A HISTOENZYMOLOGICAL STUDY OF THE PECTORAL MUSCLE FOLLOWING THORACIC X-IRRADIATION

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Histochemical changes in lactate dehydrogenase (LDH), α -glycero-phosphate dehydrogenase (α -GPDH), myofibrillar adenosine triphosphatase (ATPase), phosphorylase and glycogen were investigated in pectoral major muscle of guinea pig and albino mouse after 240 R thoracic X-irradiation. The enzymic activities of LDH, α -GPDH and ATPase showed marked reduction at 24, 48 and 72 hrs post-irradiation. Phosphorylase and glycogen, on the other hand, showed an increase in activity, but the damage was severe at 48 and 72 hrs as type I fibers also showed the presence of phosphorylase and glycogen. The phosphorylase reaction emerged from this study as the most sensitive histochemical parameter of radiation injury.

The significance of these alterations with reference to muscle glycolytic energy metabolism after thoracic X-irradiation is discussed.

Radiation-induced changes in the striated muscle have been well documented (16) but the pathogenesis of many of these changes still remains speculative and controversial. Zeman and Solomon (16) reported a spectrum of changes following relatively small doses of irradiation. Khan (8) demonstrated ultrastructural changes in rabbit pectoralis after 1300 r X-irradiation. He described muscle cell changes which were characterised by myofibrillolysis, myonecrosis followed by the appearance of muscle buds, increase in glycogen content of relatively intact fibers and myofiber atrophy and degeneration. These structural changes are directly related to altered enzymic activity and energy metabolism of muscle. No systematic histochemical study has been reported so far. We have studied the effects of thoracic X-irradiation on the histochemical distribution of some glycolytic enzymes in the pectoralis major muscle of albino mouse and guinea pig.

MATERIALS AND METHODS

Guinea pigs (*Cavia porcellus* L.), weighing 350–400 gm and albino mice (*Mus musculus* L.), weighing 25–30 gm were maintained in the laboratory on standard feeds (Hindustan Lever Ltd.). Control animals did not receive the treatment mentioned below.

X-irradiation conditions: The animals were X-irradiated in the thoracic region (ventral side facing the source) under light ether anaesthesia by Copper K alpha lines (Philips model). The skin target distance was 20 cm. The diameter of the

circular portion of the thorax exposed to X-irradiation was 1.78 cm and the total area exposed was 5.6 cm², at a voltage of 40 kV and 20 mA. The dosage employed was 24 R/s.

Experimental procedure: The guinea pigs and mice were exposed to 240 R thoracic X-irradiation. Radiation effects were studied at 24, 48 and 72 hrs after radiation exposure. The histochemical localization of lactate dehydrogenase (EC 1.1.1.27, LDH), α -glycerophosphate dehydrogenase (α -GPDH, EC 1.1.1.8), myofibrillar adenosine triphosphatase (ATPase, EC 3.6.1.3), phosphorylase (EC 2.4.1.1) and glycogen were investigated in control and X-irradiated animals.

5–10 μ m thick fresh frozen sections were cut and the following methods were used for the histochemical localization of these metabolites.

LDH: The method of Nachlas *et al.* (11). was followed.

α -GPDH: The enzyme was localised by the method of Nachlas *et al.* (11).

ATPase: The technique of Guth and Samaha (3) was used.

Phosphorylase: The method of Franko and Palkama (2) was used for histochemical localization.

Glycogen: Glycogen was stained by the PAS method (12).

RESULTS

Control animals:

LDH and α -GPDH: Type I fibers of the pectoralis major muscle were deeply stained showing high activity of LDH and α -CPDH, whereas type II fibers showed minimum enzyme activity. The intermediate fibers had medium levels of these enzymes. In guinea pig, these enzymes were localised towards the periphery of the fiber, leaving the core of the fiber almost unstained (Figs. 1, 5, 7, 11).

ATPase: The ATPase of type I fiber is acid-stable and base-labile and of type II fibers is base-stable and acid-labile. The intermediate fibers showed stability at both acidic and basic pH. The muscle fibers were characterized as moderate (M), dark (D) and light (L), on the basis of their staining intensity after the reactions for the alkali- and acid-stable ATPases (Fig. 13).

Phosphorylase and glycogen: Type II fibers showed high phosphorylase and glycogen activities, whereas type I fibers showed least activity in guinea pig and mouse muscle. The intermediate fibers had medium levels of these metabolites (Figs. 16, 19).

Effect of 240 R X-irradiation:

LDH: The distribution of LDH in guinea pig and mouse pectoralis showed a marked reduction in all three fiber types upto 72 hr post-irradiation. In mouse, type I fibers showed increased size of formazan granules at 24 hr after radiation (Figs. 2–5, 6).

α -CPDH: In guinea pig, this enzyme showed marked reduction upto 48 hr but almost normal distribution was observed at 72 hr (Figs. 8–10). In mouse, there was significant inhibition in the enzyme activity at all post-irradiation intervals (Fig. 12).

ATPase: Activities of alkali- and acid-stable ATPases also showed remarkable reduction in both the animals after irradiation except the blood capillaries which showed dark staining (Figs. 14–15).

Phosphorylase and glycogen: In guinea pig and mouse, at 24 hr the distribution of these metabolites was more or less the same as in the control. At 48 hr type I fibers also

showed the presence of these metabolites (Figs. 17, 20) in both animals. At 72 hr guinea pig muscle showed a seemingly normal pattern of phosphorylase and glycogen (Fig. 18). However, in mouse at 72 hr normal distribution was not regained (Fig. 21).

DISCUSSION

We previously reported (14) that X-irradiation induced marked changes in succinate dehydrogenase, a mitochondrial enzyme, both histochemically and biochemically. In the present study pectoral major muscle showed significant reduction in lactate dehydrogenase activity in all three fiber types in both guinea pig and mouse (Figs. 2-4, 6). The increased size of formazan granules in mouse muscle might be a compound result involving multiple factors like swelling of mitochondria, vacuole formation or fusion of mitochondria, altering normal energy metabolism of the mitochondria as well as muscle fiber. The reduction of LDH in muscle can not be easily explained but two mechanisms can be proposed: (i) The radiation-injury causes release of this enzyme from cell into blood stream. This implies that the loss of the LDH from muscle can be attributed to cellular destruction and to the increased permeability of damaged cells. Khan (8) demonstrated widespread ultrastructural changes in skeletal muscle mitochondria as well as its microvasculature and indicated that increased vascular permeability during earlier phases of injury occurs through increased pinocytotic transport across the endothelium after radiation. Hori *et al.* (5) reported that the radiation-induced elevation of plasma LDH level may be attributed mainly to the release of the enzymes from cells. (ii) Reduction in LDH activity might be due to the fact that X-irradiation inhibits NAD synthesis or activates its breakdown, as LDH is an NAD-dependent enzyme (13).

FIGS. 1-4. T.S. of pectoral major muscle of guinea pig showing localization of lactate dehydrogenase. Fig. 1. Normal guinea pig muscle.

FIGS. 2-4. Guinea pig muscle at 24, 48 and 72 hr after radiation, respectively. $\times 175$

FIGS. 5-6. T.S. of mouse muscle showing lactate dehydrogenase activity in control and at 24 hr after 240 R X-irradiation, respectively. $\times 175$ I-Type I fiber; II-Type II fiber and In-Intermediate fiber.

FIGS. 7-10. T.S. of pectoral major muscle of normal and X-irradiated guinea pig muscle showing localization of α -glycerophosphate dehydrogenase activity.

Fig. 7. Control guinea pig muscle.

FIGS. 8-10. Guinea pig muscle at 24, 48 and 72 hr after radiation, respectively. $\times 175$

FIGS. 11-12. T.S. of mouse muscle showing α -glycerophosphate dehydrogenase activity in normal and at 48 hr after radiation, respectively. $\times 175$

FIGS. 13-15. T.S. of pectoral major muscle stained for acid-stable ATPase in normal and X-irradiated guinea pig.

Fig. 13. Control guinea pig muscle.

FIGS. 14-15. At 24 and 48 hr after 240 R radiation. $\times 440$, D Dark, M Moderate and L Light.

FIGS. 16-18. T.S. of pectoral muscle of guinea pig showing phosphorylase activity.

Fig. 18. Control guinea pig muscle. $\times 175$

FIGS. 17-18. Guinea pig muscle at 48 and 72 hr after 240 R X-irradiation, respectively. $\times 440$

FIGS. 19-21. T.S. of mouse muscle stained for phosphorylase activity.

Fig. 19. Control mouse muscle.

FIGS. 20-21. Mouse muscle at 48 and 72 hr after radiation, respectively. $\times 440$

The reduced activity of α -GPDH after radiation in guinea pig and mouse muscle could reflect a slower rate of glycolysis due to more effective competition of oxidative phosphorylation for Pi and ADP, resulting in lower concentration of these compounds, and/or from a shift in the carbon source for citric acid cycle from carbohydrate (4).

ATPase activity also showed marked reduction in guinea pig and mouse muscle at all post radiation intervals studied. The reduced enzymic activity might be due to decreased ATP concentration or reduced rate of glycolysis (7, 9). After radiation blood capillaries showed dark staining which might be due to the increased levels of serum ATPase (1).

Radiation-induced glycogen accumulation is generally due to the inhibition of glycolysis without concomitant change in glycogenesis (10). The increase in phosphorylase in our experiments could reflect an attempt by the cells to deal with the accumulated glycogen. Furthermore, it is probable that the pathologically accumulated glycogen particles are of quite different molecular weight from those naturally occurring in muscle and are therefore not quite as sensitive to the action of phosphorylase as normal glycogen (6, 15). Therefore, if the newly formed glycogen is of a high molecular weight it probably would bind more phosphorylase, especially the active form, and more firmly.

Finally it should be stressed that alterations in phosphorylase activity, in contrast to all other parameters, stand out, at the light microscopic level, as the most sensitive indicator of radiation damage.

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REFERENCES

1. Albaum, G. H.: Serum enzymes following whole-body radiation in the rabbit. *Radiat. Res.* 12; 186, 1960.
2. Eranko, O. and Palkama, A.: Improved localization of phosphorylase by the use of polyvinyl pyrrolidone and high substrate concentration. *J. Histochem. Cytochem.* 9; 585, 1961.
3. Guth, L. and Smaha, F. J.: Procedure for the histochemical demonstration of actomyosin ATPase. *Exptl. Neurol.* 28; 365, 1970.
4. Holloszy, J. O. and Oscai, L. B.: Effect of exercise on α -glycerophosphate dehydrogenase activity in skeletal muscle. *Arch. Biochem. Biophys.* 130; 653, 1969.
5. Hori, Y., Takamori, Y. and Nishio, K.: The effect of X-irradiation on the lactate dehydrogenase level in plasma and in various organs of mice. *Radiat. Res.* 34; 411, 1968.
6. Ibrahim, M. Z. M., Atlan, H., Miquel, J. and Castellani, P.: Synthetic and hydrolytic enzymes of glycogen in the normal and irradiated rat brain. *Radiat. Res.* 43; 341, 1970.
7. Kay, R. E. and Chan, H.: Effect of X-irradiation on glucose metabolism in rat cerebral cortex slices. *J. Neurochem.* 14; 401, 1967.
8. Khan, M. Y.: Radiation-induced changes in skeletal muscle. An electron microscopic study. *J. Neuropath. Exptl. Neurol.* 33; 42, 1974.
9. Maass, H. and Schubert, G.: Early biochemical reactions after X-irradiation. In Proceedings of the second U.N. International Conference on the Peaceful uses of Atomic Energy, Vol. 22,

- p. 449, United Nations, Geneva, 1958.
10. Miquel, J., Lundgren, P. R., and Jenkins, J. C.: Effect of roentgen radiation on glycogen metabolism of the rat brain. *Acta Radiol.* 5; 123, 1966.
 11. Nachlas, M. M., Walker, D. G. and Seligman, A. M.: A histochemical method for the demonstration of diphosphopyridine nucleotide. *J. Biochem. Biophys. Cytol.* 4; 29; 1958a.
 12. Pearse, A. G. E.: Histochemistry, Theoretical and Applied. J. A. Churchill Ltd., London, 1968.
 13. Schneider, M., O'Bourke, F. and Redetzki, H. M.: As cited by Thyagarajan, P., Vakil, U.K. and Sreenivasan, A.: Glycolytic changes in X-irradiated rats. *Ind. J. Biochem. Biophys.* 14; 56, 1977.
 14. Shah, V. C., Bhatavdekar, J. M. and K. Aravinda Babu: Effects of low dose X-irradiation on the succinate dehydrogenase activity of guinea pig, rat and mouse tissues. *Strahlentherapie* 152; 83, 1976.
 15. Tata, J. R.: Subcellular redistribution of liver α -glucan phosphorylase during alterations in glycogen content. *Biochem. J.* 90; 284, 1964.
 16. Zeman, W. and Solomon, W.: Effects of radiation on striated muscle. In Pathology of Irradiation, ed. by Berdjis, C. C., Williams and Wilkinson, Baltimore, 1971, p 171.