KINETICS OF RADIATION-INDUCED APOPTOSIS IN NEONATAL UROGENITAL TISSUES WITH AND WITHOUT PROTEIN SYNTHESIS INHIBITION

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The difference in incidence of radiation-induced apoptosis between two neonatal urogenital tissues, kidney and testis, was analysed over a 24h period. In the rat, the two organs differ in their stage of development post-natally, with the testis approximating the adult form at birth, and the kidney remaining in a nephrogenic state until two weeks after birth. They have been shown previously to be radio-sensitive and -responsive, in specific cell populations in each organ - the nephrogenic zone in the kidney and the Sertoli cells in the testis. In the present study using 4-5 day old rats (N=4 for control or treatment sets), we have compared the kinetics of radiation-induced (5Gy of X-irradiation) apoptosis in the two tissues over a 24h period. Concurrent administration of cycloheximide (10mg/kg body weight, i.p.), a protein synthesis inhibitor, with radiation treatment was used to determine whether new protein synthesis had a role in induction of apoptosis in this *in vivo* model. Many chemotherapeutic drugs act via protein synthesis inhibition, and we believe that the results of this latter analysis may provide information for the planning of concurrent radio- and chemotherapy.

Apoptosis was quantified using morphological parameters, and verified by DNA gel electrophoresis for the typical banding pattern, and by electron microscopy. We studied the proliferative index in tissues, using [6-^{3}H]-thymidine uptake (1h prior to euthanasia and collection of tissues) and autoradiography as indicators of cell proliferation (S-phase). As well, by pulse-labelling 1h prior to radiation treatment, we able to analyse for S-phase cells undergoing apoptosis in another set of animals. Tissue was collected 2, 4, 6, 8, and 24h after radiation treatment. Expression of one of the apoptosis-associated genes, Bcl-2 (an apoptosis inhibitor/cell survival gene), was studied using immunohistochemistry. Apoptosis peaked at 4h in the testis and 6h in the kidney, emphasising the necessity of knowing tissue differences in radiation response if comparing changes at a particular time. A higher proportion (almost five fold) of the apoptotic cells died in S-phase in the kidney than the testis, over the 24h. Protein synthesis inhibition completely negated induction of apoptosis in both tissues. Necrosis was not identified at any time. In the control kidney, Bcl-2 expression was highest in the differentiated, non-nephrogenic zone, with expression increased in some cell populations of the nephrogenic zone after irradiation. In the testis, the Sertoli cells had high expression of Bcl-2 in control sections, with diminished, but not negated, levels after irradiation. Cycloheximide treatment greatly diminished Bcl-2 expression. The differences in response of the two tissues to irradiation relates to their innate cell (genetic) controls, which may be determined by their state of differentiation at time of treatment, or the tissue type. This *in vivo* study also suggests the model may be useful for analysis of other cancer therapies, for example polychemotherapies, or combinations of chemo-and radiotherapy.