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A NEW RIBONUCLEOTIDE REDUCTASE INHIBITOR, (E)-2'-DEOXY-(FLUROMETHYLENE) CYTIDINE, ACTS AS A RADIOSENSITIZER ON HUMAN COLON AND CERVIX CANCER CELL LINES

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Background : Ribonucleotide Reductase (RR) inhibitors such as Gemcitabline (dFdC) and Hydroxyurea (HU) are known to act as radiosensitizers both in vitro and in vivo. More recently, (E)-2'-Deoxy-(Fluoromethylene) cytidine (FMdC) has been developed as an RR-inhibitor. It is a cytotoxic drug *in vitro* and *in vivo*.
Purpose : To determine if at low doses of FMdC (nM concentrations) a radiation sensitizing effect can be observed in human cancer cell lines of colon (WiDr) and cervix origin (SiHa, C4-1 and C33-A) and to investigate the cell cycle effects of FMdC after irradiation by flow cytometry.
Material and methods : Postradiation surviving fraction has been evaluated by clonogenic assay. Postirradiation cell cycle distribution of WiDr has been evaluated by flow cytometry (double labelling with propidium iodine and bromodeoxy-uridine). Data were considered significantly different if a 0.05 p-value was reached (two-sided t-test).
Results :

	WiDr	C4-1	C33-A	SiHa
SF-2 (control)	77±6.8	45.7±3.9	62.6±1.9	64.9±5.5
SF-2 (FMdC)	37±2.9*	18.5±5.3#	39±4.5@	38.8±3#
DMF-50	2.3	2.5	1.7	1.7
DMF-10	1.6	1.5	1.4	1.4

SF-2 values are expressed in % and tabulated with corresponding standard error. DMF-10/50: dose-modifying factor at 10% or 50% survival level ; (*) FMdC at 50 nM; (#) FMdC at 40 nM and @) FMdC at 30 nM. The differences observed between SF-2 (Control) and SF-2 (FMdC), are all statistically significant. DMF-values were estimated from the complete dose-response curves. Flow cytometry done on WiDr, shows accumulation of irradiated cells in G2/M. This accumulation is increased by the use of FMdC.
Conclusion : In nanomolar concentrations FMdC is cytotoxic and acts as a radiosensitizer *in vitro* on colon and cervix cancer cell lines. We are currently investigating the effects *in vivo* and the cell cycle machinery regulating the G2/M transition (cyclin B, $p34^{cdc2}$, wee-1 and cdd25).

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RADIOSENSITIVITY OF MCF7 HUMAN BREAST CANCER CELLS IN MONOLAYER AND SPHEROID CULTURE: EFFECTS OF FRACTIONATED IRRADIATION.

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The aim of this study was to investigate the cellular radiosensitivity of estrogen sensitive breast cancer cells in a three dimensional model under fully oxic conditions using irradiation schedules with one and three fractions. Cellular radiosensitivity (α and β values of the linear quadratic model and 2 Gy surviving fraction SF2Gy) and radiation induced DNA damage (double strand break, dsb) studied in the spheroid model of MCF7 human breast cancer cells were compared to monolayer cultures. Grown as multicellular spheroids, MCF 7 cells showed a reduced radiosensitivity (α 0.149 Gy 1, β 0.031 Gy 2, SF2 70%; α/β 4.8; initial DNA damage 1.30 dsb/Gy/DNA unit) when compared to monolayer cultures (α 0.316 Gy 1, β 0.023 Gy 2, α/β 13.7 and SF2Gy 50%, and initial DNA damage 2.25 dsb/Gy/DNA unit). The radio protective effect of the three dimensional culture was reversed in the fractionated irradiation schedules. After a 3 x 3 Gy fractioned treatment with a 24 h interval between fraction, MCF 7 cells showed an increase in the surviving fraction (SF 0.003% for 9 Gy single dose vs. SF 0.6% for 3 x 3 Gy treatment) even though this was accompanied by an increase in cellular radiosensitivity (SF2 40%, DNA damage 2.52 dsb/Gy/DNA unit). Cell cycle analysis using flow cytometry of propidium iodure stained cell nuclei demonstraded the induction of a G2 block by a fractionated dose of three 3 Gy fractions. Our findings indicate that 1) sensitivity to radiation and the proportion of proliferating cells are probably related, and 2) differences in radiosensitivity reflect differences in radiation induced DNA damage.

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Residual DNA strand breaks and cellular radiosensitivity of 9 mammalian cell lines

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Background: The radiosensitivity of proliferating mammalian cells is mainly determined by the rate of mitotic death. X-irradiated cells ending in mitotic death die from chromosomal aberrations. Lethal aberrations arise from DNA double-strand breaks (dsb). Therefore, the number of residual dsb observed after the end of repair may be an indicator of the number of nonrepairable lethal lesions, i.e. of the cellular radiosensitivity.

Purpose: For 9 mammalian cell lines, the residual numbers of dsb as well as of single-strand breaks (ssb) were measured and compared with cell survival.

Material and methods: Human normal fibroblasts, AT fibroblasts, CHO, CHO K1, xrs1, xrs5, R1H, balb and scid cells in culture were irradiated with X-rays. The cellular radiosensitivity was measured by colony assay, dsb were determined by constant-field gel electrophoresis, ssb by the alkali unwinding method.

Results: The 9 cell lines showed a broad spectrum of cellular radiosensitivity (D_0 ranging from 0.5 to 1.3 Gy). The numbers of dsb as well as of ssb induced (immediately after irradiation) showed no difference between the cell lines. In contrast, we found a great variance for the numbers of residual breaks (measured after 24 hours of repair). The numbers were compared with radiosensitivity: in radiosensitive cells a higher number of residual breaks was found when compared to radioresistant cells. This holds for dsb as well as for ssb. However, the correlation was better for dsb than for ssb.

Conclusion: For the 9 cell lines studied, the cellular radiosensitivity is correlated with the number of residual DNA double-strand breaks as well as of single-strand breaks.

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NORMAL TISSUE RADIOSENSITIVTY AND LOCAL CONTROL IN BREAST CANCER PATIENTS WITH POSTMASTECTOMY RADIOTHERAPY

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Background : We have retrospectively investigated variations in normal tissue reactions and local control in breast cancer patients.
Materials and methods : From 1985 through 1991, 194 patients with breast cancers pT1-2pN0-2M0 received adjuvant postmastectomy radiotherapy alone without chemotherapy. The chest wall was irradiated with 9 MV-electrons with daily doses of 2 Gy up to 44 Gy, the lymphatics with an anterior 9 MV-photon field with daily fractions of 2 Gy up to 50 Gy. Side effects were evaluated once weekly during treatment and thereafter twice yearly.
Results : Skin erythema was mild in 98 patients (51%), moderate in 53 (27%) and severe in 43 (22%). 38 patients (20%) developed clinical signs of esophagitis, 13 (7%) asymptomatic and 58 (30%) symptomatic pneumonitis during radiotherapy. The frequency of late sequelae (mild to moderate, overall 17%) did not correlate with acute reactions. If patients were divided in 3 subgroups according to the degree of acute reactions, patients with minimal reactions had a significant higher probability of developing a local recurrence than patients with severe reactions (5/72 vs. 0/58 local recurrences, $p<0.05$).
Conclusions: The correlation between acute radiation reactions and local control suggests a linkage between normal and malignant tissue radiosensitivity and supports the hypothesis that genetically determined differences in radiosensitivity may be clinically relevant.