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.

99 POSTER

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

V. Strnad, L. Keilholz, R. Sauer. *Dept. of Radiation Therapy, University Erlangen-Nürnberg, Germany*

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.

100 POSTER

Radiation therapy choroidal neovascularization in age-related macular degeneration

K. Engin[1], Ö. Gelişken[2], L. Özkan[1], B. Yazıcı[2], S. Kahraman[1]. [1]*Dept. of Radiation Oncology;* [2]*Dept. of Ophthalmology Uludağ University Medical College, Bursa 16059, Turkey*

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.

101 POSTER

Extended salivary response to ionizing irradiation: An experimental study

R. Nagler, A. Kuten, D. Laufer. *The Department of maxillo-facial and oral surgery and the Department of Oncology, Rambam Medical Centre, Haifa, Israel*

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.

102 POSTER

Radiosensitizing effects of cisplatin and carboplatin in prostate cancer cell lines

A.A. Geldof[1], A. Kruit[1], D.W.W. Newling[1], B.J. Slotman[2]. [1]*Department of Urology;* [2]*Department of Radiation Oncology, Academic Hospital Vrije Universiteit, Amsterdam, The Netherlands*

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.

103 POSTER

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

Urban D. Nylén, Quimin He, Ingrid Welander, Sven Skog. *Departments of Oncology and Medical Radiobiology, Karolinska Hospital and Institute, S-171 76 Stockholm, Sweden*

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-

ing wee1 and the dephosphorylating cdc25C decide the activity in cdc2p34. When 5-fluorouracil (5-FU) is added the G2-arrest is abolished, according to our earlier studies, and the toxicity markedly increased.

Material and Methods: NMRI-mice with ascites-growing sarcoma (Bp8) were injected with CDDP or X-irradiated with 5 Gy. The agents were given single or combined with 5-FU 30 minutes later. 6 hours later tumour cells were investigated for amount and activity of cdc2p34, cdc25A and cdc25C and amount of wee1.

Results: CDDP decreases while X-irradiation increases the cdc2p34 activity. Both increase amount of the cdc2p34-inhibiting phosphatase wee1. Addition of 5-FU in both cases decreases wee1 to less than normal. The cdc2p34-activity after CDDP+5-FU is maintained as normal, whereas X-irradiation+5-FU inhibit the activity.

Conclusions: The G2/M checkpoint enzymes are affected by CDDP and X-irradiation. The mechanism by which 5-FU abolishes the G2 arrest induced by CDDP is different to the mechanism active after X-irradiation+5-FU. In both cases, however, the amount of wee1 is decreased by 5-FU. After X-irradiation an accessory regulating mechanism, except influence by cdc25C and wee1 on cdc2p34, is important for onset of mitosis.

104 POSTER

Combined effects of Ionizing radiation and 4-hydroxy-ifosfamide (IFO) in different cell lines

D. Latz[1], T. Schulze[1], P. Schraube[1], C. Manegold[2], K.J. Weber[1]. [1]*Dpt. Radiotherapy, Univ. of Heidelberg;* [2]*Chest Hospital, LVA Baden, Heidelberg, Germany*

Purpose: Combined Radiochemotherapy has gained increasing interest in clinical applications. In an in vitro study the effects of combined exposure of ionizing radiation and IFO on cell survival and DNA double-strand (dsb) induction and repair were investigated.

Methods: Clonogenic survival of log phase V79- (chin. hamster), Caski- (squamous ca.), Widr- (colon ca.) and MRI-221 cells (Melanoma) was determined after combined exposure of radiation (1–2 Gy) and IFO (1 μg/ml at 2 h exposure). Measurement of cell survival for different cell cycle phases was performed after mitotic shake off control with flow cytometry). Analysis of DNA-dsb induction and repair were carried out using pulsed field electrophoresis (PFGE).

Results: Combined exposure resulted in additive effects in all cell lines tested. IFO exposure alone resulted in a decrease of resistance for cells of the middle and late S-phase. PFGE-experiments showed a marked induction of DNA-dsb after IFO exposure alone. There was no inhibition of repair of radiation induced DNA-dsb after combined treatment.

Conclusions: The result for clonogenic cell survival revealed a purely additive mode of action for the combined exposure of ionizing radiation and IFO for all cell cycle phases. Also, the PFGE-experiments gave no indication for a synergistic mode of action. However, the marked DNA-fragmentation after IFO exposure alone is a new and interesting finding.

105 POSTER

Combined effects of Ionizing radiation and gemcitabine (GEM) in different cell lines

K. Fleckenstein[1], D. Latz, J. Blatter[2], K.J. Weber[1]. [1]*Department of Radiotherapy, Univ. of Heidelberg;* [2]*Eli Lilly, Germany*

Purpose: Gemcitabine is a new antimetabolite, structurally related to Ara-C, and is now studied for its role as a potential radiosensitizer. The present investigation focussed on the effects of combined exposure of ionizing radiation and GEM on cell survival with special emphasis on the time schedule of administration.

Methods: Clonogenic survival of log phase V79- (chin. Hamster), Widr- (colon carcinoma) and MeWo-cells (Melanoma) was determined after combined exposure of radiation (1–12 Gy) applied at different times (up to 8 hours) following GEM-exposure (2 h at 0.02 μg/ml).

Results: Supraadditive cell killing was found for all cell lines with maximal radiosensitization when irradiation was given immediately after GEM-exposure and simple additivity at later times. A half-life of 1–2 h can be estimated for this decay of the interaction phenomenon

Conclusions: These in vitro data confirm earlier suggestions of the radiosensitizing potential of GEM. The rapid decay of this effects precludes the possibility that the accumulation of cells in S-phase due to the GEM exposure accounts for the greater effectiveness of the subsequent irradiation. The inhibition of DNA-repair as an explanation for the observed phenomenon is currently under investigation.

106 POSTER

The radiosensitivity of tumor vascular endothelial cells

Ch. De Pooter[1], L. Dirix[1], E. Van Marck[1], E. de Bruijn[2], A. Van Oosterom[2]. [1]*Lab. Ca. Res. & Clin. Oncol. University of Antwerp;* [2]*Dep. Oncology. UZ K.U. Leuven, Belgium*

Purpose: Ionizing radiation (IR) kills tumor cells. In addition it also damages the vascular endothelium. Therefore, we evaluated the sensitivity of these vascular endothelial cells for IR.

Methods: Human umbilical-vein endothelial cells (HUVEC) and a mouse endothelial cell line (MEC) were used and cultured in the proper media in tissue culture flasks. Cells were incubated at 37°C and irradiated at high and low dose rate with Cobalt-60 gamma rays (dose range 1 to 15 Gy). Cell survival was measured with the cytotoxic Almar blue test. The changes in cell survival were compared to these observed in human ovarian cancer cells (AOvC-0) which are known and published previously.

Results: The acute survival curves showed a clear dose response and exhibited a broad shoulder. Cells were significantly less radiosensitive than the ovarian cancer cells. The resistance factor at the 50% survival level ranged between 5.3–6.1. The sensitivity was influenced by changing the dose rate of the IR.

Conclusion: We observed an intrinsic radiosensitivity in our tumor vascular endothelial cells which can be modified by alterations in radiation dose rate. The effect of IR on a tumor might not only be due to the cytotoxic effect on the tumor cells itself, but also due to the effect on the tumor vascular endothelial cells.

107 POSTER

Measurement of radiosensitivity in cervical tumours on the basis of the comet assay

B. Biesaga, A. Gasińska. *Laboratory of Radiation Biology, Department of Radiation Therapy, Centre Oncology Cracow, Poland*

Purpose: The aim of the study was the radiosensitivity assessment in squamous cell carcinoma (SCC) of the cervix on the basis of the comet assay in which the number of primary and residual DNA damage after 2 Gy dose of the radiation was measured.

Material: 19 SCC were studied. The patients were not treated with chemo-or radiotherapy before biopsy.

Method: Single cell suspension from a biopsy was made by digesting with collagenase. The cell suspension was irradiated with doses 0–4 Gy. After the irradiation (initial DNA damage), or after 15 and 60 minutes of incubation at 37°C (residual DNA damage) cell suspension was mixed with poliakrylamide gel. Smears were made and cells were lysed with alkali solution. Then electrophoresis was performed. The amount of damaged DNA stained with DAPI was measured with image analysis and Comet 3.0 programme. The measure of the DNA damage was tail moment, that is the length of comet tail and intensitivity of its fluorescence.

Results: The differences in the number of primary (0 Gy), initial and residual DNA damage in the examined tumours were shown. Linear relationship between number of initial DNA damage and radiation dose was obtained. Taxonomic analysis of initial DNA damage allowed for identification of 3 groups of patients of statisticaly different sensitivity. After 2 Gy dose of radiatio, statisticaly differences in residual DNA damage after 0 and 15 minutes and 0 and 60 minutes were shown. The differences between patients were shown on the basis of the efficacy of the DNA damage repair (range 8.66%–91.73%).

Conclusion: The comet assay seems to have the potential to be used as a predictive assay of individual radiosensitivity.

108 POSTER

Hyposalivation and white blood cells loss following head and neck irradiation and a mediatory role of superoxide dismutase

R. Nagler, A. Kuten, D. Laufer. *The Department of maxillo-facial and oral surgery and the Department of Oncology, Rambam Medical Centre, Haifa, Israel*

Purpose: SOD is known to act as a first line of anti-oxidant defense against oxygen free radicals that mediate cytotoxicity or cell death. Head and neck irradiation results in oropharengeal syndrome manifested by 1) mucositis, anorexia, reduction in water and food intake, weight loss, and decreased salivation; and 2) suppression of total WBC as we have previously shown. Oxygen tree radicals are believed to be involved.