# Radiobiology and Radiotherapy
## Radiobiology

1206

*IN VITRO* RADIOSENSITIVITY OF NORMAL HUMAN SKIN FIBROBLASTS CORRELATES WITH THE DEVELOPMENT OF SUBCUTANEOUS FIBROSIS AFTER RADIOTHERAPY.

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Primary skin biopsies were obtained from the upper arms of 12 women who received postmastectomy radiotherapy with a dose per fraction of about 3.5 Gy in the period 1978-1982. *In vitro* radiosensitivity was measured by a clonogenic assay. Early generations of fibroblasts in exponential growth were irradiated with 250 kV X-rays at room temperature. The fraction of colony-forming cells after doses of 0, 1, 2, 3, 4, and 6 Gy were fitted by a linear-quadratic survival curve, and from these fits the surviving fraction (SF) at 3.5 Gy was estimated. The presence of marked subcutaneous fibrosis was evaluated in a total of 36 independent treatment areas in these 12 patients after a follow-up period of 2.0 to 4.8 years (M.Overgaard et al., Radiother Oncol 9, 1-12, 1987). A logistic regression analysis showed that increasing values of $SF_{3.5}$ were statistically significantly correlated ($p=0.02$) with decreasing probabilities of developing subcutaneous fibrosis. Thus low values of $SF_{3.5}$ *in vitro* appears to be a predictor for a high probability of developing subcutaneous fibrosis.

1207

INDIVIDUAL IN VITRO FIBROBLAST SENSITIVITY PREDICTS FOR NORMAL TISSUE RESPONSE TO RADIOTHERAPY

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We have investigated the relationship between fibroblast sensitivity and normal tissue response in a group of breast carcinoma patients from clinical fractionation radiotherapy studies in Gothenburg. Fibroblast sensitivity was assessed by clonogenic assay following both high (HDR) and low (LDR) dose-rate irradiation. A correlation was observed with both early and late skin reactions which was strongest when fibroblast sensitivity was measured at LDR.

Fibroblast strains were also established from a group of individuals in Sutton, four 'normal', 3 patients who showed severe early normal tissue reactions to radiotherapy ("over-reactors") and two from Fanconi Anaemia patients who in general show slightly higher than average normal tissue radiosensitivity.

The 4 normal strains showed a similar range of both HDR and LDR sensitivity to that observed with the Gothenburg strains. Those from the FA patients fell at the sensitive end of the range of sensitivities after both HDR and LDR. Finally, the 3 strains established from "over-reactors" were not amongst the most sensitive strains after HDR. However, when compared after LDR these strains ranked as the most sensitive of all. This change in ranking was a result of a total absence of dose-rate sparing in these three strains. All other strains showed similar relative dose-rate sparing independent of radiosensitivity (mean recovery factor 1.3).

Overall our results support the hypothesis that fibroblast sensitivity measured following low dose-rate irradiation predicts normal-tissue response to radiotherapy.

1208

THE RELATIONSHIP BETWEEN CELLULAR RADIOSENSITIVITY AND DNA DAMAGE IN PRIMARY NORMAL HUMAN FIBROBLASTS

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DNA damage is widely regarded as the critical event in radiation-induced cell death. The exact mechanisms remain to be elucidated. We have investigated this relationship in primary normal human fibroblasts.

Cell survival was measured by clonogenic assay and DNA damage by pulsed-field gel electrophoresis (PFGE).

Following high dose-rate (HDR) irradiation cell survival was found to vary by a factor of 1.5 and low dose-rate (LDR) irradiation revealed differences in dose-rate sparing between these cell lines. After HDR irradiation *initial* DNA damage and its *repair* was similar in all strains. *Residual* damage was assessed following a four hour repair period after HDR and immediately after LDR irradiation. No damage was detectable up to 40 Gy. Above this threshold it increased linearly with dose and the slope of the dose response curve of the different strains showed a close correlation with cell survival.

We conclude that *residual* damage as measured by PFGE in primary normal human fibroblasts is the major determinant of the *initial* slope of the cell survival curve (i.e. LDR response). In addition, the observation that *initial* damage was similar in all cell lines whilst *residual* damage varied with sensitivity implies that cellular differences in sensitivity result from their inherent ability to process DNA damage.

1209

$pO_2$ CHANGES IN TUMOURS AFTER CARBOGEN ADMINISTRATION IN PATIENTS AND AFTER CARBOGEN AND/OR PERFLUBRON ADMINISTRATION IN MICE BEARING HUMAN TUMOUR XENOGRAFTS

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Aims: To examine the changes in tumor $pO_2$ distribution 1/ in two human tumor xenografts after carbogen inhalation with or without a perflubron emulsion (Oxygent, Alliance pharmaceutical corp.) 2/ in H&N tumors after carbogen inhalation.
Protocol: 1/ xenografts: Mice bearing HRT18 or NA11+ tumors were restrained and their body temperature was maintained at 35° ± 1°C. Carbogen was delivered at a flow rate of 9 l/min., 4 ml/kg perflubron were i.v. injected. 2/ patients: The oxygenation of head and neck metastatic lymph nodes was assessed in 20 patients before carbogen breathing and in 13 patients during carbogen exposure.(15l/mn) Patients breathed carbogen through a rubber mouth piece. Tumor $pO_2$ distribution was assessed by polarography using the KIMOC 6650 (Eppendorf).
Results. 1/ animals: The $pO_2$ values distributions were shifted to higher values in the carbogen and perflubron plus carbogen groups. The shift is greater with carbogen plus perflubron than with carbogen alone, the proportion of low $pO_2$ values (<10 mm Hg) and very low values (<2 mmHg) decreased in the two tumor cell lines. However, in the best conditions (carbogen plus perflubron), the $pO_2$ values smaller than 2 mmHg disappeared only in one tumour cell line (HRT18). 2/ patients: the median $pO_2$ readings increased during carbogen breathing. Maximal effect was obtained after 1 to 6 minutes exposure. The frequency of low values (<10 mmHg) decreased in 11 carbogen-breathing patients; however, 4 patients still exhibited very low values $pO_2$ values (<2mmHg).
Conclusions. In animals the association of carbogen plus 4 ml/kg perflubron emulsion is very efficient for increasing tumor $pO_2$; however, the effect does depend on the tumor cell lines. If the animals data are extrapolated to humans, the effect of carbogen on tumor oxygenation should be increased by perflubron administration

1210

INFLUENCE OF HEMATOCRIT ON TUMOR OXYGENATION AND SENSITIVITY TO RADIATION

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Systemic hematocrit level and intratumoral oxygen tension have both been associated with the control of tumors by radiation therapy. We investigated whether chronic changes in hematocrit influence tumoral oxygenation and sensitivity to radiation. 150 adult female Sed/Kam mice were randomly allocated to one of three groups: untreated chronic anemia, corrected anemia, or polycythemia. Anemia due to erythropoietin (epo) deficiency was induced through bilateral kidney irradiation. Anemia was corrected or polycythemia induced by SC administration of epo three times per week. The untreated anemia group received SC saline. $10^6$ FSa murine fibrosarcoma cells were then injected IM into the right thigh of each animal. The resultant hind limb tumors were given a radiation dose of 70 Gy in 10 fractions over 5 days when they reached 4-5 mm in diameter. The 150 day tumor-free survival was 0% in the untreated anemia group, 19% in the corrected anemia group, and 25% in the polycythemic group. The tumor control rate by radiation was significantly higher in the group whose hematocrit was returned to normal by erythropoietin than in the group whose anemia remained untreated ($p<0.01$). The intratumoral hypoxic cell fraction in each group is being measured to determine whether manipulation of the hematocrit affects intratumoral oxygenation in addition to radiosensitivity.

1211

INTERACTION OF ETOPOSIDE AND RADIATION IN A C3H MAMMARY CARCINOMA AND THE FEET OF CDF1 MICE

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The time-and-dose dependent interaction between etoposide and radiation was studied in a C3H mammary carcinoma and mouse feet (early skin reactions and late foot damage) *in vivo*. The assays used were local tumor control ($TCD_{50}$), moist desquamation ($DD_{50}$), and impaired joint movement. Etposide (30 mg/kg) was administered i.p. as a single dose at time intervals from 4 days before to 4 days after local irradiation. In tumors, the greatest effect of etoposide was observed when the drug was given 24 hours before irradiation. The enhancement ratio (ER) was 1.11 ($p<0.05$). In skin, the greatest enhancement was seen when the drug was given from 6 hours before to 4 hours after irradiation (ER 1.14 and 1.10, respectively; $p<0.05$). When etoposide was given 24 hours before radiation, no additional skin damage was observed (ER 1.00), and the selective tumor effect at this time interval resulted in a positive therapeutic gain ($p<0.005$). At other intervals the increase in tumor response was counteracted by a similar increase in normal tissue damage. Finally, it was found that the addition of etoposide to a fractionated regime (5f/5d) did not increase the local tumor control. The effects on late foot damage is currently being analyzed and will be presented at the meeting.

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