tumour control dose 50% (TCD50) were calculated and compared with the TCD50 values of the parental FaDu line after the same irradiation schedule.

Results: The TCD50 values after single dose irradiation were 37 Gy [95% CI 33;42], 39 Gy [33;43], 37 Gy [35;40] and 38 Gy [35;41] for FaDu-R1, FaDu-R2, FaDu-R3 and the parental FaDu, respectively. All investigated retransplanted recurrences showed a clear-cut time factor, i.e. TCD50 values after 18 fractions within 36 days were significantly higher than after 18 fractions within 18 days. The comparison of TCD50 values after the same overall treatment time revealed no significant differences between R1, R2, R3 and the parental FaDu line indicating an identical magnitude of the time factor in the retransplanted recurrences and in the original FaDu.

Conclusion: A genetically stable selection of rapidly proliferating clonogenic cells does not contribute to accelerated repopulation in poorly differentiated FaDu hSCC in nude mice.

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746 POSTER

Effect of recombinant human Keratinocyte Growth Factor (rhKGF) on proliferation, clonogenic capacity, and radiation response of human epithelial tumor cells in vitro

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Purpose: A fatal consequence of breaks in radiotherapy of head and neck cancer caused by severe mucositis may be a significant decrease in local control and cure. Amelioration of the mucosal response aiming at avoiding breaks could increase the therapeutic ratio of radiotherapy. Keratinocyte growth factor has been identified to ameliorate the acute response to radiation in animal models. The application of KGF in tumor treatment should not protect tumor cells. The purpose of this study is to investigate the in vitro effect of rhKGF on proliferation, clonogenic capacity, and radiation response of low passage human epithelial tumor cells in media containing low FCS concentration.

Material and Methods: Five tumor cell cultures derived from head and neck squamous cell carcinomas, three cultures derived from pleural effusions of lung carcinomas and normal nasal epithelial cells were analyzed. For experiments, cells in passage 2-6 were incubated with rhKGF (10;200 ng/ml) immediately after plating for clonal growth in serum-depleted media. To determine cellular radiosensitivity single doses of 1;8 Gy of X-rays were applied. Colony formation as well as the number of cell doublings was determined after 10;14 days of growth in rhKGF-treated and control cells. Each experiment was repeated twice, radiation survival curves were fitted by the linear-quadratic equation, and statistical comparison was preformed betweeen rhKGF-treated and non-treated cultures.

Results: Normal epithelial cells showed a two- to three-fold increase in the number of cell doublings due to KGF-treatment ($P < 0.0001$). In contrast, in tumor cell cultures only slight, not significant stimulation of proliferation occurred in 2 out of 8 samples ($P = 0.20$ and 0.07, respectively). This stimulation was abolished either by serum addition to the medium or in irradiated cells. In the remaining tumor cell cultures, which were not growth stimulated by KGF neither radiation-induced impairment of proliferation nor clonogenic cell survival was influenced by the addition of KGF to the medium.

Conclusion: A clinical pilot study indicate that KGF is well tolerated and effective in humans. In animal models, KGF has been shown to ameliorate the radiation tolerance of normal epithelia. Together with the minimum in vitro tumor cell response to KGF, compared to normal epithelial cells in this study, these results suggest a potential for selective protection of normal epithelia during clinical radiotherapy.

747 POSTER

The cytotoxicity of Ukrain does not involve the TP53/p21/p27-signal transduction cascade

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Purpose: Ukrain, a Chelidonium majus L.-Alkaloid/Thio-TEPA derivative, has shown cytotoxicity in vitro and in vivo. The mechanism responsible remains to be elusive. In this study the influence of Ukrain and ionizing radiation on the TP53-p21 pathway and the cell-cycle was investigated in human wild-type (wt) TP53 lung carcinoma cells (A549), TP53-overexpressing glioblastoma cells (U138MG) and normal wt-TP53 fibroblasts (HSF1).

Materials/methods: Exponentially growing cell lines/cell stem were irradiated with 1x5 Gy or treated with 1.0μg/ml Ukrain for 2, 6 or 24h. Except colony formation, TP53, p21 and p27 were examined using western blot technique. Analyses of the cell-cycle were performed by flow cytometry.

Results: Ukrain treatment demonstrated a radiosensitizing effect in A549 and U138MG cells and a radioprotective effect in normal fibroblasts. TP53 induction/stabilization (>2-fold) and subsequent induction of p21/p27 (>10-/>8-fold) could be shown in A549 cells and HSF1 after irradiation but not after Ukrain exposure. TP53-overexpression without p21/p27 induction was detected in U138MG cells. An accumulation of cells in the G2-phase after a 24h-Ukrain treatment was detected in A549 (50%) and U138MG cells (70%) whereas the HSF1 showed no alteration of the cell-cycle.

Conclusion: Ukrain did not exert its cytotoxicity via the TP53-pathway. Radioprotection of wt-TP53 cells after Ukrain was TP53/p21/p27-independent and without G1-phase block. However, in tumor cells a radiosensitizing effect was demonstrated that was possibly based on blocking cells in the G2-phase. To provide more insight into Ukrain's unique molecular mechanisms optimal for radiochemotherapeutic approaches further experiments still have to be performed.

748 POSTER

ACE-inhibition with Ramipril improves survival after thoracic irradiation in mice

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Purpose: The doselimiting effect of radiotherapy are in most clinical situations the 'late effects' of normal tissue tolerance. In this study we hypothesize that activation of matrix-metalloproteases may play a part in the pathogenesis of late effects. As Angiotensin Converting Enzyme (ACE) inhibitors have been demonstrated to inhibite at least some matrix-metallo-proteases, it was our hypothesis that they might protect against late irradiation morbidity. In addition we wanted to test if Ramipril had any effect on tumorgrowth.

Methods and materials: Single dose thoracic irradiation to the thorax in C57bl/6J mice was used as a model for late tissue tolerance. We used doses of 12, 15, 18 and 21 Gy and compared mice receiving Ramipril 30 mg/kg, continously 24 hours after irradiation in the drinking water, with mice receiving only plain water. The primary endpoint was survival, and as secondary endpoint for the 12, 15 and 18 Gy experiments we used breathrate measurements every second week for 180 days.

In a second experiment we tested the effect of Ramipril 30mg/kg in a micetumor model using the 'LPB-tumor'.

Results: Mice receiving Ramipril lived significantly longer than controls when the mice were irradiated with 18 or 21 Gy. For the lower doses the difference was not significant, but there was a trend in the same direction. The breathrate measurements supports these results. In Kaplan Meier survival plots with tumorarea $\geq$ 200 mmsq as endpoint we found a significant difference in' survival ' between mice receiving Ramipril and controls for both irradiated and non-irradiated mice.

Conclusions

The ACE-inhibitor Ramipril given 24 hours after single dose lethal thoracic irradiadition significantly prolongs lifetime in C57bl/6J mice. In addition Ramipril attenuates tumorgrowth in LPB tumors in mice.

749 POSTER

Comparison of tumor control probability and normal tissue complication probability between 3D-CRT and IMRT plans in patients with prostate cancer

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Purpose: To compare tumor control probability (TCP) and normal tissue complication probability (NTCP) between conventional three Dimensional Conformal RadioTherapy (3D-CRT) and Intensity Modulated Radiation Therapy (IMRT) in prostate cancer patients using radiobiological response models.

Methods: Ten prostate cancer patients had planning CT studies at the Houston VAMC. The prostate was immobilized using an endorectal balloon inflated with 100 cc of air. The Raptor/3D and Peacock/Corvus treatment planning systems were used to generate 3D-CRT and IMRT

plans, respectively. With 3D-CRT, six fields (laterals and obliques) were used to deliver 76 Gy at 2 Gy/fx to isocenter, ensuring complete target coverage with the 95% isodose line. With IMRT, the prescribed dose was 70 Gy (2 Gy/fx) at the 85% isodose line, limiting only 15% of rectum and 33% of bladder to exceed 68 and 65 Gy, respectively. Radiobiological response probabilities for 3D-CRT and IMRT plans were calculated using Niemierko's Equivalent Uniform Dose (EUD) model for targets and the Burman-Kutcher-Lyman model for normal tissues. TCP and NTCP between 3D-CRT and IMRT plans were compared by Student's paired T-tests.

Results: Prostate mean EUD was significantly higher for 3D-CRT plans (75.19 Gy) compared to IMRT (73.19 Gy, p=0.017), while the mean EUDs for seminal vesicles were comparable between the two techniques (74.87 Gy for 3D-CRT and 74.72 Gy for IMRT, p=0.74). Insignificant differences in TCP values were observed between 3D-CRT and IMRT plans for prostate (0.9952 and 0.9792, respectively, p=0.017) and seminal vesicles (0.9994 and 0.9995, respectively, p=0.740). IMRT resulted in significantly reduced NTCP compared to 3D-CRT for upper rectum which anatomically corresponds to the level of the seminal vesicles, (0.018 and 0.034, p=0.025) and for femurs (<.0001 and <.001, p=0.021). However, insignificant NTCP differences were observed between 3D-CRT and IMRT for both the lower rectum (p=0.939) and the bladder (p=0.137).

Conclusion: Biological response models indicate that target dose escalation by both 3D-CRT and IMRT result in comparable TCPs in the treatment of patients with prostate cancer. Moreover, IMRT also achieves superior avoidance of normal tissues as evidenced by the decreased NTCP values for upper rectum and femurs. Clinical trials are currently conducted to evaluate outcomes of patients treated with both techniques.

750 POSTER

Rapamycin increases the efficacy of fractionated radiation in malignant glioma cells

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Purpose: Repopulation of tumor clonogens during fractionated radiotherapy may adversely affect local tumor control. This suggests that pharmacologic inhibition of tumor proliferation during fractionated radiotherapy may enhance overall efficacy of treatment. As a preliminary test of this concept, we evaluated the effects of rapamycin, a novel cytostatic agent, on the proliferation of U87 and SK-MG3 malignant glioma cell lines and its efficacy when given concurrently with fractionated radiation.

Methods: Cell proliferation was evaluated by the MTS assay, which is a colorimetric assay that measures viable cell number. Cell cycle distribution was evaluated by flow cytometry after staining with propidium iodide. Cell survival was evaluated by standard clonogenic assay.

Results: Incubation with 10 nM rapamycin reduced the S-phase fraction of the SK-MG3 and U87 cells by 38 and 28%, respectively. Moreover, in an MTS assay, incubation of U87 cells with 10 nM rapamycin for six days resulted in a 41% decrease in the relative cell number compared to an untreated control. Despite the inhibition of proliferation and increased fraction of cells in G1, a 24 h incubation with 100 nM rapamycin prior to irradiation had no effect on the clonogenic survival of either SK-MG3 or U87 cells. However, when SK-MG3 cells were treated with five fractions of 3 Gy over a 96 hour period, concurrent treatment of cells with rapamycin resulted in the exclusive formation of abortive colonies containing < 20 cells. In contrast, treatment with rapamycin alone resulted in 'normal' colonies that were the same size as those treated with fractionated radiation alone. These data are consistent with the inhibition of proliferation during and after radiation. We are now testing the possibility that inhibition of proliferation by rapamycin in a U87 xenograft animal model will improve the efficacy of fractionated radiation, and the results from these ongoing animal studies will be presented.

Conclusions: Rapamycin is a promising novel therapeutic agent that is currently being investigated as a single agent in clinical trials with malignant CNS tumors. Our data suggest that concurrent administration of rapamycin may enhance the efficacy of fractionated radiation therapy and that the clinical evaluation of this combination in patients with malignant gliomas may be warranted.

751 POSTER

Epidermal growth factor as a potential early indicator of late radiation damage to the kidney

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Background and purpose: This study was designed to evaluate the proliferative response of epidermal growth factor (EGF) gene expression as an early indicator of late renal radiation damage.

Materials and Methods: EGF gene expression was measured in the irradiated left kidneys of C3H/HeSlc mice using RT-PCR 24 hours after radiation doses of 9, 12, or 15 Gy. In a second experiment, the same radiation doses were administered to the right kidney plus the lower half of the left kidney. The partly irradiated left kidneys were harvested and EGF gene expression was measured. The irradiated whole right kidneys were subjected to immunohistochemical staining for EGF protein. In a third experiment, 12 Gy was administered to the right kidney plus the lower half of the left kidney. The mice underwent left nephrectomy 24 hours after radiation, and the EGF gene expression in the kidney was correlated with blood urea nitrogen (BUN) level representing late renal functional damage.

Results: EGF expression increased in 1 of 10 control mice and in 9 of 10 mice that received 15 Gy. The extent of increase of EGF was dependent on radiation dose. In mice having an increased BUN level after irradiation, 7 of 10 had EGF positive irradiated kidneys. All six mice whose BUN levels were unchanged had EGF-negative irradiated kidneys. EGF protein staining was observed in tubule cells only, not in glomerular cells. The amount of EGF protein staining correlated with radiation dose to some extent.

Conclusions: EGF gene expression seems to be a very early indicator of late radiation damage to the kidney.

752 POSTER

Intercellular adhesion molecule-1 (ICAM-1) knockout (KO) reduces radiation induced intestinal inflammation

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Introduction: The aims of the study were to characterize the role of ICAM-1 as a mediator of radiation-induced inflammatory response.

Methods: Endothelial ICAM-1 expression were determined by the radiolabeled antibody technique (ng mAb/g tissue) in control mice or at 24 hours and 14 days following irradiation with 10 Gy. Leukocyte endothelial cell interactions (rolling and firm adhesion) were assessed using intravital microscopy in intestinal venules, in wild-type and ICAM-1 KO mice. Inflammatory infiltration was evaluated by myeloperoxidase activity. Additional survival experiments were performed in wild-type and ICAM-1 KO mice irradiated with 10 or 20 Gy; weight loss, stool consistency, occult blood, and mortality were examined daily.

Results: In wild-type and ICAM-1 KO mice, there was a similar flux of rolling leukocytes under baseline conditions, 24 hours and 14 days after radiation. In wild-type mice, leukocyte adhesion significantly increased 24 hours after radiation and was reduced at 14 days (0.6 +-0.05, 4.3 +- 0.7, 2.1 +- 0.2). Expression of ICAM-1 (829 +- 67, 2120 +-173, 881+- 39 cells/100um) and mieloperoxidase activity (46 +- 33, 263 +- 63, 9 +- 2 u/gr tissue) on the intestine was significantly increased 24 hours after abdominal radiation and returned to normal values at 14 days. Compared to wild-type mice ICAM-1 KO mice had a significantly lower number of adherent leukocyte at 24 hours and 14 days (1.9 +- 0.3, 1.2 +-0.2) and mieloperoxidase activity (111 +- 55, 4 +- 2). Irradiation with 20 Gy induced a loss in body weight and mortality that was significantly higher in wild-type than in ICAM-1 KO. No differences were observed between these two groups of animals when radiation was delivered with 10 Gy.

Conclusions: ICAM-1 plays an important role in radiation- induced intestinal injury. Modulation of ICAM-1 may protect normal tissue to radiation damage.