Radiotherapy and radiobiology

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A treatment planning comparison of conventional, 3D conformal, intensity modulated photon - and proton irradiation therapy in the treatment of paranasal sinus carcinoma

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Purpose: To determine potential improvements in patients with paranasal sinus carcinoma by comparing proton and intensity modulated photon radiotherapy (IMRT) with conventional– and conformal photon treatment techniques.

Methods and Materials: In 5 patients with paranasal sinus carcinoma comparative treatment planning was performed. Dependent on indications prescribed total doses varied from 60-70Gy (fraction size: 2Gy) with the 95% isodose including the PTV. Patient related proton plans (2-3 fields) were compared with corresponding standard (2-3 fields)-, conformal (7 fields)- and IMRT (6 fields, step and shoot technique, max. 12 segments) photon plans. All treatment plans were evaluated by DVH analyses of the target volumes and the organs at risk (OAR). Dose distributions within the PTV were analyzed with regard to mean– and maximum values, conformity indices and dose inhomogeneities. DVH analysis of the OAR (i.e. ipsi– and contralateral bulbus occuli, retinae, optic nerves, chiasm, hypophysis, glandulae lacrimales, brain) referred to the mean doses and the percentages of volumes receiving more than defined tolerance doses (i.e. glandular lacrimalis: 30Gy, lens: 10Gy, retina: 45Gy, optical pathway structures: 50Gy, hypophysis: 20Gy, brain: 50Gy). Dose exposures to non-target tissues were estimated by calculating respective volumes receiving 10%, 30%, 50%, 70%, 90% and 95% of prescribed PTV doses.

Results: Mean doses of 100% for all planning modalities were determined and maximum doses of 107% (conventional), 105% (conformal), 108% (IMRT) and 111% (proton) were assessed. Conformity indices and dose inhomogeneities were comparable for the different treatment planning techniques with values of 1,5 and 9% (conventional), 1,2 and 7% (conformal), 1,1 and 12% (IMRT), 1,2 and 9% (proton), respectively. Photon plans resulted in higher volumes of irradiated normal tissues to the 10%-70% dose levels when compared to corresponding proton plans. Volumes thereby increased by factors of 1,0–3,2 (conventional), 1,2–4,1 (conformal) and 1,1–3,8 (IMRT), respectively. In comparison to conventional techniques both conformal- and IMRT photon techniques reduced the mean doses to OARs. No additional benefit in dose reduction was found for the IMRT technique. Usage of protons further reduced the mean doses to the OARs by up to 65% and 62% in comparison to the conformal– and IMRT techniques, respectively.

Conclusion: In comparison to conventional treatment techniques conformal- and IMRT techniques similarly enabled dose reductions to non target tissues. Even relatively simple proton based techniques further reduced doses applied to the OAR and appeared superior to all photon based treatment options. Acknowledgment: This work was supported by the Government of Lower Austria and the Federal Ministry for Education, Science and Culture.

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The radiosensitising effect of difluorodeoxyuridine, a metabolite of gemcitabine, in vitro

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Introduction: Gemcitabine (dFdC) is an active antitumour agent with radiosensitising properties. dFdC is rapidly metabolised, intracellularly as well as extracellularly, by deoxycytidine deaminase to difluorodeoxyuridine (dFdU), a compound with little antitumour activity. However, plasma concentrations are maintained for a prolonged period (> 24 h) at levels known to cause growth inhibition. In this study, we investigated the radiosensitising potential of dFdU *in vitro*.

Materials and methods: ECV304, a human epidermoid bladder cancer cell line was treated with dFdU (0-0.15 mM) for 24 h prior to radiotherapy (RT) (γ -Co^{60}, 0-8 Gy, room temperature). Cell survival was determined 7 days after RT by the sulforhodamine B test. Experiments were performed at least three times. ID50, radiation dose resulting in 50% cell kill, was calculated from the survival curves, fitted according to the linear-quadratic model: survival=exp(-α D-β D^2). The radiosensitising effect was represented by the dose enhancement factor (DEF): ID50 /ID50. Synergism was determined with combination index (CI) analysis.

Results: dFdU caused a clear radiosensitising effect. DEFs increased with an increasing concentration dFdU: DEFs were 1.70 ± 0.35, 2.15 ± 0.23 and 2.83 ± 0.33 after treatment with 0.05, 0.1 and 0.15 mM dFdU, respectively, concentrations with moderate cytotoxicity (IC50=0.29 ± 0.09 mM). The CI analysis showed synergism with 0.1 and 0.15 mM and moderate synergism with 0.05 mM dFdU. The radiosensitising effect of dFdU was observed at the initial part of the dose-response curve, shown by an increase of the α value of the linear quadratic model ranging from 0.23 to 0.75. The increase in α value is statistically significant at dFdU concentrations of 0.1 and 0.15 mM.

Conclusion: dFdU, the main metabolite of dFdC, caused a clear radiosensitising effect *in vitro*. Since the metabolite is present in plasma for a long period (> 24 h) after treatment with dFdC, it might be partly responsible for the interaction between RT and dFdC. This observation might have important consequences for the optimal schedules of dFdC during radiation therapy in order to obtain the maximal radiosensitising effect.

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Transfection of human cell lines with the human multi-drug-resistence (MDR-1) gene supresses radiation-induced apoptosis and increases radioresistence

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Introduction: Radiation-induced apoptosis has only a minor influence on clonogenic survival of tumor cells from most solid tumors. However, several normal tissue cells, e.g. of hematopoietic origin, undergo apoptosis after exposure to therapeutic agents. Suppression of radiation induced apoptosis in normal tissue may therefore be a potential mechanism to increase the therapeutic gain and therefore improve tumor control rates. We studied a novel mechanism to suppress radiation induced apoptosis by transfecting human cell lines which are susceptible to radiation-induced apoptosis with the human multi-drug-resistance 1 (MDR1) gene.

Methods: The human ovarian cancer cell line A2780 and the MDR1 transfectant cell line A2780/M250 were used. The transfected cells stably overexpressed P-glycoprotein (P-gp), as monitored by flow cytometry after immunostaining, when maintained in 250 nM vincristine which was isotoxic to about 0,1 nM vincristine with the A2780 cells. Additionally, the human lymphoblastoid cell lines TK6, TK6E6, WTK1 were retrovirally transfected, followed by selection for vincristine resistance. The cell lines were irradiated with increasing X-ray doses (0-6 Gy), apoptosis was measured using the Nicoletti-assay and clonogenic survival was determined.

Conclusion: P-glycoprotein overexpression from MDR-1 gene transfer suppresses radiation-induced apoptosis. Corresponding findings were recently reported with a conditional P-gp expression system (Ruth and Roninson, 2000). With the cell systems used, MDR-1 gene transfer also increases clonogenic radioresistance, similar to the effects of PMA or caspase inhibition (in human lymphoblasts).

The P-gp inhibitor Cyclosporin A, which restores vincristine toxicity, does not prevent apoptosis suppression, indicating different pathways.

P-glycoprotein not only protects cells against chemotherapeutics but may also induce clonogenic radioresistance. This may become a novel approach to protect (hematopoetic) stem cells in cytotoxic combined modality therapy.

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Dose - dysfunction relationships within the parotid gland after radiotherapy

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Purpose: To determine salivary dysfunction of different areas within the parotid gland after radiotherapy (RT) and to evaluate dose-dysfunction relationships within the parotid glands and between patients.

Methods and Materials: Sixteen head and neck cancer patients, irradiated between September 1999 and November 2000 using a conformal parotid-sparing technique, were included in this study. Before RT and seven months after RT a salivary gland scintigraphy was performed in all patients combined with a single photon emission computed tomography (SPECT). The salivary excretion function (SEF) was measured, after stimulation, in 8-12 transversal 5 mm SPECT slices of each parotid. Loss of salivary function in these areas (dSEF%) was calculated as a proportion of the excretion function before and after RT. Because the planning CT-scan and the SPECT analysis were performed in the same treatment position, doses to areas within the parotid gland could be matched with the dysfunction of that respective area. For each patient, dose-dysfunction plots were performed and curve fitting was done.

Results: At baseline level, all but one patient had a normal salivary excretion function at the level of both parotid glands with small variation between the functionality of the different areas within the same gland. Seven months after RT, the reduction in salivary excretion function reached statistically significance for both parotids and a huge variation in functionality of the different areas within the same gland could be seen. When plotting the dysfunction of the different areas within one gland at seven months after RT against the dose these areas received, a sigmoidal function could be fit in seven (7/15) patients to the plots of both parotids and in five (5/15) patients to the plot of one parotid gland. In three (3/15) patients no dose-response curve could be fit to the plots due to a total dysfunction of all partial volumes or an absence of difference in dose between the volumes. In nine (9/15) patients a large variety of functional responses could be obtained at low irradiation doses (10-15 Gy), ranging from an improvement of function to a total loss.

Conclusion: Salivary SPECT is a useful tool for the evaluation of functional loss after RT of different areas within the parotid gland. While these areas are acting as functional sub-units under normal conditions, their dysfunction after RT does not seem to be only the result of the absolute dose these volumes received but also of the mean dose to the entire parotid gland and the dose to the surrounding areas.

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Severe radiation-induced bowel complications are not uncommon in patients with uterine cervix cancer

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Background: To evaluate the factors of severe (Grade 3-4) bowel complications after external irradiation and high dose-rate intracavitary (HDR-IC) brachytherapy among patients with cervical cancer.

Materials and Methods: We reviewed 298 patients of stage IB-IVA cervical cancer managed by curative-intent radiotherapy from May 1993 through December 1997. External irradiation to whole pelvis (34.2-50 Gy/ 19-27 fractions) was delivered to all patients initially. Two hundred and three patients received additional bilateral parametrial boost (3.6-18 Gy/ 2-10 fractions) with 4-cm midline shielding. HDR-IC brachytherapy, 16-24 Gy/ 5 fractions to Point A, was given after external irradiation. External parametrial dose < 50 Gy, 50-54 Gy and > 54 Gy were categorized as low parametrial dose (LPMD), intermediate parametrial dose (IPMD) and high parametrial dose (HPMD) group, respectively. Cumulative rectal biologic effective dose (CRBED) < 85 Gy, 85-105 Gy and > 105 Gy were categorized as low cumulative rectal biologic effective dose (LCRBED), intermediate cumulative rectal biologic effective dose (ICRBED) and high cumulative rectal biologic effective dose (HCRBED) group, respectively. The actuarial rate of bowel complications was compared among groups. We used Cox regression for multivariate analysis of bowel complications.

Results: Grade 3-4 bowel complication rates were 16%. The rates were 7%, 13%, and 34% in the LPMD, IPMD, and HPMD group (p=0.0001), respectively. The rates were 5%, 11%, and 28% in the LCRBED, ICRBED, and HCRBED group (p=0.0002), respectively. In multivariate analysis of Grade 3-4 radiation-induced bowel complications, CRBED (p=0.0010) and external parametrial dose (p=0.0007) were independent factors.

Conclusions: Radiation-induced severe bowel complications are depended on external parametrial dose and CRBED. We do not suggest external parametrial dose > 54 Gy and CRBED > 105 Gy for treatment of cervical cancer due to relatively high incidence of severe bowel complications.

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Radiation-induced changes in the cytoskeleton of human endothelial cells in relation to endothelial monolayer permeability.

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Treatment of solid tumours by radiotherapy damages the tumour cells as well as the microvasculature of the tumour and surrounding normal tissues. An increase in vascular permeability is a well known effect of radiation, which contributes toward changes in the interstitial space that lead to reduction of parenchymal cell function, necrosis and fibrosis. A response to radiation by normal tissue endothelium is thought to be a major limitation for its use in cancer treatment.

The aims of the present study were to investigate the early effects of radiation on the cytoskeleton of cultured endothelial cells and relate these to changes in endothelial monolayer permeability.

Human dermal microvascular endothelial cells (DMEC) were irradiated using a Pantak X-ray machine. Using immunofluorescence techniques, DMEC were found to respond to various doses of radiation (0.5 - 20 Gy) by a rapid (within minutes) increase in actin reorganization into stress fibers, accompanied by changes in the distribution of the adherens junction protein, VE-cadherin. Increased endothelial stress fiber formation, cell contraction and redistribution of junctional proteins can lead to intercellular gap formation and changes in monolayer permeability. Therefore, changes in permeability were quantified by monitoring the passage of fluorescently-labelled dextran through DMEC monolayers grown on microporous filters. Radiation was found to induce a significant increase in DMEC permeability suggesting that the changes in the actin cytoskeleton and distribution of cadherins were associated with increased monolayer permeability. Activation of the GTPase Rho and its associated Rho kinase have been recognised as key regulators of the actin cytoskeleton, intercellular junction integrity and permeability. Analysis of the mechanisms involved in irradiation-induced actin reorganisation, re-distribution of VE-cadherin and increased permeability revealed that these effects of radiation were dependent on activation of Rho kinase, since they were blocked by the highly specific Rho kinase inhibitor Y-27632. Simvastatin, a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor used clinically for the treatment of hypercholesterolemia, is known to inactivate Rho by inhibiting its geranylgeranylation. Simvastatin was found to inhibit the radiation mediated changes in actin and junctional proteins as well as increased permeability suggesting the involvement of Rho.

These data provide an insight into possible mechanisms involved in radiation-induced changes in vascular permeability. Further investigations are needed in order to elucidate whether compounds such as Y-27632 and simvastatin may be useful in counteracting some of the side effects of radiation *in vivo*.

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Efficacy and morbidity of linear accelerator radiosurgery for cerebral arteriovenous malformations: a comparison with the natural history

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Background: To report the results of arc-therapy radiosurgery in terms of efficacy. To compare the adverse-effects rate with the one expected from the natural history.

Material and methods: At the University Hospital of Nancy, 217 patients have been treated for cerebral ArterioVenous Malformation (AVM) by linear accelerator radiosurgery since 1992. We report here the results of a retrospective study of the 118 first patients (55 men, 63 women) treated between 01/07/92 and 30/06/98. The mean follow-up was 46 months (5-105). The mean age was 35 years (13-65). AVMs had poor prognosis features at initial presentation: existence of previous therapeutic failures (85%), high Spetzler-Martin grade (67% of grade III or higher), large size (57% > 14cc) and a high rate of initial hemorrhage (54%). Patients had already been treated by previous embolizations with a mean number of 4 procedures (1-11) in 84% of patients (99/118); 79% by embolizations alone (93/118) and 5% by partial microsurgery and embolization (6/118).