soil well accumulates radionuclides ( mainly <sup>137</sup>Cs) and the effect of influence of the NPP can be found out by the long term influence of <sup>137</sup>Cs on soil microorganisms. Based on that the soil microorganisms were chosen as the research objects for monitoring of the territory located around NPP. The necessary condition for monitoring is the presence of the same microorganisms at all points of observation. Researches have shown that the basic groups of microorganisms satisfying necessary for performance of a problem – presence at all points of monitoring – were representatives of the following sorts: bacteria of sorts *Corynebacter*, *Pseudomonas*, *Bacillus*, *Actinomyces* and also mycelium mushrooms ( *Filamentous fungi*) and yeast (*Yeasts*). Choice of points of observation was made subject to primary directions of winds in area of Armenian NPP action. It was possible to expect that in a primary direction of winds the radionuclid sedimentation on the ground will be more than in opposite direction. Our researches have shown that the quantity of bacteria in the samples of ground taken from monitoring points, located on windward side from the NPP practically was not changed. In leeward side from NPP the stimulation of microorganism growth was revealed and the maximal stimulation was revealed in the point corresponding to the maximal <sup>137</sup>Cs fall-outs.

The method developed by us permits to find out environmental radioactive pollution against a background of global radionuclid full-outs.

The results of our investigations can be considered as the base for working out the principles and methods of ecological monitoring on the territories aimed for exploitation or building of NPP as well as for organization of land tenure in area of NPP action.

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## THE NATURE OF UV-RADIATION INTERACTION WITH MEMBRANES OF DIFFERENT TISSUES OF VERTEBRATES.

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Importance of UV-radiation for functioning of various types of biological membranes is hard to overrate. In such a multicomponent systems as biological membranes are, the many photochemical reactions are taking place at the same time. But still are not clear, the changes of which structures in content of membranes play a key role in its free radical destruction under UV-radiation influence. And to us, the chemiluminescent (ChL) real time imaging of free radical formation is a very informative method for determination of biomembranes changes during of this interaction..

We isolated lipid fractions from the brain, heart, liver and muscle of vertebrates: crucian carp (*Carassius carassius*), marsh frog (*Rana ridibunda*), caucasian agama (*Stellio caucasicus*), and nonpurebred white rats by the method of Keits. The tissue homogenates and lipid fractions have been radiated by UV-lamp (MEDICOR, Q-439, Budapest); exposition 2,5 min, distance to quivette 5 cm. Chemiluminescence intensity was determined on a quantometric devise, equipped with FEU-139 photomultiplier (Zakharian A.E., 1990). Model membranes (BLM) were formed from the total lipid fraction on a teflon aperture by the method of Muller. The electrical parameters of the BLMs were determined on an electrometric devise equipped with a Keithley 301 differential feedback amplifier (USA) in a voltage-fixation mode.

Levels of homogenate's luminescence after UV-radiation increases in row of poikilotherm vertebrates: fishes – amphibian – reptiles and slightly decrease in homogenates of mammal's tissues. The ChL response of brain homogenates is noticeably low compare to heart and muscle tissue homogenates. Only ChL intensity of liver tissue is lower than the level of its for brain. We think it depend of very low content of integral proteins in liver of vertebrates. The mechanisms of photodestruction of peptides are known.

Intensities of ChL of phospholipids, purified from same tissues of vertebrates are distinguish not to a marked degree, what, probable, testify about key role of protein components of cell membranes as emitters of quantum during UV-induced ChL.

Moreover, the electrical properties of BLMs from lipids after UV-radiation influence are similar to resistance and conductivity of BLMs from normal lipids. Thus, we can conclude, that condition of lipid components of biomembranes in course of UV-radiation changes very slightly.

## A COMPARISON OF THE RADIATION SENSITIVITIES OF EMBRYOS AT THE GASTRULA STAGE IN TWO MOUSE STRAINS.

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Objective : Gastrulation in mice is known to be associated with a period of extreme proliferation and differentiation. According to Heyer et al, the potential cost to the embryo of a very rapid proliferation rate is a high production of damaged cells (Heyer et al, 2000). The present research was performed in order to characterise the effects of X-irradiation at the gastrula stage.

Methods : Mouse gastrulas (7 days *post conception*) were irradiated with 1 or 2.5 Gy of X-rays. A comparison was performed between the "radiation-resistant" C57BL and the "radiation sensitive" BALB/c mouse strains. A series of parameters was investigated 6 and 24 hours after X-irradiation, *e.g.*: total area of the embryonic part of the whole embryo, cell and nuclear morphology (chromatin condensation at the nuclear periphery, breaking up of the nucleus, production of apoptotic bodies), cell size, cell cycle and caspase 3 activity.

Results : X-irradiation did not induce a change in the total area of the embryo. Furthermore, our first results suggest a decrease in cell size correlated to an increase in granularity (both described in the literature as a characteristic of apoptosis) 24 hours after irradiation of BALB/c or C57BL gastrulas. The proportion of cells in the sub-G1 fraction (corresponding to the apoptotic cells) was also increased after 24 hours and the cell cycle was modified. However, in contrast with the results of Heyer et al (2000), no change in cell size and granularity, cell cycle and sub-G1 fraction was observed 6 hours after irradiation with a high dose of X-rays. This could be partially due to the fact that total gastrulas were collected with no distinction made between extra-embryonic and embryonic parts, knowing that radiation-induced apoptosis has been described to be related to the embryonic part (in particular the ectoderm). 6 hours after irradiation, the apoptosis degree would still be "diluted" and a longer time (24 hours) will be needed to observe significant changes in the apoptosis degree.

Conclusion : Further characterization enabling us to distinguish between apoptosis and necrosis are currently being performed (in particular, the annexin V-PI test). We also plan to develop TUNEL and caspase 3 assays on gastrulas collected at various times following irradiation with different doses.

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## EPIGALLOCATECHIN GALLATE AFFECTS THE X-RAY INDUCED APOPTOSIS IN HUMAN IM-9 CELLS.

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Objective: Recent studies in human and mammalian cells have suggested that epigallocatechin gallate (EGCG) could interfere with several biological processes, including the regulation of carcinogenesis. However, the molecular mechanisms underlying its antitumorigenic activity are still not defined. The aim of the present study was to characterize the influence of EGCG on a number of physiological parameters following exposure of cultured cells to X-irradiation.

Methods : Cells of the multiple myeloma derived cell line IM-9 were exposed to X-rays (doses from 0 to 8 Gy) and/or EGCG (concentrations from 0 to 200  $\mu$ M) and analysed at successive sampling times (from 1 up to 3 days). The parameters investigated included the general physiology ( $\text{pH}_{\text{int}}$  and  $\text{Ca}^{++}_{\text{int}}$ ), the cell cycle, the induction of apoptosis (annexin V-propidium iodide, membrane potential, esterase activity) as well as the estimation of induced oxidative stress (reactive oxygen species and  $\text{H}_2\text{O}_{2\text{int}}$  concentration) through cytofluorimetric analysis.

Results : Our results suggest that EGCG *per se* does not influence the cell cycle but exerts a dose-dependent increase of apoptosis coupled with a concomitant decrease of the general health status of the IM-9 line. Furthermore, EGCG prevents the cell cycle arrest and polyploidization induced by X-rays stimulating the apoptotic clearance of irradiated cells.

Conclusion : These results may open a perspective of synergical therapy (radiation + EGCG) at the condition to also address the intrinsic toxicity of EGCG on normal cells.



## RADIOMODIFYING AND CYTOGENETIC ACTIVITY OF GREEN TEA EXTRACT

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Objective: Cytogenetic investigation of bone marrow cells (BMCs) of animals was carried out to study the mutagenic and radio modifying activity of green tea extract in rats.

Methods: The green tea extract was prepared according to the following method: Fresh tea (*Camellia sinensis*) leaves were treated with 130° C water steam for 2-3 min to inactivate enzymes. Then the leaves were crushed in CTC type machine and extracted with 80°C hot water at a ratio 1:10 (w/w). The liquid extract was filtered, concentrated up to 25% dry matter and spray-dried. The ready green tea extract powder contained the following: 4.0% caffeine, 18.0% amino acids and other nitrogen containing substances, 10.0% pectic substances, 29.0% sucrose and reducing sugars, 22.0% polyphenols, among them 3.0% flavonols and 13.2 % catechins (including 5.28 % (-) epigallocatechingallate).

White inbred rats obtained 1 ml 0.5% water solution of green tea extract *per os* during 7 consequent days. BMCs of the first group of tea-pretreated rats were examined in order to reveal the mutagenic activity of green tea extract. The second group of tea-pretreated rats was exposed to single total body  $\gamma$ -irradiation with  $^{60}\text{Co}$  at the dose level of 8 Gy.

The cytogenetic investigation of BMCs of animals was performed in 24 hours after irradiation to study the radio modifying action of green tea extract. The cytogenetic studies involved analysis of the BMCs' chromosome apparatus with the evaluation of the frequency of non-stable chromosome aberrations (ChA) per 100 dividing cells, determination of the proliferative activity (PA) of the BMCs (the number of dividing cells), and the quantity of polyploid cells.

Results: Weekly per oral administration of green tea extract to animals caused the increase of ChA level (8.00 vs 1.74% in Norm), stimulation of PA of BMCs (2550 vs ~1500 in Norm), and the appearance of polyploid cells (1.07% vs 0%); thus signifying to the mutagenic activity of green tea extract.

On the background of pretreatment with green tea extract, the cytogenetic responses of BMCs of white rats to ionizing radiation were modified: as compared with BMCs in animals exposed only to irradiation the frequency of ChA was reduced (8.00% vs 43.01%), PA was increased (703.00 vs 60.90), and the level of polyploid cells was decreased.

Conclusion: The green tea extract possesses the high biological activity and improves the cytogenetic indices of irradiated animals.

## THE EFFECT OF MM-RANGE ELECTROMAGNETIC WAVES OF LOW INTENSITY ON STRUCTURE - FUNCTIONAL PROPERTIES OF RED BLOOD CELLS MEMBRANES

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Objective: The research was aimed to study such indices of structure-functional state of erythrocyte membranes as the activity of lipid peroxidation processes,  $K^+$  permeability and deformity under the exposure of animal organism to mm-range electromagnetic radiation of low intensity.

Methods: The research was conducted in white non-linear pubescence rats exposed daily to 30 minutes total irradiation with mm-range coherent electromagnetic waves (EMWs) during 4 subsequent days.

“Yav-1” device generating EMWs with 5.6 mm wavelength and 10 mWt emission intensity was the source of non-ionising radiation.

On day 1, 7, 14 and 30 post irradiation the animals were sacrificed and analyses were performed. Erythrocyte membrane lipid peroxidation intensity was determined using the TBA-test.  $K^+$  permeability was studied based on the results of K-ions concentration increase in medium in 1 hour after incubation of erythrocytes in 0.9% NaCl isotonic solution. The deformation ability of red blood cells was evaluated as follows: (1) the number of erythrocytes in saline suspension was calculated before the filtration of the solution through milliporous filter, then (2) the number of cells was counted in resulting filtrate, and the ratio of (2) to (1) obtained. The results were compared with data obtained in animals exposed to the same manipulations with the device turned off.

Results: All the studied indices underwent significant changes beginning from the 1<sup>st</sup> day after the cessation of the last irradiation séance. The most expressed changes were revealed on day 7, including enhancement of lipid peroxidation intensity in erythrocyte membranes, increase of  $K^+$  outflow and reduction of deformation ability of red blood cells. After the 7<sup>th</sup> day, the indices manifested the tendency to be recovered. Analyses performed on day 30 indicated that the values of studied indices became closer to those of untreated animals.

Conclusion: After a 4-day exposure to mm-range coherent EMWs, significant changes were recorded in the following indices of structure-functional state of erythrocytes in rats: lipid peroxidation processes intensity in erythrocytes' membranes,  $K^+$  permeability and deformation ability of red blood cells. Changes were observed till day 30 with the most evident expression on day 7 of post-exposure period.

## RADIOPROTECTIVE, ANTIOXIDANT AND CYTOGENETIC ACTIVITY OF COMPOUND # 3998

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Objective: The goal of research was to study the radioprotective capacity of arylsubstituted acetic acid amide, encoded as the compound #3998, which was selected amongst other arylsubstituted acetic acid amide derivatives based on expressed antioxidant and cytogenetic activity.

Methods: Antioxidant capacity of the lipid-soluble compound #3998 was evaluated with the help of a chemiluminescent analyzer PHOTOCHEM (AnalyticJena Co., Germany) and appropriate ACL-kit according to its inhibitory effect on luminescence generation by comparison with the standard substance (generation of a calibration curve with Trolox).

The cytogenetic studies involved the analysis of chromosome apparatus of the bone marrow cells (BMCs) of white non-linear rats treated intraperitoneally with the single dose of compound #3998 at 10 mg/kg. The animals were sacrificed in 24 hours and the appropriate BMCs preparations obtained. The frequency of non-stable chromosome aberrations (ChA), proliferative activity (PA) of BMCs as well as the quantity of polyploid cells (4n) was taken into account.

To determine the radioprotective properties of the compound tested, the rats were treated with compound #3998 at the dose level of 20 mg/kg in 0.5 ml of water suspension 1 hour prior to X-irradiation at dose level of radiation exposure that corresponded to LD<sub>90-100/30</sub> (~7 Gy). Indices of animal survival and mean lifetime were determined in 30 days of post-radiation period.

Results: Compound #3998 possessed an antioxidative capacity calculated in equivalent units of Trolox: the extent of inhibition of the measured detector signal caused by 1 nanomole of this substance was equal to the degree of signal suppression produced by 1.12 nanomoles of the standard.

Compound #3998 did not affect the level of ChA (0% as in Norm), but it specifically influenced the mitotic spindle of animals' BMCs; this latter was manifested in decrease of their proliferative activity. In addition, compound #3998 caused the appearance of 4n cells (2.78% vs 0% in Norm).

On the background of preliminary administered compound #3998, the number of surviving animals 30 days after radiation exposure reached up to  $55.0 \pm 5.5\%$ , and their mean lifetime was equal to 20.5 days.

Conclusion: Compound #3998, as a representative of aryl substituted acetic acid amides, exhibits highly expressed radioprotective activity.

## SUNBED USER'S MOTIVATIONS, KNOWLEDGE AND HABITS IN BUDAPEST, HUNGARY

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### Objective

The aim of this study was: to recognise the main motivations of sunbed use of the Hungarian public, the user's knowledge of health effects and their habits related to tanning in sunbed and/or by sunlight. Being in possession of this knowledge it will be possible to establish better education programs for the public in order to be able to reduce the potential health consequences of excess artificial UV exposure.

### Methods

The study was performed in five sunbed salons in Budapest. Sunbed users were asked to complete a questionnaire containing 59 questions, covering the following main topics: personal data(age, gender, profession, education), data of skin(skin type, number of moles, allergy), habits in sunbed use(age at first use, seasonal frequency, use of cosmetics and goggles, frequency of erythema), motivations of sunbed use, opinion about health effects of sunbed use and UV radiation, habits related to exposure to natural sun radiation, health awareness(body weight, sport activity, smoking, drinking), information sources about health effects of sunbed use.

The data will be stored on computer and statistically analysed by EPI INFO 6.04 software intended to epidemiological use.

### Results

443 of 500 questionnaires (88,6 %) were completed by customers of five sunbed salons in the Hungarian capital between 24/03/2004 and 31/05/2004. Preliminary statistics of the first 100 completed questionnaires are the following: 55 % of all users were woman. The average age was similar in both gender: 28 and 29.2 years in case of men(M) and women(W), respectively. The average age of first sunbed use was 21/22 years M/W, respectively. Most of the participants (72 %) completed secondary school. Men use sunbed more in yearly average, than women (78/45, M/W). 54 % classified her/his skin into the Type 3 (well tanning) within the 4 levels scale. Although, nobody(!) classified her/his skin into Type 1 (the most sensitive to burn) 48 % of individuals reported rarely occurring "sun"burn due to sunbed (19 % once, 22 % never) and 3 %(3 men) frequent burning.

Final results will be presented at the conference.

## DOSE AS A KEY PARAMETER AND THE LNT HYPOTHESIS IN RADIATION RESEARCH

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### Objectives

A basic question of current radiation biology, radiation protection and cancer research is the shape of the dose – biological effect curve at low doses. In the literature, significant efforts have been made to answer this query. The reply of the international radiation protection organizations is that the linear-nonthreshold (LNT) relationship should be applied as long as there is not enough information for the exact form of the dose – effect curve. Based on the NCRP 139 Report (“Evaluation of the Linear No Threshold Hypothesis in Radiation Protection”) there is not enough arguments and proofs to deviate from the LNT approximation. There are some new arguments that under a given radiation exposure one should neglect the health effects of radiation. The present lecture points to the multi-parameter nature of the analyzed relationships and demonstrates that in general a unified dose – effect curve cannot exist.

### Methods

Three possible methods will be presented and compared for the description of the dose – effect relationship. The introduction of several dose definitions such as the equivalent and effective doses could be the direction of one of these methods, but we should continue with all the parameters which affect the health consequences.

### Results

The lecture demonstrates that the dose – effect relationships cannot be described by a single curve because they form a multidimensional surface. The reason why the measured parameters show high uncertainty at low doses originates from this feature. At low doses, not the dose but other parameters have or may have much higher role than the dose in the biological responses. Thus, there is no sense to search for a unique dose – effect curve. Under a particular dose, it is not the dose but other physical, chemical and biological parameters, which basically determine the health effects. Even, this dose limit may depend on the other parameters. The search for these dose limits is an important task.

### Conclusions

The uncertainties in dose – effect relationships at low doses do not originate from the shortage of our knowledge but from the improper expectations associated with the LNT hypothesis.

## WHOLE BODY AND SPECT EXAMINATIONS IN ONCOLOGICAL DOG PATIENTS BY TC-99M DMSA(V) INJECTION

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Objective: <sup>99m</sup>Tc-DMSA(V) oncological scintigraphy only at a few nuclear centers is a clinical routine in detecting, staging and follow-up oncological patients. The aim of this study was to prove the tumor-affinity characters of <sup>99m</sup>Tc-DMSA(V) in vivo, in canine oncological patients.

Methods: Altogether 121 dog patients with the history of soft tissue (sarcomas, mastocytomas, mammary tumors, histiocytoma and schwannoma) and bone (osteosarcomas, fibrosarcoma and metastatic) tumors were referred to our laboratory for scintigraphical examination. One (early) and four hours (late imagination) after administering 80-120 MBq <sup>99m</sup>Tc-DMSA(V) (Penta-DMSA<sup>®</sup>, N.R.I.R.R., Hungary) per 10 bodyweight kgs, animals were sedated, thereafter wholebody, static and SPECT gamma camera (Nucline X-ring, Mediso Ltd, Hungary) images were taken. Pictures were evaluated visually and quantitative data e.g.: tumor uptake of ID and ROI rate (tumor/contralateral site) were calculated. Group I: clear vizualization of primary tumor and metastases, uptake>3%, ROI-rate>1.5. Group II: tumors are visible, 3%>uptake>1%, 1.5>ROI-rate>1.2. Group III: tumors are not visible, uptake <1%, ROI-rate<1.2.

Results: All the bone tumor (n=20), mastocytoma grade III (n=16), soft tissue sarcoma (n=12), histiocitoma (n=3) and schwannoma (n=2) bearing dogs, mammary carcinomas (16 out of 19), mammary fibrosarcomas (8 out of 10) and mastocytoma grade I – II (3 out of 13) belong to Group I. Remaining 3 mammary carcinomas, 2 mammary fibrosarcomas, all the perianal gland adenocarcinomas (n=8) mastocitoma grade I – II (10 out of 13) malignant lymphomas (2 out of 6) stay at Group II. In Group III. <sup>99m</sup>Tc-DMSA(V) oncological scintigraphy has basically failed in detecting all of the liposarcomas (n=3), and mammary fibroadenomas (n=9), furthermore most of malignant lymphomas (4 out of 6). In Group I and II all the therefore known tumors were vizualized by scintigraphy, and there were 17 cases in Group I where SPECT imagination was able to detect small, earlier not known metastases.

Conclusion: <sup>99m</sup>Tc-DMSA(V) oncological scintigraphy proved to be helpful in localizing a variety of soft tissue-, and bone tumors in canine oncological patients. Further studies needed to elucidate the sensitivity of the method in veterinary patients.

## HEALTH EFFECTS IN NUCLEAR INDUSTRY WORKERS OF UKRAINE: PROBLEMS AND PERSPECTIVES

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**Objective.** Professional hazards in staff of nuclear power plants include high psycho-physiological loads especially in operators and possibility of overexposure. These factors are accompanied by a complex of physical factors, hypoxia, work regimens, radioactive dust environment for the staff of Shelter object.

**Methods.** A study of 2200 radiation workers from 2 Ukrainian NPPs (operative, administrative staff) and Shelter object was performed. Investigation protocol included a set of clinical, instrumental and laboratory studies.

**Results.** Biological effects of radiation exposure at doses not exceeding limits for professionals included a set of sub-threshold anon-specific reactions of adaptive origin such as activation of DNA synthesis, calcium channel, elevated expression of immune cells activation antigens, cholesterol synthesis and lipids peroxidation. Radiation induced changes (chromosome aberrations, TCR mutations, micronuclei) were shown only in the cases of overexposure. The development of the exhaustion is long and associated with the violations of the oxidative homeostasis, neuro-humoral regulation and immune function with the threshold value of 100-150 mSv. Object "Shelter" staff was heterogeneous by the revealed health effects. Health problems were revealed in over-exposed during the first year of the Chernobyl clean-up works with the progression of psychosomatic, respiratory and digestive systems pathology, vascular and immune function changes. In recovery operation workers exposed over 250 mSv psycho-neurological changes were revealed in 80.5%, in exposed below 250 mSv – in 21.4% ( $p < 0.001$ ).

**Conclusion.** Adoption of the ICRP controlled dose strategy led to the changes of the implemented practice of radiation protection with the attention to the individual radioprotection and re-evaluation of the health care survey protocols and legislation.

## ONCOHEMATOLOGICAL EFFECTS IN ACUTE RADIATION SICKNESS SURVIVORS

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Several issues of the hematological diseases origin in persons having high radiation doses remain disputable. Any studies in this field are priority-driven. Elaborated health monitoring system for the acute radiation sickness (ARS) survivors after the Chernobyl NPP accident provided opportunity to analyze in them the oncohematological morbidity. Individual absorbed doses in 96 ARS survivors were more than 2.5 sGy. Origination of tumors resulted from the hemopoietic cell neoplastic transformation in ARS patients just can be the consequence of radiation exposure. There were so far 5 cases of hematological diseases revealed in the described group i.e. 2 MDS, 1 CML, 1 AML and 1 hypoplastic anemia ones; 3 solid tumors (sarcomas) were diagnosed too. Four diagnoses were fixed in convalescents of ARS grade 2–3 corresponding to the actual opinions on dose-effect under impact of ionizing radiation. High ratio of MDS cases to all the myeloid leukemia ones (1.5:1) was found in ARS grade 1–3 convalescents, being comparable to that in persons receiving thorotrast (4:6) and much higher than in Japanese (1:10) exposed mainly to  $\gamma$ -radiation after the A-bombing in 1945. MDS in ARS grade 1–3 convalescents were peculiar with long-term benign period with unstable leuko-, neutro- and thrombocytopenia on background of bone marrow hypocellularity as against hypercellularity in the initial period in the control group. Hypocellularity remained in 2/3 of patients along all the survey period upon diagnosis established. Adipose tissue prevailed over the hemopoietic one in bone marrow trepanobiopsy material. Reticular fibrosis was found. Progressing of clinical and laboratory findings both with primary resistance to applied therapy and short life-span were peculiar to AL and CML cases.



## SYSTEM OF MEDICAL REHABILITATION FOR PERSONS, WHO HAD SUFFERED IN RADIATION ACCIDENTS

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A system of medical rehabilitation was elaborated, above all, for persons, who had suffered from acute radiation sickness (ARS), and clean-up staff of Chernobyl Nuclear Power Plant. We took in account gradual forming a non-specific long-term radiation pathology in victims, which is connected with destructive processes in slowly proliferating and non-proliferating cell systems (brain, parenchymatous organs, endocrine glands etc.) and with special type of their reparation namely compensatory hypertrophy.

During 10 years after the Chernobyl accident we were studying health condition in 90 ARS convalescents, 168 irradiated victims with absorbed doses higher than 0.25 Gy, and on this basis the rehabilitation system was worked out. From 1996 to 2004 the rehabilitation system was being improved permanently due to use new drugs and methods of non-drug treatment.

All rehabilitation measures were divided on the three stages. On the first stage, in hospital, patients are examined comprehensively in order to determine both the type of pathological changes and the developing of diseases in comparison with previous years. The scheme of pharmacological treatment is chosen according to terms passed from the moment of irradiation. Such drugs as antioxidants, radioprotectors, adaptogenes, hemostimulators, immunomodulators, membrane defending remedies, hepatoprotectors, nootropics and vasoactive substances play the main role in the therapy on this stage. In hospital we also use massage, balneotherapy and therapeutic physical training.

On an outpatient stage of rehabilitation the patients visit periodically SCRM outpatient department where their pharmacological course of treatment is being done. They received reflexotherapy and hypnosis in order to refuse from bad habits (tobacco smoking, alcohol consumption) and learned the self-control methods for use therapeutic physical training at home. The health style of life is being propagandized as well.

The third stage of the rehabilitation system is a sanatorium-and-spa treatment. There are many different health-resort zones in Ukraine where natural and preformatted physical factors (balneotherapy, air-cure, mud cure and phytotherapy), therapeutic physical training and walk, dietotherapy and psychological relief are being used widely.

The rehabilitation measures in hospital, outpatient department and sanatorium are based on the principle of continuity, self-descriptiveness, complex use of all medicinal factors. A long-term use of the rehabilitation system resulted in steady remission of different somatic and neurological diseases in ARS convalescents and patients with high-absorbed doses but it does not stop stochastic pathology.

## DYNAMIC CLUSTERING AND CO-LOCALIZATION OF 53BP1/ $\gamma$ -H2AX FOCI

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The residual DNA double strand breaks (DSBs) may be useful in assessment of radiosensitivity in the dose range that is relevant for radiotherapy. A novel assay for radiosensitivity has been recently suggested as based on measurements of residual foci (RF) produced by 53BP1 and  $\gamma$ -H2AX that co-localize with DSBs (1, 2). Here, we investigated time kinetics for  $\gamma$ -ray-induced 53BP1 and  $\gamma$ -H2AX RF in human normal fibroblasts (VH-10) and cancer cell line (HeLa). DSBs and foci were studied by pulse field gel electrophoresis and confocal laser microscopy, respectively. The linear dose response was observed for foci at 30 min after irradiation. Dose of 1 cGy induced significant amount of foci. The amount of these primary foci decreased with time after irradiation. Despite this decrease, the area of foci as measured by the dedicated software did not change. The foci become larger in size. These data suggested that primary foci clustered. We speculate that slow repair of DSBs occurs in foci following their clustering. RF remained 24 h after irradiation of VH-10 cells. No foci were observed 24 h post-irradiation in HeLa cells. The data suggested that radiation-induced foci do not enter the next cell cycle following irradiation. Linear-quadratic or linear response was observed dependent on cell type and the time after exposure, 12-24 h. The co-localization of residual  $\gamma$ -H2AX and 53BP1 foci was dose- and time-dependent. RF remained in nuclear matrix upon extraction of proteins and were observed in metaphases. The dose responses for RF in HeLa and VH-10 cells 12 h post-irradiation correlated with dose responses for clonogenic survival. We conclude that for radiosensitivity assay, the time for analysis of RF should be less than duration of cell cycle or cells should be kept in confluence.

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## MICROWAVES FROM MOBILE PHONES AFFECT HUMAN LYMPHOCYTES FROM NORMAL AND HYPERSENSITIVE PERSONS

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We have recently described frequency –dependent effects of mobile phone microwaves (MWs) of Global System for Mobile Communication (GSM) on chromatin conformation and DNA double-strand break (DSB) co-localizing proteins 53BP1/ $\gamma$ -H2AX in human lymphocytes. Contrary to GSM phones, mobile phones of the 3<sup>rd</sup> generation irradiate UMTS (Universal Global Telecommunications System) signal at one from several possible frequency bands. MWs representing wide-band signal may result in higher biological effects because of eventual “effective” frequency windows. Here, lymphocytes from persons reporting hypersensitivity to MWs and matched healthy persons were exposed to GSM (905 MHz and 915 MHz) and UMTS (1947.4 MHz, middle channel), output power being the same, 0.25 W. Chromatin condensation indicative of stress response and genotoxic effects and DNA double strand break (DSB) co-localizing 53BP1/ $\gamma$ -H2AX foci were analysed using method of anomalous viscosity time dependence and laser confocal microscopy immediately, 24 h and 72 h following 1 h exposure. UMTS MWs induced significant stress response or/and DNA damage in human lymphocytes from both normal and hypersensitive subjects. UMTS MWs affect human lymphocytes stronger or in the same manner as GSM MWs. Remarkably, the effects of MWs on 53BP1/ $\gamma$ -H2AX foci persisted up to 72 h following exposure of cells. In conclusion, we confirmed our previous observations regarding frequency-dependent effects of MWs from GSM phones and report for the first time that UMTS MWs induce significant and rather stable biochemical responses in human lymphocytes.

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## STUDIES OF RADIATION INDUCED BYSTANDER EFFECTS IN 3D TISSUE MODELS

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The overall objective of this ongoing research is to study mechanisms of bystander effects induced by ionising radiation in three-dimensional (3D) tissue systems. Bystander effect is a non-(DNA)-targeted phenomenon whereby cellular responses are expressed in non-irradiated neighbouring cells near to an irradiated cell or group of cells. The final goal of this study is to develop a unifying hypothesis for the role of non-targeted effects in radiation-induced tissue responses and, ultimately, carcinogenesis.

Studies of bystander effects using *in vivo* like tissue systems were carried out during several years in Gray Cancer Institute (GCI), UK, Columbia University (RARAF), USA and Radiation and Nuclear Safety Authority (STUK), Finland. We started this work with *in vitro* microbeam irradiations of primary porcine and human ureter explant systems, continued with *ex vivo* primary porcine ureter 3D tissue systems and *in situ* microbeam irradiation. Later, this line of research was expanded to *in vivo* like artificial human 3D tissue systems. Microbeam is a powerful tool for investigating mechanisms of bystander effects, which allows irradiation of individual cells with a precise numbers of particles with *micrometer* precision. In our research we were using GCI ( $^3\text{He}^{2+}$  ions, protons) and RARAF ( $\alpha$ -particles, protons) charged particle microbeams. We evaluated bystander induced cell survival assays, estimated DNA and chromosomal damage, studied changes in gene expression, differentiation and proliferation.

We demonstrated a direct evidence for the bystander effect in 3D tissue systems, established a dose dependency and estimated the role of differentiation vs. damage induction processes. A gross bystander induced differentiation in the urothelial explant outgrowth after microbeam irradiation was observed indicative of a protective response. We found a strong proliferation-dependent bystander effect in urothelial explants after microbeam irradiation. Finally, studies with artificial human 3D tissue systems demonstrated that bystander induced apoptosis could be detected at the distance over 900 micrometers from irradiated cells, which suggest that hypothetical bystander factor has a long range, could travel through 3D tissue and possibly gap junctions mediated.

Our studies are relevant to several areas related to radiation protection. In particular, bystander effect is potentially significant for radiation regulation issues and may have implications for the applicability of the Linear-No-Threshold (LNT) model in extrapolating radiation risk data into the low-dose region.

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## "CONTRIBUTION OF THE VASCULATURE RESPONSE TO THE ACUTE RADIATION SYNDROME : POSSIBLE THERAPEUTIC WINDOW"

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Evidence from accidental situations shows that acute exposure can be total or localised but for the majority of cases the dose distribution is heterogeneous. However, the physical parameter “dose” is not sufficient to predict the clinical evolution of damage in an individual patient. One conclusion of METROPOL team was to point out the integrative and dynamic impairment of the organism by ionising radiation and that this must be the rational basis for therapeutic strategies. Moreover, a consensus exists to indicate that the clinical management of haematopoietic aplasia, a predominant factor in patient survival, is now possible. Progressive, sequential and single versus multiple systems failure have now to be considered, with regard to the most critical organ systems (hematopoietic, cutaneous, gastrointestinal and pulmonary systems). The vascular reaction can be considered as a “critical check-point” for individual and multiple organ responses so providing a multi-organ “therapeutic window”.

Experimental data on three critical organ systems with regard to the acute radiation syndrome (skin, lung, intestine) have been obtained to support the view that the acute response to tissue injury is intimately linked to the functional and structural damage to the vascular system. Depending on the dose, the irradiated volume and the exposed tissue, the vasculature elicits differential responses in the irradiated area which may play a central role in initiating and perpetuating local as well as distant organ responses. Thus the vasculature should be considered as a target, not the sole, but rather integrated with other entities participating in organ dysfunction. Possible therapeutic windows will be discussed which target vascular dysfunction and/or activation. A combination of the pleiotropic cytokines interleukins 4 and 11 with thrombopoietin were shown to improve survival by limiting vascular leakage, which in turn could limit inflammatory reactions and the ensuing tissue damage. The use of statins will be also discussed. For doses of irradiation that cause vascular destruction, progenitor cells may provide an opportunity for therapeutic intervention in order to restore vascular integrity as well as the use of growth factors.

Integrated, dynamic biological responses now have to be considered, instead of a “system-by-system” approach. It is clear from data obtained from radiation accidents that multi-organ dysfunction or failure has a pivotal role in patient survival. This concept will be discussed on the basis of some of our experimental data.

## FOLLOW-UP OF PLASMA FLT3 LIGAND CONCENTRATION IN HEMATOPOIETIC STEM CELL TRANSPLANTED PATIENTS.

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*Objectives:* Previous works indicated that Flt3 ligand (FL) concentration was increased in patients suffering from aplasia either acquired (such as aplastic anemia or fanconi anemia) or induced by chemotherapy, suggesting a possible use of FL as a biological indicator of bone marrow aplasia. The aim of the present work was to follow-up variations in plasma FL concentration during radiation or chemotherapy induced aplasia, in order to compare the influence of conditioning regimen on FL concentration modifications and to define the role of circulating cells in the regulation of FL during aplasia.

*Material and methods:* 10 patients undergoing conditioning regimen including BEAM, cyclophosphamide + total body irradiation (TBI) or cyclophosphamide + anti-lymphocyte serum (ALS) and followed by haematopoietic stem cell transplantation were enrolled in this study. Plasma FL concentration was measured by ELISA, membrane-bound form of FL (mFL) on the surface of circulating white blood cells (WBC) was determined by FACS analysis and FL mRNA expression in WBC by RT-PCR.

*Results:* We showed that FL concentration increased rapidly during conditioning regimen in all patients. At any time point, FL concentration was negatively correlated with the number of WBC. Area under the curve (AUC) of FL according to the time was directly correlated with the duration of aplasia, indicating that blood FL concentration was a reflect of aplasia. However, in ALS treated patients, a reduction of FL AUC was observed. This reduction was due to a rapid decrease of blood FL concentration down to normal values, before the WBC recovery. As a consequence, FL AUC did not correlate with duration of aplasia.

*Conclusion:* Our results indicate that FL concentration variations in the blood are the reflect of either radiotherapy- or chemotherapy-induced aplasia. Moreover, these results showed that the regulation of FL concentration in the blood of patients by WBC is mainly implicated during the recovery period of aplasia.

## APPLICATION OF AUTOLOGOUS CELL THERAPY IN THE CONTEXT OF ACCIDENTAL HETEROGENEOUS IRRADIATION

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Although rare, accidentally irradiated victims remain difficult to treat, mainly due to the complexity of radiation-induced physiopathology. In fact, the acute radiation syndrome (ARS) appears as the addition of several pathologies such as the hematopoietic syndrome, the gastro-intestinal syndrome and the cerebro-vascular syndrome, but also kidney and lung disease or cutaneous syndrome. As a result, the overall long term survival of victims irradiated at whole body doses above 5 Gy is about 16%, highlighting the need for new therapeutic approaches of accidentally irradiated victims.

Over the past decade, increasing amount of data indicated that *ex vivo* expanded hematopoietic cells are highly efficient in the treatment of chemotherapy-induced aplasia. Thus, it was proposed that autologous hematopoietic cell therapy could be applied to the treatment of accidental radiation-induced hematopoietic syndrome. Such an approach is based upon two hypothesis. The first hypothesis is the heterogeneous nature of accidental irradiation, which suggests the existence of bone marrow sites that are protected from irradiation, as it was demonstrated in several radiation accidents. The second hypothesis is the possibility to expand hematopoietic stem cells that were previously irradiated. This was also demonstrated by several teams, although with a limited efficiency. As compared to classical stem cell transplantation used in recent radiation accidents, such a cell therapy approach might have several advantages, among which the autologous context of cell therapy, which avoid risks of GVHD, graft rejection, and conditioning regimen. However, such a protocol needs important developments, including the technical requirements for hematopoietic cell expansion. Moreover, these studies need to be developed in a large animal model such as non-human primates with an heterogeneous irradiation that might be representative of an accidental irradiation situation. Such a model may help to define the limits of cell therapy, both for dose range and therapeutic efficiency. Despite these limitations, autologous cell therapy appears as a promising approach, that may be applied with other cell types such as mesenchymal stem cells or multipotent adult stem cells. Such protocol may be useful also for the study of physiopathology of the ARS.

## CYTOGENETIC EFFECTS IN BLOOD LYMPHOCYTES OF PEOPLE RESIDING OR WORKING IN CHORNOBYL NPP EXCLUSION ZONE

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**Objectives.** The dynamic cytogenetic examination of people residing without permission in the villages of a 30-km ChNPP Exclusion zone and of the personnel working on “Shelter” Object was conducted. As a respective control the group of village inhabitants of Kiev region, not contaminated with radionuclids of Chornobyl origin, and groups of residents from Kiev and Slavutych (the place of residence of ChNPP personnel and their families) pertained to radiation control zone were examined.

**Method.** The method of conventional chromosomal aberration (CA) analysis was used for this study.

**Results.** Zone self-settlers were shown to have a significantly higher interindividual variability and mean group CA frequency compared to the control ( 5,7% versus 3,5%) not only of a chromosome-type (unstable exchanges – 2,5-fold) but also of chromatid-type aberrations caused mainly by effects in people younger 60. It was found a higher sensitivity *in vitro* to bleomycin correlated with CA frequency *in vivo*. From 1998 it was revealed no alterations of total CA along with a decrease of chromosome-type aberrations owing to fragments reduction. Chromosome exchange frequency and “rouge” cell (0,10 %) frequency did not significantly altered. It was pointed on repeated finding of “rouge” cells in single individuals.

In Slavutych residents, having no occupational contact with radiation, it was revealed a higher CA frequency compared to Kiev inhabitants caused by internal irradiation at the expense of local foodstuff consuming. In “Shelter” Object personnel working directly on 4<sup>th</sup> block and adjacent areas cytogenetic effects frequency was significantly higher than that of Slavutych residents (chromosome exchanges – 2-fold), in personnel working in administrative buildings of free access area cytogenetic effects frequency was similar to Slavutych group's. "Rouge" cell frequency in contamination control zone workers was 0,2%, in free access area group 0,12% and in Slavutych group 0,09%. Occurrence of such cells is connected, probably, with getting into the body of  $\alpha$ -emitting transuranium elements not only by aerosol inhalation but also with local foodstuff.

**Conclusion.** Obtained results testify to the general stabilization of cytogenetic effects in blood lymphocytes of Exclusion zone self-settlers in remote period after the accident. Cytogenetic effects in the personnel group caused not only by specific radiation conditions on “Shelter” Object but also radio-ecological situation in Slavutych.



## GENOTOXIC EFFECTS IN PERIPHERAL LYMPHOCYTES OF PATIENTS TREATED WITH ANTINEOPLASTIC DRUGS

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**Purpose:** The aim of this study was to determine the changes in somatic cells after chemotherapy.

**Patients and methods:** This prospective study included 60 patients. According to the histological results and underwent various therapies they were divided into four groups: 1.Group I - All patients underwent orchidectomy of the affected testis and then had one, two or three cycles of Paraplatinum at doses from 450 mg to 750 mg, depending on the body surface area. 2.Group II - Patients with nonseminoma tumors, who underwent orchidectomy, had two, three or four cycles of BEPV – Bleomycin, Etoposide, Cisplatin, Vinblastin. 3.Group III - Patients with Hodgkin's disease, who had 6-9 cycles MOPP/ABV – Mechlorethamine, Vincristine, Procarbazine, Pronison/Doxorubicin, Bleomycin, Vinblastine. 4.Group IV - All patients underwent orchidectomy of the affected testis and then surgery lymph nodes or only observation.

The changes in the genome of individual cells were detected by *Structural chromosomal aberrations* (CA). Structural chromosome damage was categorized as chromosomal breaks, acentric fragments, dicentrics and ring chromosomes. Gaps were not included in the total number of CA. Mutagenetic test were performed at diagnosis, immediately after the completion of treatment and six month after it. To represent differences in the genome picture between Group I, Group II and Group III the Kruskal-Wallis (KW) test and Mann-Whitney (MW) test and Bonferroni's correction ( $p < 0,017$ ) were used. Group IV was the control group.

**Results:** Before treatment, the patients showed no deviations in the genome picture in comparison with the control group as to the number of CA, chromatid breaks, dicentrics and acentric fragments. After treatment we observed a strong inhibition of the mitotic activity of lymphocytes in the Group II and III. It was determined that this phase ended within 6 months and the mitotic activity of lymphocytes returned to normal. The number of cytogenetic changes was lower, but still considerably higher than before treatment. The percentage of CA was higher after chemotherapy MOPP/ABV than after chemotherapy paraplatinum ( $p = 0,001$ ), although after chemotherapy according to BEPV schedule and chemotherapy with paraplatinum, the percentage of CA was not significantly different ( $p = 0,146$ ). The types of chromosomal changes in the observed patients were different and the number of dicentrics were significantly higher ( $p = 0,005$ ). Six months after the completed treatment the chromosome damage occurred. CA did not differ much from the values measured immediately ( $p = 0,175$ ).

**Conclusion:** These statistically significant values determined for CA may be associated with the predominance of the cells afflicted with different types of genome changes, having a causative relationship with secondary tumors.

## BYSTANDER EFFECTS IN A HELA $\times$ FIBROBLAST CELL LINE FOR NEOPLASTIC TRANSFORMATION AND CELL KILLING.

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The existence of the Bystander effect is now indisputable however the extent of its implications with regards to radiotherapy is still unknown. Radiation induced bystander effects are observed when neighbouring cells are irradiated, or when media from irradiated cells is transferred to unexposed cells. In this study the bystander signal carried in the medium, and passed between cells (not in contact) has been investigated.

Cultures of CGL1 (human HeLa  $\times$  skin fibroblast hybrid cell line) cells were irradiated using a high LET  $\alpha$  particle Pu-238 source and a low LET  $\beta$ -particle Sr-90 source. Two different methods and end points were used in this investigation. The potential for  $\alpha$ -irradiated cultures to induce neoplastic transformation in non-irradiated cells was assessed. A cell survival end point was used to investigate the Bystander effect instigated by medium transfer. Neoplastic transformation frequency for the  $\alpha$ -irradiated cultures where cells could communicate, and survival fraction (SF) for the  $\beta$ -irradiated cultures where medium was transferred from irradiated cells to unexposed cells.

The results showed a consistent reduction in plating efficiency (16-31%) and SF (17-20%) for the bystander cells, when compared to parallel control cells, with an independence of dose above 1 Gy. For the first time a bystander effect for the induction of neoplastic transformation has been demonstrated. CGL1 cells exposed to a dose of 1 Gy of  $\alpha$ -particles produced a significant enhanced (above the spontaneous level) transformation frequency in neighbouring but unexposed CGL1 cells. The observed transformation frequency in the bystanding cells was equivalent to that for cells exposed to an acute dose of  $\alpha$ -particles of 0.6 Gy. These studies will be extended to lower doses of  $\alpha$ -particles. These results may have important implications for the risk assessment of low doses of  $\alpha$ -particles.

In conclusion, bystander effects have been shown to exist for both cell killing and neoplastic transformation. The relevance of these results for radiotherapy – in terms of induction of cancers to exposed normal tissue – and for radiation risk will be discussed.

## SAMPLE PREPARATION METHODS FOR THE MEASUREMENT OF RADIUM VIA ALPHA-SPECTROMETRY

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One of the most difficult ones from naturally occurring isotopes is the determination of radium isotopes. It has several causes: for example the low activity concentration in the environmental samples and the complicated source preparation due to alpha spectrometry.

The most popular and most simple methods is using selective reagent to concentrate radium isotopes.

One of these methods is the utilization of the high selectivity of the  $\alpha$ -MnO<sub>2</sub> crystal structure.

It has been reported by several investigators that the MnO<sub>2</sub> coated manganese fibres can be used to pre-concentrate radionuclides from large water samples and other researchers applied MnO<sub>2</sub> coated polyamide disks for source preparation.

Another way is using selective organic materials, for example 3M Rad Disk. The problem of this method is the high cost.

In this work we would like to present our attempts on new methods of the source preparation.

Various MnO<sub>2</sub> surfaces were used, on the one hand polished MnO<sub>2</sub> surfaces and thin MnO<sub>2</sub> films and on the other hand membrane filters coated with some organic reagents for source preparation for alpha spectrometry.

In case of MnO<sub>2</sub> surfaces the executed experiments showed that the method can be applied for the detection of <sup>226</sup>Ra, however the resolution was low. It can be caused by the surface roughness and by the width of the MnO<sub>2</sub> coat. Whereas the surface roughness of MnO<sub>2</sub> cannot be smoothened more, we had to find other ways to produce smooth MnO<sub>2</sub> surfaces on other materials, like stainless-steel, nickel, copper and chrome plate.

In case of organic membrane filter measurements some new organic complexing agents with large molecules were used for source preparation, for example modified calixarenes.

The source preparation methods examined were found to be good for the determination of radium isotopes, but unfortunately these methods are not as effective as the already used methods. Therefore, we would like to carry on with the examination of these methods in order to improve them as they make source preparation methods rather simple.

## COMBINED EFFECTS OF RADON, ASBESTOS, MINERAL DUSTS AND HEAVY METALS AT CELLULAR LEVEL

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Radon, asbestos, mineral dusts and heavy metals are some of the important environmental pollutants. The alpha-particles irradiation, asbestos, mineral dusts and heavy metals act by generation of reactive oxygen species (ROS). The biological effects observed are: DNA damage, mutation induction, micronucleus induction, changes in gene expression or cell killing. Our objectives were to study the DNA damage and cytotoxicity induced by the combined effects of these pollutants.

Different cells (HFL-1, CHO) were irradiated with 2 and 10 mGy alpha particles and treated with 2, 5, 8 and 10 mcg/cm<sup>2</sup> asbestos, glass fibres (biosoluble and non biosoluble) or 0.0005-10 mM heavy metals (Cd, Ni, As). To study the DNA damage and repair the treated cells were subjected to alkaline Comet Assay in different time after the exposure (0'; 5'; 20'; 1h, 4h and 24h). For studying the cytotoxicity, the treated cells were incubated for 1, 24, and 48 hours and the survival was checked with MTT technique.

Asbestos and heavy metals alone induced the decreased cell survival as the dose increased. The combined treatment of radon and asbestos increased the cell death. The 2 and 10 mGy alpha irradiation and the low concentration of asbestos (1-2 mcg/cm<sup>2</sup>) alone were not enough to enhance the DNA fragmentation, compared to control group. Raising of asbestos concentration up to 5 and 10 mcg/cm<sup>2</sup> resulted in a higher DNA fragmentation. The biosoluble glass fibres (*bgf*) caused less DNA damage than the non biosoluble glass fibres (*nbgf*). *Nbgf* were longer fibres than *bgf* and hereby were more DNA damaging. Combination of *nbgf* and alpha particles induced a lower DNA damage than *nbgf* alone. The combined effect of *bgf* and alpha particles resulted in a higher DNA damage, initially. The rejoining of the breaks started after 20 minutes repair and was almost completed at the end of 4<sup>th</sup> hours. Increased cytotoxicity was found after treatment of irradiated cells by toxic metals at higher concentrations. Concentration under 0.01mM of Cd and Ni increased the survival of irradiated cells compared to cells exposed to 10 mGy alone.

The understanding of the exact mechanism of asbestos fibre carcinogenesis is still obscure today. One of the strong hypotheses is that fibres cause DNA damage through oxidant mediated mechanism. The result presented here show that another physical carcinogen i.e. low dose alpha particles enhanced the initial DNA damage caused by asbestos and its potential substitutes and also modified the kinetics of repair. Lower concentrations of heavy metals decreased the cytotoxicity of 10 mGy in case of Cd and Ni and enhanced it in case of As.

## THE NATURE OF PSYCHOPHYSIOLOGICAL DISORDERS AMONG CHERNOBYL NUCLEAR STATION ACCIDENT RECOVERY WORKERS.

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Asthenic and asthenoneurotic syndromes are the basic for development of chronic diseases of central nervous system in recovery workers after Chernobyl accident. They have been formed long before main disease became nosologically defined. The mechanisms underlying clinical and behavioral manifestations of chronic exhaustion and asthenia among recovery workers of young and middle age have been investigated. On the data of long-standing examinations ( 1991 - 2004 ) it has been observed intensive increase of irritative and dystrophic changes, as well as depletion of functional activity of CNS. Relatively young age of recovery workers (35 - 45) points to an early beginning and unfavourable course of vessel changes, that is the index of accelerated aging of recovery workers organisms.

Object: correction of the workers psychophysiological condition in the postextremal period for the medical – rehabilitation process and its effective assess by the objective controlling methods of psychophysiological condition.

Methods: EEG and evoked potential (EP), computer analysis of the bioelectrical brain activity, the psychodiagnostical questionnaires, confrontation of the complex psychophysiological examine results with the clinical – laboratory data.

Results: the psychoemotional strain and the reactive uneasiness were demoted to the norm, state of health and mood were evidently improved, the tone of a sympathetic department of vegetative nervous system was demoted, the parameters of the EEG and the EP became normal, the structure of the field of biological potentials of the cerebral cortex was restored, the functional condition of a brain was optimized without a sedation, dream became normal.

Deduction: The mental condition of the workers is instability. It cause an attrition of organism's resources under action of the strong psychological and physiological stress. It has the neurophysiological picture, received by means of the modern electrophysiological diagnostics methods. Pathogenesis of the psychophysiological disorders is defined by the systemic infringements in brain functional activity. Dysfunction of cortical and subcortical structure can be subject to the adequate psychophysiological correction. In the course of the correction it is objectively observed ordering of the structure of the brain biological potential field. It correlates with normalization of the workers clinical condition.

Key words: EEG, EP of the brain, computery methods of the analysis, diagnostic, psychophysiological condition, correction, postextremal period.

Shot name: «Diagnostic and correction of psychophysiological condition»

## GENE EXPRESSION CHANGES IN RAT SKIN AT CANCER INHIBITING LEVELS OF DIETARY RETINYL ACETATE AFTER EXPOSURE TO A 56FE ION BEAM.

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**Objective:** Cancer chemopreventive activity by dietary retinyl acetate has been established in rats exposed either to low or high LET radiation. The present study was an effort to learn how gene expression patterns differ at chemopreventive levels of the retinoid given as a 300 ppm dietary supplement.

**Methods:** Rats were randomly assigned to 2 groups of 48 each to be exposed or not to 3 Gy in the plateau region of a 56Fe ion beam. The initial groups were further subdivided into 4 equal groups receiving the dietary supplement or not. For irradiation a 4 x 6 cm area of dorsal skin was pulled up into the beam and held in place by surgical thread, while the body was shielded with a 33 cm thickness of polystyrene. Subsequent to exposure the rats were observed for onset of skin cancers and other evidence of tissue alterations, including suppression of hair growth and desquamation.

**Results:** The results indicate that the 56Fe beam is about 3 fold more effective than electrons for producing the skin responses and cancer induction. The retinoid was more effective for preventing electron-induced cancer (90%) in comparison to 56Fe-induced cancers (60%).

**Conclusion:** Expression microarray analysis indicated that alteration of proliferation and/or inflammation-related genes explain much of the observed preventive activity of the dietary retinoid. Work supported by NASA.

## GERMLINE MUTATION RATES AT ESTR LOCI IN P53 DEFICIENT MICE.

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### Objective.

To analyse the effects of p53 deficiency on mutation rates in the mouse germline. p53 tumour-suppressor protein is directly involved in cellular response to various genotoxic stresses, including cell cycle arrest, DNA repair and apoptosis. Given the important role of p53 in numerous cellular signalling pathways, it is therefore conceivable that the p53 deficiency could affect spontaneous and radiation-induced mutation rates. The *p53* knock-out mouse strain provides a useful model *in vivo* model for the analysis of the effects of p53 deficiency on somatic and germline stability.

### Methods.

Mutation rates at hypervariable Expanded Simple Tandem Repeat (ESTR) DNA loci were analysed in the germline of non-irradiated and irradiated (1 Gy of acute X-rays) *p53*<sup>+/+</sup>, *p53*<sup>+/-</sup> and *p53*<sup>-/-</sup> male mice (C57BL background).

### Results.

Mutation rates in the germline of non-exposed *p53*<sup>+/+</sup>, *p53*<sup>+/-</sup> and *p53*<sup>-/-</sup> male mice were similar and did not significantly differ from those in other wild-type inbred strains. Acute X-ray exposure to pre-meiotic spermatogonia resulted in similar increases in ESTR mutation rates in the germline of wild-type and p53-deficient mice. ESTR mutation spectra in the *p53*<sup>+/-</sup> and *p53*<sup>-/-</sup> strains did not differ from that in the isogenic wild-type *p53*<sup>+/+</sup> mice.

### Conclusions.

p53 deficiency does not affect spontaneous and radiation-induced ESTR mutation rates in the mouse germline. The effects of deficiencies at various DNA-damage response genes on ESTR mutation rates in the mouse germline will be discussed.

## PERIPHERAL BLOOD CD34<sup>+</sup> CELLS AT THE STAGE OF LATE RADIATION EFFECTS DEVELOPMENT

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**Objective.** Chernobyl recovery radiation workers exposed over 300 mSv demonstrate prevalence of immune function changes and are referred as a risk group of late radiation pathology development. CD34<sup>+</sup> hematopoietic stem cells seem to be key factors at the development of stable immune deficiency and leukemia.

**Methods.** Study of peripheral blood CD34<sup>+</sup> cells surface phenotype was performed by flow cytometry. Study groups included recovery operation workers 15-18 years after exposure – group 1 (n-16) – suffered from acute radiation syndrome, grade 2-3 (doses higher than 2 Sv); group 2 (n-20) – without history of bone marrow syndrome (doses lower than 1 Sv) and controls (n-16) irradiated at natural radiation levels.

**Results.** Number of CD34<sup>+</sup> cells in both groups of exposed was increased. This increase was significant after acute radiation syndrome ( $p < 0,001$ ). In both groups an increase of peripheral blood CD34<sup>+</sup> cell counts was associated with the most primitive progenitor cell subsets - CD34<sup>+</sup>CD90<sup>+</sup>CD45<sup>-/low</sup> and CD34<sup>+</sup>CD45<sup>-</sup>CD38<sup>-</sup>. Correlation analysis showed correlations between dose of exposure and CD34<sup>+</sup> cell counts only in exposed under 1 Sv (Spearman  $r = 0,56$ ) while in recovery workers after acute radiation syndrome no relationships were seen ( $r = 0,11$ ). Increased proliferation potencies were shown for hematopoietic progenitors in this group of exposed by the earlier study (N.Bilko, 1998).

**Conclusion.** High proliferation ability and accumulation of CD34<sup>+</sup> cells after high dose exposure seem to reflect the adaptation process. Its conservation at the level of primitive progenitors combined with the decreased apoptosis could be the background of the late radiation effects formation especially for leukemia.



## CHANGES IN CYTOPLASMIC ORGANELLES FOLLOWING IRRADIATION OR MEDIUM-TRANSFER TREATMENT

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Bystander effects in unirradiated neighbours of irradiated cells have often been related to changes in the cells' nuclear capability, observed for example as induction of chromosomal instability. There have been few reports of bystander effects in cytoplasmic organelles, despite the importance to the cell of proper functioning of this compartment.

The current study<sup>1</sup> uses two cell types, fibroblasts and neurons, with different functions and organelle profiles. The two cell types have been used as fibroblast/fibroblast and neuron/fibroblast 'pairings', with the first cell type named exposed to irradiation or sham treatment and the second being unirradiated but receiving medium from irradiated or sham treated cells. Alpha irradiation (0.5Gy) was used on MEF 348 fibroblast and NIE 115 neuroblastoma cell lines. There were eight groups; unirradiated and irradiated fibroblasts and neurons; and fibroblasts after incubation with medium transferred from irradiated or sham-treated fibroblasts or neurons. The times involved were 5 minutes between radiation and subsequent treatment and 60 minutes for incubation of transferred medium. Thereafter lysosomal and mitochondrial probes were added and images recorded with a confocal scanning laser microscope. At least 60 cells per group were analysed using an ImageProPlus image analysis package and quantitative data were collected for parameters relating to cell and nuclear dimensions and also area, number and intensity of lysosomes and mitochondria. The experiment was carried out twice, although sham groups were included in only one study.

In overall terms, untreated neurons and fibroblasts differed greatly. In the treated groups, changes related to fibroblasts were more often lysosomal, while those associated with neurons were more commonly mitochondrial. Alterations were seen after all types of treatment, including radiation and transfer of medium from irradiated and sham-treated cells, although neurons produced many fewer sham-associated effects than fibroblasts. For analysis of individual parameters, after statistical analysis, parameters were excluded where aspects of the data called for further study. The bystander response was different in neurons and fibroblasts for the percentage of cytoplasm taken up by lysosome probe-positive material. The proportion of heavily probe-positive mitochondria increased after irradiation and medium transfer for both irradiated cell types.

The study confirms that non-nuclear changes are seen in bystander cells, as reported earlier for changes in protein levels. It also shows that different cell types respond differently with respect to the level of change and to the organelle affected. Further work is needed on several aspects of the results, in particular to explain the effects produced by sham-treated medium.

<sup>1</sup> Supported by UK Department of Health

## LOW-LEVEL EXPOSURES TO IONISING RADIATION MODULATE THE ANTI-TUMOUR ACTIVITY OF MURINE NK CELLS

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The most important late effect of an exposure to ionising radiation is increased incidence of cancer in the exposed population. On the other hand, experimental evidence from the recent years indicates that low-level irradiations with X or gamma rays may inhibit the development of both primary and secondary tumours. It has been suggested that this beneficial effect is causatively related to stimulation of DNA repair processes, scavenging of free radicals, and/or stimulation of anti-tumour defence mechanisms. In fact, the results of our previous experiments demonstrated that whole body irradiation of mice with 0.1 or 0.2 Gy of X-rays led to the significant inhibition of the development of the induced pulmonary tumour colonies.

In the present study, 6-8-week old male BALB/c mice were exposed to a whole body irradiation with 0.1, 0.2, or 1.0 Gy X-rays and then NK cell-enriched suspensions were prepared from the spleens. FITC-tagged anti-mouse Pan-NK (DX5) antibody and PE-tagged anti-mouse FasL antibody were used to label NK cells. Anti-asialo GM<sub>1</sub> antibody was injected i.p. to block the NK cell-mediated activity. Cytotoxic activity of NK cells was estimated in vitro using the classical <sup>51</sup>Cr-release assay. Production of IFN-γ was examined with use of the ELISA test.

The results demonstrate that cytotoxic activity of NK cells collected from the irradiated mice is significantly stimulated compared to that of the NK effectors obtained from the sham-exposed, control mice. This effect was totally abrogated by the injection of the anti-asialo GM<sub>1</sub> antibody. Moreover, NK cells obtained from the irradiated mice exhibited reduced surface expression of the Fas ligand.

The obtained results suggest that the inhibitory effect of the low-level exposures to X-rays on the development of pulmonary tumour nodules may be causatively associated with stimulation by such exposures of the anti-neoplastic activity of NK cells.

## IONIZING RADIATION CAN UTILIZE A POSITIVE FEEDBACK REGULATION OF PKC $\delta$ FOR AMPLIFYING THE MITOCHONDRIAL ACTIVATION-MEDIATED APOPTOSIS MACHINERY IN NON-SMALL CELL LUNG CANCER CELLS

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**Objective:** The functional role of PKC $\delta$  in the process of apoptosis varies considerably with cell types as well as the apoptotic stimuli. Here, we show that PKC $\delta$  mediated-p38 MAPK activation is required in part for the induction of apoptotic cell death in human non-small cell lung cancer cells treated with ionizing radiation.

**Methods:** Radiation-induced apoptotic cell death was determined by flow cytometric analysis. Involvement of the mitochondrial pathway was examined by monitoring of the mitochondria membrane potential, cytochrome *c* release and Bax translocation.

**Results and Conclusion:** Ionizing radiation induced activation of PKC $\delta$  and p38 MAPK, dissipation of mitochondria membrane potential, activation of caspase-9 and -3 and subsequent cell death. Inhibition of PKC $\delta$  effectively attenuated ionizing radiation-induced p38 MAPK activation, mitochondria membrane potential loss and caspase-dependent cell death. Furthermore, inhibition of p38 MAPK suppressed ionizing radiation-induced loss of mitochondria membrane potential and activation of caspase-9 and -3. Ionizing irradiation also induced cleavage of PKC $\delta$  and its translocation to the mitochondria. Pretreatment of caspase-3 specific inhibitor attenuated radiation-induced cleavage of PKC $\delta$  and subsequent translocation to the mitochondria. Moreover, inhibition of p38 MAPK significantly blocked radiation-induced caspase-3 activation and cleavage of PKC $\delta$ , indicating that PKC $\delta$ /p38 MAPK pathway is required for caspase-3 dependent cleavage of PKC $\delta$  and its translocation to the mitochondria. Taken together, these results suggest that ionizing radiation can utilize a positive feedback regulation of PKC $\delta$  for amplifying the mitochondrial activation-mediated apoptosis machinery, and could serve as potential targets for strategies that take advantage of signaling-based apoptosis to enhance cell killing in radiation therapy.

## ABOUT POSSIBLE DIRECT INFLUENCE OF IONIZING IRRADIATION ON CYTOMEGALOVIRUS INFECTION REACTIVATION IN THE VICTIMS OF CHERNOBYL NPP ACCIDENT

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Our investigations of 1987-2003 revealed significant prevalence of cytomegalovirus (CMV) infection among the acute radiation syndrome convalescents (ARSC), clean-up workers and inhabitants of contaminated territories. Taking into account possible influences of immune deficiency and CMV reinfection during transfusion of blood products to ARSC we try to elucidate possible direct influence of ionizing radiation (IR) on the reactivation of latent viral infections.

Strategy of CMV infection is directed on the destruction of nuclear bodies and a PML protein, which is needed for cell cycle stopping, DNA reparation and apoptosis activation. The same mechanism is involved in the response to IR, as a genotoxic agent, which induces p53 acetylation, stopping of cell cycle, allowing reparation of genetic disturbances. It is possible to imagine that changes in the structure of nuclear domain (ND) and involvement of proteins, contained in ND10 in the realization of reparation processes promote an activation of the locus of super early CMV genes.

Second possible mechanism of CMV reactivation under the influence of IR consists of activation of CMV genes transcription as a result of increased activity of intracellular ways of signal transduction. Among them those which are mediated by mitogene activation protein (MAP) kinases support vital capacity of cells and provide for repopulation effects after irradiation. While MAP-kinases way activation the quantity of NF- $\kappa$ B nuclear transcription factor is increased in the cell. But the target for the NF- $\kappa$ B nuclear transcription factor are both cellular genes and IE promotor of CMV that leads to transcription of super early genes of virus. Besides IR induces the synthesis of TNF, which after interaction with corresponding receptor of surface membrane activates NF- $\kappa$ B and provides its translocation to nucleus where the latter interacts with promoters of sensitive genes and super early genes of CMV.

In spite of the reason of CMV reactivation its activity is harmful for health state of patients. We found out in preliminary investigation an existence of certain correlations between CMV reactivation and chronic gastritis, chronic obstructive bronchitis, bronchial asthma, arterial hypertension and diabetes.

## BONE MARROW CELL DEATH AND RECOVERY FOLLOWING EXPOSURE TO AN ACUTE LEUKAEMOGENIC DOSE OF X-RAYS.

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### Objective:

Studies of A-bomb survivors and radiotherapy patients indicate that whole body exposure to ionising radiation results in a large excess relative risk of radiation-induced acute myeloid leukaemia (r-AML). Although whole body exposure results in approximately equal DNA damage in all cells in the body, why is there such a high risk of r-AML and not other cancers?

r-AML is a haemopoietic bone marrow stem cell (HSC) malignancy. As the HSC is particularly susceptible to radiation-induced malignant transformation *in vivo*, we have analysed the *in vivo* effects of a single acute leukaemogenic dose of X-rays on white bone marrow cells (WBMC) and phenotypically defined long-term (Lin<sup>-</sup> Sca-1<sup>+</sup> and c-kit<sup>+</sup>) or short term (Lin<sup>-</sup> Sca-1<sup>++</sup> Flk-2<sup>++</sup>) repopulating HSCs in the mouse.

### Methods:

WBMC numbers were counted at various times after *in vivo* exposure to 3 Gy X-rays. Lineage positive (Lin<sup>+</sup>) cells were removed by immunomagnetic depletion using biotin-conjugated antibodies to CD3e, CD11b, CD45R, Ly-6G and Ly-6C, and TER-119. Lin<sup>-</sup> bone marrow cells were stained with FITC-conjugated anti Sca-1 and RPE-conjugated anti *c-kit* or Flk-2 and analysed by flow cytometry.

### Results:

*In vivo* exposure to a single acute dose of 3 Gy X-rays kills 80% of (Lin<sup>+</sup>) WBMC and 90% of Lin<sup>-</sup> bone marrow cells within two days. Bone marrow cellularity recovers over the next 10-14 days and this is driven by the massive amplification of Lin<sup>-</sup> Sca-1<sup>++</sup> c-kit<sup>++</sup> and Lin<sup>-</sup> Sca-1<sup>++</sup> Flk-2<sup>++</sup> stem cells 0-3 days post irradiation.

Unlike the vast majority of relatively radioresistant cells in the body, a moderate dose of ionising radiation will kill a large proportion of the haemopoietic cells required for survival. Long-term survival therefore requires that the haemopoietic tissue be regenerated rapidly. In bone marrow, and unlike most other tissues, this involves the stimulation of proliferation of HSCs 0-3 days post exposure so that they can both self-renew and differentiate to repopulate the haemopoietic system.

### Conclusions:

Cancer is the net result of the accumulation of gene mutations in a single cell over time. A mutation is defined as a heritable change to DNA that is fixed by DNA replication. The genotoxic effects of ionising radiation will therefore be most apparent in cells that must proliferate in response to radiation-induced cell death to ensure survival of the individual. This proliferation also occurs shortly after exposure before some of the DNA damage can be repaired. We therefore propose that what distinguishes HSC from the majority of other cells in the body, is that bone marrow cell death leads to HSC proliferation and the fixing of radiation-induced DNA damage (or radiation-induced genomic instability) as mutations. This could explain why HSC are particularly susceptible to radiation-induced malignant transformation (r-AML).

## BONE MARROW DERIVED STEM CELLS REDUCE RADIATION-INDUCED DAMAGE TO SALIVARY GLAND.

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The salivary glands are often included in the irradiation field during radiotherapy of head and neck cancer. This can result in severe side effects that reduce the patient's quality of life and that may even limit the treatment dose. Late damage to the salivary glands is mainly caused by exhaustion of the tissue's stem cells. Post-irradiation replacement of salivary gland stem cells with healthy donor stem cells may ameliorate radiation-induced complications. Bone marrow derived stem cells (BMSC) have been shown to be able to engraft in many tissues after injury. In this study, we assessed the potential of BMSC to reduce irradiation-induced salivary gland damage. eGFP bone marrow chimeric C57Bl/6 mice were locally irradiated with 15 Gy of X-rays. Bone marrow derived stem cells were mobilised to the blood 10, 30, 60 days (or a combination) after irradiation by s.c. injection of G-CSF.

Pilocarpine induced saliva secretion was determined up to 90 days after irradiation every 30 days. Hereafter, the glands were extirpated and examined for eGFP-expression. Of every animal, one gland of the parotid and the submandibular gland was suspended into a single cell suspension and flow cytometry was used to detect eGFP positive cells. The other glands were analysed using confocal laser fluorescence scanning microscopy and light microscopy.

Most importantly, G-CSF treatment resulted in an increase of saliva flow for all time points of injection. The optimum time-point of mobilisation was 30 days after irradiation and improved salivary flow from 5 to 30%, when compared to irradiation alone. Flow cytometry showed that 10% of the isolated cells were eGFP-positive. Microscopy revealed a similar amount of positive cells appearing in clusters and an improved morphology. Immuno-histochemistry using  $\alpha$ -SM-actin antibodies showed the close vicinity of actin and eGFP within the cells, demonstrating the occurrence of BMSC derived myoepithelial cells in irradiated salivary gland. On going studies using cell-type specific antibodies will be used to more specifically characterize the eGFP<sup>+</sup> cells.

In conclusion, the results show that bone marrow-derived cells home to severely damaged salivary glands after mobilisation. Hence, BMSC transplantation could become a promising modality to ameliorate radiation-induced complications in salivary glands after radiotherapy.

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## MECHANISMS OF CHROMOSOMAL ABERRATION FORMATION WITH LOW AND HIGH LET RADIATION: STATE OF ART

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Chromosomal aberrations are among the most important biological effects observed following exposure to ionizing radiation *in vivo* and *in vitro*. The frequencies of chromosomal aberrations induced by various doses and qualities of radiation were the basis for the early radiobiological concepts such as target theory. However, the process by which chromosomal aberrations are formed is complex and involves a chain of events. Premature chromosome condensation technique is applied to define the initial DNA damage and the repair kinetics. Fluorescence *in situ* hybridization (FISH) technique using chromosome, chromosome arm, chromosome region, centromere and telomeres specific DNA libraries has improved the resolution of detecting all classes of radiation induced chromosomal inter- and intra-changes. Multi-colour FISH has opened the possibility to determine accurately complex chromosome exchanges in whole genome.

In the present work studies were designed to elucidate the mechanisms of chromosome aberration formation following exposure to X-rays, neutrons, heavy ions and ultra-soft X-rays. On the basis of newly obtained data new insights into the mechanisms of chromosome aberration formation with low and high LET radiation are reviewed.

Experiments were designed to define: (a) the initial frequency of chromosomal aberrations, (b) repair kinetics, (c) modulating effect of DNA repair inhibitors, differential repair at the chromosome level and among different chromosomes, (d) influence of chromatin packing (i.e. hetero- versus eu-chromatin), (e) influence of chromatin structure, (f) the proximity effect of chromosomal regions in interphase nucleus, (g) the notion of lesion-lesion and lesion-nonlesion interaction in normal as well as Chinese hamster ovary repair deficient cell types and (h) the hall-mark of high LET radiation.

## ASSESSMENT OF FISH-TRANSLOCATION ASSAY FOR BIOLOGICAL DOSEMETRY: STATE OF THE ART AND CURRENT VIEWS

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In case of radiation accidents, it is essential to estimate the absorbed dose of the victims to help plan their therapy regimen. Even in situations in which the physical measurement is feasible, an independent estimation by biological dosimetry can be very helpful. Furthermore, there is a great need for reliable methods to assess past radiation overexposure (accidental or natural) and to assess human risk.

The most fully developed biological indicator of exposure to ionising radiation is the study of chromosomal aberrations, in particular dicentric in the peripheral blood lymphocytes of exposed individuals. However, difficulties for dose estimation arise with old acute or chronic exposures, due to a decline in cells containing unstable dicentric aberrations. However, the fluorescence in situ hybridisation (FISH) technique employing chromosome specific DNA libraries to “paint” individual chromosomes has opened new perspectives for rapid and reliable detection of stable chromosome aberrations such as translocations. The inherent stability of translocations over cell generations has enabled them to be used as a biodosimeter.

For accurate assessment of ionising radiation induced chromosomal aberrations immediately following exposure and to determine whole and partial body exposure to proceed with treatment of victims technique of premature chromosome condensation is introduced and standardised using *in vivo* and *in vitro* assays. Furthermore, two novel FISH assays were developed and standardised: 1. Combining chromosomes, centromeres and telomeres specific DNA libraries for detecting unstable and stable (complete and incomplete) chromosome exchanges, and 2. A multi-colour FISH assay, so called COmbined Binary Ratio labeling (COBRA) for simultaneously visualisation of all human chromosomes with 23/24 different colours. This assay was further modified in which p and q arms were also painted in different colours that enabled to detect pericentric inversion.

These assays were employed to detect spontaneous frequency and X-ray-induced chromosomal exchanges in human lymphocytes following *in vitro* and *in vivo* exposure (victims of radiation accident).

The results of these studies will be presented and for the assays developed, their application for dosimetry and retrospective biological-dosimetry in case of immediate and past exposure will be discussed.



## CELLULAR RESPONSES TO NON-TARGETED EFFECTS OF LOW DOSE RADIATION

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The main objectives of our work (i) to obtain experimental comparative data on the contribution of non-targeted effects in radiation carcinogenesis by measuring bystander effect, adaptive response and genome instability in vitro. (ii) to answer whether bystander signal transmitted to the neighbouring cells may also involved in radio-adaptive response (iii) to estimate the genomic instability induced in the progeny of irradiated and bystander cells.(iv). to assess the contribution of non-targeted effects to environmental carcinogenesis.

Cellular effects were examined in confluent cultures of HFL1 (Human foetal lung fibroblast). Cells exposed to low doses of alpha particles, and challenged by gamma rays later on. The bystander effect was detected after transfer of medium from irradiated cells and also on double-layers of cells hold in the same medium where only one layer was exposed to radiation. HPRT mutations and DNA damage and repair were examined. The delayed effect of radiation was followed for approx. 40 generation of cells. The involvement of cellular factors such as antioxidant defence and stress protein expression was also studied.

The bystander effect was shown at both experimental setup, indicating that the damage signals was transmitted to neighbouring non irradiated cells. Mutational events arising in bystander cells were less frequent than in direct hit cells. Mutagenic adaptive response was detected in pre-treated cells by scoring HPRT<sup>-</sup> mutant colonies and also in their bystanders. Pre-exposure to alpha irradiation (10-50 mGy) conditioned medium did not result in adaptive response to the subsequent alpha irradiation (2 Gy). The mutation frequency showed 2-3 fold increases in twice irradiated as well as medium exposed and later on challenged cells. DNA damage and repair measured by Comet assay, showed significant differences in its magnitude and kinetics. Cumulative cell number of the irradiated cells was lowered after 10 passages in a dose dependent manner. Cumulative plating efficiency was also dependent on radiation dose and bystander effect was expressed at doses below 2 Gy. Apoptosis/Mitosis index was higher in direct hit cells than in the bystanders. At 6-9 passages there was an increase of MN positive cells especially in bystander cells.

Our results showed that the non targeted effects induced by low dose radiations are complex cellular phenomena and should taken into consideration in estimating of the risk from combined environmental hazards.

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## LOW ENERGY (0.5 – 500 eV) ION DAMAGE TO DNA BASES

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Energetic ions represent the most efficient and volume selective mode of therapy for deep-seated and non-operable tumors, and are abundant in space environments, or supersonic altitudes. Moreover, low energy secondary ion fragments are ubiquitous along radiation tracks in cells, where they enhance clustered DNA lesions, mutations, or cell death. While knowledge of ballistic ion effects on DNA is essential for radiation risk assessment, dosimetry, or efficient use of radiotherapy, little data exists on mechanisms of secondary ion damage at the molecular level, or ion energy depositions at track ends.

Therefore, we have begun a series of studies focused on low-energy ion damage to solids of DNA, or its components, using novel ultra-high-vacuum ion beam techniques developed in-house. The apparatus allows sample irradiation with a highly focused, mass and energy selected ion beam in 0.5 – 500 eV energy range. A mass spectrometer monitors desorbing ionic or neutral species during ion impact, and afterwards via SIMS or TD-MS. DNA bases are evaporated (at 80 C) *in vacuo* onto atomically clean Pt held at room temperature, and film deposition is calibrated to within 0.5 ng/cm<sup>2</sup> by a quartz crystal microbalance.

Here we present measurements on films of DNA bases irradiated with He<sup>+</sup>, Ar<sup>+</sup>, Ar<sup>++</sup>, D<sup>+</sup>, D<sub>2</sub><sup>+</sup>, D<sub>3</sub><sup>+</sup>, N<sup>+</sup>, N<sub>2</sub><sup>+</sup>, and CO<sup>+</sup> at ion energies below 500 eV. For example, in thymine films, we observe formation of at least 22 different positive and negative ion-fragments, including exo- and endocyclic fragments, such as H<sup>-</sup>, O<sup>-</sup>, CN<sup>-</sup>, OCN<sup>-</sup>, H<sup>+</sup>, CH<sub>3</sub><sup>+</sup>, CO<sup>+</sup>, OC<sub>2</sub>CH<sub>3</sub><sup>+</sup>, C<sub>4</sub>H<sub>6</sub>NO<sup>+</sup>, and (T-O)<sup>+</sup>, as well as (T-H)<sup>-</sup>, and (T+H)<sup>+</sup>. In particular, for He<sup>+</sup> impact on thymine films below 100 eV, we find that exocyclic bond cleavage is strongly enhanced by a factor of 10 or more compared to Ar<sup>+</sup> or Ar<sup>++</sup>, which we attribute to resonant core level excitations of N or C atoms in thymine. Conversely, fragmentation of N<sub>2</sub><sup>+</sup> projectiles appears to enhance some endocyclic bond cleavage channels. Finally, measurements of thymine survival in the films, for increasing ion doses, allow us to determine the total degradation quantum yield for specific ion projectiles; for example, a single 100 eV Ar<sup>+</sup> can deactivate about six thymine molecules in the solid, mainly by fragmentation. In order to further identify bond cleavage sites, experiments on partially deuterated thymine, and 5-Br-uracil have also been performed, and will be presented. The latter species is a radiosensitizing thymine analog easily incorporated into cellular DNA, however its sensitivity to ion damage, and the involved mechanisms, are unknown at this time.

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## CHROMOSOMAL RADIOSENSITIVITY AND SNP ANALYSIS IN THE XRCC1 AND XRCC3 GENES IN PATIENTS WITH RADIATION INDUCED DAMAGE TO NORMAL TISSUES FOLLOWING RADIOTHERAPY FOR GYNAECOLOGICAL CANCERS

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**Objective.** About 5% of oncology patients treated by radiation therapy develop acute or late radiotoxic effects whose molecular mechanisms remain poorly understood. There is a large body of evidence suggesting a genetic basis for the predisposition to side effects of radiotherapy in healthy tissues. Single nucleotide polymorphisms (SNPs) in genes related to the biological response to radiation injury may affect clinical normal tissue radiosensitivity. This study investigates whether 7 SNPs selected in two DNA repair genes XRCC1 and XRCC3 are related to an increased risk for adverse reactions after radiotherapy. Furthermore, the correlation between clinical radiosensitivity and in vitro chromosomal radiosensitivity was assessed.

**Methods.** The study population consists of 54 women suffering from cervix or endometrium cancer treated with fractionated external radiotherapy followed by brachytherapy. Patients were evaluated with respect to several different normal tissue reactions according the CTC scale. CTC0-1 comprises patients with no adverse reactions to radiotherapy (n=36), CTC2 contains patients with intermediate but distinct reactions (n=10) and CTC3-5 reflect patients with severe reactions following radiotherapy (n=8). At least 6 months after therapy, blood samples were collected. Chromosomal radiosensitivity was measured using the G<sub>2</sub>-assay. SNPs in XRCC1 (194 Arg/Trp, 280 Arg/His, 399 Arg/Gln, 632 Gln/Gln) and in XRCC3 (5'UTR, IVS5-14, 241 Thr/Met) were analysed by PCR-RFLP.

**Results.** The average yield of chromatid breaks per cell for patients in group CTC0-1, group CTC2 and group CTC3-5 was 1.16 breaks/cell, 1.33 breaks/cell (p=0.047) and 1.39 breaks/cell (p=0.030) respectively. Of 14 patients with reactions to radiotherapy, 7 could be judged as highly radiosensitive with respect to chromosomal radiosensitivity (p=0.002). For XRCC1, variant alleles in codon 280 (OR:2.2), 399 (OR:1.7) and 632 (OR:1.9) correlated positively with increased risk for normal tissue reactions (CTC2-5). The same is true for variant alleles in IVS5 (OR:3.1) and codon 241 (OR:1.7) for XRCC3. In contrast, polymorph genotypes in codon 194 (OR:0.0) for XRCC1 and 5'UTR (OR:0.6) for XRCC3 are negatively correlated with increased risk for reactions to radiotherapy. None of these results are however significant. Combined analysis of multiple SNPs in XRCC1 and XRCC3 demonstrated that the risk for radiotherapy reactions correlated with the number of risk alleles in such a manner that patients with 4 risk alleles exhibited a remarkable degree of radiosensitivity (OR:35, p=0.001).

**Conclusion.** These findings support the assumption that normal tissue radiosensitivity should be regarded as a complex trait caused by the combined effect in several DNA repair genes. The G<sub>2</sub>-data were found to be highly correlative with the severity of late clinical radiosensitivity.

## RADIATION INDUCED APOPTOSIS IN THE E15 DAY EMBRYONIC MOUSE BRAIN.

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Apoptosis is a fundamental aspect of morphogenesis of embryonic tissues. In the developing nervous system, apoptosis results in naturally occurring cell death, a necessary process that eliminates neurons that have made faulty synapses or have not reached appropriate targets. Apoptosis is also often a response to many stimuli like ionizing radiation and factors into many neurodegenerative diseases.

It is however not clear whether the apoptotic pathway in radiation induced apoptosis or during normal development is similar. The present study using p53 wild type and knocked out mice aims at identifying the possible mechanism involved in radiation induced apoptosis.

Apoptosis in E15 mouse brain was evidenced by DNA ladder Electrophoresis, after 60 cGy exposure. Experiment with non-NMDA glutamate receptor inhibitor (dizocilpine), and with inhibitors (MK801) of calpain, a proteolytic  $\text{Ca}^{++}$  dependant enzyme, have indicated the possible role of the non-NMDA receptors in a radiation-induced apoptosis in the developing brain. DNA ladder were not evidenced in p53 knocked out mice. It clearly suggested p53 apoptotic pathway dependence.

To further identify the radiation response mechanism, the genes possibly involved were screened by DNA micro-array analysis. using a 21.572 mouse gene library (VIB Mouse 21K (Incyte, NIA) Library).

In this first approach, Expressed Sequence Tags (EST) were neglected. Only genes with identified function and a minimal 2 fold amplification were further considered. Our preliminary results indicate that genes activated in p53  $+/+$  and in p53  $-/-$  mice appear quite different. Many genes activated in p53  $+/+$  mice are guanine nucleotide binding proteins (G-proteins) related. G-proteins couple extracellularly-activated integral-membrane receptors to intracellular effectors such as ion channels and enzymes that on their turn may alter the concentration of second messenger. Other activated genes were typically apoptotic pathway related genes including caspases, Cdkn1/p21 and Ccng1. In the p53  $-/-$  mice the activated genes (msh4, Mash2) are related to cell differentiation while other show correlation with  $\text{Ca}^{2+}$  level regulation (Sos2).

However such data are preliminary and require to be further investigated. The genes expressed in association with irradiation indicates that, in the developing brain, ion transport and ion receptors could play a role in the evidenced radiation induced apoptosis and also supports the data from non-NMDA receptor inhibition experiments. Altered gene expression related to individual brain regions (hippocampus, cortex, cerebellum) and to developmental stages (E12 to E17) will be analyzed soon.

## DETECTION AND EXAMINATION OF BACTERIAL BIOFILMS AND THEIR EFFECT ON THE MATERIALS OF NUCLEAR WASTE CONTAINERS

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In order to obtain information on the effect of the microbial metabolism at a potential nuclear waste repository, the corrosive and migrative effects induced by the potentially developing biofilms on the surface of the containers must be considered. Traditional culturing methods underestimate the total number of microorganisms in a sample due to the selective nature of the media employed, lack of detection of non-viable microorganisms, and failure to count microorganisms that are present as aggregates or associated with particles. The need to count the total population of microorganisms may be important since the presence of large numbers in some samples may be significant, regardless of viability.

Our work was to examine different biofilms formed by bacteria from pure and mixed cultures on the surface of glass, copper and stainless steel coupons. The study of these bacteria and biofilms included several imaging techniques such as epifluorescent microscopy, which allows the direct observation of morphology and total enumeration of viable and nonviable organisms. Another visualizing method was confocal microscopy which has a unique optical sectioning capability. This optical sectioning makes it possible to record images of layers within the biofilm. By collecting a group of such images from different depths, it is possible to display the three-dimensional structure of a biofilm. These methods are, however, qualitative and do not provide information on the effect that the biofilm exerts on the underlying substratum. Aspects of biofilm/substratum interactions were examined by atomic force microscopy (AFM). Molecular biological techniques such as polymerase chain reaction (PCR) and Denaturing gradient gel electrophoresis (DGGE) were very useful in biofilm studies because of their ability to determine the diversity of mixed microbial communities.

With the help of these techniques we will be able to prepare a workplan the results of which will provide information to develop a model predicting the consequences of microbial processes at a potential nuclear waste repository site.

## USE OF THE COMET-FISH ASSAY TO MEASURE RADIATION-INDUCED DNA DAMAGE AND REPAIR IN SPECIFIC GENE REGIONS OF CELLS DEFICIENT IN TRANSCRIPTION COUPLED REPAIR.

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Transcription coupled repair (TCR) is a mechanism for preferential repair of DNA damage in actively transcribed genes. Patients with Cockayne's syndrome (CS) are known to be defective in this repair pathway. We have used a combination of the alkaline Comet Assay (ACA) and fluorescent in situ hybridisation (FISH) to investigate TCR in normal fibroblasts (GM38) and CS A and CS B fibroblasts. In order to observe DNA damage and repair in individual gene regions, fluorescent DNA probes which hybridize to an actively transcribed gene (p53) and an inactive gene (hTERT) were hybridized to the cells following ACA electrophoresis. Since each probe had a different fluorophor (spectrum orange for p53 for spectrum green for hTERT), we were able to identify the amount of DNA damage and rate of repair in the selected gene regions of the same cells.

Cells were exposed to 5Gy gamma irradiation and analysed after 0 – 60 min incubation at 37°C. Repair was stopped by placing slides in alkaline lysis buffer before analysis using the Comet/FISH protocol. At each time point examined, the numbers of p53 and the hTERT spots, per cell, were recorded for 50 cells per slide. Simultaneously, the normal comet parameters were measured in the same (DAPI stained) cells and the location of hybridisation spots within the comet head or tail was recorded. The position of the hybridisation spots in the comet head or tail indicates whether the sequence of interest lies within or in the vicinity of a damaged region of DNA. Significant damage was seen in both gene regions, and there was clearly preferential repair, in the GM38 cells, in the actively transcribed p53 gene region relative to both the inactive hTERT domain and the overall genome. However CS A and CS B cell lines were unable to preferentially repair the p53 region, consistent with being deficient in TCR. This is the first demonstration of the use of multi-colour Comet-FISH to follow repair in cells.

## SIMPLE METHODS FOR THE MEASUREMENT OF URANIUM IN ENVIRONMENTAL SAMPLES

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Numerous methods have been published for the determination of uranium in environmental samples. Two of them were adopted successfully in our laboratory at University of Veszprém. These two determination techniques are electrodeposition and microfiltration.

Before the determination the samples were prepared by ion-exchange resin process. The procedure to separate uranium from other elements is based on its different adsorption onto anion exchangers (ion exchange separation method). The strongly anionic resin DOWEX 1x8 (100-200 mesh) was used for this purpose. A further purification step was performed before the ion exchange separation method: co precipitation with iron(III)-hydroxide by adding ammonia. In order to quantify the possible losses during the preparation process,  $^{232}\text{U}$  tracer must be added at the beginning of the procedure. The purified uranium fractions were electrodeposited onto stainless steel plates or filtrated using 0,1  $\mu\text{m}$  filter-disks.

During the electrodeposition the sample solution which is suitable for electroplating (we have to adjust the pH accurately) is placed into a deposition cell. The solution is the electrolyte, the stainless steel planchet (negative lead) and the platinum wire (positive lead) are the electrodes. We need to continue the electrodeposition with 0.5 A current for 2 hours and the platinum wire is approximately 11 mm from the planchet. After the deposition process we remove the steel disk from the cell, dry it and then submit to count room for alpha spectrometric measurement.

The second method we used is microfiltration. At first the Nd carrier is added to the sample solution. Then a solution of 15%  $\text{TiCl}_3$  in 10 % HCl is added. After the purple color persisted for 2 minutes, 40 % HF is added to precipitate  $\text{NdF}_3$ . The U co-precipitated with neodymium fluoride  $\{\text{NdF}_3 (\text{UF}_4)\}$  is filtered through a 25 mm diameter, 0.1 $\mu\text{m}$  pore size membrane filter. The filters were dried and analyzed by alpha spectroscopy.

Both processes are effective if we want to know the uranium content of environmental samples. The main advantage of the electrodeposition compare to micro-precipitation is the two times better energy resolution FWHM (Full Weight at Half of Maximum) of the alpha peak. On the other hand the electrodeposition is more time-consuming method than the other one. Before beginning any measurements we need to size up the situation and select the most economical procedure.

## THE IMPORTANCE OF MOLECULAR SIZE AND CHARGE IN DESIGNING POLYMERIC RADIOPHARMACEUTICALS TO TARGET NEOPLASTIC DISEASE IN THERAPY

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Water-soluble polymers are under development as vehicles for systemic drug and radionuclide delivery due to the effect of enhanced permeability and retention whereby neoplastic tissue selectively accumulates macromolecules. The 10-30 kDa fraction of the polymer <sup>99m</sup>Tc-PEI-MP previously presented promising biodistribution and pharmacokinetics in primates and dogs and good tumour location in natural canine osteosarcoma. PEI-MP can also be labelled with therapeutic radionuclides such as <sup>186</sup>Re and <sup>117m</sup>Sn. Molecular size and charge are however known to affect its biodistribution and pharmacokinetics. The purpose of this study was therefore to investigate the effect of charge of the PEI-MP moiety and molecular size on the pharmacokinetics and biodistribution of <sup>99m</sup>Tc-PEI-MP in six normal dogs.

PEI-MP was sized into small (3-10 kDa), medium (30-50 kDa) and large (50-100 kDa) fractions, which were further separated into positive and negative charged fractions by ion-exchange on CM- and DEAE- Sephadex, and incorporated into <sup>99m</sup>Tc labelling kits. Each of the dogs under an i.v infusion of pentobarbitone anaesthesia received an i.v administration of <sup>99m</sup>Tc-PEI-MP (5mCi) of a particular size fraction and a particular charge while in the supine position under a gamma camera for a scintigraphic dynamic study of 60x1 min frames for the first hour followed by single static two minute frames at two and three hours. From the dynamic studies regions of interest were drawn over the cardiac bloodpool, lungs, kidneys, liver, cortical bone and background regions. The average counts per pixel for each region were used to draw time-activity-curves from which the biodistribution and pharmacokinetics of the variously sized and charged fractions of the <sup>99m</sup>Tc-PEI-MP can be assessed and compared.

Substantial differences in the in vivo behaviour of these fractions were observed, related both to molecular size and charge. In the liver, lung and cardiac blood pool the uptake/retention decreased with a decrease in molecular size with the negatives throughout tending to higher uptake and slower washout than positives, while the opposite behaviour was observed in the bone, with higher uptake of the small fraction, more so for negatives. No uptake or retention of the large negative fraction was observed in the kidneys while for the other fractions there was a higher uptake and retention for the positive fractions, even influencing the effect of size.

These results indicate that by manipulating the molecular size and charge of polymeric delivery vehicles for radionuclides, their in vivo behaviour can be directed eg. to avoid radiosensitive non-target organs or tissues, which also allows more freedom with therapeutical dosages.



## VALIDITY OF THE LATENT TIME CONCEPT

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Radiation effects in normal tissues are depicted as "early" or "late" with a cut-off for the time of first occurrence at 90 days after the onset of radiation treatment. This cut-off is required for statistical reasons, but is purely arbitrary. Organ-specific cut-offs might be preferable.

Early radiation effects are predominantly seen in turnover tissues, where permanent cell loss is precisely counterbalanced by cell production in the germinal tissue compartments. Radiation-induced impairment of cell production results in progressive tissue hypoplasia and eventually in a loss of functional cells. Latent times for the clinical manifestation of early radiation effects are – over a wide range of doses – independent of radiation dose, and are related to normal tissue turnover times. In contrast, the time to tissue restoration increases with increasing dose. The radiation response in these turnover tissues is likely to be affected by parallel responses in other tissue compartments, i.e. the vascular response or radiation effects in associated connective tissue, transmitted by paracrine mediators.

Latent times to chronic clinical effects can be as long as 10 years or even longer. They are the shorter the higher the dose was. This relationship, however, is non-linear. Late radiation effects are usually irreversible and progressive in nature, with a dose-dependent progression rate. Therefore, the tolerance doses for late effects clearly decrease with an increase in follow-up time.

Radiobiological studies demonstrated in a variety of organs that the development of late radiation effects is a continuous process, starting immediately at the time of irradiation. These early and intermediate changes are usually not associated with clinical symptoms. They comprise responses in a variety of cell populations, including parenchymal cells, fibroblasts and vascular endothelial cells. Each of these populations is likely to respond specifically to radiation exposure, with a specific time course. Interactions between the different cell populations, including those involved in the early radiation response in the same organ (consequential component of late effects), render the pathogenetic pathways even more complex, and may also impact on the time course of the response, i.e. the latent time to clinical manifestation.

In conclusion, the clinical manifestation of late deterministic radiation effects is based on continuous processes progressing – at a dose-dependent rate - from at the time of radiation exposure. Therefore, definition of a "latent time" is highly dependent on the endpoint studied, i.e. the quality and/or severity of changes analysed, as well as on radiation dose and further factors.

## DNA DAMAGE AFTER LOW DOSE IONIZING RADIATION

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**Introduction:** Over the years number of non-targeted effects of radiation exposure have been described including bystander effect and genomic instability. Radiation induced genomic instability (GI) manifests in the progeny of irradiated cells, multiple generations after radiation exposure resulting in a number of deleterious alterations in their genome. Based on recent data it appears to involve interactions among oxidative stress induced free radicals, cell signal transduction pathways, and also various epigenetic factors. We report here our experimental data by which we attempt to access mechanisms involved in and the relative magnitude of these phenomena.

**Methods:** RPMI 8322 human melanoma cells and human lung fibroblast of fetal origin (Hfl-1) cells were examined. The DNA damage (dsb and ssb) and the DNA repair were examined with alkaline Comet Assay. The cells were processed for comet slides 0, 1, 24 hours after irradiations. Genomic instability was examined in irradiated (0.05-5.0 Gy) and non-irradiated bystander cells. The plating efficiency, the proliferative capacity, frequency of the micronucleated cells and apoptosis-mitosis index were followed up to approx. 40 generation of cells. Measurement of superoxide-dismutase (SOD) activity was performed using a RANDOX kit, glutathione-peroxidase (GPx) activity was measured using an OXIS GPx-340 colorimetric assay following the suppliers instructions.

**Results and conclusions:** Reduction in cumulative plating efficiency of the direct hit cells was dose dependent. Delayed reproductive death in bystanders was observed at doses below 2 Gy. At doses 0.05 Gy and 0.5 Gy the cumulative plating efficiency of the bystander cells was lower than that of irradiated cells. It had been shown that genomic instability was induced in targeted cells and also their non-irradiated bystanders. Our data on MN induction showed good correlation with apoptosis studies. A/M index was much higher in the irradiated cells than in the control cells at the early passages. Bystander effect experienced also as early as the first passage after exposure to medium harvested from irradiated cells. The rate of apoptotic fragmentation decreased as the passage number increased. Delayed apoptosis, the second wave in programmed cell killing was shown after the later (8-9th passages) in direct hit cells and also in lesser extent in their bystanders. The tail extent moment (TEM) as a sign of the DNA damage was dose dependent and had not been repaired completely as much as 24 hours after irradiation. TEM was in good correlation with the activity of the antioxidant enzymes. Our results supported the theory of the newly improved mechanisms of the carcinogenesis.

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## SHORT-TERM EFFECTS OF URANIUM ON MUCOSAL IMMUNITY IN RAT INTESTINE.

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**Objective.** In case of chronic ingestion, the digestive tract is the first biological system exposed to daily intake of uranium in intestinal lumen. The increased uranium concentration in intestinal lumen and/or uranium accumulation in some intestinal cells may lead to functional modifications of intestine, notably in the intestinal barrier and the mucosal immunity. A recent study has demonstrated that contamination by heavy metal (e.g. lead) ingestion led to modifications of the gut immune system. However, no data are available in the literature on the effect of uranium ingestion on the immune intestinal functions. The objective of this study was thus to determine if mucosal immunity of intestine is modified in the first days following an acute ingestion of a sublethal dose of uranium.

**Methods.** The experiments are performed in Sprague-Dawley rats, which received an intragastric administration of depleted uranium (74 mg, pH = 3). The animals were euthanased at one and three days after uranium administration. Intestinal segments were taken from distal ileum for i) standard histological analyses (HES staining) ii) immunohistochemical analyses of immune cells (macrophages, CD163 and intraepithelial lymphocytes, CD8) iii) tissue cytokine (IFN $\gamma$ ) expression by RT-PCR and iv) cytokine (IFN $\gamma$ , IL-2, IL-6, IL-12) and chemokine (MIP-2, MCP-1) production by ELISA.

**Results.** Pictures of macrophage staining in lamina propria and intraepithelial lymphocyte staining showed no modifications in localization and density of these two immune cell populations at day 1 and 3 post-contamination. Few changes in cytokine/chemokine production are observed at 1 and 3 days post-contamination. Only a 50% diminution of production of MCP-1 ( $p=0.05$ ) and IL-12 (non significant) was observed at day 1. Tissue IFN $\gamma$  level was slightly increased at day 1 and 3. However, the IFN $\gamma$  expression measured 3 days after uranium contamination indicated a marked increased ( $\times 3.5$ ) in mRNA level of this cytotoxic molecule.

**Conclusion.** These results indicated that uranium contamination at sublethal dose induced slight modifications of molecular and cellular actors implicated in intestinal immune response in the first days following uranium contamination. However, increased expression at day 3 of IFN $\gamma$ , a cytotoxic molecule notably for enterocytes, suggested that uranium may have deleterious effects at longer term (5 or 7 days) in intestinal functions, such as transport and immunity.

## TRANSFER OF URANIUM THROUGHOUT THE ENTIRE GASTROINTESTINAL TRACT IN RAT.

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**Objective.** The presence of uranium in environment may lead to contamination of human population throughout the entire life mainly by chronic ingestion. The mechanisms of uranium transfer from alimentary bolus to blood are still not well known. The aim of this present study was thus to determine the preferential digestive segments for uranium absorption throughout the entire alimentary tract (oral cavity, small intestine, colon) with *in vivo* and *ex vivo* approaches. In addition, some information about the nature of transepithelial pathways (para- or trans-cellular) was done.

**Methods.** The experiments are performed in Sprague-Dawley rats. After euthanasia (overdose of sodium pentobarbitone), samples of digestive segments (duodenum, jejunum, ileum, proximal and distal colon) were placed in Ussing chambers to measure tissue transepithelial resistance ( $R_T = PD/I_{sc}$  in  $\Omega/\text{cm}^2$ ) and apparent permeability of uranium ( $P_{app}(U)$  in  $\text{cm/s}$ ) with mucosal-serosal  $^{233}\text{U}$  fluxes (2 kBq/4 ml). These experiments were completed by *in vivo* experiments performed in anaesthetized rat after *in situ* deposit of uranium (50 kBq/ml) in specific parts of digestive tract and measurements of uranium apparition in peripheral blood (up to 3 hours after) to estimate uranium absorption by each studied segment.

**Results.**  $P_{app}$  values in small intestine were of 3.0  $\text{cm/s}$  for duodenum, 1.6 for jejunum and 2.4 for ileum. In large intestine, values were very different from proximal colon (3.7  $\text{cm/s}$ ) to distal colon (1.7  $\text{cm/s}$ ). A negative correlation was found in large intestine between  $R_T$  and  $P_{app}(U)$  ( $r^2=0.88$ ), which suggests a paracellular pathway for uranium in this part of digestive tract, unlike in small intestine. Results of *in vivo* experiments indicated uranium absorption by ileum and no uranium absorption by mouth and proximal colon.

**Conclusion.** The results obtained in the present study indicated no uranium absorption by buccal cavity. Concerning the small intestine, different parts (duodenum, jejunum and ileum) of this segment had the capacity to transport uranium, with a more important passage observed in duodenum. In large intestine, the marked value of  $P_{app}(U)$  obtained *ex vivo* in proximal colon suggested uranium absorption by this segment, which was not corroborated with *in vivo* experiments. In conclusion, only small intestine seems to participate to the gastrointestinal absorption of uranium.

## IONISING RADIATION AND TRANSGENERATIONAL INSTABILITY IN MICE

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**Objective.** To analyse transgenerational changes in the germline and somatic tissues of non-exposed offspring of irradiated male mice. **Methods.** Mutation rates at tandem repeat DNA loci and protein-coding genes were studied in the germline and somatic tissues of first- and second-generation offspring of inbred male CBA/H, C57BL/6 and BALB/c mice exposed to either high-LET fission neutrons or low-LET X-rays. Germline mutation rates at tandem repeat DNA loci were analysed using the pedigree approach as well as Single-Molecule PCR technique; the same PCR technique was used to estimate mutation rate in somatic tissues. **Results.** Our results showed that radiation-induced instability can be transmitted for at least two generations after initial paternal exposure to either high-LET fission neutrons or low-LET X-rays. Germline mutation rates in the offspring of irradiated parents were detected in three inbred strains CBA/H, C57BL/6 and BALB/c, demonstrating that transgenerational instability is not restricted to one particular inbred strain of mice. ESTR mutation rates were persistently elevated in the germline and somatic tissues of first-generation offspring irradiated male mice. A similar increase in the frequency of somatic mutations at the *hprt* locus was also detected in the first-generation offspring of irradiated mice. **Conclusions.** Our data suggest that germline instability is caused by some DNA-dependent signal transmitted from the irradiated father and implicate an epigenetic mechanism for the transgenerational instability. The potential implication of these results for the estimates of genetic risks for humans will be discussed.

## QUANTITATIVE ANALYSIS OF CONCENTRATION AND RATIOS OF URANIUM ISOTOPES IN THE US MILITARY PERSONNEL AT SAMAWAH, IRAQ DURING OPERATION ENDURING FREEDOM

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**OBJECTIVE:** The aim of this study was to analyze the concentration and isotopic composition of four uranium isotopes ( $^{234}\text{U}$ ,  $^{235}\text{U}$ ,  $^{236}\text{U}$  and  $^{238}\text{U}$ ) in the urine samples of US soldiers deployed in Samawah, Iraq during Operation Iraqi Freedom.

**METHODS:** Nine US soldiers, the members of Military Police unit 442, deployed during the Iraq military operations in March 2003, presented with non-specific symptoms of headaches, fatigue, fever, musculoskeletal pains, respiratory impairment, neurological, and affect alterations. 24-hour urine samples of each subject were analyzed together with control samples consisting of internal urine standards. The analytical methodology included preconcentration of the urine samples using either co-precipitation or evaporation, oxidation of organic matter, uranium purification by ion-exchange chromatography, and mass spectrometry analysis. For determination of total uranium concentration a 2ml aliquots, precisely weighed to 0.1%, were spiked with a certified  $^{233}\text{U}$  tracer solution. A larger aliquot of 500ml unspiked urine were used for precise determination of the  $^{238}\text{U}/^{235}\text{U}$ ,  $^{234}\text{U}/^{238}\text{U}$  and  $^{236}\text{U}/^{238}\text{U}$  ratios. All specimens were analyzed in duplicate, using an Aridus desolvation system and a double-focusing Thermo Finnigan Neptune multi-collector ICP-MS equipped with a retarding potential quadrupole lens and a secondary electron multiplier for ion counting. The reproducibility of the  $^{238}\text{U}/^{235}\text{U}$ ,  $^{234}\text{U}/^{238}\text{U}$  and  $^{236}\text{U}/^{238}\text{U}$  ( $6.8 \times 10^{-8}$ ) for an 8ppb NBS950a solution ( $n=14$ ) over two days were, before applying any corrections, 0.13, 0.6 and 2.6%, respectively. Limits of detection for  $^{238}\text{U}$  are about 1 pg/L and analytical blanks were below 6 pg.

**RESULTS:** The mean concentration of total uranium was  $3.2 \pm 0.6$  ng/L. Five of the nine soldiers have a  $^{238}\text{U}/^{235}\text{U}$  ratio of natural uranium. Three subjects of this group had detectable levels of  $^{236}\text{U}$ . Four soldiers were clearly identified as positive for depleted uranium excretion. The  $^{234}\text{U}/^{238}\text{U}$  ratio varied from  $5.7 \times 10^{-5}$  to  $7.2 \times 10^{-5}$  and correlates negatively with the  $^{238}\text{U}/^{235}\text{U}$  ratio. Urinary  $^{236}\text{U}$  concentrations of these four individuals vary from 1.4 to 12.2 femtograms/L and their  $^{236}\text{U}/^{238}\text{U}$  ratio correlates positively with the ratio of  $^{238}\text{U}/^{235}\text{U}$ .

**CONCLUSION:** Our findings demonstrate depleted uranium contamination of military personnel deployed in the radioactive battlefield and suggest a need of sustained follow up for potential somatic and genetic consequences. Our current studies of military and civilians contamination with isotopes of uranium and plutonium are in progress for the risk assessment of actinides in the biosphere of post-conflict Iraq.

## DECREASED DIFFERENCE IN RADIOSENSITIVITY BETWEEN DIFFERENT HUMAN CELL LINES AFTER EXPOSURE TO HIGH LET RADIATION

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The influence of linear energy transfer (LET) on cell survival and the dose and time dependence on the induction of apoptosis were compared in a panel of different human malignant and normal cells exposed to accelerated light boron (<sup>10</sup>B) ions, accelerated heavy nitrogen (<sup>14</sup>N) ions and photons.

Normal human skin fibroblasts, proficient (GSH<sup>+/+</sup>) or deficient (GSH<sup>-/-</sup>) in glutathione, a human melanoma cell line (AA), a small cell lung carcinoma cell line (U-1690) and the human glioma cell lines M059J (DNA-PKcs<sup>-/-</sup>) and M059K (DNA-PKcs<sup>+/+</sup>) were used. The cells were exposed *in vitro* to different doses of nitrogen ions (140 eV/nm), boron ions (40, 80, 125 and 160 eV/nm), and photons (0.2 eV/nm). The radiosensitivity of the different cell lines was based on the clonogenic cell survival assay. We also measured the induction of apoptosis up to nine days after irradiation using morphological characterisation of apoptotic cells and bodies. In parallel, measurements of cell-cycle distribution, monitored by DNA flow cytometry and were performed.

The SF<sub>2</sub> values varied between 0.02-0.7 for photons and 0.02-0.08 for nitrogen ions (140 eV/nm). The lowest values were obtained with the DNA repair deficient M059J cells given an RBE of 1.03 (at 10% survival).

The loss of clonogenic ability was dose and LET dependent after exposure to boron ions (AA cells). There was a significant increase in apoptosis as compared to photons at all time-points studied. It was not possible to quantitatively measure an LET dependent apoptotic response due to its complexity. The early premitotic apoptotic cells disappeared at 24 h following exposure to the highest LET (160 eV/nm). A postmitotic apoptotic response was seen after the release of the G<sub>2</sub>/M accumulations, which were dose, time and LET dependent. This biphasic response was also seen after exposure to nitrogen ions (140 eV/nm) for all cell line studied. For M059J cells even low LET radiation induced a significantly elevated apoptotic response as compared with M059K cells at 144 h post-irradiation. However, following high LET radiation exposure, there was no difference in the level of apoptosis between the cell lines at this late time point.

## NBS1 PROTEIN AS REGULATOR OF APOPTOSIS

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**Objective:** *Nijmegen Breakage Syndrome* (NBS) is a rare genetic disorder characterized by pleiotropic defects including radiation hypersensitivity, immune deficiency and genomic instability. The responsible *NBS1* gene which is homozygously mutated in NBS patients is known to play an important direct or indirect role in DNA double strand break (DSB) repair. The Nbs1 protein forms a complex together with the hMre11 and hRad50 proteins. This trimeric complex is thought to regulate the processing of broken DNA ends. Beyond Nbs1's essential role in DSB repair, an additional role in signal transduction has been proposed. We were interested in elucidating this putative role of Nbs1 in other cellular functions besides DSB repair.

**Methods:** Our functional analyses were done with two NBS<sup>-/-</sup> patient cell lines derived from two families and their consanguineous NBS<sup>+/-</sup> controls. We have investigated the effects of genotoxic and apoptosis-inducing agents on cell survival and on the regulation of gene expression on both the transcriptional and translational levels. We performed microarray analyses using an in-house cDNA array. We concentrated on genes involved in DNA repair, downstream signalling, apoptosis, ubiquitin metabolism and some other processes.

**Results:** NBS<sup>-/-</sup> cells are *more* sensitive to DNA damaging agents like  $\gamma$ -irradiation or the crosslinking agent cisplatin than NBS<sup>+/-</sup> cells. In contrast, NBS<sup>-/-</sup> cells are *less* sensitive to apoptosis-inducing agents that do not damage DNA, like TNF $\alpha$  or hyperthermia (43°C). Furthermore, inhibition of the ubiquitin-dependent proteasomal protein degradation is more harmful to the heterozygous NBS<sup>+/-</sup> cells than to the homozygous NBS<sup>-/-</sup> cells. Western blot analyses of some important proteins involved in apoptosis (tumor necrosis factor receptor 1, TNFR1, and its anti-apoptotic regulator BAG-4) and in ubiquitin metabolism revealed significant differences between the homozygous and heterozygous cells. The regulation of most of these factors does not occur on the transcriptional level.

**Conclusion:** The increased sensitivity of NBS<sup>-/-</sup> cells to DNA damaging agents on one hand and the decreased sensitivity of these cells to DNA damage-independent apoptosis induction on the other hand suggests additional roles for Nbs1 as a regulator of cell death pathway(s) besides the described function(s) in DSB repair processes. Furthermore, we have identified a few genes which seem to be differentially expressed in a *NBS1*-dependent manner.



## COMPARISON BETWEEN HIGH DOSE RATE X-RAY RADIATION AND STATIC GAMMA IRRADIATION EFFECTS ON HUMAN LYMPHOCYTES IN VITRO (BASING ON MICRONUCLEI TEST RESULTS)

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The biological effects of high dose rate X-ray radiation and gamma-radiation from  $^{60}\text{Co}$  isotope source have been compared basing on micronuclei count results for lymphocytes of peripheral human blood. A 1 MB small-size ARSA accelerator was a source of pulse X-rays. This is a direct-action accelerator with a sealed-off accelerating tube providing a uniform irradiation field. The X-radiation pulse width at half maximum is 4 ns. The accelerator operates either in a single-pulse mode, or frequency mode according to the preset program. The weights of the accelerator's high-voltage unit and its charging device are 55 kg and 18 kg, respectively. Test-tubes with blood were irradiated: on ARSA accelerator. Bilateral irradiation was conducted with dose 28 cGy per pulse, with the absorbed dose distribution non-uniformity not exceeding 10 %. The mean dose rate within a pulse was  $7 \cdot 10^7 \text{ Gy} \cdot \text{s}^{-1}$ . The pulse frequency was 1 Hz. in a uniform field of the certified  $^{60}\text{Co}$  isotope source with the absorbed dose rate  $8 \cdot 10^{-3} \text{ Gy} \cdot \text{s}^{-1}$ . The absorbed doses were measured using the selected thermoluminescent detectors based on LiF monocrystal with errors within 5 %.

Blood drawn from five donors (men and women, 23 to 53 years old) was irradiated in the modes above with doses 0.5 Gy, 1 Gy, 2 Gy, and 3 Gy. The irradiated and non-irradiated (control) blood preparations were examined by micronuclei test. The standard method using cytochalasin B was used. No less than 1000 binuclei cells per each dose were examined. The spontaneous level of cells with micronuclei in control samples was 2 % to 5 %. When blood has been irradiated with the doses above using ARSA accelerator, the percentage of picknotic cells doesn't exceed this parameter values obtained for  $^{60}\text{Co}$  isotope source. The maximum percentage of picknotic cells (42 % at dose 3 Gy) was found in blood irradiated by  $^{60}\text{Co}$ . The dose-effect dependences were obtained for each kind of irradiation. For each dose value, the mean percentages of cells with micronuclei are the same (within 0.05 significance level) for the both irradiation modes. It has been shown that the small-size, inexpensive and environmentally safe high-dose rate X-ray irradiator can be used in radiobiological investigations instead of isotopic sources.

## TOWARD AN AUTOMATIC SYSTEM FOR THE ANALYSIS OF CYTOGENETIC ABNORMALITIES USING FLUORESCENCE *IN SITU* HYBRIDIZATION TECHNIQUE

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The analysis of chromosomal aberrations (CA) in peripheral blood lymphocytes has been widely accepted as a useful biomarker both in pre-natal diagnoses and cancer cytogenetics, as well as to determine the biologically significant dose of specific genotoxic agents (both physical, such as ionising radiations, and chemical) to which an individual is exposed.

A particularly useful cytogenetic technique for the analysis of CAs is *Fluorescence in situ Hybridization* (FISH) which simplifies the automatic identification and characterisation of aberrations, allowing the visualisation of chromosomes as bright signals on a dark background, and a swift analysis of stable aberrations which are particularly interesting for late effects.

The main limitation of CA analysis is the rarity with which these events occur and therefore the time necessary to single out a statistically significant number of aberrant cells. In order to address this problem a prototype system (RAIC) capable of automatically searching, acquiring, and recognising chromosomal images of samples prepared using FISH has been developed. The system is able to score a large number of samples in a reasonable amount of time using a predefined search criteria.

The system is based on the appropriately implemented and characterised automatic metaphase search engine Metafer4 (MetaSystems, Germany), coupled with a specific module for the acquisition of high magnification metaphase images with any combination of fluorescence filters. These images are then analysed and classified using the original software. The prototype is currently capable of separating normal metaphase images from presumed aberrant ones.

This system is currently in use in our laboratories both by us and by other researchers not involved in its development in order to carry out analyses of CAs induced by ionising radiation.

RAIC allows the simple acquisition and management of large quantities of images and makes it possible to carry out methodological studies –such as the comparison of results obtained by different operators– as well as increasing the degree of standardisation of the criteria upon which analyses are carried out.

This paper analyses the system's main characteristics and limitations, presenting the results of the tests performed during the implementation and the use of the Metafer4 together with a comparison with the results obtained with manual scoring, as well as the results of the test of the algorithms we wrote to automatically analyze acquired images.

## CFD SIMULATION OF ACTIVITY DISTRIBUTIONS OF DEPOSITED RADON PROGENIES IN CENTRAL HUMAN AIRWAYS

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### Objectives

The general objective of this research is the modelling of biophysical effects of inhaled radon progenies especially their deposition patterns in the central airways where most of the adverse health effects have been found in case of uranium miners. One of the weakest points of current lung dosimetry models is that they cannot take into consideration the local inhomogeneity of cellular burden. Our effort is also related to the question of the LNT (linear no threshold) dose-effect hypothesis and may serve as a useful tool for lung cancer modelling.

### Methods

A three-dimensional physiologically realistic geometry of the 1-5<sup>th</sup> generations of human tracheobronchial tree (leading to the right upper lobe) has been simulated, then the user enhanced FLUENT CFD (computational fluid dynamics) code has been applied to compute the airflow field at different flow rates and the trajectories of injected radioactive particles have been traced to determine the local deposition patterns. Activity distributions of the deposited radionuclides have been computed based on the deposition patterns.

### Results

The airflow fields and deposition patterns are highly sensitive to the shape of the geometry and breathing conditions. Computed deposition patterns of attached and unattached radon progenies are strongly inhomogeneous at all flow rates creating hot spots, mainly at the carinal regions. The related activities are also much higher in the hot spots than the average values in these airways.

### Conclusions

The local inhomogeneities of the activities of inhaled radon progenies suggest that the inhalation low average doses may present quite high doses in case of several large clusters of cells. The elaborated model is an improvement compared to the presently available micro-dosimetry models, which can describe only the average burdens.

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## MICROBIAL PROCESSES IN RADIOACTIVE WASTE REPOSITORY

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The proportion of nuclear energy in the energy production of industrialized countries makes up about 70 % (France), 35 % (Japan), 30 % (Germany), and 11-15 % (USA and Russia). The development of nuclear industry is closely related to the solution of the problem of nuclear waste disposal. Large national and international research programs are currently under way in Canada, Finland, France, Great Britain, Japan, Spain, Sweden and the United States of America in which different questions concerning the safety of future underground repositories for nuclear waste are being studied. High level waste or so called long-lived high-level waste consists primarily of spent nuclear fuel. After 1000 years, less than 1 % of the radioactivity remains. The fuel must, however, be kept isolated for a long period (10 000 years) of time, since the remaining radionuclides can be harmful if they enter the human body.

The main purpose of this work was to study the microbial activity in the Hungarian Upper Permian Siltstone (Aleurolite) Formation from the aspect of the safety of future underground repositories for HLW (high-level nuclear waste). Air, groundwater, technical water, rock and surface samples were collected aseptically from different depths. The number of morphologically different isolates was 300. The gases produced by the isolates were CO<sub>2</sub>, H<sub>2</sub>, NH<sub>3</sub>, N<sub>2</sub>, H<sub>2</sub>S. About 20% of the aerobic isolates produced siderophores. The highest proportions of acid producers in the aerobic and anaerobic isolates from the air samples were 63% and 54%. Altogether 84 of the aerobic isolates and 80 of the anaerobic isolates were sporeformers. The radiosensitivity of the aerobic and anaerobic isolates was also determined: the D<sub>10</sub> values of the aerobic spore-formers were ranging between 0.8-2.44 kGy, and those of the anaerobic spore formers were 1.86-4.93 kGy. Our results indicate that the sulfate-reducing bacteria and the production of complexing agents (siderophores) may contribute

to the mobilization of radionuclides from underground repositories. As well, microbial gas production can influence the environmental conditions. These facts must be considered during the planning of a nuclear waste repository.

## THE EFFECT OF SIMULATED EXTRATERRESTRIAL CONDITIONS ON BACTERIOPHAGE AND DNA THIN FILMS

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The experiment “Phage and uracil response” (PUR) will be accommodated in the EXPOSE facility of the ISS to examine and quantify the effect of specific space conditions on bacteriophage T7 and isolated T7 DNA thin films. To achieve this a new method was elaborated for the preparation of DNA and nucleoprotein thin films (1). During the EXPOSE Experiment Verification Tests (EVT) the samples were exposed to vacuum ( $10^{-6}$  Pa), to monochromatic (254 nm) and polychromatic (200-400 nm) UV radiation in air, in argon atmosphere, as well in simulated space vacuum. Using neutral density (ND) filters dose-effect curves were performed in order to define the maximum doses tolerated, and we also studied the effect of temperature in vacuum as well as the influence of temperature fluctuations. We obtained substantial evidence that DNA lesions (e.g. strand breaks, DNA-protein cross-links, DNA-DNA cross-links) accumulate throughout exposure. DNA damage was determined by quantitative PCR using 555 bp and 3826 bp fragments (2) of T7 DNA and by neutral and alkaline agarose gel electrophoresis; the structural/chemical effects were analyzed by spectroscopic and microscopical methods. Characteristic changes in the absorption spectrum, in the electrophoretic pattern of DNA and the decrease of the amount of the PCR products have been detected indicating the damage of isolated and intraphage DNA. Preliminary results suggest a synergistic action of space vacuum and UV radiation with DNA being the critical target.

1. Fekete et al. J. Luminescence 102-103, 469-475, 2003

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## IRRADIATION OF LIVING CELLS WITH HIGH ENERGETIC PROTONS

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Our objective is the study of the cellular response to targeted irradiation of living cells with high energy protons with special emphasis to the Bystander effect. For the targeted irradiation we use our high energy ion nanoprobe LIPSION. It provides a focused ion beam ( $H^+$  or  $He^+$ ) of energies up to 3 MeV with a beam spot of less than 50 nm in diameter (LET for 2 MeV: 10 keV/ $\mu$ m for  $H^+$ , 100 keV/ $\mu$ m for  $He^+$ ). The ion beam can be moved to the target position (cells or even cell compartments) by a magnetic scanning system. Furthermore, the number of ions per target position can be chosen.

The cells are seeded into small Petri dishes of 35 mm in diameter with a  $2 \times 2$  mm<sup>2</sup>, 200 nm thick  $Si_3N_4$  irradiation window in the center of the Petri dish bottom. During the irradiation we measure the energy loss within the cells by analyzing the transmitted energy of the ions using a particle detector installed behind the cells. With the information of the energy loss the applied dose can precisely be determined. However, we have to avoid energy loss in the surrounding medium by removing the medium as much as possible. The procedure of irradiation where the cells are without medium takes ca. 10 min. Tests showed a good acceptance by the cells.

After irradiation we study the cellular response (survival fraction, apoptosis, micronuclei, cytokines) of different cell lines (HUVEC, HeLa, COS7, CHO). For the survival test the cells are stained with fluoresceindiacetate (FDA) and propidium iodide (PI). First tests showed that the special cytokines interleukin-1 $\alpha$  and integrin- $\alpha_1$  are suitable response markers. The patterned irradiation of semi-confluent cells did not show a pattern related distribution of responding cells. These findings lead to the assumption of paracrine signaling pathways supporting a possible Bystander effect.

## STUDIES ON THE CELLULAR BYSTANDER RESPONSE AFTER EXPOSURE TO LOW FLUENCES OF HEAVY IONS

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Objective: To investigate if bystander radiation responses of non-targeted cells can be observed after heavy ion exposure of human fibroblasts. Although increasing evidence for a bystander effect has been reported following  $\alpha$ -particle exposure, for heavier ions this has not yet been clearly established.

Methods: Confluent monolayers of three normal human fibroblast strains were used, and the intercellular communication was assessed using fluorescent dye-transfer. Irradiation with low fluences of heavy ions (LET between 33 and 15000 keV/ $\mu$ m) was carried out, so that only 1% to 10% of the cells were traversed by a particle. At several time intervals after exposure, the overall induction of the cell cycle regulator CDKN1A (p21) was assessed by Western blot and immunofluorescence (IF). In parallel, the amount of sister chromatid exchanges was quantified and the differentiation pattern was determined by morphological features. Additionally, experiments were done using a heavy ion microbeam developed at GSI allowing for the targeted irradiation of single cells.

Results: Intercellular communication was observed for all fibroblast strains used. A slight overall induction of CDKN1A was found up to 24 hours after exposure to low fluences of carbon ions, but not after helium and uranium exposure. In IF studies a considerable variability of the protein levels of control populations was shown. Nevertheless, the Western blot results could be confirmed regarding the overall induction. No distinct clusters of cells bearing an elevated CDKN1A expression level in the direct neighbourhood of the hit cells were noticed during the single cell irradiation experiments. Remarkably, quite different levels of CDKN1A protein were found even in the traversed cells.

In contrast to the weak but consistent effect regarding CDKN1A induction, the differentiation pattern reflecting the long-term radiation response was not different from the unirradiated populations two weeks after low fluence carbon irradiation. Furthermore, in ongoing studies no significant excess formation of SCEs, which would indicate a potentially induced chromosomal damage, was detected in low fluence irradiated cells.

Conclusion: After heavy ion irradiation a slight induction of CDKN1A in non-hit cells is observed, but is so far not indicative for a clear bystander effect. The CDKN1A induction is not restricted to the surrounding cells. It is likely to be an early effect which is not reflected in the longterm cell response of the bystander fibroblasts.

## HUMAN MESENCHYMAL STEM CELLS HOME SPECIFICALLY TO RADIATION INJURED TISSUES AND EVIDENCED PHENOTYPICAL VARIATION IN A NOD/SCID MOUSE MODEL.

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Stem cell plasticity has increasingly been reported in a wide range of adult tissues. Mesenchymal Stem Cells (MSCs) might proliferate and differentiate in a site-specific manner. We hypothesized that radiation-induced tissue injuries might play a role in the recruitment of MSC for tissue repair. In order to determine factors involved in MSC recruitment, migration, and homing kinetics, human MSC were injected following global or local (abdomen) irradiations to an immunotolerant NOD /SCID mouse model. We have tracked human MSCs through the use of real time PCR assay that specifically amplify human genes. Systemic delivery of human bone marrow expanded MSCs into sublethally irradiated NOD/SCID mice recipients resulted in homing to liver (0,4%), bone (0,35%), bone marrow (0,3%), gut (0,2%), hearth (0,1%), kidney (0,03%), brain (0,02%), lung (0,01%), stomach (0,01%) 15 days post irradiation. Human MSCs were found for up to 3 months post irradiation in gut, lung, bone marrow, and heart. After abdominal radiation exposure human MSCs engrafted preferentially in the abdomen: liver (1%), spleen (0,5%), stomach (0,25%), kidney (0,2%) and gut (0,02%) at 15 days. By contrast human MSCs were scarcely detectable in other body areas. Tissue localization was performed using immunohistochemical staining with human  $\alpha$ -2-microglobulin antibody. Immunohistochemical staining revealed a perivascular MSC implantation. MSCs were also implanted in functional units such as kidney glomerulus. These observations support our hypothesis that MSCs might have an important potential of *in vivo* specific tissue-differentiation. Preliminary results on human MSC gene expression in tissues confirmed these data. These results support the hypothesis that MSCs can be recruited to the radiation exposed sites and might have potential of *in vivo* transdifferentiation into injured tissues. In conclusion the use of MSC as a source of cells able to repair the damages induced by radiation could be an interesting strategy to limit the deleterious effects to normal tissues -thus allowing dose escalation- during radiotherapy.



## ROLE OF BAX/VDAC1 INTERACTION IN IR-INDUCED APOPTOSIS

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The mitochondrial (MT) cell-death pathway that involves cytochrome c (Cyt c) release under regulation by pro- and anti-apoptotic Bcl-2 family proteins plays a pivotal role in IR-induced, p53-dependent apoptosis. The Cyt c release pore remains still controversial, despite proposal of several models in vitro such as permeability transition pore, oligomeric Bax (Bak) pore, and lipidic pore. We aimed to investigate the role for Bax (Bak)/VDAC1 hybrids in Cyt c release after treatments with IR and/or modifiers of Bax (Bak)/VDAC1 interaction.

[Material and Methods] The human B-cell lines used were Black93 (expressing p53, Bax and Bcl-XL but not Bcl-2 and Bak) and Reh (expressing Bcl-2 and Bak but not p53 and Bax). Apoptosis was assayed by annexin V-FITC/PI and LDH. Protein expression and Cyt c release were by immunoblotting (IB). Following cross-linking of proteins in MT isolated from the cells, Bax (Bak) oligomers and Bax/VDAC1 hybrids were identified by IB and the 2-step IP, respectively.

[Results and Conclusion] p53/Bax-expressing Black93 cells showed hypersensitivity to IR-induced Cyt c release and apoptosis through the MT death pathway, but p53/Bax-lacking Reh cells were completely resistant without Cyt c release. IR induced the increased translocation of Bax to MT in Black93, but unaltered Bak in Reh MT. The protein cross-linking and the 2-step IP (1<sup>st</sup> anti-VDAC; 2<sup>nd</sup> anti-Bax or anti-Bak) studies indicated the IR induced mono- and dimeric Bax/VDAC1 hybrids in MT from sensitive Black93 cells and only monomeric Bak/VDAC1 hybrid in MT from resistant Reh cells. The Bax and Bak oligomers in Black93 and Reh MT respectively were rather invariable with time after irradiation. In Black93 cells, further, (i) clotrimazole that displaces hexokinase and recruits Bax to VDAC1 enhanced the IR-induced Cyt c release and apoptosis, (ii) a cell-permeable synthetic peptide of VDAC1-binding domain of hexokinase induced formation of Bax/VDAC1 hybrid dimer, Cyt c release and death, but (iii) a VDAC inhibitor DIDS suppressed the hybrid dimer formation without affecting Bax oligomers, Cyt c release and apoptosis after IR. On the other hand, a chemical agonist HA14-1, that binds to the Bcl-2 surface pocket and acts like a BH3-peptidic apoptosis-sensitizer, induced the Bak/VDAC1 hybrid dimer in resistant Reh cells, which, otherwise not, rendered the cells highly susceptible to Cyt c release and apoptosis. Together, the above results suggest that the MT death pathway under p53-Bax regulation is indispensable for IR apoptosis, and a new finding that, although Bax (Bak) oligomers have been proposed in vitro, the Bax (Bak)/VDAC1 hybrid dimer in the outer MT membrane also constitutes a Cyt c-conducting channel within cells at an important death control point in MT.

## MUTATION PROCESS IN CHRONICALLY IRRADIATED BANK VOLE POPULATIONS INDICATES THE TRANSGENERATIONAL GENOMIC INSTABILITY INDUCED BY CHERNOBYL FALLOUT

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The objective of this investigation is analysis of mutagenesis dynamics in bank vole populations chronically exposed to low doses of ionizing radiation in connection with the absorbed dose dynamics and the number of affected generations over 1986–1996. Frequencies of different end-points (chromosome aberrations in bone marrow cells and embryonic mortality) as well as the dose rate and absorbed doses of external and internal irradiation from caesium isotopes were determined for four populations inhabiting the sites with different ground deposition of  $^{137}\text{Cs}$  (8–1526 kBq/m<sup>2</sup>). It has been first revealed that the main feature of mutagenesis dynamics in populations of mammals chronically exposed to very low doses of ionizing radiation is a gradual increase in the rate of somatic mutagenesis and embryonic lethality over 1–22 generations. At the same time, the dose rate and whole body absorbed dose decreased in every consecutive generation after the primary radiation insult in 1986. The data on chromosome aberrations and embryonic lethality were fitted by the exponential and linear functions respectively. It means that genomes of animals from distant generations are more sensitive to the impact of very low radiation doses in comparison with those of animals of prior generations. The fact that dynamics of somatic mutagenesis (by the chromosome aberration frequency in bone marrow) and embryonic lethality during the period of the study closely resemble each other is an additional proof for the persistence of the delayed response. Thus, enhanced response of distant generations of mammals to low doses of ionizing radiation is likely to be due to transgenerational genomic instability.

## REALISTIC APPROXIMATION OF KÖVÁGÓSZÖLŐS RADON PROBLEM

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The health hazard of radon problem in Kövágószőlős village was overestimated and improperly interpreted by media in the beginning of that year. The realistic interpretation is given in our presentation.

Kövágószőlős village is mostly exposed to the effect of abandoned uranium mining, however natural ore outcrops are situated in the neighbouring area as well. An extra radiation dose to inhabitants arises from a mixture of natural and artificial origin, although the latter sources has been practically remedied. The most important question wheather the radon can migrate into the human residences from the abandoned underground mining cavity system. The results of indoor radon measurements indicate that the radon concentration exceeds the intervention level recommended by ICRP in the nearly 10 % of dwellings, however the distribution of anomaly pattern doesn't follow the artificial objects.

A complex survey was elaborated to reveal the conditions. All the dwellings were radon monitored above the possible disturbed zone of underground mining cavities and the possible migration zones are investigated by soil gas radon measurements and surface radon exhalation studies. In the case of mining origin would be justified, an efficient intervenion is neccessary to decrease the radon levels (by the ventillation of underground system). Otherwise the proper management of high radon levels is a general public health task.

## POST-IRRADIATION RECOVERY IN HUMAN GLIOMA M059K CELL LINE AND ITS DNA-PK<sub>CS</sub> DEFICIENT COUNTERPART M059J: EFFECT OF SIGNALING PATHWAYS INHIBITORS

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**OBJECTIVE:** To study mechanisms underlying radiosensitisation of human glioma cells by inhibitors of growth factors-dependent signaling pathways.

**METHODS:** The subjects of the study were M059K and J cell lines, the latter deficient in the catalytic subunit of DNA-dependent protein kinase, DNA-PK<sub>CS</sub>. The cells were pretreated (or not) with tyrphostin AG 1478 (a specific inhibitor for epidermal growth factor receptor, EGFR) or PD 098059 (acting on MEK 1/2 kinases) and subsequently X-irradiated. The cell survival was assessed with clonogenicity tests. DNA break rejoining was estimated by alkaline comet assay (single- and double- DNA breaks, SSBs and DSBs) and pulse-field gel electrophoresis, PFGE (DSBs). In parallel, cell death processes were monitored by determining early and late apoptotic DNA fragmentation (PFGE and conventional DNA electrophoresis respectively) as well as counting percentages of apoptotic and necrotic cells.

**RESULTS:** M059J cells were much more sensitive to X-irradiation than M059K cells, which was in agreement with post-irradiation slower SSBs and DSBs rejoining in these cells. M059J cells were also more sensitive to PD 098059 but more resistant to tyrphostin AG 1478 than M059K cells. Within an X-ray dose-range corresponding to 50 % or less survival, both inhibitors exerted a radiosensitising effect. Below this range, no effect or even protection were observed. DSBs rejoining after concomitant treatment with X-rays (10 Gy) and the inhibitors was overlapped by the appearance of rare DNA cuts characteristic for early apoptosis.

**CONCLUSIONS:** Cell signaling inhibitors: tyrphostin AG 1478 and PD 098059 radiosensitise human glioma M059K and J cells at X-ray dose range corresponding to 50 % survival or less, but had no effect or a protecting effect at lower doses, possibly due to upregulation of growth factor receptors. Treatment with the inhibitors accelerated the onset of apoptosis in both cell lines. Early appearance of rare DNA cuts of apoptotic origin is a confounding factor in determination of the influence of the inhibitors on post-irradiation DSB rejoining rate.

## NEW APPROACHES TO PREDICT THE SEVERITY OF THE HEMATOPOIETIC SYNDROME AFTER ACUTE IRRADIATION

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*Objective:* Based on our previous studies showing that G-CSF mobilises to peripheral blood (PB) the complete repertoire of mouse bone marrow (BM) progenitors, we hypothesized that the number of progenitors that can be mobilized to PB after irradiation would predict the total reserve of hematopoietic progenitors that survive the radiation exposure.

*Methods:* Initially, B6D2F1 mice were total body irradiated with doses of up to 3 Gy of X-rays and then treated with G-CSF either immediately after irradiation, 3 days later or 6 days later. The content of hematopoietic progenitors (CFCs) was then assessed in the PB of the animals according to standard procedures. Also other mouse strains and partial body irradiation regimens were used thereafter.

*Results:* Mobilisations initiated the day of the irradiation or 3 days later, resulted in a dose-dependent reduction in the number of progenitors mobilised to PB. When animals were treated with G-CSF 6 days after irradiation, no evident correlation between the radiation dose and the number of mobilised progenitors was seen. Similar dose-dependent relationships were observed in Balb/c and C57Bl mice, compared to B6D2F1 mice. By means of the evaluation of the total number of hematopoietic progenitors that survived to increasing doses of radiation we determined that the number of mobilised progenitors was highly dependent on the total reserve of bone marrow progenitors that survived the irradiation. A similar observation was made in SCID/NOD mice, highly sensitive to ionising radiation. To investigate whether our approach could also be useful to predict the HSC reserve after partial body radiation exposures, a high dose of X rays (10Gy) was given to mice in which different proportions of the BM were shielded. As happened in total body irradiated mice, a good relationship was observed between the number of mobilised progenitors and both the reserve of BM progenitors and the severity of the hematopoietic syndrome.

*Conclusions:* Our results show that the number of progenitors that can be mobilised to PB after irradiation is indicative of the global reserve of hematopoietic progenitors that survive the radiation exposure, suggesting that the evaluation of mobilised progenitors would constitute a new approach to directly predict the severity of the hematopoietic syndrome of victims exposed to unknown doses of radiation.

## COMBINATION WITH p53 GENE THERAPY ENHANCES THE ANTITUMOR EFFECT OF RADIATION IN A GLIOMA MODEL

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*Objective:* The overall goal of this study was to analyze the effect and mechanism of radiation in combination with vaccinia viruses (VV) carrying the p53 gene against glioma. *Methods and Results:* Comparison of two alternative treatments of cultured C6 (p53<sup>+</sup>) and 9L (p53<sup>-</sup>) rat glioma cells showed significantly reduced survival for both cell lines, especially 9L, when radiation was applied prior to virus versus radiation alone. High p53 protein expression mediated by VV-TK-p53 was measured in infected cells. Single modality treatment of C6 cells with psoralen and UV (PUV)-inactivated VV-TK-p53 (PUV-VV-TK-53) or radiation significantly decreased survival compared with PUV-inactivated L-15 (PUV-L-15) control virus. However, no difference was observed between radiation and combination treatments of C6 cells. In contrast, radiation followed by PUV-VV-TK-53 resulted in dramatic reduction of 9L cell viability, compared to single modality treatment. Flow cytometry analysis of Annexin-V-stained 9L cells showed that radiation and PUV-VV-TK-53 caused a significant decrease in live cells (17.2%) as compared to other treatments and control (61.6 - 98.3%). Apoptosis was observed in 37.2% of cells, while the range was 0.7-7.8% in other treatment groups; maximal p53 level was measured on day 7 post-infection. In athymic mice bearing C6 tumors, VV-TK-53 plus radiation in both single and multiple therapies resulted in significantly smaller tumors by day 30 compared to the agents given only once. Immunohistochemical analysis of tumor sections demonstrated p53 protein expression over 20 days after VV-TK-53 treatment. Analysis of blood and spleen cells of mice given multiple combination treatments showed significant splenomegaly, leukocytosis, and increased DNA synthesis and response to mitogen. Multiple combination treatments were also associated with significantly elevated natural killer and B cells in the spleen. There were no overt toxicities, although depression in red blood cell and thrombocyte parameters was noted. *Conclusion:* The data demonstrate that radiation significantly improves the efficacy of VV-mediated tumor suppressor p53 therapy and may be a promising strategy for glioma treatment. Furthermore, the results support the conclusion that the mechanisms underlying the enhanced anti-tumor effect of combination treatment include apoptosis/necrosis and up-regulation of innate immune defenses.

## GENETIC POLYMORPHISMS OF DNA REPAIR GENES AND RADIOSENSITIVITY OF CERVICAL CANCER CELL LINES

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**Objective:** Cancer of the cervix is the third most common cancer affecting women worldwide. Radiation therapy has been successfully used for the treatment of this kind of cancer, however, despite the use of near-tolerance doses of radiation, a significant number of patients will experience relapse within radiation field. For that reason the identification of factors, that help to predict which patients are at risk for radiation failure, is essential. A potentially important source of inter-individual variability in response to radiation is DNA-repair capability. Our project is aimed to investigation of the biological responses of peripheral blood lymphocytes of cancer patients with adverse reaction to irradiation *in vitro*. Presented results form introductory part of the project. **Methods:** In cervical cell lines CaSki, C33, HeLa and SiHa with different origin and phenotype, the alkaline comet assay, as a predictive assay of radiosensitivity, was used. Both the initial DNA damage, a level of strand breaks immediately after irradiation at doses ranging from 0.5 Gy to 4 Gy, and the residual DNA damage at 15, 30, 45 and 60 min after irradiation were assessed. Genotypes of DNA repair genes (*XRCC1*, *XRCC4*, *XPB*, *hOGG1* and *P53*) and genes for xenobiotic-metabolizing enzymes (*GST* and *ExH*) were analyzed in all four types of cervical cell lines by PCR combined with RFLP assays. **Results:** The comet data clearly indicate a variable but dose-dependent increase in the initial DNA damage in all cell lines. The results have shown that the level of DNA strand breakage has returned near the background level within 30-60 min after irradiation. Nevertheless, radiation induced DNA strand breaks and their rejoining were found to be cell line-dependent. In the most radiosensitive cell line C-33 A, polymorphisms of *XPB*-23 and *hOGG1* genes were found. **Conclusions:** The ability to repair DNA lesions induced by radiation could be affected by polymorphisms in various DNA repair genes. Therefore the main idea of our study is the correlation between DNA repair capacity and polymorphisms of repair genes.

## INVESTIGATION OF TUMOR PROGRESSION MARKER EXPRESSION IN HDC KO MICE ON EXPERIMENTAL DERMATOFIBROSARCOMA MODEL

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**Objective:** The complex chemical and photocarcinogenesis is a classical experimental model for studying development of tumours. Histamine seems to be an immunomodulator and also could have an important role in angiogenesis regulation associated with promotion of tumour progression.

**Methods:** In our study, we used transgenic mice genetically lacking histidine decarboxylase (HDC<sup>-/-</sup>), to evaluate the role of endogenous histamine synthesis on tumour growth and spreading. Invasion markers and in general the tumour development have been measured. To examine the alterations in expression of uPA, uPAR and VEGF their relative mRNA levels were measured from the tumour tissue by quantitative real time RT-PCR. Angiogenic markers as PAI-1/2, angiopoietin1/2, HGF, bFGF, and VEGFs were analysed by pathway specific gene expression profiling system (GEArray<sup>TM</sup>Q Series Superarray KIT).

**Results:** The chronic suberythral UV-B radiation together with croton oil treatment accelerated CD34, CD44 positive dermatofibrosarcoma (DFS) genesis in this model. Tumour locations were similar in both wild type and transgenic animals, but the tumour incidence was significantly higher in HDC<sup>+/+</sup> mice. Controversially, the tumours appeared earlier in the HDC<sup>-/-</sup> mice and their survival were worse than the wild types.

The expression of uPA, uPAR and VEGF were significantly reduced in the HDC<sup>-/-</sup> mice, the H1R and H2R were unchanged and the array data showed elevated mRNA level of PAI-2. We have established in vitro culture from the tumours (DFS<sup>HDC+/+</sup> and DFS<sup>HDC-/-</sup>) for further investigations

**Conclusion:** We showed that DFS was induced with this carcinogenesis protocol on the histamine lacking, as well as, the wild type mice. Plasminogen activator system, and vascular endothelial growth factor could be involved in the tumour growth regulation by histamine mediated way.



## RECOMBINANT HUMAN EMBRYONIC KIDNEY CELLS AS SENSORS FOR ACTIVATION OF THE NUCLEAR FACTOR $\kappa$ B PATHWAY BY HEAVY ION EXPOSURE

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**Objectives:** Cellular stress protection responses lead to increased transcription of several genes via modulation of transcription factors. Activation of the Nuclear Factor  $\kappa$ B (NF- $\kappa$ B) pathway as a possible antiapoptotic route represents such an important cellular stress response. For identifying conditions which are capable to modify this pathway, a screening assay for detection of NF- $\kappa$ B-dependent gene activation using the reporter proteins Enhanced Green Fluorescent Protein (EGFP) and its destabilized variant (d2EGFP) had been developed.

**Material and Methods:** Human Embryonic Kidney (HEK/293) Cells were stably transfected with a receptor-reporter-construct carrying EGFP or d2EGFP under the control of a synthetic promoter containing four copies of the NF- $\kappa$ B response element. Treatment with Tumor Necrosis Factor  $\alpha$  (TNF- $\alpha$ ) gave rise to substantial EGFP/d2EGFP expression of the cells and was therefore used to select for a stably transfected clone for performing experiments with accelerated heavy ions. The time course of d2EGFP expression and thereby activation of NF- $\kappa$ B dependent gene expression was measured after treatment with TNF- $\alpha$  or after heavy ion exposure using flow cytometry.

**Results:** Cellular response to TNF- $\alpha$  was much more pronounced (up to 90 % of the cells) than to heavy ion exposure. After exposure with particle fluences over  $1 \times 10^6$  particles  $\text{cm}^{-2}$  of 95 MeV argon ions (LET  $\sim 230$  keV/ $\mu\text{m}$ ) activation of the NF- $\kappa$ B pathway is significant. For doses from  $2 \times 10^6$  to  $10^8$   $\text{cm}^{-2}$  d2EGFP expression starts and can be seen 3 hours after exposure; d2EGFP fluorescence reaches its maximum after 12 to 24 hours with up to 40 % of the cells expressing d2EGFP. Fluorescence stays on a high level for several days, while only about 1 percent of untreated cells fall in the gate for EGFP(+) cells. At  $2 \times 10^7$  particles  $\text{cm}^{-2}$ , when a survival level of 0.5 is reached, maximal NF- $\kappa$ B activation takes place. With greater doses, overall fluorescence yield is reduced, in parallel with a reduction of cellular growth.

**Conclusion:** The reported experiments have clearly shown, that accelerated argon ions (95 MeV, LET  $\sim 230$  keV/ $\mu\text{m}$ ) induce the NF- $\kappa$ B pathway already at low particle densities (1-2 particle hits per nucleus), which result in as less as 10 induced DSBs per cell.

## EVALUATION OF THE RETICULOCYTE MICRONUCLEUS ASSAY IN PERIPHERAL BLOOD OF PATIENTS TREATED WITH RADIOIODINE

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In mice micronucleus (MN) assay in immature erythrocytes of the blood is acceptable for assessing MN induction in bone marrow. The assay is widely used in toxicology studies and radiobiology. Because the human spleen captures and destroys micronucleated erythrocytes, the MN assay is only used in splenectomized persons. Liliane Abramsson-Zetterberg et al. (Environ. Mol. Mutagen. 36 (2000) 22-31) could show that the MN assay can also be applied in healthy humans, if it is restricted to the youngest reticulocyte cohort of peripheral blood, the transferrin positive reticulocytes (TF-Ret). In this presentation results will be given on dose and time dependence of the frequency of micronucleated TF-Ret (MN-Tf-Ret) in blood of patients during radioiodine therapy (RIT) for thyroid cancer.

Most patients (n=58) were young adults from Belarus, who suffered from thyroid cancer in consequence of the Chernobyl accident. Before RIT patients were thyroidectomized. Several RIT were needed to eliminate cancer. In the first RIT about 50 MBq I-131 per kg BW were administered for ablation of remanent thyroid tissue, and about 100 MBq in the following for elimination of metastases. In RIT red marrow is exposed to an exponentially decreasing low dose rate irradiation of  $\beta$ - and  $\gamma$ -rays for 2 to 4 days. The total dose varied between 0.1 to 0.5 Sv. It was determined according to the recommendations of the MIRD committee. For the determination of MN-Tf-Ret frequency Tf-Ret were isolated immunomagnetically from peripheral blood, and the frequency of MN-Tf-Ret was determined by flow cytometry.

In humans spontaneous MN-Tf-Ret frequency was 1 o/oo. In RIT patients MN-Tf-Ret frequency rose after a latent period of about 3 days after radioiodine administration, reached its maximum about one day later, and declined in the following 2 to 4 days to its initial value. The length of the latent period corresponds to the transit time required by a late erythroblast for nucleus extrusion and reticulocyte maturation in marrow. The increase in MN-Tf-Ret frequency corresponds to the radiation dose during the last erythroblast cell cycle. In patients after RIT the doubling dose was <50 mSv.

In summary the MN-Tf-Ret assay has some features that make it a most useful biological dosimeter in humans. It is very sensitive, since a dose as low as 50 mSv can be detected. The short memory of the assay allows the retrospective comparison between the spontaneous and the radiation induced MN-Tf-Ret frequency on an individual base. Finally, the assay can be performed within three days. Thus, the method may be used for monitoring individuals after suspected accidental radiation exposure, provided blood samples can be obtained during a few days after the accident.

This investigation was supported by the Bundesamt für Strahlenschutz, Grant StSch 4371.

## IDENTIFICATION OF CANDIDATE GENES IN RADIATION-TRANSFORMED HUMAN BREAST EPITHELIAL CELLS (B42) BY POSITIONAL CLONING

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**Objective:** Genetic changes in radiocarcinogenesis were studied using an immortalised cell line derived from primary cultures of human breast epithelial cells. Following fractionated exposure to  $\square$ -rays anchorage independent cell lines were established and analysed for chromosomal aberrations. To identify genes involved in the process of radiation-induced transformation, chromosomal breakpoints were further characterised by positional cloning.

**Methods:** Human mammary epithelial cells were immortalized using the catalytic subunit of telomerase (hTERT). After exposure to fractionated doses of  $\square$ -rays, a series of neoplastically transformed cell lines derived from anchorage independent cell clones have been established. These cell lines were investigated for chromosomal aberrations by spectral karyotyping analysis (SKY). FISH (fluorescence *in-situ* hybridisation) with YAC clones was applied to identify breakpoint-spanning clones.

**Results:** From the 15 neoplastically transformed cell lines analysed a total of 32 different breakpoints have been identified by SKY analysis. A series of breakpoints occurred recurrently in independent cell lines. There was a clustering of breakpoints on chromosome 10, and in addition all transformed lines show an isochromosome 8. Up to now, six breakpoints on chromosome 10 (10p22.1), (10p11.2), (10q11.1), (10q22), (10q23) and (10q4) were selected for positional cloning by FISH analysis. The identification of breakpoint-spanning clones revealed candidate genes (PTEN, HOX11, Caveolin, frizzled-8) that are located on the respective clones.

**Conclusion:** Human cell lines immortalised by hTERT are powerful model systems to study chromosome aberrations and molecular genetic changes after neoplastic transformation by ionising radiation. Sorting of marker chromosomes by FACS of specific marker chromosomes - just established in our laboratory - will further accelerate the breakpoint cloning procedure since they will be used on BAC arrays to identify breakpoint-spanning clones more efficiently.

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## MODULATION OF HAEMATOPOIESIS BY ACTIVATION OF ADENOSINE CELL SURFACE RECEPTORS: A NEW POSSIBILITY FOR TREATMENT OF RADIATION-INDUCED MYELOSUPPRESSION?

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### Objective

Radiation-induced myelosuppression presents a serious medical problem. In the laboratories of the authors, studies have been performed in which pharmacologically induced activation of adenosine cell surface receptors has been tested from the point of view of its effects on either normal or suppressed murine haematopoiesis. The receptors were activated non-selectively (by elevating extracellular adenosine) or selectively (using synthetic agonists of adenosine receptors specific for individual receptor subtypes).

### Methods

(CBAX57BL)F<sub>1</sub> mice were used. Myelosuppression was induced with a <sup>60</sup>Co  $\gamma$ -ray source and/or with cytostatic drugs. Effects of adenosine monophosphate (AMP, adenosine prodrug) given jointly with dipyridamole (DP, inhibitor of the cellular uptake of adenosine), and actions of synthetic adenosine receptor agonists NECA (non-selective), CPA (selective for A<sub>1</sub> receptors), CGS 21680 (selective for A<sub>2A</sub> receptors) and IB-MECA (selective for A<sub>3</sub> receptors) were tested. Complex analysis of haematopoiesis was performed.

### Results

Elevation of extracellular adenosine obtained by combined action of AMP + DP was found to support haematopoiesis and act radioprotectively if the drugs were given before irradiation. The AMP + DP combination stimulated haematopoiesis also when given repeatedly following induction of severe myelosuppression evoked by combined action of  $\gamma$ -radiation and carboplatin. In experiments employing 5-fluorouracil, a cycle-specific cytotoxic agent, activation of A<sub>3</sub> receptors by IB-MECA was observed to enhance cycling of hematopoietic progenitor cells.

### Conclusions

The findings of the effects of pharmacological activation of adenosine receptors on murine haematopoiesis may have also clinical impact in treatment of radiation injuries, and in supporting haematopoiesis damaged by cytotoxic drugs.

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## REESTABLISHMENT OF A G1 CHECKPOINT IN MOUSE ES CELLS FOLLOWING EXPOSURE TO IONIZING RADIATION

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**Objective:** Induction of double strand breaks by ionizing radiation causes somatic cells to arrest at multiple cell cycle checkpoints, but primarily in G1. Embryonic stem (ES) cells, display a distinct G2 checkpoint, but lack a G1 arrest and are hypersensitive to DNA damage. ES cells, like germ cells, are pluripotent and therefore, must have exquisite mechanisms to protect their genomes beyond those utilized by somatic cells. Our goal is to determine the basis for the absent G1 arrest in ES cells.

**Methods and Results:** There are at least two ways that this can be accomplished. 1) ES cells can suppress mutation, and we have reported that ES cells have a mutation frequency about 100-fold lower than somatic cells. 2) They can facilitate apoptosis, a mechanism that we now describe. We postulate that since ES cells lack a G1 checkpoint, cells with damaged DNA following irradiation proceed into S phase where the damage is exacerbated and undergo apoptosis. This provides a mechanism that can remove cells with an unwanted mutational burden from the population. We hypothesize that if the G1 arrest were re-established, ES cells would be protected from cell death. We have identified the signaling pathway that is compromised in mouse ES cells and that leads to a natural absence of a G1 arrest after exposure to ionizing radiation and consequent DNA damage. In somatic cells ATM is phosphorylated and phosphorylates Chk2 on serine 68. The activated Chk2, in turn, phosphorylates the Cdc25A phosphatase and causes its ubiquitination and degradation. The lack of Cdc25A results in an inability to dephosphorylate Cdk2 with consequent arrest in G1. In ES cells, this pathway is compromised. Chk2 is not intra-nuclear as it is in somatic cells, but it is modified and targeted to centrosomes. This centrosomal localization apparently renders it unavailable to phosphorylate its downstream target Cdc25A. We have reconstituted this pathway and have demonstrated by flow cytometry that after ionizing radiation the ES cells now arrest in G1.

**Conclusion:** Consistent with our hypothesis, there is a significant reduction in cell death using AnnexinV as a marker for apoptosis. We suggest that ES cells have lost the capacity to arrest in G1 to facilitate apoptosis and to maintain genomic integrity within the cell population.

## FACTORS INFLUENCING THE LATENT PERIOD FOR ACUTE RADIATION DAMAGE TO THE SKIN AND IMPLICATIONS FOR SO CALLED CONSEQUENTIAL LATE EFFECTS IN CUTANEOUS TISSUES.

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The outermost surface of the skin, the epidermis, is composed of a number of layers. The deepest of these, that the junction with the dermis is the basal layer where most cell proliferation is to be found although not all proliferating cells are stem cells. Above this single layer of cells is the spinous and granular layers, plus the stratum corneum, which is composed of flattened dead cells. The more superficial layers, above the basal layer, are associating with the functional integrity of the epidermis. Collectively the cellular organisation of the epidermis is referred to as 'hierarchical' in the sense that under steady-state conditions the rate of cell production in the basal layer is matched by the rate of cell loss from stratum corneum. Cell transit through the various layers is controlled and it is this transit time, the total turnover time that determines the latent period for the time of onset of the most significant acute radiation reaction of the epidermis, moist desquamation. Either the death of stem cells and transit proliferating cells in the basal layer or simply the inhibition of cell proliferation (cell production) is associated with continued differentiation and cell loss at the normal rate so that moist desquamation results over a timescale associated with the total turnover time of the epidermal structure under investigation. In thinner rodent skin, turnover times are shorter than in human and take skin and so is the time of onset of moist desquamation. Differences in turnover time exist between different pig strains and this influences the time of onset of desquamation.

For a given system that time of onset of desquamation is independent of the total radiation dose and the dose fractionation schedule used. However, for a given system the severity or extent of desquamation is dose-related since this depends on the extent to which stem cells of reproductively sterilised. Also the duration of moist desquamation is related to total dose since the rate of epidermal reconstitution will depend to the greatest extent on the number of viable stem cells available for the task. In areas of total stem cell denudation, where a new epidermal covering relies on cell migration from field edges there will, as with superficial thermal or chemical induced desquamation, be secondary damage even tissue loss from the deeper layers of the skin. In this situation tissue healing is frequently delayed and leads to scarring. Such changes are frequently referred to as 'consequential late effects' since the detriment is persistent. However, these detriments are in reality 'consequential' to a severe acute radiation reaction.

## CHARACTERIZATION OF THE WR-2721 INDUCED HEMOPOIETIC STEM CELL MOBILIZATION IN MICE

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Radioprotection provided by sulphydryl related compounds is most significant in cell renewal systems and closely related to the drug induced tissue hypoxia. In bone marrow both were found the most pronounced in areas colonized by hemopoietic stem cells (HPSC). As tissue hypoxia is a known inducer of HPSC mobilization, WR-2721 was also assumed to have such an effect. The main goal of this study was therefore to justify and characterise the WR-2721 induced HPSC mobilization in BD2F1 mice by the use of in vitro HPSC plating and mononuclear blood cell transplantation techniques. Blood mononuclear cells (MNC-s) isolated from male mice, previously subjected to a single ip. dose of WR-2721, were cultured for CFU counting. Pooled blood MNC-s containing HPSCs were used for transplantation at different time points after exposure of female mice to 9 Gy lethal dose of gamma radiation. Hemopoietic recovery was followed in the survivors, and also Y chromosome was demonstrated by DNA hybridization procedure.

WR-2721 increased the ratio of CFU-s both in the bone marrow and peripheral blood MNC samples, and in a dose- and time-dependent manner, reaching a 5-10 fold increase after two hours and at 300 mg/kg WR-2721 dose. MNC transplantation of irradiated female mice with grafts from males challenged by WR-2721 resulted better survival percentage and shorter recovery due to the higher initial HPSC count. Better results were achieved when MNC transplantation was performed 48 hrs after the irradiation than when it was done after one day, indicating the importance of the recipient's actual hemopoietic, immunological and cytokine status. Plasma samples obtained from the gamma-irradiated females inhibited the in-vitro clonogenicity of HPSC-s only on the first day. Recovery from the hemopoietic depletion was significant but not complete after 30 days. Long-term HP recovery resulted no further death after 20 days. Several months later bone marrow cellularity and HPSC levels returned to the control. Hemopoietic chimerism was justified in all survivors by the demonstration of the Y chromosome in the blood MNC-s of the grafted females.

In vitro incubation of blood MNC suspensions with WR-2721 and WR-1065 resulted in a temporary and time-dependent delay in the in-vitro colony formation, and also in the micromorphological appearance of colonies. At this moment however, this phenomenon is subject of further and thoroughful analysis.

## EFFECT OF $^{60}\text{Co}$ -GAMMA IRRADIATION ON IL-3 RECEPTOR EXPRESSION AND BONE MARROW REPOPULATING ABILITY OF HISTIDINE DECARBOXYLASE KNOCK OUT (HDC-KO) AND WILD TYPE MICE

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**Objective:** Earlier it was found that there was a difference in the percentages of immunophenotypically characterised bone marrow (BM) cell populations between HDC-ko and wild type (WT) mice. In the following study the effect of 4 Gy whole body irradiation on BM cell populations and IL-3 receptor expression was compared in WT and HDC-ko mice.

**Methods:** HDC-ko and WT mice were subjected to a total dose of 4 Gy whole body gamma-irradiation. Bone marrow samples were obtained on the 1<sup>st</sup>, 3<sup>rd</sup> and 7<sup>th</sup> days. Using flow cytometry, regenerating cell populations were immunophenotypically characterised: Lin- population, CD34+ cells, ckit+ cells, long-term (CD34-/ckit+/Sca1+/Lin-) and short-term (CD34+/ckit+/Sca1+/Lin-) repopulating cells were distinguished. Also, the surface expression of IL-3R  $\alpha$  and  $\beta$  chains on these cell populations was determined. In WT mice intracellular HDC and histamine content in the BM cells was measured. Serum level of IL-3 protein was measured using ELISA.

**Results:** The results show that the irradiation-induced decrease in the defined cell populations is to a greater extent in HDC-ko mice. While WT bone marrow is almost recovered by day 7, HDC-ko regeneration is delayed. Also, intracellular HDC and histamine content of BM cells of WT mice is increased during regeneration compared to control animals. Moreover, the increase of IL-3 receptor expression on regenerating BM cells lags behind in HDC-ko mice. In accordance with BM results, the serum level of IL-3 protein is also decreased in HDC-ko mice.

**Conclusions:** It can be concluded that irradiation-induced BM depression is greater and BM regeneration is delayed in the absence of histamine. Moreover, IL-3 receptor expression on regenerating BM cells is reduced as well as serum IL-3 concentration. These findings support the role of histamine in the regulation of hematopoiesis and BM regeneration processes.



## SINGLE, DOUBLE, AND MULTIPLE DOUBLE STRAND BREAKS INDUCED IN DNA BY 3 – 100 eV ELECTRONS

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Although radiation therapy is a common mode of cancer treatment, there exists a persistent lack of fundamental knowledge of the complex, ultra-fast reaction cascades that unfold on femto-second time scales after the interaction of ionizing radiation with living tissue. Non-thermal secondary electrons with initial kinetic energies below 100 eV are a ubiquitous transient species created in irradiated cells, and thermalize within picoseconds through successive multiple energy loss events.

Here we present measurements of DNA damage induced by 3 – 100 eV electrons. Pure films of plasmid DNA are irradiated with a monochromatic electron beam in ultra high vacuum [Huels, *et al. JACS* 2003, 125, 4467]; after irradiation, DNA is recovered into a buffer, and analyzed by gel electrophoresis.

We show that 3 - 15 eV electrons induce SSB and DSB in plasmid DNA [*Science* 2000, 287, 1658] exclusively via formation and decay of molecular anions (resonances) localized on DNA components (base, sugar, phosphate, etc.). The strand break quantum-yields due to resonances occur with similar intensity than those which appear between 25 - 100 eV electron energy, where non-resonant mechanisms related to ionizations & dissociations dominate the yields, with some contribution from multiple electron scattering. We also present measurements of the electron energy dependence of multiple DSB (MDSB) induced in DNA by electrons with energies below 100 eV. Unlike the SSB and DSB yields, which remain relatively constant above 25 eV, the MDSB yields show a strong monotonic increase above 30 eV, however with intensities at least one order of magnitude smaller than the combined SSB and DSB yields. The observation of MDSB *above* 30 eV is attributed to strand break clusters (*nano-tracks*) involving *multiple successive interactions* of one single electron at sites which are distant in primary sequence along the DNA double strand, but are in close contact; such regions exist in cellular DNA where the double helix crosses itself, or is in close proximity to another part of the same DNA molecule.

Much like molecular excitation or ionization, the basic *resonant* mechanisms involved here are observable in any molecule, in any state of aggregation, but are modulated by the particular physico-chemical environment, in the present case the DNA plasmid. Thus, they are expected to occur in living cells as well, and a full understanding of the biological effects of ionizing radiation must incorporate detailed knowledge of their action. (*Funded by CIHR*)

## INTELLECTUAL DEVELOPMENT OF THE PERSONS EXPOSED IN UTERO: 10-YEARS FOLLOW-UP INVESTIGATION

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Purpose: Prospective investigation of the intellectual development at the age of 6–7 years till age 15–16 years among persons exposed *in utero* following the Chernobyl accident.

Material and methods: 250 persons from Belarus exposed *in utero* following the Chernobyl accident and a control group of 250 persons from non- and slightly contaminated regions. Psychological examinations were performed among persons of both groups at the age 6–7 years, 10–12 years, and 15–16 years as well as dosimetrical analysis.

Results: Mean antenatal internal dose to thyroid gland arising from the intake of <sup>131</sup>I among persons of exposed group is  $390 \pm 550$  mGy (maximal dose – 4100 mGy), in control group –  $35 \pm 65$  mGy. Mean antenatal external dose among persons of exposed group is  $10 \pm 13$  mGy. At the age of 6–7 years the persons in the exposed group had a mean Full Scale IQ lower than the control group ( $89.6 \pm 10.2$  vs  $92.1 \pm 10.5$ ,  $P=0.007$ ). At the age of 10–12 years there was no statistically significant difference between the two groups ( $94.3 \pm 10.4$  vs  $95.8 \pm 10.9$ ,  $P=0.117$ ). Positive dynamics of intellectual development in persons of both groups has been observed up to age of 15–16 years ( $98.7 \pm 10.2$  и  $99.5 \pm 10.5$ ,  $P=0.171$ ). No statistically significant correlation was found in exposed group between individual thyroid dose as well as individual antenatal external dose and IQ at the different ages. In both groups we notice a positive moderate correlation between IQ of persons and the educational level of their parents.

Conclusion: A significant role in the genesis of borderline intellectual functioning and emotional disorders in the exposed group of persons was played by unfavorable social-psychological and social-cultural factors.

Key words: antenatal exposure, intellectual development, dosimetrical analysis.

## RADIATION INDUCED DNA DOUBLE STRAND BREAK SIGNALING AND CELL CYCLE RESPONSE

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Endogenous cellular processes and exogenous factors such as ionizing radiation (IR) can generate double strand breaks (DSB) in the DNA that undermine genomic integrity. Two enzymatically distinct processes, homologous recombination repair (HRR) and non-homologous end joining (NHEJ) can, in principle, repair DNA DSBs. DNA DSBs induced in the DNA after exposure of cells to IR activate checkpoint pathways that inhibit progression of cells through the G1- and G2-phase and induce a transient delay in the progression through S-phase. These checkpoints together with repair and apoptosis are integrated in a circuitry that determines the ultimate response of a cell to DNA damage. Checkpoint activation typically requires sensors and mediators of DNA damage, signal transducers and effectors. In this presentation, I will briefly review the current state of knowledge regarding mechanisms of checkpoint activation and proteins involved in the different steps of the process. Emphasis will be placed on the role of ATM and ATR, as well of Chk1 and Chk2 kinases in checkpoint response. The role of downstream effectors such as p53 and the Cdc25 family of proteins will also be described, and connections between repair and checkpoint activation attempted.

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## IN VITRO GENOTOXIC EFFECTS OF RADIOFREQUENCY ELECTROMAGNETIC FIELDS

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**Objectives:** The increase in the use of mobile phones has brought about an urgent need to determine whether their microwave radiation (radio-frequency-modulated electromagnetic fields, RF-EMF) may cause health hazards. Therefore we have performed in vitro genotoxic studies with cultured human fibroblasts and rat granulosa cells.

**Methods:** Cells were exposed to intermittent or continuous radiofrequency electromagnetic fields (RF-EMF) used in mobile phones (GSM basic 1950 MHz) with different specific absorption rates (SAR 0.1-2 W/kg) and different exposure times (4-24 h). Genotoxic effects were determined using alkaline comet assay, micronucleus test and chromosome analysis. All experiments were performed under blind conditions and each exposure level was tested in duplicate. In addition, a reproduction of key data has been obtained by independent laboratories under blind conditions.

**Results:** RF-EMF exposure induced DNA single and double strand breaks in a dose-dependent manner, starting at an exposure level as low as 0.3 W/kg. Effects occurred at an exposure time of 8 hours, in both cell types, showing a stronger effect at intermittent exposure. In addition an increase in micronuclei frequencies and chromosomal aberrations could be demonstrated. RF exposed fibroblasts showed a 4-fold increase in chromosome breaks and elevated incidences of dicentrics and acentric fragments.

**Conclusion:** In summary, our data strongly indicate a genotoxic action of RF-EMF. These effects cannot be based on thermal effects due to temperature controlled exposure conditions.

## RADIATION-INDUCED CHANGES IN UROPLAKIN III EXPRESSION, AND UROTHELIAL CELL NUMBERS IN MOUSE URINARY BLADDER

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**Objective:** Bladder dysfunction is a frequent side effect of radiotherapy of pelvic tumours. Early effects are associated with changes in prostaglandin metabolism. Moreover, an impairment of the urothelial barrier must be considered. Since the apical uroplakin-containing membrane on the surface of the superficial umbrella cells is one of the components of this permeability barrier, the present study was initiated to quantitate radiation-induced changes in uroplakin III (UP-III) expression and in cell numbers in the urothelium of mouse urinary bladder during the early radiation response phase.

**Methods:** 33 female C3H mice were subjected to single dose irradiation with 20 Gy. Groups of 3 mice were sacrificed in 2-3 days intervals between days 0-31 after irradiation. The bladders were excised, and processed for histology and UP-III immunohistochemistry. Cell numbers in different layers were counted in 5 randomly taken microscopic fields over a defined length of urothelium. UP-III expression was determined using an arbitrary score ranging from 0 (no staining) up to 3 (strong staining).

**Results:** Irradiation resulted in a progressive decrease in total cell numbers over the entire acute phase. The most pronounced decrease was seen in the number of cells in the superficial layer, i.e. in umbrella cells, resulting in  $0,27 \pm 0,12$  cells (mean 3 animals  $\pm$  SD) at day 31 after irradiation, compared to  $4,11 \pm 0,38$  cells in control animals ( $p=0,00007$ ). In unirradiated bladders, a thin intensely stained superficial layer of UP-III was found on the luminal membrane of the umbrella cells. Additionally, diffuse but weak cytoplasmatic UP-III staining was seen in the cytoplasm of umbrella and intermediate cells, while the basal cell layer was completely negative for UP-III. After irradiation, a progressive loss of the UP-III layer on top of the umbrella cells was detected, correlating with the decrease in the number of umbrella cells ( $p=0,002$ ). In contrast, a progressive increase in cytoplasmatic staining of UP-III in the upper intermediate layers of urothelium was seen. The decrease in the superficial layer of UP-III was significantly negatively correlated with the cytoplasmatic staining intensity ( $p=0,001$ ), suggesting that the loss of the superficial, protective layer of UP-III was the trigger for synthesis of cytoplasmatic UP-III in the underlying cells.

**Conclusion:** Irradiation of the urinary bladder results in significant changes in urothelial UP-III expression, as well as in urothelial cell numbers, suggesting that UP-III and loss of the bladder permeability barrier maintaining umbrella cells are involved in the pathogenesis of acute radiation effects in the urinary bladder.

## STUDIES ON THE RADIOSENSITIVITY OF THE MOUSE OOCYTE USING AN EARLY PREANTRAL FOLLICLE CULTURE SYSTEM

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Objective : *In vivo* studies have shown that the frequency of mutational events recovered following irradiation of mouse oocytes greatly varies with the time interval between irradiation and ovulation of the oocyte. The present experiments aimed at characterizing the radiation sensitivity of mouse oocytes at two different stages of oocyte maturation, using a recently developed *in vitro* system allowing to cultivate follicle/oocyte complexes from the mid-growth stage up to ovulation.

Methods : ovarian follicles from prepuberal F1 (C57BL/6J X CBAca) females were collected and selected according to their intactness, diameter (100 to 130  $\mu\text{m}$ ), presence of 1-2 granulosa cell layers and a clear central oocyte. Follicles were grown individually for 13 days (37°C, 5% CO<sub>2</sub> in air). Every 2 days, half of the medium was replaced. On day 12 of culture, follicles were stimulated to ovulate by addition of 1.5 IU/ml hCG and 5 ng/ml EGF to the culture medium. Colchicine was added at the same time to block ovulated oocytes in MI. *In vitro* ovulation was assessed on day 13, 16 h after hormonal stimulation. Ovulated oocytes were examined for germinal vesicle breakdown (GVBD) and absence of the first polar body (PB) and then prepared for cytogenetic examination. X-irradiation with 2 or 4 Gy occurred *in vitro* either on day 0 or on day 12 of the culture, 3 h after hormonal stimulation.

Results : X-irradiation did not alter the developmental capacity of the follicle/oocyte complexes, whatever the time of irradiation, but had a dose-dependent effect on the GVBD. Mainly chromatid type aberrations (breaks, fragments and chromatid interchanges) were observed in fixed oocytes and their frequencies increased with the dose of X-rays. The frequencies of chromosome aberrations observed in oocytes irradiated 2 weeks before ovulation were in good agreement with those reported by others, after *in vivo* irradiation of mouse females at a similar time interval before ovulation. High levels of chromosome aberrations were found in oocytes irradiated 3 h after hormonal stimulation of ovulation, although their frequencies were lower than those reported earlier by others, after *in vivo* irradiation at a comparable time. This is probably due to a difference in the oogenetic stages at the time of irradiation (late diplotene vs. diakinesis).

Conclusion : The *in vitro* system used for these experiments offers several advantages (irradiation of homogenous populations of follicle/oocyte complexes, analysis of the detrimental effects of the treatments on oocyte and follicle development). It will be used in a next future to study the influence of various agents on follicle/oocyte radiation sensitivity (EDF contract RB-2003-05).

## HAEMOPOIETIC STEM CELLS AND RADIATION-INDUCED AML – A GENETIC APPROACH IN THE MOUSE

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### Objectives:

There is a strong genetic component that determines the risk of radiation-induced acute myeloid leukaemia (r-AML). As r-AML is a bone marrow haemopoietic stem cells (HSC) malignancy, differences in the risk of r-AML may be determined by genetic factors that modify the biology of the target HSC and/or its response to ionising radiation.

Inbred mouse strains can be used to dissect complex genetics traits and thus determine whether two phenotypes are genetically related. The inbred CBA/H mouse is susceptible to r-AML, but the C57BL/6 mouse is resistant. It is also well established that there are also genetically determined differences in the control of mouse HSC numbers, their proliferative status and response to cytokines. We have employed genetics to define Quantitative Trait Loci (QTL) that determine r-AML- susceptibility and HSC numbers.

### Methods:

r-AML: 1150 (CBA/HxC57BL/6)F1 x CBA/H backcross mice were exposed to 3 Gy X-rays and 78 affected mice (r-AML) diagnosed. Affected mice were genotyped in a genome wide screen to assess linkage between genotype and phenotype.

HSC: Lin<sup>+</sup> cells bone marrow cells from CBA/H, C57BL/6 and 124 (CBA/HxC57BL/6)F1 intercross (F2) mice were depleted Lineage positive cells by immunomagnetic depletion. Lin<sup>-</sup> bone marrow cells were stained for Sca-1 and *c-kit* and analysed by FACS. The F2 mice were genotyped across the genome at 20 cM intervals.

### Results:

r-AML: In the analysis of affected (CBA/HxC57BL/6)F1 x CBA/H mice, we have identified a CBA/H r-AML susceptibility gene on distal chromosome 1 (100 cM;  $p = 0.00026$ ). A QTL that determines HSC numbers maps to this region suggesting HSC numbers may be a risk factor in r-AML.

HSC numbers: Using flow cytometry, we have observed that r-AML susceptible CBA/H mice have a higher number of a discrete population of phenotypically defined Lin<sup>-</sup> Sca-1<sup>++</sup> c-kit<sup>++</sup> HSC than C57BL/6 mice. Genetic analysis of 124 F2 mice revealed suggestive QTL on chromosomes 1 (60 cM), 17 (20 cM) and 18 (16 cM) that determine the frequency of Lin<sup>-</sup> Sca-1<sup>++</sup> c-kit<sup>++</sup> HSC. All three QTL have been previously implicated in determining the frequency of long-term repopulating HSC in the mouse, but neither maps to the r-AML susceptibility locus.

### Conclusions:

HSC numbers in control unirradiated mice may not be a risk factor in r-AML as it is the number of surviving HSC following exposure to a leukaemogenic dose of X-rays that would in theory determine risk. Nevertheless, our data illustrate the tremendous power of mouse genetics in the study of radiobiology.

## RADIATION EXPOSURE OF PATIENTS AND OPHTHALMOLOGISTS DURING A DACRYOSCYNTYGRAPHY AND A DACRYOCYSTOGRAPHY

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The aim of the study was to estimate radiation risk to the patients and ophthalmologists involved in dacryocystographic and dacryoscyntygraphic examinations. Based on the values of equivalent doses, eye-lenses and thyroid glands were chosen as organs particularly exposed during such examinations. For the estimation of the absorbed doses thermoluminescent (TL) dosimeters and the TL RA94 dosimeter reader-analyser were used. The measurements were carried out in a group of 20 subjects.

During a dacryocystography, TL dosimeters were placed in the vicinity of the doctor's and patient's eye-lenses and thyroids as well as on the doctor's hands. During a dacryoscyntygraphic examination (utilizing the Tc-99m-tagged radiopharmaceutical) exposure of the patient's eye-ball was estimated from the 10-minute measurement of the absorbed dose using the dosimeters placed on the eyelids.

Effective doses absorbed by the lenses of patients undergoing dacryocystography ranged from 20 to over 60 mSv per one examination whereas the doctors' lenses received approx. 2 mSv per one procedure; slightly smaller doses were absorbed by the thyroid glands. The eye-lenses of patients undergoing dacryoscyntygraphy received doses ranging from a few to several dozen mSv (during the 10-minute measurement the absorbed dose equalled to 1 mSv).

The present results indicate that ophthalmologists performing dacryocystography should obligatorily use radio-protective equipment such as lead aprons, glasses and thyroid screens; monitoring of the absorbed doses is also necessary. The patients should be covered with lead aprons and other appropriate shields for eye-lenses, thyroid glands, and gonads. Upon completion of the dacryoscyntygraphic examination, the patient should carefully wash his/her eyes to remove the residues of the radiopharmaceutical.



## RADIATION EXPOSURE OF THE PATIENTS' EYES DURING X-RAY EXAMINATIONS

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Common utilization of X-ray tests and the need to comply with the dose limits set by radiation protection regulations justify the necessity of the assessment of radiation risks associated with such tests.

The aim of the present study was to estimate the level of exposure to ionizing radiation of the patients' lenses and eyeballs during routine radiological examinations. For measurements of the absorbed doses thermoluminescent dosimeters, a dosimeter reader, and a human body phantom were used. The measurements were carried out during tomography, vascular examinations, and X-ray tests of the teeth and chest. From these measurements, values of the equivalent doses received by the eyes during each type of the examination were determined.

The results indicate that, depending on the type of the examination, the lenses are exposed to doses ranging from natural background level to several dozens of mSv per one examination. The level of exposure to the eye can be decreased by modifying the parameters of the X-ray equipment as well as by use of the protective screens.

## ESTIMATION OF DOSES OF IONISING RADIATION RECEIVED BY THE MEDICAL STAFF AND PATIENTS DURING VASCULAR PROCEDURES

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During vascular examinations and operations both medical staff and patients are exposed to relatively high doses of ionising radiation. Estimation of the doses is necessary to ensure radiological safety of the staff as well as to limit the X-ray exposure of the patients.

In the present study radiation risk was assessed to medical staff and patients during vascular examinations and operational radiology. For the measurements of the absorbed doses either during the real operations and examinations or using a human body phantom thermoluminescent dosimeters were employed.

Effective doses received by the medical staff during one procedure ranged from background level to fractions of mSv while those contracted by the patients equalled to 10 or more mSv per operation. Calculated values of the equivalent dose in the critical organ received by the medical staff and patients ranged from fractions of mSv to several mSv and from several mSv to scores of mSv, respectively. The patients undergoing vascular operations on the abdomen and skull as well as the staff involved in these procedures were exposed to the highest doses of ionising radiation as a result of the protracted exposure to X rays.

Utilizing multislice tomography for vascular examinations led to reduction of the doses received by the patients and medical staff involved in these procedures.

## THE INFLUENCE OF DNA REPLICATION BYPASS ON THE LETHAL EFFECTS OF UV AND $\gamma$ RADIATION

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### Objective

The capacity to rescue stalled replication forks is an important mechanism to maintain cell viability and genome integrity. Two pathways for replication bypass has been suggested, translesion synthesis (TLS), utilizing a switch from high to low fidelity polymerases, and homologous recombination (HR). The question that has been addressed here is how replication bypass influences the lethal effect of radiation and in what way other DNA repair pathways are involved to restore stalled replication forks.

### Methods

We here developed a method to study replication fork progression (RFP) and how DNA lesions and DNA repair mechanisms are involved during the replication process. The method is based on the principle that each replication fork is expected to provide a pair of single strand ends. By pulse labeling followed by measuring single strand breaks, using alkaline DNA unwinding technique, we could analyze the lethal effect of radiation on fork progression. Chinese hamster ovary (CHO) cells deficient in nucleotide excision repair (NER), base excision repair (BER), non-homologous end-joining (NHEJ) and homologous recombination (HR) were investigated in their response to ultraviolet light (UV) or ionizing radiation ( $\gamma$ ) by investigating DNA repair activities, formation of double strand breaks (DSBs), rate of RFP and survival.

### Results

It was found that after UV exposure, cells deficient in above mentioned DNA repair pathways were influencing the rate of RFP although differently to what was expected from survival. The cyclobutane pyrimidine dimers (CPD) adducts were more efficiently bypassed as compared to the 6-4 photoproducts ((6-4)PP). Deficiency in NER delayed the rate of RFP significantly after UV-induced damage suggesting that this repair pathway takes part in replication bypass. In the case of  $\gamma$  radiation, the replication bypass is much less influenced of DNA repair, but rather contributing differently to a delayed lethal effect.

### Conclusion

Our observations support a model suggesting that the mechanism(s) involved in the restoration of stalled replication forks induced by UV operate(s) together with DNA repair processes, which has implications for the lethal effect of this agent. The conclusion from irradiation with  $\gamma$  suggests a late lethal effect possibly generated during replication bypass.

## APPLICABILITY OF INDUSTRIAL BY-PRODUCTS IN BUILDING INDUSTRY AND THEIRS RADIOLOGICALLY QUALIFICATION

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Recently the interest is focused on the elimination and utilisation of the environmental damage caused by industrial by products, so red mud origins from the bauxite processing, fly ash, slag from coal burning and the uranium tailings. In the course of our work we set ourself an aim to find out the possibilities of the utilization of these wastes in the building industry or in other fields of application radiologically. We applied the European Union index classification concerning building materials.

The activity-concentration of these samples change between 3-200 times higher (e.g.  $^{226}\text{Ra}$ : 150-18000 Bq/kg) as compared to the average radionuclide concentration of the building materials ( $^{226}\text{Ra}$ : 50 Bq/kg  $^{226}\text{Th}$ : 50 Bq/kg  $^{40}\text{K}$ : 670 Bq/kg).

After the evaluations the radon emanation coefficient( $\epsilon$ ),-which is very important factor for the released radon fraction- was determined in some cases. We found that emanation coefficient changes in wide range ( $\epsilon=0,5-30\%$ ). The variation of the emanation coefficient depends on the material and the origin of the sample.

It was found that red mud and some fly ash are allowed to use as an additives. The byproduct content of the mixture is allowed to be maximum 20% according to the EU recommendations. Slags and uranium tailings on the contrary mustn't be used as a component of the building materials because of the very high content of gamma emitter-radionuclides and the  $^{226}\text{Ra}$ . These materials could be used as a filling material in the course of road construction if it is allowed to do. Unfortunately in most cases these slags and uranium tailings have as high activity so can't be reutilize. That's why these materials should be left in the deposits or the valuable components should be extracted in the end the area should be recultivated.

## PROBABILITY FOR LATE NORMAL TISSUE REACTIONS

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Objective: An increasing number of patients survive cancer after having received radiation therapy. Therefore, the occurrence of late normal tissue complications among long-term survivors is of particular concern.

Methods: A review is given on the distribution of latent times to late effects in normal tissue occurring after radiation therapy. It is based on the analysis of our own data (Svoboda et al., Radiother. Oncol. 1999; 53: 177-187) and numerous data sets from published reports.

Results: When the percentage of patients being free from late effects is plotted as function of time after treatment, three types of kinetics could be identified for the incidence of late normal tissue complications occurring after radiation therapy (Jung et al., Radiother. Oncol. 2001; 61: 233-246). Type 1 denotes purely exponential kinetics; this type is mainly observed when relatively large volumes were exposed to relatively high doses. Type 2 means exponential kinetics, the slope of which decreased exponentially with time. Alternatively, this type may also be described by one exponential component and a constant fraction, in particular when the total doses applied were relatively inhomogeneous. The constant fraction may indicate, that a portion of the patients received relatively small doses for which the risk of developing late effects was virtually zero, whereas in the other subgroup of the patients, late effects occurred at exponential kinetics. Type 3 means curves composed of two components, a fast initial decline followed by an exponential decrease. This type is frequently observed for late effects in the lung and in the head and neck region.

Conclusion: Our results indicate that the risk for the occurrence of late complications after irradiation may remain constant for many years, either for all patients treated or for a subgroup exposed to doses exceeding the tolerance limit of the tissue under consideration. – *Supported by Roggenbuck Foundation, Hamburg.*

## HIGH SUSCEPTIBILITY OF ATM-NULL MICE TO SPONTANEOUS AND X-RAY INDUCED MICRONUCLEUS FORMATION IN ERYTHROBLASTS

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[Objective] To evaluate the role of *atm* gene as a defense of somatic cells from chromosome damage arising spontaneously and after radiation.

[Methods] Null (-/-) and heterozygous (+/-) for *atm* or *p53*, and the wild-type (+/+) mice were obtained as F1 males of intra-strain cross of C57BL mice heterozygous for *atm* or *p53*. At an age of 14 weeks, a drop of blood was sampled on an acridine-orange coated slide from the tail of each mouse for measurements of spontaneously arising micronuclei (MNs) in peripheral reticulocytes. Two weeks later, the mice were whole-body exposed to X-rays at a dose of 0.5 or 1 Gy and blood samples were obtained at 12- or 24-h intervals starting at 0 h and ending at 120 h after exposure. Sampled blood was subjected to the micronucleus (MN) assay under a fluorescence microscope (1000 x). The MN frequency was calculated as the number ratio of MNs detected to reticulocytes examined.

[Results] The ratios of spontaneous MN frequencies in *atm*(-/-), *atm*(+/-) and *atm*(+/+) were  $1.8 \pm 0.3 : 1.2 \pm 0.2 : 1$ . The frequency ratios in *p53*(-/-), *p53*(+/-) and *p53*(+/+) mice were  $2.2 \pm 0.3 : 1.0 \pm 0.2 : 1$ . Thus, *atm*-null and *p53*-null mice were found to have high susceptibility to spontaneous MN formation with comparable extent.

To compare X-ray sensitivities for MN induction, the area beneath the time-course curve of MN frequency (*A*) was calculated for each mouse and used as an index of the total amount of induced MNs. The ratios of mean *A* values for *atm*(-/-) and *atm*(+/-) mice relative to that for *atm*(+/+) mice after 0.5 Gy of X-rays were  $2.9 \pm 0.8$  and  $1.4 \pm 0.3$ , respectively; those after 1 Gy were  $1.8 \pm 0.3$  and  $1.0 \pm 0.2$ , respectively. The relative ratios for *p53*(-/-) and *p53*(+/-) mice after 0.5 Gy of X-rays were  $2.7 \pm 0.5$  and  $1.3 \pm 0.3$ , respectively; those after 1 Gy were  $2.0 \pm 0.4$  and  $1.1 \pm 0.2$ , respectively. Thus, both *atm*-null and *p53*-null mice were 2-3 fold more sensitive than the wild-type to X-ray induction of MNs in erythroblasts, whereas both types of heterozygous mice were near normal with respect to X-ray sensitivity for MN induction.

[Conclusion] *atm* is involved in defense of somatic cells from chromosome damage arising spontaneously and after radiation, most probably via *p53*-dependent pathway.

## MATERNAL EXPOSURE TO LOW-DOSE X-RAYS INDUCES ACTIVITY OF AUTOPHAGIC CELL DEATH IN UNEXPOSED OFFSPRINGS, WHICH CAN BE MODULATED BY TRANSPLANTATION OF MITOCHONDRIA DNA

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In recent years there has been growing concern over the biological effects of low-dose X-rays. Our laboratory had observed flies (*Drosophila melanogaster*) irradiated with low dose X-rays tend to emerge earlier than normal flies. This observation led us to quantitatively examine the effects of low dose X-irradiation on development in the fly. Following exposure of prepupal flies to 0.5 Gy X-rays, the time to emergence was shorter than in the sham controls. This tendency was increased when the X-ray exposure came during the pupal stage. In these flies, the time to eclosion decreased significantly, by an average of 30 hours sooner than sham controls. Interestingly, increasing radiation dose to 1 Gy did not result in any changes compared to sham controls.

At the onset of metamorphosis, most of the obsolete larval tissues and organs like salivary gland become committed to programmed cell death, while imaginal discs and histoblasts will undergo proliferation into imaginal structures. In this study, the larval salivary glands of animals exposed to 0.5 Gy were completely destroyed by 10 hours after puparium formation (APF), and the condensed larval midgut of exposed animals could be clearly seen inside the developing adult midgut 5 hours APF. Furthermore, large autophagic vacuoles appeared to fragment rapidly following 0.5 Gy X-irradiation, and acid phosphatase activity associated with an autophagy in exposed animals was increased significantly compared to sham controls. These results indicate that the animals subjected to low dose X-rays can acquire the activating of autophagic programmed cell death, resulting in leading to significant faster adult eclosion.

A further experiment examined whether such radiation effects could be observed in the unexposed F1 generation of exposed individuals. Greater radiation effects on early F1 emergence were seen when the time between exposure and mating was 3 days, indicating an effect on early spermatid development. Early F1 emergence was also observed after exposure of female flies to X-rays during late previtellogeny. Furthermore, rapid emergence could be induced in the F1 embryos of unexposed parents by transferring the mitochondria (mt) DNA from F1 embryos of exposed flies. In transferring F1 5 hours APF, larval salivary glands possessed DNA fragmentations as indicated by the TUNEL staining, and showed that acid phosphatase activity was increased significantly. These results show that radiation-induced effects can be transmitted to the next generation through the mtDNA.

## MODULATION OF CIRCULATING CD34<sup>+</sup> AND TdT<sup>+</sup> CELLS NUMBER BY MEANS SKIN-CONTACT MICROVIBRATIONS ON BONE MORROW WITH CHERNOBYL'S CLEAN UP WORKERS

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**Objective:** In preliminary investigation with 9637 Chernobyl's clean up workers (CUW) divided on two groups we have found the lower (-7%,  $p=0,02$ ) number of leucocytes (L) for CUV with reconstructed doses  $>25\text{cGy}$  in comparing those with  $\leq 25\text{cGy}$ . This difference was valid among CUW at 35-50 years old only. The aim present study was to develop noninvasive technology for increasing of CD34<sup>+</sup> cells in blood with skin contact mechanical microvibrations at zone of spine projection on the back.

**Methods:** The CD34<sup>+</sup> and TdT<sup>+</sup> subpopulations of blood cells were measured with 5 healthy donors and 25 CUW ( $49 \pm 0,7$  years old) using DAKO EnVision System, HRP (DAB) or MAT CD34<sup>+</sup> ClassIII/RPE and TdT/FITS (DABCO) by means UV-microscope "Opton" joint with programmed PC, counting  $5 \cdot 10^5$  cells per each point. Microvibrations were induced with standart medical equipment "Vitaphon" through 8 vibrophones operated at mode: amplitude 12 mkm, modulation 0,02-20 kHz, cycle' time  $\cong 1$  min., duration 10 min.

**Results:** For healthy donors the average CD34<sup>+</sup> cells number was increased from  $0,07 \pm 0,01\%$  to  $0,11 \pm 0,01\%$  ( $p=0,01$ ) in 40 min and to  $0,25 \pm 0,05\%$  ( $p<0,01$ ) in 210 min after single procedure. In group of CUW  $0,16 \pm 0,024\%$  CD34<sup>+</sup> and  $8,6 \pm 1,17\%$  TdT<sup>+</sup> cells were registered before procedure. Repeated (5 times, daily) procedures with duration 10 min each one have arisen the level of CD34<sup>+</sup> cells to  $0,27 \pm 0,026\%$  ( $p=0,004$ ) without any sufficient change of TdT<sup>+</sup> cells-  $6,5 \pm 0,6\%$ .

**Conclusion:** Noninvasive skin- contact microvibrations are able to bring into action the process of moderate enrichment the number of circulating stem cells.

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## AUTOMATIC ANALYSIS OF URINARY 8-HYDROXYDEOXY-GUANOSINE BY HPLC-ECD

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Reactive oxygen species (ROS) generated by environmental agents or endogenous ROS, due to various lifestyles, may induce cancer and many adult diseases. Although the pathway of urinary 8-hydroxydeoxyguanosine excretion is not clear, its analysis may be useful for the assessment of oxidative stress in vivo. We developed a new method to measure urinary 8-OH-dG by an HPLC-ECD system. This method is unique in that, 1) the urine is first fractionated by anion exchange chromatography prior to analysis by reverse phase chromatography; and 2) the 8-OH-dG fraction in the first HPLC run is precisely and automatically collected, based on the added ribonucleoside 8-hydroxyguanosine marker peak, which elutes 4-5 min earlier. Up to 1000 human urine samples can be continuously analyzed with high accuracy within a few months. Using this method, the urine samples of 500 employees of a company were analyzed. Alcohol drinking, cigarette smoking and difficulty in mental diversion (non-smoker) showed positive correlations with the 8-OH-dG level, while combinations of various foods, physical activity and fruit intake showed negative correlation. This method will be useful for studies in radiotherapy, molecular epidemiology, risk assessment, and health promotion.

### (Reference)

Kasai, H., A new automated method to analyze urinary 8-hydroxydeoxyguanosine by a high-performance liquid chromatography-electrochemical detector system.

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## RADIOPROTECTIVE EFFECT OF GREEN TEA POLYPHENOL IN MICE

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[Objective] Green tea polyphenol (GTP) shows a radioprotective effect when it is administered to mice prior to exposure to ionizing radiation and the post-irradiation survival is monitored. Our present study was carried out to examine the effect of GTP on radiation-induced chromosome damage by administering it to mice before and both before and after ionizing radiation.

[Methods] Polyphenon 70S (P70S, Tokyo Food Techno co., Ltd., Japan), 78.4% of which was GTP, was given at 0.01% in DW to ICR female mice *ad libitum* for 4 weeks starting at an age of 7 weeks prior to X-ray irradiation. P70S-treated and -untreated mice were exposed to an X-ray dose of 1 Gy. Following exposure, a fraction of P70S-pretreated mice were continually treated with P70S in the same way as used for the pretreatment. Peripheral blood sample was obtained from each of mice at 24 h intervals beginning 0 h and ending at 96 h after exposure; the sampled blood was subjected to the reticulocyte micronucleus (MN) assay for monitoring chromosome damage induced by irradiation.

[Results] The time course curves of X-ray induced MN frequency obtained for P70S-treated and -untreated mice were almost indistinguishable from each other, showing no evidence of radioprotective effect of pretreated GTP on X-ray induction of MN formation. On the other hand, the curve for mice that were treated with P70S both before and after irradiation was clearly distinguishable from that for P70S-pretreated mice, with relatively lower MN frequencies at time points 24, 48 and 72 h after irradiation.

[Conclusion] Treatment of mice with GTP both before and after irradiation can reduce the severity of radiation effect in somatic chromosomes.

## RADIATION EXPOSURE OF MEDICAL STAFF DUE TO $^{131}\text{I}$ THERAPY FOR HYPERTHYROIDISM AND THYROID CANCER

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**OBJECTIVE:**  $^{131}\text{I}$  therapy for hyperthyroidism and thyroid cancer has an increasing tendency in Hungary. Because of the high radionuclide activities administered and the number of treatments per year we have investigated the radiation exposure of the medical staff.

**METHODS:** The external doses were measured by thermoluminescent and electronic personal dosimeters, while the estimation of internal doses was based on the radioiodine air activity concentration measurement.

**RESULTS:** The annual effective external doses to the medical staff were below 1 mSv for diagnostic investigation and therapy for hyperthyroidism, as well. External doses to the hands, fingers of the staff did not exceed 6 mSv annual value. The internal radiation burden of the staff is expected to remain below 0.005 mSv annual dose.

The external effective annual dose to the medical staff was 0.3 mSv both for diagnostic investigation and therapy for thyroid cancer. Annual external dose to the hands, fingers was estimated as 20 mSv per diagnostic and therapeutic application, too. The annual internal radiation burden of the staff was estimated as 0.03 and 1.1 mSv for the diagnostic and therapeutic practice, respectively.

**CONCLUSION:** The doses to the medical staff due to the therapeutic use of  $^{131}\text{I}$  radionuclide remain well below the annual dose limits in Hungary.

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## ACTIVATION OF DEOXYCYTIDINE KINASE BY GAMMA-IRRADIATION

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Objective: Deoxycytidine kinase (dCK, EC 2.7.1.74) is the principal nucleoside salvage enzyme in lymphoid cells, responsible for the activation of natural deoxynucleosides and several nucleoside analogues widely used in the chemotherapy of leukemias and solid tumours. Previously, we found that the activity of dCK was enhanced by incubating primary cultures of human lymphocytes with various drugs such as 2-chloro-deoxyadenosine (CdA, Cladribine), etoposide and other genotoxic agents. The aim of this study was to establish a direct correlation between dCK activation and DNA repair induced by gamma-irradiation of human tonsillar lymphocyte cultures.

Methods: Enzyme activities of dCK and thymidine kinase 1 were measured in cellular extracts based on phosphorylation of radiolabelled substrates. Reparation of DNA damage following 0.5 – 2.0 Gy irradiation was detected by thymidine incorporation and by the Comet assay. dCK protein levels were determined by western blot and deoxyribonucleoside triphosphate (dNTP) pools were assessed by the DNA polymerase test.

Results: Exposure of short-term cultures of human lymphocytes to gamma-irradiation led to a dose- and time-dependent enhancement of dCK activity whereas dCK protein levels and thymidine kinase activity remained unchanged. Irradiation of cells caused a dramatic increase in DNA fragmentation; however, DNA damage was completely repaired within 1 hr as monitored by thymidine incorporation and the Comet assay. DNA repair coincided with the nearly fourfold activation of dCK. We detected a marked elevation of the dATP-pool upon gamma-irradiation while the rest of dNTP pools was not affected significantly.

Conclusions: The increased activity of dCK upon irradiation-induced DNA damage seems to be a compensatory cellular mechanism since dCK might supply all four natural deoxynucleotides for elevated rates of DNA repair. In seriously damaged cells, however, stimulated dCK activity might contribute to the activation of the apoptotic cell death machinery by producing dATP that is known to be a toxic and proapoptotic deoxynucleotide if accumulated in lymphocytes.

## MUTATION INDUCTION IN HUMAN CELLS BY $^{60}\text{Co}$ GAMMA RAYS AND 30 KV X-RAYS

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Mutations were studied at the HPRT-locus in MGHU-1 (human male bladder carcinoma) cells after exposure to filtered 30 kV X-rays and  $^{60}\text{Co}$  gamma rays. Mutant frequencies were found to increase in a linear-quadratic fashion with dose in both cases. If only the low dose part is analysed: a RBE of  $2.05 \pm 0.46$  is obtained. PCR analysis was also here performed. There is a clear tendency that the fraction of deletions is increased with lower photon energies but the effect is not significant. Translocations of the q-arm of the X-chromosome where the HPRT-gene is located were also determined. With 30 kV X-rays 17% out of 53 analysed mutant clones showed translocations (95% confidence interval: 9..30). The respective numbers for gamma-induced mutants (25 clones) are 12 (confidence interval: 4.6..31). Although the lower photon energy induces more translocations the difference is again not significant. The low fraction with translocations leads to the conclusion that only a minor part of mutants with no detectable deletions can be attributed to translocations.

## TRANSGENERATIONAL EFFECT OF IONISING RADIATION IN MICE

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**Objective:** Ionizing radiation can induce genetic instability and mutations both in somatic and germ line cells. Mutations in germ cells might be inherited by the next generations. The inherited mutations may induce cancer in the affected population. Exposure of the affected population to a second environmental carcinogen might further increase the cancer incidence. This process was studied in an experimental system.

**Methods:** Male mice (DBA2) were irradiated with either  $^{60}\text{Co}$ - $\gamma$  (dose rate 1.013 Gy/min) or fission neutron (1 MeV; dose rate 2.57 mGy/s;  $\gamma$  component 15%) radiation. Irradiated mice were mated with unirradiated females (C57Bl/6) during the 1<sup>st</sup>, 3<sup>rd</sup> and 11<sup>th</sup> weeks after irradiation. DNA was isolated from tail pieces of F1 mice. The number of minisatellite mutations has been determined by Southern blot hybridization to mouse minisatellite DNA probe M and Pc-1.

Cancer incidence was also followed in the offspring. To investigate the effects of a second environmental carcinogen paternally irradiated mice were treated with a chemical carcinogen (ENU) on the 15<sup>th</sup> prenatal day.

**Results:** When mating was performed during the first week after irradiation (spermatozoa stage) only high dose neutron radiation caused a significant decrease in litter size. Male germ cells were the most sensitive for irradiation at the spermatid stage (third week after irradiation), when a dose-dependent decrease in the litter size has been detected. When mating was performed during the eleventh week after irradiation (sperms were irradiated at spermatogonium stage) the litter size went back to the normal level. Exposure to ionizing radiation at the spermatozoa stage doubled the mutation incidence in the offspring. When exposure occurred at the spermatid stage the mutation at the investigated minisatellite loci increased in a dose dependent manner peaking at 3-4 times above the control level after 2 Gy neutron irradiation. Exposure at the spermatogonium stage hardly increased mutation rates. The tumor incidence also increased in mice paternally irradiated at the spermatid stage. Exposure to ENU further increased the cancer incidence in prenatally irradiated mice.

**Conclusions:** Paternal irradiation will increase the minisatellite mutation rates in the F1 offspring and the cancer incidence is also enhanced

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## COMPARISON OF DIFFERENCES IN THE EFFECTIVENESS BETWEEN CONVENTIONAL DICENTRIC ASSAY AND TRANSLOCATION ANALYSIS FOR BIODOSIMETRY IN CULTURED PERIPHERAL BLOOD LYMPHOCYTES OF KOREAN INDIVIDUALS

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**Objective:** Fluorescence *in situ* hybridization (FISH) is a powerful method largely used for detecting chromosomal rearrangements, translocations in particular, which are important biomarkers for dose assessment in case of human exposure to ionizing radiation. To test the possibility of using the translocation analysis by FISH-painting method for biodosimetry assessment in Korean, we carried out *in vitro* experiments in  $\gamma$ -ray irradiated human peripheral blood lymphocytes from healthy Korean individuals.

**Methods:** Human peripheral blood lymphocytes and Radiation exposure, Culture conditions and chromosome preparations, Conventional dicentric assay Fluorescence *in situ* hybridisation method, Statical analysis

**Results and Conclusion:** The *in vitro* dose-response curves for the genomic translocation frequencies (FGs) and conventional dicentric assay fit a linear quadratic model, according to the equation each:  $y = ax^2 + bx + c$ . The values of FGs and dicentric assay were also calculated for the exposed range from 0.00 to 6.00 Gy for HPBL of the individuals exposed to <sup>137</sup>cesium on same samples, taking the opportunity to test the validity of translocation analysis in biodosimetry. A tentative of retrospective dosimetry was performed, indicating that the method is feasible only for some exposed range (below 2 Gy), while for higher doses there is a need to apply appropriate correction factors, which take into consideration mainly the persistence of chromosomal translocations along with time, and the influence of endogenous and exogenous factors determining the inter-individual variability in the cellular responses to radiation.

**Keywords:** Conventional dicentric assay, Chromosomal translocations; Fluorescence *in situ* hybridization (FISH); Ionizing radiation; Retrospective biological dosimetry

\* These two authors contributed equally to this work.

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## A NEW MONTE CARLO CODE ETMICRO FOR TRACING ELECTRONS IN LIQUID WATER MEDIUM

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**Introduction:** Ionizing radiations of high energy produce a considerable number of secondary electrons at energy below 100 eV along their tracks. In the biological cell, on the other hand, water is a major component. Understanding the sequence of the interactions between low-energy electrons and water is, therefore, the very first requisite for theoretical radiation biology study. In this paper, a new Monte Carlo code ETMICRO (Electron Transport code for MICROdosimetry) is introduced to be utilized for obtaining microdosimetric quantities from the interactions of low-energy electrons with the liquid water medium.

**Methods:** Total 11 scattering modes (5 ionization, 5 excitation and 1 elastic) were considered in following the electron movements in liquid water. The inverse mean free paths (IMFPs) and the corresponding differential values (DIMFPs) were constructed as functions of the electron energy and the energy loss, by employing the fitting parameters provided by Dingfelder et al (*Radiat Phys Chem* 53; 1-18: 1998), to describe the collective response of the liquid water. The cross sections for elastic scattering were collected from various literatures. The electron tracing cutoff was set at 10 eV in kinetic energy.

**Results:** The Monte Carlo code ETMICRO provides the user with the record on the numbers of each scattering mode occurring in a target volume per primary electron emission and the corresponding energy depositions. It differentiates the primary and the secondary electrons in recording. Demonstrational calculations have been made for the primary electrons at energy from 100 eV to 10 keV. The record has been also utilized to produce 2-dimensional drawings of the electron tracks.

**Conclusion:** ETMICRO has been proven to be an efficient tool for simulating the interactions of low-energy electrons with the liquid water through the comparisons with the previous work. The approximate data of the elastic scattering cross sections for the electrons employed in the transport codes seem to be a critical factor for the differences in the simulation data especially for a micro-scale target.



## DOWN-REGULATION OF ERK PATHWAY IS REQUIRED FOR CASPASE-8 DEPENDENT MITOCHONDRIAL ACTIVATION-MEDIATED CELL DEATH BY RADIATION IN HUMAN CERVICAL CANCER CELLS

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**Objective:** Exposure of cells to ionizing radiation induces simultaneous activation or down regulation of multiple signaling pathways. These signals play critical role in controlling cell death, cell survival and repopulation after irradiation, in a cell type specific manner. In this study, we investigated the role of MAPKs in radiation-induced apoptotic cell death/survival signaling in human cervical cancer cells.

**Methods:** Radiation-induced apoptotic cell death was determined by flow cytometric analysis. Involvement of the mitochondrial pathway was examined by monitoring of the mitochondria membrane potential, cytochrome *c* release and Bax translocation. Subcellular redistributions of AIF were detected using Western blot analysis after subcellular fractionation and confocal microscopic analysis.

**Results and Conclusion:** Ionizing radiation caused induction of caspase-8 activity and Bid cleavage, loss of mitochondrial membrane potential, increase of cytosolic cytochrome *c*, translocation of apoptosis inducing factor to the nucleus and subsequent apoptotic cell death after irradiation. We also found that decrease of phosphorylated-ERK1/2 and increase of p38 MAPK phosphorylation after irradiation. Activation of ERK1/2 by pretreatment with PMA or forced expression of ERK1/2 attenuated radiation-induced caspase-8 activation, cytochrome *c* release, AIF translocation and apoptotic cell death, indicating that down regulation of ERK is required for the caspase-8-dependent mitochondrial activation-mediated cell death pathway. Moreover, inhibition of p38MAPK by pretreatment of SB203580, or by expressing a dominant negative p38MAPK potentiated radiation-induced caspase-8 activation and mitochondria-mediated cell death, suggesting that activation of p38MAPK is involved in cell survival after irradiation. In addition, we found Src activation following irradiation. Inhibition of Src attenuated radiation-induced p38MAPK activation, and efficiently enhanced radiation-induced caspase-8-dependent cell death, indicating Src is located upstream of p38MAPK in cell survival signaling after irradiation. Molecular dissection of the signaling pathways that regulate the apoptotic cell death machinery is critical for both our understanding of cell survival/death events after ionizing irradiation and development of molecular target for cancer treatment.

## INDIVIDUAL RADIATION SENSITIVITY IN CANCER PATIENTS UNDERGOING RADIOTHERAPY

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**Objective:** Very serious radiation-induced side effects will develop in about 5-10% of cancer patients undergoing radiation therapy. The aim of our experiments is to establish screening methods to identify radiation sensitive patients before the onset of radiation therapy.

**Methods:** Blood samples and skin biopsies were taken from cancer patients undergoing radiation therapy. The in vitro radiation sensitivity of peripheral blood lymphocytes was studied by single-cell electrophoresis (comet) and micronucleus assays. Primary fibroblast cultures were established from skin biopsies and the radiation sensitivity of fibroblasts was investigated by comet assay and by determining the survival fraction after 2 Gy irradiation (SF2 value). The in vitro data were correlated to the clinical symptoms of the patients. The gene expression patterns of radiation sensitive and resistant patients were studied by macro array analysis (3000 genes).

**Results:** The comet and micronucleus assays were not informative. The SF2 values of control patients ranged between 26-40%. The SF2 values of patients with radiation-induced late toxic reactions in the central nervous system moved toward lower ranges and peaked between 8-15%. Similar alterations have been observed in patients with early and late radiation-induced toxic reactions in the skin and mucosa. There the SF2 values peaked between 15-20%. The gene expression analysis revealed different expression patterns in radiation sensitive and resistant patients.

**Conclusion:** In vitro assay might be applied to estimate the radiation sensitivity of cancer patients before the start of radiation therapy. This might be used for the individualization of radiotherapy protocols.

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## RADIOLOGICAL EMERGENCY PREPAREDNESS DEVELOPMENT FOR HOSPITALS BASED ON THE BALANCED SCORECARD APPROACH

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**Objective:** The purpose of this work is the description of conceptual a study for radiological emergency preparedness for hospitals. Most hospitals are not prepared for radiological or even CBRN (nuclear, biological, chemical and radiological) disasters. Disaster procedure manuals for hospitals are usually not read or transformed into practical preparedness strategies of the hospital management. New organizational learning and management concepts have to deal openly with possible radiological scenarios that have become globalized threats due to the stockpile and proliferation of radiological and nuclear weapons as well as rising political tensions worldwide. Only a continuous and systematic approach which is educationally and financially viable can transform existing administrative and work procedures into true learning organizations with a high degree of practical implementation.

**Method:** The balanced scorecard (BSC) is a practical change management tool that has been successfully applied in this field of hospital management. It has the capacity to implement new goal oriented values linked with strategies (management by objectives, MBO) as well as to control (monitor / regulate) the change process which is usually initiated by teams. Since each area has individual tasks and needs to fulfill an overall set of values defined for disaster preparedness, it has to be redefined on each particular organizational level. A top-down approach also needs participation, i.e. involvement of employees (bottom-up) refining strategies according to their situation which also ensures a high degree of acceptance of change and success. Vision and strategy of the BSC refer to 5 areas of hospital management: 1. Patients / victims (e.g.: speed of triage and treatment), 2. Organizational processes (e.g.: disaster response, setup of deco-zones, etc.), 3. Finance (e.g.: synergies among and extra budget for specific areas of development), 4. Employees (training, learning and development: e.g. electronic learning, courses, drills with other agencies, etc.), 5. Future / development (e.g.: degrees of preparedness). Each area consists of a list of various aims that fulfill a major mission of that particular field. Each aim is scored (evaluated with a number in the team with a consultant), for the evaluation of performance.

**Results:** The specific development process of a balanced scorecard for the purpose of emergency preparedness in the hospital is highly self-reflective with regards to possible scenarios of disaster and the individual's personal role in it. Only if personnel holding key positions within the organization have achieved a sound level of awareness of basic categories of disaster and their potential impact, optimal change strategies can be developed together with consulting experts and put in practice before, during and after casualties. This awareness of the dimensions and consequences of radiological disasters is a key educational issue on a personal as well as organizational level. It can unfold particularly during team sessions and workshops especially if a conceptual framework for self- and organizational reflection of possible scenarios precedes the development of the categories of the balanced scorecard. Such a complementary concept functions as a scenario-based subjective work analysis (swa) and prepares the design and implementation of the balanced scorecard (BSC) in the organization helping to acquire the potential to perform according to the needs of disaster.

**Conclusion:** The BSC performance measurement concept provides an innovative approach to a unique value-based concept of organizational change in hospital management of the current reality of CBRN disasters.

## A FLUORESCENCE SPECTROSCOPY STUDY OF MEMBRANE STRUCTURAL ALTERATIONS IN BLOOD LYMPHOCYTES OF RATS EXPOSED TO LOW DOSE RATE $\gamma$ -RADIATION

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**Objectives.** The peculiarities of low dose rate radiation impact are supposed to be concerned mainly with secondary oxidizing processes development, first of all, in membrane structures. It is also well known that blood as a whole system and, particularly, immunocompetent cells are of the most radiosensitive organism components. Furthermore, it has been found a relationship between oxidative homeostasis imbalance and immune response reduction. Imperfection of lymphocyte membrane function is likely to be an important link in this process. Since membrane functional activity to a certain extent depends on lipid-protein interactions investigation of lymphocyte membrane structure under long term low dose rate radiation exposure is of a great importance.

**Methods.** The investigation was carried out on cytoplasmic membranes of isolated intact blood lymphocytes of rats  $\gamma$ -irradiated from  $^{60}\text{Co}$  with dose rate of 0,72 cGy/day during 2, 21, 42, 84 and 139 days. The total absorbed doses amounted to 1,5, 15, 30, 60 and 100 cGy respectively. Physicochemical parameters of cytoplasmic membranes were studied using two fluorescent probes. Pyrene located within the lipid phase was used to estimate microviscosity and micropolarity of bilayer lipids. The membrane outer surface condition was evaluated through modifications of 1-anilinonaphthalene-8-sulphonate (ANS) binding parameters to lymphocytes.

**Results.** For the most part, it was found significant spectral changes of both probes compared to the control in the lymphocytes of rats exposed to the doses of 1,5, 15, 60 and 100 cGy indicated membrane structure alterations. However, there were found no significant spectral shifts compared to the control after a 30 cGy dose accumulation. It was significant that after radiation exposure at the doses lower than 30 cGy the substantial spectral changes became apparent not in every particular experiment, sometimes being of mismatched directed nature from one experiment to another. This fact implies unstable, probably oscillatory, nature of shown changes related to individual low dose radiation response dynamics during initial period of exposure. The changes revealed at 60 cGy and 100 cGy doses possessed a clear unidirectional nature. In particular, after a 1,5 cGy exposure more typical was small, but significant, reduction of pyrene microenvironment polarity in lipid bilayer. For the other doses tested (except 30 cGy) there was shown an increase of micropolarity, making 10% in nonpolar and 15% in more polar membrane zones after 60 cGy and 100 cGy exposures. There was also observed for the lymphocytes of rats exposed to 15, 60 and 100 cGy a significant microviscosity elevation for more polar domains of lipid bilayer running its maximum (20%) at a 60 cGy radiation dose. Simultaneously, there was revealed a significant microviscosity reduction to 30% for the hydrophobic membrane sites at this dose. Thus, changes of the whole membrane microviscosity profile occurred after a 60 cGy exposure. Membrane outer surface study also revealed irregular conflicting nature of ANS binding to lymphocytes at a dose of 1,5 cGy. However at a 15 cGy dose accumulation there was observed a general decrease of an ANS binding constant to 40-60% and, accordingly, a membrane surface charge decrease, 2-3-fold binding sites number elevation and also a quantum yield reduction. Further exposure to 60 cGy resulted in only a quantum yield reduction that was related most probably to increased water molecule penetration in polar zone of bilayer.

**Conclusion.** The results of our study testify to the structural alterations in lymphocyte membranes under permanent low dose rate  $\gamma$ -irradiation, to the different patterns of radiation response development on different exposure stages. Also oxidative process intensification in the remote period of exposure is indicated based on the complex analysis of fluorescent probes spectral changes.

## RESIDUAL RADIATION: SPECIAL RADIATION PROTECTION ASPECT ON MEDICAL LINEAR ACCELERATORS

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Medical linear accelerators (LINAC-s) are the most common radiation treatment units today. They contain no radioactive isotopes, but their high-energy ( $X_{\text{high}}, E > 10 \text{ MeV}$ ) photon radiation and neutron capture can produce short-living radioactive isotopes in the unit, head, beam track, and in the air outside. There is an increased background radiation level which dependent on time about units after terminating patient treatment radiation. Technicians and other workers who enter the treatment room and manipulate on unit will be exposed to gamma and beta radiation, the residual radiation has radiation protection significance on them. In this study: Creation of residual radiation in LINAC is explained. - Residual dose output and cumulative dose measurements in the isocentre of LINAC Type Philips SLI Plus after it was in use. Dose-dependence on treatment dose (Monitor Units) and time elapsed. Residual dose output dependence on time elapsed after terminating treatment radiation. - Low-resolution (50 channels in 0-3 MeV range) gamma-spectrometry to identify some isotopes created by activation in the LINAC - Special measurements to identify gaseous components. Basic dose assessment for technical and medical staff. *Results* : first half-time of residual radiation is about 7 minutes, 5 nuclides identified in spectra, and  $^{13}\text{N}$  in the air. Cumulative added dose for health care staff appr. 2-3 mSv/year from external gamma component.

## STRUCTURE OF THE PATIENTS WITH ACUTE RADIATION DISEASE TREATED IN THE DEPARTMENT OF ACUTE RADIATION PATHOLOGY IN THE PERIOD OF 1996-2002

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Since 1996 till 2002 six patients were admitted into the department of acute radiation pathology of the Clinical branch of the Institute of Biophysics – 6 patients (on 2 in 1996, 1997, 2000 years) with suspicion of ARD. The jobs of all the patients were connected with the sources of ionizing radiation (3 – the personnel of nuclear power plants, 1 – scientist, 2 – members of defectoscopic team). Patient B.V.A., 51 and B.V.O., 28, who were observed in our department in December, 1996, were the Kursk nuclear power plant workers and they were admitted because of the excess of radiation dose found during a control check of individual dosimeters B.V.A. dose 74 and 80 Gy, B.V.O. - 177 and 200 Gy. Clinical investigation and clinical dosimetry (neutrophiles, platelets and chromosome aberrations in lymphocytes peripheral blood count) did not disclose any evidences of presence of acute local or total radiation affect. The estimation of possible dose of radiation by means of electron spin resonance of dental enamel was not performed because of the patients' refuse. A similar situation took place in 1997 – the patient G.D.V., 22, a defectoscopist of the Kursk nuclear power plant, was found the dose of 139 and 133 B. According to the results of clinic and dosimetric research did not confirmation ARD. In 1997 patient Z.A.N., 44, a scientist of Russian Federal Nuclear Center (the city of Sarov), on June 17, 1997 was performing an experiment upon a model of uranium construction when a spontaneous chain reaction (SCR) occurred. Complex dosimetric, clinical and laboratory estimation of this case allowed concluding that the patient was affected by extremely uneven gamma-neutron radiation with average body dose of 8-11 Gy. The dose at the palms of the hands was 200-250 Gy. The patient passed over on the third day since the event. The syndrome of direct general radiation damage of heart, vessels and the early radiation pulmonitis development played the leading role in tanathogenesis. In the accident in 2000 near the city of Samara during the work with a defectoscope (the source – Yridium-192) three patients were damaged. The patient E.I.I., 65 had ARD mild degree – dose total gamma-irradiation 0,8-1,5 Gy. Second patient S.E.V. had ARD medium degree (dose total gamma-irradiation 3,3 Gy0). This patient had clinical problems - necrotic and hemorrhagic tonsillitis and pharyngitis, herpes simplex on the lips, skin hemorrhages, sepsis, phlebitis with signs of phlegmon of left thigh, septic retinitis of right eye, pneumonia in low lobe of left lung. The case of problems was a later hospitalization.

## THE USE OF THE TOTAL GAMMA-THERAPEUTIC RADIATION AS A MODEL OF THE ACUTE RADIATION DISEASE

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Radiation accidents are rather rare all over the world. Since 1954 till now in the USSR and then in Russia there took place over 160 accidental situations which were the cause of more than 300 cases of ARD. For the last five years only five patients with ARD of different degree of severity were treated in the Clinic of the Institute of Biophysics. In these conditions the problem of the Clinic personnel training for the treatment of ARD patients appears to be important, especially in case of massive admission of patients. Partially this problem can be solved by means of the use of the total gamma-therapeutic radiation in the treatment of haemoblastoses as the model of ARD.

During the last 20 years in our department we use the conditioning regime in autologic and allogeneous bone marrow or peripheral blood stem cells transplantations: the infusion of cyclophosphane 60 mg/kg for 2 days followed by the TTR of 12 Gy (six fractions of 2 Gy each for 3 days).

When we have it's model, we can decide that's questions of treatment ARD: The training of the skills of treating the patients with bone marrow syndrome: the use of programs of infusion of modern antibacterial, antifungal, antiviral medicines, the use of colony-stimulating factors, the use of immune globulins in the treatment of patients with haemorrhagic syndrome. 2. The training of the skills of treating the patients with intestinal syndrome (the treatment of mucositis including the ones complicated with fungal or viral infection, the use of different programs of parenteral feeding). 3. The training of the skills of treating of complications (viral hepatitis's, liver failure, renal failure etc).

Except the training of physicians there are the matters of great importance such as the training of nurses' skills of the patients care in the conditions of aseptic regime and intensive care, ready for action of the laboratories and paraclinic services; the reciprocity with the blood transfusion department; constant availability of necessary medicines and equipment. The time limit for a department to get ready and the economic disadvantage of having a department in a "stand-by" mode are also advantageous for this approach. At the same time there are some negative points: the fractionating of radiation and addition of cytostatics, the absence of local radiation damages meanwhile in practice the incidence of combined trauma is high, the previous treatment of the majority of patients.

### THREE CASES OF ACUTE RADIATION DAMAGE OF HUMANS FROM GAMMA SOURCE (IRIDIUM-192): DOSE RECONSTRUCTION AND CLINICAL PICTURE

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Purpose: To describe three cases of acute radio disease developed due to the external radiation from a gamma-source <sup>192</sup>Irin on August 15-23, 2000.

Subjects and methods: To reconstruct the dosage of external gamma-radiation were used modern methods of physical and biological dosimetry including the dosage estimation of ESP teeth of dental enamel and the frequency of chromosomal aberrations in blood and bone marrow lymphocyte culture.

Results: Three males (defectoscopist – 33 years, major defectoscopist – 65 years, driver – 26 years) of a line team of gas-pipe control were affected by radiation of gamma-source of a defectoscope which was left unshielded after the day shift. The team members spent the night (August 15-16, 2000) in a car where they got maximal total radiation. In 8 days during the work with the same defectoscope it was found that the gamma-source was unshielded and trying to fix it manually the defectoscopist got severe radiation of both palms (30-70 Gy). The facts of the defectoscope unshielded and the radiation leak were kept in secret by the team, and they started seeking for medical assistance as the clinical features appeared – on the 20<sup>th</sup> day (palm burns) and on the 27<sup>th</sup> day (agranulocyte sore throat) after the first radiation attack. Total dosage of gamma-radiation were about 1.0, 1.5 and 2.5 Gy in the major defectoscopist, defectoscopist and driver with the development of light ARD without clinical features, light ARD with severe local radiation defects of the palms and medium ARD with several episodes of severe infection and hemorrhages, respectively.

Conclusions: Now the acute radiation disturbances are usually observed due to radiation of uncontrolled gamma-sources of defectoscopes. The methods of physical and biological dosimetry allow estimating accurately the total gamma-radiation dosage. The severity of system and local radiation disturbances correlates well with the dosage of gamma-radiation.



## MODIFICATION OF “UV RESPONSE”: A NOVEL PHOTOPROTECTIVE MECHANISM

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Skin cancer, the most common malignancy in Caucasians, affects more than 3 million people worldwide yearly. BGP-15 (R,S-O-(3-piperidino-2-hydroxy-1-propyl)nicotinic acid amidoxime) a new type sun protective agent having moderate poly(ADP-ribose)polymerase inhibitor activity is effective against both acute and chronic UV light damage and blocks UV induced carcinogenesis. UV irradiation causes a complex cellular adaptive reaction called “UV response”.

We analyzed the effect of BGP-15 on major UV light fraction B (B) induced signaling pathways in human HaCaT keratinocytes exposed to 12.5-100 mJ/cm<sup>2</sup> irradiations. UVB induced activation of mitogen-activated protein kinase pathways (Jun NH<sub>2</sub>-terminal kinase, JNK; p38; and extracellular-regulated kinase 1/2, ERK1/2), activation of Akt and p53 were monitored by measuring their phosphorylated forms. Effect on terminal differentiation was estimated by the expression level of the differentiation marker involucrin.

BGP-15 treatment moderately increased GSH level 3 hours after UVB irradiation. Activation of JNK showed similar pattern in the presence and absence of BGP-15 after low dose, 25 mJ/cm<sup>2</sup> irradiation, however a faster decrease of activated JNK was observed in the presence of BGP-15 after high dose, 100 mJ/cm<sup>2</sup> UVB. BGP-15 treatment significantly stimulated the phosphorylation of p38, ERK1/2, Akt, and p53 at low UVB dose while at high UVB dose BGP-15 facilitated only the activation of ERK1/2. BGP-15 treatment partially restored the diminished involucrin expression in UVB irradiated cells.

Data show that BGP-15 can modify crucial UVB induced signaling pathways, it improves oxidative balance and terminal differentiation. Facilitation of UV-response can be a basis of in vivo photoprotective and anti-carcinogenic effect of BGP-15. The described activity of BGP-15 opens a new way for skin protection through modifying UV response.

## THE ROLE OF MICROORGANISMS IN THE MOBILITY OF RADIONUCLIDES IN CHERNOBYL SOIL

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Many reports in the literature, most in Russian, are available concerning the consequences of radioactive release to environment from the Chernobyl Nuclear Power Plant. These results have not covered the role of microorganisms in the mobility of radionuclides in soil. The aim of this paper is to understand how soil bacteria will interact with radionuclides in soil. It concerns several major items that may have an influence on the mobility of radionuclides in direct or indirect ways thereby being important for the analysis.

Soil samples were analysed layer by layer from two contaminated sites near Chernobyl Nuclear Power Plant 17 years after the accident. Physico-chemical characteristics and radioactive concentrations of the soil samples were determined. The results show that radiocaesium migrates very slowly downwards. Microbiological analyses were performed; number, diversity and activity of soil microorganisms were assessed. The average CFU counts of soil samples were  $10^5 \cdot g^{-1}$ . We have isolated 88 bacteria from the two soil samples, among them being aerobe, anaerobe, sulfate-reducing, ammonia-producing, nitrate-reducing and nitrifying bacteria. Isolated bacteria were analysed to determine their metal mobilising capacities (acid production, siderophore-production), metal immobilising capacities (biosorption, bioaccumulation), resistance to heavy metals and radiation.

## CORRELATED AND INDEPENDENT CHROMOSOMAL ABNORMALITIES IN ROOT MERISTEM CELLS OF PLANT SEEDS IN THE LAB AND IN NATURE

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Objective: To investigate the correlations between the chromosomal aberrations (CAs) in root meristem cells of seeds irradiated or not. Methods: The CAs consisted of chromosome bridges, and acentric fragments were studied in root meristem cells of seeds. We used seeds of radioresistance (*Pisum arvense*) and radiosensitive (*Pisum sativum*) lines of pea as well as plantain (*Plantago major*) population growing around the NPP (~10-15  $\mu\text{R/h}$ ). Pea seeds were gamma-irradiated ( $^{60}\text{Co}$ ) with 7 cGy at 0.3-19.1 cGy/h and with acute doses 0.26; 0.56 Gy and 25; 50 Gy. The statistical simulation of CAs appearance used  $\chi^2$  and clusterization criterions. The mathematical modelling used the equations of death/birth processes. Results: The statistical modeling showed that CA number is geometrical (G)-distributed in meristem cells of pea seeds without irradiation; cells with CAs that are G-distributed with increased sample mean are produced at irradiation with 7 cGy at 0.3 cGy/h. At 1.2 cGy/h the Poisson (P) -distributed CAs appeared in some subpopulation of cells; at 19.1 cGy/h the P-distribution dominates, whereas G one comes into negligible. The irradiation with 7 cGy decreases G subpopulation and increases its sample mean with dose rate. We can presuppose that the primary DNA injuries are P-distributed. The correlative G subpopulation corresponds to enhanced CA number together with cells elimination from proliferation pool. At the acute irradiation (25 and 50 Gy) G-distributed CAs in cells are observed. We can suggest that this irradiation did not induce cell elimination, and G subpopulation masked minority P one. Statistical simulation showed that G subpopulation dominates in distant plantain populations, whereas P one is observed near the NPP. The correlative mathematical model is based on the following hypothesis: 1) primary DNA damages can appear; 2) correlative DNA damages can appear; 3) repair processes. Correlative model predicts that at low primary DNA damages' intensities the probability of CAs appearance is exponent whereas at a high one it is binomial. The intensity of both correlated CAs emerging and repair processes do not change the general regularities. The acute primary dose increases the probability of G distributions. Conclusion: The statistical simulation showed that CAs are G-distributed in root cells of unirradiated seeds. Low dose-rate irradiation increases CA number together with cells elimination in G-subpopulation. P subpopulation describes the primary DNA damages. The correlative mathematical model predicts that CAs probability appearance depends on intensity of primary DNA injuries emerging.

## INTEGRAL GENOTYPE STRUCTURE, INDIVIDUAL RADIOSENSITIVITY AND HEALTH STATUS OF THE PERSONNEL, CHRONICALLY EXPOSED TO GAMMA- AND NEUTRON IRRADIATION

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### ABSTRACT.

Epidemiological and Radiation-Monitoring Register of Russian Federal Nuclear Center (RFNC-VNIIEF), Sarov contains dosimetry, clinical, epidemiological and genetic data on the personnel, occupationally exposed to different types of ionizing radiation. Here we present preliminary data on the effects of genotype and individual radiosensitivity (micronuclei test, MN) on health status of the group of nuclear reactors' workers, chronically exposed to gamma- and neutron irradiation. Within this cohort of 75 men, 10 polymorphic loci were typed and the frequency of micronuclei was analysed.

The analysis of blood samples irradiated ex-vivo did not reveal significant correlation between the occupational dose and MN induction, therefore indicating the lack of adaptation (hormesis) or increased radiosensitivity within this cohort.

The analysis of association between genotypes and morbidity revealed: (1) higher incidence of endocrine diseases among homozygotes EsD 2-2 and Gc 1-1; (2) elevated frequency of urological disorders among homozygotes Gc 1-1; (3) elevated incidence of benign (non-malignant) tumors among homozygotes Gc 2-2 and also GLO 2-2, EsD 1-2 or 2-2, showing differences in the frequencies of spontaneous and radiation-induced MN; (4) elevated incidence of cardio-vascular diseases among homozygotes ACP 1-1 and EsD 1-2 or 2-2, also mediated by the differences in MN frequency.

The contribution of genotype and individual radiosensitivity (MN test) to the health status of occupationally exposed individuals will be discussed.

## LOW FRACTION-SIZE (3 GY) HDR AFTERLOADING BREAST BOOST IRRADIATION RESULTS IN AN INCREASED RATE OF GRADE $\geq 2$ TOXICITY. EVIDENCE BASED ON A 3-YEAR FOLLOW-UP OF 93 PATIENTS

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**Purpose:** To investigate the radiation-induced late toxicity of a high-dose-rate (HDR) afterloading (AL) brachytherapy (BT) boost in early-stage breast cancer.

**Materials and Methods:** 93 women diagnosed with stage I or II breast carcinoma (94 breasts) at the University of Pécs between November 1996 and January 2003 underwent breast-conserving surgery with unknown (24 breasts), close or affected (22 breasts) or safe (48 breasts) surgical margins. 14% of the patients (13/93) received chemotherapy and 80% of them (75/93) hormonal treatment. The postoperative whole-breast irradiation was performed with a daily fraction of 2 Gy with either telecobalt (40 breasts) or 6 MV photons (54 breasts). The median delivered dose was 51 Gy (mean: 50 Gy, range: 48-60 Gy) for telecobalt, and 53 Gy (mean: 54 Gy, range: 40-56 Gy) for the 6 MV photons. External irradiation was followed within a week by interstitial HDR AL (Nucletron, 192-Ir) tumor bed irradiation in a single plane (89 breasts) or double plane (5 breasts, plane distance 10 mm) using flexible (27 cases) or rigid (67 cases) catheters. The median number of inserted catheters was 5 (mean: 5, range: 1-10) with 14-mm center-to-center spacing. The reference BT dose was specified at 5 mm from the surface of the catheters. The median delivered dose was 10.5 Gy (mean: 11.7 Gy, range: 3.5-32 Gy) with a median fraction dose of 3.0 Gy (mean: 3.6, range: 2.0-7.0 Gy) twice a day, 8 h apart. In the assessment of the skin and subcutaneous toxicity, the RTOG late radiation morbidity scoring system was applied. Tabar score system was applied for breast parenchyma scoring.

**Results:** By the end of the median follow-up of 38 months (range: 12-85 months), all the patients were tumor-free. 0.6% (1 breast) of the cohort had grade 1, 6.4% (6 breasts) had grade 2, and 0.6% (1 breast) had grade 3 telangiectasis. 29% (27 breasts) exhibited grade 1, 8.5% (8 breasts) grade 2 and 4.3 % (4 breasts) of them grade  $\geq 3$  localized fibrosis. 18% (17 breasts) of the cohort displayed some form of fat necrosis. 4 of 5 breasts with grade 2 fat necrosis was associated with Tabar 4-5 score.

**Conclusion:** Following whole-breast external irradiation, an interstitial HDR AL boost BT of the tumor bed, a low median total dose of 10.5 Gy and a small median fraction size of 3.0 Gy resulted in a relatively high rate of grade  $\geq 2$  radiation-induced toxicity by the end of the median 3-year follow-up. Tabar 4-5 type breasts seems to be associated with high risk of fat necrosis.

## THROUGH THE HILLS AND VALLEYS OF RADIATION BIOLOGY IN HUNGARY

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The radiation biology as a discipline is on the borderline of several scientific branches. Developing in interactions with them, the knowledge on radiation effects – as an aim for studies as well as a tool to study others like structure and function of biomolecules, immunology, DNA-repair, cellular, tissue and organ reactions, effects on organisms – depends also on the progress of others studies. Accordingly, the “hills” and “valleys” appear in each laboratory when they select, initiate or change research topics. In the present review examples of the “hills”, i.e. results which have reached the international literature and interest, in Hungary are demonstrated in the radiobiological research on the cellular membranes, cytogenetics, antioxidant defense, regulation as well as practical applications in microbiology, like corrosion, sterilization and radiopharmacy. A few notes on the biological basis of radiation protection are included.

## RADIOBIOLOGY FOR ION BEAM THERAPY

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The use of ion beams in tumor therapy has two rationales: On the physical side the greater precision in dose combined with an inversed dose profile having the maximum at the end of the range. This allows to increase the dose to the tumor without increasing the side effects. On the biological side the change in the biological effectiveness of the ion beams compared to photons gives a greater efficiency for the tumor cell inactivation when the regions of elevated RBE can be restricted to the tumor only. The greater RBE is the main argument for the heavier ions like carbon where clinical RBE values up to 5 can be reached for radio-resistant tumors. But also for protons elevated RBE values can be found in cell experiments. In the clinical experience a constant  $RBE = 1.1$  is used for protons.

However, using the most precise beam delivery system for the ion treatment, the Intensity Modulated Particle Therapy (IMPT), the RBE is an additional variable over the treatment field that has to be optimized from pixel to pixel for both: protons and carbon ions.

Therefore, the exact knowledge of RBE and its dependence on biological and physical parameters will be discussed, as well as possibilities how to include it into treatment planning.

## CLINICAL AND HYGIENIC CONCEPTION OF PROPHYLACTIC TREATMENT FOR ACCIDENTAL RADIONUCLIDE INCORPORATION

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According to the IAEA document (Intervention Criteria in a Nuclear and Radiation Emergency. Safety series No 109, page 41) prophylactic treatment with stable iodine will be recommended if the dose in the thyroid gland avertable by this protection action is 100 mGy or more. In Russia this generic intervention level is confirmed in the following details: 100 mGy for children and 250 mGy for adults per 10 days. If we take the weighting factor for the thyroid gland as 0.05, so the committed effective doses will be 1.25 cSv/10 days for adults and 0.5 cSv/10 days for children.

If we agree with the international general principles of protection in radiation accidents we can use these committed effective doses for the most radioisotopes. We propose to use a two level intervention conception to make decision on prophylactic treatment for accidental radionuclide incorporation. Intervention level to begin a specific antidote therapy of patients with accidental radionuclide incorporation is at an avertable effective dose interval from 1.25 cSv to 12.5 cSv per 10 days. Intervention level to use both an intensive antidote therapy and preventive nonspecific methods radionuclide decorporation is a avertable effective dose more than 12.5 cSv per 10 days.

We have checked this two level intervention conception of prophylactic treatment with both our experience and some cases published by other authors and come to the conclusion this general conception is suitable for the most cases of accidental radionuclide incorporation in the human body.



## DIFFERENT ASPECTS “LOW DOSE” PROBLEM : DOSE RATE AND IRRADIATION CONTINUANCE

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Objective: Assessment of low dose biological efficacy is complex and polysemantic problem. Chernobyl Accident initiated studies of low doses efficacy in different systems and objects that are irradiated *in situ* as well as in controlled conditions of laboratory experiment. No wonder that the properties of chromosomal aberration formation are the most investigated now, as long as appearance of cytogenetic anomalies is common organism reaction to man-caused pollution, including radionuclide pollution. Appearance of chromosomal aberrations is the example of stochastic radiation effects, hence investigation of radiosensitivity of this process gives information about other possible stochastic consequences of low dose irradiation (cancer, genetic disorders). Thus, estimation of possibility of cytogenetic anomalies appearance for different objects is necessary to investigate in a broad range of practical and theoretical issues, including reliability of cytogenetic tests usage for human biodosimetry and advisability of usage of linear, “convex” and “concave” model of cancer and inherited anomalies risks extrapolation. However, results that were obtained at the conditions of acute short-term experiment don’t reflect human reaction to irradiation and the reaction of biota *in situ*, when irradiation is of very low intensity. The problem of low doses has two fundamentally different aspects connected with dose level as well as with formation time. First aspect affects the issue of organisms’ radiosensitivity difference at different dose intervals. Second aspect concerns with the estimation of prolonged action cumulative effect for low dose irradiation. Low dose irradiation could lead to gradual modification of radiosensitivity in all dose range, in other words it could lead to organism’s radiosensitization as well as to organism’s radioadaptation. The process of radiosensitivity modification is protracted, it consists of several phases and it could change sign during its development.

Results: As an example of prolonged irradiation efficacy we could consider obtained experimental data. Risk calculations (i.e., possibilities of chromosomal aberration appearance for dose unit) for corn germ of different sorts are listed at the four tables. As it is illustrated by obtained results, risk of cytogenetic anomalies of one corn sort is much higher for irradiation of 30 mP/h during several months than for acute irradiation. As times goes by, risk of cytogenetic anomalies is constant for first corn sort, while for the second sort possibility of cytogenetic anomalies for dose unit increases. It could be inferred that: risk of stochastic effects could be much higher at the conditions of chronic irradiation its value could change with chronic irradiation duration.

Conclusion: Thus, while utilizing traditional approach to estimation and forecasting of irradiation consequences, we could see that: Dose formation occurs at the condition of prolonged low dose irradiation that leads to the modifications of radiosensitivity and, formally, to modifications of risk coefficient. Dynamics of cancer and heritable disease occurrences will be modified greatly in this case.

## EVALUATION OF DNA DAMAGE BY ALKALINE COMET ASSAY AFTER IN VITRO EXPOSURE TO ELF MAGNETIC FIELD AND IONISING RADIATION

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**OBJECTIVE:** The aim of our study was to determine if exposure to 500  $\mu$ T magnetic field for 2, 4 and 24 hours on human lymphocytes can cause DNA damage, and can magnetic field alter the DNA repair if the samples were exposed to it before the ionising radiation exposure.

**METHODS:** Whole venous blood was taken by vein puncture and dispensed to Eppendorf tubes. The samples in Eppendorf tubes were exposed in thermostat on 37°C to vertical 50 Hz 500  $\mu$ T magnetic field, between two horizontally placed Helmholtz coils (1), for 2, 4 and 24 hours. Sham exposed controls were placed in the same thermostat, where the lowest stray magnetic field was measured ( $\sim 1,3 \mu$ T) for 2, 4 and 24 hours respectively. Positive control was exposed to ionising radiation ( $^{60}\text{Co}$ ; 4Gy). The alkaline single-cell gel electrophoresis - Comet assay was used to investigate the DNA damage (single and double strand brakes) on lymphocytes, 0, 30, 60, 120 and 240 minutes after the exposure to magnetic field or 4 Gy. The comets were analysed with the Komet 4.0 (Kinetic Imaging, Ltd, UK) image analysis system with respect to their tail DNA (tail factor %). Exactly the same cells that were seen on screen during the computer analysis were classified in five categories according to Anderson et al (2).

**RESULTS AND CONCLUSION:** It is well known, that ionising radiation damages the DNA. In our assay the tail factor of the lymphocytes immediately after the 4 Gy exposure was 4 fold the control, but after 120 minutes it returned to the control level.

In our experiments, 24 hours ELF MF exposure significantly increased the DNA damage, but if the blood was exposed to 4 Gy after 24 h ELF MF, there were no significant difference between the results of ELF MF exposed and not ELF MF exposed samples.

It seems, that visual analysis of comets overestimates the damage of the cells, especially at those points where the DNA damage of the lymphocytes were higher.

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## REGULATION OF P53 IN RESPONSE TO IONIZING IRRADIATION

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The p53 protein accumulates in response to DNA damage due to rescue from proteasomal degradation. Currently, several mechanisms are discussed that are activated in response to ionising irradiation and that interfere with p53 degradation. Among these mechanisms are phosphorylation of Mdm2 and phosphorylation of p53. More recently, we showed an additional possibility, which is hypophosphorylation of Mdm2. Ionising irradiation causes hypophosphorylation of several contiguous and constitutively phosphorylated residues in the central domain of the Mdm2 protein. Phosphorylation of these amino acids is absolutely required for p53 degradation. In search for kinases that phosphorylate these sites in proliferating cells, we came across glycogen synthase kinase 3 (GSK-3), a protein with a prominent role in sugar metabolism and Wnt signalling.

The central domain of Mdm2 possess two consensus sites for Glycogen synthase kinase 3. Data will be presented showing that glycogen synthase kinase 3 phosphorylates Mdm2 in vitro and in vivo and that it regulates p53 degradation. Importantly, ionising irradiation leads to rapid phosphorylation at Ser9 of glycogen synthase kinase 3 with subsequent inactivation of the kinase. Replacing Ser9 by Ala reduces the accumulation of the p53 protein after ionising irradiation. These data provide strong evidence that GSK-3 is a component of the signalling pathway leading to p53 accumulation in response to ionising irradiation via hypophosphorylation of Mdm2.

## PROBABILISTIC TWO-STAGE MODEL OF CELL INACTIVATION BY LIGHT IONS

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Mathematical modelling of the biological effects of protons and light ions plays a crucial role in treatment planning in hadron radiotherapy. It might also contribute to understanding better the underlying radiobiological mechanism. Up to now, the models of cell inactivation that have been used in radiobiology and radiotherapy, such as the linear-quadratic model, have described the biological effects in a phenomenological way only. They have not been very helpful in understanding the underlying mechanism.

We will present the probabilistic two-stage model of cell inactivation that represents a more realistic description of the radiobiological mechanism. It is based on distinguishing single-particle and collective effects of the mechanism: Processes from initial energy transfer events to the formation of DNA damages run practically immediately after the passages of individual particles through the cell nucleus. On the other hand, the final response of the given cell, leading eventually to its inactivation, has to be classified as a response to the total damage caused by all particles traversing the nucleus. The model takes into account the actual number of particles traversing individual nuclei, the amount of transferred energy, and the probability of forming lethal and sublethal damages. The effects of cellular repair processes are respected, too.

The basic features of the model will be explained. Results of analyses of experimental cell survival data for different ions will be shown. Differences in damage induction and repair after irradiation by diverse ions will be discussed.

The probabilistic two-stage model opens the way towards connecting cell survival characteristics with the outputs of e.g. track-structure models or models of DNA damage induction. In its simplified versions, the model can be used in treatment planning procedures in hadron radiotherapy. The model enables to represent not only the global shape but also the detailed structure of cell survival curves. This is important when evaluating different fractionation schemes, as in such a case even small deviations of survival curves from their global shape might yield large differences in the cumulative biological effect.

## URANIUM AND FERTILITY: OOCYTE DYSMORPHY AND APOPTOSIS IN CUMULUS CELLS.

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### Objective:

Exposure to environmental toxicants have been documented in the contemporary literature that could alter human reproductive function and affect fertility. IRSN has started in 2001 the ENVIRHOM' program. The objective of this program is study the radionuclides effects on the human health at low concentrations and chronic expositions. In this context, the objective of this work is to evaluate the uranium (U) effects on fertility in an animal model.

### Methods:

In one hundred and fifteen hybrid females of mice (CBA x C57 Bl) the super ovulation was induced. Sixty-one were controls and 54 were exposed at chronic concentrations of U in drinking water - uranyl nitrate - with final U concentrations 107, 40 and 20 mgU/l during 48 days. At the end of contaminations periods, they were sacrificed before super ovulation and the oocyte number, oocyte morphology and apoptosis in *cumulus* cells (CC) were evaluated.

### Results:

No significant differences were observed between number of oocytes ovulated by females of control group ( $29.40 \pm 10.17$ ) and contaminated group ( $30.13 \pm 9.29$ ;  $34.93 \pm 10.69$ ;  $36.11 \pm 8.30$  respectively).

2843 oocytes from 80 ♀ were classified. The percentage of oocyte dysmorphism (OD) was statistically different between control group ( $22.27 \% OD \pm 6.47$  /1125 oocytes / 27 ♀) and contaminated group: 107 mgU/l ( $66.90 \% OD \pm 19.08$  /904 oocytes / 30 ♀) 40 mg U/l ( $59.69 \% OD \pm 11.36$  / 489 oocytes / 14 ♀) and 20 mgU/l ( $59.99 \% OD \pm 6.36$  / 325 oocytes / 9 ♀).

The apoptosis in CC has increased the way dose dependent: control apoptosis mean  $1.44 \% \pm 0.38$  (411 oocytes / 10 ♀) 107 mgU/l apoptosis mean  $79.18 \% \pm 5.72$  (69 oocytes / 3 ♀) 40 mgU/l apoptosis mean  $28.29 \pm 4.19$  (489 oocytes / 12 ♀) 20 mgU/l apoptosis mean  $13.42 \pm 1.17$  (325 / 9 ♀).

### Conclusion:

This study has showed that U doesn't alter the number of oocytes ovulated by female but it causes OD and apoptosis in CC in all the concentrations of U employed.

In the human species the OD and apoptosis in CC affect: fertilization, the first stages embryonic development and implantation.

The analysis of the *cumulus* oocyte complex (COC) constitute a new element of study in reproductive toxicology. The COC of mice revealed to be a sensible indicator of U contamination and could be employed to evaluate concentrations inferior to those used in this study.

## URANIUM AND DEVELOPMENT: EFFECTS ON PREIMPLANTED EMBRYOS: DELAY, DYSMORPHISM AND APOPTOSIS.

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### Objective:

The uranium exposure increases embryo-fetal toxicity, teratogenicity and reduces the offspring growth. The IRSN has started in 2001 the ENVIRHOM' program. The objective of this program is study the radionuclides effects on the human health at low concentrations and chronic expositions. In this context, the objective of the present study is to evaluate the direct effects of uranium (U) on mouse preimplantation embryos, a period corresponding to the first 2-4 days after human conception.

### Methods:

Embryos were obtained from hybrid females CBA x C57 Bl following induction of superovulation. 1292 embryos in the one-cell stage were placed in the culture medium at 37 °C in 5% CO<sub>2</sub> air. The final U concentrations were 6.5; 13; 26; 52 and 104 µgU/ml and each experiment count with its own control. They were observed each 24 hs to 96 hs of cultured. The embryo were classified as two and four - cell stage, morula and early - expanded blastocyst. By apoptosis evaluation, embryos were stained with supravital colorants DAPI + PI according protocol.

Each 24 hs the grade delay, dysmorphism embryoner and apoptosis presence were valued.

### Results:

The observation of grade delay in mean control embryos (DC) and grade delay in mean embryos cultured with U (DU) showed: a) at 48 hs of cultured (< 4 cells) DC was 4,42 % ± 0.64 (N° of embryos = 181) vs DU 16.87% ± 2.6 (615); b) at 72 hs (< 8 cells) 5.76 % ± 1.08 (71) vs 16.34 % ± 5.18 (247); c) at 96 hs (≤ morula) 51.85% (27) vs 72.8% ± 3.2 (151) respectively.

The observation of dysmorphism embryoner mean control (DEC) and dysmorphism embryoner mean in U cultured (DEU) was: a) at 48 hs of cultured DEC 0.32 % ± 0.56 (181) vs DEU 8.38 % ± 2.15 (615); b) at 72 hs 13.5 % ± 5 (181) vs. 22,5 ± 5.19 (247); c) at 96 hs 0 % (27) vs. 14 ± 4.04 (151).

The apoptosis was positive in 100% embryo dysmophy (15), 0% in good morphology embryos controls (15) and 20 % in the good morphology embryos cultured with U (10).

### Conclusion:

The toxicity of uranium produce severe alterations from 4 cells stage (48 hs post conception) in all concentrations of U employed. Embryo development was delayed, apoptosis was present and dysmorphism embryoner was the highest in the embryos exposed to the uranium as compared to the controls throughout the cultured period. In the human specie the dysmorphism embryoner is the most important reason for no implantation. The mouse embryo assay demonstrated to be a valuable tool to study the firsts U toxicity effects in early mammalian embryos and could be employee to evaluate inferior concentrations to those used in this study.

## A SUBPATHWAY OF NON-HOMOLOGOUS END-JOINING REPAIR INVOLVES FACTORS OF THE ATM SIGNALLING PATHWAY

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The ATM protein, which is mutated in individuals with ataxia telangiectasia (AT), is central to a network of cell cycle checkpoint responses initiated by DNA double-strand breaks (DSBs). However, the role of ATM and its substrates BRCA1, 53BP1, H2AX, and NBS1 in DSB repair is currently unclear as is the basis underlying the radiosensitivity of cells with mutations in any of these factors. We applied immunofluorescence detection of  $\gamma$ -H2AX nuclear foci and/or pulsed-field gel electrophoresis to quantify the repair of DSBs in confluence-arrested fibroblasts with mutations in ATM, 53BP1, H2AX or NBS1. In contrast to the pronounced repair defect that is observed in cells with mutations in the classical non-homologous end-joining (NHEJ) factors DNA ligase IV, XRCC4 and DNA-PK, fibroblasts with defects in the ATM signalling pathway repair the majority of DSBs with normal kinetics. However, a subset of DSBs exists that is repaired by a process requiring both classical NHEJ and ATM signalling factors. This subset of induced breaks depends on the kind of agent generating DSBs and correlates with the complexity of the lesions induced. It is small for etoposide that leads to enzymatically-induced DSBs by topoisomerase inhibition, intermediate for sparsely ionizing X- or  $\gamma$ -radiation and greatest for densely ionizing  $\alpha$ -particles that are known to cause the most complex forms of DSBs. Significantly, cells deficient in Artemis, a nuclease involved in V(D)J recombination and known to interact with DNA-PK, show the same DSB repair defect as cells deficient in ATM signalling factors, suggesting that Artemis operates in the same subpathway of NHEJ repair. Our data suggest a model in which repair of a certain class of DSBs involves, in addition to classical NHEJ factors, components of the ATM signalling pathway. These lesions likely represent complex DSBs that may require a sophisticated ensemble of proteins for their faithful repair, including nucleases for the processing of break ends (Artemis and NBS1), factors for the retention of repair enzymes at the break site (H2AX), scaffold proteins for the coordinated assembly of the repair complex (53BP1 and BRCA1) and a factor for the regulation of this process (ATM).

## HIGH THROUGHPUT ANALYSIS AND NEW INDICATORS OF RADIATION EXPOSURE IN HUMAN SKIN CELLS.

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Since a long time, biological markers have been sought in radiobiological studies in order to identify individuals exposed to ionising radiation in human populations. With recent developments in high-throughput gene expression screening, it is possible to develop gene expression profiles that may correlate with the timing and dose of radiation exposures. The identification of such a set of genes and proteins would enable more rapid testing of potentially exposed populations. The aim of this study was thus to define such sets for the skin, which is the first organ damaged by radiation exposure, and more specifically for the keratinocyte, which is the main target cell for radiation in skin. In a first study, highly differentiated HaCaT cells have been studied. The most significant novel findings concern genes induced by moderate (2 Gy) and high doses (15 Gy) at 3 hours after exposure. A hundred of genes have been identified by microarrays as new markers of exposure to  $\gamma$  rays. Functional classification of these genes reveals induction of markers from basal proliferating keratinocytes, activation of ATP producing pathway, absence of pro-apoptotic response, induction of chromatin remodeling and surprising down-regulation of specific DNA repair genes. Microarray results were confirmed by RT-PCR and correlated to changes in protein level by western-blot for a series of genes. Moreover, the regulation of early markers in cultured cells has been confirmed by *in vivo* studies in irradiated human skin. Finally, some markers were studied in late radiation sequelae of patients, that developed after radiation accidents or radiotherapy. These experiments show that genes induced rapidly within hours in skin cells can remain overexpressed for years in the irradiated skin, as long term signatures of radiation exposure. In a second study, specific effects of low dose (10 mGy) were studied in primary cultures of human keratinocytes. DNA microarrays containing 9500 probes were used to assess transcriptional changes in a time-course between 3 and 72 h post-irradiation. Keratinocytes were studied at an advanced stage of differentiation in order to mimic the supra-basal layers of the epidermis. We identified 423 genes differentially expressed by the low-dose (10 mGy) versus 573 genes for the therapeutic dose (2 Gy) at least at one time point of the kinetics. A major finding of this study was the identification of an important number of low-dose specific genes (183), most of which were modulated at 48 h. By hierarchical clustering, probes specifically modulated by the 10 mGy dose were classified into 3 clusters. In order to identify common regulatory sequences in the promoter of 17 co-expressed genes from one of these clusters, we analysed these sequences using a probabilistic Gibbs Sampling algorithm. This led to the identification of putative human binding sites such as AP-2, GATA-1, GATA-3, IL-6 RE and SP-1. The role of the corresponding transcription factors is now under investigation. In summary, these results show for the first time that low-dose ionizing irradiation induces a specific transcriptional response in human keratinocytes.



## RADIOPROTECTION BY THE SOY ISOFLAVONE GENISTEIN

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There is a need to develop medical countermeasures to protect first-responders and remediation workers from the biomedical effects of external ionizing radiation. Agents that can protect against ionizing radiation and that can be administered before radiation or shortly after irradiation would permit rescue workers to provide the services needed. The ideal radioprotector would be nontoxic and would not degrade performance. In addition to their use in the context of radiological terrorism, chemical radioprotectors may have utility in clinical oncology and space travel. Soy phytoestrogens have been reported to have many beneficial health effects including a reduction in osteoporosis and some types of cancer. The most plentiful isoflavone from soybeans is genistein (4', 5, 7-tri-hydroxy-flavone). Genistein has been shown to provide protection against non-ionizing ultraviolet radiation but not against the more damaging effects of ionizing radiation

In the present study, the radioprotective and behavioral effects of an acute administration of the isoflavone genistein were investigated in adult CD2F1 male mice. Mice were administered a single subcutaneous (sc) dose of genistein either 24 hr or 1 hr before a lethal dose of gamma radiation (9.5-Gy cobalt-60 at 0.6 Gy/min). Mice received saline, PEG-400 vehicle, or genistein at 3.12, 6.25, 12.5, 25, 50, 100, 200, or 400 mg/kg of body weight. For mice treated 24 hr before irradiation, there was a significant increase in 30-day survival for animals receiving genistein doses of 25 to 400 mg/kg ( $p < 0.001$ ). In contrast, the 30-day survival rates of mice treated with genistein 1 hr before irradiation were not significantly different from those of the vehicle control group. Additionally, the acute toxicity of genistein was evaluated in non-irradiated male mice administered a single sc injection of saline, vehicle, or genistein at 100, 200, or 400 mg/kg. At these genistein doses, there were no adverse effects, compared with controls, on locomotor activity, grip strength, motor coordination, body weight, testes weight, or histopathology. These results demonstrate that a single sc administration of the flavonoid genistein at nontoxic doses provides protection against acute radiation injury.

## $\gamma$ -IRRADIATION-INDUCED DNA DAMAGE AND REPAIR IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES TREATED WITH MICROCYSTIN-LR

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Ionizing radiation induces various types of DNA damage that include single strand breaks (SSB), double strand breaks (DSB) and base alterations. These lesions are repaired by distinct DNA repair mechanisms. Disturbances in the repair processes may lead to chromosome rearrangements and mutations which are common events during the development of cancer. Although the process of reversible phosphorylation has been shown to be essential for repair mechanisms, corresponding phosphatases have not been fully identified.

The objective of this study was to investigate whether protein phosphatases PP1 and PP2A are included in ionizing radiation-induced DNA repair.

Human peripheral blood lymphocytes were separated from healthy donors and were treated in the G<sub>0</sub>/G<sub>1</sub> phase of cell cycle with the inhibitor of protein phosphatases PP1 and PP2A - microcystin-LR at a dose of 0.5  $\mu$ M for 3 hours. Thereafter cells were irradiated with 2 Gy gamma radiation (<sup>60</sup>Co, Siemens Theratron Elite 80). The level of DNA damage and the kinetics of DNA repair were assessed in cells sampled 0, 15, 30, 60 and 120 minutes post exposure by the comet assay. Simultaneously the frequencies of chromosomal aberrations in the first division metaphases were determined by light microscopy in cells harvested after 50 hours from the beginning of the experiment. The results obtained with the comet assay correlate with the cytogenetic results and indicate that microcystin-LR inhibits DNA repair. Moreover protein phosphatases PP1 and PP2A are probably involved in this process.

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## CHARACTERIZATION OF NOVEL TRANSCRIPTIONAL TARGETS IN THE UV RESPONSE

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Solar ultraviolet (UV) radiation harms skin cells by damaging cellular macromolecules, one of the most relevant being DNA. UV radiation-induced DNA damage has several effects in skin cells both in short and long term, the most persistent being promotion of skin tumorigenesis. To protect themselves from the harmful effects of UV radiation, skin cells employ stress responses which involve several signaling cascades. These responses aim at adaptive changes in cellular functions. These include halting of cell cycle progression ensuring time for DNA damage repair, or apoptosis in case of very severe damage. If the repair mechanisms fail, carcinogenesis may be provoked.

We have studied the transcriptional response of human skin fibroblasts to UV radiation in large scale. To limit the damaging effects of the radiation mostly to DNA, UVC radiation was used. By DNA microarray technology, we compared changes in gene expression upon low ( $10 \text{ J/m}^2$ ) and high ( $50 \text{ J/m}^2$ ) doses of UV radiation, inducing a transient cell cycle arrest or apoptosis, respectively. We aimed to find genes common, and specific, to these cellular responses, and to identify previously unknown UV-regulated genes (Gentile et al. 2003). 460 genes out of 12 000 underwent regulation by UVC with over 3 fold changes. The data showed highly divergent transcriptional responses in cells undergoing a transient cell cycle arrest or apoptosis upon UV radiation-induced DNA damage.

We found several interesting novel UV-regulated genes, which are implicated to play a role in the DNA damage response and may have a role in UV-promoted tumorigenesis. Several of these transcripts represent previously identified genes, however, with unknown functions in the damage response. In addition, a number of UV-regulated targets are genes with unknown functions. Currently we are studying further three genes, namely CRP1, ISG15, and KIAA1100. Data will be presented to discuss roles of these proteins in the UV-response.

## RADIO-INDUCED DNA DAMAGE IN SKIN FIBROBLASTS TREATED BY THE COMBINATION OF PENTOXIFYLLINE AND $\alpha$ -TOCOPHEROL

Carine Laurent, Philippe Voisin and Jean-Pierre Pouget

If clinical symptoms induced by ionizing radiations (IR) on skin have been described in detail, the cellular and molecular mechanisms at the origin of these lesions and the kinetic of their appearance have not been clarified. It has been for long considered that radio-induced cutaneous late injury was only due to the delayed mitotic death of parenchymal or vascular cells explaining the lesions were progressive and inevitable. Moreover, recent studies have demonstrated an active role of these cells in response to IR exposure. Therefore, these cells appeared as interesting candidates for pharmacological treatments by directing on events that lead to late injury as production of ROS could be. Many works have thus focused on antioxidants therapy such as using the detoxification enzyme, superoxide dismutase (SOD), which was shown to induce a fast radio-induced skin fibrosis regression but could not be produced for a safe clinical treatment. Interestingly, the combination of pentoxifylline (PTX), antioxidant phytochemical, and  $\alpha$ -tocopherol ( $\alpha$ T), antioxidant nutrient, showed a similar effectiveness in reducing the late radio-induced skin damage with a longer treatment period. This work aims to investigate the molecular and cellular mechanisms involved in the effects of this combination. Primary cultures of dermal fibroblasts were gamma-irradiated at confluence and incubated in presence of PTX and Trolox (Tx), the water-soluble analogue of  $\alpha$ T, either before or after radiation exposure. Antioxidant capacity of drugs was assessed. Viability, survey, cell cycle distribution and ROS production were measured. DNA damage formation was assessed by the comet and micronuclei (MN) tests and 8-oxodGuo level was measured by HPLC-EC. The combination PTX/Tx was shown to reduce both the immediate and the late ROS productions observed after irradiation. DNA strand breaks and alkali-labile sites yield were decreased whenever the treatment was added. For the highest irradiation doses, an increase in the late production of MN and a decrease of clonogenicity values were observed when PTX/Tx was administered before or even 24 hours after irradiation although the G1-arrest observed after irradiation was not abrogate. In conclusion, PTX/Tx was shown to have an antioxidant effect which could not explain alone the observed DNA damage decrease since the treatment had the same effects whenever it was administered. Moreover, for the highest irradiation doses, the combination led to a decrease in survey that could be linked to the increase in MN frequency. This would suggest that PTX/Tx combination could interfere with DNA repair mechanisms.

## MOLECULAR DETECTION OF FIELD CANCERIZATION IN HEAD AND NECK CANCER PATIENTS

**Nongnit Laytragoon-Lewin, Juan Castro, Britt Nordlander, Jan Lundgren and Freddi Lewin**

**Objective:** Locoregional disease recurrence remains the dominant form of radiotherapy failure for patients with head and neck squamous cell carcinoma (HNSCC). Recent molecular studies have suggested that a tumour could be surrounded by a mucosal field consisting of genetically altered cells. The detection of pre-neoplastic lesion in HNSCC would be of the great value for treatment strategies.

**Material and methods:** Nine biopsies obtained from 3 head and neck cancer patients were used for our study. Three biopsies were from the tumour phenotype and 6 from morphologically normal tissue. Nuclear DNA content was analysed by cytometry. The cancer related gene expression profiling was analysed by cDNA microarray.

**Results:** All 3 biopsies with tumour phenotype consist of aneuploid DNA content. The 5 morphologically normal biopsies of these patients also showed aneuploid DNA pattern. The abnormal content of DNA in morphological normal tissue suggests a subclinical cancerization field outside the clinical evident tumours.

With cDNA microarray analysis, each biopsy has their unique pattern of gene expression when compared with their housekeeping gene in the same array filter.

**Conclusion:** Our investigation indicates that varies genetically altered cells might occur prior to appearance of morphological hyperplasia, dysplasia or malignant phenotype in HNSCC patients. Thus, diagnosis and treatment of epithelial cancers should not only be focused on the tumour but also from the theoretically primed mucosal tissue. Analyses of DNA content and gene expression profiling of normal mucosal field surrounded the tumour might open a possibility to select patients who benefit from more extensive combined radiotherapy rather than single mode treatment.

## NITRIC OXIDE GENERATED BY IONIZING RADIATION AND EGF IS IMPLICATED IN EGF RECEPTOR PHOSPHORYLATION IN A549 LUNG CARCINOMA CELLS

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**Objective:** Although it has been demonstrated that nitric oxide (NO) controls various cell functions in a different cell types, the mechanisms of its action are not well understood. In this present study, we investigated the possible role of NO in epidermal growth factor (EGF) signaling.

**Methods:** Serum-starved A549 human lung carcinoma cells were exposed to ionizing radiation (IR) and EGF. Intracellular concentration of NO was measured with Griess reagent, the NO-sensitive fluorescent probe, DAF-FM diacetate, and confocal microscopy. EGF receptor and its downstream signal activation were determined by immunoblot and immunoprecipitation.

**Results:** Treatment of A549 human lung carcinoma cells with ionizing radiation (IR) and EGF resulted in an increase in the intracellular concentration of NO. The scavenging of NO by 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) inhibited the IR- and EGF-induced generation of NO, EGF receptor phosphorylation at tyrosine 1173 site, Shc binding, a downstream of EGF receptor phosphorylation, and ERK1/2 activation. NO donor S-nitroso-N-acetylpenicillamine (SNAP), in itself, could induce EGF receptor dimerization and phosphorylation in A549 cells.

**Conclusion:** These results suggest that IR- and EGF-induced NO formation is required in the EGF receptor phosphorylation and its signal activation.

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## PET FROM THE ASPECT OF RADIATION PROTECTION

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The only PET diagnostic centre in Hungary and in Central–Europe as well, is working in Debrecen. The number of diagnostic cases has been increasing year by year. Production of radio–pharmacons also takes place here.

As compared to other isotope–diagnostic methods the appearance of annihilatic energy levels (PET) called attention to new radiohygienic aspects.

In PET diagnostic processes in each step characteristic dosis / dosis-rate can be detected.

Where radiation protection in PET diagnostic process can have special importance – among others – is connected with hand-dose.

## PET INVESTIGATIONS IN FULLY OR PARTIALLY REVERSIBLE RADIATION MYELOPATHY

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**Objective:** Tissue-level phenomena of patients with fully or partially reversible radiation myelopathy were investigated by positron emission tomography (PET).

**Methods:** PET studies have been carried out on 5 patients by measuring glucose metabolism (<sup>18</sup>F-fluorodeoxyglucose, FDG), amino-acid (<sup>11</sup>C-methionine) uptake and blood perfusion (using <sup>15</sup>O-butanol). Case 1 irradiated with a subthreshold biologically effective dose (BED) of 80 Gy<sub>2</sub> at  $\alpha/\beta=2$  Gy had no toxicity during the 4 years follow-up according to CTC Version 2.0. Case 2 had originally grade 2 (BED=94.8 Gy<sub>2</sub>), Case 3 (BED=88 Gy<sub>2</sub>) and 4 (BED=103.8 Gy<sub>2</sub>) grade 3, and Case 5 grade 4 (BED=165 Gy<sub>2</sub>) peak toxicity and they were investigated 6-25 years after radiotherapy. At that time, Cases 2-5 expressed some degree of reversibility with respect to the original clinical symptoms. To check whether increased individual radiosensitivity could contribute to the development of late effects, survival rates of the fibroblasts of the patients were compared in clonogenic assays with those of healthy controls.

**Results:** All patients exhibited temporarily (Case 1, for 4 years) or permanently (for 6-25 years) increased FDG accumulation indicating elevated levels of glucose metabolism in the irradiated spinal cord segments. Tissue perfusion positively correlated with FDG accumulation, excluding severe deterioration of microcirculation. The amino acid uptake was negligible in the irradiated spinal cord segments in all patients. Radiobiological investigations showed increased fibroblast radiosensitivity only in one patient (Case 4). Autopsy revealed extensive demyelination and only minimal vascular injury in Case 5.

**Conclusion:** The substantial FDG uptake indicated a need for extra energy which could not be attributed to cell proliferation on the basis of either the anamnestic data or the pathologic findings (Case 5). This conclusion was further supported by the low <sup>11</sup>C-methionine accumulation. We suggest an alternative conduction mechanism of increased energy demand brought about by the transitory (Case 1) or permanent (Cases 2-5) overexpression of Na<sup>+</sup>-channels along the demyelinated segments resulting in the restoration of axonal conduction. This restoration explains the improvement of the clinical symptoms. Our results document that severe spinal cord injuries can be induced by irradiation with sub- or near-threshold dose in patients with normal radiosensitivity.



## INFLAMMATORY EVENTS AFTER $\gamma$ -IRRADIATION: PIVOTAL ROLE OF PPARS

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*Objective:* Because irradiation can cause acute enteritis that leads to reduced motility and in a later phase to fibrosis, the small bowel is an important dose-limiting organ following radiotherapy. Pathologic changes may be caused by the early stage of an inflammatory process; we therefore studied the irradiation effect on the imbalance between pro and anti-inflammatory and apoptotic events. We focalized our attention on peroxisome proliferator-activated receptors (PPARs), which have been recently implicated as regulators of inflammatory responses.

*Methods:* PPARs, cytokines (TNF- $\alpha$ , IL-6, IFN- $\gamma$ , IL-10), neutrophil chemoattractant interleukin 8 (CINC), monocyte chemotactic protein-1 (MCP-1), anti apoptotic Bcl-2 and apoptotic Bax mRNA levels were analyzed by RT-PCR assay at 6 h and 3 days after 10-Gy  $\gamma$  whole body irradiation in the ileum mucosa of Wistar rats. Neutrophils infiltration was characterized by myeloperoxidase immunohistochemical assay and apoptotic cells were detected by labeling of DNA strand breaks (TUNNEL).

*Results:* The mRNA for cytokines TNF- $\alpha$ , IL-6 was significantly increased at 6h and persisted at 3 days post irradiation. The expression of IFN- $\gamma$  was significantly elevated (2.4 fold;  $p < 0.01$ ) at 3 days post irradiation. On the other hand, the anti-inflammatory cytokine IL-10 mRNA was markedly lower as early as 6h post irradiation. Analysis of PPARs implicated in the anti-inflammatory and anti-apoptotic events show a drastic decrease of the mRNA levels for PPAR $\alpha$  (80%) and PPAR $\gamma$  (63%) at 3 days post-irradiation. PPARs repression contributes to overexpressions of MCP-1 and CINC with a 6 fold ( $p < 0.005$ ) and 20 fold ( $p < 0.01$ ) increase respectively. CINC expression correlates with an increase of MPO-positive cells at 3 days post irradiation. TUNNEL assay showed an increasing number of apoptotic cells at the bottom of the villi at 6h. No or very few apoptotic cells were observed at day 3. Interestingly, anti-apoptotic Bcl-2 mRNA level was significantly increased (2.5 fold;  $p < 0.005$ ) whereas apoptotic Bax expression remained unaffected, at 3 days after irradiation.

*Conclusion:* Irradiation induced an early imbalance between pro-inflammatory (TNF- $\alpha$ , IL-6, IFN- $\gamma$ , CINC, MCP-1) and anti-inflammatory (IL-10, PPARs) mediators in the ileal mucosa. Infiltration of inflammatory cells is enhanced following irradiation. These molecular and cellular changes are correlating to an up regulation of an anti-apoptotic gene at day 3. Our work suggests that PPARs may be serving as key regulators in the irradiation-induced inflammatory response. Further investigation are undertake to confirm our hypothesis.

## EFFECT OF VARIOUS DOSES OF X-RAYS ON PRO-ANGIOGENIC PARAMETERS OF MURINE ENDOTHELIAL CELLS

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Recently, much attention has been paid to the possibility of thwarting cancer progression by inhibition of angiogenesis in the growing tumours. However, even though general mechanisms of this process have been elucidated, virtually nothing is known about the effects of ionising radiation on the pro-angiogenic characteristics of endothelial cells.

Thus, the aim of the present study was to compare the effects of low (0.2 Gy), intermediate (1.0 Gy) and high (4.0 Gy) doses of X rays on the selected angiogenesis-related parameters of isolated murine pulmonary endothelial cells. The results indicate that 24 to 48 hours after the irradiation with 0.2 Gy cell proliferation was inhibited to a higher extent than following exposure to 1.0 Gy X-rays. This effect was accompanied by the increased number of apoptotic/necrotic cells at 48 hours after the irradiations (250, 140, and 150% of the control value for exposures to 0.2, 1.0 and 4.0 Gy, respectively). Results of the colony formation assay suggested the existence of “hyperradiosensitivity” of the endothelial cells in the low-dose region (0.2-0.5 Gy). Additionally, although adhesion of the cells to both gelatin and plastic was disturbed by all the exposures, the most pronounced effect was detected two hours after the irradiation with 0.2 Gy. Finally, during the first hours after the exposures to all doses of X-rays a transient down-regulation of the surface expression of beta3 integrin subunit (one of the most important pro-angiogenic adhesion molecules) was observed. These results indicate that proliferating endothelial cells are sensitive in vitro to the relatively low, non-cytotoxic doses of ionising radiation. This sensitivity might appear useful for designing new anti-neoplastic therapies aimed at reduction of the formation of new blood vessels in the growing tumors without significantly affecting the viability and function of the surrounding healthy tissues.

## INCREASED FREQUENCY OF RADIATION-INDUCED CHROMOSOME ABERRATIONS IN PERIPHERAL BLOOD LYMPHOCYTES OF PATIENTS WITH LARYNX CANCER

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There is data suggesting that the sensitivity to ionising radiation of peripheral blood lymphocytes of cancer patients is higher than in healthy donors. This effect is especially prominent when chromosomal aberrations induced in S/G<sub>2</sub> phase of the cell cycle are analysed. The aim of our study was to investigate if the S/G<sub>2</sub>- aberration frequencies in lymphocytes of patients with larynx cancer were higher than in control individuals. In addition, the multiple fixation regimen was applied in lymphocytes of the cancer patients. The aim of this was to check if the aberration frequencies scored in cells harvested at one time point were representative for a larger fraction of the cell cycle.

Peripheral blood of 31 patients was collected before the onset of radiotherapy, cultured and irradiated with Co-60 after 67 hours of culture time. Irradiation was performed in the Świętokrzyskie Oncology Center which is located in a different part of Kielce. Therefore, blood cultures were transported to and from the Center and irradiated on ice. Chromosome specimens were prepared from cells fixed at three time points after irradiation: 5, 7 and 9 hours. Colcemid was always added for 2 hours before harvest. Lymphocytes of 28 healthy donors were cultured and irradiated in the same way like in the case of patients with cancer, however, they were only harvested at one time point (5 hours p.r.).

No statistically significant differences in aberration frequencies were observed between lymphocytes harvested at the 3 time points. In both donor groups, individual differences in aberration frequencies were observed. Despite this, the aberration frequencies in lymphocytes of patients were in average higher than in the healthy donors. This suggests, that the radiation sensitivity of lymphocytes of patients with larynx cancer may be a marker of cancer predisposition. More patients must be analysed to confirm this hypothesis.

## INHIBITION OF 12-LOX AND IN VITRO RADIOSENSITIVITY OF HUMAN PROSTATE CANCER CELL LINES

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**Objectives:** Products of 12-lipoxygenase (LOX) metabolic pathway act as survival factors for prostate cancer in stress situations like serum retrieval. Radiation is also a considerable stress to cancer cells. In this study we examined how whether 12-LOX inhibition modifies the radiation sensitivity of human prostate cancer cell lines.

**Methods:** Proliferation, clonogenic survival and apoptosis of human prostate cancer cell lines (LnCaP, DU-145 and PC3) were examined following exposure to different doses of radiation, various concentrations of 12-LOX inhibitors Baicalein and BHPP and 5-LOX inhibitor Rev-5901. Combination treatments with LOX inhibitors and radiation was performed. Add-back experiments with 5-, 12- and 15-HETE were carried out too.

**Results:** 12-LOX inhibition sensitizes certain prostate cancer cell lines to radiation in vitro. Add-back of 12-HETE suspends the effect of 12-LOX inhibition while 5- and 15-HETE have no effect. Effect of radiation on proliferative capacity was not affected significantly while apoptosis was modified by the various LOX inhibitor treatments.

**Conclusion:** Metabolites of 12-LOX pathway are survival factors for prostate cancer cell lines, receiving radiation. 12-LOX inhibitors may have radiosensitizing properties, especially with tumors with excessive 12-LOX expression. Since 12-LOX expression is uniform in various forms of epithelial malignancies, in vivo studies are urgently needed to analyze the radiosensitizing effect of 12-LOX inhibition.

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## ANTIANGIOGENIC EFFECTS OF RADIOTHERAPY PREDICTS SURVIVAL OF INOPERABLE HEAD AND NECK SQUAMOUS CELL CARCINOMA

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*Objective:* To evaluate the predictive value of intratumoral microvessel density (MVD) in patients with locally advanced primarily inoperable head and neck cancer (HNC).

*Methods:* Tumor samples of 55 patients with HNC treated with radiotherapy were taken with punch biopsy before and after 20 Gy irradiation. Histological grade, mitotic activity index and MVD of HNC were determined. Correlations with response and survival were analyzed.

*Results:* With a median follow up of five years we found that, stage, low post-treatment MVD and decrease in MVD following 20 Gy irradiation of HNC (>50%) significantly affected objective response. Overall and progression free survival was significantly influenced by response to irradiation, and therapy induced decrease in tumor-MVD or low postirradiation MVD.

*Conclusions:* We have shown that the anti-angiogenic effect measured in the form of decrease in MVD of irradiation is an indicator of the success of radiotherapy and may serve as a predictive factor thus helps to select patients who may benefit from more aggressive treatment.

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## PROGNOSTIC VALUE OF LYMPHOCYTES IN CERVICAL CARCINOMA

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**Objective:** The aim of our study was to test whether DNA damage in peripheral blood lymphocytes may be a factor predicting development of cancer and results of anticancer treatment.

**Methods:** The tested group consisted of 56 patients with cervical cancer in clinical stage IIB- IIIB and the control group of 38 healthy women. Lymphocytes were isolated from fresh blood, taken from patients before treatment (radiotherapy or radiochemotherapy). DNA damage and its repair were measured after irradiation with 2Gy of  $\gamma$  rays using the alkaline comet test. Lymphocyte subpopulations were detected by monoclonal antibodies and flow cytometry.

**Results:** Lymphocytes of patients with cancer presented a higher level of background DNA damage ( $p < 0.00001$ ), increased irradiation induced damage ( $p < 0.00005$ ) and reduced repair capacity ( $p < 0.00001$ ) in comparison with healthy donors. In both groups broad interindividual differences in DNA repair kinetics were observed.

Blood of patients and normal controls contained comparable numbers of T, B, CD4+, CD8+ and NK lymphocytes. Testing the relationship between the distribution of subpopulations and DNA repair kinetics we found that patients who were characterized by more efficient repair in lymphocytes (lower level of residual DNA damage after 180 min of repair) had a higher number of T, CD4+ lymphocytes and a lower number of NK cells than patients with less efficient DNA repair ( $p = 0.021$ ,  $0.021$ ,  $0.005$  respectively).

The data obtained by the comet test were compared with clinical results of treatment. A higher efficiency of DNA repair in lymphocytes was characteristic for patients with faster regression of tumour. About 92.9% of persons whose lymphocytes repaired DNA damage better exhibited complete or almost complete reduction of tumour mass after termination of radiotherapy. A faster regression of tumour was characteristic for patients with a higher number of T lymphocytes ( $p = 0.013$ ) and a lower number of NK cells ( $p = 0.017$ ). This suggests that lymphocytes, which repair DNA damage more efficiently probably better survive genotoxic effect of treatment and participate in tumour elimination.

**Conclusions:** DNA repair capacity of lymphocytes may be an important factor in determining susceptibility to cancer and response to cancer therapy. Differences in response of lymphocytes to genotoxic factors may result from different sensitivity of their subpopulations.

## COMBINED TREATMENT OF MURINE GLIOMAS WITH RADIOTHERAPY, RADIOSENSITIZING AND CHEMOSENSITIZING GENE THERAPY

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**Objectives:** The aim of this work was to improve tumor radiosensitivity by increasing the intratumoral activation of gemcitabine. Our hypothesis was that by increasing the deoxycytidine kinase (dCK) enzyme level that activates gemcitabine within the cells will lead to an increased Gemcitabine activation, which could improve the efficacy of radiotherapy.

**Methods:** We used the murine glioma 261 (Gl261) tumor model. The dCK gene was cloned in an adenovirus vector (Ad-dCK). The level of dCK enzyme in the virus infected cells was measured. For the in vitro proliferation assays cells were transduced with Ad-dCK at different multiplicities of infection (MOI) 24 hours after seeding, treated with Gemcitabine 24 hours after virus infection and irradiated with 4 Gy 24 hours after Gemcitabine treatment. Cell number was determined one week after seeding. For the in vivo experiments subcutaneous tumors were established in C57BL/6 mice. One week later Ad-dCK was injected in the palpable tumor, followed by intraperitoneal injection of Gemcitabine 24 hours later and local irradiation of the tumors 24 hours after Gemcitabine treatment. This treatment schedule was repeated again one week later. Tumor growth and survival of animals were followed.

**Results:** The dCK enzyme activity levels in vitro in the Ad-dCK transduced Gl261 cells at 20 MOI were 4 fold and at 100 MOI were 7 fold higher than in uninfected cells. Virus infection at 100 MOI was toxic to cells. Gemcitabine at 5 nM produced 85% survival rate, but 10 nM was toxic. Irradiation resulted in 40% survival rate. Irradiation and Gemcitabine treatment led to 25% survival rate. The combined Ad-dCK infection and Gemcitabine treatment resulted in 12% survival rate. The combination of Ad-dCK and Gemcitabine treatment and 4 Gy X-irradiation resulted in 4% survival rate. The animal experiments show, that Ad-dCK treatment alone has no therapeutic effect. Gemcitabine treatment or irradiation has a tumor growth retardation effect. Combining Gemcitabine treatment with irradiation or Ad-dCK treatment with irradiation or Gemcitabine and Ad-dCK treatment and irradiation resulted in a significant tumor growth retardation and a prolonged survival compared to non-treated animals.

**Conclusions:** Both Ad-dCK gene therapy and Gemcitabine therapy can increase the radiosensitivity of murine gliomas. The radiosensitizing effect of Ad-dCK was seen in vitro, and in vivo, as well.

## ESTABLISHING A TREATMENT PROTOCOL FOR SEVERELY IRRADIATED PERSONNEL IN THE RADIATION ACCIDENT SCENARIO: THE EVOLVING PRECLINICAL DATA BASE.

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Several prevailing themes are present throughout the radiation accident scenario. Namely, the radiation environment is likely to be ill-defined and uncontrolled. Also, the exposure may be nonuniform, partial-body, and of variable dose rate and exposure time. The time interval between exposure and treatment is usually less than optimal and it is difficult to establish accurate absorbed dose. The only reasonable aspect of the radiation exposure is that its uncontrolled and ill-defined nature will forecast a variable dose distribution and possible sparing of hematopoietic stem cells.

The definition of an effective treatment strategy for radiation-induced myelosuppression will depend on the aforementioned conditions. We propose that there is only one treatment strategy available for irradiated personnel. It is available now and focuses on the prevention of radiation-induced neutropenia and infection. Its two components are aggressive supportive care and administration of recombinant cytokines as soon as possible after radiation exposure and triage.

We support our proposal with a consistent and substantial data base in preclinical, large animal (canine and rhesus monkey) models of severe radiation-induced myelosuppression. The data base underscores the efficacy of supportive care and recombinant cytokines in enhancing the recovery of myelopoiesis, as well as increasing survival after lethal doses of acute, total-body irradiation.

This data base is concordant with requirements under the FDA Rule for approval of new drugs and biological products when efficacy studies in humans are not ethically feasible. In this regard, there is a reasonably well understood mechanism of radiation effects and prevention. The treatment effects are substantiated in two relevant species and the response is predictive for humans. New directions in growth factor therapies will also be discussed.



## AN ADAPTIVE RESPONSE IN UNIRRADIATED HPV-G CELLS INITIATED BY A BYSTANDER SIGNALING MECHANISM.

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It has been shown in recent years that in both *in vivo* and *in vitro* irradiation of cells give rise to signaling mechanisms, which affect unirradiated cells. This process is known as “the bystander effect”. The presently unknown factor causes chromosomal aberrations, initiation of apoptosis and reduced clonogenic survival. Using a medium transfer method to study the adaptive response, this study investigated the effect of two exposures to bystander medium, (generated by a low dose followed by a higher dose) to investigate if the first dose makes the cells more resistant to the second exposure to bystander medium. Experiments have shown that when cells are exposed to low level of actual irradiation and then to a higher dose there is a reduction in chromosomal aberrations and a reduction in micronuclei and sister chromatid exchanges. Medium from irradiated (0.5Gy to 5Gy) human HPV-G keratinocytes was harvested one hour after irradiation, sterile filtered and transferred on to unirradiated HPV-G cells. HPV-G cells were exposed to 0.5Gy bystander medium for 24 hours and then exposed to bystander medium obtained from cells exposed to 5 Gy. The medium exposed cells were assayed for apoptotic markers and signs of cellular responses such as a calcium flux, mitochondrial membrane permeability alterations, cytochrome c release, increase in mitochondrial numbers, changes in distribution of the mitochondria, bcl-2 expression, caspase 2, 8, 3 activity and apoptosis / necrosis.

Data indicate that exposure to two doses of bystander medium can activate a response in HPV-G cells that is not as great as that following a single exposure. It has also been observed that this adaptive response follows a very non linear pattern with some cells responding and some not. This is in contrast to the all or nothing response observed with one exposure to bystander medium.

This may help clarify how cells sector to death or survival following receipt of a radiation borne signal.

## TRANSITION METALS COMPLEXES OF N-SUBSTITUTED AMINO ACID SCHIFF BASES: ANTIOXIDANT, CYTOGENETIC AND RADIOPROTECTIVE ACTIVITY

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**Objective:** The research was aimed to study the newly synthesized complexes of Mn(II), Zn(II), Co(II), Fe(III) with ethyl esters of nicotinoil -tyrosine, -tryptophane and -GABA for probable antioxidant and radioprotective properties.

**Methods:** Antioxidant (AO) activity of the synthesized compounds was evaluated with the help of a chemiluminescent analyzer and appropriate ACL-kit according to their inhibitory effect on luminescence generation.

The primary decision about the possible radioprotective capacity of the metalocomplexes was made on the basis of cytogenetic indices of bone marrow cells (BMCs) of white non-linear rats. Animals were sacrificed 24 hours post single administration of the compounds studied at 10 mg/kg and the BMCs preparations were obtained. The frequency of non-stable chromosome aberrations (ChA) and the proliferative activity (PA) of BMCs were evaluated.

The radioprotective activity of the substances was figured out by determination of animal survival 30 day post radiation exposure performed on the background of preliminary administration of 20 mg/kg tested substances to animals 1 hour prior to X-irradiation at LD<sub>100/30</sub> (7.2 Gy).

**Results:** Amongst the complexes studied, Co(II) complex with ethyl ester of nicotinoil-tyrosine and Zn(II) complex with ethyl ester of nicotinoil-tryptophane revealed high AO activity. Generally all compounds caused an expressed increase of the spontaneous level of ChA and a significant inhibition of PA of BMCs: in some cases 10-fold. However, complexes of Mn(II) and Zn(II) with ethyl ester of nicotinoil- tryptophane and Co(II) with ethyl ester of nicotinoil –tyrosine made some exception: on the background of these compounds ChA of BMCs increased only 2 – 2.5 times, while the PA was extremely stimulated. On the background of these compounds, the number of animals surviving 30 days after radiation exposure reached up to 60%.

**Conclusion:** Transition metals complexes with ethyl esters of nicotinoil -tyrosine and -tryptophane exhibit expressed radioprotective properties. The cytogenetic and AO activities might be essential for radioprotective action exerted.

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## HAIR-CYCLE-DEPENDENT SUSCEPTIBILITY TO IONISING RADIATION INDUCED BASAL CELL CARCINOMA IN PATCHED HETEROZYGOUS KNOCK-OUT MICE

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**Objectives:** Basal cell carcinoma (BCC) is the most common skin cancer, accounting for about 70% of all skin malignancies. BCCs show frequent mutations in the tumor suppressor gene *Patched* (*Ptch1*) and a strong correlation with UV and ionising radiation (IR) exposure. We developed a model of BCC induction by IR, based upon *patched* (*ptch1*) gene knock-out mice, which develop a high incidence of BCC-like tumors in response to a single dose of IR. Previous data showing that induction of infiltrative BCC by ionising radiation is dependent upon the mouse age at irradiation suggest that susceptibility correlates with the hair growth cycle, and with the proliferative state of hair precursor cells. The main objective of this study was to investigate further age-related responsiveness to IR-induced BCC tumorigenesis. Our analysis was extended to microscopic BCC precursor lesions, *i.e.*, basaloid hyperproliferation areas arising from both follicular and interfollicular epithelium, and nodular BCCs. **Methods:** *Ptch1*<sup>neo6-7/+</sup> mice were irradiated with a single dose of 3 Gy of X rays at different ages, corresponding to different phases of the hair growth cycle. Ages at irradiation were 35 and 60 days, corresponding to G2 growth phase and R2 resting phase, respectively. The relative time of appearance of microscopic skin lesions (*i.e.*, basaloid hyperproliferation areas and nodular BCCs) was determined in a retrospective manner in tissue samples collected from sacrificed mice. For a better characterization of BCC incidence, mice were subdivided into three groups (*i.e.*, 15-30, 30-45 and 45-60 weeks) depending on the time elapsed from radiation exposure and mouse sacrifice. **Results and Conclusion:** We observed a higher nodular BCC yield after irradiation in active hair growth phase (G2) compared to resting phase (R2). *Ptch1*<sup>neo6-7/+</sup> mice irradiated in G2 showed earlier occurrence of nodular BCC (10% at 15-30 weeks) compared to mice irradiated in R2 that only developed such lesions starting from 32 weeks post-irradiation. Mice irradiated in G2 showed a constantly higher incidence of nodular BCC compared to mice irradiated in R2, *i.e.*, a 1.8-fold and a 1.5-fold incidence at 30-45 and 45-60 weeks, respectively. These findings were consistent with the results of tumor multiplicity analysis. These observations suggest the existence of age-related windows of susceptibility within which the epithelium is more competent to develop basal cell tumors, and that these windows are related to the Shh-dependent proliferative state of hair progenitor cells at the time of irradiation. (Partially supported by the Radiation Protection Action of the EC, contract FI6R-CT-2003-508842)

## NO EFFECT OF MODELLED MICROGRAVITY ON THE CHROMOSOME ABERRATION DOSE-RESPONSE CURVE FOLLOWING LOW-LET IRRADIATION

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**Objective:** To study the influence of simulated microgravity on repair of radiation-induced damage

**Methods:** G<sub>0</sub> human lymphocytes were irradiated with 60 MeV protons and allowed to repair DNA damage for 24 h under either normal gravity or microgravity modelled by means of the NASA-designed rotating-wall bioreactor. For comparison, X-ray irradiation was performed. After phytoemagglutinin-induced stimulation for 48 h under normal-gravity conditions, chemically condensed chromosomes were harvested and radiation-induced structural aberrations were analysed by fluorescence *in situ* hybridisation of chromosomes 1 and 2. Cell growth curves, bromodeoxyuridine labelling and morphological evaluation of blastogenesis were used to assess gravisensitivity of proliferation kinetics.

**Results:** The response to mitogen was profoundly depressed by microgravity but all aberration dose-response curves measured under normal gravity or microgravity conditions were identical within experimental errors.

**Conclusions:** The yield of radiation damage in the form of chromosome aberrations is not modified by bioreactor-based microgravity, which did reproduce space-related inhibition of mitogen stimulation in human lymphocytes.

## RESIDUAL 53BP1, $\gamma$ -H2AX FOCI ASSAY FOR BIOLOGICAL DOSIMETRY

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We have suggested and evaluated a novel residual foci assay for radiosensitivity that is based on measurements of DSBs co-localizing proteins such as 53BP1 and  $\gamma$ -H2AX (1-3). Here, we studied possibility to use residual foci assay for biological dosimetry. Kinetics of radiation-induced 53BP1,  $\gamma$ -H2AX foci were studied in human G0-lymphocytes 24 and 96 h, 1, 2, and 4 weeks following irradiation with  $\gamma$ -rays at doses 0.5-10 Gy. Similar to our previous results (2), linear dose dependence was observed for 53BP1 foci and also  $\gamma$ -H2AX foci 24 h following irradiation. At all later time points, the saturation in dose response for 53BP1 foci and  $\gamma$ -H2AX was seen beginning with dose 3 Gy. The number of foci normalized per cell and per Gy was significantly lower at higher doses. These effects are attributed to either dose-dependent clustering of residual foci (1) to apoptotic elimination of lymphocytes containing higher amount of foci. The linear dose response up to 2 Gy was very similar between all time points. The co-localization of residual 53BP1 and  $\gamma$ -H2AX foci did not exceed 15%. The results obtained suggest that the 53BP1/ $\gamma$ -H2AX residual foci assay may be used for biological dosimetry at least 24 h – 4 weeks post-irradiation.

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## MICROWAVES FROM MOBILE PHONE AFFECT 53BP1/ $\gamma$ -H2AX FOCI IN HUMAN LYMPHOCYTES DEPENDENT ON CARRIER FREQUENCY

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It has been shown that non-thermal effects of microwaves (MWs) occur dependent on physical parameters including frequency (*f*). Here, we investigated effects of MWs of Global System for Mobile Communication (GSM) at different carrier frequencies, 905 and 915 MHz, on human lymphocytes from 5 healthy persons and from 5 persons reporting hypersensitivity to MWs. The exposure, 1 h, was performed using a GSM test-mobile phone, specific absorbed rate (SAR) being 37 mW/kg. The changes in chromatin condensation were measured by the method of anomalous viscosity time dependencies (AVTD). Statistically significant changes in condensation of chromatin were observed at 915 MHz in cells of 6 from 10 persons. Similar variability was seen after mild heat shock. MWs at 905 MHz resulted in significant AVTD changes in cells from 2 donors. 53BP1 and  $\gamma$ -H2AX proteins, which co-localize in distinct foci with DNA double strand breaks (DSBs), were analysed by confocal laser microscopy. The exposure at 915 MHz, similar to heat shock at 41-43°C, induced statistically significant decrease in 53BP1 and  $\gamma$ -H2AX foci in cells from all persons. In contrast, exposure to 905 MHz induced either decrease or increase in 53BP1 and  $\gamma$ -H2AX foci. The majority of 53BP1 and  $\gamma$ -H2AX did not co-localize. Based on pooled data, the effects of MWs at 905 and 915 MHz on 53BP1 and  $\gamma$ -H2AX foci were statistically significantly different. For the first time, we report that GSM MWs affect 53BP1/ $\gamma$ -H2AX foci dependent on carrier frequency.

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## HUMAN EPIDERMIS STEM CELLS AND RADIATION DAMAGE

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Epidermis can be damaged as an acute effect of radiation. During the course of radiotherapy, some radiosensitive patients may develop radiation burns, reflecting the death of differentiated keratinocytes, basal cells and stem cells. It is thought that surrounding stem cells and deep stem cells from hair follicles permit the re-epithelisation. Years after radiotherapy, late complications may develop in skin, including atrophies, necrosis and hyperplasia. The fate of stem cells in these irradiated tissues is completely unknown. Do atrophy and necrosis result from a lack of stem cells? Do hyperplasia result from increased number of stem cells or deregulation of the activity of these cells? What could be the use of stem cell grafting to reconstitute such tissues ? To answer these questions, research in basic science is necessary. Since isolation of epidermis stem cells was so far difficult, these cells have been poorly described and the lack of molecular markers hampered until recently real progress. Methods can now be proposed to isolate cell populations enriched in progenitors from human epidermis, allowing their molecular characterisation and the study of their grafting potential. In our group, normal keratinocytes are isolated from adult mammary skin and foreskin. Two sorting methods are currently used. Progenitors are directly isolated from human skin using membrane markers and flow cytometry (MM cells). These membrane markers are both classical published proteins and new markers that we isolated by proteomic studies. In primary cultures of keratinocytes, the Hoechst labelling method permits to select cell populations enriched in progenitors (SP cells). Both MM and SP keratinocytes exhibit an increased growth potential in tissue culture as compared to the initial cell populations and a higher clonal potential. The molecular signature for SP cells has been searched using the microarray technology. Grafting adult human stem cells to reconstitute tissues severely damaged by radiotherapy or radiation accidents can be now tested in animal models. We assess the potential of human stem cells to reconstitute irradiated skin tissue in NOD/SCID mice. Bone marrow mesenchymal stem cells have been shown to be multipotent cells and to be able to engraft in many tissues after injury. These cells have been i.v. injected in irradiated mice to test their capacity to home to the irradiated skin tissue. Similarly, cell populations enriched in keratinocyte stem cells will be i.v. injected in irradiated NOD/SCID to test their potential to home to the damaged skin and to other mouse tissues. A successful replacement of stem cells in irradiated normal tissues may open the road to completely new strategies in radiotherapy and help combating cancer.

## PROTEOLYTIC CLEAVAGE OF CYCLIN E DURING APOPTOSIS REVEALS A P18-KD DERIVATIVE FRAGMENT THAT BINDS TO KU70 AND RELEASES BAX TO INDUCE APOPTOSIS

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Cyclin E (CycE)/Cdk2 is a critical regulator of cell cycle progression from G1 to S phase in mammalian cells and has an established role in oncogenesis. Examination of the role of deregulated CycE expression in apoptosis following ionizing radiation (IR), revealed a substantial increase in p50-CycE protein in multiple tumor cell types. Particularly in hematopoietic cells, the levels of p50-CycE initially increased (Mazumder et al, Oncogene 2000), followed by a decrease starting at 8 h following treatment with genotoxic stress agents, such as IR, coinciding with a time-dependent expression of a novel p18-CycE (Mazumder et al, MCB 2002). Moreover, caspase-mediated Cyclin E cleavage abrogated its interaction with Cdk2 and therefore inactivated the associated kinase activity. Overexpression of p18-CycE but not of several other CycE mutants, specifically induced all the apoptosis-associated processes examined. These and generation of p18-Cyclin E were significantly inhibited by overexpressing a cleavage-resistant CycE mutant. These data indicate that CycE has a dual role in apoptosis induced by genotoxic stress. CycE activation as an early response is likely to play a critical role in the initiation of cell death, with a distinct role for p18-CycE in the amplification of the apoptotic process. Since p18-CycE no longer interacts with Cdk2, it is likely that it induces amplification of apoptosis by interacting with novel partners. Yeast 2-hybrid screening, mass-spec analyses, and pull-down assays have identified Ku-70, a critical component of DNA repair (NHEJ), as a p18-Cyclin E-interacting protein. None of the p18-CycE-interacting proteins we have identified are unable to interact with p50-CycE. This suggests that p18-CycE acquired an ability to associate with new partners which may translate into a novel function. IR or p18-CycE expression greatly inhibited NHEJ, as well as the interaction of Ku70 with Ku80. A Ku70 domain (amino acids 219-235) was found to be critical for in vitro p18CycE binding. The Ku70 domain that interacts with p18CycE is distinct from the one reported for Bax. Importantly, interaction of p18CycE or irradiation led to release of Bax from the Ku70 complex its activation and subsequent apoptosis.

The interaction between p18-CycE and Ku70 in B and T cells, which have high NHEJ activity, could be an important mechanism for a full execution of the apoptotic program by activating apoptosis while preventing any chromosomal translocations which may lead to generation of neoplastic cells that could thus escape cell death. Our findings identify CycE as a critical regulator of cell cycle, DNA repair, and apoptosis activation following cellular genotoxic stress.



## DIFFERENT MOLECULAR PATHWAYS FOR APOPTOSIS IN CELLS EXPOSED TO HIGH AND LOW LET RADIATION

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There are several advantages in using high LET charged particles in radiation therapy. One is the achieved increase of RBE for cell-killing. In addition it has been shown that accelerated charged particles not only induce a faster apoptotic response than low LET photons, but also that this process can occur independently of the p53, ATM (in ataxia telangiectasia mutated) and DNA-dependent protein kinase (DNA-PK) status of the cell. This is important since approximately 50% of all tumours have mutated p53, and since p53, ATM and DNA-PK are important 'safeguards' in recognition and restoration of DNA damage. In previous studies we also observed that the apoptotic course differs between high and low LET. Following high LET irradiation we have seen a biphasic apoptotic response, i.e. a pre- and a post-mitotic. This seems to be a general phenomenon for high LET irradiation in cells with different origin and gene status. One putative candidate that may be involved in these apoptotic responses is ceramide, a sphingolipid derived second messenger which expression has been shown to increase in a time-dependent pattern in lymphoblastoid cells exposed to low LET radiation.

To explore whether ceramide and sphingomyelinases (SMase) are involved in the biphasic apoptotic response observed following high LET irradiation (accelerated boron and nitrogen ions), we determined the activities of neutral SMase (NSMase) using the Amplex Red kit from Molecular Probes, and of the acidic SMase (ASMase). Ceramide endogenous levels were determined by HPLC in tumour cells of different origins and displaying defined gene status. mRNA differential display studies were also performed in an attempt to shed new light on the molecular pathways that may be involved in the cell fate reported herein.

Previous results show that both M059K and M059J glioma cell lines, that either express or not the catalytic subunit of DNA-PK (DNA-PKcs), display an early (1-10 h) increase in NSMase activity following exposure to nitrogen ions (140 eV/nm, and that it correlates with induction of apoptosis at that times. A biphasic pattern was observed in M059J within 48 h post-irradiation while a similar phenomenon occurred within 72 h in M059K. Upon exposure to low LET radiation, only M059J cells displayed this enhanced activity that was sustained for 1-10 h before it decreased at 24 h. A secondary increase was observed 48-72 h post-irradiation. This cell response was much more sustained but to a lower extent when compared to the one reported after high LET irradiation. The activity of ASMase and the expression of ceramide levels are under investigation.

## CHANGE OF CARDIOVASCULAR STATUS OF PATIENTS WITH ACUTE RADIATION DISEASE AND CHERNOBYL LIQUIDATORS

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The purpose of the study was to assess the condition of health in patients with acute radiation disease and personnel of the Chernobyl Nuclear Power Plant after the 1986 accident in dynamic (1986-1997) at Clinic of Institute of Biophysics.

The cardiovascular system function for 511 patients has been analyzed. Patients were investigated with ECG, echocardiography, ECG monitoring.

High percent of arterial hypertension (44,2%), arterial hypotension (38,3%), disturbance of cardiac rhythm and conduction in patients with acute radiation disease were detected earlier for six – eight year. Frequency of an arterial hypertensia at liquidators I, II and III groups exceeded 40 % a level revealed at separate contingents of adult population of Russia in the age of than 30 years (45,2 % are more senior; 43,4 %; 41,1 %). In a basis of occurrence of an arterial hypertensia and a hypotension at patients of group OLB, and also arrhythmies hearts, it is especial in the age of 20-30 years and 31-40 years the stress from an emergency and vegetative-endocrinosis of an exchange from the transferred sharp radiation sickness and local radiation injuries, at patients I, II and III groups - stress from postemergency factors.

The data obtained are of great importance for young persons involved in clean-up activities of the Chernobyl accident.

## EXTREMELY LOW FREQUENCY MAGNETIC FIELD EXPOSURE AND CHILDHOOD LEUKEMIA – A REVIEW OF EPIDEMIOLOGIC STUDIES

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In 2001, the International Agency for Research on Cancer (IARC) classified exposure to extremely low frequency magnetic fields as possibly carcinogenic to humans (Group 2B). This classification was primarily based on epidemiologic evidence linking childhood leukemia to residential exposure to power frequency magnetic fields. The evidence has been deemed insufficient to establish an association between magnetic field exposure and other types of childhood or adult cancers or non-cancer health outcomes.

Since 1979, dozens of epidemiologic studies have been conducted to examine a potential association between childhood leukemia and magnetic field exposure. In my presentation, I will review the evolution of the epidemiologic evidence, describe the most influential epidemiologic studies, and discuss how exposure assessment developed, and how interpretation changed over past 25 years. The epidemiologic association between childhood leukemia and magnetic field exposure, however, remains difficult to interpret without clear supporting evidence from experimental laboratory work and without known biophysical mechanism. Possible interpretations and potential future research directions will also be discussed.

## RETROSPECTIVE CYTOGENETIC ANALYSIS OF URANIUM MINERS

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In our examination we aimed to detect the radiation burden of radon exposed miner population. The analyses were performed from blood samples of 165 active underground uranium miners between 1981 and 1985. After decommissioning the mine in 1997 aberration analyses were also included into the medical laboratory investigations on health conditions of 141 subjects between 1998 and 2002 within the framework of a follow-up-study. For the detection of cytogenetic damages different biological dosimetric methods were used, namely lymphocyte micronucleus frequency assay and chromosomal aberration analysis.

Additionally measurements of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and total antioxidant status (TAS) values were also performed.

The frequencies of the cytogenetic aberrations were higher than in the unexposed population, and ascended parallelly with the increase of exposure. These values remained higher than the average for 15-20 years after the ceasing of the underground work, although slight decreases could be observed with time. By the measurement of the TNF- $\alpha$  values, it is ascertainable that the TNF- $\alpha$  as a clastogenic agent may contribute to the long-lasting existence of the cytogenetic damages. The TAS values in sera showed reduced protective antioxidant ability.

On the basis of the cytogenetic parameters it is observable, that in the former uranium miners the previously increased risks persist even after years, and the possibility is suggested that for the long-term persistence of cytogenetic alterations the permanent production and presence of clastogenic factors might be responsible accompanied with lower protection.

## CANADIAN CYTOGENETIC EMERGENCY NETWORK (CEN) FOR BIOLOGICAL DOSIMETRY FOLLOWING RADIOLOGICAL/NUCLEAR ACCIDENTS

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**Objectives:** We are developing a network of laboratories across Canada to provide the capacity for rapid biological dose estimates using the dicentric chromosome assay (DCA) for emergency response. The DCA uses the frequency of dicentric and ring chromosomes to estimate the radiation dose.

**Methods:** In a major emergency situation, even with the combined capacity of the four core labs (HC, DRDC, AECL and McIARS), only a limited biological dosimetry service would be available due to limitations in both equipment and expertise. Therefore, to increase Canada's response capacity, we are expanding the network to include cytogenetic laboratories across the country.

A workshop on biological dosimetry was held in May 2004 in Toronto, which was attended by directors of interested cytogenetic laboratories. Blinded slides, prepared for DCA analysis following *in vitro* irradiation of blood from a healthy volunteer to a range of gamma-ray doses, were distributed to the workshop participants and to the four core laboratories. Fifty metaphases will be analysed per slide, to mimic triage scoring, and dose estimates will be calculated. Results from all laboratories will be collated and analysed. Following this initial scoring exercise, interested laboratories will receive slides on an ongoing basis to maintain their expertise in the DCA and to ensure their readiness to respond to local and national radiological/nuclear emergencies.

**Results:** Preliminary results in our laboratory show that analyses of 50 metaphases follow the dose response curve established in our laboratory. We expect to have preliminary data from the workshop participants and core laboratories.

**Conclusions:** When this network is formed and operational, Canada will be better prepared to provide triage quality biological dosimetry in response to radiological/nuclear emergencies.

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## FUNCTIONAL ROLE OF THE CHANGES IN THE NUCLEIC COMPONENT COMPOSITIONS DURING SEEDS GERMINATION.

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Plants have a complex battery of genes encoding putative DNA methyltransferases, which perhaps play a role in the methylation of heterochromatin. Our investigation have been shown changes in some physicochemical characteristics of cereal seeds DNA and chromatin during genome activation. We have suggested that the high methylated region of repeated DNA commonly lies in heterochromatin region adjacent to the nuclear envelope (NE).

Recent studies have also shown that tethering a boundary between a gene and surrounding silenced heterochromatin. This is support that the NPC through association with the underlying chromatin regulates gene expression. Particularly have been obtained the changes in the RNA, protein and in phospholipid content of the NE, chromatin and soluble nuclear fraction during germination of cereal seeds embryos and under influence of phytohormon. The characteristic trait for growing seed nucleus is a rising in phosphatidic acid in nuclear membrane on the third day of growing. Phospholipids small headgroups characterizes by a high charge density and modification in composition of NE content can influence on total charge and in particularly on permeability of membrane. The goal of this work is to show correlation between genome activation and the change in content of the nuclear membrane during genome expression. Nevertheless much about the relationship between chromatin organization and the NE remains to be discovered.

## EXPOSURE SYSTEM, RF DOSIMETRY AND THERMAL IMAGING FOR HUMAN STUDIES ON POTENTIAL HEARING EFFECTS OF CELLULAR PHONE WITHIN THE GUARD EU-FP5 PROJECT

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The main aim of GUARD project is to assess potential changes of the hearing function of animals and humans after exposure to electromagnetic fields produced by cellular phones. The specific aim of this study was to provide exposure system, relevant dosimetry and thermal imaging for the human studies.

The Nokia 6310i mobile phone was chosen within the GUARD project which provides all special requirements for the study: The exposure system was developed with the assistance from the Nokia Research Center in Helsinki. The RF power of phones was measured using the external RF antenna output. The long term output power stability measurement was made by PC data acquisition during the whole lifetime of the battery. In order to develop a comfortable holder of the phone, a system of phone fixation have been designed with a possibility of freely moving of the subjects head. External RF loads were designed connected to the phone's external antenna output for sham exposure situations. The SAR measurements were made according to the CENELEC requirements using the "touch position" of the phone. A Janoptik-VarioScan high resolution compact (Infratec) thermocamera was used to assess potential changes of the phone and head skin temperature after exposure. The phone was operated at maximal power level (900 MHz, 2W peak; 1800 MHz, 1W peak). The warming up of the human head skin was measured after 10 minutes use of mobile phone.

The RF power uncertainty of the phone during the whole lifetime was below 1 % using the highest peak power level at 900 MHz, 2W, 1800 MHz, 1W respectively. In the first 10 minutes the uncertainty was below 0,4%. No radiated RF fields were measured using the RF load connected to the external antenna output. The SARs at the cochlea region were 0,41 W/kg and 0,19 W/kg at 900 MHz and 1800 MHz respectively. The maximum temperature rise of the mobile phone surface was 6,2 °C and 3.2 °C, at 900/1800 MHz respectively. The warming up of the earlobe and anywhere on the skin of the face was less than 1 °C in all cases.

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## BIOKINETICS OF URANIUM IN RATS CONTAMINATED BY REPEATED INHALATIONS : IMPLICATIONS FOR THE MONITORING OF NUCLEAR WORKERS

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Inhalation of airborne uranium compounds is one of the major health risks for nuclear workers. For radiation protection purposes, the Human Respiratory Tract Model (ICRP Publication 66) is applied together with systemic models (ICRP Publication 69) to calculate dose coefficients, retention and excretion functions for workers exposed to uranium. These functions and the biological distribution of uranium in the body do not only depend on the quantity and physicochemical properties of the inhaled particles but also on the duration of inhalation (acute or chronic). The ICRP dosimetric model for chronic contamination is represented by the addition of successive acute intakes. The aim of our work is to test this hypothesis, using as a model a rat contaminated by repeated inhalations. The main stages of this study are to (1) determine the biokinetics of acute inhalation of insoluble uranium dioxide  $\text{UO}_2$ , (2) determine the biokinetics of repeated inhalation of  $\text{UO}_2$  and (3) investigate the biokinetic consequences of acute or repeated  $\text{UO}_2$  inhalation on subsequent acute inhalation of moderately soluble uranium peroxide  $\text{UO}_4$ .

Male Sprague-Dawley adult rats were exposed to uranium aerosols using a nose-only inhalation system. For  $\text{UO}_2$  repeated inhalation, the rats were exposed 1 h per day, 2 days per weeks for 3 weeks. Three rats per condition (acute or repeated intake) were sacrificed 1, 3, 7, 16 and 30 days after the end of the repeated contamination period. The different organs and urine and faecal samples were mineralised and the uranium content was determined by kinetic phosphorescence analysis (KPA).

The biokinetics of uranium after acute inhalation of  $\text{UO}_2$  allowed to designing a rat inhalation model, using IRSN software Cyclomod. The theoretical biokinetics of the repeated  $\text{UO}_2$  inhalations were computed with this model. The theoretical and experimental biokinetics have been compared and were found to be similar. In this case, the addition of successive acute intakes may be used to simulate the actual repeated contamination. A more detailed analysis of the minor discrepancies between the model and the experimental results, as well as a new experiment involving more frequent repeated inhalations are in progress. The comparison between  $\text{UO}_4$  biokinetics preceded or not by repeated  $\text{UO}_2$  inhalation allowed to appreciate the importance of the history of exposure. Preliminary results showed that urinary excretion of uranium from  $\text{UO}_4$  compounds seems to be modified by previous repeated  $\text{UO}_2$  inhalations. The influence of the  $\text{UO}_2$  inhalation time pattern is now investigated by comparing the effects of repeated or acute  $\text{UO}_2$  inhalation on an acute  $\text{UO}_4$  inhalation.

The knowledge of the biokinetics of repeated contaminations by uranium will allow to better understand the effects of uranium inhalation on the respiratory tract clearance and absorption to blood, and to improve the monitoring of workers exposed to contamination by airborne uranium.



## INVOLVEMENT OF CYTOKINES IN THE INITIATION OF RADIATION INDUCED GENOMIC INSTABILITY IN PRIMARY HUMAN LYMPHOCYTES

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Introduction: Non-targeted effects of ionizing radiation such as genomic instability and bystander effects have become increasingly important in determining human health risk from environmental, medical and occupational exposures. Although several factors (eg. cytokines, ROS) have been postulated to be involved in the bystander response, factors involved in the initiation of genomic instability remain somewhat elusive. Furthermore, radiation quality has been confirmed to be an important factor in the induction of instability.

Objectives and Methods: To investigate the possible involvement of cytokines in the induction of radiation-induced instability, primary human lymphocytes were irradiated in the presence and absence of antibodies against the pro-inflammatory cytokine, tumour necrosis factor alpha (TNF- $\alpha$ ). The entirety of the cellular population, or fractions thereof, was targeted for traversal by a single high LET  $^3\text{He}^{2+}$  ion using the Gray Cancer Institute microbeam facility. Secondly, to test the hypothesis that the same cytokines are involved in the initiation of instability irrespective of radiation quality, we irradiated primary human lymphocytes from the same donor with 0.1, 0.5, 1, 2, and 3 Gy low LET X-rays (250 KeV) with and without TNF- $\alpha$  antibody (TNF- $\alpha$  AB). Chromosomal aberrations were scored 13-15 population doublings after irradiation.

Results: Metaphase analysis after microbeam irradiation demonstrates that incubation with TNF- $\alpha$  AB during irradiation leads to a significant reduction in delayed chromosomal instability when all cells are irradiated, but not when fractions (50% or 15%) of the population are targeted for irradiation (Moore et al. submitted). Inactivation of TNF- $\alpha$  during X-irradiation did not significantly affect the expression of the instability phenotype as measured by delayed chromosomal aberrations. Interestingly, instability was moderately increased in some cases when cells were irradiated with X-rays in the presence of TNF- $\alpha$  AB.

Conclusion: Inactivation of TNF- $\alpha$  during high LET irradiation can substantially reduce the level of radiation induced genomic instability, suggesting that 1) ROS are critical to the initiation of genomic instability and/or 2) some physiological function of TNF- $\alpha$  itself is critical to the initiation of genomic instability. Experiments are ongoing to further interrogate the role of LET, to determine the temporal nature of the inactivation of TNF- $\alpha$ , and to examine the role that ROS may have. Finally, experiments are underway to determine the contribution that individual lymphocyte-donor variation may have in the initiation of genomic instability after microbeam and X-irradiation.

## DELAYED EFFECTS OF RADIATION EXPOSURE

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Cellular exposure to DNA damaging agents like ionizing radiation can result in mutations, gene amplifications, chromosomal rearrangements, carcinogenesis, and even cell death. The paradigm for understanding how induced damage results in these cellular endpoints dictates that cellular responses to the induced damage, e.g., DNA repair, and cell cycle arrest fix the damage and thereby seal the fate of the irradiated cell. This presentation will focus on delayed genetic effects occurring in the progeny of cells after exposure to ionizing radiation, including delayed chromosomal rearrangements, and recombination events as determined by a plasmid based assay system for homologous recombination. We will present new data on how changes in gene expression as measured by differential display and DNA microarray analysis provides a mechanism by which cells display a memory of irradiation, and introduce candidate genes that may play a role in initiating and perpetuation the unstable phenotype. In addition, we will describe how the cellular micro-environment can perpetuate instability in clonally expanded populations of cells surviving irradiation. These results will be discussed in terms of non-targeted bystander like effects where by cells that themselves were not irradiated exhibit many of the same detrimental effects as irradiated cells and what implication these effects may have for radiation carcinogenesis.

This work was supported by the Biological and Environmental Research Program, U.S. DOE, DE-FG02-01ER63230, and NIH Health Awards CA73924 and CA 83872.

## LIFESPAN OF *DROSOPHILA MELANOGASTER* MUTANTS AFTER GAMMA-IRRADIATION

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It was investigated the influence of chronic low doze and acute height doze gamma-irradiation at pre-imago stages on the life span of *Drosophila* imago.

Shown, that the life span in unaffected strains with apoptosis deregulations (mutations *grim*, *hid*, *reaper*, *Dcp-1*, *dArk*, *th*), DNA repair defects (*rad54*, *mus210*, *mus209*, *mei-9*, *mei-41*) and red-ox system weakening (*Sod*) lower than in wild type strain *Canton-S*. But the low doze chronic irradiation (60 cGy per generation) led to significant increasing of the life span (in strains with the mutations *grim*, *hid*, *reaper*, *Dcp-1*, *dArk*, *th*, *rad54*, *mus210*, *mus209*, *Sod*). In some cases the level of the life span exceeded that in intact strain *Canton-S* (*Sod*, *th*, *Dcp-1*, *dArk*). In the sensitive to apoptosis induction strains (mutants on apoptosis and red-ox system), possibly, this is due to induced elimination of the radiosensitive cells that will be subjected to accelerated aging. The life span in homozygous and hemizygous flies with the mutation of the gene *mei-41* (homolog of the human ATM) after irradiation was decreased with comparison to the control, but in heterozygous was increased. Obviously this is because of the major role of *ATM/mei-41* in sensing of DNA defects after irradiation. Thus, it is demonstrated the recessive effect of *mei-41* mutation in the control of radio-induced life span alteration. The chronic irradiation of wild type strain *Canton-S* during 5 generations led (60 cGy per generation) to increase of life span only in first generation, in the following it was no effect and in 5<sup>th</sup> generation it was the decrease. After acute irradiation in height doze (10 and 30 Gy) life span in most cases is significant lower that in control.

It is shown, that lines with various genotypes react in the different way to an irradiation. The results are discussed from the point of view of the role of apoptosis and genomic instability in radio-induced aging.

## IMPLICATIONS OF RADIATION-INDUCED BYSTANDER EFFECTS AND OTHER NON-TARGETED RADIATION EFFECTS FOR PROTECTION OF NON-HUMAN BIOTA AND FOR REGULATION OF MULTI POLLUTANT ENVIRONMENTAL EXPOSURES

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Environmentally relevant low doses of ionizing radiation are now accepted to induce a variety of biological effects at levels where it is difficult to implicate direct (targeted) DNA damage. These effects include bystander effects, genomic instability, adaptive responses and low dose hypersensitivity. The importance of these effects is that all are induced at very low doses. Typically one track of high LET radiation or less than 5mGy of low LET radiation can trigger these effects. Once induced the level of effect does not increase with increasing dose and is persistent. The mechanisms underlying these effects are not known but it is accepted that genetic background is crucial in determining what the consequences of the exposure will be. I will discuss evidence that chemical pollutants (heavy metals and micro-organics) can also induce these low dose responses, that these effects can be induced in vivo as well as in vitro using mouse models exposed to whole body doses, that the mechanism involves persistent elevation of ROS and that the effect can persist over many cell generations, that chronic low dose exposures may actually be more effective than acute doses at inducing certain types of response, that combinations of these inducers (whether radiation or chemical) and classical mutagens, can enhance the frequency of mutations due to the mutagen.

There are implications for radiation and environmental protection which at present treat radiation as a “stand alone” agent and assume a linear correlation between radiation dose and effect.

While it is easy to point out the problem, it is far more difficult to suggest a new framework for regulation. This is because we do not know how to distinguish “effect” from “harm” at these low doses using cellular markers. There is a dearth of data spanning from cellular effects in non-human species, to population or ecosystem effects. There are few people with the expertise to bridge the gap. Population effects are difficult to detect when the frequency of damage is low and when causation cannot be definitely attributed to radiation.

## RADIOTHERAPY AND THE POTENTIAL EXPLOITATION OF BYSTANDER EFFECTS

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Radiation-induced bystander effects (RIBE) are the subject of intense investigation at present in radiation protection. The field has been reviewed recently by our own group and also by two other prominent laboratories working in the field. Because the effects predominate at low doses they have been discussed in terms of their potential impact on low dose risk assessment and while the possible therapeutic implications have been alluded to they have not been discussed in any great detail. The purpose of this review is to consider areas of major importance or interest in radiotherapy where bystander effects might be important. These include consideration of RIBE during the cell cycle, under hypoxic conditions, where fractionated therapy modalities are used or where combined radio-chemotherapy is given. Also discussed are individual variation in toxicity of bystander factors and normal tissue "collateral" damage. The importance of considering the tumour in the context of the organ and even the organism which supports it, is also discussed. Direct clinical radiotherapy studies which consider bystander effects are not in the public domain at the time of writing but many in vitro studies are available which are relevant and some preliminary animal data have also been published. Since RIBE appear to challenge many of the central assumptions which underlie radiotherapy practice, it is important to consider what unexplored treatment avenues might result from a consideration of these effects. The final part of this presentation is devoted to this point.

## RADIATION DOSE ASSESSMENT BY FLUORESCENT MICROSPHERE-BASED IMMUNOASSAYS.

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The need to rapidly assess radiation dose in mass casualty and population-monitoring scenarios prompted an evaluation of suitable molecular biomarkers that can provide early diagnostic information after exposure. Using an *in vitro* model system of human peripheral lymphocytes as well as an *in vivo* murine model, we demonstrated radiation-responsive changes in the expression of the proto-oncogene proteins *ras*-p21 (Blakely *et al.*, Proc. 36<sup>th</sup> Midyear Topical Meeting, Health Physics Society, pp. 231, 2003; Blakely *et al.*, Adv. Space Res. 31(6): 1487, 2003) and recently as well *raf*-1, GADD45 DNA repair protein p21Waf1Cip1, all with a progressive time- and radiation-dose-dependent increase.

In our strategy to identify, optimize, and validate radiation-responsive molecular biomarkers, we are testing a microsphere-based multi-analyte assay system (Luminex-100<sup>TM</sup>). This technology is based on microscopic spherical polystyrol particles that serve as a solid phase for molecular detection reactions measured by a flow cytometer equipped with a 96-well microtiter plate platform. Current studies focus on the use of commercially available cytokine-antibody-conjugated Luminex beads as well as reagents prepared in-house by conjugating various antibodies (e.g., *ras*-p21, amylase, and GADD45) to Luminex microspheres.

Preliminary results from commercial human cytokine multiplex immunoassay kits (BioSource International, Inc. and Linco Research, Inc.) showed that, at 24 and 48 hours after irradiation of human blood with 0.1 to 6.0 Gy of <sup>60</sup>Co-gamma rays at 0.1 Gy/min, there was no significant change in IL-6, IFN-gamma, and TNF-alpha levels in both serum and cell-pellet samples. However, radiation-responsive changes in the level of GADD45 DNA repair protein occurred with a progressive time- and radiation-dose-dependent increase in the range of 0.15 to 6.0 Gy. Overall, these results demonstrate that our protocol is a feasible biodosimetry approach for radiation dose assessment.

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## PROTON RBE AT LOW ENERGIES

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**OBJECTIVE:** In heavy-ion therapy, protons are produced in addition to various other light ions by fragmentation of the projectile or target nuclei. The damage caused by these fragments needs to be taken into consideration when calculating a treatment plan. In proton therapy a general RBE of 1.1 to 1.2 is used. To verify this for the treatment planning of the carbon ion therapy at GSI, first track segment and passive deceleration irradiation experiments have been conducted, the energy ranging from 2.5MeV up to 20MeV.

**METHODS:** CHO-K1 cells were grown on Petri dishes or mylar foil and irradiated in air. The Petri dish samples were then immediately put back into medium, whereas the cells on mylar foil were covered by another foil to prevent the cells from drying out. The cells were then plated in culture flasks so to form 100 colonies within one week. During this time, the cells were kept in an incubator at 37°C and 5% CO<sub>2</sub>. The proton energy distribution was measured at target position with a silicon detector. For 20MeV protons, the Bragg curve was measured by comparing the electric charges produced in two ionization chambers with PE foils of different thickness between them.

**RESULTS & CONCLUSION:** First results show the expected higher cross sections for the track segment irradiations than for the same energy achieved by passive deceleration. Due to the non-linearity of the LET, a higher overall LET can be assumed for the energy spectra resulting from the passive deceleration, compared to the LET of the mean energy of each spectrum. This assumption is currently being examined and will be discussed.

## RADIATION-INDUCED ACTIVATION OF PROTEIN KINASE C $\delta$ IN RADIOSENSITIVE AND RADIORESISTANT MOUSE THYMIC LYMPHOMA CELL LINES

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(Objective) Protein kinase C (PKC) plays an important role in radiation-induced apoptosis. However, each function of PKC subtypes remains unclear. In this study, expressions and functions of PKC $\delta$ , which is known to be involved in radiation-induced apoptosis, were assessed in radiosensitive and radioresistant cell lines derived from mouse thymic lymphomas

(Methods) A radiation-sensitive mouse thymic lymphomas, 3SBH5 and its radio-resistant derivative, XR223, were used. PKC $\delta$  distribution was evaluated using Western blot analysis. Percentage of apoptosis was evaluated by FACS using PI staining. PKC $\delta$  activity was measured by PKC $\delta$  immunoprecipitation kinase assays using MBP<sub>4-14</sub> as the substrate. Caspase-3 activity was measured using CaspACE<sup>TM</sup> Assay System (Promega, Madison, WI).

(Results and Conclusion) Rottlerin, a PKC $\delta$  specific inhibitor, abolished the radiation-induced apoptosis in 3SBH5 completely and PKC $\delta$  degradation was observed in a dose dependent manner after irradiation, indicating that PKC $\delta$  is an important regulator in the radiation-induced apoptosis in the cells. Indeed, radiation increased PKC $\delta$  activity in a dose dependent manner in 3SBH5. In addition, it was demonstrated that the radiation-induced PKC $\delta$  activation is correlated to the degradation. In XR223, PKC $\delta$  was less activated than that in 3SBH5 by irradiation with the dose even as high as 2 Gy. PKC $\delta$  signal detected by Western blot in cytosols of 3SBH5 cells was remarkably decreased after irradiation, reflecting PKC $\delta$  translocation to membranes and degradation. In contrast, in XR223, the PKC $\delta$  signal in cytosols was decreased very slightly. Interestingly, the basal signal of PKC $\delta$  in cytosols in XR223 by Western blot was much stronger than that in 3SBH5 cells. Caspase-3, by which PKC $\delta$  is degraded, was activated by irradiation in both cell lines and the activity in XR223 by irradiation with 2Gy appeared to be sufficient to degrade PKC $\delta$  in spite of the less activity of PKC  $\delta$ . Overall, the regulation of PKC $\delta$  distribution may determine radiation sensitivity in these 3SB cell lines. We will also discuss comparative data of PKC  $\delta$  distribution between mouse thymic lymphoma cells derived from the wild (+/+) and null (-/-) mouse of *Atm*, the gene mutated in the human genetic disorder, ataxia telangiectasia.



## OXIDATIVE STRESS SIGNALING IS RESPONSIBLE FOR TNF-ALPHA INDUCED GENETIC DAMAGE IN PRIMARY HUMAN AORTIC ENDOTHELIAL CELLS

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Genomic instability, an increased rate of accumulation of new mutations in tumor cells or cells surviving irradiation, has been demonstrated in progeny of irradiated cells, and unirradiated bystander cells. Bystander responses are thought to depend on activation of cellular communication processes. In this study we examined one such mediator of cellular communication, the pro-inflammatory cytokine tumor necrosis factor alpha (TNF- $\alpha$ ). In this study, we have directly investigated the role of TNF- $\alpha$  on cell survival, free radical generation, and DNA damage in human aortic endothelial (HAE) cells, by incubating cultures of HAE cells with varying concentrations of TNF- $\alpha$  protein. To determine the effect of TNF- $\alpha$  on cell survival, HAE cells at passage five were incubated with 0, 1 and 10 ng/ml recombinant human TNF- $\alpha$  (rTNF- $\alpha$ ). Cells exposed to 2 Gy X-rays (250 KeV) were used as positive controls. Survival was estimated by bulk doubling at three days post-exposure, and was also measured by clonogenic plating efficiency measured at day 14. Compared to untreated controls, both survival assays showed no cytotoxicity in TNF- $\alpha$  treated samples at concentrations of 1 or 10 ng/ml. Next, to determine whether TNF- $\alpha$  generates free radicals in HAE cells, the cells were incubated with an oxidative-sensitive probe 2',7'-dichlorofluorescein (DCFH-DA) in phenol red-free medium and then treated with 0, 1 and 10 ng/ml rTNF- $\alpha$ . The cells were harvested at 30 min and examined for oxidative stress by FACS and analyzed with Cell Quest software program. Cells treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 15 min were used as positive controls. A 2-fold increase in intracellular ROS was noted in rTNF- $\alpha$  treated cultures compared to untreated controls. Third, to examine whether TNF- $\alpha$  induces genetic damage, HAE cells were treated with 0.1, 1 and 10 ng/ml of rTNF- $\alpha$ , harvested after 5 h, and analyzed for DNA damage by the COMET assay. Cells exposed to 0.1 or 2 Gy or treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> were used as positive controls. The results showed that TNF- $\alpha$ , at concentrations as low as 0.1 ng/ml, could initiate a 5-fold increase in DNA damage compared to untreated controls. In sum, these results clearly indicated that TNF- $\alpha$ , at sub-cytotoxic levels, can induce genetic damage through free radical generation. These experiments are currently being repeated and extended using the more sensitive DNA strand break indicator,  $\gamma$ H2AX, instead of the COMET assay. Furthermore, cultures will be incubated with the free radical scavengers SOD and Catalase in order to more clearly define the pathway. Finally, chromosomal analysis at delayed times, to assess genomic instability, will be performed on all groups using conventional staining methods as well as multicolor fluorescence in situ hybridization (M-FISH). The results of these ongoing studies will be reported and discussed.

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## RECENT ADVANCES IN RADIATION CYTOGENETICS

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Ionising radiation (IR) is very efficient in inducing chromosomal aberrations and this aspect has been studied for more than 100 years. Basic concepts on the formation of chromosome aberrations were formulated already in 1930s (Sax, Lea) and are still valid. Among the lesions induced by IR in DNA, double strand break (DSB) has been shown to be the most important lesion for the formation of chromosome aberrations. Employing radio-sensitive cell lines defective in different pathways of DSB repair, it has become evident that non-homologous end rejoining (NHEJ) pathway is the most predominant one and homologous recombination repair (HRR) pathway is mostly active in S and G2 phases of the cell cycle.

Fluorescence in situ hybridisation (FISH) employing chromosome specific DNA painting probes, arm specific probes, region specific probes, centromere and telomere specific probes has given very valuable information on the nature and formation of IR induced CAs. There is heterogeneity between chromosomes and chromosome regions for involvement of IR induced CAs. Intra-chromosomal exchanges appear to be formed more frequently than inter-chromosomal exchanges. Telomeric regions in general and interstitial telomeric regions (in rodents) are preferentially involved in exchanges. These aspects will be discussed.

## EFFICACY OF DIFFERENT UV EMITTING SOURCES IN THE INDUCTION OF T CELL APOPTOSIS

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**OBJECTIVE:** one of the major mechanisms of UVB immunosuppression in the treatment of different skin diseases is thought to be an apoptosis-inducing effect on T cells infiltrating the skin. We examined the T cell apoptosis-induction capacities of different UV light sources.

**METHODS:** 4 different polychromatic UV light sources with and without filters were used to irradiate peripheral blood mononuclear cells. The extent of apoptosis was measured by Apo2.7 antibody staining and flow cytometry.

**RESULTS:** the xenon chloride (XeCl) laser proved to be the strongest apoptosis-inducer. The use of a phthalic acid filter eliminated UV radiation almost completely below 300 nm, which resulted in a severe decrease in the apoptosis-inducing capacity of different UVB sources. Using the results of the measurements with polychromatic UV light sources, the wavelength dependence of UVB light for the induction of T cell apoptosis was also determined. The regression line of the action spectrum demonstrated a continuous decrease from 290 nm to 311 nm.

**CONCLUSION:** the decreasing trend of this action spectrum is similar to that for erythema induction and thymine dimer formation. The explanation for the similarities might be that all of these processes are mostly mediated by UV-induced DNA damage.

## EFFECTS OF IRRADIATION WITH 25 kV OR 200 kV X-RAYS IN MAMMARY EPITHELIAL CELLS 184A1

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**Objective:** A relative biological effectiveness (RBE) not substantially higher than unity is usually assumed for soft X-rays (up to approximately 50 keV) that are applied in diagnostic radiology such as mammography. On the other hand, there have been recent reports of an RBE of more than 3 for low energy X-rays, mainly based on studies in fibroblasts or fibroblast-tumor-hybrid cells. However, target cells for carcinogenesis in breast tissue are mammary epithelial cells. Therefore the present study has been initiated to quantitate the RBE of low energy X-rays in mammary epithelial cells for a variety of biological endpoints.

**Methods:** In the present study, non-malignant human mammary epithelial cells 184A1 were used at early passages (P8-P14). Clonogenic survival, micronucleus induction (cytochalasin B assay) and DNA damage, assessed as Olive tail moment (ratio of tail length to tail DNA content) or head DNA/tail DNA-ratio, with the alkaline comet assay (microgel electrophoresis) were chosen as endpoints. Experiments were performed with 25 kV X-rays, using therapeutic X-ray machine (Darpac 150-MC, Forward Raytech, UK), and compared to 200 kV X-rays (Isovolt 320/20, Seifert Roentgenwerke Ahrensburg, Germany) as reference radiation.

**Results:** Irradiation with 25 kV X-rays, compared to the effect of 200 kV X-rays, did not yield significant differences in the parameters for clonogenic survival, indicating a RBE of 1. Micronucleus induction, assessed as frequency of binucleated cells (BNC) with micronuclei, was slightly more pronounced with 25 kV X-rays, resulting in a RBE-value of 1.3. Similarly, the average number of micronuclei in micronucleated BNC was slightly higher after irradiation with 25 kV X-rays (RBE=1.3). The amount of DNA damage was assessed in a alkaline comet assay. The preliminary data show that the number of damage per unit dose is approximately the same after 25 kV and 200 kV X-rays irradiation, again indicating a RBE of 1.

**Conclusion:** In conclusion, the RBE of soft X-rays for clonogenic survival and DNA damage (comet assay) is not significantly higher than 1 in 184A1 mammary epithelial cells. In contrast, the micronucleus test revealed a significantly higher RBE of 1.3. These results are in good accordance with previous studies in murine and human non-epithelial cell lines and skin keratinocytes. The higher number of micronuclei in micronucleated cells suggests that the quality of damage is different for the two radiation qualities. This, however, has to be validated in further studies, including fluorescence in-situ hybridisation (FISH) of the micronucleus content.

## STIMULATORY EFFECT OF SINGLE EXPOSURES TO LOW-LEVEL X-RAY IRRADIATIONS ON ANTITUMOUR FUNCTIONS OF MURINE PERITONEAL MACROPHAGES

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A number of epidemiological and experimental data indicate that exposures to low doses of low-LET ionising radiation inhibit tumour growth by triggering the activity of natural anti-tumour immune mechanisms. Previously, we showed that whole-body irradiation of mice with a single low (0.1 and 0.2 Gy) but not higher (1.0 Gy) dose of X-rays resulted in the significant reduction of the development of pulmonary tumour metastases induced by i.p. injection of syngeneic L1 sarcoma cells.

In the present experiments, 6-8-week-old BALB/c mice were exposed to a whole body irradiation with 0.1, 0.2, or 1.0 Gy X-rays and then peritoneal macrophages were collected. Cytotoxic activity of these cells was estimated in the *in vitro* assays using the [<sup>3</sup>H]thymidine-labelled L1 and P815 neoplastic cells as targets. Colorimetric assay with the Griess reagent and the NBT-reduction assay were used for the detection of nitric oxide (NO) and superoxide anions' production in the collected macrophages, respectively. Finally, synthesis of TNF- $\alpha$  by these cells was examined in the ELISA tests.

The results indicate that all the tested parameters are significantly up-regulated in macrophages obtained from mice exposed to 0.1 or 0.2 Gy X-rays compared with the cells obtained from non-irradiated and 1.0 Gy-exposed mice.

These results clearly suggest that the inhibitory effect of small doses of X-rays on the induction of pulmonary tumour nodules may be causatively related to stimulation by such exposures of the natural anti-tumour defence mechanisms mediated by macrophages.

## CYTOGENETIC ANALYSES IN PERIPHERAL LYMPHOCYTES OF PERSONS LIVING IN HOUSES WITH INCREASED LEVELS OF INDOOR RADON CONCENTRATIONS

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### Objectives:

The study was performed to evaluate cytogenetic damage in peripheral lymphocytes of individuals living in houses with elevated levels of indoor radon concentrations.

### Methods:

Four exposure groups were established in relation to the indoor radon concentration measured (group I: < 200 Bq/m<sup>3</sup>, mean 140 Bq/m<sup>3</sup>; group II: 200 - 1000 Bq/m<sup>3</sup>, mean 450 Bq/m<sup>3</sup>; group III: 1000 - 5000 Bq/m<sup>3</sup>, mean 1900 Bq/m<sup>3</sup>; group IV: > 5000 Bq/m<sup>3</sup>, mean 8100 Bq/m<sup>3</sup>). The fluorescence plus Giemsa (FPG)-technique was used to study the frequency of dicentric chromosomes (dic) and centric rings (cr) and the fluorescence in situ hybridization (FISH)-technique was applied in order to analyse translocations.

### Results:

In exposure group II the number of cells containing dicentrics and/or centric rings ( $C_{dic+cr}$ ) ( $(2.45 \pm 0.50) \times 10^{-3}$ ) was significantly increased ( $p < 0.05$ ) in comparison to the control level ( $(1.03 \pm 0.15) \times 10^{-3}$ ). However there was no difference in the mean frequency of  $C_{dic+cr}$  between the groups living in dwellings with higher radon concentrations. The translocation frequency observed in exposure group IV was  $(3.9 \pm 0.64) \times 10^{-3}$ . In comparison to the control group ( $(2.02 \pm 0.18) \times 10^{-3}$ ), there was a slight but not statistically significant increase in the exposed group ( $p = 0.055$ ). If, however, the age of the examined persons is taken into account, the values are significantly increased ( $p < 0.05$ ) in the exposed persons older than 40 years in comparison to the age-matched controls.

### Conclusion:

The data demonstrate that chronic exposure of persons to an elevated radon concentration in dwellings causes an increased level of cells containing dic and/or cr in peripheral blood lymphocytes. The increased level of translocations is mainly due to translocations in stable cells, which may be a hint that they should be induced in blood forming tissue.

## REPAIR MECHANISMS INVOLVED IN PROCESSING OF COMPLEX DNA DAMAGE

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Objective: Ionizing radiation (IR) is known to produce different types of DNA lesions such as single strand breaks, base losses, modified DNA bases and double strand breaks. When clustered these lesions give rise to multiple damaged sites (MDS). Our main objective was to study the role of homologous recombination (HR), non-homologous end joining (NHEJ) and base excision repair (BER) in processing repair of MDS.

Methods: We have investigated the repair strategy for MDS by examining the growth inhibition after IR exposure of Chinese hamster ovary (CHO) cell lines, which are deficient in different repair pathways. We also used the V79 derived SPD8 Chinese hamster cell line that has a tandem duplication of exon 7 in its *hprt* gene. This duplication is shown to be a useful tool for determination of intra-chromosomal homologous recombination. Levels of HR were determined in parallel with survival. The repair deficient CHO cell lines used were *irs1SF*, V3 and EM9, defective in HR, NHEJ or BER, respectively. The parental wild-type cell line AA8 was used in comparison. Irradiation was performed in the presence or absence of the radical scavenger dimethylsulfoxide (DMSO). DMSO scavenges IR produced free radicals and in this way the indirect effect of IR is reduced. Irradiations were performed with gamma radiation ( $^{137}\text{Cs}$ , 0,56 Gy/min) at doses ranging from 0 through 20 Gy.

Results: Clonogenic survival for SPD8 cells at the dose giving 50 % toxicity was elevated 2.7 times in the presence of DMSO while homologous recombination decreased significantly by 2.0 times at doses above 3 Gy. The effect of DMSO at 50 % growth inhibition (IC<sub>50</sub>) was measured in the CHO cell lines 24 or 120 hours after irradiation. The results suggest a late effect of IR, e.g. the 24 hour time point showed no effect of IR, while for 120 hours post-exposure all three repair deficient cell lines showed enhanced toxicity compared to wild-type suggesting that all types of lesions contributed to the lethal effect of IR. DMSO counteracted the growth inhibition in the HR defective cell line by 45 % compared to 53 % for the cell lines defective in NHEJ or BER and 57 % in the wildtype.

Conclusion: Decreasing the indirect effect of IR by DMSO also decreased HR, suggesting that HR is involved in the repair of MDS. The data from the CHO cell lines indicate that HR is a crucial repair pathway for MDS caused by IR since the HR deficient cell line was the most sensitive to IR exposure and did benefit the least from the protecting effect of DMSO.

## ROLE OF TYPE II PNEUMOCYTES IN PATHOGENESIS OF RADIATION PNEUMONITIS

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**Objective:** We studied the dose response of radiation-induced changes at 3 weeks after 1-25 Gy irradiation and we investigated the anti-inflammatory therapy influence.

**Methods:** Wistar rats were given 1-25 Gy of thoracic irradiation. The first group was non-treated, while the second group was administered subcutaneously twice per week by a combination of pentoxifyline (35 mg/kg) and dexamethasone (1 mg/kg). Lungs were examined histochemically and number of neutrophile granulocytes, alveolar septal thickness, air/tissue ratio, number of alveoli per field, number of type II pneumocytes per alveolus, and occludin 1 expression were measured.

**Results:** A significant dose-dependent type II pneumocytes depletion was found after irradiation from 1 Gy. A higher neutrophils number after 1 Gy with dose-dependency after 10-25 Gy and higher alveolar septal thickness after 5-25 Gy were measured. A lower occludin 1 expression was noticed in 5-20 Gy irradiated animals. Used therapy partially inhibited the increase of neutrophils number at the whole-radiation-dose range and the type II pneumocytes depletion after 1, 10, and 15 Gy. In treated rat lungs an occludin 1 expression was significantly enhanced.

**Conclusion:** We suggest that the pneumocytes depletion is a major factor responsible for the radiation pneumonitis development and the observable post-radiation lung changes are compensable up to the threshold dose irradiation.

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## INDIVIDUAL RADIOSENSITIVITY OF PATIENTS WITH LUNG CANCER AND HEALTHY DONORS: ANALYSIS OF DNA DAMAGE AND REPAIR IN PERIPHERAL BLOOD LYMPHOCYTES

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Individual radiosensitivity of the human population is heterogeneous. In case of patients undergoing radiotherapy an enhanced curability could be achieved by adjusting the treatment scheme to the individual sensitivity of the patient. There is data suggesting that the radiosensitivity of peripheral blood lymphocytes (PBL) may serve as a model cell system for determining the individual sensitivity to ionising radiation. An interesting question is if the radiosensitivity of PBL also correlates with the individual sensitivity towards chemotherapy drugs. Also, an open question is which biological test is most suitable for determining the sensitivity of lymphocytes towards radiation.

The aim of our study was to investigate 1: the correlation between the frequency of chromosomal aberrations induced in G<sub>0</sub> phase and the kinetics of DNA repair in PBL of healthy donors and lung cancer patients estimated by the comet assay; 2: the possible correlation between the radiosensitivity of PBL of cancer patients with their sensitivity towards chemotherapy drugs estimated by the therapy outcome.

Peripheral blood lymphocytes were collected from cancer patients (before therapy with VP16 + DDP) and from healthy donors and irradiated with 2 Gy <sup>60</sup>Co. Chromosome slides were prepared from cells fixed at 50 hours after irradiation. The frequency of chromosome aberrations was observed in cells before the first mitosis division. The level of DNA damage was estimated by the alkaline comet assay after 0, 15, 30, 60 and 120 minutes post exposure.

Preliminary results suggest that no correlation exists between the frequency chromosomal aberrations and the kinetics of DNA repair. The analysis of a bigger number of donors is necessary to confirm these results. More experiments are under way.

## SPACE AND TIME CLUSTER ANALYSIS OF MORBIDITY FROM CHILDHOOD LEUKAEMIA IN HUNGARY

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**Objective:** The spatial and temporal changes of childhood leukaemia morbidity was assessed by the method of space and time cluster analysis between 1980 and 2001. The generated time clusters were compared to the regions of different categories of excess doses of radiation due to the Chernobyl accident in 1986.

**Methods:** Childhood leukemia morbidity data were obtained from the National Pediatric Cancer Registry of Hungary. Time clusters were identified using SCAN method. Time clusters were defined by each possible time aggregation within the period examined. From these aggregations in time, 3-year long time periods were selected to follow the changes of excess of morbidity for the total period. The generated consecutive 3 year long time clusters were compared to each other and the regions of different categories of excess doses of radiation by the method of two field contingency table analysis. Relative excess morbidity was computed for the regions of different categories of excess of radiation in relation to the expected morbidity based on the average value of the time period of 1980-1985.

**Results:** The analysis of the consecutive 3-year long time clusters between 1980 and 2001 revealed a systematic 8-year long decrease of correlation after the accident. Before and after that systematic decrease the correlation changed randomly. This means that the spatial distribution of the areas of excess of morbidity changed after the Chernobyl accident. Comparing the consecutive 3-year long time clusters of 1980 and 2001 with the regions receiving the greatest excess doses of radiation the results showed significant relation in two periods. The first 3-4 year long period was after the accident, the second 3-4 year long period was 10 years later. The relative excess in standardised morbidity ratio computed for the regions exposed to the greatest excess doses of radiation showed similar results. However similar analysis of the other regions exposed to less excess doses of radiation or using rougher categories of excess doses of radiation did not show significant relation between them.

**Conclusion:** These results of the applied sensitive method showed an insignificant excess of morbidity due to Chernobyl accident in the regions exposed to the greatest excess doses of radiation. Examining regions of rougher categories or regions exposed to lower levels of radiation this excess of morbidity can not be detected.

## DETERMINATION OF RBE OF 10 KV AND 25 KV X-RAYS

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X-rays in the range  $\sim 10 - 50$  keV are widely applied in the diagnostic radiology (particularly mammography) and radiotherapy (e. g. brachytherapy). However, the published data about their relative biological effectiveness (RBE) allow up to now no definitive conclusions about the action on different biological systems. Moreover, the biological effects depend on the spectral distribution of the photon source. In order to study this dependence, the RBE of 10 kV and 25 kV X-rays has been determined relative to 200 kV X-rays by X-ray tube irradiation.

The studies were carried out on the mouse fibroblasts NIH/3T3 and the human mammary epithelial cells MCF-12A. For the soft X-rays irradiation, a tungsten-anode X-ray tube operated at 10 kV (no filtration) or at 25 kV (0.3 mm Al filtration) was used. The reference irradiation was performed with a 200 kV X-ray tube with 0.5 mm Cu filter. The dose rate for all irradiations was in the range  $0.3 - 1.9$  Gy/min. The spectral dose distribution of all radiation qualities was also determined. Cell survival was studied after irradiation with  $0.5 - 10$  Gy by the clonogenic assay. Chromosomal damage was assessed by the cytokinesis-block micronucleus (MN) test in the dose range  $0.2 - 5$  Gy. In addition, first results for the RBE determination by chromosomal aberrations induction in MCF-12A, will be presented.

The cell survival data were fitted to the linear-quadratic model, resulting in an RBE value of  $1.1 - 1.3$  at the 10% survival level, depending on the used radiation quality and cell line. For both cell lines, an increase of RBE was found with decreasing dose after 10 kV X-rays, whereas a decrease of RBE of 25 kV rays was observed. The MN test results for the fraction of binucleated cells (BNC) with MN and the number of MN per BNC were fitted to a quadratic dependence, resulting in an RBE of  $1.1 - 1.4$ .

The data obtained in the present work are in good agreement with observations of other authors as well as with theoretical predictions for this photon energy range. However, the detailed RBE dependence on photon energy can be determined only at a monochromatic X-ray source. Currently, the installation of an intensive, tunable, quasi-monochromatic source for cell irradiation in the energy range  $10 - 100$  keV is under progress at the ELBE accelerator at Forschungszentrum Rossendorf. The experimental verification of the theoretical calculation of the spectral distribution and intensity of this novel photon source will be presented.

## UV INDUCED P53 CLONE FORMATION IN MOUSE SKIN

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Non-melanoma skin tumors show the highest prevalence among human malignancies with their incidence dramatically increasing. Tanning and natural aging with a rising number of elderly contribute this increase and make the burden higher on healthcare systems worldwide. Ultraviolet radiation (UVR) is undoubtedly the most important environmental risk factor in the development of non-melanoma skin cancer. UVR is thought to induce skin tumors by creating mutations throughout the genome. Mutations in tumor suppressor genes or oncogenes may furnish cells with growth advantage over normal cells thus enabling uncontrolled clonal proliferation. P53 is most frequently effected with a mutation rate of up to 90% in skin tumors. Acute UV radiation causes p53 induction in a large portion of human keratinocytes. Within days after a single UV irradiation p53 levels return to normal in most cells, except for those that suffered mutations in the gene. Furthermore chronic UVB radiation has been shown to create cell groups with mutated p53 which increase in size and number with growing cumulative doses of UVB in animal experiments. These p53 positive cell groups were also found in sun exposed human skin, thus suggesting a role for these mutant cell groups in the development of human skin tumors.

Studying the changes in the clonal proliferation of p53 positive cells in murine skin enables us to study the very early steps of UV carcinogenesis, which is invaluable in developing new prevention methods.

## UNDERSTANDING THE RADIOSENSITIVITY / RADIORESISTANCE OF STEM CELLS BY MICROARRAYS TRANSCRIPTION PROFILING

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Eradication of hematopoietic cells is the first noticeable somatic effect following total body ionizing radiation (IR) exposure. Although marked differences in sensitivity of these cells have been largely noticed, little is known of the underlying mechanisms. To understand at the molecular level the cell-dependent IR response, transcriptional changes occurring in stem cells and mature B-lymphocytes were monitored using a microarray-based strategy. Comparisons of the responses were followed from 1h to 15 days post-irradiation at the clinically relevant dose of 2 Gy.

Flow cytometric analysis of purified marrow cells clearly showed the differential radiosensitivity of both populations. Only 3.5% of mature B-cells survived at 48h following irradiation, whereas the number of stem cells was unchanged during this period. Stem cell numbers decreased transiently 7 days after irradiation, reflecting presumably their capacity to restore the eradicated lymphoid compartment. The data show a high transcriptional activity in the stem cell enriched population as early as 1h following IR exposure. Analysis of expression profiles revealed a set of 249 genes clustered in 7 different groups only modulated in stem cells. Assignment of these genes to functional categories revealed that the stem cell elicits a specific damage response mostly triggered by the modulation of DNA repair enzymes, protein transport, pro- and anti-apoptotic signals and a variety of yet uncharacterized stress signals that were not found in mature lymphocytes. In contrast, the B-mature cell response was mostly characterized by the modulation of known cell cycle checkpoints (such as cyclin A2, PCNA) and transcription factors never described as involved in this process. This is particularly interesting considering that to date, very few transcription factors appear to be responsive after physiological doses of IR. Few genes were modulated in the same manner in both cell populations. Examples are the up-regulation of heat shock responsive genes.

Altogether our strategy allowed defining novel and specific actors in the IR-response of stem cells. The comparison of transcription profiles with those obtained for the mature B-lymphoid cells clearly demonstrated that the elicited response is highly dependent on the stage of differentiation of the cell. This approach has the potential for finding novel molecules to treat bone marrow failure and unraveling molecular networks involved in the IR sensitivity/resistance at clinically relevant doses.

## A COMPARATIVE ANALYSIS OF PATIENT DOSES OF THE MOST COMMON DIAGNOSTIC X-RAY PROJECTIONS BASED ON THE SURVEYS PERFORMED IN 1993 AND 2003

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According to the Order No. 31/2001. (X. 3.) EüM which complies with the Council Directive 97/43/EURATOM a wide range of patient dose investigation, establishment and adaptation of guidance levels in diagnostic radiology and nuclear medicine are required in Hungary.

Investigations of patient doses arising from the diagnostic X-ray and nuclear medicine procedures began in 1989 in the framework of the National Patient Dose Evaluation Program controlled by the NPHC National Research Institute for Radiobiology and Radiohygiene.

In 1993 a regional study of the most common X-ray diagnostic procedures was performed. We studied the patient doses and technical parameters on 107 diagnostic X-ray equipment operated in 71 X-ray or other (pulmonary, surgery, dentistry etc.) departments. The NEXT method for data collection, processing and evaluating the results were applied. We concluded that the studied region belongs to the first health-care level according to the WHO classification. Accordingly, the chest and spine projections were the most frequently performed. Technical parameters affecting on the patient exposures varied rather widely and very often were not complied with international recommendations and standards, resulting in unnecessarily large variations in patient doses. The mean values of the skin doses were one and a half times higher than the international reference levels, and higher than typical for the first health-care level.

Last year we performed a nationwide study of the technical parameters and patient doses of the most commonly performed diagnostic X-ray projections. Data collection was based on a special questionnaire applied earlier in different countries of the EU and the entrance skin doses were measured by TL dosimeters. 30 diagnostic X-ray equipment were involved in the program and the entrance skin dose measurements were performed on 390 average sized patients. On the base of our investigation we have information about the patient doses of 13 types of commonly performed X-ray projections.

The authors present a comparative analysis of the most important results of the two surveys.

## RADIATION INDUCED MYELITIS IN THE RAT SPINAL CORD AFTER SINGLE AND SPLIT DOSES OF PHOTONS AND CARBON IONS

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**Objective:** Clinical studies at the GSI in Darmstadt are accompanied by a biological program to assess the relative biological effectiveness (RBE) of carbon ion irradiations (12-C) related to photons with special emphasis on late complications of the central nervous system (CNS).

**Methods:** The cranial part of the cervical spinal cords of 180 rats were irradiated with 1 and 2 fractions of 12-C or photons. The target volume (field size: 10x15mm) was positioned either in the plateau-region of a 270 MeV/u 12-C-beam or in the middle of an extended Bragg-peak of a 140 MeV/u 12-C-beam. Photons were delivered by a linear accelerator (Siemens MPX, 15 MeV). Treatment fields were defined by a multileaf collimator with an aperture of 10x15mm. Thirty rats subdivided into 5 groups (n = 6) per treatment arm were irradiated with different doses (range: 12 Gy - 61 Gy). Two groups with 6 animals each served as controls. Animals were kept under daily observation with a special focus on radiation-induced acute reactions, latency and incidence of neurological disorders. Dose-response curves for the endpoint symptomatic myelopathy (paresis II) were established and the resulting values for ED<sub>50</sub> (dose at 50% complication probability) for radiation myelopathy were used to determine RBE-values.

**Results:** The overall median latency of myelitis was 167 days (range, 121-288 days). 80% of all symptomatic animals developed symptoms within 136 to 212 days after irradiation. The median latency after carbon ion irradiation was shorter in animals irradiated in the Bragg peak (T<sub>50</sub> = 157 days) than in those irradiated with plateau ions (T<sub>50</sub> = 178 days). The ED<sub>50</sub>-values for irradiation within the plateau-region were 17.1±0.8 Gy for single dose and 24.9±0.7 Gy for split dose experiments. Irradiation of spinal cords in the Bragg peak yielded ED<sub>50</sub>-values of 13.9±0.8, and 15.8±0.7 Gy for 1 and 2 fractions, respectively. The corresponding RBE-values were 1.43±0.08, 1.37±0.12 (for 1 and 2 fractions, plateau) and 1.76±0.05, 2.16±0.11 (for 1 and 2 fractions, peak), respectively.

**Conclusion:** In the split dose experiments, a clear fractionation effect was observed in the plateau phase, which allows more sparing of normal tissues. In contrast, no clinically relevant effect of fractionation was obtained for spinal cords when carbon ion irradiation was performed in the Bragg maximum.

## CHROMOSOMAL RADIOSENSITIVITY IN SECONDARY-PROGRESSIVE MULTIPLE SCLEROSIS PATIENTS

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### Abstract

*Objective:* To investigate chromosomal radiosensitivity of secondary progressive (SP) phase multiple sclerosis (MS) patients in comparison to a group of healthy individuals.

*Material and methods:* Chromosomal radiosensitivity was assessed in vitro with the G2 assay and the G0-micronucleus (MN) assay. For the G2 assay PHA stimulated blood cultures were irradiated with a dose of 0,4 Gy <sup>60</sup>Co γ rays in the G2 phase of the cell cycle. For the MN assay unstimulated blood cultures were exposed to 3,5 Gy <sup>60</sup>Co γ rays delivered at a high dose - rate (HDR = 1 Gy / min) or low dose – rate (LDR = 4 mGy / min).

*Results:* No significant differences in the number of chromatid breaks were observed between MS patients and healthy individuals. With the G0 – MN assay a higher spontaneous MN yield was found in MS patients. At HDR irradiation no significant differences were shown, while at LDR irradiation, MS patients were found less sensitive than healthy controls. The higher dose – rate sparing index obtained pointed to a better repair capacity in MS patients.

*Conclusions:* The radioresistance observed at LDR irradiation in the lymphocytes of SPMS patients may be due to an adaptive like response induced by the in vivo protracted oxidative stress exposure of lymphocytes associated with the SP stage of the disease. The modulation of radiosensitivity could also be due to abnormalities in cytokine signaling characterising the MS pathology.



ADVANCES IN DIAGNOSIS AND PATHOPHYSIOLOGICAL  
UNDERSTANDING OF RADIATION INJURIES. PROGRESS OF THE NAIMORI  
PROJECT OF THE EC

R. U. Peter

## ALTERED GENE EXPRESSION AFTER IRRADIATION WITH 25 kV VS. 200 kV X-RAYS: IN VITRO STUDIES

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Objective: For low-energy X-rays, e.g. used in mammography, a relative biological effectiveness (RBE) of 1 is generally assumed. Recently, however, several investigations reported a significantly higher RBE after exposure of cells to 25 kV X-rays compared to 200 kV X-rays for several endpoints. The present study was initiated to investigate changes in the expression level of a number of genes related to proliferation (PCNA) and DNA repair/apoptosis (PARP-1, p53, MRE-11, BAX, Ku-70) in human mammary epithelial cells (184 A1; ATCC, USA) after irradiation with 25 kV vs. 200 kV X-rays.

Methods: Radiation doses administered were 1, 2 or 5 Gy at dose rates of 1.8 Gy/min for 25 kV (Darpac 150-MC, Forward Raytech, UK) and 1.3 Gy/min for 200 kV X-rays (Isovolt 320/20, Seifert, Germany), respectively. Irradiation was carried out at room temperature. The analyses were performed after fixation of the cells at 1 h or 24 h after exposure. After isolation of total RNA and synthesis of cDNA with Oligo-dTs, gene expression levels were analysed by real time polymerase chain reaction (iCycler, Bio-Rad, Munich, Germany), detected by Sybr Green I and normalized to the expression of GADPH as a housekeeping gene.

Results: One hour after irradiation with 25 kV PCNA, PARP-1, BAX, and Ku-70 were down regulated to about 15-20%, and MRE-11 to about 5% of the control level (0 Gy). In contrast, P53 showed no significant change in expression level. After 24 h, P53 expression was also decreased compared to control cells. At this time, no further changes compared to 1 h were observed for the expression levels of the other genes studied. The changes were independent of the radiation dose.

After 200 kV X-irradiation, both at 1 h and 24 h, the selected genes displayed variations in the expression level similar to those observed after irradiation with 25 kV with the exception of MRE-11, for which up-regulation was observed at 1 h after exposure. Dose-dependence of the changes in expression levels was observed only at one hour after 200 kV irradiation

Conclusion: These preliminary data suggest that, using expression of the selected genes (except MRE-11) as an endpoint, the RBE of 25 kV X-rays is not significantly different from the RBE of 200 kV X-rays.

## ROLE OF TELOMERES IN THE FORMATION AND THE TRANSMISSION OF RADIATION-INDUCED CHROMOSOMES DAMAGES

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Alteration of telomere maintenance can lead to major consequences. We have shown the involvement of telomere loss in chromosomal instability, chromosome imbalances and gene amplification. In the long-term progeny of irradiated cells we observed telomere fusions and chromosome imbalances indicating that this chromosomal instability could be one step towards radiation-induced cell transformation. The two **objectives** of our current studies are : to characterize the interactions between telomere loss and radiation-induced breaks and to study the role of telomerase overexpression in the radiation-induced chromosomal instability

### Methods

The consequences of telomere loss were studied in a human cell model where a plasmid containing telomeric repeat sequences had integrated at a chromosome end. We analyzed how chromosome lacking one telomere could interact with radiation-induced chromosome breaks (2Gy Gamma Co60) and the mechanisms involved in the addition of telomeres onto the end of a broken chromosome.. The effect of telomerase over-expression on radiation induced chromosomal instability was tested in human fibroblasts.

### Results

After irradiation all the marker chromosomes having lost their telomeres get a new telomere via dicentric or translocation formations These first result shows an interaction between spontaneous and radiation induced breaks.

The ectopic over-expression of telomerase is associated with unusual spontaneous as well as radiation-induced chromosome stability. Long-term studies illustrated that human fibroblasts immortalized by telomerase presented an unusual stability for both chromosomes and for plasmid integration sites, both with and without exposure to ionizing radiation.

### Conclusion

The radiation-induced chromosome breaks can interact with the DNA free end generated by spontaneous telomere loss inducing a huge instability. Moreover, these results confirm a role for telomerase in genome stabilisation by a telomere-independent mechanism .Thus lost telomeres and telomerase activation show opposite effects on chromosome stability.

## ABERRANT GENOMIC DNA METHYLATION AND RADIATION-INDUCED GENETIC INSTABILITY

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### **Objective:**

Exposure to ionizing radiation results in a significant increase in the risk of radiation-induced acute myeloid leukaemia (r-AML). Ionizing radiation also induces genetic instability, and oxidative stress has been implicated. However, there is no direct evidence that radiation-induced genetic instability is involved in the radiation-leukaemogenic process, and this is in part due to the fact that the molecular mechanism(s) underlying radiation-induced genetic instability remains unclear.

Aberrant DNA methylation is associated with chromosomal instability, increased mutation rates, and a predisposition to cancer. Using a mouse model of radiation-induced r-AML, we are testing the hypothesis that ionizing radiation directly induces aberrant DNA methylation and genetic instability *in vivo*. The DNA methylation status of normal haemopoietic tissues before and after exposure to 3 Gy X-rays has been assessed, and compared to that of r-AMLs.

**Methods:** Genomic DNA methylation was assessed by HPLC analysis, by Southern blot analysis of HpaII digested genomic DNA and/or by bisulfite sequencing. Mice were exposed to a single acute leukaemogenic dose of 3 Gy X-rays.

**Results:** The DNA methylation status of a cancer is compared to that of control tissue, and it has been assumed that the methylation status of somatic cells is constant. However, the methylation status (% <sup>me</sup>CpG) of control spleen and kidney is 25% higher than control bone marrow, suggesting that more immature cells have a lower <sup>me</sup>CpG content than terminally differentiated tissues. The definition of DNA hypomethylation in cancer thus depends on what is used as a control. As r-AMLs are a bone marrow stem cell malignancy, normal bone marrow is the control.

The <sup>me</sup>CpG content of r-AMLs is highly variable and ranges between 86% to 112% of control, indicating that the r-AML genome can be hypo- and hyper-methylated. In contrast, the <sup>me</sup>CpG content of the Intra Cisternal A Particle (IAP) genomic DNA repeat sequence in the r-AMLs is 10% lower than control and the methylation of the Pax5 gene promoter (83%) much higher than control (0%), strong evidence that aberrant methylation plays a key role in radiation-leukaemogenesis. We have also assessed the <sup>me</sup>CpG content of normal bone marrow post *in vivo* exposure to 3 Gy X-rays, and detect a 6% decrease in genomic DNA <sup>me</sup>CpG 6-14 days post exposure indicating radiation directly induces aberrant DNA methylation *in vivo*.

**Conclusions:** We propose a hypothesis that ionizing radiation induces DNA hypomethylation and/or incorporation of uracil into DNA due to the oxidative cleavage of folate, and hypermethylation through the dis-regulation of cellular DNA methyltransferase (*Dnmt*) gene expression and/or a cellular response to radiation-induced DNA hypomethylation.

## MEDIUM-DOSE-RATE (MDR) INTERSTITIAL PARTIAL BREAST IRRADIATION RESULTS IN FREQUENT GRADE 3 OR 4 TOXICITY

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**Objective:** To investigate the radiation-induced toxicity of brachytherapy (BT) alone in early-stage breast cancer.

**Methods:** 70 women with stage I-II breast carcinoma participated in a BT study between 1987 and 1992. Following breast-conserving surgery, the tumor bed was irradiated with interstitial <sup>60</sup>Co sources, with an active length of 4 cm, with 10-mm center-to-center spacing, arranged in a single plane. The median number of inserted sources was 5 (range: 2-8), with a linear activity of 133-137 MBq/cm at the beginning of the study. The 50 Gy delivered dose at 5 mm from the surface of the <sup>60</sup>Co sources was administered during 10-22 h to the virtual postoperative lumpectomy cavity. For radiobiological considerations, the clinical target volume (CTV) was calculated with a 10-mm safety margin, resulting in a 72-cm<sup>3</sup> median CTV (range: 36-108 cm<sup>3</sup>) irradiated with a dose of 28 Gy. In the assessment of the skin and subcutaneous toxicity, the RTOG late radiation morbidity scoring system was applied. The radiosensitivity of the cultured fibroblasts was determined by clonogenic assay to check whether individual radiosensitivity played a role in the development of radiation-induced side-effects.

**Results:** The median follow-up was 12 years (range 10-15 years). The population of the final study comprised all survivors with tumor-free breasts (27 cases) and patients with breasts erroneously ablated/excised for misinterpreted radiation-induced sequelae (7 patients). 97% of the cohort (33/34) had grade  $\geq 2$ , and 59% (20/34) of them had grade  $\geq 3$  radiation-induced toxicity. By the end of the follow-up, 85% of the patients experienced grade  $\geq 2$  telangiectasis and 41% had grade 3 telangiectasis. 88% had fibrosis of some form, and 35% had grade  $\geq 3$  fibrosis. 41% of the cohort displayed fat necrosis, which was always accompanied by grade  $\geq 3$  fibrosis and/or telangiectasis. The radiosensitivity of the fibroblasts was increased in only 2/24 patients (in agreement with data published for the general population). Comparisons of our fibrosis prevalence data with those of others allowed an estimate of 0.47 h<sup>-1</sup> for the rate of recovery of DNA damage in the fibroblasts.

**Conclusions:** Interstitial MDR BT of the breast tumor bed with a limited CTV (median 72 cm<sup>3</sup>) and a total dose of 28 Gy is associated with a high rate (59%) of grade  $\geq 3$  radiation-induced toxicity at the end of a median follow-up of 12 years. A relatively high BT dose rate applied during a short overall treatment time and a possible geographical miss (close to skin implantation) might have contributed to the development of these sequelae.

## RADIATION HORMESIS IN THE RUSSIAN POPULATION AFTER THE CHERNOBYL ACCIDENT BY USING THE RANDOM COINCIDENCE MODEL

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### **Abstract:**

It is unclear whether cancer rate of the Russian population is increased after the Chernobyl accident. The Random Coincidence Model is used to calculate the radiation induced cancer caused by radiation levels from the Chernobyl accident in the Russian population. It involves the damage of nucleotides caused by spontaneous damage and radiological induced damage. The enhanced repair capacity related to enzyme production and scavenger mechanisms is also included in the model. Beside this, it has been approved to explain hormesis of the late effect of radiation. The data of exposure dose rate and number of population were arranged into pixels. Programming with adjustable interfaces was special written for the calculation which was done in 2,500 pixels matrices. For low LET radiation, parameters obtained from fitting of the epidemiological study of ARIP were used. The dose rates above background radiation from the Chernobyl accident in each time interval were applied into these epidemiological data fits. By conservative estimation, the generation rate of malignant cells and the tumor mortality of the Russian population can be inferred from that of the U.S. population. An excess of dose rates in a range of 0.01-14.31 mGy·a<sup>-1</sup> above background radiation can reduce about 0.03 % to 36.30% of the generation rate of malignant cells. The dose rate in the first time interval must be high enough to stimulate the repair system for resisting the damage in the next exposure. At low doses and low dose rates, cellular repair mechanisms are enhanced to compensate for the damage. The number of the generation rate of malignant cells is affected by not only dose rates but also the enhanced repair capacity. The reduction of the generation rate of malignant cells exists in the Russian Population after the Chernobyl accident.

## CYTOGENETIC LABORATORY AUTOMATION AND HIGH-THROUGHPUT ANALYSIS FOR RADIATION DOSE ASSESSMENT.

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To aid clinical management, early and individualized biological dose estimates are required in radiation disasters involving a large number of victims. The conventional lymphocyte metaphase-spread-based chromosome aberration analysis is time consuming and laborious. This study's objective is to systematically evaluate our needs so that a plan can be developed and implemented for an automated cytogenetic laboratory that triages chromosome aberration analysis and differentiates between those exposed to radiation and the "concerned public" following a disaster. We performed a detailed review of existing, commercially available, off-the-shelf technologies and equipments suitable for customization and/or reengineering to meet the needs of an automated cytogenetic biodosimetry laboratory. This review included studies addressing concept feasibility, work flow analysis, required possible process reengineering, bottleneck elimination in manual processing, and proof-in-principle experiments conducted for cytogenetic laboratory automation. We have already established metaphase-spread-based dicentric assays in accordance with international harmonized protocols. To enhance throughput and reduce costs, the components of an automated cytogenetic biodosimetry laboratory include sample and reagent tracking by bar coding, an automated high throughput lymphocyte isolation system for short term culturing, sample processing for metaphase spread preparation using an automated metaphase harvester and a spreader, an automated microscope slide washing module, an automated slide stainer and integrated coverslipper, a high throughput metaphase finder, and multiple satellite microscope scoring systems for scoring and analysis. In addition, quality control and quality assurance can be achieved by using instruments equipped with data loggers. Using an automated cytogenetic laboratory will increase throughput by at least ten-fold and reduce cost per sample analysis for radiation dose assessments. Up to 500 samples per week can be analyzed in triage mode where chromosome aberration analysis is restricted to 20-50 metaphase spreads per sample compared to the conventional approach of 500-1000 spreads.

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## SEQUENTIAL IMPLICATION OF LYMPHOID AND STROMAL CELLS IN FL VARIATIONS AFTER IRRADIATION IN MICE.

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**Objective:** We previously showed that plasma FL concentration was increased after irradiation and that this increase was correlated to the numbers of bone marrow surviving progenitor, reflecting bone marrow function during radio-induced aplasia. We thus investigate which organ or cells are implicated in FL concentration increase post irradiation.

**Methods:** Balb/C mice and NOD/SCID mice were irradiated at doses of 6 Gy and 4 Gy respectively. At various time after irradiation, bone marrow, spleen, liver, brain, thymus and blood cells were analysed for FL mRNA expression by RT-PCR. We also quantified the FL protein in each tissue.

**Results:** We showed that in bone marrow, spleen, liver and brain of Balb/C mice, only weak variations in FL mRNA expression were observed. Major changes were observed in WBC with a  $6,33 \pm 0,68$  fold increase on day 3 after irradiation, and in thymus with a first  $3,68 \pm 0,88$  fold increase on day 3 followed by a second  $10,8 \pm 4,3$  increase on day 21. In order to confirm the implication of these tissues in FL regulation after irradiation, we performed FL quantification at day 3 in these organs. After irradiation, no significant variation were observed in brain and liver, while a significant decrease in FL quantity was observed in the bone marrow. By contrast, an increase in FL quantity was observed in spleen, thymus and WBC. These results suggest that T lymphocytes are strongly implicated in radio-induced plasma FL increase. We thus investigate variation in FL concentration in irradiated NOD/SCID mice, which are devoided of T lymphocytes. FL mRNA expression was different as compared to Balb/C mice with a 4-fold increase in FL mRNA expression at day 28, and a higher FL mRNA expression in WBC. A weak expression was observed in the thymus. Despite these major changes of FL mRNA expression as compared to Balb/C mice, the quantity of FL detected on day 3 in these organs was similar in the 2 strains of mice. Thus, the lack in T lymphocytes modified the pattern of FL mRNA expression in the organs without affecting the FL production.

**Conclusions:** Our results suggest that T lymphocytes are not solely implicated in radiation induced increase in FL concentration, and that other cell types, such as endothelial cells or stromal cells might be strongly implicated.



## PLASMA FL CONCENTRATION VARIATION AFTER HETEROGENEOUS IRRADIATION IN MICE.

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**Objective:** We previously showed that plasma FL concentration was bio-indicator of the number of surviving progenitor both in the bone marrow and in the spleen after homogenous total body irradiation. In order to determine how FL can be used as a bio-indicator of heterogeneous bone marrow damage, we used a model of heterogeneous irradiated Balb/C mice.

**Methods:** Mice were irradiated with 25%, 50%, 75% or 100% of bone marrow exposed to 4 Gy, 8 Gy, or 12 Gy of gamma rays ( $^{60}\text{Co}$ ). Twice a week during four weeks, 5 mice of each configuration were euthanased and blood cell count, formula, plasma FL concentration, exposed and non exposed bone marrow Colony Forming Cells (CFC) and spleen CFC were defined.

**Results:** An early decrease in WBC numbers was observed after irradiation whatever the dose and the configuration. This decrease and the recovery were dose dependent and depended on the size of the exposed medullar territory. Results also showed an increase in plasma FL concentration after irradiation whatever the dose and the configuration. In fact, the plasma FL concentration increased with the dose and the size of the exposed medullar territory. As for WBC, a decrease in absolute CFC numbers that depends on the irradiation dose and the size of the exposed medullar territory was observed in exposed bone marrow. By contrast, in unexposed bone marrow as in spleen (not exposed) results indicates that the absolute CFC numbers could decrease after irradiation. These results suggest a modification of the hematopoietic progenitor distribution after irradiation. We then correlated the total CFC numbers in mice (ie irradiated bone marrow CFC plus non exposed bone marrow CFC plus spleen CFC) to the plasma FL concentration. Results demonstrate that plasma FL concentration was negatively correlated to the total CFC numbers whatever the dose and the percentage of exposed bone marrow.

**Conclusion:** These results demonstrate that plasma FL concentration reflect the number of residual CFC in mice after irradiation whatever the dose, the size of the exposed medullar territory and the time after irradiation. It thus reflects the total bone marrow damage and the ability of hematopoietic recovery. This bio-indicator would be useful both in accidental irradiation situations to help in the therapeutic choice and in the follow-up of the radiotherapy patient.

## BYSTANDER AND ADAPTIVE RESPONSES IN TARGETED CELLS

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The use of microbeam approaches has been a major advance in probing the relevance of bystander and adaptive responses in cell and tissue models. Our own studies at the Gray Cancer Institute have used both a charged particle microbeam, producing protons and helium ions and a soft X-ray microprobe, delivering focused carbon-K, aluminium-K and titanium-K soft X-rays. These radiations can be easily targeted to individual cells within populations, or subcellular locations. Using these techniques we have been able to build up a comprehensive picture of the underlying differences between bystander responses and direct effects in cell and tissue-like models. What is now clear is that bystander dose-response relationships, the underlying mechanisms of action and the targets involved are not the same as those observed for direct irradiation of DNA in the nucleus. Our recent studies have shown bystander responses even when radiation is deposited away from the nucleus in cytoplasmic targets.

Adaptive responses follow similar phenomenology to bystander responses in that they are observed after low dose exposures. We have performed studies comparing bystander-mediated cell killing with adaptive responses. The interaction between bystander and adaptive responses may be a complex one related to dose, number of cells targeted and time interval. Overall, however, pre-treatment of cell populations with adaptive doses leads to protection from a subsequent bystander treatment. Adaptive responses are known to involve stimulation of DNA repair processes. Importantly, we have good evidence that the ability to efficiently repair DNA damage also influences the level of bystander effect which is observed.

## STUDY ON THE RATE OF RECOMMENDED STANDARDS AT THE DIAGNOSTIC RADIOLOGY UNITS OF THE HOSPITALS AFFILIATED TO THE MAZANDARAN UNIVERSITY OF MEDICAL SCIENCES

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**Background and Purpose:** The medical services which are given to the people, in production of medical equipments aim is not only manufacturing good quality instrument, but also providing proper of medical care and services. The sensitivity of diagnosis is giving medical services is such a way that in case of lack of accuracy would be hazardous. In assessment of existing hygienic and treatment conditions the first crucial step is evaluation of the medical equipment the methods, efficiency and productivity and their combined performance in treatment of each disease. For this reason, evaluation of the obtained results should be compared with commended standards. Aim of this study is to evaluate the condition of radiology units of the hospitals affiliated to the Mazandaran University of Medical Sciences and compare with the standards recommended by (ICRU, NCRP, ICRP) since the radiologic units are the most expensive because of provided equipment. Specialists and the other staff and the need space, on the other hand radiology equipments are daily changing due to development of technology, and radiology devices should be the advanced one, because of the real need to meet the proper diagnosis.

**Materials and methods:** In this study different variables for the evaluation of diagnostic radiology condition were studied, Using the newest standards recommended by recognized organization, questionnaire was designed, comprising three main sections first section about space condition, second, about personal information and protective barrier for the patients and the staff, the third, about the condition of radiology equipment and dark room devices. In this study, in addition to filling of questionnaires, electronic dosimeter and thermometer was used. The obtained data were compared with the standards rate and the conditions of radiology unit for medical equipments with proper quality and quantity were evaluated.

**Results:** Aim of this study was to know the existing conditions of radiology units of the hospitals affiliated to the Mazandaran University medical sciences, for having regarding space, equipment and staff, which was done by referring to the educational and non educational hospitals of Mazandaran province. After direct observation interviewing questionnaire was filled. The obtained data showed that, the radiologic units under study were beyond the recommended international standards, to such an extent that, such hospital 50% of the recommended standards.

**Conclusion:** Considering the data from this study, it was shown that no one of the dark room was standard and enough alerting device in 63% of these units there was no filing system about protection barrier against radiation protective barrier. Rate of defect was observed 60%, 51% and 47% for radiology room, protective barrier and lack of radiation absorption respectively. Considering the obtained data, periodic and having recommended standard is much required.

**Key words:** Diagnostic radiology, dose meter, radiation standards, protection against radiation, ionizing ray.

## SUSCEPTIBILITY TO UVB CARCINOGENESIS

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Nonmelanoma skin cancers are the most frequent human malignant tumors. Their multiplex development is common. The importance of environmental and genetic factors in tumor development has been proven by epidemiological studies. The most important environmental factor was found to be the UVB irradiation. The proper function of proteins essential in defending cells in the skin from UVB is necessary to prevent tumor formation. Thus polymorphisms of these proteins can increase one's susceptibility to this low wavelength portion of sunlight. Polymorphisms of genes that alter control and correction of UVB induced DNA damage (nucleotide excision repair, detoxification, melanin synthesis), cell cycle regulation (proliferation, apoptosis) or immune elimination are the best candidates for studying the genetic background of increased susceptibility.

We studied the incidence of multiplex NMSCs at our clinic in the last 5 years to collect a cancer population for studying the genetic susceptibility caused by polymorphism of XPD, glutathione transferase, catalase, myeloperoxidase, melanocortin 1 receptor, p53-72P, IL10 and TNF-alpha.

Over the examined period (29.06.1999-12.04.2004.) 2621 tumors of 1734 patients were removed by surgery and were examined histopathologically. 453 patients showed multiplex tumors and 1347 tumors were collected from these patients. The incidence of tumors increased with time and age of patients. A population of patients with multiplex tumors (66 patients) developed in younger ages was collected and further examined by a questionnaire to study the possible effect of environmental factors. Blood samples were also collected to investigate genetic factors.

Studying individual susceptibility to the effects of UVB irradiation may help design a better individual prevention.

## RADIATION-INDUCED CNS DAMAGE IN RATS AND ITS AMELIORATION BY STEM CELL TRANSPLANTATION

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Radiation damage to the central nervous system involves defects in neurogenesis, possibly due to high radiosensitivity of the neural stem cells, which is now well accepted to persist in the adult brain. Therefore, stem cell therapy can be a potential modality in modification of radiation-induced neural lesions. The influence of the transplantation of stem cells on the development of radiation myelopathy has been examined in rats. A 12 mm section of the cervical spinal cord of five week old female rats was locally irradiated with a single dose of 22 Gy of <sup>60</sup>Co  $\gamma$ -rays. This dose of radiation is known to produce myelopathy in all animals within 180 days after irradiation. After irradiation the animals were subdivided into two groups and at 90 days after irradiation neural stem cells or saline (controls) were injected into the spinal cord at two sites, 6 mm apart, at the centre of the irradiated length of spinal cord in a 2 $\mu$ L volume. Controls received two injections of 2 $\mu$ L saline. All rats received 10mg/kg Cyclosporin (10mg/ml) daily IP. All animals in control group developed paralysis within 167 days after irradiation while paralysis-free survival of the rats in stem cell transplanted group was 34% at 183 days.

In a separate study the influence of the transplantation of stem cells, isolated from bone marrow, on the development of radiation myelopathy was examined. Stem cells were isolated from bone marrow and expanded in vitro. Stem cells were transplanted via sublingual vein 90 days after spinal cord irradiation. Transplantation procedure was repeated for three consecutive days. Paralysis-free survival for stem cell transplanted animals was 66.7% at 210 days after irradiation.

It was concluded that stem cell transplantation significantly ameliorated radiation-induced myelopathy in rats.

## DOSE DEPENDENCE OF LATENT TIMES AFTER RADIOTHERAPY

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The expression of radiation injury is a time dependent process and the latent period is referred to the time from irradiation until a specific endpoint is expressed. Expression of clinical endpoints is dependent on the cell type in first instance. Normal tissues can be divided into two main categories: hierarchical and flexible.

In hierarchical tissue systems, which usually have rapid cell turnover, latency time is determined by the lifespan of the mature cells. This may vary from a few days in case of intestinal epithelium to more than 100 days in erythrocytes. The tissues with rapid cell turnover such as intestine, skin demonstrate shorter latent periods than tissues with slow cell turnover such as lung kidney and spinal cord.

There is no dose dependency for latent period in early responding hierarchical tissues, however, the intensity and duration period of the lesions are dose dependent.

The flexible tissue types (those with a slow rate of renewal) such as kidney, liver, lung and CNS are usually not well characterised as the hierarchical tissues. The rate of development of radiation reactions in these tissues depends on the radiation dose. The intensity of the lesions is also dose dependent.

Late effects are not confined only for late responding tissues. They can develop in early responding tissues as well and there are usually more than one type of lesion that can develop in the same organ, albeit with different underlying mechanism and latency period. For example pulmonary fibrosis follows radiation pneumonitis of lung; skin fibrosis, atrophy or telangiectasia follow moist desquamation of the skin. Overall, the latent times for late effects show a great variation and it can even be years after irradiation as in cases of telangiectasia, lymphedema or radiation myelopathy.

The knowledge of latent times is crucial with important clinical implications that can influence clinical decision-making.

## BRONCHOPULMONARY SYSTEM OF THE COPD PATIENTS LIVING UNDER THE INFLUENCE OF INDOOR RADON-222

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The study contains complex clinical characteristics of a bronchopulmonary systems of 372 inhabitants of the Rokitnanskiy district of the Kyiv region with COPD. Mild equivalent exposure doses of bronchopulmonary tissue for the explored persons have made from 13 up to 260 mSv per one year, in more than 70 % cases mild dose value is more than in the Ukraine (31mSv/y). In 11 % of cases equivalent exposure doses on bronchopulmonary tissues are more than 100 mSv/y. Methods: clinical, screening, function, roentgenological, endoscopical. Among the surveyed inhabitants of Rokitnanskiy district of the Kyiv region the considerable abundance of COPD takes place. The basic nosological form of COPD among the surveyed inhabitants of Rokitnanskiy district of the Kiev region is chronic obstructive bronchitis. Primary chronic development of COPD was typical for this category of patients. In the structure of ventilation violations bronchoobstructive are dominated. An appreciable expressiveness pneumofibrotic and emphysemic changes of bronchopulmonary tissue by results of X-ray inspections is marked. The endoscopical data testify to the expressed catarrhal-sclerotic changes of a mucosa of bronchuses and, more than in 75 % of cases, - purulent changes of a bronchial secret. COPD are combined from significant pathology of cardiovascular, digestive and endocrine (thyroid) systems. Under the pulmonological screening of the population lung cancer for the first time was diagnosed in 4 cases.

## RELATIONSHIP BETWEEN CELL CYCLE ARREST AND ABERRATION YIELD IN NORMAL HUMAN FIBROBLASTS AFTER X-RAY AND C-ION EXPOSURE

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*Objective:* To study the influence of cell cycle arrest(s) on the expression of aberrations in normal human fibroblasts after X-ray and C-ion irradiation.

*Methods:* Confluent G<sub>0</sub>/G<sub>1</sub>-phase AG1522 cells were exposed to X-rays, 195 MeV/u C-ions (LET=16.6 keV/μm) and 9.5 MeV/u C-ions (LET=170 keV/μm). Directly after irradiation cells were reseeded in medium containing BrdU. At multiple time points post-irradiation the cumulative BrdU-labeling index, mitotic index and aberration frequency were measured. Based on these data the total amount of damage induced within the entire cell population was estimated by means of a mathematical analysis (Scholz et al., IJRB 74, 325-331, 1998).

*Results:* All radiation types have a pronounced effect on the cell cycle progression of fibroblasts. They result in delayed entry of cells into S-phase and into the first mitosis, and they cause a dramatic reduction in the mitotic activity. Furthermore, a large proportion of cells suffers a chronic arrest in G<sub>0</sub>/G<sub>1</sub>. With increasing dose and LET these effects are more pronounced. Analysis of chromosomal damage in first-cycle cells at multiple time-points post-irradiation shows, that the frequency of aberrant cells and aberrations increases with sampling time, but this effect is less pronounced than in other cell systems. More importantly, when the data for the whole cell population are analysed, it becomes evident that only a few damaged fibroblasts are able to progress to the first mitosis, a response attributable to a longterm arrest of injured cells in the initial G<sub>0</sub>/G<sub>1</sub>-phase. For all endpoints studied the effectiveness of 195 MeV/u C-ions was similar or slightly higher than X-rays leading to RBEs in the range of 1 to 1.4, while for 9.5 MeV/u C-ions RBEs in the range of 2 to 4 are estimated.

*Conclusions:* The obtained RBE values are consistent with those reported for cell inactivation and fibrosis related parameters in the same cells (Fournier et. al., IJRB 77, 713-722, 2001). Thus, with respect to the application of C-ions in radio-oncology, there is no indication of a higher RBE for the induction of cytogenetic damage in healthy tissue surrounding a tumor than for other effects that have been investigated up to now. Furthermore, the data obtained for the fibroblast population as a whole clearly demonstrate, that injured cells are rapidly removed from the mitotically active population through a chronic cell cycle arrest. This observation is in line with the view that the chronic arrest is a specific strategy of fibroblasts to minimize the fixation and propagation of genetic alterations.



PP06/0258: OVEREXPRESSION OF EPIDERMAL GROWTH FACTOR PREDICTS REDUCED SURVIVAL IN PATIENTS WITH STAGE III AND IV HEAD AND NECK CANCER

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Objective: The elucidation of molecular pathways and possible biological mechanisms in determining DFS and OAS have been investigated in patients with advanced SCCHN

Materials and Methods: 58 patients with T3/T4 stage SCCHN, who were treated with hyper fractionated radiation, were studied. Using immunohistochemical staining, EGFR, TGF-alpha and Erb B-2, Ki67, topoisomerase II alpha, apoptosis (bc 12, Bax, Bel-X, Mcll, p53 and Bag-1) and angiogenesis, were studied.

Results: Overexpression of EGFR noted in 29 (50%) cases. 17 out of 29 (57%) developed recurrence after 3 yrs. compared to 6 out of 29 (21%) with negative or weak EGFR expression. Four out of 29 (15%) with EGFR overexpression lived over 5 yrs. compared to 12 out of 29 (42%) with negative or weak expression. Multivariate analysis showed overexpression of EGFR was a strong independent predictor of both reduced DFS ( $p < 0.0005$ ) and OAS ( $p < 0.0001$ ). TGF-alpha overexpression was associated with reduced DFS and OAS with  $p < 0.059$ .

Conclusion: Cell signal transduction mediated by the EGFR tyrosine kinase pathway is a dominant biological mechanism governing progression of SCCHN. Strategic use of agents blocking EGFR overexpression should be considered as part of standard treatment of advanced SCCHN.

Keywords: SCCHN

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## SOLAR UV RADIATION -- EXTENSION TO THE EARTH'S ORBIT AND BEYOND

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Solar UV radiation is a driving force of the life on the solar system, selected photons of the radiation participate in specific beneficial chemical reactions, however, UV photons serve also as constraints in certain biological processes. In the evolution of the solar system the ozone layer in the Earth's atmosphere exerted a protective effect against the damages due to the UV (mainly shorter wavelength UVB) photons by attenuating and modifying the solar spectral irradiance. On the Earth's orbit or on the surface of an other planet the atmosphere contains no ozone at all or it exists in a very low concentration. Extraterrestrial solar radiation possesses about 3 orders of magnitude higher irradiance, thus the biological effect (both damaging and beneficial) of the short wavelength components in the solar (extraterrestrial) radiation has of specific interest.

Nucleic acids are essential components of the living systems and proved to be the major targets for the UV photons. The structural and functional stability of nucleic acids plays very important role in several problems of the recently established astro/exobiology, searching e.g. the early evolution of life on the Earth, the possibility of the interplanetary transport of life or the planetary safety. In addition, studies of the stability of nucleic acids in extraterrestrial conditions can provide information on the future of the Earth's biosphere in the progressive ozone reduction.

Aiming to assess the human UV hazard on the Earth and to elucidate the role of UV photons in the problems of astro/exobiology, attempts have been made in wide international cooperations to quantify the biologically effective UV dose with DNA based biological UV dosimetry. For this purpose simple microorganisms and/or model molecules have been used as detectors: bacteriophage T7, isolated phage-DNA and polycrystalline uracil. Measuring results obtained in short-term and long-term monitoring of the terrestrial solar radiation (diurnal profile and annual profile respectively) will be presented. In addition the problems of biological UV dosimetry in space simulation conditions will be discussed.

## HETEROGENEOUS CYTOGENETIC DOSE ASSESSMENT OF PATIENTS TREATED BY RADIOTHERAPY

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**Objective :** Biological dose estimate following exposure to ionizing radiation is based on the yield of dicentrics observed in the lymphocytes of patients. This technique is especially precise if the exposure is recent and homogeneous. But in most of accidental overexposure cases, irradiation is heterogeneous leading to an under estimation of the dose received by the fraction of irradiated lymphocytes. Therefore some mathematical models have been developed to evaluate dose heterogeneity (QdR, Dolphin). They have been successfully validated by *in vitro* studies. The objective of this study is to test these models on an *in vivo* study.

**Methods:** blood samples were collected from patients undergoing fractionated radiotherapy for cervical cancer before starting the treatment, after the first 2 Gy fraction, after the 12 Gy fraction, at the end of their treatment and 6 months after. Six patients were treated with a large field radiotherapy whereas two others had a reduced one. Dicentrics were observed on these samples.

**Results:** An increase in the whole body dose is observed after the 2 Gy fraction with an average biological dose of 0.21 Gy for the large field patients and 0.10 for the reduced irradiation field. At the end of the treatment, a whole body dose of 3-4 Gy is measured. Using the mathematical models Dolphin and QdR, the dose delivered to the irradiated fraction of lymphocytes is between 5 and 6 Gy for the large field radiotherapy whereas it is only 2 Gy for the two other patients. The percentage of irradiated lymphocytes is estimated to 80 % for the large field irradiation and only 15 % in the other configuration.

**Conclusion:** the use of mathematical models allows the detection of dose heterogeneity. A strong difference was observed between the two types of irradiation as dose heterogeneity is detected after the 12 Gy fraction for large field exposed patients whereas it is only detected at the end of the treatment for the other kind of exposure. This is explained by a reduction of the irradiated lymphocytes percentage when the treatment is applied to a reduced volume of the neck.

## NON-TARGETED EFFECTS OF IONIZING RADIATION - IMPLICATIONS FOR RADIATION PROTECTION

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A basic paradigm in radiobiology is that, after exposure to ionising radiation, the deposition of energy in the cell nucleus and the resulting damage to DNA, the primary target, are responsible for the harmful biological effects of radiation. The radiation-induced changes are thought to be fixed already in the first cell division following the radiation exposure and health effects are considered to result as a consequence of clonal proliferation of cells carrying mutation in specific genes.

These basic assumptions have now been challenged by the new findings on radiation-induced genomic instability and bystander effects showing that deleterious effects can be observed also in cells that were not irradiated. Genomic instability and bystander effects are observed already after very low doses. In fact, some dose response data indicate that the relative contribution of these indirect effects as compared to damage caused by direct hits may well be more pronounced in the low dose region, thus giving some support for a potential supralinear response at the low-dose region. The non-targeted effects also provide a potential mechanistic explanation for the development of non-cancer diseases.

The cancer risk of low doses of ionising radiation will probably never be fully elucidated by epidemiological studies, as this would require very large populations and accurate dosimetry. The dosimetry of protracted exposures is even more demanding than the dosimetry for single exposures. Uncertainties in dosimetry of epidemiological studies make it more difficult to observe a dose response, which in turn tends to lead to lower risk estimates. Biological modelling of radiation carcinogenesis may offer a tool to study the risk in the low-dose region. The input data should contain not only the conventional direct effects, but also the non-targeted effects which may be important modifiers of radiation response at the low dose region.

The genomic instability and bystander endpoints are both transmissible (mutational) and non-transmissible (lethal). The balance of these in the different cellular systems may lead either to an increased or decreased risk. Some scientists indeed argue that these non-targeted radiation effects are in fact part of the adaptive response to ionising radiation. More research is needed on the delayed damage response systems, such as adaptive response and premature differentiation.

Individual sensitivity seems to play a role both in genomic instability and bystander effects. Genotypes that have a more effective apoptotic response seem to be less predisposed to the development of malignancy. The genetic basis for this variability requires further research.

## RADIOBIOLOGICAL MODELLING OF THE CONFORMAL RADIOTHERAPY OF INFILTRATIVE TUMOURS BASED ON CLONOGENIC CELL DENSITY

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The object of the study was to compare by modelling the predicted effectiveness of conventional strategies for the radiotherapy of infiltrative tumours and a novel strategy conforming radiation dose to clonogenic cell density.

Three treatment strategies were compared: uniform irradiation at full dose to full volume; uniform irradiation at full dose to an inner volume and at reduced dose to an outer volume; uNon-uniform irradiation with dose conforming to the estimated distribution of clonogenic cell density. Both deterministic modelling and Monte Carlo simulation were used. The strategies were compared for an idealised scenario of a small brain tumour diffusively infiltrating adjacent tissues.

The non-uniform strategy is optimal under ideal conditions and provides significant therapeutic advantage. This strategy is also superior when the cell density function is only approximately known and varies between patients but is inferior to conventional strategies if the extent of tumour cell infiltration is seriously underestimated.

Good prospects exist for improved radiotherapy of infiltrative tumours by conforming radiation dose to tumour cell density. Methods are required to provide approximate estimates of the tumour cell density distribution for particular tumour types and for individual patients.

## MULTISTEP MODEL FOR RADIATION INDUCED BCC TUMORIGENESIS IN *PTCH1*<sup>NEO67/+</sup> HETEROZYGOUS MICE

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**Objectives:** Loss-of-function mutations in *Patched* (*Ptch1*) are implicated in constitutive activation of the Sonic hedgehog pathway in human basal cell carcinomas, and inherited *Ptch1* mutations underlie basal cell nevus syndrome (BCNS), in which a typical feature is multiple basal cell carcinoma (BCC) occurring with greater incidence in portals of radiotherapy. Mice in which one copy of *Ptch1* is inactivated show increased susceptibility to spontaneous tumor development, and hypersensitivity to radiation-induced tumorigenesis, providing an ideal *in vivo* model to study the typical pathologies associated with BCNS. **Methods:** We examined BCC development in control and irradiated *Ptch1*<sup>neo67/+</sup> mice. *Ptch1*<sup>neo67/+</sup> mice and wild type littermates of both sexes were whole-body irradiated with 3 Gy of X rays as newborns (4 days) or adults (90 days). In addition, two months old mice were subjected to local irradiation of the dorsal skin with a single dose of 4 Gy of X rays. **Results and Conclusion:** We show that unirradiated mice develop putative BCC precursor lesions, *i.e.*, basaloid hyperproliferation areas arising from both follicular and interfollicular epithelium, and that these lesions progress to nodular and infiltrative BCCs only in irradiated mice. Data of BCC incidence, multiplicity, and latency support the notion of epidermal hyperproliferations, nodular and infiltrative BCC-like tumors representing different stages of tumor development. This is further supported by the pattern of p53 protein expression observed in BCC subtypes, and by the finding of retention of the normal remaining *Ptch1* allele in all nodular, circumscribed BCCs analysed compared to its constant loss in infiltrative BCCs. Our data suggest chronological tumor progression from basaloid hyperproliferations to nodular and then infiltrative BCC occurring in a step-wise fashion through the accumulation of sequential genetic alterations. (Partially supported by the Radiation Protection Action of the EC, contract FI6R-CT-2003-508842)

## ACTIVITY OF CELLULAR ANTIOXIDANT ENZYMES AFTER LOW DOSE IONIZING RADIATION AND OTHER ENVIRONMENTAL TOXIC AGENTS

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**Objective:** The antioxidant enzymes are involved in the defence against reactive oxygen species (ROS) and may also contribute to the protection against cellular oxidative stress. The purpose of our study was to examine the activity of various antioxidant enzymes after exposure to ionizing radiation and different chemical agents. We studied also whether the antioxidant enzymes are involved in the prevention of the DNA damage and apoptosis caused by environmental agents.

**Methods:** Cells were exposed alone and combined to alpha or gamma irradiation and cadmium ( $\text{CdCl}_2$ ) or glass fibre (GF). The activity of antioxidant enzymes was measured: superoxide-dismutase (SOD)-RANSOD-, glutathione-peroxidase (GPx) – Bioxytech-kits, spectrophotometrically, catalase activity (CAT) that was measured by the decrease of  $\text{H}_2\text{O}_2$  on 240 nm. The glutathione (GSH) level was measured according Tietze. DNA breaks were detected by the alkaline Comet-assay was performed by Tice. Apoptotic cells were detected by the TUNEL assay using the In situ cell death detection kit AP (Roche).

**Results and conclusions:** The activity of the antioxidant enzymes decreased in the function of the dose and the time after irradiation. The damage of the antioxidant enzyme system developed in the first 3 hours but did not normalize even 24 hours after irradiation. Extracellular ascorbic acid modified the activity of antioxidant enzymes in a dose dependent manner.

In primary cultures of pneumocytes and macrophages reduced SOD activity was found after one week treatment of rats instilled by glass fiber. In contrary the SOD activity increased after 1 month treatment with GF more in the macrophages and less in the pneumocytes. These results suggest that cells adapted to the glass fibres less than one month. SOD activity decreased in the pneumocytes and macrophages treated with various concentrations of cadmium-chloride. Cadmium modifies DNA damage repair. The level of the DNA breaks and their repair were in negative correlation with the level of the antioxidant enzymes. We found a good linear correlation between the apoptotic index measured after 24 h and the antioxidant enzyme activities measured immediately after irradiation. At low doses of radiations or low concentrations of chemical agents antioxidant defence could have a greater role as in case of higher doses.

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## ENHANCEMENT OF TUMOR RADIOSENSITIVITY BY ELECTROPORATION: A PROMISING STRATEGY FOR CANCER RADIOTHERAPY

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Investigations in our laboratory on  $\gamma$  radiation effects on liposomal and intact cell membrane have shown radiation dose dependent changes in lipid peroxidation and in lipid bilayer fluidity as measured by MDA and fluorescence probe methods. *In vitro* studies on radiation effects on thymocytes obtained from mouse have shown a loss of membrane permeability with the increasing radiation dose as measured by FDA probe. Results on radiation induced loss of membrane permeability and lipid peroxidation showed a similar pattern of induction of apoptosis in thymocytes as observed by internucleosomal breakage reflected in regular DNA fragmentation by gel method. These studies have suggested that a correlation existed between radiation induced membrane oxidative damage and induction of apoptotic death in irradiated thymocytes. Studies were further extended to investigate the radiosensitivity of thymic lymphoma cells from mice and viability of cells was found to decrease with the increase in radiation dose within therapeutic range as determined by dye exclusion method. Exposure of cells to high intensity brief electropulses is known to induce permeabilization of membrane and therefore, it was considered a potential strategy to enhance the radio-cytotoxicity of tumor cells by electroporation. *In vitro* studies have shown that combined treatment of tumor cells like fibrosarcoma, EAC and TL to radiation and electroporation significantly increased the cell killing suggesting a membrane injury based new approach to increase tumor cytotoxicity for more effective treatment of cancer. The efficacy of the combined treatment of radiation and electroporation was examined *in vivo* on growth of transplanted fibrosarcoma tumor on mouse and results have shown remarkable tumor regression. This talk will focus on targeting membrane as a promising strategy for enhanced tumor cytotoxicity by radio-electroporation for improvement of cancer radiotherapy.

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## BONE MARROW VEGFs AND VEGF RECEPTORS mRNA EXPRESSION AFTER RADIATION-INDUCED APLASIA IN MICE.

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**Objective :** Some vascular endothelial growth factor isoforms (VEGF120 and VEGF164) are regulators of haematopoiesis. The functional role of type 2 VEGF receptor (VEGFR-2) expression on haematopoietic stem cells (HSC) in adult bone marrow remains unclear whereas a role for type 1 VEGF receptor (VEGFR-1) during haematopoietic reconstitution after bone marrow suppression in adults has been identified. The role of neuropilin-1 (NP-1), a VEGFR-2 co-receptor has never been investigated. The goal of this study was to evaluate whether there is a relationship between the expression of VEGFs and their receptors (VEGFR-1, VEGFR-2 and NP-1) and bone marrow haematopoietic regeneration after radiation-induced aplasia in mice.

**Methods :** Mice were submitted to a whole body irradiation of 4 Gy. VEGFs, VEGFR-1, VEGFR-2 and NP-1 mRNA levels were evaluated by RT-PCR at several delays after irradiation : on days 3 and 5 when the depletion of haematopoietic cells was almost complete, on day 7, when bone marrow recovery started and on days 10 and 15, when bone marrow cellularity was almost normal.

**Results :** The levels of VEGFs isoforms 120 and 164 mRNA were similar in control and in irradiated mice, whatever the delay tested. VEGFR-1 mRNAs were not detected in control bone marrows and were present in 2 of 6 irradiated mice at day 3 after irradiation. A weak expression of VEGFR-2 mRNA was present in control mice and increased in irradiated animals between day 3 and day 7 after irradiation. The levels of NP-1 mRNA were very high at day 3 and 5 post-irradiation as compared to control mice.

**Conclusion :** These data indicate that an increase of NP-1 and VEGFR-2 mRNA levels precedes the haematopoietic recovery. Whether these VEGF receptors are involved in bone marrow reconstitution after irradiation and the identification of cells responsible for their expression are under investigation.

## BIOLOGICAL DETECTION OF IONIZING RADIATION USING INDUCTION OF REPORTER GENES IN *DROSOPHILA MELANOGASTER*

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The phenomenon of activated gene expression in response to irradiation was shown for some DNA repair and apoptosis genes. The purposes of our investigation were to study radiation activated sequences in *Drosophila* and to design radiation sensitive test-system for low-dose environmental detection.

We screen P-element enhancer-trap insertions in *Drosophila melanogaster* genome from our collection. These constructs contain a reporter beta-galactoisidase gene reflecting expression patterns of tissue's enhancer activity. We have found few insertions of reporter genes that in further investigation were elucidated as induced by gamma irradiation. As it shown for the P[1ArB] insertion it is possible to detect doses about 1 Gy with confidence and to estimate doses about 5 cGy using these flies.

As a result of advances in cDNA microarray technology, whole genome studies of radiation activated reading frames were carried out in various organisms. This allowed us to develop strategy of designing transgenic flies capable to detect low doses more precisely. The technique of genetic engineering allows to amplify a signal of the reporter gene using cascade of Gal4-UAS and to design constructs in which activity of the reporter gene will be kept in generations after induction. In addition there are genes inducible (including in specific way) in response to chemical mutagens. It defines essential advantages of biological mutagen detection as mutagenic activity of many substances may be estimated by universal way in a uniform scale. It will be discussed a problem of most advisable strategy of biological dosimetry.

## ATM: NEW INSIGHTS INTO THE DNA DAMAGE RESPONSE

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The cellular response to DNA damage is critical for cellular life and homeostasis. The intricate signaling network mobilized by DNA damage is perhaps one of the broadest and most vigorous cellular responses to external or internal stimuli. It spans numerous pathways linking DNA repair, cell cycle checkpoints and stress responses that affect almost every aspect of cellular metabolism. One of the best documented DNA damage responses is that activated by DNA double strand breaks (DSBs). Formation of DSBs in the DNA leads to rapid recruitment of sensor/activator proteins to the damaged sites. Among them are the MRN complex whose core contains the Mre11, Rad50 and Nbs1 proteins and the BRCT proteins Brca1, 53BP1 and Mdc1/Nfbd1. This process is required for the next step in the DSB response, the activation of the nuclear protein kinase ATM - the primary transducer of the DSB alarm. ATM, a member of the PI3-kinase-like protein kinase (PIKK) family, is activated via autophosphorylation, and a portion of it binds to the DSB sites. Activated ATM then phosphorylates key players in a wide range of processes. Repeated cycles of phosphorylation at the DSB sites, which include as target the histone H2AX, lead to further recruitment of the sensor/activator proteins and the formation of prominent protein foci at these sites. Deficiencies of ATM or components of the MRN complex lead to genomic instability syndromes such as ataxia-telangiectasia (A-T), A-T-like disease (A-TLD) and the Nijmegen breakage syndrome (NBS). We are investigating the early events that precede ATM activation and are seeking novel downstream processes represented by new ATM substrates. Two new ATM substrates will be discussed: the COP9 signalosome (CSN) complex and the co-repressor KAP-1. These new functional links in the web of the DNA damage response add further dimension to the increasing complexity and richness of this process.

## AGE RELATED FEATURES OF LYMPHOPOIESIS AND MORBIDITY FOR CHERNOBYL' CLEAN UP WORKERS

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**Objective:** In previous study with untreated oncological patients ( $\approx 1 \pm 8$  years old), we showed that the number of circulating immature lymphocytes ( $CD4^+Leu8^+$ ) had been changed periodically ( $T \approx 8-9$  month), as the life span had been shortened during five finale years. Moreover current cells level was closely proportional (positive correlation) to the death rate levels that regularly had been registered in each 3 months intervals [Shoutko A. et al., 2002 ESRB Meeting]. This might reflect a pathological mechanism to support of tumor development via natural morphogenetic function of lymphocytes (MFL). In order to evaluate if the expected mechanism of MFL is active toward normal tissues as well, we studied of circulating lymphocytes among Chernobyl' clean up workers (CUW) with different age and compared it with their rate of morbidity (MR) taking the age as a time scale (t).

**Objects and methods:**  $CD2^+$ ,  $CD3^+$ ,  $CD4^+$ ,  $CD8^+$ ,  $CD4^+Leu8^+$ ,  $CD8^+,11b^+$ ,  $CD21^+$  and  $CD19^+$  positive cells were estimated in blood of CUW using MAT "DACO" and UV-microscope "Opton". Morbidity rate was taken from register of Medical Military Academy.

**Results:** It was found that common MR (for 15 classes of diseases according to ICD-9, %o per year) reveals two maximums around 43 and 64 years old. The curve is described by 6th degree polynomial approximation ( $R=0,75 \pm 0,0036$ ;  $n=14448$ ). The average curve of  $CD4^+Leu8^+$  cells blood content according the same approximation has showed two minimums also at 43-44 and  $\geq 64$  ( $R=46 \pm 0,07$ ;  $n=122$ ). The average slow age' trend for cells is formed on the base of more rapid number' pulsation, resembling the  $CD4^+Leu8^+$  level' fluctuations for oncological patients. There is strong negative dependency MR (y) from  $CD4^+Leu8^+$  level (x):  $y=750x^{-0,28}$ ;  $R=0,79 \pm 0,09$ .

**Conclusion:** The reverse correlation between rate of common morbidity and average level of circulating immature T-cells points out two most dangerous age' periods for CUW around 40-45 and  $\geq 65$  old, where the morphogenetic lymphocyte' support of tissues became weakest. First of them should be expected as result of natural thymus' age involution.

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## PHOSPHORYLATION OF HISTONE H2AX IN HUMAN CELLS AFTER EXPOSURE TO $\gamma$ -RAYS AND CARBON IONS

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### OBJECTIVE

Development of methods to predict cellular response to radiation represents an important approach to improve response to radiation therapy. Of particular importance is the measurement of repair of radiation induced DNA double strand breaks (DSB), because of their relevance to cell death.

### METHODS

Primary human fibroblasts were irradiated with  $\gamma$ -rays or 62 MeV/u carbon ions (LET in water: 40 keV/ $\mu$ m) and DSB induction and rejoining were analysed by the evaluation of the phosphorylation at serine 139 of histone H2AX. Phosphorylation-dephosphorylation kinetics and DSB rejoining, as measured by Pulsed Field Gel Electrophoresis (PFGE), were also performed after  $\gamma$ -irradiation in the presence of calyculin A, a protein phosphatases inhibitor.

### RESULTS

The number of  $\gamma$ -H2AX foci rapidly increased after 1Gy of  $\gamma$ -rays, with the maximum reached at 20 min from irradiation. Then, the foci number decreased, reaching 10% of the maximum after 2 hour repair. In the presence of calyculin A, no significant dephosphorylation was found up to 20 min from irradiation and no further induction of  $\gamma$ -H2AX foci appeared at longer times. Using PFGE we found that the rejoining kinetics were not affected by the presence of calyculin A.

After 1Gy of carbon ions, a time delay in H2AX phosphorylation was observed with respect to  $\gamma$ -rays and the maximum foci number was comparable to that of particle traversals. Moreover, a longer persistence of  $\gamma$ -H2AX foci was found.

### CONCLUSION

In this work we confirmed that histone H2AX phosphorylation analysis can be an useful approach for DSB induction and repair studies. Our data also suggest that the maintenance of the phosphate group at serine 139 in  $\gamma$ -H2AX does not represent an obstacle for recruitment and activity of proteins involved in DSB repair. Moreover, the longer damage persistence in cells irradiated with carbon ions with respect to  $\gamma$ -rays is consistent with the notion that high LET radiation induces clustered damage that is more severe and difficult to repair.

## EFFECT OF SPINACIA OLERACEA ON RADIATION INDUCED BIOCHEMICAL CHANGES AND LEARNING ABILITY IN BRAIN OF SWISS ALBINO MICE

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Hazardous effects of free radicals can be decreased by radioprotectors. In this context search for such natural compounds is on which could quench the reactive energy of free radicals and also have low/no toxicity at the effective dose. The present study has been undertaken with *Spinacia oleracea* L. (Eng:-Spinach), a commonly occurring herb reported to be good source of antioxidants.

Thirty healthy Swiss albino mice (6 weeks old) were selected from an inbred colony, maintained under controlled conditions of temperature ( $25 \pm 2^{\circ}\text{C}$ ). Fresh spinach leaves collected locally were air dried, powdered and extracted with methanol by refluxing for 48 hr. 1/2 kg spinach yields about 100 gm powdered form when dried, yields 20 gm crude extract, which is dissolved in equal volume of double distilled water (DDW) just before oral administration.

Animals were divided into two groups; group I (control) were given DDW for 15 days and then exposed to single dose of 5 Gy whole-body irradiation. Group II (Experimental) mice were gavaged orally spinach extract for 15 consecutive days once daily and were then exposed with single dose of 5 Gy of gamma radiation. Learning ability and various biochemical parameters viz. Lipid peroxidation, Protein and GSH were estimated in whole brain by adopting standard procedures.

Mice supplemented with SE extract prior to irradiation took lesser time to reach the goal and the deficit in learning was completely reversed by the last interval studied i.e. day 30<sup>th</sup>, whereas, in irradiated group normalcy could not be achieved though recovery was noticed from day 15<sup>th</sup> onwards.

Protein concentration reduced continuously up to day 7<sup>th</sup> *post-irradiation* followed by an increase at later intervals in both the groups. At day 30<sup>th</sup> in the experimental group protein content reached almost normal level (98.62%) but in the control group it was 91.5% of the normal. Glutathione levels increased by 17.78% after oral spinach supplementation prior to irradiation. Percentage protection observed in LPO level in experimental group was 14.38%, 17.72%, 19.98%, 15.24% and 12.21% at 1,3,7,15,30 days *post exposure* respectively.

Nutritional intervention with spinach may play an important role in reversing the deleterious effect of radiation on biochemical parameters and learning ability.

## MODULATION OF RADIATION INDUCED STRESS IN MICE CREBELLUM BY MELATONIN

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Melatonin an important hormone of pineal gland now known to be an antioxidant has been shown to participate in number of physiological processes like regulation of reproduction, sleep, mood, behaviour etc. It also crosses all morphophysiological barriers, including blood brain barriers and distributes throughout the cell increasing efficacy as an antioxidant.

Therefore, the present study aimed to investigate the protective effect of melatonin against radiation induced oxidative stress in the cerebellum of Swiss albino mice selected from inbred colony maintained in air-cooled laboratory. Mice were sacrificed at various autopsy intervals viz. 1-30 days. Biochemical parameters included Lipid peroxidation (LPO) and Glutathione (GSH) were taken as endpoints. Quantitative study included alterations in number and volume of Purkinje cells.

Fifteen days oral administration with melatonin (0.25 mg/kg.b.wt) followed by single exposure of 4 Gy of gamma-radiation at the dose rate of 1.072 Gy/min (source to surface distance of 77.5 cm) inhibited, the radiation induced augmented level of LPO by approximately 50% at day 30 post exposure. Furthermore, melatonin augmented glutathione level by 68.9% in cerebellum on day 30<sup>th</sup> the last interval studied.

It also significantly increased the number and volume of Purkinje cells, which was significantly decreased by radiation exposure.

In conclusion the melatonin treatment may provide neuroprotection against radiation-induced neurotoxicity by increasing the survival of Purkinje cells and their volume and inhibition LPO possibly by directly scavenging reactive oxygen species and by indirectly augmenting their antioxidative capacity.

## POSTRADIATION DAMAGE IN CHICKENS – APOPTOSIS

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Although radiation induced disorders are known to be dependent on immunocompetent cells, many factors can influence the course of the damage to the organism (source of radiation, dose, dose rate, stress, nutritional status, coincidence with infection and non-infection agents, etc.). The exact mechanism, by which the radiation damage develops, is insufficiently understood. Bursa cloacalis and thymus, the primary immune organ are essential for the function of the immune system to control humoral and cell – mediated immunity in avian species. Peripheral lymphoid tissues include the unique role of the spleen, the Harderian gland, the caecal tonsil, the lymph node, the pineal gland, Neckel diverticulum, Peyer's patches and intestinal lymphocytes. The present work is dealing with the influence of gamma radiation and related postradiation damage in chickens. The postradiation immunosuppression in chickens and radiation induction of apoptosis in B and T lymphocytes, proliferation and necrosis was monitored using  $^{60}\text{Co}$  source, SSD: 95 cm, 0.3 Gy / min., the total dose: 4.5 Gy. The Flow – cytometer (488 nm argon line excitation) were used for the detection of the content of DNA in the B and T cells. The cells showing less content of DNA than  $G_0/G_1$  phase of the cell cycle were counted as apoptotic cell. Cells showing more DNA than  $G_0/G_1$  were countered as proliferating cells –  $G_2/M$ . Irradiation had no effect on the proportion of proliferating cells, and resulted to the increase of apoptosis. The total antioxidant status (TAS) increase was found. It could be a result of release of intracellular antioxidants from damaged cells in the necrotic tissue as oxidative stress as an initial insult as well as the signals transduced by reactive oxygen species which play an important role in radiation induced apoptosis.



## THE BYSTANDER EFFECT IN CELLS; SMALL DOSES AND MEDIUM TRANSFER

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### Introduction

Previously introduced terms “low-dose hyper-radiosensitivity” and “increased radioresistance” have been described after low LET irradiation. A threshold of transition between the two is seen generally above 0,5 Gy. The bystander effect has been found as an excess of damage at the low doses detected in cells which have not been irradiated. The aim of the study was to investigate the influence of medium transfer and the possibility of detection of certain cytokines within the irradiated as well as bystander cell populations.

### Materials And Methods

The hybrid cell line E.A.hy. 926 was irradiated with 0; 0.5; 1; and 5 Gy. Bystander cell populations were established via medium transfer 45 min or 4 hours after irradiation from the irradiated to un-irradiated cell populations. Specific antibodies were used for immunohistochemistry, flow cytometry or fluorescence microscopy detections of Interleukin 1 alpha (IL-1 $\alpha$ ) and  $\beta_1$ -integrin (CD29) at different time points after (150 kV) X-ray irradiation.

### Results

Detection of IL-1 $\alpha$  and  $\beta_1$ -integrin on irradiated cells by fluorescence microscopy showed the maximum presence of cytokines after 0,5Gy, as early as 30 min and 1 hour after irradiation (the same for 1 and 5Gy). Using immunohistochemistry, both cytokine over-expressions were observed in irradiated cell cultures after 1 and 5 Gy as well as in the bystander populations with the maximum at 1 and 24 hours after irradiation/medium transport. Flow cytometry method shows only significant shift of the shoulder (20%) of  $\beta_1$ -integrin less positive or negative cells from the left to the right side of a diagram after the irradiation. Again, the dose of 0,5Gy and the time 30 min post-irradiation showed the biggest activation. Clonogenic survival in irradiated cells was decreasing with the dose but in all bystander populations remained at control levels. No influence of irradiated medium itself has been found.

### Conclusions

Maximum expressions of cytokines were found very early after the application of small and high doses. If an appropriate method of detection is used, cytokines could serve as even more sensitive marker of post-irradiation response of irradiated and bystander populations than the other classical markers.

### Acknowledgements

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## RADIOTOXICITY ALONG THE BRAGG CURVE FOR A 200 MeV CLINICAL PROTON BEAM

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### Objective:

To determine the variations in biological effectiveness with depth for a high-energy proton beam to estimate the potential influence for clinical radiotherapy.

### Methods:

Human lymphocytes and V-79 fibroblasts have been exposed at various positions along the Bragg curve of a 200 MeV clinical proton beam. This includes the plateau region, middle and distal positions within a 5 cm SOBP as well as the distal edge. Micronuclei formations in binucleated lymphocytes and cell survival for fibroblasts were quantified in each instance.

### Results and Conclusion:

With respect to the plateau region the lower proton energies with depth result in a continuous increase in the biological effectiveness. For the fibroblast both the  $\alpha$ - and  $\beta$ - parameters describing the survival curves increase with LET. The RBE increases along the Bragg curve from 1.07 in the middle of the SOBP to 1.16 in the distal SOBP and to a value of 1.5 in the distal edge (32 % of dose maximum). With T-lymphocytes the RBE increases from 2.1 in the middle SOBP to 2.7 in the distal SOBP and a dose limiting value of 3.2 is noted in the distal edge at (12 % of dose maximum). All observations are significant at the 95 % confidence level.

As larger RBE values are calculated at lower doses for any of the irradiated positions it is concluded that the changes noted is the result of higher ionisation densities. Also that a RBE value larger than the generic value of 1.1 be used when planning proton therapy where a critical structure is positioned close and distal from the SOBP. Furthermore, as lymphocytes are generally more radioresistant than fibroblasts, it is concluded that some potential for therapeutic gain may be implicated by these measurements.

## TELOMERE MAINTENANCE AND DNA DOUBLE STRAND BREAK REPAIR

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Telomeres are physical ends of chromosomes responsible for chromosome stability maintenance. Some proteins involved in DNA double strand break (DSB) repair are located at telomeres and these include Ku and DNA-PKcs. Lack of these proteins causes telomere dysfunction. We have also shown that the above proteins may have effect on telomere length. For example, cells from scid (severe combined immunodeficiency) mice, defective in DNA-PKcs, show abnormally long telomeres. Telomere maintenance abnormalities have been observed in human diseases such as Nijmegen breakage syndrome (NBS) and ataxia telangiectasis (AT). We have recently started investigating telomere maintenance in BRCA1(Breast Cancer 1) and BRCA2 defective cells. Functions of BRCA1 and BRCA2 is not completely clear but they behave as tumor suppressors and are involved in homologous recombination most likely through interactions with RAD51. BRCA1 and BRCA2 defective cells are radiosensitive and show genomic instability. Our preliminary results indicate telomere dysfunction in BRCA1 and BRCA2 deficient rodent and human cells. We also observed accelerated telomere shortening in mouse embryonic stem cells deficient in BRCA1. These results argue that both BRCA1 and BRCA2 affect telomere maintenance. It is not clear if this is direct effect or result of genomic instability generated by BRCA1 or BRCA2 deficiencies. Overview of the role of telomeres in DNA damage response will be presented.

## COMPARISON OF RADIATION-INDUCED BYSTANDER EFFECTS IN NEURONS AND FIBROBLASTS\*

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Radiation-induced bystander effects are defined as effects observed in cells which have not themselves been irradiated, but have been in communication with irradiated cells. These bystander effects can be relayed by the release of medium-borne factors and/or via direct intercellular communication through gap junctions. Several endpoints have been used to assess this phenomenon including cell survival, mutation induction, chromosomal instability and changes to protein levels within a cell. If these effects occur in the human population at environmentally relevant doses, they will affect current risk estimates obtained by extrapolation from higher doses. Current data on bystander effects have mostly related to communication between cells of one type, although there have been reports of asymmetric signalling between, for example, epithelial cells and fibroblasts. There may be a difference in production of, and response to, the bystander effect both in, and between, different tissue types. The current project tests the hypothesis that fibroblasts and neurons might also trigger, or respond to, bystander effects to a different extent.

In this study, low doses (0.5Gy) of high LET alpha radiation and medium transfer from irradiated (either fibroblast or neuronal) to unirradiated (fibroblast) immortalised cell line populations were used. Eight groups were investigated - sham irradiated and irradiated samples of both cell types, as well as unirradiated fibroblasts incubated for 60 minutes with medium from either sham or irradiated fibroblasts or neurons, transferred 5 minutes after irradiation. Cells were analysed for chromosomal damage at early passage (P2) to examine immediate damage, and late passages (P9 & P20) to investigate induction of the instability phenotype.

Results from these experiments show that direct irradiation of fibroblasts increased chromosomal aberrations at both early and late time-points, suggesting the induction of chromosomal instability. Medium transfer from irradiated neuronal cells produced a bystander effect at early passages, which persisted to later passage analysis, whereas irradiated fibroblasts produced a bystander effect only at later passages.

These data support the hypothesis that neurons, as specialised cell types for communication, have increased signalling ability after exposure to external irradiation. Ongoing work includes the use of different types of neuron and fibroblast cultures, the use of different culture systems and the correlation of chromosome abnormalities with other subcellular changes.

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## ANALYSIS OF THE FREQUENCIES OF EXCHANGE TYPE ABERRATION IN CHROMOSOMES 2, 8 AND 14 IN LYMPHOCYTES OF SEVEN DONORS BY CHROMOSOME PAINTINGS

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Chromosome painting allows the analysis of frequencies of all types of exchange type aberration, not only dicentrics but also translocations. The advantage of analysing translocations is that they do not decline with time. Chromosome painting allows to identify exchanges as colour junctions between painted chromosomes. Usually selected chromosomes are painted and total genomic translocation frequency is calculated using Lucas equation. Such calculations relies on the assumption that the probability of radiation induced aberrations is directly proportional to the DNA content of each chromosome. The published data suggest that this may not be so. However, there is a lack of consensus with regard to which chromosomes are more or less radiosensitive than expected on the basis of their DNA content. The majority of studies were performed with cells from a single donor. Hence, a possible source of variability is an individual variation in chromosomal sensitivity.

The aim of the study was to assess the possible extent of individual variability in the involvement of selected chromosomes in radiation-induced aberrations. We irradiated human lymphocytes of five female and two male donors with various doses of gamma rays (Co-60). The frequencies of aberrations were analysed in painted chromosomes 2, 8 and 14. The total genome frequencies of translocations, dicentrics as well as of all aberrations were calculated. In lymphocytes of some donors higher or lower than expected frequencies of aberrations were found in some chromosomes. Generally, the sensitivity of chromosomes 2 was lower than expected and that of chromosomes 8 or 14 was higher than expected.

In order to check if there are any differences in radiosensitivity between lymphocytes of individual donors we pooled the aberrations scored in chromosomes 2, 8 and 14 for each donor. The data were fitted to linear-quadratic dose-response curves by the method of maximum likelihood. Differences were found between the curves, suggesting the presence of individual variation of lymphocyte sensitivity to radiation.

Work supported by the Polish Ministry of Scientific Research and Information Technology (grant number: 6 P05A 119 20).

IN CELLS OF THE PERSONS WITH HEREDITARY MUTATION IN BRCA1 AND BRCA2 GENES CENTROMERIC LOCI OF CHROMOSOMES UNLIKE NORMAL NOT CAPABLE TO DISPLACEMENT IN SPACE OF CELLS NUCLEUS UNDER ADAPTING DOSE OF X-RAYS.

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Previously, using non - radioactive in situ hybridization and computer image analysis, we have shown a changing of distribution of 1q12 chromosome 1 centromeric loci radius vector (distance between loci position and nucleus center) values in human lymphocytes nuclei after irradiation by adapting (3 - 25 cGy) doses of X-rays. This changing can be interpreted as a induction of displacement or motion of 1q12 loci from nuclei peripheral to internal nuclei positions caused by adapting doses irradiation. This effect was been dose dependent and was observed between 0,5 and 5 hours after radiation exposure. Similar induction of 1q12 loci displacement was partially and fully blocked in cells from individuals with inheritable mutations of BRCA1 and BRCA2 genes accordingly but it is not disturbed in cells from their sisters whose genomes did not bear BRCA mutations. These BRCA mutations was selected as a examples of DNA double strands breaks (DSB) reparation disturbance. The detaching of some chromosomal loci from nuclei matrix is probably required for loci displacement. It is possible, that adapting doses irradiation can induce loci detaching process. And the absence of induced loci displacement in cells from patients with BRCA mutations can caused by loci detaching disturbance. It is can be closely related with DSB reparation disturbance because the loci displacement (and detaching accordingly) is required for homological recombination that is necessary for DSB reparation. On the other hand, it is possible, that products of BRCA and some others genes that related to DSB reparation (ATM, NBS, e.t.c) can be involved in regulation of loci displacement or/and detaching.

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## HE2100 AND HE3204 PROTECT RHESUS MACAQUES FROM RADIATION-INDUCED MYELOSUPPRESSION

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**Objectives:** HE2100, androst-5-ene-3 $\beta$ ,17 $\beta$ -diol, and HE3204 are natural and synthetic adrenal steroids, respectively. HE2100 studies of lethally irradiated rodents demonstrated a significant survival advantage for treated groups (Whitnall et. al., Int. J. Immunopharm.2000). This study investigates bone marrow protective effects in sub lethally irradiated non-human primates.

**Methods:** Rhesus macaques were total body irradiated with sub-lethal, 4.00 Gy, <sup>60</sup>Co ionizing radiation. Four hours after sublethal total body irradiation exposure, animals were treated with either 5 once daily or 4 once weekly subcutaneous (sc) injections of HE2100 or HE3204. Blood counts were measured daily during severely neutropenic days. Additional studies on primitive (Long-Term Culture-Initiating Cells-LTC-IC), lineage committed (Granulocyte-Macrophage Colony-Forming Cells, GM-CFC) and bone marrow mesenchymal progenitor cells were performed.

**Results:** HE2100, at a dose of 15 mg/kg given for 5 days, significantly reduced the number of days of severe neutropenia (<500cells/uL) in the sub-lethally irradiated macaques, from 12 days (vehicle) to 3 days and 4-7 days for those treated once weekly. Three of the four treated groups were significantly different from placebo (p<0.05). HE2100 had an effect on platelets, with placebo animals having 8 days of grade 3 or 4 thrombocytopenia, compared to treated 0-3 days (p<0.05). Grade 3 or 4 anemia occurred for 7 days in placebo treated animals, compared to treated 0-4 days. HE3204 at a dose of 5 mg/kg once weekly for 4 sc injections resulted in 2.5 days of neutropenia, with control groups exhibiting 13.3 days (p<0.05). An average of 4.5 days of grade 3 or 4 thrombocytopenia was observed in controls versus 1.8 days in treated animals. Grade 3 or 4 anemia was observed for 11.8 days in controls versus 1.0 day in treated animals (p<0.05). HE2100 increased (1) GM-CFC-derived colony numbers increased by 36% using standard *in vitro* assays (p= <0.001); (2) the GM-CFC number by 280% at wk 4 in Long Term Bone Marrow Culture (LTBMC) (p=0.035) and (3) Bone marrow mesenchymal progenitor cells *in vitro*.

**Conclusions:** This class of compounds expands committed and primitive bone marrow progenitor cells and affords significant protection from radiation-induced myelosuppression in non-human primates.

## BIOCHEMICAL AND MORPHOLOGICAL EFFECTS OF RADIATION TRAVERSED HUMAN MELANOMA CELLS ON NONTRAVERSED NEIGHBOURS

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**Objective:** The bystander effect is an biological effect induced by irradiated cells in neighbour not traversed radiation cells. Mechanisms of this effect induction are caused by clastogenic and toxic factors released from irradiated cells.

**Methods:** In the present study, the multicellular megacolonyes of human melanoma Me-45 line growing on one part of the bottom of culture flasks were irradiated with 5 Gy (Co-60) whereas megacolonyes growing on the second part of the bottom were shielded. The bystander effect of radiation traversed cells on non traversed was studied during postradiatio co-cultivation. Activity of superoxide dismutase (Mn and CuZn isoenzymes), glutathione peroxidase (GSH-Pox) and malonaldehyde (MDA) concentration as a biochemical markers of bystander effect were monitored for a period of 72 h. The DNA damage was measured by comet assay. Micronucleus induction, mitotic index and cellular death as apoptosis or necrosis were simultaneously estimated based on morphological criteria.

**Results:** The bystander effect of irradiated cells on their neighbours was observed as a slight increase of MDA concentration, comparable decrease of GSH-Pox activity, and some fluctuation of mitochondrial and cytoplasmic isoenzymes of SOD. DNA strand breaks and rejoining measured by comet assay as mean tail lenght, demonstrated clearly the bystander effect for nontraversed radiation cells, additionally verified by tail moment. There was also significant increase of micronucleation and apoptosis generated by radiation traversed cells in shielded neighbours. Furthermore, significantly higher increase of necrosis in shielded neighbour cells compared to radiation traversed cells were observed. Proliferative activity showed a suppression in both, radiation traversed and shielded neighbour cells in all measured time points.

**Conclusion:** In summary, the present data provide different evidences that, the radiation traversed cells secreted some signals or long lived modifiers which influence the neighbour cells inducing lethal lesions and clastogenic damage. These factors are probably of oxidizing species, however further studies are required to define the chemical nature of these species.



## SECONDARY ELECTRON EMISSION YIELD OF THE MYLAR MATERIAL FOR BEING UTILIZED IN REAL-TIME MONITORING OF ELECTRON BEAM

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Microbeam irradiation facilities, which enable cells *in vitro* to be irradiated individually, have been utilized for investigating the cellular effects of radiation. A new microbeam irradiation system utilizing electrons has been under development at Korea Institute of Radiological and Medical Science (KIRAMS) since 2002. The KIRAMS microbeam irradiation system is designed to provide a 5  $\mu\text{m}$ -diameter electron beam of energy up to 100 keV. The system is going to be devoted to the low-dose (less than 0.2 Gy) radiation effect studies for the genomic instability, bystander effects and adaptive response-mechanisms. The beam characteristics at the cell entrance has been measured by using the passivated implanted planar silicon (PIPS) detector. In low-dose electron beam irradiation, however, the real time monitoring is required for accurate estimation of the number of electrons incident on the individual target cell. The secondary electron emission (SEE) from the vacuum window can be a good probe for the real time monitoring of the primary electrons leaving the vacuum chamber toward the individual target cell. The mylar foil, which is the vacuum window material for the KIRAMS microbeam system, is considered to provide the secondary electrons with a sufficient yield. In this paper, we discuss the measurements and calculations of the forward and backward SEE from the primary electron beam of 10 to 100 keV in energy normally or slantingly incident on the mylar material of 0.9, 1.5 or 2.5  $\mu\text{m}$  in thickness. A center-hole chevron type micro-channel plate (MCP) and a simple hole-cup have been used in measurement.

## THE PROBLEM OF DEFINING WEIGHTING FACTORS FOR THE ABSORBED DOSE TO BIOTA IN VIEW OF NON-TARGETED EFFECTS.

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For the past decade the requirement for an internationally agreed rationale to the protection of the environment to ionising radiation has been recognised. The FASSET project was launched under the EC 5<sup>th</sup> Framework Programme (November 2000) to develop a Framework for the ASSESSment of Environmental impacT of ionising radiation in European ecosystems. In order to develop a framework it is essential to establish the relationship between exposure and the relevant biological effects. In human radiation protection dosimetry, radiation quality is considered by applying radiation weighting factors to the absorbed dose. Similar procedures have been suggested for biota but the challenge is however to define relevant values on the weighting factors for the radiation regimes in contaminated environments, and for the biological endpoints of concern. Experimentally obtained RBE (relative biological effectiveness) values for critical end-points will give guidance to the selection of factors. Environmental risk assessments focus mainly on end-points relevant to effects on the populations rather than individuals. These end-points have been assumed to be deterministic in nature and related to impairment of reproductive capacity. Experimental studies on RBE for effects on reproduction are therefore important in order to select radiation weighting factors. These effects are induced at low dose levels and can, in some cases, be regarded as stochastic effects. The gametogenesis has been found to be extremely radiosensitive in mice and studies have shown a 50 % reduction in the number of primary oocytes after irradiation with a dose of 80 mGy of low LET radiation. For low LET, at this dose level, all cells are traversed by radiation tracks. Inversely, at high LET radiation, e.g.  $\alpha$ -particles (at an initial energy of 5.5 MeV), one track crossing each nucleus (at 8  $\mu$ m diameter) results in an average cellular dose of 370 mGy. Assuming an RBE of 1 for high-LET radiation, 22 % of the primary oocytes will then be hit by radiation. Concurrently, studies have indicated RBEs for killing of oocytes of 50 or more for high LET radiation compared to  $\alpha$ -rays. Such high RBEs result in only few percent of the oocytes are directly hit by high LET radiation although 50 % of the cells would be killed. A tentative explanation to these findings may be the existence of some bystander mechanisms within the cell systems. The presentation will elaborate on the problem with selection of radiation weighting factors for doses to biota considering the mechanisms that may be involved in the various responses to high LET or low energy photons (tritium).

## RADIATION-INDUCED PATHOMORPHOSYS OF COPD IN LIQUIDATORS OF CHERNOBYL CATASTROPHE

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During 1988-2003 complex clinical-morphological investigation of special features of COPD in 2113 liquidators of Chernobyl nuclear power plant accident and 309 persons of nosological control group was carried out. The doses of external exposure were found in interval from 2 to 76 cSv. The inhalation influence of radionuclides on the bronchopulmonary system are taken into account too. Due to the special wholebody counter incorporation of the “hot particles” with the different activities to the lung tissue were determined in 10 patients. All patients have not bronchopulmonary pathology before 1986 year. Above this, the group that contains 82 reconvalescents of acute light syndrome was investigated. Complex of contemporary research methods (clinical, peak flow-volumetry, bronchofiberscopy, laboratory, morphological, immunological, microbiological etc.) was used. Argued, that treatment and clinical examination of liquidators and determination of expert questions had to take into account a presence of radiation induced pathomorphosys of COPD in this cohort of patients. The main clinical features includes characteristics of cough syndrome, course of the disease. Bronchoobstructive syndrome has undergone an interesting dynamics from very often hypotonic dyskinesia of membranous parts of trachea to total obstruction. Endoscopic research of the mucous membrane shows the development of deep atrophic and sclerotic changes. Morphological and laboratory patterns and structural equivalents of this phenomenon are also represented in the study. Developed diagnostic algorithm and methodological basis of medical and prophylactic tactics for these patients, which are integrated into corresponding conception.

## INFLUENCE OF CHROMATIN STRUCTURE ON YIELDS OF RADIATION INDUCED 8-OXO-dG AND DNA STRAND BREAKS.

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Radiation-induced formation of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG) and DNA strand breaks (SB) was studied in human fibroblast cells with normal or modified chromatin structure. The aim was to study the protection provided by the chromatin structure alone or in combination with added radical scavengers against these two types of DNA damage where the contribution of indirect and direct effects of radiation differ.

VH 10 fibroblasts were irradiated as cellular monolayers (intact cells), nuclear monolayers (permeabilized cells with intact chromatin structure) and nucleoid monolayers (permeabilized and salt treated cells to obtain histone free DNA). Formation of 8-oxo-dG was assayed with reversed phase HPLC coupled to an electrochemical detector and SB with the alkali unwinding assay.

Depletion of low molecular weight nuclear components, increased the radiation-induced formation of 8-oxo-dG 5-fold compared to 2-fold for the formation of SB. Removal of both low molecular weight components and histones increased the yield of 8-oxo-dG 46-fold and the yield of SB 43-fold. The increase in yield due to the removal of histones only, is thus 2 times higher for the formation of SB as compared to the formation of 8-oxo-dG. Addition of radical scavengers (ethanol or thiols) to nuclear and nucleoid monolayers provided a significantly better protection against the formation of 8-oxo-dG relative to the formation of SB. The results show that the protective role of chromatin organization is more pronounced for the formation of SB as compared to the formation of 8-oxo-dG. Since both alcohol and thiols provided efficient protection against the formation of 8-oxo-dG, the mechanism of protection is likely due to radical scavenging rather than chemical repair.

In conclusion, this suggests that in intact cells, 8-oxo-dG is preferentially formed in structures of chromatin not associated with histones, indicating a significantly larger role of the indirect effect of radiation on the formation of 8-oxo-dG than on the formation of SB

## CHROMOSOME ABERRATIONS IN CELLS OF THE SNAIL (*LIMNEA STAGNALIS* L.) EMBRYOS FROM WATER BODIES WITHIN THE CHERNOBYL NPP EXCLUSION ZONE

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The structural damages of DNA, arising due to ionising radiation impact, are the basic reason of reproductive cells death are registered as chromosome aberrations. The analysis of such cytogenetic effects of irradiation on biosystem are extremely important at study and forecasting of the remote consequences of the Chernobyl NPP accident.

The rate of chromosome aberrations in cells of freshwater snail (*Lymnaea stagnalis* L.) embryos was carried out. The mollusc's embryos have taken in different seasons of 2002–2003 in reservoirs of the Chernobyl NPP exclusion zone (Azbuchin Lake, Dalekoye-1 Lake, Glubokoye Lake, Yanovsky Creek, Uzh River and Pripyat River), characterised by various levels of radioactive contamination and, accordingly, dose rate for hydrobionts. The chromosome aberration rate was registered by anaphase method. The results of the analyses compared to the data received for molluscs from Goloseevo lakes located within Kiev City territory.

The absorbed dose rate for snails, living within littoral zone of the researched water objects, due to external irradiation and radionuclides incorporated in snail tissue was in a range from  $2.5 \times 10^{-4}$  to  $3.4 \text{ Gy year}^{-1}$ . The highest value was found for hydrobionts from lakes within the embankment territory on the left-bank flood plain of Pripyat River, the lowest – for specimens from the running water objects and lakes of Kiev City. The high level of chromosome aberration in of snail's cells from water objects within the Chernobyl exclusion zone has been registered in comparison with Goloseevo lakes. The molluscs from Dalekoye-1 Lake and Glubokoye Lake were characterised by the maximal rate of chromosome aberration – about 20–25%, that in 10 times exceeds a level spontaneous mutagenesis for hydrobionts. A little bit less rate is registered for snails from Azbuchin Lake and Yanovsky Creek. The chromosome aberration rate of molluscs from Goloseevo lakes on average was about 1.5 %, and the maximal rate did not exceed 2.5 %.

## PILOT SURVEY OF ELF MAGNETIC FIELD PERSONAL EXPOSURE IN BUDAPEST

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### Objective

The aims of this study were: 1) to measure the 24 hour extremely low frequency (ELF) personal magnetic field exposure on two different occasions, to find out whether the repeated exposures correlate with the previous ones, 2) to reveal the main components of overexposure ( $>0,2 \mu\text{T}$ ).

### Methods

24 hour measurements were performed by EMDEX PAL magnetic field loggers. The loggers were attached to the belt and for the night they were put near the bed. Participants were asked to fill out a diary covering the main activities of the day (home, bed, work, travel).

The data were stored on computer and statistically analysed by EPI INFO 6.04 software.

### Results

40 personal magnetic field exposure measurements of 20 participants were performed between January-December 2003. Participants were recruited from the Institute. The mean personal exposure was  $0,158 \mu\text{T}$  ( $0,049 \mu\text{T} - 0,622 \mu\text{T}$ ). Average BED exposure was  $0,112 \mu\text{T}$  ( $0,009-0,843 \mu\text{T}$ ). Average HOME exposure was  $0,125 \mu\text{T}$  ( $0,021 \mu\text{T} - 0,649 \mu\text{T}$ ). Average WORK exposure was  $0,201 \mu\text{T}$  ( $0,036 \mu\text{T} - 1,895 \mu\text{T}$ ). Average TRAVEL exposure was  $0,181 \mu\text{T}$  ( $0,052 \mu\text{T} - 0,753 \mu\text{T}$ ).

The repeated exposure data significantly related to the previous ones ( $P < 0,01$ ). The WORK exposure correlated best ( $P < 0,001$ ).

Five participants had magnetic field exposures higher than  $0,2 \mu\text{T}$ . Three persons acquired the overexposure from HOME exposure (kitchen appliances, electric radiator below the bed, 120kV power line within 25 meters) and two persons from both WORK and TRAVEL exposures (electric appliance use at the workplace: sewing machine, densitometer, travelling by train).

## THE COMBINED THERAPEUTIC EFFECT OF LOCAL TUMOR IRRADIATION AND IFN- $\gamma$ VACCINATION ON MURINE BRAIN TUMORS

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**Objectives:** The aim of this study was to investigate the combined antitumor effect of radiation therapy and adenovirus-mediated IFN- $\gamma$  vaccination therapy on experimental brain tumors.

**Methods:** An adenoviral vector encoding the murine IFN- $\gamma$  cDNA was constructed (AdIFN- $\gamma$ ).

For B7 and MHC expression studies glioma 261 (G1261) cells were either irradiated or transduced at different multiplicities of infection (MOI) with AdIFN- $\gamma$ . Total RNA was isolated then B7 and MHC expressions were studied by semi-quantitative RT-PCR.

For in vitro proliferation studies, G1261 cells were infected with AdIFN- $\gamma$  at different MOI and cell growth was monitored for 5 days.

Intracranial tumors were established in C57Bl mice by transplantation of G1261 cells. To produce IFN- $\gamma$  secreting vaccines in vitro growing G1261 cells were transduced with AdIFN- $\gamma$  and irradiated 48 hours later to stop cell division. The irradiated cells were used to vaccinate brain tumor bearing mice, three days after tumor inoculation. In some experiments the vaccination therapy was combined with local tumor irradiation. Survival of mice was followed.

**Results:** MHC I expression was low in uninfected cells and it increased 4-10 fold after AdIFN- $\gamma$  transduction. Wild type glioma cells had undetectable level of MHC II expression. Transduced cells exhibited elevated expression of MHC II antigen. We have not observed any changes in the B7-2 expression level. Irradiation of G1261 cells did not change MHC or B7-2 expression levels.

Infection of glioma cells with AdIFN- $\gamma$  led to a slower growth rate of glioma cells under in vitro conditions. In vivo vaccination studies showed moderate therapeutic effects in mice. This effect was significantly increased when IFN- $\gamma$  vaccination therapy was combined with local tumor irradiation.

**Conclusion:** Our results showed that AdIFN- $\gamma$  gene-therapy has only a limited effect on malignant gliomas, but its combination with tumor irradiation is promising.

## RADIOBIOLOGICAL EFFECT OF NEOADJUVANT BRACHYTHERPY IN STAGE ONE ENDOMETRIAL CARCINOMA

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It is still questionable whether there is any survival advantage attributable to preoperative intrauterine brachytherapy in early stage endometrial cancer. The prognostic tumor biological factors can be used in the indication of the neo adjuvant brachytherapy.

Retrospective analysis **1019** endometrial cancer was performed. Five year disease-free survival of stage I patients with and without preoperative brachytherapy was analyzed according to the grade and depths of myometrial invasion in the Radiotherapy and Gynaecological Department of Debrecen University between 1978-1993. **526** patients received 3 times 6 Gy intrauterine MDR treatments before Hysterectomy, while in 291 cases neo adjuvant treatment was not performed. 91.5 % of the irradiated patients underwent 50 Gy adjuvant external beam irradiation, while 70.1% of them received 3x6 Gy vaginal application as well. 93,1% of previously not irradiated cases got postoperative irradiation, 77.3% was treated with combined postoperative radiotherapy modalities. The life table method was used for survival analysis. Cumulative disease-free survival probabilities were calculated and survival curves were compared by long-rank test.

The frequency of sterilized operative specimens was 10 % in the neo adjuvant group. There was no significant difference between the preoperatively irradiated and not irradiated patients survival in poorly differentiated (G1) and well-differentiated tumors (G3). Patients with moderately differentiated tumors (G2) treated with neo adjuvant brachytherapy had significantly better disease-free survival than those without preoperative radiotherapy.

Our results demonstrate important role of the tumour grading in radiosensitivity of early stage endometrial tumors.



## ALPHA-HITPROBABILITY DISTRIBUTIONS OF DEPOSITED RADON PROGENIES IN CELL NUCLEI, CELLS AND CELL SURROUNDINGS OF THE CENTRAL AIRWAY EPITHELIUM

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### Objectives

Based on several experimental findings, deposition of inhaled particles is strongly non uniform within airway bifurcations. As a consequence, the deposited radon progenies emitted alpha-radiation may cause significant cell damage in the nearby cells especially in areas with high deposition densities. The general objective of this research is the construction of a mathematical model to characterise and quantify the local damages caused by alpha particle hits of inhaled radon progenies.

### Methods

The FLUENT<sup>TR</sup> computational fluid dynamics (CFD) programme package has been applied to determine the deposition patterns in a realistic airway geometry model of airway generations 1-5 constructed by the help of our own developed surface generator code. The interaction of the alpha particle tracks and the epithelial cells has been analysed using a current model of our research group. The output of this approach contains details on the number of alpha-hits and several microdosimetric parameters in cell nuclei, cells and cell surroundings with different radii. The cells of the epithelium have been generated taking into account the depth-distributions, surface density distributions, cell and cell nuclei diameters from the actual literature. Specifying other parameters as mucus thickness, alpha-energy and the range of the alpha particles the alpha tracks can be simulated.

### Results

Even at low average deposition densities, multiple hits have been located in the vicinity of the carina ridge although the number of exposed cells rapidly decreases with the number of hits in a cell.

### Conclusions

Applying these results and in vitro transformation data, we hope to receive useful information for the study of the linear-non-threshold (LNT) hypothesis.

### *Acknowledgements*

This research was supported by the Hungarian NKFP-3/005/2001 and NKFP-1/008/2001 Projects.

## G<sub>2</sub>-CHECK POINT ABROGATION AS A CANDIDATE MECHANISM FOR THE ENHANCED G<sub>2</sub> CHROMOSOMAL RADIOSENSITIVITY IN AT CELLS.

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G<sub>2</sub>-chromosomal hypersensitivity and its dependence on G<sub>2</sub>-checkpoint response following irradiation were investigated in Ataxia Telangiectasia (AT) cells. Lymphocytes obtained from AT-homozygotes, heterozygotes and from AT-unrelated donors as well as EBV-transformed lymphoblastoid cell lines (LCL) with different status of ATM, were irradiated with  $\gamma$ -rays in G<sub>2</sub>-phase. To examine whether DNA repair processes or G<sub>2</sub>-checkpoint abrogation and the rapid entry of cells with DNA damage into mitosis play a key role in the mechanisms underlying the enhanced G<sub>2</sub>-radiosensitivity of AT-cells, chromatid breaks were analyzed at metaphase using the G<sub>2</sub>-assay and compared to those scored directly in G<sub>2</sub>-phase using premature chromosome condensation (PCC). The importance of cdk1/cyclinB activity, a key regulator in G<sub>2</sub> to M-phase transition and conversion of DNA damage into chromatid breaks, was also examined. AT patients and ATM<sup>-/-</sup> (GM15786) lymphoblastoid cells showed an increased chromosomal G<sub>2</sub>-radiosensitivity compared to controls and carriers (ATM<sup>+/-</sup>). G<sub>2</sub>-radiosensitivity for carriers and ATM<sup>+/-</sup> LCL (GM03188A) was in between ATM<sup>-/-</sup> and controls. However, when the analysis was carried out directly in G<sub>2</sub>-phase by means of PCC, no differences in G<sub>2</sub>-radiosensitivity were observed between ATM<sup>-/-</sup>, ATM<sup>+/-</sup> and controls. These interesting findings together with data on mitotic indexes and cell cycle kinetics provide direct evidence to support the hypothesis that G<sub>2</sub>-checkpoint abrogation plays a key role in the mechanisms underlying enhanced G<sub>2</sub>-chromosomal radiosensitivity in AT cells. Furthermore, when LCL mitotic cells were fused to irradiated Go primary lymphocytes, the conversion of DNA damage into chromatid breaks was found higher when ATM<sup>-/-</sup> mitotic cells instead of ATM<sup>+/-</sup> or control mitotic cells were used. This suggests that in addition to G<sub>2</sub>-checkpoint abrogation, an increased cdk1/cyclinB activity of ATM<sup>-/-</sup> cells may also contribute to the observed G<sub>2</sub>- chromosomal hypersensitivity during G<sub>2</sub> to M-phase transition in AT patients.

**EU FP6 COORDINATION ACTION - EMF-NET “EFFECTS OF THE EXPOSURE TO ELECTROMAGNETIC FIELDS: FROM SCIENCE TO PUBLIC HEALTH AND SAFER WORKPLACE”**

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The European Coordination Action EMF-NET (<http://emf-net.isib.cnr.it>) is financed by the 6<sup>th</sup> Framework Program of the European Union. The aim is not to produce new studies, but to ensure the best use of existing data on exposure to electromagnetic fields by identifying all relevant studies and analysing their findings. The focus of the work is not only exposure associated with cell phones, but also exposure to many other sources such as power lines, broadcasting antennas, and electric household appliances such as induction ovens, mixers, washing machines and televisions.

The project, which will last four years (2004-2008) and involves 40 partner organisations. It includes all the coordinators of previous projects supported by the EU on these topics, representatives of the main National research activities in the field, the coordinators of other European and International research projects, industrial partners from mobile phone operators and the electrical and electronic industries, and representatives from trade unions, regulatory bodies and other stakeholders.

(EMF-NET Coordinator: Dr. Paolo Ravazzani, e-mail: [emf-net@polimi.it](mailto:emf-net@polimi.it))

## RADIOBIOLOGICAL MODELS OF TUMOR CONTROL AND NORMAL TISSUE COMPLICATION PROBABILITY FACILITATE QUALITY MANAGEMENT OF IMRT FOR HEAD AND NECK CANCER

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**Objective:** In Intensity Modulated Radiation Therapy (IMRT), doses arise nonuniform in target volumes with high gradients between normal tissues. This work aims to help interpret IMRT plan quality using the volumes and dose distributions of irradiated tissues embodied in models of Normal Tissue Complication Probability (NTCP) and Tumor Control Probability (TCP).

**Methods:** A Corvus system generated IMRT plans for 11 cases of head and neck cancer using 7 gantry angles for Varian 6Ex linac equipped with Millennium 80 and Brainlab micro-multileaf collimators. Plan safety and effectiveness assessed by TCP and NTCP considered factors including (i) achievable gradients depend on leaf size, (ii) target dose nonuniformity vs. partial volumes of irradiated tissues, and (iii) fractionation schemes. Effective volume DVH reduction method permitted NTCP calculation by Lyman's model. Equivalent Uniform Dose (EUD) method permitted TCP calculation by Poisson cell survival model. NTCP is a function about TD50(1) dose where 50% probability of complication occurs for a reference tissue volume (vref), then corrected to effective partial volume (veff),  $TD50(veff) = TD50(1) [veff^{*-n}]$ . TD50(1) is corrected by n at each dose level, and m is analogue to variance coefficient. Integrated over limits  $-\infty$  to  $t=(D-TD50(veff))/(m*TD50(veff))$  yields

$$NTCP = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^t \exp(-x^2/2) dx. \text{ TCP is a function of EUD and parameters } D_{50}, \text{ dose}$$

equals 50% tumor control, and  $\gamma_{50}$ , analogue to maximum slope of cell survival. Use of parameters yields  $TCP = 0.5^{\exp[(2\gamma_{50}/\ln 2)(1-EUD/D_{50})]}$ . First leaf size effect is reviewed. Next probability models are analyzed for primary target and spinal cord. Last fractionation effect on TCP and IMRT is analyzed where patient started immediately with conventional technique, followed by IMRT plan when ready, and completed with IMRT primary boost.

**Results:** Achievable penumbra between structures influences protection of normal tissue relative to high target dose. Using 3 mm micro-multileaf collimator, 90% to 10% penumbra shows 23.5% dose/mm gradient, but between 95% to 90% gradient shows 5% per 1.5 mm. For primary target, average (+/- 1 std. dev.) prescribed dose over cases is 65.18 +/- 2.36 Gy. EUDs average 66.96 +/- 2.57 Gy extrapolated to TCP average 0.71 +/- 0.04. Distinct from Poisson model, graph of EUDs vs. TCP shows linear correlation (99.9%). Spinal cord sparing reveals dependence of maximum dose (Dmx) and veff, Dmx average 40.84 +/- 3.13 Gy but percentage veff average 0.024 +/- .023. Following multiple regression analysis showed veff a significant linear predictor of NTCP combined with Dmx where  $F=110.3$  at (2,8) degrees of freedom ( $p<0.001$ ). In fractionation of nasopharynx carcinoma wrapping optic chiasm, TCP ranges from 0.654 to 0.675 regardless of number of fractions conventional treatment. On the other hand, complications increase to spinal cord, brainstem, and optic chiasm of 4%, 5%, and 4%, respectively. Overall, complication rate increases by about 1% per 2 fractions waited before starting full field IMRT.

**Conclusion:** To achieve highest TCP but maintain lowest NTCP, IMRT should begin as soon as reasonably achievable. Sooner IMRT begins, better the patient will fare for target coverage and critical structure sparing!

## PROTEIN SURFACE MAPPING BY HYDROXYL RADICAL INDUCED $^1\text{H}/^3\text{H}$ EXCHANGE

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Proteins surfaces are important in most biological processes including protein-protein or protein-ligand interactions. We have developed a methodology with the potential to analyze the whole surface of proteins whatever their size and at micromolar concentrations. This strategy is based on a non labile  $^1\text{H}/^3\text{H}$  exchange on amino acid side-chains. The amino-acids within a protein directly in contact with water incorporate more radioactivity than the buried ones.

The methodology can be resumed as followed:

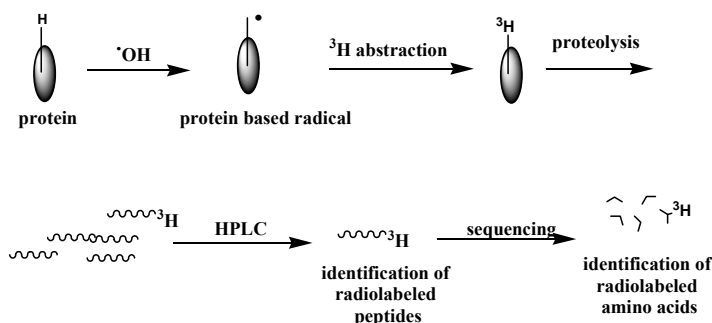
Hydroxyl radicals are cleanly produced in the presence of the protein under anaerobic conditions.

These radicals react with amino acid side-chains to lead carbon-centered radicals.

A judiciously chosen tritiated compound then transfer its tritium atom in place of the previously abstracted hydrogen.

Localisation of the tritium incorporation is obtained after proteolysis, HPLC purification of the labelled peptides followed by sequencing and radioactive counting.

Scheme 1: Rapid strategy for probing protein surface



Apomyoglobin was chosen as a model protein. The radioactive incorporation within each amino acid fully correlates with its solvent accessibility.

In conclusion we have developed a very sensitive methodology for the surface mapping of proteins. This strategy should be applied to micromolar concentrations of proteins without size restriction.

## ACTINIDE ANALYSIS IN ENVIRONMENTAL HOT PARTICLES BY SYNCHROTRON RADIATION

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### Objectives

Radioactive hot particles have been dispersed into the environment during atmospheric nuclear tests, accidents of the nuclear fuel cycle and authorised discharges from nuclear reprocessing plants (1). It is necessary to perform detailed studies in order to understand their behaviour in the environment to understand their mechanisms of transport and distribution as well as their bio-availability. The paper proves detection capability for actinide analysis by X-ray micro-fluorescence and absorption technique using synchrotron sources.

### Methods

Sea sediment core samples were collected at a shore of the Irish Sea near the Sellafield reprocessing plant (2). Specimen fractions collected at 22–30 cm depths were prepared on Nuclepore filters. The position of hot particles was determined by autoradiography. Based on the radiograph hot particles were measured at the micro-fluorescence beamline L at HASYLAB (3). Particles were scanned by a 15  $\mu\text{m}$  diameter X-ray beam formed by polycapillary. Uranium-containing sediment and soil particles of 20–40  $\mu\text{m}$  diameter were selected for XANES (micro X-ray absorption near-edge structure) measurements.

### Results and conclusions

Detection limits obtained for X-ray microanalysis were close to that of nuclear analysis. For long half life nuclides (more than  $10^5$  years), X-ray spectrometry was more sensitive, while being non-destructive and offering additional information on oxidation states using X-ray absorption. Using the combination of autoradiography and  $\mu\text{-XRF}$ , identification and quantitative analysis of individual radioactive particles of 20  $\mu\text{m}$  diameter were possible. Despite the strong spectral overlap with the Rb- $K\alpha$  characteristic line, the oxidation state of  $15 \mu\text{g g}^{-1}$  U in a single hot particle could be determined using fluorescence mode  $\mu\text{-XANES}$ .

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## CHILDHOOD LEUKAEMIA INCIDENCE IN HUNGARY. IMPACT OF THE CHERNOBYL ACCIDENT

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**Objective:** To report incidence data of childhood leukaemia in Hungary (1973-2002), and to investigate the impact of the Chernobyl nuclear accident during the observed period.

**Methods:** Retrospective, population based study using a new approach called 'Hypothesized Impact Period Interpolation'-model. Data of the National Paediatric Cancer Registry of Hungary were analysed (n=2204).

**Results:** The incidence of childhood leukaemia shows a statistically significant, moderate increase (0,71 % annually,  $p=0.0105$ ) during the observed period. The incidence rates were elevated by 6.0 % in the period of hypothesized impact, but this change was not statistically significant ( $p=0.25$ ).

**Conclusion:** The incidence, age distribution, and gender ratio of childhood leukaemia in Hungary are similar to other European countries. We did not find a statistically significant increase of incidence in the period of hypothesized impact of the Chernobyl accident.

## TRANSCRIPTIONAL DOWN-REGULATION OF ATAXIA-TELANGIECTASIA MUTATED PROTEIN (ATM) RADIOSENSITIZES HUMAN PROSTATE CANCER CELLS

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**Objective:** The goal of this study is to explore the mechanism/s by which the phorbol ester 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) induces apoptosis as a single drug and in combination with radiation treatment (RT) in the human prostate tumor cell lines.

**Materials and Methods:** Using plateau-phase cultures, we investigated the response of these cells to TPA, RT and the combination by measuring apoptosis using the bisbenzimidazole method. Ceramide generation was measured using the DAG kinase assay and activation of ceramide synthase (CS) enzyme, the enzyme responsible for ceramide generation in these cells. ATM levels were measured by western blot analysis, and ATM mRNA levels by RT-PCR assay. Sp1 binding to the ATM promoter was measured by gel shift analysis. We targeted ATM directly using oligonucleotides antisense technology (AS-ODN).

**Results:** We demonstrated that TPA reduces the ATM protein level in both LNCaP and CWR22-Rv1 cells. Radiation alone had no significant effect on ATM levels in these prostate cancer cell lines, nor when applied together with TPA. TPA-induced reduction of ATM protein correlated with increased apoptosis in these cell lines, while quantitative RT-PCR showed a 50% reduction of ATM mRNA between 8-16 h of TPA treatment. Gel-shift analysis showed a significant reduction in the amount of Sp-1 transcription factor binding to the ATM promoter, as early as 4 h post TPA-treatment for LNCaP, but with slower kinetics for CWR22-Rv1. The use of morpholino-antisense ATM (AS-ODN-ATM) replaced the TPA effects by significantly reducing the levels of ATM protein levels, and conferred substantial radiation-induced activation of CS, ceramide generation and apoptosis. Furthermore, AS-ODN-ATM conferred CS-mediated radiation-induced apoptosis also in TPA-resistant prostate cancer cell lines, PC-3 and DU-145.

**Conclusions:** These data demonstrate that in both androgen-dependent and-independent human prostate cell lines, the radiation apoptotic response is mediated through ATM/CS pathway. This investigation defines a new approach to overcome radiation resistance in human prostate cancer cells. (Supported by CaPCURE and NCI Prostate Spore Grant # CA92629-01 to A H-F.)



## INTERNATIONAL CO-OPERATION FOR, AND THE LESSONS TO BE LEARNT FROM THE MEDICAL MANAGEMENT OF RECENT RADIATION ACCIDENTS

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Radiation accidents with severe health consequences are rare. Nevertheless, there is a need for global collaboration by relevant international organizations for prevention, preparedness and medical management of nuclear accidents and radiological emergencies. There is a growing concern and need today to prevent malevolent use of radioactive materials by terrorists and for early recognition of possible radiation injuries.

The International Conventions on "Early notification of a nuclear accident" and on "Assistance in case of a nuclear accident or radiological emergency" (1986) provide the prime legal instruments that establish an international framework to facilitate exchange of information and prompt provision of assistance in the event of radiation emergencies, with the aim of minimizing the health consequences. The WHO acceded to the Conventions in 1988, after the establishment of its Radiation Emergency Medical Preparedness and Assistance Network (REMPAN). Authority, responsibility and response functions of the international organizations (EC, FAO, ICAO, IAEA, NEA/OECD, OOSA, PAHO, UN OCHA, WHO and WMO) cooperating in the "Joint Radiation Emergency Management Plan of the International Organizations" were published by the IAEA, Vienna, in Dec. 2002.

The lessons to be learnt from the medical management of radiation accidents in the last decade drew attention for strengthening international cooperation in development of infrastructure and manpower training. It resulted in 30 IAEA-WHO publications in the last eight years including the first joint training material in radiation medicine (CD-ROM for 5-day courses, Vienna, 2002).

As a result of strengthening WHO/REMPAN in the last two years, it has developed by June 2004 to a worldwide network of 28 Collaborating Centres (CCs) and Liaison Institutes (LIs) in 17 countries: ARG, ARM, AUS, BRA, CAN, CHI (2), FIN, FRA (2), GER (2), HUN, IND, JAP (3), KOR (2), RUS (4), UK, UKR & USA. The WHO Operational Guidance for coordination of the medical management and public health actions in case of a radiation emergency was first published in Oct. 2002. Its updated version titled "Medical Response to Radiation Emergencies" has been completed in May 2004 for publication by the WHO.

Understanding the importance of dissemination of relevant information to the emergency medical responders, the physicians (GPs) and the public, the WHO maintains a homepage [http://www.who.int/ionizing\\_radiation/en/rempan](http://www.who.int/ionizing_radiation/en/rempan) with links to updated Facts sheets.

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(\*in Jan.1995-May 2002: Radiation Safety Section of the International Atomic Energy Agency, Vienna and in June 2002-June 2004: Radiation and Environmental Health Unit of the World Health Organization, Geneva)

## M-FISH-ANALYSIS OF RADIATION-INDUCED CHROMOSOME ABERRATIONS IN PRIMARY CULTURED HUMAN SKIN FIBROBLASTS FROM AN ACCIDENTALLY IRRADIATED PATIENT

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### **Objective:**

Following accidental radiation exposure dose estimation is of major importance concerning the choice of treatment of the victim. For this reason, usually a cytogenetic dose estimation is done, using chromosome spreads of the patient's blood lymphocytes. Unfortunately, in cases where a cutaneous radiation syndrome evolves through inhomogenous partial body irradiation, often an underestimation of the radiation dose in the skin organ occurs using this method. Therefore, we tried to develop a technique to estimate the radiation damage leading to cutaneous radiation syndrome directly in the target where the damage occurred, namely the skin cells.

### **Methods:**

Skin biopsies of locations showing the cutaneous radiation syndrome and of normally appearing skin were obtained from a patient irradiated during the radiation accident in Lilo/Georgia 1996/97. Skin fibroblast cultures were established and chromosome aberrations in cells were analysed using the technique Multicolour Fluorescence in situ Hybridisation (M-FISH).

### **Results:**

M-FISH chromosome aberration analysis succeeded in seven primary fibroblast cultures of two different body locations. Of each location chromosome analyses both of healthy appearing and of pathologically altered skin were done. In all preparations of obviously damaged skin areas multiple chromosome aberrations were found. Interestingly, chromosome breaks could also be detected in preparations of visually normal appearing skin, indicating radiation damage due to subclinical doses. A comparison of chromosome breakage events in fibroblasts of damaged and normal appearing areas of skin-location 1 in 20 metaphases each revealed in average 9,5 and 2,7 breaks per cell, respectively.

### **Conclusion:**

Stable chromosome aberrations are present even seven years after irradiation in skin fibroblasts. The presence of chromosome aberrations also in fibroblasts of healthy appearing areas surrounding damaged skin helps to identify the critical dose threshold for the development of the chronic cutaneous radiation syndrome.

## CHANGES IN GENE EXPRESSION IN HUMAN LYMPHOBLASTOID CELLS EXPOSED TO EITHER 0.02 OR 2 GY GAMMA RADIATION

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**Objective** For a long time in radiobiology it was very difficult to assess the effects of low doses of ionizing radiation because we did not have biological tools to measure the effects objectively. Transcriptome analysis now permits to explore the field of low doses of radiation and potentially it can provide a global view of radiation responsive pathways

**Method** We have exposed a human lymphoblastoid cell line to either 0.02 or 2 Gy of ionizing radiation that yielded relatively little or faint cytotoxicity and little or no apoptotic DNA fragmentation. We used cDNA microarrays (8000 probes corresponding to 6000 genes) to examine the modulation of gene expression at various time points within 72 hours following gamma radiation exposure at either 0.02 or 2 Gy.

**Results:** We observed that 1) a lower number of genes are deregulated after 0.02 compared to 2 Gy, 2) some genes are specifically deregulated according to the dose while others are similarly deregulated whatever the dose, 3) all responsive genes after both doses and those specifically deregulated after 2 Gy are mainly involved in signal transduction, cytoskeleton, protein metabolism and catabolism, intracellular trafficking and transcription factors whereas genes specifically deregulated after 0.02 Gy are mainly related to signal transduction, cytoskeleton, stress response, ionic transport and channel 4) after both doses, responsive genes related to cell survival and death are in good agreement with data obtained on cell survival and death and 5) overall results support the hypothesis that low doses of ionizing radiation lead to a typical stress-induced translation inhibition and RNA processing alteration.

**Conclusion:** This work emphasizes the usefulness of DNA microarray to obtain an integrated view of the radiation response and underlines the need of further efforts to explore the effects of low doses of radiation since it highly suggests that part of the response at low doses cannot be predicted by extrapolation from data obtained at high doses.

## ROLE OF HOMOLOGOUS RECOMBINATION IN THE PRODUCTION OF X-RAY-INDUCED CHROMOSOME ABERRATIONS DURING THE G<sub>1</sub> AND G<sub>2</sub> PHASE OF THE CELL CYCLE

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*Objective: To define the role of the DNA-repair pathways non-homologous end joining (NHEJ) and homologous recombination (HR) in different stages of the cell cycle.*

*Methods:* V79 mutant cell lines XR-V15B and V-C8, deficient in respectively Ku80, which is part of NHEJ and Brca2 as part of HR, were X-irradiated at the G<sub>2</sub> or G<sub>1</sub>-S phase of the cell cycle and analysed for the induction of structural chromosomal aberrations. To obtain irradiated G<sub>1</sub> cells two methods i.e multiple sampling times or synchronization using isoleucine depletion were used. In addition an other HR mutant, CL-V4B, deficient in Rad51C and displaying impaired sisterchromatid cohesion (SSC) was used for G<sub>2</sub> experiments.

*Results:* Due to the very short G<sub>1</sub> phase in normal growing V79 cells, isoleucine depletion turned out to be the best method to obtain G<sub>1</sub>-S phase enriched populations of cells. The results of the lightmicroscopical chromosomal analysis in the the cell lines show that:

Frequencies of spontaneously occurring aberrations were much higher in the HR deficient mutants than in the the NHEJ deficient cells.

2. The ratio of chromosomal exchanges/breaks was similar for all cell lines indicating that both repair pathways have the same mis-repair versus non-repair ratio.

Both repair pathways lead to comparable radiosensitization with a factor 3-4 in G<sub>2</sub> but in G<sub>1</sub>-S, the contribution of NHEJ (factor 13) seems to be much higher than HR (factor 5).

G<sub>2</sub> chromosomal radiosensitivity in the HR mutants was not correlated with impaired SSC.

*Conclusion:* From a mechanistic point of view it remains difficult to explain a contribution of HR in the prevention of radiogenic chromosomal aberrations during the G<sub>1</sub> and G<sub>2</sub> phase of the cell cycle.

## THE LATENT TIME FOR CELLULAR AND TISSUE RESPONSES IN THE SPINAL CORD

Albert J. van der Kogel

The spinal cord, as part of the central nervous system, is a classical “late responding tissue”, with latent times in human patients typically between one and two years for the most devastating syndrome of radiation myelopathy. In rodents the most extensively studied endpoint is that of paralysis due to white matter necrosis, which develops after 5-6 months after a single dose of >20 Gy megavoltage photons to at least a 10 mm length of cervical cord. The so-called latent period is highly reproducible at specified dose levels, and generally decreases (log) linearly with increasing doses. Since the damage at the tissue level is mostly restricted to the white matter, it has long been assumed that oligodendrocytes and their progenitors are the key cellular targets of this lesion, with a gradual loss of mature oligodendrocytes and a lack of regeneration due to depletion of the stem cell pool. The early loss of oligodendrocytes by apoptosis has indeed been well documented even at dose levels well below the tolerance dose for white matter necrosis, and is clinically associated with the Lhermitte syndrome of demyelination. This self-limiting syndrome generally occurs after 2-4 months and is thought to be due to demyelination followed by proliferation of glial precursors and differentiation into mature myelinating oligodendrocytes.

In rat spinal cord, a dose- and time-dependent loss of oligodendrocyte precursor cells (O-2A progenitors) has been observed, followed by recovery to different (dose-dependent) levels after 2-3 months. Only after a paralytic dose of 22 Gy a secondary loss of O-2A progenitors was observed starting at approximately one month before the development of white matter necrosis and neurologic impairment. Various investigations now indicate that this second wave of oligodendrocyte loss is most likely the result of a cascade of inflammatory changes leading to blood-barrier disruption, tissue hypoxia and necrosis, with the initial events originating in the microvascular endothelium.

In a recently completed series of experiments of high precision proton irradiations of rat spinal cord, it has been shown that the latent time to white matter necrosis is not only dependent on dose but even more on the irradiated length, irradiated region (lateral vs. central) and the addition of a low dose bath surrounding the target volume. These results provide further insight into the potential mechanisms of radiation-induced injury in the central nervous system.

## PROGESTERONE PREVENTS RADIATION-INDUCED APOPTOSIS IN BREAST CANCER CELLS

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**Objective:** Sex steroid hormones play an essential role in the control of homeostasis in the mammary gland. Although the involvement of progesterone in cellular proliferation and differentiation is well established, its exact role in the control of cell death still remains unclear. As dysregulation of the apoptotic process plays an important role in the pathogenesis of breast cancer, we investigated the regulation of apoptosis by progesterone in breast cancer cell lines.

**Methods:** We used various breast cancer cell lines, expressing or not the progesterone receptor (PgR+ and PgR-). Effect of progesterone treatment on cell growth, survival and apoptosis was analyzed. Cell cycle distribution was studied by FACS analysis. Indexes of proliferation were determined by the cytochalasin-B blocked assay, and chromosome aberrations were estimated by scoring micronuclei in dividing cells.

**Results:** Progesterone treatment protects against radiation-induced apoptosis. This prevention appears to be mediated by the progesterone receptor and is unrelated to p53 status. There is also no correlation with the intrinsic hormonal effect on cell proliferation, as the presence of cells in a particular phase of the cell cycle. Surprisingly, progesterone partly allows bypassing of the irradiation-induced growth arrest in G2/M in PgR+ cells, leading to an increase in cell proliferation after irradiation. One consequence of this effect is a higher rate of chromosome damage in these proliferating progesterone-treated cells compared to what is observed in untreated irradiated cells.

**Conclusion:** We propose that progesterone, by inhibiting apoptosis and promoting the proliferation of cells with DNA damage, potentially facilitates the emergence of genetic mutations that may play a role in malignant transformation.

## CAFFEINE-INCREASED RADIOSENSITIVITY IS DEPENDENT ON PREVENTION OF G<sub>2</sub>/M ARREST AND INDUCTION OF APOPTOSIS IN HL-60 CELLS

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**Objective:** We have examined the effect of ATM kinase inhibitor caffeine on the radio-sensitization of HL-60 (human promyelocyte leukemia cell line) after gamma irradiation with high (HDR=0.6 Gy/min) and low (LDR=3.9 mGy/min) dose rate.

**Methods:** The influence of caffeine on radiotoxicity has been assessed by clonogenic survival assay. The G<sub>2</sub> phase arrest abrogation and apoptosis (subG<sub>1</sub>) has been measured by flow-cytometry. Western blotting has been used for determination of phosphorylation of Chk1 and presence of Cdc25A phosphatase.

**Results:** Most cell lines that lack functional p53 protein are arrested in the G<sub>2</sub> phase of the cell cycle due to a DNA damage. We have found that the human promyelocyte leukemia cells HL 60 (TP53 negative) exposed to a ionizing radiation in the doses up to 10 Gy were arrested in the G<sub>2</sub> phase for the period of 24 hours. When the HL-60 cells have been exposed to a low dose-rate 3.9 mGy/min (LDR) gamma irradiation, which resulted in a pronounced accumulation of the cells in the G<sub>2</sub> phase during the exposure period, their radioresistance has increased in comparison with the cells irradiated with a high dose-rate 0.6 Gy/min (HDR). The D<sub>0</sub> value (a dose of radiation, after which 37% of cells survive) has been 3.7 Gy for LDR and 2.2 Gy for HDR. The prevention of the G<sub>2</sub> phase arrest by caffeine (2mM) and the irradiation of the cells with HDR and LDR caused radiosensitization (D<sub>0</sub> = 1.5 Gy resp. 2.1 Gy).

**Conclusion:** The irradiation in presence of caffeine has decreased the phosphorylation of Chk1 kinase and increased the amount of Cdc25A phosphatase. Therefore, the cells in presence of caffeine have entered the cell cycle with an unprepared DNA, resulting in a radiosensitizing effect. Programmed cell death (apoptosis) has appeared in later intervals after irradiation.

## RADIATION-INDUCED KIDNEY DAMAGE: PATHOGENESIS AND INTERVENTION STRATEGIES

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Radiation-induced renal injury is the major dose-limiting factor in radiotherapy of the abdomen. Its clinical significance has been recognized in many experimental and clinical studies since the beginning of the 20<sup>th</sup> century. One of the first reports on radiation-related effects on the human kidneys was presented in 1907, but it took almost another 50 years before the “limit of renal tolerance to radiation” was defined by Kunkler *et al.* Current treatment protocols that place the kidney(s) at risk, include radiotherapy for gastric NHL, Hodgkin’s disease, testicular seminoma and, more recently, gastric carcinoma.

Following irradiation of the kidney, a progressive decrease in both glomerular and tubular function is observed, which is dose- and volume-dependent. After unilateral kidney irradiation, a gradual compensatory hyperactivity develops in the contralateral organ. In a prospective study with a follow up of more than 10 years, we found that 56% (14/25) of the patients with renal functional impairment developed hypertension after a mean interval of 45 months (range 12-224). In 50% (7/14) of these hypertensive patients, a renovascular component was identified by captopril-renography and confirmed by selective renal angiography.

Despite extensive research, the pathogenesis of radiation nephropathy (RNP) is still not fully understood. Histologically, an early phase of predominantly glomerular vascular damage is seen, in particular after low and intermediate radiation doses, suggesting that vascular injury plays a prominent role in the onset and progression of RNP. We hypothesize that the vascular endothelium is a critical target in the development of RNP. We and others have observed several pivotal events occurring in cultured endothelial cells after irradiation: (1) an increased release of von Willebrand Factor (vWF), a critical mediator of platelet adhesion in small blood vessels; (2) a decreased production of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation; (3) an enhanced expression of tissue factor, an activator of the blood coagulation cascade. We also observed an increased glomerular vWF deposition in the mouse kidney *in vivo* after irradiation. These effects may trigger intravascular thrombosis ultimately leading to vascular occlusion and organ dysfunction.

Several pharmacological strategies have been tested to influence the process of RNP, including ACE inhibitors, angiotensin II blockers and anti-platelet agents. We found that long-term oral administration of acetylsalicylic acid reduced the renal functional impairment in mice that developed after split-dose irradiation, but not after fractionated irradiation. Effective amelioration of renal failure in experimental RNP has been achieved by the ACE inhibitor captopril, even when started after the onset of renal toxicity.

Another approach that has been pursued to reduce renal doses to radiation involves the introduction of improved radiation techniques. For example, the respiration-correlated cone-beam CT scan generates high-quality 3D anatomical information during treatment that allows us to significantly reduce the radiation dose to the left kidney in case of adjuvant chemoradiation in gastric cancer.

**Conclusions:** The progressive functional impairment of the kidney requires long-term follow up. Radiation-induced injury of small blood vessels is an important factor in the pathogenesis of RNP. Both pharmacological and technical approaches may prove successful in limiting renal injury after treatment.



## DOSE RESPONSE FOR T-CELL RECEPTOR (TCR) MUTANTS IN PATIENTS REPEATEDLY TREATED WITH I-131 FOR THYROID CANCER

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### **Objective:**

The main risks of radioiodine treatment are the induction of lung fibrosis and secondary cancers in consequence of the genotoxic effects of the radioiodine radiation. In the present investigation the TCR assay was applied to quantify the genotoxic effect of I-131 therapy and to detect patients who might have an increased cancer risk. This is a part of a bigger project where by the same patients not only TCR assay but also Micronuclei assay in reticulocytes was done.

### **Methods:**

The T-cell receptor mutant frequency (TCR-Mf) was measured in 80 thyroid cancer patients, who were treated with radioiodine. Patients came from the southern part of Belarus, which had suffered heavily from the Chernobyl disaster. TCR-Mf was determined by flow cytometry before and after one to maximal ten treatments. A test takes about 6 h and not more than 3-6 ml blood is required. Also in 27 patients TCR-Mf was measured by lymphocytes incubated for 6 days. Incubation was done to overrun the latent period after exposure needed for mutation to arise.

### **Results:**

Before first treatment TCR-Mf of patients was  $2.0 \times 10^{-4}$ . This Mf value is in the range as that of healthy donors of the same age. After I-131 therapy TCR-Mf increases within about half a year to a maximum. The increase per 1 mGy to red marrow was  $8.66 \times 10^{-7}$ , which corresponds to a doubling dose of about 250 mGy. After the maximum TCR-Mf declines exponentially with a half-life of approx. 3 years. On the base of these data a calibration curve for the use of TCR-Mf as a biological dosimeter is given.

In the case of incubation the significant increase in the MF's before and after irradiation was found. This difference was more evident in the case of the first time treated patients.

### **Conclusions:**

The TCR-Mf test is a reliable biological dosimeter. The calibration curve found in I-131 treated patients can be used if radiation quality, dose and dose-rate are in the same range as in radioiodine therapy. For the shortening the latent period needed for mutation to appear incubation of lymphocytes can be used.

## BIOLOGICAL INDICATORS OF RADIOLOGICAL ACCIDENT FOR THE PRESENT AND THE FUTURE

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### Objective

Exposure to ionising radiation can induce significant alterations in some biological parameters produced in different tissues. With respect to their intrinsic properties (specificity, sensitivity, delay) biological indicators can constitute an useful complement of the clinical symptomatology and of the physical reconstruction in case of accidental radiation overexposure suspicion. In addition, the evidence of dose-effect relationships discriminates the biological dosimeter from the biological indicator. A particular interest of biological dosimetry estimation is to take into account the susceptibility of people to ionizing radiation. The next question should be: is biological dosimetry sufficient to characterize radiation extent ?

### Methods & Results

For many years, the scoring of unstable chromosome aberrations (dicentric & centric ring) in peripheral blood lymphocytes was considered as the 'gold standard' of biological dosimetry in case of acute, whole-body and homogeneous irradiation. However, most of radiation overexposure are heterogeneous, fractionated or their evaluation delayed. New methods were thus added to the conventional one in order to set up a multiparametric panel of biological dosimeters. They have been first developed in cytogenetic area (translocations, micronuclei, prematurely condensed chromosomes) on circulating lymphocytes but also on resident cells, in order to assess the dose locally received. Even if the dose is useful to the medical team in charge of patients to establish the damage level, the dose by itself is not sufficient to set up organ dysfunction as a consequence of radiation injury. In fact at the level of the organ, biological dosimeters should be completed by bioindicators of prognosis and of diagnosis. Some of these indicators looked promising and are presented in this paper (FLT-3, Citrulline, Oxysterols). They refer to either structural or functional alterations.

### Conclusion

The final purpose is to establish a cartography of the doses and of the damages received by various organs as soon as possible in order to evaluate Multiple Organ Dysfunction Syndrome leading to an eventual Multiple Organ Failure.

## BIOLOGICAL EFFECTIVENESS OF THE 150 MEV PROTON MEDICAL BEAM

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The effectiveness of the lethal action of protons with energy 150 MeV on C3H10T1/2 cells in four points of the spread out (3.7 cm wide) Bregg-peak (SOBP) has been studied. Cell suspension with concentration  $1-5 \times 10^5$  cells/ml in 1 cm diameter test-tubes and also a cell monolayer in the wall's surface of the 25 cm<sup>2</sup> plastic flasks was irradiated with 2 Gy dose. Three of them were in the plateau region of Bragg peak and the fourth was in the entrance. Significant differences were not obtained in the different points of mid-SOBP region. Cell survival was noticeably high in the entrance point.

We also carried out a research on the determination of RBE for the 150 MeV proton medical beam. The effectiveness of the protons action was compared to the effectiveness of the <sup>60</sup>Co  $\gamma$ -rays. The conditions of the experiment were maximally approximated to the conditions during radiotherapy. It is known that C3H10T1/2 cells are sensitive to postconfluence inhibition of division, that is why after achieving the saturation density equal to 2.9 till  $3.8 \times 10^4$  cells/cm<sup>2</sup> they stop dividing. This property allowed using them for long fractionated irradiation of this cells' monolayer, which grows on the walls of the 25 cm<sup>2</sup> plastic dishes.

The fractionated irradiation was carried out with an interval between fractions of 24 hours with 4 Gy dose in one fraction (4-40 Gy dose range). Cells were exposed to irradiation 5 days per week. The obtained results showed that protons and  $\gamma$ -rays have similar radiobiological effectiveness in the case of cell killing. The RBE value for all the survival levels, i.e. calculated for LD<sub>50</sub>, LD<sub>10</sub> and LD<sub>1</sub> is always equal to one.

## SIGNALING PATHWAYS INVOLVED IN HEMATOPOIETIC STEM CELL PROLIFERATION, DIFFERENTIATION AND RECONSTITUTION FOLLOWING WHOLE BODY IRRADIATION

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The microenvironmental stimuli and growth factors that control the proliferation and differentiation of hematopoietic stem cells are largely unknown. Growth factors implicated include thrombopoietin, c-kit ligand, flt-3 ligand, interleukins-3 and interleukin-6, with thrombopoietin an initially unexpected major factor as evidenced by TPO and c-mpl knock-out mice, reconstitution kinetics following total body irradiation in both mice and nonhuman primates, as well as in NOD/SCID recipients of human stem cells. Signaling pathways operational and determining the balance of proliferation and differentiation in hematopoietic stem cells include the transcription factor HOXB4, and the wnt/ $\beta$ -catenin pathway. Since many of the growth factors stimulating stem cells activate the STAT5 pathway, we examined its role in the reconstitution of hematopoiesis in irradiated recipient mice following retrovirus-mediated transduction of wild type (wt) STAT5. Following transplantation, using a competitive set-up in which the STAT5 expressing stem cells need to compete with both endogenous as well as transplanted normal stem cells, ectopic expression of wt STAT5 appeared to confer an approximately 20-fold multilineage repopulating advantage to long term repopulating stem cells relative to controls, untransduced cells, and endogenous stem cells. Production of red cells, platelets, neutrophils/monocytes and T cells was normally regulated with normal multilineage growth factor responses (+ increased life span of B cells). Immature cell numbers as measured by marrow repopulating ability and the spleen colony assay stayed within normal ranges, showing that STAT5 did not influence self-replication of stem cells. The ectopic wt STAT5 phenotype is inversed to the STAT5a/b KO mouse and demonstrates that the repopulation capacity of stem cells is not fixed but can be quantitatively modulated. This opens new avenues to design strategies to modulate and increase hematopoietic stem cell repopulating capacity following total body irradiation.

## RADIATION EMERGENCY MEDICAL DIAGNOSIS AND TREATMENT ENTERING THE POST-GENOMICS ERA

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The outcome of the acute radiation syndrome largely depends on controlling radiation induced inflammatory reactions, on the reconstitution capacity of the stem cells of vital organs such as the blood producing bone marrow and the intestines, and on recovery from damage to more composite organs such as the skin. It is well recognized that the symptoms and life-threatening complications following accidental exposure evolve both dynamically with time as well as in interactive patterns of reaction to damage at cellular, tissue, organ and organism levels. The current diagnosis and treatment state-of-the-art consists of early diagnosis based on evolving symptoms in the first hours to days, and rapid intervention so as to curtail further aggravation and ameliorate symptoms, as has been recently summarized in a detailed protocol<sup>1</sup> developed in a European concerted action. Despite vast advances in biomedical knowledge, markers to identify and quantify the radiation damage at the required very early time interval after accidental exposure still do not surpass the highly appraised diagnostic value of early shifts in white blood cell counts, while treatment options remain limited, including those for the bone marrow syndrome, where neither stem cell transplantation nor growth factor treatment have fulfilled the promises due to respectively, lack of suitable donors to reduce the risks and insufficient responses at life-threatening radiation exposures, as has been amply documented in limited clinical experience and extensive experimental animal data. Therefore, an entirely new approach is an imminent requirement. The mapping of the human genome and the ensuing science of genomics and proteomics with high throughput analysis of gene expression profiles may well provide the opportunity to reach a breakthrough in rapid diagnosis of radiation damage and tailored intervention. By the same token, the identification of signaling pathways and downstream genes controlling inflammatory reactions and the reconstitution capacity of stem cells will result in the development of small molecules that electively activate desired and/or disrupt detrimental protein functions, while the rapid advances in automated genome sequence data will allow the generation of new proteins to serve as much more effective medicines than their native originals. And finally, introducing temporarily expressed defined genes into transplantable stem cells may tailor their expansion, migration, differentiation and elimination in vivo, according to the individual's unique medical condition. Also, novel effector functions can be introduced into cells to protect against infectious agents.

Fliedner TM, Friesecke I, Beyrer K. (Eds.), Medical Management of Radiation Accidents: Manual on the Acute Radiation Syndrome. 2001 Published by the British Institute of Radiology. Supported by Euratom under contract number FI4PCT970067.

## RADIOPROTECTION BY NATURAL AND SYNTHETIC ANTIOXIDANTS

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The potential of antioxidants to reduce the cellular damage induced by ionizing radiation has been investigated for more than 50 years. The application of antioxidant radioprotectors to various human exposure situations has not been extensive, although it is generally accepted that endogenous antioxidants, such as cellular non-protein thiols and antioxidant enzymes, provide some degree of protection. This review compares effects of synthetic and naturally-occurring antioxidants on various endpoints of radiation damage. Although amifostine (WR-2721) has been approved as an adjunct to specific radiotherapy procedures, it has not yet been approved for use in nuclear emergency scenarios because of adverse side effects. Similarly, the use of phosphorothioates as antimutagenic or anticarcinogenic agents in the context of long-term radiation effects has not progressed because of the absence of human data supporting this application. Results from animal experiments indicate that antioxidant nutrients, such as vitamin E and selenium compounds, are protective against lethality and other radiation effects but to a lesser degree than most synthetic protectors. Naturally-occurring antioxidant nutrients and phytochemicals have the advantage of low toxicity although they are generally protective only when administered at pharmacological doses. These antioxidants may also provide an extended window of protection against low-dose, low-dose-rate irradiation, including therapeutic potential when administered after irradiation. A number of phytochemicals, including caffeine, genistein, and melatonin, have multiple physiological effects, as well as antioxidant activity, that can enhance *in vivo* radioprotection. Many antioxidant nutrients and phytochemicals have antimutagenic properties, and their modulation of long-term radiation effects, such as cancer, needs further examination. Additional studies are required to determine the potential value of specific antioxidant nutrients and phytochemicals during radiotherapy for cancer.

## DOUBLE-STRAND BREAK REPAIR BY HOMOLOGOUS RECOMBINATION – MECHANISMS AND CONTROL

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In recent years, the DNA repair field has become aware of the relationship between genome instability and predisposition to cancer. The mechanisms by which cells repair potentially mutagenic lesions and DNA breaks caused by radiation or DNA damaging agents are now known to be critical for the faithful reproduction of the genome and tumour avoidance. Our particular interest lies in the mechanisms by which chromosomal breaks are repaired by homologous recombination.

It is known that some homologous recombination proteins (e.g. RAD51) are conserved from simple organisms to man, whereas other vertebrate proteins have no obvious prokaryotic homolog. One example of the latter is BRCA2, a tumour suppressor protein that is often mutated in individuals predisposed to breast and ovarian cancer. that is thought to control RAD51 activity through direct protein-protein interactions. The *BRCA2* gene encodes a large (384 kDa) protein containing 3418 amino acids. Although the sequence of BRCA2 has revealed few clues to its cellular function, a role in DNA repair and replication fork maintenance was indicated by the hypersensitivity of BRCA2-defective cells to ionizing radiation and other DNA damaging agents. Most importantly, BRCA2 mutant cell lines were shown to exhibit defects in the repair of DNA double-strand breaks by homologous recombination.

The role that BRCA2 plays in homologous recombination is likely to be mediated through its interactions with RAD51 recombinase. In new studies, we have shown that the C-terminal region of human BRCA2 is phosphorylated by the cell cycle regulatory kinase CDK2-cyclin A. Inhibition of cell cycle progression at the G2/M boundary by nocodazole treatment results in phosphorylation of the S3291 CDK2 target site in BRCA2 and disrupts interactions between the C-terminus of BRCA2 and RAD51. Conversely, ionising radiation leads to reduced phosphorylation of S3291 — thereby stimulating interactions between BRCA2 and RAD51. These results show that the phosphorylation status of S3291 is critical for modulating interactions between the C-terminus of BRCA2 and RAD51, and provide new insight into why C-terminal deletions of *BRCA2* result in radiation sensitivity and cancer predisposition.

## DOSE RESPONSE RELATIONSHIP FOR TRANSLOCATIONS IN RETIRED RADIATION WORKERS

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**Objective:** To establish the dose-response relationship for translocation frequencies in peripheral blood lymphocytes from occupationally exposed radiation workers with well documented radiation dosimetry records.

**Methods:** Chromosome aberration analysis using the FISH technique was undertaken on 294 retired workers from the British Nuclear Fuels plc facility at Sellafield, UK, 95 with cumulative occupational external exposures <50mSv, 108 with 50-499mSv and 91 with >500mSv. All were male, aged over 50 years.

**Results:** Univariate analysis of translocation frequency was statistically significant for both external dose ( $P = < 10^{-5}$ ) and age ( $P = 0.0075$ ) but no effect was found for smoking status. Multivariate analysis with age and dose as continuous variables revealed slopes of  $0.017 \pm 0.0075 \times 10^{-2}$  translocations per cell per year for age ( $P = 0.024$ ) and  $1.11 \pm 0.190 \times 10^{-2}$  translocations per cell per Sv for external dose ( $P < 10^{-5}$ ).

**Conclusion:** The dose response for translocation induction in these workers is similar to the linear component of published *in vitro* dose response curves. Consideration of the exposure pattern as a series of daily increments of <0.4mSv indicates an additive effect with no suggestion of a deviation from a linear response at very low doses. Thus there is no evidence for novel mechanisms influencing the response to occupational radiation and the work supports the use of translocation frequency for retrospective dosimetry in situations of chronic exposure over many years. The dose response is lower than the linear component of the dose response for stable aberrations obtained for the Japanese atomic bomb survivors suggesting that if chromosome frequencies are indicative of cancer risk then low-dose risks derived from the Japanese survivor data will overestimate the risks associated with occupational exposure at the levels encountered by the men in this study.



## 5-ANDROSTENEDIOL: A LONG-ACTING NATURAL SYSTEMIC RADIOPROTECTANT WITH LOW TOXICITY

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5-Androstenediol (5-AED) is a natural circulating steroid originating from the adrenal cortex. Subcutaneous (sc) injections of 5-AED 24 h or 48 h before whole-body gamma-irradiation enhance survival and stimulate hematopoiesis in mice. Its radioprotective effects are observed mainly in the radiation dose range characteristic of the hematopoietic syndrome, and diminish rapidly as radiation doses are elevated into the range causing the gastrointestinal syndrome. The dose reduction factor (DRF) in mice is approximately 1.3. In mice treated with 5-AED, we have observed increases in marrow granulocyte-macrophage progenitors by histology and clonogenic assays, and elevations of circulating neutrophils, monocytes, and natural killer (NK) cells, but not B cells or T cells. We have also observed amelioration of radiation-induced decreases in circulating platelets and red blood cells. Functional activation of innate immune cells in circulation is indicated by elevation of surface CD11b on NK cells, stimulation of phagocytic activity in granulocytes, and enhancement of oxidative burst in monocytes. Pharmacokinetic studies after sc or buccal administration indicate the radioprotective effects of 5-AED depend on events triggered within hours after administration, but do not depend on the plasma concentration present at the time of irradiation. Comparisons with other steroids show that oxidation of the 17-hydroxyl to a keto group, or addition of an hydroxyl group at the 7 position, is deleterious, and radioprotective efficacy is probably not due to activation of sex steroid receptors. However, some of these results may have been due to differences in pharmacokinetics, rather than specific interactions with steroid receptors. Treatment after exposure to radiation has been successful with 5-AED and water soluble analogs 5-AED sulfate and HE3204 (17 amino analog). Radioprotection can be achieved after oral administration. 5-AED exhibits extremely low toxicity as demonstrated by blood chemistry, histology, and behavioral assays.

5-AED has a number of characteristics that make it attractive as a radioprotectant for field use: it is a small molecule that is stable at environmental temperatures, has moderate radioprotective efficacy, and has extremely low toxicity. We have demonstrated alleviation of radiation-induced neutropenia in dogs and nonhuman primates, and are in pre-Investigational New Drug (pre-IND) discussions with the U.S. Food and Drug Administration (FDA).

## RISK OF SOMATIC LATE EFFECTS IN HUMANS AFTER THERAPEUTICAL INJECTION OF THE SHORT-LIVED $\alpha$ -EMITTER RADIUM-224

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An epidemiological study has been carried out at the GSF – National Research Center for Environment and Health – on 1460 ankylosing spondylitis (AS) patients. The aim of the study was to ascertain the late health effects suffered by these patients who had received repeated intravenous injections of the short lived  $\alpha$ -emitter <sup>224</sup>Radium between 1948 and 1975 (excluding radiation therapy with X-rays). The usual therapeutic plan consisted of a total of 10 to 12 injections of 1.036 MBq (28  $\mu$ Ci) of <sup>224</sup>Ra each, given at weekly intervals; this would result in a cumulative  $\alpha$ -dose of 0.56 to 0.67 Gy to the marrow-free skeleton (bone surface dose:  $\sim$  5.5 Gy) of a 70-kg-man (standard man). These patients have been followed together with a control group of 1323 ankylosing spondylitis patients not treated with radioactive drugs and/or X-rays. Causes of death have been ascertained for 842 exposed patients and 861 controls (mean follow-up time of 24.6 yr in the exposed group or 23.1 yr in the control group). In the exposed group there has been a total of 219 malignant diseases (vs. 252.2 expected cases) and 206 cases among the controls (vs. 278.1). In particular, we observed 15 cases of leukaemia in the exposure group (vs. 5.9 cases expected,  $p = 0.001$ ) and 8 cases of leukaemia in the control group compared to 6.5 cases expected ( $p = 0.3$ ). Further subclassification of the leukaemias demonstrated a high increase of myeloid leukaemia in the exposure group (9 cases obs. vs. 2.5 cases exp.,  $p = 0.001$ ), and out of these especially the acute myeloid leukaemias (6 cases observed vs. 1.6 expected,  $p < 0.01$ ), whereas in the control group the observed cases are within the expected range (3 myeloid leukaemias vs. 2.6 cases).

Out of these 6 cases of myeloid leukaemia, 3 cases have been observed at doses comparable to those of the currently applied <sup>224</sup>Ra treatment with the preparation *SpondylAT*® (10 injections each of 1 MBq), in one case the <sup>224</sup>Ra-dose was the 0.6fold, in another case 1.6fold, whereas in one case the total dose could not be verified exactly.

Similar higher incidences of leukaemia have not been found in another group of patients who have been treated at higher doses or dose rates of <sup>224</sup>Ra, observed by Spiess and co-workers. However, the enhanced leukaemia incidence in our exposed group is in line with results from animal experiments in mice having been injected with bone seeking  $\alpha$ -emitters given at low dose rates.

## *IN VIVO* CHROMOSOME ABERRATIONS IN RETIRED RADIATION WORKERS: NO EVIDENCE FOR INTERACTION OF DNA REPAIR GENE POLYMORPHISMS WITH RESPONSE TO RADIATION

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**Objective:** To investigate the influence of seven DNA repair gene variants on inter-individual response to ionising radiation in a sample of retired radiation workers.

**Methods:** Variation at seven polymorphisms in four genes involved in the base excision repair (*XRCC1* R194W, R399Q and microsatellite) and double strand break repair (*XRCC3* T241M and microsatellite, *XRCC4* I134T, *XRCC5* microsatellite) pathways was analysed in a sample of 291 retired radiation workers who received cumulative occupational external radiation doses of between 0 and 1873 mSv. The influence of DNA repair gene polymorphisms on the response to radiation (translocation frequency in peripheral blood lymphocytes determined using FISH) was analysed using linear regression. Radiation dose and smoking were independently added to the linear regression model to test for interaction between each factor and genotype on the outcome of chromosome aberration frequency.

**Results:** No evidence was found to indicate that any of these polymorphisms have impacted upon the *in vivo* response to occupational radiation exposure when chromosome translocations are the measured endpoint. In addition, no evidence of an interaction with radiation dose has been found for any combination of variant alleles. A positive interaction between genotype at a microsatellite locus in the *XRCC3* gene and smoking status was demonstrated, indicating that this DNA repair gene variant might be associated with a sub-optimal response to smoking induced DNA damage.

**Conclusion:** Variation at these seven loci does not influence the response to occupational radiation as determined by chromosome aberration analysis.

## BRDU AND RADIATION-INDUCED SISTER CHROMATID EXCHANGES (SCE): THE ROLE OF INTERSTRAND CROSSLINKS (ICL) AND RAD51C

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It was suggested that interstrand cross-links (ICL) are formed in cells exposed to 5-bromo-2'-deoxyuridine (BrdU) plus UVC radiation. Experiments were performed to determine if cells hypersensitive to ICL (Rad51C mutated CL-V4B cells) were also sensitive to BrdU+UVC. Mutant and wild-type cells were exposed to mitomycin C (MMC) and BrdU+UVC, and chromosomal aberrations, cell survival and sister chromatid exchanges (SCE) were analysed. MMC-induced SCE were scored in the first (M1) and second (M2) posttreatment mitoses. The results confirmed that CL-V4B cells are sensitive to MMC and BrdU+UVC, indicating that the latter treatment may lead to formation of ICL. CL-V4B cells showed the same frequencies of MMC-induced SCE as wt cells, but a reduced level of SCE was observed following exposure to UVC with or without BrdU. This indicates that (a) the DNA damage induced by MMC and UVC is processed differently in the course of SCE generation and (b) Rad51C may be less important for SCE formation after MMC treatment in contrast to that after UVC. In the CL-V4B cells the SCE were higher in M2 than in M1 suggesting that ICL are either not removed completely or are transformed into another form of DNA damage that persists until the next cell cycle.

## THE ROLE OF POLY(ADP-RIBOSYLATION) IN DOUBLE STRAND BREAK FIXATION IN L5178Y AND CHO CELLS

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**OBJECTIVE:** To investigate the role of poly(ADP-ribosylation) in DNA double strand break (DSB) repair in L5178Y murine lymphoma sublines, LY-R and LY-S, and a pair of Chinese hamster ovary (CHO) lines, wild type (WT) and mutant xrs6 cells. The radiosensitive cell lines have impaired non-homologous end-joining (xrs6 - Ku80 mutation, LY-S- molecular defect undefined [Radiat.Res., 115, 566, 1988, Mutat.Res., 409, 31, 1998]).

**METHODS:** Cells were incubated with 2mM poly(ADP-ribose) polymerase inhibitor, 3-aminobenzamide (AB) at 37°C for 2 h, X-irradiated with 10 Gy and allowed to repair DSB for 15, 60 and 120 min) at 37° C or 25° C. DSB (initial and remaining after repair intervals) were estimated by the neutral comet assay.

**RESULTS:** At 37°C no effect of AB treatment on the repair kinetics was observed either in xrs6 or CHO (WT) cells. In contrast, AB inhibited the repair of DSB in LY-S but not LY-R cells, in agreement with the previously observed sensitisation of LY-S, but not LY-R cells to X-rays by poly(ADP-ribosylation) inhibition [Acta Radiol.Oncol. 24, 451, 1985]. However, DSB rejoining in the repair competent cell lines, CHO and LY-R, also was affected by AB when the post-irradiation incubation was carried out at 25° C. Analysis of these results together with some earlier data on LY-S cells allowed to interpret these results in terms of Radford's [Int.J.Radiat.Biol., 78, 1081, 2002] model that predicts competition between radiation damage repair and fixation within transcription factories. Fixation is due to topoisomerase I and is prevented by its ADP-ribosylation. Hence, poly(ADP-ribosylation) inhibition favors damage fixation.

**CONCLUSION:** The slow repair of DNA breaks in LY-S results in an enhanced fixation of a sector of DSBs (presumably in transcription factories) both at 37°C and 25° C. In the repair competent cell lines, CHO and LY-R, slowed down rejoining is achieved by incubation at 25° C. Then, fixation of DSB enhanced by poly(ADP-ribosylation) inhibition is revealed in these cell lines. The results indicate that poly(ADP-ribosylation) can be an important modulator of the conversion of DNA damage to lethal events.

## TIME COURSE OF MOLECULAR AND STRUCTURAL CHANGES IN THE HEART AFTER LOCAL IRRADIATION

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During radiotherapy of mediastinal tumours (lymphomas, breast cancer, etc.) frequently the entire heart or part of the heart is included in the treatment field and may receive the full tumour dose. High cure rates of disease and long follow-up times revealed that irradiation of the heart can cause radiation-induced heart disease (RIHD) ranging from asymptomatic pericarditis up to impairment of cardiac pump function or even to cardiac related death. In patients clinical symptoms of RIDH develop over a wide range of latency times. Most of the cases of pericarditis occur within 2 years after radiotherapy. Coronary artery disease, less common compared to pericarditis, develops later and the incidence shows a near linear progression with post irradiation time. Conduction defects and Myocardial fibrosis are relatively rare and develop very late during the course of RIDH.

Follow up studies using experimental animals (rats, rabbits and dogs) show similar phases of RIDH. In our laboratory the time course of both functional, structural and molecular changes in the rat heart after local irradiation was studied. Early changes in cytokine mRNA expression occur immediately after irradiation. Within a few months post treatment, damage to capillary endothelium (increase Von Willebrand factor expression; decrease capillary density) was observed. Deterioration of capillaries was progressive with time interval and was followed by progressive alterations in composition (i.e. severe fibrosis and myocytolysis) of the different cardiac layers.

The delayed appearance of myocardial degeneration compared with the early decrease in capillary density supports the hypothesis that primary damage to the vasculature is responsible for the secondary observed degeneration of dependent myocardial tissue. Experiments with *in vitro* cultured cardiac myocytes which show that these cells are highly resistant to ionising radiation further support this hypothesis.

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## GENOMIC INSTABILITY AND BYSTANDER EFFECTS: RELATED MANIFESTATIONS OF UNTARGETED RADIATION RESPONSES

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The major adverse consequences of radiation exposures are attributed to DNA damage in irradiated cells that has not been correctly restored by metabolic repair processes. However, the dogma that genetic alterations are restricted to directly irradiated cells has been challenged by observations in which effects of ionizing radiation arise in non-irradiated cells. These, so called, untargeted effects are demonstrated in cells that are the descendants of irradiated cells (radiation-induced genomic instability) or in cells that have communicated with irradiated cells either directly or via media transfer (radiation-induced bystander effects). Radiation-induced genomic instability is characterized by a number of delayed responses including chromosomal abnormalities, gene mutations and cell death. Bystander effects include cell death, cell differentiation, radioadaptation, induction of mutations and chromosome aberrations and chromosomal instability. There is increasing evidence that instability and bystander effects are linked untargeted processes mediated by free radical-mediated signalling mechanisms. At present it is not known to what extent these untargeted effects contribute to overall cellular radiation responses, especially *in vivo*. Their expression and potential consequences will reflect a balance between the type of signals produced and the responses of cell populations to such signals, both of which may be significantly influenced by cell type and genotype. Thus, as well as targeted effects of damage induced directly in cells by irradiation, a variety of untargeted effects may also make important short-term and long-term contributions to determining overall outcome after radiation exposures.

## THE EFFECT OF SUPEROXIDE DISMUTASE ON THE ADAPTIVE RESPONSE INDUCED BY IONIZING RADIATION IN MOUSE BONE MARROW CELLS *IN VIVO*

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In recent years, a variety of biologically active substances of different origin possessing the antioxidant activity are recommended not only as nutritional additives for elderly people and persons with various pathologies but also as prophylactic means for various groups of population. The goal of our experiments was to examine the effect of compounds containing the enzyme superoxide dismutase (SOD) on the radiosensitivity of mouse bone marrow cells *in vivo* using the scheme of adaptive response (AR). This scheme was chosen because it was shown previously that low doses of  $\gamma$ -radiation induce AR in mice, which persists almost throughout the life of the animal.

Experiments were carried out on male SHK mice. The nutritional additive containing SOD (General Nutr.Corp., USA) was administered to mice *per os* for two weeks at a dose of 170 unit/kg per day. Then mice were irradiated with an adaptive dose ( $D_1$ ) of 10 cGy (0.125 Gy/min) and after 24 h, with a challenging dose ( $D_2$ ) of 1.5 Gy (1 Gy/min). SOD isolated from bovine erythrocytes (ICN Biomed. Inc., USA) served as a positive control. The animals were sacrificed 28 h after the irradiation with  $D_2$ , and then cytological samples were prepared by the standard method. On the average, 2000 polychromatophilic erythrocytes (PCE) from the bone marrow of each mouse were analyzed. The level of cytogenetic damage was defined as the percentage of PCE with micronuclei. It was also found that in mice receiving a SOD preparation prior to irradiation with  $D_1$  or with  $D_1$  and  $D_2$ , no AR was observed. If inactivated SOD solution was administered in the same variants of the experiment, the level of cytogenetic damage was two times lower than in animals irradiated with  $D_2$  only; i. e., a clearly pronounced AR was observed. Since it is known that AR depends on the level of primary DNA damage, it can be assumed that, as a result of additional damage caused by treatment with SOD, the level of damage falls outside the range of exposures that lead to the induction of AR. These data indicate that, upon screening of preparations like nutritional additives, it is necessary to examine their combined effect with possible unfavorable environmental factors, in particular IR, because it may have a latent negative action.



## ROLE OF HOMOLOGOUS RECOMBINATION IN PROVIDING PROTECTION AGAINST RADIATION-INDUCED DNA DOUBLE-STRAND BREAKS AND DNA CROSS-LINKS IN MAMMALIAN CELLS

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*Objectives:* To examine the role of homologous recombination (HR) in the cellular response to ionising radiation (IR) and DNA interstrand crosslinks (ICLs) in mammalian cells.

*Background:* HR plays an essential role in the maintenance of genome integrity and in the cellular response to DNA damage, such as DNA double-strand breaks (DSB) that are the most lethal lesions induced by IR. These DNA lesions are repaired by the non-homologous end-joining (NHEJ) and HR pathways but despite that both pathways are responsible for DSB repair, the final responses clearly differ for each pathway. Both pathways protect genetic stability although most cells defective in NHEJ do not show spontaneous instability, while high levels of spontaneous and induced instability occur in HR-deficient mammalian cells. Recently compelling evidence suggest that HR also plays an important role in processing ICLs that are induced by commonly used anti-cancer drugs (e.g. cisplatin or mitomycin C). In consequence, mammalian cell lines defective in HR display hypersensitivity to mitomycin C (MMC) and spontaneous chromosomal instability.

*Methods:* We analysed cellular responses induced by IR in HR-defective mutants, such as clonogenic survival, Rad51 foci formation in response to DNA damage, chromosomal aberrations, centrosome abnormalities and X-ray induced mutagenesis at the endogenous *hprt* gene.

*Results:* Cell lines defective in the *Brca2* or *Rad51C* genes showed primarily hypersensitivity to CL-inducing agents and only a mild increase in IR sensitivity, measured as clonogenic survival, although e.g. Rad51 foci formation in response to X-ray or MMC was similarly defective. The radiation-induced S-phase checkpoint and centrosomes were abnormal in *Brca2*-defective cells. The spontaneous mutation rate was about 4-fold increased in these cells. The rate of base substitution was similar to that observed in wild-type cells, while the rate of deletion was 14-fold increased.

*Conclusions:* Our results underscore the role of HR in maintaining genome stability by controlling several different processes, such as mutagenesis, cell cycle progression and centrosomes. *Brca2* mutations predispose cells to an increased risk of mutagenesis after exposure to IR, thus pointing out the importance of *Brca2* in the prevention of X-ray-induced mutagenesis. Our data suggest that IR might be a potential risk factor by mammography for BRCA2 patients.