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High levels of radiation-induced excess acentric fragments in cells deficient in DNA-PK, p53, and p21

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Purpose: DNA-damage activates complex regulatory response pathways and several of the proteins involved have already been characterized. In the smooth muscle cell line SMCM IX, originally derived from the vein of a healthy individual, we analysed radiation-induced chromosomal aberrations, the expression of p53 and p21, and the activity of the DNA-dependent protein kinase DNA-PK.

Material and Methods: SMCM-cells in a later passage were grown in SMCM-specific medium, 10% FCS in a 5% CO2-atmosphere at 37°C. Plateau-phase cells were irradiated with graded doses of 1 Gy to 5 Gy of 200 kV X-rays. Chromosome aberrations were determined as genomic yields of dicentric chromosomes and excess acentric fragments, scored in Giemsa-stained metaphases. Protein expression of p53 and p21 was determined by Western blot analysis. DNA-PK activity was determined using the SigmaTECT(TM) DNA-Dependent Protein Kinase Assay System.

Results: We observed neither a DNA-PK-activity nor a radiation-induced expression of p53 or p21. In irradiated as well as in control cultures, a surprisingly high proportion of metaphase cells, 18± 3%, were aneuploid. Only diploid cells were analysed with respect to radiation-induced chromosomal aberrations. Here, a high proportion of excess acentric fragments (yac(ex)) was observed. The ratios of (yac(ex))/ydic were 11.5, 19.0, 19.3 and 30.0 for radiation doses of 2 Gy, 3 Gy, 4 Gy, and 5 Gy, respectively. This was a marked increase compared to yac(ex)/ydic of other normal tissues, like 0.98 for lymphocytes, 1.52 for dermal fibroblasts, and 3.64 for endothelial cells from umbilical cord veins, after a radiation dose of 4 Gy.

Conclusion: Deficiencies in proteins involved in the control of genomic integrity and DNA-dsb repair were observed in a cell line together with remarkably high levels of unrepaired DNA-dsb's manifested as excess acentric fragments. Moreover, our observation of the high proportion of aneuploid cells in the deficient cell line indicates the participation of p53/p21 in control of chromosome segregation.

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Radiation enhancement and modulations of cell cycle distribution induced by Gemcitabine (dFdC)

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Purpose: Among various mechanisms responsible for the radiation enhancing effect of low-dose Gemcitabine (2',2'difluoro desoxycytidine), cell cycle modulations may play a role. It was our aim to evaluate this effect and the underlying changes in cell cycle distribution in #4197-cells (human oropharyngeal squamous cell carcinoma).

Methods: In 96-well-plates, cells were exposed to dFdC (0-3,0 μM) for 24 hours (h). After drug removal, immediate irradiation (0-10 Gy), and incubation (120 h), viability was determined fluorometrically. For cell cycle analysis by flow cytometry (FACS), cells were irradiated (0-40 Gy) or treated with dFdC (0,012-1,0 μM) (6-well-plates). Additionally, cells were exposed to dFdC (2,0 μM) for 0-6 h. FACS-analysis of propidium iodide stained cells was performed immediately or 16-63 h later. Cell cycle kinetics were evaluated using BrdU (10 μM) S-phase labeling, given either 30 minutes prior to or in the last hour of dFdC treatment (0-6 h; 2,0 μM).

Results: The fluorometric assay revealed that dFdC enhances radiation induced cytotoxicity at marginally toxic or untoxic concentrations (<37 nM). Radiation resulted in the anticipated G2/M-arrest. DFdC induced concentration and exposure time-dependent cell cycle changes that were better resolved using BrdU demonstrating a pronounced S-phase arrest already at 12 nM. BrdU-puls labeling revealed that the cell cycle block occurred at the G1/S-boundary.

Conclusion: Our data reconfirm the already known radiation enhancement and S-phase specific activities of dFdC. In literature data, it is discussed that the progression of cells through the S-phase seems to be important for the radiosensitizing properties of dFdC. However, we could demonstrate that before progressing into the S-phase, cells were blocked at the G1/S-boundary which is a more radiation sensitive cell cycle stage. We conclude that dFdC enhances the radiation effect by accumulating cells in this stage. Furthermore, cells progressing past the block might accumulate pro-apoptotic signals caused by both, radiation and dFdC which will also results in cell death.

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The relationship between Fludarabine-induced radiosensitisation and apoptosis in six human squamous cell carcinoma lines

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Purpose: To determine the relationship between Fludarabine-induced radiosensitisation, and apoptosis in six human squamous cell carcinoma lines.

Methods: Head and neck cancer (ZMK-1, OH-65, GR-145, BW-225-l); lung cancer (A-549), or cervical cancer (CaSki) cells were treated with escalating doses of Co-60-g-irradiation, different doses of Fludarabine (0.5 - 0.005μg/ml), or a combination of both. Cell survival was measured by a standard colony-forming assay; apoptosis was evaluated morphologically in acridin-orange-stained cells.

Results: Radiosensitisation was only observed for the more fludarabine-resistant ZMK-1, CaSki, and A-549 cells. The corresponding SER values at the 37% survival level were 1.5, 1.3, and 2.5, respectively. For, ZMK-1 and CaSki cells a fludarabine-induced doubling of the amount apoptotsis was observed, whereas A-549 cells showed a marked radiosensitisation and no apoptosis. For the OH-65, GR-145, and BW-225-l cell lines, neither radiosensitising effects, nor an increase of the amount of apoptosis was found.

Conclusions: We could demonstrate a fludarabine-induced radiosensitisation in 3 out of 6 cell lines tested, which for two cell lines seems to be related to the degree of apoptosis-induction of fludarabine alone.

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Identification of target cells for CNS radiation injury: Is apoptosis of neural stem cells a pathogenesis of radiation-induced leukoencephalopathy?

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Purpose: Pathophysiological mechanisms of radiation-induced leukoencephalopathy are yet to be clarified. Subependymal zone of the lateral ventricles and dentate gyrus in the hippocampus are unique regions of the CNS where majority of neural stem cells exist. We demonstrate that ionizing radiation induces apoptosis of the stem cells and then accelerates differentiation of stem cells into oligodendrocyte lineage leading to myelin synthesis disorder.

Materials and Methods: Adult C57BL/6J female mice were irradiated at 10 Gy and was sacrificed 4 h, 8 h, 24 h, 7 days, 14 days, and 28 days after irradiation. Whole brain samples were dissected into three portions (the ventricle-hippocampal region, the rest of forebrain, and the cerebellum). Apoptosis was assessed by the in situ immunohistochemical analysis (Tunel assay). An anti-nestin antibody (a marker for neural stem cells), an anti-O4 antibody (a marker of premature oligodendrocytes), an anti-NeuN antibody (a maker for neurons), and an anti-GFAP antibody (a marker for astrocytes) were used. The O4 protein and myelin basic protein (MBP) (a marker of mature oligodendrocytes) were consecutively assessed by western blotting. The brain sections of each time point were evaluated using Luxol Fast Blue (a method for myelin staining).

Results: (1) In both the subependymal zone and the dentate gyrus, the number of apoptotic cells increased at 2 h and reached its maximum (20%) at 6-8 h. The apoptotic cells were also detected by the anti-nestin antibody suggesting neural stem cells. (2) Characteristics of the apoptotic stem cells at subependymal zone and those in dentate gyrus were different (subependymal:GFAP-positive, dentate gyrus:O4positive). (3) The O4 protein and MBP syntheses in the ventricle-hippocampal region showed different style consecutive changes. The changes of the O4 protein and MBP synthesis were not observed from the samples of the rest of forebrain and those of cerebellum. (4) Myelin synthesis decreased sporadically 28 days after receiving 10 Gy irradiation.

Conclusion: We demonstrate that neural stem cells in the ventricle-hippocampal region are highly radiosensitive and that apoptosis of the stem cells could be an initial step of radiation injury. Ionizing radiation accelerates differentiation of stem cells into oligodendrocyte lineage leading to myelin synthesis disorder. These evidences pave the way for the understandings of a mechanism of radiation-induced leukoencephalopathy.

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Intraluminal application of potential radioprotectors in an animal model of localized hypofractionated small bowel irradiation

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Purpose: The risk of normal intestinal toxicity is a major dosis-limiting factor during radiation therapy for abdominal and pelvic malignancies. Investigations have been directed toward increasing normal tissue tolerance by using radioprotectors, however, intraveneous administration of potential radioprotectors, i.e. amifostine, has produced limiting nausea and vomiting. The presented animal model was developed for intraluminal application of potential radiation biology modifiers and localized fractionated small bowel irradiation.

Methods: Fourty two male Sprague-Dawley rats were orchiectomized and a 5 cm segment of small bowel was sutured to the inside of the scrotum to form an artificial "scrotal hernia". In addition, a proximal Bishop-Koop ileostomy was fashioned for intraluminal drug application; small intestine was re-anastomosized end-to-side using 6-0 absorbable interrupted sutures. After 4 weeks postoperative recovery small intestine in the scrotal hernia was sham-irradiated or exposed locally to hypofractionated orthovoltage radiation of daily 5 x 5 Gy or 5 x 7,5 Gy. In treatment groups 10 min. before irradiation 50 mg Ethyol (Amifostine) dissolved in 9M buffer was administered intraluminally. Specimens of sham-irradiated or irradiated intestines were procured at 2 weeks after the end of irradiation and assessed for morphologic changes by semiquantitative histopathology and for extracellular matrix-associated pan-TGFbeta by immunohistochemistry. In addition, the enteric nerve system (ENS) was assessed using electron microscopy.

Results: Surgery and anaesthesia related mortality rates were 5% and 2%, respectively. Irradiated animals exhibited characteristic dose-dependent intestinal mucosal denudation, inflammation, subserosal thickening, differences between the irradiated groups were significant (p=0.02). Amifostine-treated animals in the 7.5 Gy group showed a slight but not significant reduced intestinal injury at 2 weeks than irradiated animals treated with buffer. Using electron microscopy irradiated specimens exhibited characteristic alterations of the ENS.

Conclusion: This animal model allows the application of fractionated small bowel irradiation in combination with local testing of potential radioprotectors locally. Amifostine and other locally acting radioprotectors should undergo further testing, particular as modifiers of chronic intestinal radiation toxicity.

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Chromosomal damage and survival of keratinocytes and fibroblasts after irradiation with 200 kV and 25 kV X-rays

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Purpose: A relative biological effectiveness (RBE) of 1 is generally accepted for soft X-rays (25-30 kV), which are applied in diagnostic radiology (mammography). However, it has been shown, that soft X-rays can be more effective in cell killing and chromosomal damage. The present study was initiated to define biological effects of low-energy X-rays in vitro.

Methods: Experiments were performed with 25 kV X-rays and 200 kV reference X-rays on neonatal human keratinocytes (HEKn), human fibroblasts (HFIB) and NIH/3T3 mouse fibroblasts. Cell survival was studied with graded doses in a clonogenic assay, chromosomal damage in a micronucleus (MN) assay.

Results: The surviving fraction at 2 Gy for keratinocytes was 46±5% after 200 kV and 33±11% after 25 kV X-rays. Linear-quadratic cell survival analysis yielded a=0.31±0.03 Gy-1 and b=0.048±0.011 Gy-2 for 200 kV and a=0.40±0.10 Gy-1 and b=0.048±0.054 Gy-2 for 25 kV. For 3T3 fibroblasts SF2 of 53±3% after 200 kV and 61±18% after 25 kV were observed. Values of a=0.24±0.02 Gy-1 and b=0.022±0.002 Gy-2 for 200 kV and a=0.10±0.05 Gy-1 and b=0.070±0.010 Gy-2 for 25 kV X-rays were derived. The induction of binucleated (BN) cells in the MN assay was highly dependent on the cell line studied, but independent on radiation quality. Compared to the effect of conventional, 200 kV X-rays, 25 kV X-rays resulted in an increased number of chromosomal damages expressed as either the percentage of BN cells with micronuclei (%BNC + MN) or the number of micronuclei per BN cell (MN/BNC).

Conclusion: Cell survival after 25 kV and 200 kV X-irradiation was similar, although for 3T3 fibroblasts, a reduction in survival at higher doses was observed after 25 kV X-rays. Induction of micronuclei after irradiation with 25 kV X-rays was significantly higher than with 200 kV, resulting in a RBE value of about 1.2. This indicates a higher potential of the soft X-rays for the induction of genetic damage.

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Tumor interstitial fluid pressure in patients: possible correlation with tumor size

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Purpose: Interstitial fluid pressure (IFP) is determined by the volume of free interstitial fluid and the distensibility of the interstitium. Normal tissues have low vascular permeability and an extensive lymphatic network, and therefore contain only small quantities of interstitial fluid at low pressure. IFP in normal tissues is between -5 and +5 mmHg. Malignant tumors are very permeable and lack functional lymphatics, which allows free fluid to accumulate in the interstitium, producing a high tumor interstitial fluid pressure (TIFP). A high TIFP may be associated with hypoxia and poor prognosis in radiotherapy. TIFP may increase with tumor size. In this study, we evaluated whether TIFP is correlated with tumor size.

Materials and Methods: From August 1998 to December 2000, we measured TIFP using a modified wick-in-needle technique in 33 biopsy-proven uterine cervical cancer patients and 33 primary or metastatic head and neck cancer patients in whom the tumor was accessible by direct inspection and palpation and was sufficiently thick (>1 cm) to permit accurate needle placement. Blood pressure was checked before TIFP measurement. Tumor size was measured by clinical and radiological methods.

Results: In cervical cancer, the mean TIFP was 29.1 mmHg and had no significant relationship with tumor size (p = 0.59). In head and neck cancer, the mean TIFP was 26.5 mmHg and was significantly related to tumor size (p = 0.03).

Conclusion: The mean TIFP was elevated at 29.1 mmHg in cervical cancer and 26.5 mmHg in head and neck cancer. TIFP was significantly related to tumor size in head and neck cancer.

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Influence of percutaneous radiotherapy on skin microcirculation

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Purpose: Acute and chronic skin reactions represent serious side effects in radiotherapy (RT). Both are accompanied by histologically proven changes of capillary vessels. The aim of this investigation was to show evidence for these changes under in-vivo-conditions.

Methods: Morphologic modifications of the nutritive skin capillaries have been investigated by means of the capillary microscopy in eight irradiated patients with different malignant tumours. Treatment has been delivered by linear accelerator with 6 or 15 MV photons. Measurements were performed before, during, at the end of treatment and twice in follow up. An investigation of the deeper plexus of thermal regulation of the skin took place with the laser doppler flowmetry (LDF). Investigation areas were the irradiated field and un-irradiated skin (controls). Acute and chronic skin reactions were scored by RTOG/EORTC toxicity criteria respectively LENT/SOMA tables.

Results: An edema formation was shown in all patients during RT leading to reduced skin transparency. Therefore capillary density could be