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

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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

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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

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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

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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

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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.

Materials and Methods: we evaluated the protective effect of MnSOD and Cu/ZnSOD following 15Gy irradiation (IR) of the head and neck in male Wistar rats.

Results: SOD treatment did not protect the animals against irradiation induced reduction in oral intake and weight loss as well as against submandibular hypofunction. In contrast, MnSOD had a protective effect against irradiation induced hypofunction of the parotid gland. The mean parotid saliva flow rates were 84.1 6.5 ml/30 min in the controls, 22.2 4.3 ml/min following irradiation, and 38.5 4.5 ml/30 min following MnSOD therapy, respectively (n = 10) (P°0.05). Both MnSOD and Cu/ZnSOD demonstrated a protective effect against irradiation induced WBC suppression as the WBC of the controls was 15.5 × 10^9/L; 6 days post (IR), the counts fell to 3.2 × 10^9/L (n = 10) (P°0.01)., while following MnSOD or Cu/ZnSOD therapy, the WBC were 7.5 × 10^9/L and 6.3 × 10^9/L respectively (n = 10) (P°0.01).

Conclusion: these results indicate that SOD partially protects against head and neck irradiation induced injury while MnSOD may be superior to Cu/ZnSOD.

109 POSTER

Effect of hypoxia on tumour growth and metastasis formation studied in chick embryo

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Purpose: Tissue oxygen supply influences the proliferation kinetics and vascularization of tumours. We studied the effect of hypoxia on tumour growth and metastasis formation in the early chick embryo.

Materials and Methods: For this study fertilised were incubated in an upright position in a commercial incubator at 36.8 ± 0.1°C and 60–65% relative humidity. The daily weight loss of the eggs was 0.3 ± 0.05 g per day which was considered to be within the normal range. The eggs were either incubated in air (20.9% oxygen, normoxia) or in 5% oxygen (hypoxia). After 48 hr approximately 4 × 4 mm of the inner egg shell were removed. In this area 4 × 10^5 glioblastoma cells (human, U-138 MG) were implanted. After implanting the tumour cells the eggs were investigated by videomicroscopy. At the end of the experiment tissue specimen were taken and analysed for proliferating cell nuclear antigen (PCNA).

Results: Tumour growth was accelerated in hypoxia compared to normoxia. Furthermore, the shape of the tumour was different under these conditions. While during normoxia the growing tumours had a relatively sharp border, during hypoxia this was not the case. Metastasis were found only under hypoxic conditions. The determination of PCNA also showed clear differences between normoxic and hypoxic tumours.

Conclusions: Hypoxia itself obviously induces tumour cell proliferation. Whether or not this is due to autocrine or paracrine stimulation cannot yet be answered. Furthermore, hypoxia favours the formation of metastasis.

110 PUBLICATION

Continous in vivo measurement of local mitochondrial metabolism after radiation

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Purpose: Different *in vitro* tetrazolium/formazan assays to determine radiation or drug effects on cell growth have been previously described. The aim of our study was to evaluate a new *in vivo* assay for continous measurements of local mitochondrial metabolism and cell proliferation.

Methods: For this study fertilized eggs were used. Irradiation of the area vasculosa (A.V.) was performed with a linear accelerator on day two of incubation. The eggs received doses from 2 to 10 Gy. For investigations of the local mitochondrial metabolism of the A.V. a micro-light guide spectrophotometer with modified light guides was used to measure the cleavage of a tetrazolium salt. 50 μm of the reagent (WST-1) were filled into the tip of the light guide. The WST-1 diffuses into the tissue and is cleaved to formazan. This is accompanied by a change in the absorption, which is measured as a calorimetric assay. In vitro calibration was performed with irradiated and non-irradiated single cell suspensions.

Results: There was a significant increase in absorption (optical density) measured by the calorimetric assay 1 hour after radiation with 10 Gy. Within 48 hours a significant decrease was observed compared to controls. After radiation with doses <10 Gy there was also a distinct, significant increase in absorption 1 h after irradiation. But then, optical density came to a normal range compared to the age matched control eggs.

Conclusion: The results show that it is possible to determine local metabolic activity and cell proliferation after irradiation continuously *in vivo*.

111 PUBLICATION

Effect of ionizing radiation on cell-cycle progression as an expression of intrinsic radiosensitivity

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Background: The clinical outcome of radiotherapy is associated with the differences in the intrinsic radiosensitivity of human tumours. The amount of initial DNA double strand breaks is directly related to cellular radiosensitivity DNase when added fixes the damage and therefore modifies the pattern of progression through the cell cycle.

Purpose: To assess the *in vitro* intrinsic radiosensitivity in two cell lines derived from human tumours by flow cytometry after DNase was employed to fix the initial DNA damage induced by ionizing radiation (XRT).

Methods: Two cells line A549 (lung carcinoma) and A375 (melanoma) with different radiosensitivity $SF2_{A549}$ = 0.85 and $SF2_{A375}$ = 0.75 were irradiated at doses ranging of 2 Gy and incubated with various concentration of DNase over a wide range of time. After the enzyme was removed the cells stained with propidium iodide were analysed for DNA content by flow cytometry.

Results: There is a difference in the redistribution of cell lines XRT dose dependent. Whereas the more radioresistant A549 shows little and transient alteration of DNA content at low doses. A375 cell line exhibits a delay of progression through the cycle and an accumulation at the interfaces G1/S and S/G2. These alterations may be related to a greater inhibition of cyclins which control the cell cycle check-pints.

Conclusion: The method might be used as a simple and quick test to approximate radiosensitivity in limited tumour samples

112 PUBLICATION

Radioperoxidation of human low-density lipoproteins (LDL): Antioxidant activity of a vitamin E derivative (MOH) -effect of the dose rate

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Purpose: The possible role of fatty acid peroxidation by free radicals is suggested by many epidemiologic and laboratory studies. We have evaluated the antioxidant effect of MOH and the effects of the dose rate on the peroxidation of LDL by free radicals produced by gamma radiolysis.

Methods: The effects of increasing doses have been followed by measuring the evolutions of the concentrations of endogenous vitamin E and "thiobarbituric acid-reactive substances" (TBARS).

Results: When MOH is in the solution, the consumption of endogenous vitamin E and TBARS are delayed, a higher dose is necessary to decrease the concentration of these compounds. Further more, the increase of the dose rate products a decrease of the yields of the two measured parameters of oxidation of LDL.

Conclusion: MOH exhibits antioxidant properties and prevents LDL against peroxidation by free radicals ($RO_2°/O_2°^-$) produced by gamma radiolysis. It is well know that these produced radicals are low initiators of lipid peroxidation. We can admit that these radicals react together in a preferential way. These reactions compete with the reactions of oxidation of LDL. The prevalence of the reactions of recombinaison leads to a decrease of the consumption of vitamin E and the formation of TBARS.