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Renal toxicity after total body irradiation

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Purpose: To evaluate the incidence of renal dysfunction after total body irradiation (TBI) using the protocol developed at the Transplant Unit, Royal Adelaide Hospital, South Australia.

Methods: Between July 1990 and February 1997, 64 patients of median age 40 years, with malignant haematological conditions, Ewing's sarcoma and neuroblastoma received TBI as part of their conditioning regimen with high dose chemotherapy prior to allogeneic bone marrow transplant. All patients with normal renal function at commencement of treatment were included in this study. Conditioning included high dose Cyclophosphamide (CTX) alone (39), CTX with Melphalan (15), high dose Melphalan alone (4), high dose CTX with Busulphan (3),high dose Melphalan with Busulphan, or high dose VP16 with CTX (1). All patients recieved 12 Gy in 6 fractions prescribed to the highest lung isodose (corrected for lung transmission), treating twice daily in 3 consecutive days, at a dose rate of 7.5 cGy/min, using CT scan planning; renal shielding was not utilised. All patients received Acyclovir, Cyclosporin and Amphotericin. The blood pressure of each patient prior to transplant was normal in all patients except in 5, where this was elevated. Renal dysfunction was assessed based on serum creatinine levels measured at the start of TBI, at the end of TBI, 6 months following completion of treatment, at 12 months and at 18 months. The influence of chemotherapy, antibiotics, antifungals, antivirals, infection, hypertension and graft versus host disease (GVDH) on TBI related renal dysfunction was also assessed.

Results: In only 4 patients did renal dysfunction deteriorate by the end of TBI. Of these serum creatinine levels normalised within 6 months in 2 patients. The other 2 patients died secondary to multisystem failure as a result of infection. At respectively 6, 12, and 18 months, none of the surviving patients recorded elevated serum creatinine levels. Death secondary to renal dysfunction was only recorded in 1 patient, with multiple myeloma, who succumbed to widespread systemic disease progression. There was no correlation between TBI related renal dysfunction and the development of GVDH, hypertension, type of chemotherapy or antibiotics used.

Conclusion: A dose of 12 Gy at 2 Gy per fraction, delivered in 3 days, did not result in renal dysfunction in the patients studied, up to 18 months after completion of TBI.

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Overcoming mutant p53 with hypoxic cell radiosensitizer, PR-350

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Purpose: Tumor hypoxia is common and often associated with resistance to chemotherapy and radiation therapy. PR-350, 2-nitroimidazol nucleoside analog hypoxic cell sensitizer, is now in the way of phase I/II clinical trials combined with conventional radiotherapy or IORT in Japan. The p53 status also has a dramatic effect on tumor response to irradiation. PR-350 can sensitize not only wtp53 cells but also mp53 cells to irradiation. Hypoxic fraction (HF) in tumors is the critical factor in evaluation of the efficacy of hypoxic cell sensitizers. In this study, HF and sensitizer enhancement ratio (SER) of PR-350 were evaluated in solid tumors with different p53 status.

Materials & Methods: The human squamous cell carcinoma, SAS/wtp53 and SAS/mp53, with identical genetic background except for the p53 status were used in this study. Irradiation was given locally when tumors, growing subcutaneously, became 50-100 mm3 in their volume. Tumor growth delay time and cure rate were obtained to compare the effects. The hypoxic marker pimonidazole was used to measure tumor hypoxia.

Results: SER of PR-350 in wtp53 cells was superior to that in mp53 cells in vitro. Differently from the results in vitro, the magnitude of sensitization of PR-350 seemed to be bigger in SAS/mp53 than in SAS/wtp53. Cure rates at 110 days after treatment of SAS/wtp53 (saline), SAS/wtp53 (PR-350), SAS/mp53(saline) and SAS/mp53(PR-350) were 55, 60, 40, 50%, respectively. Tumor hypoxia was evaluated in three different growing conditions; (a):intra-tumory with sufficient oxygen and nutrient supply, (b):subcutaneously (normal condition), (c):subcutaneously under inferior circumstances (tumor growth time became longer). When tumors were 50-100 mm3 in their volume, HF increased in inverse relation to oxygen supply; HF(c) > HF(b) > HF(a). In good growing conditions(a), HF was almost same between SAS/wtp53 and SAS/mp53. In subcutaneous growing conditions(b), where SER of PR-350 was obtained, HF/SAS/mp53 was 1.6 times bigger than HF/SAS/wtp53. This difference was amplified in the condition (c), that was 1.83.

Conclusion: The resistant response of mp53 tumors to irradiation in comparison with wtp53 tumors might be attributed to the different HF between them as shown in our results. Fortunately, PR-350 can overcome this disadvantage of mp53 tumors.

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Multicellular megacolonies of tumour cells can be useful experimental model for fractionated radiotherapy

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Purpose: For studying an influence of fractionation regimens or radiomodifying agents on tumour response, the transplantable solid tumours are the best model. However, using plenty of animals in a such experiments bears some objection for ethical reasons. The multicellular megacolonies of tumour cells in vitro can in some respects replace the animal tumours. The aim of our study was to evaluate an effectiveness of two different fractionation regimens on clonal regrowth, cellular damage and cell cycle redistribution in megacolonie's system.

Materials and Methods: Murine SCC cell line AT478 and human lung adenocarcinoma, A549 growing as megacolonies (ca. 1cm in diameter) were used. Conventional fractionation, CF (2Gy/fr, 5day/week) and continuous accelerated irradiation, CAIR (2Gy/fr,7day/week) were applied up to total doses 20-80 Gy.

Results: TCD50 estimated on the basis of clonal regrowth were 47 Gy (CAIR) vs 56 Gy (CF) for murine and 56 Gy vs 68 Gy for human lines. TCD50 estimated for single dose, unexpectedely showed higher radiosensitivity for human cells (18 Gy) than for mice (21,6 Gy), suggesting that alpha/beta for adenocarcinoma must be higher. Flow cytometric analysis performed on AT478 megacolonies irradiated with CF and CAIR up to the same dose, 22 Gy, did not show significant differences in cell cycle distribution. However, it was observed that this tumour line contains two aneuploid populations (DI=1.96 ~92%, and DI=1.3 ~8%) and after two day-break in conwentional scheme this near-teraploid population increased up to 97%. It may be more resistant population which repopulate during the break. Cellular damage estimated on the basis of morphological criteria as apoptosis, necrosis and micronuclei indicated higher level of damage, particularly apoptosis frequency, in CAIR than in CF.

Conclusion: If some cells can be adapted to grow as multicellular megacolonies, they may serve as useful model for radiobiological studies, specially concerning fractionation effects.

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Whole brain radiotherapy for cerebral metastases: relation between survival and primary tumour site in 110 cases

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Purpose: The discovery of cerebral metastases (CM) modifies the prognosis of patients presenting a cancer. We present a retrospective study of 110 patients treated in the Centre Antoine Lacassagne in Nice, having presented single or multiple CM treated by surgery and/or radiotherapy. The purpose of this study was to obtain a description of the population presenting this kind of pathology and an overview of the relation between primary tumor site and survival.

Methods and materials: 77 men and 33 women presenting CM from September 1998 to December 2000 were analysed, the average age was 62 years [36-86]. Treatment consisted of: 1) a complete surgical resection (21%), 2) a partial resection or biopsy (5%), 3) an exclusive radiotherapy of the whole brain (74%). All patients received whole brain irradiation (WBI). Total doses ranged from 18 to 40 Gy, administered with 2 to 6 Gy. per fraction. Approximately 2/3 of the patients received 30 Gy. in 15 fraction. The localization of the CM was supratentorial in 30%, infratentorial in 30%)

and supra and infratentorial in 40%. Patients presented metastases from primitive tumor of the lung (74%), the breast (9,1%), colorectal origin (9%), cutaneous origin (6,4%) head and neck origin (5,3%), other origin (7%) and unknown origin (7,3%).

Results: Survival of patients with CM was dependent on the type of treatment tumour, it was about 339 days [28-662], 222 days [90-390] and 64 days [8-678], respectively in the event of complete surgical resection, of biopsy or partial resection or exclusive radiotherapy. In addition survival was also conditioned by the type of primary tumour, it was 197 days, in case of non small cell lung cancer and 119 days for the small cell lung cancer. In case of breast cancer, colorectal cancer, cutaneous cancer, head and neck, other origin or unknown, survival was respectively 106, 90, 63, 120, 226, 174 days.

Conclusion: Survival was dominated by the achievement of a surgical resection and by the aggressive nature of the primary tumor. It seem possible to use different radiotherapy scheme according to primary tumor site

Radiobiology

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Role of Bcl-2 subcellular localization for radiation-induced apoptosis

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Introduction: The anti-apoptotic proto-oncogene Bcl-2 is expressed in membranes of mitochondria and endoplasmic reticulum and mediates resistance against a broad range of apoptotic stimuli. Although several mechanisms of Bcl-2 action have been proposed, its role in different cellular organelles remains elusive.

Material and Methods: We analyzed the function of Bcl-2 targeted specifically to certain subcellular compartments in Jurkat lymphoma cells. Bcl-2 expression was restricted to the outer mitochondrial membrane by replacing its membrane anchor with the mitochondrial insertion sequence of ActA (Bcl-2/MT) or the ER-specific sequence of cytochrome b5 (Bcl-2/ER). Additionally, cells expressing wildtype Bcl-2 (Bcl-2/WT) or a transmembrane domain-lacking mutant (Bcl-2/DTM) were employed. Apoptosis induced by ionizing radiation was quantified using scatter characteristics and by determination of the mitochondrial membrane potential (DYm) using FACS Calibur flow cytometer. Furthermore activation of different caspases was analysing by western blotting.

Results: Bcl-2/WT and Bcl-2/MT strongly inhibited radiation-induced apoptosis and caspase activation, whereas Bcl-2/DTM had completely lost its anti-apoptotic effect. Interestingly, Bcl-2/ER conferred protection against radiation-induced mitochondrial damage and apoptosis similarly to Bcl-2/MT.

Conclusion: Here we show for the first time that not only mitochondrial Bcl-2 but also ER-targeted Bcl-2 interfered with mitochondrial DYm breakdown and caspase-9 activation. Our finding therefore indicates the presence of a crosstalk between both organelles in radiation-induced apoptosis.

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Immunohistochemical study on reoxygenation of FaDu-tumours during fractionated radiotherapy

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Purpose: Previous investigations indicated that reoxygenaton might be the stimulus for accelerated repopulation of FaDu-tumors during fractionated radiotherapy. In addition to these experiments immunohistochemical studies on the oxygenation status and the tumormicromilieu during radiotherapy were performed.

Methods: Tumorbearing mice were irradiated with 3 to 15 daily fractions (3 Gy) under normal blood flow and clamp hypoxia. Mice were sacrificed one day after end of irradiation after injection of different histological markers and tumors were stained and evaluated. Vascularization (ERMP-12), perfusion (Hoechst) and the amount of cellular hypoxia (Pimonidazol) was quantified by multiparameter image analysis.

Results: Vascular density in the vital tumor area was constant with increasing number of fractions (5-8%). The perfused fraction of vessels decreased considerably after irradiation with 3 and 6 fractions compared to unirradiated controls from 37% to 7% but increased after 12 to 15 fractions to values comparable to unirradiated tumors. The amount of cellular hypoxia in the vital tumor area decreased with increasing number of fractions from 17% to 2%.

Conclusion: From these immunohistochemical and morphometric studies we conclude that there is a high degree of hypoxia during the initial part of radiotherapy in FaDu-tumors. After 12 fractions reoxygenation occurs. These data are in good agreement with our functional studies on radiobiological hypoxia.

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Comparison of biodistribution of two hypoxia markers [18F]fmiso and [18F]fetnim in an experimental mammary carcinoma

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Nitroimidazole compounds labelled with positron emitting radionuclides such as fluorine-18 offer a means for non-invasive detection of tumour hypoxia with positron emission tomography (PET). A good marker for clinical use would apparently be one with a high hypoxia-specific signal-to-background ratio in target tissues. Our goal was to compare the intratumoural biodistribution of [18F]fluoromisonidazole ([18F]FMISO) with that of [18F]fluoroerythronitroimidazole ([18F]FETNIM) in carbogen treated and untreated mice, in order to compare the hypoxia-specificity of the tracers. Female CDF1 mice with a C3H mammary carcinoma grown on the backs were used. Tumours were size matched and animals breathed either normal air or carbogen gas (95% O2 + 5% CO2). The gassing procedure was started at least 5 min prior to the intravenous injection of either [18F]FMISO or [18F]FETNIM and continued throughout the experiment. A minimum of six mice were used for both gas conditions with each tracer. The hypoxia markers were allowed to distribute for 120 min. Blood, tumour, muscle, heart, lung, liver, kidney, fat, and bone tissues were immediately removed, counted for 18F-radioactivity and weighed. Tumour and muscle were frozen in dry ice/isopentane and cut with a cryomicrotome into 20 μm thick slices. The spatial distribution of 18F-radioactivity from the tissue slices was determined with digital autoradiography.

The treatment had no effect on the biodistribution of either tracer in the normal tissues, but had an effect on the tumours. Autoradiography results showed that the whole tumour-to-muscle 18F-radioactivity uptake ratios were significantly higher in untreated mice as compared to carbogen treated mice for both [18F]FMISO ($p = 0.004$) and [18F]FETNIM ($p = 0.004$). The autoradiograms showed that the 18F-activity was heterogeneously distributed within tumours showing regions with high and very low uptakes. These uptakes will be correlated to the histological status of the tumour slices.

In conclusion, our study shows that both [18F]FMISO and [18F]FETNIM uptake correlates with the oxygen status in tumours.

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Does selection of rapidly proliferating clonogenic tumour cells contribute to accelerated repopulation during fractionated RT? A study on human squamous cell carcinoma in nude mice

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Purpose: FaDu hSCC exhibits a clear-cut time factor of fractionated irradiation due to accelerated repopulation of clonogenic tumour cells during treatment. The underlying mechanisms of accelerated repopulation are not fully understood. Beside other mechanisms genetically stable selection of rapidly proliferating clonogenic tumour cells may be involved in this phenomenon.

Materials and methods: Three FaDu tumours (R1, R2, R3) that recurred locally after fractionated RT with high doses and long overall treatment times were retransplanted s.c. into the right hind leg of NMRI nude mice. Human origin was confirmed by LDH isoenzym pattern. Six millimeter tumours were irradiated either with single dose, 18 fractions of 3 Gy within 18 days, or 18 fractions of 3 Gy within 36 days. To obtain complete dose effect curves, graded top-up doses were given after fractionated RT. All irradiations were applied to anaesthezised animals under clamp hypoxia. For data evaluation