EFFECT OF AUTOGRAFTING OF MINCED MUSCLE TISSUE FOLLOWED BY LASER THERAPY ON REGENERATION OF IRRADIATED TRAUMATIZED MUSCLE

V. S. Azarova, M. F. Popova, and Sh. G. Il'yasova

UDC 616.74-018-001.29-02:615.849.19]-07

KEY WORDS: regeneration; autografting; helium–neon laser; x-ray irradiation; skeletal muscle.

The action of a low-energy helium–neon laser, used under continuous and pulsed regimes, may lead to restoration of regeneration in an irradiated and transversely divided gastrocnemius muscle both in young, mature rats and also in old rats [2, 8]. In this case laser therapy has the same stimulating action on an irradiated traumatized muscle as grafting regenerating muscle tissue taken from the same animal, from an unirradiated part of the body, into the region of injury of an irradiated muscle [4-6, 9, 10]. The beneficial effect of continuous and of pulsed low-intensity laser radiation on the transplantation activity of irradiated (10 Gy) rat skeletal muscle also has been observed [3, 7, 10]. An increase in the dose of local x-ray irradiation of an animal's limb leads to more profound disturbances of structural, metabolic, and regulatory processes in the regenerating muscle, and the use of the method of grafting minced muscle tissue into the defect of an irradiated skeletal muscle does not always give the desired results, especially in the late period after irradiation and operation [1]. The study of the conditions leading to more complete regeneration of a traumatized skeletal muscle, irradiated with a high dose, and also of the skin of the limb which has undergone surgery.

The aim of this investigation was a morphological study of regeneration in an irradiated skeletal muscle exposed to the combined action of grafting a defect with minced, unirradiated muscle tissue, in conjunction with laser therapy in the postoperative period.

EXPERIMENTAL METHOD

The experiments were carried out on 65 noninbred male albino rats weighing 150-170 g. There were five series of experiments. Rats irradiated in a dose of 30 Gy, and with their gastrocnemius muscles divided transversely (series I) served as the control. In experimental series II and IV, after irradiated of the rats' right hind limbs in doses of 30 (series II) and 40 Gy (series IV), their gastrocnemius muscles were completely divided and muscle tissue obtained from the left, unirradiated gastrocnemius muscle, minced with scissors, was transplanted into the defect thus formed. The conditions of x-ray irradiation were: RUP-200 apparatus, voltage 190 kV, current 15 mA, dose rate 0.67 Gy/min; filters: 0.75 mm Al + 0.5 mm Cu. In series III and V, after irradiation of the limb in doses of 30 and 40 Gy, respectively, and after the operation described above, laser therapy was applied to the lower limb on the side of operation, from the dorsal aspect, under the following conditions: OKG-12 laser, dose rate 1-3 mW/cm^2, beam defocused to 3 cm in diameter by means of a lens, duration of session 1-3 min, number of sessions from 5 to 10. The regenerating tissues of the gastrocnemius muscles were studied 2 weeks and 1 and 2 months after irradiation and the operation. Histologic sections were stained with Regaud's hematoxylin and counterstained by Mallory's method; the regeneration process was studied in different parts of the muscle, and a morphometric analysis was made of the relative percentages of muscle, connective, and adipose tissues and fibrin; the areas occupied by these tissues in sections of the gastrocnemius muscles also was determined. The state of the skin of the lower limb and of the fur was monitored by means of an ISM-50A scanning electron microscope.

Evolutionary Histology Group, A. N. Severtsov Institute of Evolutionary Morphology and Ecology of Animals, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 9, pp. 313-316, September, 1990. Original article submitted November 30, 1988.

0007-4888/90/0009-1271$12.50

Fig. 1. Series I, control. Vacuolar degeneration of damaged ends of irradiated muscle fibers 2 weeks after irradiation (30 Gy) and operation. Here and in Figs. 2 and 3, Regaud's hematoxylin with counterstaining by Mallory's method. 100×.

EXPERIMENTAL RESULTS

In animals of the control series the skin on the dorsal aspect of the lower limb was thin and wet, the edges of the wound were slowly knitting together, to form deep, indolent skin and muscle ulcers (in 72% of the animals). Phagocytic activity of macrophages was suppressed in the irradiated divided gastrocnemius muscle, and unresorbed fibrin was deposited in the region of trauma, and interfered with the timely development of granulation tissue; a zone of coarse fibrous connective tissue was formed around the edges of the defect. The ends of the irradiated traumatized muscle fibers did not proliferate but developed vacuoles (Fig. 1), and underwent necrosis for a considerable distance. After 1 month the regenerating tissues consisted of muscle and connective-tissue organs with a narrow constriction band in the region of trauma and an atrophied distal part, which after 2 months was replaced by dense connective tissue with regions of fatty degeneration.

After transplantation of muscle tissue, minced into small fragments, into the region of division of the muscle, irradiated in a dose of 30 Gy (series II), ulcers affecting skin and muscle were formed on the lower limb in 25% of cases. On the ends of the divided fibers 2 weeks after irradiation and the operation there were few if any vacuoles, concentrations of muscle nuclei were observed, together with the formation of muscle buds, some of which were elongated and growing into the defect in the form of myosyncytia. In the zone of regeneration, part of the transplanted muscle was undergoing necrosis and disintegration, but most of the fragments were giving rise to myosyncytia, growing singly among the fibroblasts or in small groups. Some areas were packed with dense connective tissue. After 1 month the ends of many of the muscle fibers of the stomachs were firmly "welded" into the fibrous connective tissue. At the site of trauma there were many myosyncytia, as well as young muscle fibers growing in various directions. After 2 months the process of regeneration of the muscle was already virtually complete. In the zone of the defect, bundles of muscle fibers and areas of dense connective tissue were closely intertwined, with predominance of the latter, greatly reducing the quality of the regenerating tissues and leading to partial atrophy of the distal stump.

Fig. 2. Series III. Muscle buds on ends of irradiated traumatized muscle fibers of proximal stump 2 weeks after irradiation (30 Gy), operation, and five sessions of laser therapy. 280×.

With a combination of the method of grafting muscle fragments into the defect of the gastrocnemius muscle, irradiated in a dose of 30 Gy, and subsequent laser therapy to the limb, in the postoperative period (series III) suppuration of the suture tracks was observed with the formation of skin ulcers in 13% of cases, and the fur was restored more rapidly on the lower limb. Regenerative processes developed in the gastrocnemius muscle in all parts after 2 weeks (five sessions of laser therapy): in the defect against the background of an active inflammatory reaction myoblasts could be distinguished from the muscle fragments, and on their fusion with each other myosyncytia and young muscle fibers were formed; proliferation was marked on the traumatized ends of the irradiated muscle fibers and muscle buds with numerous nuclei were formed (Fig. 2). There were many myosyncytia in the connective-tissue septa of the proximal stump. After 1 month (10 sessions of laser therapy) almost the whole defect was filled with muscle tissue at various stages of differentiation: myosyncytia formed from muscle fragments, with many nuclei, young muscle fibers, and also regenerating fibers from the stumps. At the ends of some of them, the formation of muscle buds and myosyncytia continued. After 2 months mainly bundles of muscle fibers growing in various directions were seen in the zone of the defect. Often muscle bands connected to the stumps, thus enabling the distal part to preserve its muscular type of structure.

Irradiation of the limb before the operation in a dose of 40 Gy led as a rule to severe aftereffects for the traumatized muscle and skin of the lower limb. Starting with the 2nd week the epithelium was desquamated, the skin was wet, the hair dropped out, and the edges of the suture did not knit together for a long time. In 48% of the animals deep ulcers were subsequently formed. In the gastrocnemius muscle 2 weeks after irradiation (40 Gy) and transverse division with transplantation of minced muscle tissue (series IV) the fibers of the stumps were strongly vacuolated, hemorrhages and much fibrin were present in the defect. Much of the autograft had undergone necrosis, but in a narrow band close to the stump, myogenic cells were undergoing separation from fragments of grafted muscle. Connective tissue differentiated quickly, and after 1 month a dense connective-tissue scar was formed in the defect, in which the ends of the divided muscle fibers were apposed. By 2 months there was almost complete replacement of the distal stump by coarse connective tissue with large areas of fatty degeneration.

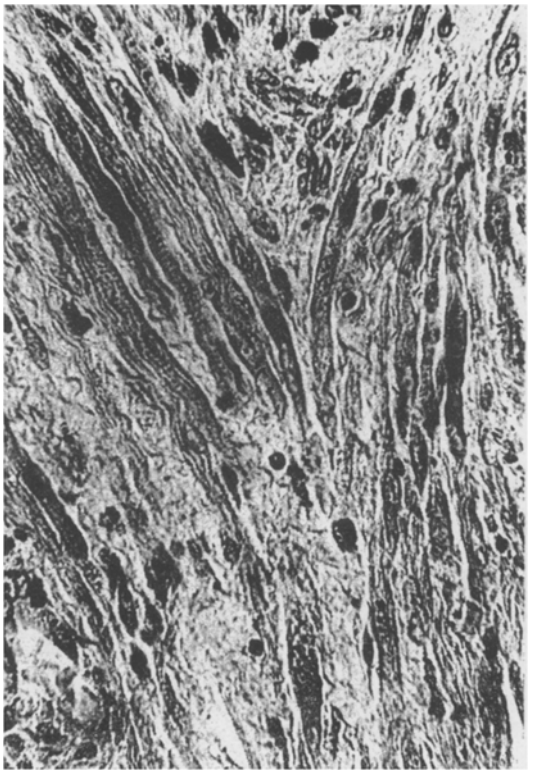

Fig. 3. Series B. Myosyncytia and young muscle fibers formed from underlying muscle fragments in defect of gastrocnemius muscle 2 weeks after irradiation (40 Gy).

TABLE 1. Comparative Quantities of Muscle Tissue in Regenerating Muscles (in % by area occupied by muscle tissue in projections of sections)

Time	I	II	III	IV	V
2 weeks	44,4±1,6	67,9±1,5	68,2±1,5	60,7±1,2	61,3±1,8
1 month	39,1±1,5	71,6±1,2	77,7±1,0	57,4±1,5	67,9±1,3

Exposure of the irradiated (40 Gy) and traumatized limb to the action of a helium–neon laser (series V) greatly alleviated the aftereffects of radiation trauma. Ulcers were formed in 35% of animals. In the muscle in the early stages some autografted fragments were undergoing restructuring (Fig. 3), and even after 1 month, mitotically dividing cells could be observed in the defect, i.e., proliferation of the tissues was continuing. Besides differentiated muscle fibers, myosyncytia also were found. The number of myogenic elements diminished in the direction of the distal stump. Large areas of the defect were occupied by dense connective tissue with diffuse scatterings of fat. After 2 months, as a result of laser therapy, the fur of some of the animals began to be restored on the lower limb, but in the zone of trauma to the muscle and in its distal part processes of connective-tissue and fatty degeneration were still continuing, although sometimes a pattern of myogenesis could be observed.

The morphometric analysis showed a statistically significant difference ($p \leq 0.01$) in the content of muscle tissue in the regenerating muscles of all series 1 month after irradiation and the operation (Table 1).

Thus, a combination of the method of stimulation of the regenerative capacity of a muscle, when depressed by irradiation, suggested by the writers previously, involving the use of autografting of unirradiated minced muscle tissue with laser therapy of the injured lower limb, gives an appreciable improvement of the results of regeneration, especially in the case of irradiation of the limb in a dose of 30 Gy, as shown on the diagrams of projections of sections through the regenerating muscles (Fig. 4). Preservation of the integrity of the skin helps to promote regeneration in the gastrocnemius muscle.

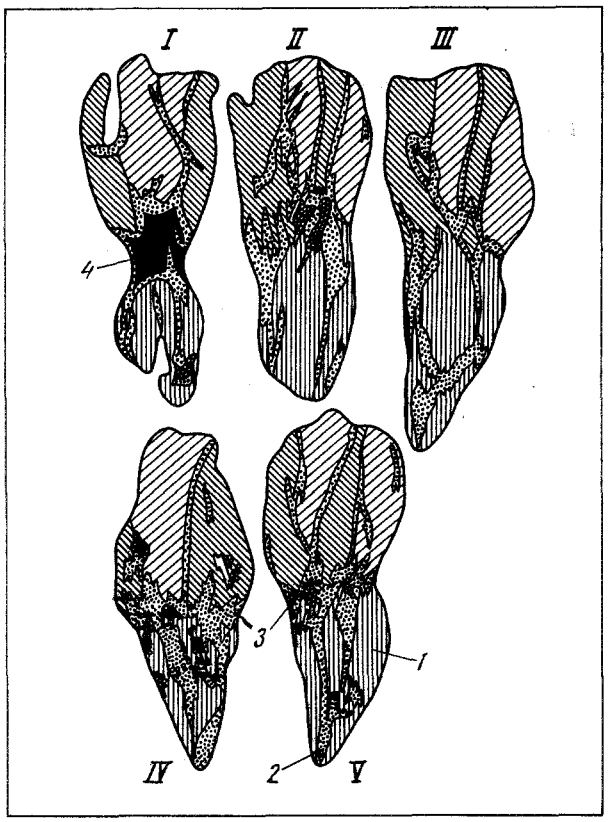

Fig. 4. Series I-V. Diagram showing relations between muscle (1), connective (2), and adipose (3) tissues and fibrin (4) on projections of sections through regenerating muscles after 1 month.

LITERATURE CITED

1. C. S. Azarova, Dokl. Akad. Nauk SSSR, **298**, No. 2, 470 (1988).
2. N. V. Bulyakova and M. F. Popova, Byull. Éksp. Biol. Med., No. 4, 481 (1987).
3. Sh. G. Il'yasova and M. F. Popova, Byull. Éksp. Biol. Med., No. 2, 222 (1980).
4. M. F. Popova, Regulatory Mechanisms of Regeneration [in Russian], Moscow (1973), pp. 216-225.
5. M. F. Popova and V. S. Azarova, Arkh. Anat., **68**, No. 1, 92 (1975).
6. M. F. Popova, "Plastic defensive and restorative state of regenerating tissue," Doctoral Dissertation, Biological Sciences, Moscow (1976).
7. M. F. Popova and Sh. G. Il'yasova, Byull. Éksp. Biol. Med., No. 7, 64 (1978).
8. M. F. Popova, N. V. Bulyakova, and V. S. Azarova, Radiobiologiya, No. 1, 50 (1983).
9. M. F. Popova, Radiosensitivity and Stimulating Properties of Regenerating Mamallian Tissues [in Russian], Moscow (1984).
10. M. F. Popova, Sh. G. Il'yasova, and V. M. Inyushin, Dokl. Akad. Nauk SSSR, **296**, No. 5, 1248 (1987).
11. A. N. Studitskii and M. F. Popova, Dokl. Akad. Nauk SSSR, **145**, No. 1, 198 (1962).