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TIME AND DOSE-RELATED CHANGES IN THE EXPRESSION OF NEUROPEPTIDES IN SALIVARY GLANDS IN RESPONSE TO IONISING IRRADIATION.

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BACKGROUND: It has recently been demonstrated an enhanced expression of substance P (SP) and leu-enkephalin (L-ENK) in the parasympathetic innervation of the rat submandibular gland 10 days after fractionated irradiation to the head and neck region. In the present study, the expression of different neuropeptides in the submandibular and parotid glands were examined two and five days after initiation of treatment as well as 10 and 180 days following the termination of irradiation. The irradiation was given in a 5 days schedule, 6-9 GY daily up to a total dose of 30-45 Gy. Immunohistochemical and radioimmunological methods were used for identification of neuropeptides.

RESULTS: In the parenchyme of the submandibular and parotid glands, nerve fibres showing SP- and L-ENK-like immunoreactivity (LI) were fewer in number after two days of irradiation than in controls. After five days treatment, the pattern of peptide expression had returned to approximately that seen in controls. Ten days after cessation of treatment, a marked radiation dose-dependent increase in the number of fibres showoing SP- and L-ENK-Li in the parenchyme was observed. 180 days later no obvious differences were seen between control and irradiated animals. The pattern of expression of other peptides tested (CGRP, NPY) in gland parenchyme was not changed at all. Furthermore, the pattern of SP- and L-ENK-expression around large ducts and blood vessels was similar at all stages.

CONCLUSION: The study demonstrated a radiation dose-dependent change in the expression of SP- and L-ENK in the submandibular- as well as in the parotid gland following irradiation. Thus, a plasticity of peptidergic innervation after irradiation is evident. The results may give a new approach to the inheritent radiosensitivity of salivary glands and physiological alterations in normal tissues following radiotherapy.

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HEAT SHOCK PROTEIN HSP70 PROTECTS CELLS FROM HEAT SHOCK, OXIDATIVE STRESS AND X-RAY IRRADIATION

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Heat shock induces the preferential synthesis of a group of proteins termed the heat shock proteins (hsps), and the development of a transient resistance to heat termed thermotolerance. There is much data supporting the hypothesis that hsps play important roles in modulating cellular response to heat shock or other environmental stresses. Here, we report our studies on the expression of a cloned human hsp70 and its mutant derivative in rodent cells and its effects on cells' thermal sensitivity, cells' transcriptional and translational activity and their recovery kinetics after heat shock. Our data show that expression of intact human hsp70 or some mutant hsp70 in rodent Rat-1 cells, confers heat resistance in terms of cell survival. On the other hand, expression of only the intact human hsp70, can afford translational tolerance and facilitate cells' ability to recover from heat-induced transcriptional and translational inhibition.

Furthermore, we have examined the role of hsp70 in protecting cells from oxidative stress and x-ray irradiation. Our results show that constitutive expression of human hsp70 also confers resistance to hydrogen peroxide and x-ray irradiation. Our data, thus, implies a direct link between expression of a functional mammalian hsp70 and cell survival during heat shock, oxidative, or radiation stress.

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TISSUE CHANGES IN THE NORMAL AND SURGICALLY MANIPULATED CANINE LIVER FOLLOWING INTRAOPERATIVE RADIOTHERAPY (IORT)

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Introduction: After liver resection for colorectal metastases microscopic residual disease is often left behind. With external beam radiotherapy (EBRT) a tumoricidal dose can't be applied without damaging normal tissues. Theoretically IORT might be an alternative. However, before introducing IORT clinically, dose guidenes concerning the tolerance of liver tissue to single high dose irradiation have o be provided. The histopathological tissue changes of normal and surgically manipulated canine liver following different IORT-doses, was experimentally nvestigated.

Materials & Methods: In 25 Beagles a partial liver resection was performed. The resection plane as well as a normal non-surgically manipulated part of the liver was intraoperatively irradiated with single doses of 0 (sham),10,20,25 or 30 Gy IORT with an energy of 10 MeV.

Results: There was no peri- and postoperative morbidity during a follow-up of 2 years. Elective sacrifice for histopathological purposes was performed 3 months, 1, and 2 years following IORT. Resection planes appeared to be uncomplicatedly healed. With Light Microscopy and Electron Microscopy histopathological alterations were seen: capsular thickening, subcapsular fibrosis, bridging portal fibrosis and liver cell atrophy were especially prominent in the higher dose groups, minimal after 3 months but more severe after 1 and 2 years of follow-up. Vascular alterations were less distinct.

Conclusion: Two years following IORT, doses up to 30 Gy to normal and surgically manipulated canine liver are well tolerated without any morbidity, and resulted in parenchymal fibrosis and atrophy.

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RADIATION-INDUCED CHANGES IN THE DIFFERENTIATION PATTERN OF FIBROBLASTS: THE MAJOR CAUSE OF RADIATION FIBROSIS ?

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The cellular basis of radiation induced fibrosis is not understood. Fibroblasts as the major collagen producing cell type seem to be primarily involved in fibrotic reaction and have recently been described to differentiate from mitotically active progenitor cells into postmitotic fibrocytes. By means of cell and molecular biological parameters (colony formation, cytomorphology, gene expression, collagen production) investigations using cultured human fibroblasts were performed in order to elucidate whether changes in the differentiation pattern of these cells occur after irradiation. Single doses of 1-7 Gy induced the premature terminal differentiation process from progenitor fibroblasts to postmitotic fibrocytes. The radiation induced fibrocytes synthesized the various collagen types at significantly elevated rates resulting in an enhanced deposition of interstitial collagen. Thus, it can be postulated that the fibrotic reaction after irradiation is predominantly caused by the radiation induced premature differentiation of progenitor fibroblasts into postmitotic fibrocytes which are highly active in collagen production.

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IN VITRO ASSESSED SYNERGIC EFFECT OF FOTEMUSTINE AND IONIZING RADIATION ON 3 CELL LINES OF NON SMALL CELL LUNG CARCINOMA (NSCLC)

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Synergic effect of fotemustine (FT) and radiotherapy (RT) was tested with a MTT assay on 3 human lines (A549-SKLU-SKMES) of NSCLC in order to select suitable conditions for its clinical use. **Materials and methods** : range of concentrations of FT (eight levels from 10^{-7} to 10^{-4} M) and doses of ionizing radiation (from 2 to 16 Gy) were firstly tested to select the most appropriate range according to the rate of cytotoxicity fixed at 15-50 % for FT and 15-25 % for RT. RT was performed using a ^{60}Co source. For each cell line, FT was tested according to 5 timings, 2 before radiation (-24h and -1h), 2 after radiation (+ 1h and + 24h) and one with concomittent treatment. MTT assay was performed 7 days after with absorbance (DO) readings at 540 nm. Each experiment, including 8 measures, was performed in triplicate. Analysis of variance were performed to study interaction between radiotherapy or not and the fotemustine doses.**Results** : FT was tested at 10^{-7}, 5.10^{-7}, 10^{-6}, 5.10^{-6} M and RT at 2 Gy. Whatever the cell line and the timing between FT and RT (excepted SKMES line + 1 hour), a significant interaction was noted ($p < 0.05$) on percentage of survival according to the FT concentration. The most important benefit was observed at 5.10^{-7}M for 7 experiments and 4 at 10^{-6}M. The gain in % compared to a simple additive effect was as below:

TIMING	SKMES %	SK LU %	A 549 %
- 24h	104	116	17
- 1h	155	42	5
0	0	44	66
+ 24h	100	27	71

The clinical situation with 1 hour infusion of 5.10^{-7}M corresponds to a dose of c.a. 7 mg/m². A clinical study with low dose of FT -24 or -1 hour before each fraction of RT might be of value in combined therapy of NSCLC.

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RADIOSENSITIVITY TESTING OF HUMAN TUMOR PRIMARY CULTURES

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The inherent radiosensitivity of primary cultures directly obtained from 11 ovarian cancer and 6 malignant melanoma patients was determined by a soft-agar clonogenic assay. No correlation between tumor cell radiation response and clonogenic capacity was observed. Melanomas were generally more radioresistant than ovarian cancers, as demonstrated by the higher median surviving fraction at 2 Gy (0.47 vs 0.32). The induction and repair of radiation-induced DNA double-strand breaks (DSB) was determined by neutral filter elution. In individual tumors, inherent radiosensitivity was not related to initial DNA DSB frequency. In contrast, the surviving fraction at 2 Gy directly correlated with the rate of DNA DSB rejoining 2 h after irradiation. Moreover, melanomas were generally more efficient than ovarian cancers in repairing DNA damage (median percentage of DNA DSB repaired, 90% vs 59%). In accord with that previously reported for human tumor established cell lines, our results indicate the ability to repair DNA DSB as an important determinant for radiation response of human tumor cells growing as primary cultures.

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