STIMULATION OF POST-TRAUMATIC REGENERATION OF SKELETAL MUSCLES OF OLD RATS AFTER X-RAY IRRADIATION

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Stimulation of the regenerative capacity of tissues when depressed by ionizing radiation is of great importance in the treatment of radiation trauma. One method of restoring regeneration processes is by grafting a small quantity of minced, unirradiated muscle tissue, taken from the same animals, in the irradiated part of the body [6]. At the same time, exposure to a helium-neon laser (HNL) beam has been shown to have a restorative action on regeneration in irradiated muscles [9, 10]. These results have been obtained on young, sexually mature rats.

The aim of this investigation was to seek a method of stimulating restorative processes in irradiated muscles of old animals. It was shown previously that local x-ray irradiation in large doses impairs the ability of muscles to undergo post-traumatic regeneration by a greater degree in old animals than in young [8].

EXPERIMENTAL METHOD

Experiments were carried out on 40 noninbred albino rats aged 2-2.5 years. There were three series of experiments. I) complete transverse section of the gastrocnemius muscle after local x-ray irradiation in a dose of 20 Gy (conditions of irradiation: RUP-200 apparatus, voltage 190 kV, current 15 mA, dose rate 0.75 Gy/min, filters 0.5 mm Cu + 1 mm Al); II) laser therapy of the transversly divided gastrocnemius muscle in the same dose (conditions of laser treatment: OKG-12 apparatus, wavelength 632.8 nm, power flux density 2.5-3 mW/cm^2). In the course of 2 weeks, 8 or 9 sessions of laser therapy were given. It was shown previously that laser therapy of the cornea after x-ray irradiation is more effective under pulsed than continuous conditions [1], and for that reason pulsed laser irradiation was used in this investigation. The total exposure per session was 3 min (60 times, 3 sec each time, with intervals of 7 sec); III) grafting of a small quantity of minced muscle tissue from the left, unirradiated limb, into the region of the defect in the divided gastrocnemius muscle of the right limb of the same animal, irradiated in a dose of 20 Gy. The regenerating muscles were studied 14 and 30 days after trauma. Histological sections were stained with Regaud's hematoxylin. From each regenerating muscle 5 sections were cut at different levels and the relative percentages of muscle and connective tissue and of fibrin were determined in them, as reflected in the area occupied by them on the section.

EXPERIMENTAL RESULTS

Post-traumatic regeneration of the gastrocnemius muscle of old rats is illustrated schematically in Fig. 1. In the experiments of series I erythema and exudative edema of the skin of the limb, slow healing of the edges of the skin wound, and the frequent formation of deep cutaneomuscular ulcers were observed after irradiation and transverse division of the muscles. The hair did not grow. The muscle stops were poorly united 14 days after trauma. Fibrin was present in the region of injury. The inflammatory response was sluggish in its course. Phagocytic activity of the macrophages was depressed. Few fibroblasts and collagen fibers were present in the newly formed connective tissue. Many muscle fibers showed vacuolar degeneration. There were few muscle nuclei, which varied in size, and were arranged singly or in

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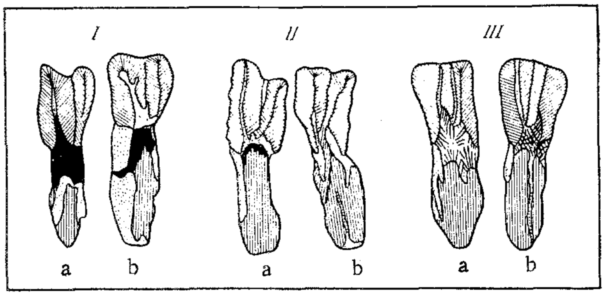

Fig. 1

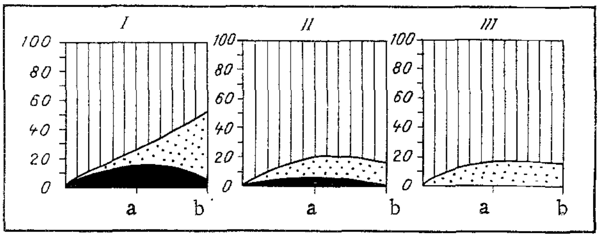

Fig. 2

Fig. 1. Scheme of regeneration of gastrocnemius muscle of old rats after transverse section. I) transverse section of gastrocnemius muscle after irradiation in a dose of 20 Gy; II) Laser therapy of divided muscle, irradiated in a dose of 20 Gy; III) Grafting of minced, unirradiated muscle into region of defect in divided muscle irradiated in a dose of 20 Gy. a) 14 days, b) 30 days. Shaded area – muscle tissue, dotted – connective tissue, black – fibrin.

Fig. 2. Ratio between quantities of muscle and connective tissue and fibrin in regenerating gastrocnemius muscle of old rats after transverse section. Ordinate, quantity of tissues (reflected in area occupied by them on the section, in percent; total area of regenerating muscle taken as 100%). Remainder of legend as to Fig. 1.

groups of 2 to 4. The quantity of fibrin in the region of the defect was reduced 30 days after trauma. With removal of the breakdown products regions of loose and dense fibrous connective tissue, containing few cells, were formed. Many muscle fibers contained vacuoles. A concentration of polymorphic nuclei (up to 6-8) could be seen in single muscle fibers. Muscle fibers in the distal stump were reduced in thickness or dead, and replaced by adipose tissue and coarse fibrous connective tissue

In the experiments of series II the process of healing of the wound in the irradiated limb was stimulated by laser therapy. During the first week after the operation erythema of the skin and scab formation were observed; the scab subsequently separated and thin but coarse hair grew in its place. Cutaneomuscular ulcers formed extremely infrequently. Both muscle stumps were firmly united by regenerating bands 14 days after the operation. Resorption of fibrin and necrotic masses took place rapidly. Blood vessels grew actively into the region. Granulation tissue formed and mitoses appeared. Vacuoles still remained at the ends of some divided muscle fibers. However, in many fibers an accumulation of regeneration nuclei could be clearly seen, and in many of them there were large nucleoli. Muscle buds, myosyncytia, and muscle tubes, containing small chains of pale, round nuclei, grew out of the muscle fibers. The defect one month after trauma was filled with loose and fibrous connective tissue, in which mitoses were seen. Young muscles growing out from both stumps joined the stumps together in places, although in the region of the wound dense connective tissue was still present and prevented the growth of muscle fibers. Macrophages with lipofuscin were present among the young muscle fibers.

In the experiments of series III on the first day after injury erythema of the skin and moistening of the suture were observed, but no deep cutaneomuscular ulcers were formed. The two muscle stumps quickly joined together. After 14 days no fibrin was present in the region of trauma. The defect was filled with young granulation tissue. Mitoses were frequent. In the proximal stump there were far fewer muscle fibers with vacuoles than in the animals in the experiments of series I. The newly formed muscle tissue consisted of myosyncytia, muscle tubes, and young muscle fibers. In the central part of the zone of injury they were formed from the grafted regenerating minced muscle tissue. At the boundary between the proximal and distal stumps, and also in the septa of the gastrocnemius muscle, they grew out of the previously irradiated muscle fibers. After one month wide and narrow muscle fibers interwove in the region of trauma. The connective tissue showed considerable recovery and created a firm basis for growth of the regenerating muscle fibers.

Analysis of the relative quantities of the tissues in the regenerating muscles (Fig. 2) showed that in the animals in the experiments of series II and III fibrin was more rapidly absorbed and functional muscle tissue was formed in a larger quantity (85.6 ± 2.7 and 85.0 ± 1.2%, respectively) than in the animals in the experiments of series I (48.8 ± 4.2%).

The experimental data thus showed that pulsed laser therapy or grafting of minced unirradiated muscle tissue can largely restore the regenerative capacity of the gastrocnemius muscle of old rats when depressed by x-ray irradiation; the method of grafting minced unirradiated muscle tissue, however, was more effective. In that case, in particular, necrotic masses were more quickly resorbed, thus contributing to the development of granulation tissue. In an attempt to explain the mechanism of action of these stimulating factors on regeneration of the irradiated muscles it can be postulated that postradiation regeneration of the muscle is stimulated, and this requires intensification of energy and protein metabolism [2, 12]. After exposure to HNL radiation, DNA, RNA, and ATP synthesis in the tissues is intensified [3, 4, 11]. Meanwhile, it has been shown that antioxidants and other biologically active metabolites, capable of regulating metabolism and the reparative activity of irradiated tissues, accumulate in regenerating unirradiated minced muscle tissue [5, 7].

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