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Radiation responses of the gastrocnemius muscle in the WAG/Rij rat

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'Radioresistant' tumours frequently give rise to problems in administering a sufficiently high cumulative dose of radiation which offers the largest probability of tumour cure and avoids unbearable early and/or late normal tissue complications. In order to fulfil especially the latter requirement, data on radiation 'tolerance' doses must be available, preferably for all the normal tissues expected ever to be exposed to ionizing radiations clinically. However, reports of data on radiation tolerance of skeletal muscular tissue in the literature are scarce. A reason for this lack of information may be the fact that it is common clinical experience, supported by earlier literature (Zeman & Solomon, 1971; Faes, 1973), that the skeletal muscle can sustain doses of 20 to 30 Gy of low LET X-rays without showing overt signs of impairment, whereas more recently reported data on late radiation effects in legs of rodents are still inconclusive as to the question whether indeed this type of tissue is relatively radioresistant (Utley *et al.*, 1981; Hunter & Milas, 1983; Stone, 1984). In contrast to this current view, results of experiments are presented, providing evidence of significant muscular wasting of the calf musculature in young rats which can already be observed after irradiation of their hind legs with single doses of only 15 Gy of 300 kV X-rays.

Materials and methods

Type of experiments

In order to obtain a first set of data on radiation induced muscular damage, which should serve as a baseline for a comprehensive investigation into the tolerance of skeletal muscular tissue, two groups of animals, comprising male WAG/Rij inbred rats differing in age, were given single doses of 300 kV X-rays. At the time of irradiation the rats of one group were 62 to 65 days old and those of the other 382 to 390 days. Animals of each group were randomly assigned to one of five subgroups for treatment with single doses of either 0, 15, 30, 45 or 60 Gy, each subgroup comprising five animals. Both the hind legs of each rat were irradiated from heel to knee while the remainder of its body was shielded* with lead. During irradiation the animals were anaesthetized with pento-barbital sodium.

Measurements

Animals were submitted to measurements, first twice weekly, beginning two weeks before, and up to about three months after irradiation and, subsequently, at intervals of one to two weeks until almost the end of their life span. During this period changes in the mass of the gastrocnemius muscle were assessed as well as reactions of the overlying skin. Assessment of the mass of the target muscle was performed by employing an indirect method, requiring measurements of the circumference of the calf at three predeterminated positions between heel and knee and of the tibia length. From these data the volume, V_{mc}, of the gastrocnemius muscle was calculated according to a simple geometric equation describing the shape of this muscle. Comparison of data for V_{mc} with those for wet-weight, W_{mw}, of the excised muscle was determined in a separate experiment for over 200 non-irradiated legs using animals varying in age from 21 to 210 days old. This showed a linear relationship between calculated and actual mass of the target muscle; i.e., $V_{mc} = 2.03 \times W_{mw} + 0.115\,\text{cm}^3$.

Reactions of the skin covering the leg between heel and knee were scored by a method in which two factors for scoring skin damage have been combined: one being a factor of quality for scoring the 'severity' of damage, i.e. the degree of expansion into the histological structures, and a second for scoring the percentage of skin surface showing damage relative to the total surface exposed to irradiation. This procedure allows for expressing the skin damage as a percentage of the total amount of tissue irradiated, i.e. comprising the entire structure of the epidermis.

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*The design of the radiation set-up was made in collaboration with Dr J. Zoetelief, who also performed the dosimetry. The average dose measured in the shielded body e.g., the sacrum and the central abdomen, ranged from 2% to 8%, respectively, of that measured in the target tissue.

At the end of the observation period the animals were euthanised in the course of a perfusion-fixation procedure which was performed in order to prepare tissue of the gastrocnemius muscle for examination by means of light microscopy, LM and electron microscopy, EM.

Finally, tissue was also dissected from the gastrocnemius muscle for biochemical determinations of its content of specific muscular proteins (Unsworth *et al.*, 1982).

Results and discussion

Figure 1 illustrates examples of radiation effects which developed in the gastrocnemius muscle and in the overlying skin after these tissues were irradiated in 62–65 day old rats. The open and closed circles represent measurements performed in ten legs per point and correspond to time course variations in muscle responses of hind legs irradiated with doses of 0 and 60 Gy, respectively. It is of interest to note that the increase in volume of non-irradiated muscles does not proceed smoothly. Three phases may be distinguished in the growth pattern of the calf musculature as reflected by the sequence of data on volume shown in Figure 1. During each phase the growth rate is initially fast, it slows down near the end of its phase and then it speeds up again at the transition to the next phase. However, the average growth rate of the muscular volume also slows down with passage through subsequent phases until the time when the animals have reached an age of ~220 days. Then the growth rate which has become quite slow continues slowing down smoothly. This pattern of volume increase seems to reflect oscillations in the net effect of influences on muscular growth exerted by stimulatory and inhibitory factors. A similar but more pronounced pattern of changes in dimensions is reflected by data on changes in tibia length (data not shown) which influence the results of calculations of V_{mc}. Changes observed in V_{mc} of muscles irradiated with different doses employed so far in young rats, all show a pattern qualitatively similar to that shown in Figure 1 for muscles irradiated with 60 Gy. The pattern depicted in Figure 1 may be characterised by four subsequent phases, *viz.*: (a) a 30 to 50 day period of increase in V_{mc} indistinguishable from the increase in mass of non-irradiated controls; (b) a 60 to 90 day period of unstable or absent increase in V_{mc}; (c) an 80 day period (or longer) of rapid decrease in V_{mc}; and (d) a final period of slow decrease in V_{mc} if any. In general, phases b and c will be longer for muscles irradiated with doses of <60 Gy. Values obtained for V_{mc} corresponding to phase b may be influenced by the development of subcutaneous oedema, which becomes more prominent with increasing dose. Accordingly, the time of onset of rapid muscular wasting, though clearly expressed during phase c, cannot be derived precisely from patterns like those shown in Figure 1. However, by comparing the patterns of radiation responses observed in muscle and skin as shown in Figure 1 the possibility might be considered that muscular wasting and late skin damage starts developing at about the same time; i.e., at ~40 days after treatment with a single dose of 60 Gy of X-rays. After the rapid wasting has ceased the average muscular volume does not seem to increase again in either of the dose groups studied. This suggests that in the irradiated muscle the full somatic damage is expressed only at a late stage. Comparison of the levels of wasting expressed during phase d observed in muscles irradiated with different radiation doses in the young animal suggested that a maximum degree of wasting may be attained with doses in excess of 30 to 45 Gy. More accurate data on dose-response relationships determined for late damage induced by irradiating muscles of young and old animals are presented in Figure 2, panels a and b, respectively. Interestingly, these data show that muscles of young rats suffer about twice as much damage when irradiated with doses of <45 Gy than do muscles of much older animals. At doses of 45 Gy and over the differences become smaller. Furthermore, since the data presented in Figure 2 specify radiation effects of the integral tissue in terms of (late) damage per unit tissue mass, it may be stated that in the rat the skeletal muscle seems to experience at least as much damage, if not more, from a given dose of X-rays as does the skin.

As for the mechanism of muscular damage, studies with LM and EM revealed that (i) significant amounts of collagen are observed in muscles irradiated with 45 and 60 Gy, and only little amounts in muscles irradiated with smaller doses; (ii) the Z-bands are wider and sometimes arranged in a fork-like fashion in muscles irradiated with 45 and 60 Gy, whereas this is not the case in muscles treated with smaller doses. Occasionally, satellite cells were observed to have entered mitosis, which supports the view that some degree of regeneration may take place in muscles which experience physical or genetical mediated damage (Wirtz *et al.*, 1982). Finally, it was observed that the content of muscle-specific proteins per mg protein, does not change significantly in the irradiated gastrocnemius muscle.

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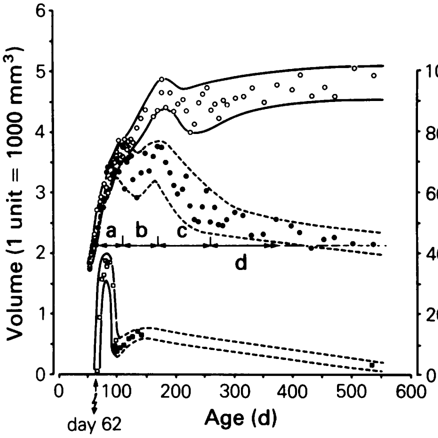

Figure 1 Comparison of time course variations in normal tissue damage observed after irradiation of the hind legs of 62–65 day old WAG/Rij rats with single doses of 300 kV X-rays. Left hand scale: ○—○ calculated muscle volume for non-irradiated controls; ●---● ibid for irradiated muscles. Right hand scale: □—□ acute skin reaction; ■---■ late skin reaction. Each symbol represents the mean value of calculations performed with data obtained from 10 legs. The distances between pairs of both solid and interrupted lines indicate the range of variations between subsequent measurements including the s.e. of the mean corresponding to each datum point shown.

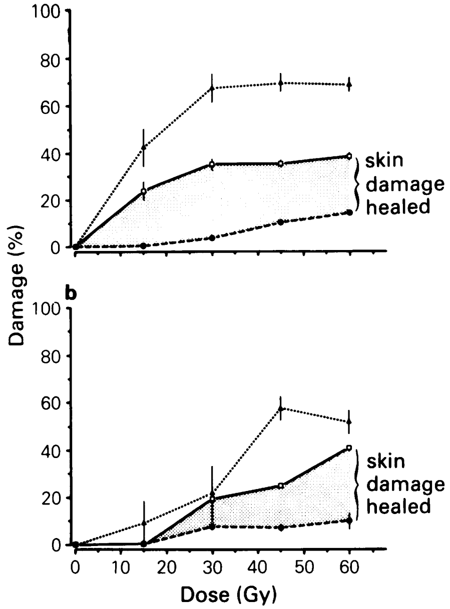

Figure 2 Relationships between dose and percent damage calculated for effects observed in normal tissues of the hind legs of rats irradiated with different doses of 300 kV X-rays. Panels a and b: results obtained with WAG/Rij rats submitted to irradiations when they were 62–65 and 382–390 days old, respectively. ▲---▲ damage calculated from data on wet-weight of muscular tissue excised from the animal; □—□ damage calculated from data on acute skin effects; ●--● damage calculated from data on late skin effects. Each symbol represents the mean of 10 sets of data used for the calculations. Vertical bars: s.e.m.

$$\text{percent damage} = \left(1 - \frac{\text{amount of tissue remaining intact}}{\text{amount of tissue irradiated}}\right) \times 100$$

References

FAES, M.H. (1973). Pathological aspects and treatment possibilities of striated muscle exposed to gamma radiation. *Thesis,* Catholic University Louvain: Belgium.

HUNTER, N. & MILAS, L. (1983). Protection by S-2-(3-aminopropylamino)-ethylphosphorothioic acid against radiation-induced leg contractures in mice. *Cancer Research,* **43,** 1630.

STONE, H.B. (1984). Leg contracture in mice: an assay of normal tissue response. *Int. J. Radiat. Biol. Phys.,* **10,** 1053.

UNSWORTH, B.R., WITZMANN, F.A. & FITTS, R.H. (1982). A comparison of rat myosin from fast and slow skeletal muscle and the effect of disuse. *J. Biol. Chem.,* **257,** 15129.

UTLEY, J.F., QUINN, C.A., WHITE, F.C., SEAVER, N.A. & BLOOR, C.M. (1981). Protection of normal tissue against late radiation injury by WR-2721. *Radiation Res.,* **85,** 408.

WIRTZ, P., LOERMANS, H. & RUTTEN, E. (1982). Effects of irradiation on regeneration of dystrophic mouse leg muscles. *Br. J. Exp. Pahtol.,* **63,** 671.

ZEMAN, W. & SOLOMON, M. (1971). Effects of radiation on striated muscle. In *Pathology of Irradiation,* Berdjis, Ch.C. (ed) Chapter 10. The Williams and Wilkins Cy: Baltimore.