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.

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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.