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The relationship between normal tissue radiosensitivity and radiation induced cell-cycle delays in asynchronous populations of normal fibroblasts analysed by bivariate flow cytometry

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Introduction: It is possible to study irradiation induced cell-cycle delays in asynchronous populations with bivariate flow cytometry.

Materials: Twelve normal human fibroblast cell strains were studied, including, two genomic radio-sensitive cell strains, a radioresistant cell strain and nine strains derived from vaginal biopsies from pre-therapy patients with carcinomas of the cervix.

Methods: Confluent cells were irradiated at 0, 2 and 4 Gy, and then plated out at low cell density with the addition of bromodeoyxuridine for 72 hrs. and then lysed and double-labelled with Hoechst 33258-ethidium bromide for bivariate flow cytometry.

Results: Cell-cycle analysis showed emptying of the G_2 compartment which indicates a G_2 block for cells irradiated in G_2. This was maximal in the most radioresistant cell strains (occurring at a dose of 2 Gy), whereas in the most radiosensitive cell strains this does not occur after a dose of 4 Gy. The proportion of cells which cycle following irradiation correlates with the cell surviving fractions at 2 Gy, SF_2, $r = -0.86$, $p = 0.026$, after a dose of 2 Gy. The accumulation of cells in the first G_1 compartment following irradiation is greater in radioresistant cells than in radiosensitive cells: after 4 Gy this relationship reaches significance when correlated with SF_2, $r = 0.81$, $p = 0.05$. The percentage of cells entering the G_2 compartment in the first cycle is the same irrespective of whether the cells are irradiated or not. In cycling cells G_2/M delay measured by the ratio of the percentage of cells in G_2 in the first cycle, to the percentage of cells in G_1 in the second cycle, correlates with SF_2 $r = -0.89$, $p = 0.03$ at 2 and 4 Gy.

Conclusions: Irradiation induced cell-cycle delays correlate with normal tissue radiosensitivity.

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Fibroblast contamination in assays of tumour radio-sensitivity

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Cellular *in vitro* radiosensitivity was measured in 69 tumour biopsies from SCC of the head and neck using the modified Courtenay-Mills soft agar clonogenic assay. In the cultures of all tumour biopsies, stromal fibroblasts colonies were obtained (range 14–100%), determined by immunocytochemistry. The tumour cell and fibroblast radiosensitivities were uncorrelated. In an attempt to compare the growth and radiation characteristics of fibroblasts with different origin, skin, mucosa and stromal tumour fibroblasts were obtained from 7 of the patients, and cultured in culture flasks and in soft agar. Plating efficiencies were significantly higher for fibroblasts originating from normal mucosa ($p < 0.012$) than for the stromal tumour fibroblasts. In this small sample, the SF_2 values of the normal tissue fibroblasts did not correlate significantly with the SF_2 values of stromal tumour fibroblasts, which fell into the range of SF_2 values obtained from 36 skin biopsies from women who received postmastectomy radiotherapy in a previous study in the lab. This study illustrates once more the problem with fibroblast contamination in assays of tumour radiosensitivity. A pilot study on SCC of the uterine cervix indicates that the problem with stromal fibroblast contamination exists in this case also. The growth and radiation characteristics of the stromal tumour fibroblasts did not differ considerably from the normal tissue fibroblasts. A more definitive study investigating these characteristics would include many patient biopsies and would be very time consuming and expensive.

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Chromatin structure in human glioma cell lines with different radiosensitivity

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Purpose: To study the correlation between cellular radiosensitivity and chromatin organisation.

Methods: Two human glioma cell lines, U87 and EA14, of differing radiosensitivity were examined. Radiation induced changes in chromatin organisation were assessed on histone-depleted nuclei (nucleoids) following exposure to high-salt buffers. The ability of propidium iodide (PI) to introduce positive supercoils in nucleoids was measured as forward light-scatter by using flowcytometry analysis.

Results: Cell survival varied by a factor of 1.4: U87 (a 0.19 Gy-1; b 0.011 Gy-2) and EA14 (a 0.27 Gy-1, b 0.041 Gy-2). Both cell lines expressed similar maximum loop relaxation at comparable PI concentrations indicating no differences in DNA loop size or native supercoiling. As a result of irradiation nucleoids from EA14 displayed more forward light-scatter than those from U87.

Conclusion: These results suggest the involvement of differences in the arrangement of supercoiled nuclear DNA into loop domains anchored to the nuclear matrix in the ability of these two cell lines to express radiation-induced DNA damage.

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Effects of fractionation and dose rate in PDR-brachytherapy (PDR-BT) of B14-fibroblasts

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Purpose: The aim of our study was to evaluate the impact of different pulse doses and dose rates of pulsed brachytherapy on cell survival under clinically conditions.

Methods: Hamster fibroblasts were exposed to a radiation source at a distance of 9 mm with following RT-schemes: dose per pulse: 1/2.5/5 Gy (PDR); 2.5/5 Gy (HDR); total dose: 5–30 Gy (PDR/cLDR), 5–20 Gy (HDR); dose rate: 50 cGy/h (cLDR); 100–300 cGy/h (PDR); 1500–2000 cGy/h (HDR); pulse repetition: 1 Gy/1 h or 1 Gy/2 h (PDR); 2.5 Gy/2.5 h and 5 Gy/5 h (PDR/HDR). Cell survival was measured by dye exclusion test and clonogenic survival assay.

Results: Cell survival decreased for pulse doses of 5 Gy compared to 2.5/1 Gy (PDR/HDR) or when using HDR- brachytherapy. No differences were observed with dose rates of 100–300 cGy/h with a biological equivalence of cLDR- and PDR- BT, keeping the total exposure time constant.

Conclusion: Radiobiological effects of PDR-BT are dependent on the total dose, the dose rate, the dose per pulse and the total exposure time.

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PR-350, the least toxic 2-nitroimidazole hypoxic cell radiosensitizer for clinical use

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Purpose: PR-350 is a newly developed 2-nitroimidazole nucleoside analogue radiosensitizer which is now undergoing clinical trials. The purpose of this study was to extensively evaluate its efficacy, toxicity and suitability for clinical studies.

Methods: In vitro sensitizing activity was assessed by colony and micronucleus assays in various murine and human cancer cells. In vivo effects were assessed by tumor growth delay and in vivo-in vitro assay of SCCVII tumors. The drug was combined with a single 20 Gy dose or 5 doses of 4 Gy. The 50% lethal dose in ICR mice and the concentrations in the sciatic nerve of C3H mice were determined to evaluate toxicity. Etanidazole was used for comparison.

Results: In vitro enhancement ratio of PR-350 was 1.4–1.6 at 1 mM, which was similar to that of etanidazole. In vivo, PR-350 was as efficient as etanidazole. The 50% lethal dose in mice was ≥ 5.8 g/kg. The concentration of PR-350 in the sciatic nerve was as low as that of etanidazole.

Conclusion: PR-350 is as efficient as but less toxic than etanidazole. Clinical studies of this compound, especially in combination with intraoperative radiotherapy or radiosurgery, seem to be warranted.

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Oxygen tension in metastatic lymph nodes and the changes during acute respiratoric hypoxia

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Purpose: The radiosensitivity of tissues is influenced by acute and chronic hypoxia. Based on the oxygen effect a new therapeutic modality has been developed to protect healthy tissues while hypoxic breathing during irradiation the hypoxyradiotherapy.

Patients and Methods: The effect of hypoxic breathing (8.1% O_2) on the pO_2 in metastatic lymphnodes was studied in 14 patients. Tissue oxygenation was assessed using a polarographic electrode system.

Results: The median pO_2 was 19.6 mm Hg prior to hypoxic breathing with a great intra- and intertumoral variability. The relative frequency of pO_2-values <5 mm Hg was between 0 to 88%. During breathing of hypoxic gas mixture we registered no significant changes in the mean, the median or in the pO_2-values <5 mm Hg.

Conclusions: In metastatic lymphnodes can be found chronic hypoxia with great inter- and intratumoral pO_2-variability. The hypoxic breathing (8.1% O_2) shows no significant changes in the tumor oxygenation. This fact explains the experimental and clinical experience, that the hypoxic breathing (8–10% O_2) protects the healthy tissue without changes in the radiosensibility of chronic hypoxic tumor tissue.

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Radiation therapy choroidal neovascularization in age-related macular degeneration

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Purpose: A prospective Phase I/II study was designed to determine the toxicity and efficacy of external beam radiation therapy in patients with age-related macular degeneration (ARMD) complicated with choroidal neovascular membranes (CNVM).

Methods: Patients older than 55 years with progressive vision loss who had been treated with laser or who were assessed as not suitable for laser treatment were included in the trial. Submacular degeneration was detected using FFA. Patients with diabetic or hypertensive retinopathy were excluded. Patients who refused the radiation treatment were included in the control group. Biomicroscopy and FFA were performed and visual acuity was determined just before the commencement of radiation therapy. A single lateral 6 MV photon beam portal with a field size of 3 × 4 cm was used. It was angled 50 posteriorly to avoid the anterior segment of the contralateral eye. Using asymmetric collimation, isocenter was placed just posterior to the lens of the involved eye. Computerised planning was done for all patients. Dose was normalised to the posterior segment of the involved eye. Total radiation dose was 15 Gy with 3 Gy per fraction in 5 elapsed days for the first part of the study and 20 Gy with 4 Gy per fraction in 5 days for the second part. Subretinal neovascularization and size of scar field were determined with FFA 1., 3., 6., 12. and 18. months after radiation therapy. Orbital CT with high resolution was taken.

Results: To date 34 patients were included in the study and 3 in the control group. Mean age was 71 with a range of 55 and 86 (23 male; 11 female). Duration of symptoms ranged between 1 and 45 months with a mean of 7 months. Subjective improvement or stabilisation have been achieved in the great majority of the patients. No acute or subacute side-effect has been observed. Detailed analysis with respect to patient and radiation therapy factors will be presented.

Conclusion: External beam radiation therapy appears to be effective in achieving improvement or stabilisation for the patients with ARMD complicated with CNVM without any acute or subacute side-effect.

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Extended salivary response to ionizing irradiation: An experimental study

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Purpose: previous studies have examined the acute effects of head and neck radiation (IR) in rats, but none have reported salivary function at later time points post IR.

Methods: in this study, mature male Wistar rats were given a single exposure of 0, 2.5, 5, 7.5, 10, or 15 Gy head and neck X-irradiation. Animals were provided with food and water ad libitum. Body weight, parotid (P) and submandibular (SM) gland weights, and P and SM salivary flow rates were determined at 6, 9, and 12 months following irradiation.

Results: At 6 months there were dose-related reductions in gland weight which were significant at 7.5 Gy and above for P and 5 Gy and higher for SM. Significant reductions in salivary flow were found only in the 15 Gy groups for both P and SM glands. A similar picture was seen at 9 months. Of greatest interest were results at 1 year following irradiation. There was late mortality, between 9 and 12 months, with death of all 15 Gy rats. Body weight of animals in the 7.5 and 10Gy groups was significantly decreased. At 12 months, P gland weight was significantly less at all radiation doses examined (2.5, 5, 7.5, and 10 Gy) compared to controls. P salivary flow also was significantly decreased in every dose group.

Conclusion: these data demonstrate that 1) there are significant late effects of head and neck X-irradiation an the salivary glands and survival in rats; 2) at 12 months, P weight and function are more significantly affected by X-irradiation than SM glands; and 3) a single dose of head and neck X-irradiation as low as 2.5 Gy can significantly affect P function and result i the death of rats between 9 and 12 months post-exposure.

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Radiosensitizing effects of cisplatin and carboplatin in prostate cancer cell lines

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Purpose: The radiosensitizing effect of cisplatin has been described in various tumors. In prostate cancer however, this concept has not yet been validated in clinical nor in experimental settings.

Methods: Cisplatin was added to cultures of human (DU-145) and rat (R3327 MATLy-Lu) prostate cancer cell lines, maintained in RPMI 1640 medium supplemented with 10% fetal calf serum. The final concentrations were 0.33, 1.67 and 3.30 μM for cisplatin and 0.167, 0.33 and 1.67 μM for carboplatin. Immediately after plating and addition of the drug irradiation was given to doses of 2, 4, 6 and 8 Gy. The surviving fraction was determined by counting cell numbers after 3 days. In addition, a semi-solid agar assay was performed and the number of colonies was determined after 7 days.

Results: At various combinations of cisplatin and radiotherapy, a supra-additive effect was observed in both assays. Similar effects were observed with carboplatin. The addition of glutathione (1 g/l) was shown to protect against radiation effects. Co-incubation with cisplatin and glutathione resulted in inactivation of the biological effect of cisplatin, presumably be precipitation. Pretreatment of cultures with glutathione did not influence the pattern of supra-additivity, as observed without glutathione.

Conclusion: A supra-additive effect was observed for various combinations of platinum compounds and irradiation. The present results suggest that glutathione is not a major factor in the radiosensitizing effects of cisplatin and carboplatin.

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5-Fluorouracil abolishes cell cycle arrest and increases cytotoxicity by different mechanisms when added after cisplatin or X-irradiation

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Introduction: DNA-damaging agents, such as cisplatin (CDDP) and X-irradiation, inhibit cell cycle progression from G2 to mitosis. When the G2 arrest is abrogated the toxicity of DNA damage is increased. For mitosis the cdc2p34 enzyme has to be active. The balance between the phosphorylat-