6. P. P. Rumjantsev (P. P. Rumyantsev), Int. Rev. Cytol., 51, 187 (1978).
7. P. P. Rumyantsev and V. O. Mirakyan, Tsitologiya, No. 10, 1276 (1968).
8. D. S. Sarkisov, Arkh. Anat., No. 10, 3 (1963).
9. I. V. Uryvaeva and M. A. Lange, Ontogenez, 2, 26 (1971).
10. W. Grabner and P. Pfitzer, Arch. path. Anat. Abt. B, Zellpath., 15, 279 (1974).
11. A. Katzberg, B. Farmer, and R. Harris, Am. J. Anat., 149, 489 (1977).
12. O. Klinge, Fortschr. Med., 88, 101 (1970).
13. C. Nadal and F. Zajdela, Exp. Cell Res., 42, 99 (1966).
14. P. Pfitzer, H. J. Knieriem, and H. Schulte, J. Med. Primatol., 6, 349 (1977).
15. R. Zak, Circulat. Res., 35, Suppl. 2, 17 (1974).

EFFECT OF THE HELIUM-NEON LASER BEAM ON POSTRADIATION REPAIR IN SKELETAL MUSCLE TISSUES

Sh. G. Il'yasova and M. F. Popova

UDC 616.74-001.29-085.849.19

Three series of experiments were carried out on the rat gastrocnemius muscle. Whole muscles were autografted in series I. Both hind limbs of the animals were irradiated in a dose of 1000 R before autografting of the muscles in series II. In series III x-ray irradiation in the same dose was followed by exposure of the hind limbs for 10 days to the action of a helium-neon laser, after which the muscles were autografted. The process of transplantation regeneration was investigated histologically 2 weeks and 1 and 2 months later. Exposure to the laser beam was shown to stimulate repair in skeletal muscle tissues and to normalize the process of post-traumatic regeneration when depressed by x-rays.

KEY WORDS: transplantation regeneration; x-ray irradiation; postradiation repair; helium–neon laser.

Ionizing radiation in a dose of 1000-2000 R has been shown to depress the ability of skeletal muscles to undergo posttraumatic regeneration for a long time [1, 2, 5, 7, 8]. The writers showed previously that the action of light from a helium-neon laser on a limb previously irradiated (2-3 h before transplantation) with x-rays can restore much of the ability of the gastrocnemius muscle to undergo transplantation regeneration, terminating with the formation of a contractile organ composed of muscle and connective tissue [6]. The mechanism of the stimulating action of red laser radiation on processes of regeneration of irradiated tissues is not yet clear: does recovery of the muscle from the structural changes taking place after transplantation take place purely on account of the more rapid elimination of muscle cells most severely damaged by x-rays, or is intracellular repair from radiation injury stimulated, i.e., does postradiation regeneration affect all the tissues composing the muscle? The investigation described below was carried out to shed light on this basic question.

EXPERIMENTAL METHOD

The method chosen to study this problem was one of those widely adopted in radiobiology to study intracellular repair after exposure to ionizing radiation. Essentially, radiation injury, revealed by inhibition of mitotic activity, chromosomal aberrations, depression of regenerative power, and by other indices, is recorded immediately after irradiation and also when a certain time interval has elapsed, during which the consequences of radiation injury have cleared up. According to the writers' own observations and also to data in the literature, radiation injury to skeletal muscles, reflected as a disturbance of posttraumatic regeneration, is very

Laboratory of Evolutionary Histology, A. N. Severtsev 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. 89, No. 2, pp. 222-224, February, 1980. Original article submitted June 24, 1979.

 0007-4888/80/8902-0212$07.50

conservative and persists for many months. If, as a result of certain stimulating actions, the consequences of radiation trauma could be eliminated, the muscle must reacquire ability to regenerate. As the therapeutic agent, the cold light of a helium–neon laser was chosen.

Experiments were carried out on noninbred male albino rats aged 2-3 months. In three series of experiments the gastrocnemius muscles were autografted in both hind limbs. In the experiments of series I (control) the autografted muscles were not subjected to any additional treatment, but in series II both of the animal's hind limbs were irradiated before autografting of the muscles with x-rays in a dose of 1000 R (conditions of irradiation: RUP-200 apparatus, tube voltage 190 kV, current 15 mA, dose rate 67 R/min, filters: Al 0.75 mm and Cu 0.5 mm). In the experiments of series III, the rat's limbs irradiated in the same dose were treated for 10 days by exposure to laser radiation (LG-75 apparatus, power 30 mV) before autografting of the gastrocnemius muscles. Hence, unlike in the previous investigation, the two procedures to which the muscle was subjected – transplantation and laser irradiation – were separated in time, and transplantation regeneration took place after and not during application of the laser. Regeneration of the muscles was investigated histologically after 2 weeks and 1 and 2 months, and five or six animals were used each time. Before the animal was killed contractions of the grafts in response to indirect stimulation by an intermittent direct current with a frequency of 60 pulses/min and a voltage of 2 V, were recorded on a kymograph. The regenerating muscles were fixed in Carnoy's solution or 12% neutral formalin. To reveal the network of blood vessels, before fixation the animal was perfused through the heart with a solution of gelatin with ink. Histological sections through the muscles were stained with Regaud's hematoxylin and counterstained by Mallory's method, or impregnated with silver by the Bielschowsky–Gros method followed by gilding by Lavrent'ev's method.

EXPERIMENTAL RESULTS

The results of the control series of experiments showed that the muscle tissue in the center of the graft had not yet undergone structural changes after 2 weeks. In the direction toward the periphery the process of regeneration of the muscles took place more actively. Necrotic areas of muscle fibers were resorbed, and myoblasts building muscle tubes were liberated. The wide peripheral zone of the graft contained many muscle syncytia and also young muscle fibers, lying in loose connective tissue. After 1 month, as a result of development of regeneration, large areas of differentiated muscle tissue were formed in the graft. In other unsuccessful cases of transplantation, when intensive septic inflammation developed, dense connective tissue was formed at the site of the graft.

Preliminary irradiation of the animal's limb in a dose of 1000 R almost completely suppressed the transplantation activity of the muscles. Results obtained 2 weeks after autografting showed that reconstruction of the graft took place very slowly, mainly in the proximal and distal ends of the muscle, in the regions of its excision and attachment. Most muscle fibers had undergone necrosis and replacement by connective tissue. A few muscle syncytia and single muscle tubes were visible in the peripheral zone. Towards the end of the first month of regeneration the grafts consisted chiefly of connective tissue, but here and there at their periphery there were thin, newly formed muscle fibers, whose nuclei occupied a central, axial position. After 2 months the grafts consisted of connective-tissue formations without contractile activity. In the experiments of series III, in which the irradiated limbs were exposed to the action of laser light before grafting of the muscles, the process of transplantation regeneration in most grafts was hardly distinguishable from normal. After 2 weeks many blood vessels resembling lacunae, running mainly around the periphery and along the septa, and penetrating into the central regions of the grafts, could be observed in the grafted muscle. In these places the process of regeneration was most actively developed. The muscle fibers preserved their cross-striation and contained large nuclei with two or three nucleoli. Newly formed muscle syncytia lay between the muscle fibers. Not only blood vessels but also axons could now be seen in the middle part of the graft. The central zone of the graft 1 month after transplantation of the muscle was filled with muscle tissue of varied degrees of maturity (Fig. 1). The newly formed muscle fibers were arranged in bundles in the loose connective tissue, rich in fibroblasts. Mitoses were seen in the freely lying cells. Most of the grafts after 2 months consisted chiefly of differentiated tissue. Muscle fibers of various diameters lay in bundles, usually dense but sometimes loose, with narrow bands of connective tissue containing delicate collagen fibers arranged between them. The muscle nuclei, long and densely stained, sometimes lay in chains. The blood vessels showed a considerable degree of differentiation. However, thin-walled blood vessels resembling lacunae, giving off numerous branching capillaries which formed a dense network around the muscle fibers, could still be seen. Reinnervation of the graft continued. The axons had varicosities, they were arranged along the muscle fibers, and they terminated on the surface in small neuromuscular plaques containing five or six nuclei (Fig. 1b). The grafts possessed contractile activity.

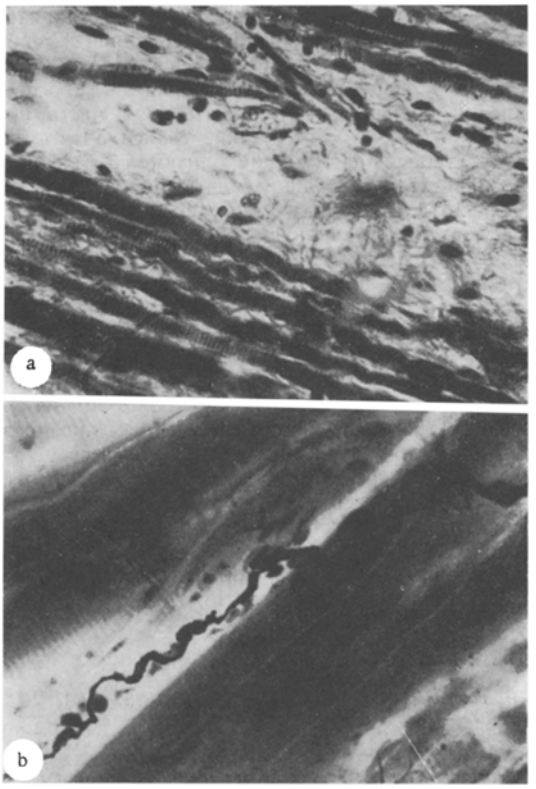

Fig. 1. Muscle tissue in autograft of irradiated (1000 R) muscle, treated by exposure to laser beam before transplantation. Fixation in Carnoy's solution. Iron hematoxylin, counterstained by Mallory's method. a) Young muscle fibers in month-old graft (400×); b) differentiated muscle fibers with motor innervation in graft 2 months after transplantation (600×).

The investigations showed that exposure of the rat limb, irradiated with x-rays in a dose of 1000 R, to the action of a helium–neon laser beam for 10 days, considerably restores the regenerative capacity of the gastrocnemius muscle disturbed by the previous irradiation. Comparison of these data with the results of exposure of an irradiated muscle to a helium–neon laser after transplantation [6], obtained previously, shows that laser therapy, if used before transplantation of the irradiated muscle, gives better results. Revascularization and reinnervation of the transplanted muscle takes place much quicker. As a result a considerable part of the tissues of the graft remains alive and takes part in regeneration. Such grafts after 2 months were similar in their structure and function to 3-month-old grafts from the comparable experiments of the previous investigation. Since laser therapy was given before transplantation of the muscle irradiated with x-rays, restoration of its transplantation activity can be regarded solely as the result of postradiation repair of the muscle fibers and cells of the muscle under the influence of laser radiation. The molecular mechanism of the normalizing action of cold light of the helium–neon laser with a wavelength of 632.8 nm on irradiated tissues is not quite clear. It can be tentatively suggested that in this case laser light stimulates energy processes in the cells and thus facilitates the formation of ATP, which plays a direct part in postradiation repair of membranes, chromosomes, and other organelles of the injured cells, and also in the restoration of their functional properties [3, 4, 9].

LITERATURE CITED

1. N. V. Bulyakova, Dokl. Akad. Nauk SSSR, 202, 729 (1972).
2. Sh. G. Il'yasova, Dokl. Akad. Nauk SSSR, 243, 771 (1978).
3. V. M. Inyushin, Laser Light and the Living Organism [in Russian], Alma-Ata (1970), pp. 30-31.

4. E. V. Kozyreva, P. N. Eliseenko, and M. V. Tikhomirova, Radiobiologiya, 17, 733 (1977).
5. M. F. Popova, "The plastic defensive-reparative state of regenerating tissue," Author's Abstract of Doctoral Dissertation, Moscow (1976).
6. M. F. Popova and Sh. G. Il'yasova, Byull. Éksp. Biol. Med., No. 7, 64 (1978).
7. T. N. Tuzhilkova, Med. Radiol., No. 4, 14 (1956).
8. M. M. Umnova, "Tissue interaction after autografting of skeletal muscles in rats and frogs after x-ray irradiation," Author's Abstract of Candidate's Dissertation, Moscow (1973).
9. M. Elkind, C. Kamper, V. Moses, et al., in: Regeneration and Reparative Mechanisms in Radiobiology [Russian translation], Moscow (1972), pp. 69-96.

MIGRATION AND DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS IN AUTOIMMUNE MICE OF DIFFERENT AGES

R. M. Khaitov, I. N. Shatalova,
A. M. Nazhmetdinov, T. V. Dikaya,
S. Yu. Pchelintsev, G. N. Pleskovskaya,
and V. A. Nasonova

UDC 612.419-053

Migration and differentiation of hematopoietic stem cells were studied in autoimmune (NZB × NZW)F_1 mice of different ages. Migration of stem cells was shown to be reduced in old (NZB × NZW)F_1 mice. Irrespective of age, inhibition of differentiation of stem cells along the granuloid path of development was observed in (NZB × NZW)F_1 mice. It is suggested that in (NZB × NZW)F_1 mice there is either a defect of development of the T-lymphocyte subpopulation influencing differentiation of stem cells along the granuloid pathway or a genetic defect at the level of precursors of the granulocyte series (CFU_c).

KEY WORDS: stem cell; hematopoietic colony; differentiation.

It was shown previously that during aging migration of hematopoietic stem cells from the bone marrow diminishes in normal mice of several lines [4]. In old animals inhibition of differentiation of stem cells along the granuloid path is observed under these circumstances [5].

Inhibition of migration of granuloid differentiation of stem cells has also been observed in thymectomized mice [2, 8]. Since these processes are restored to normal by injection of thymocytes or peripheral T-cells from young donors into thymectomized (artificial T-deficiency) or old (age T-deficiency) mice, it has been suggested that thymus-dependent lymphocytes have a determining (inducing) effect on differentiation of the stem cell along the granuloid pathway.

In the investigation described below migration and differentiation of hematopoietic stem cells were studied in (NZB × NZW)F_1 mice, which are used as a model of systemic lupus erythematosus in man. As a rule an autoimmune state, associated with a deficiency of the T-system of lymphocytes, develops in (NZB × NZW)F_1 mice at the age of 4-5 months.

EXPERIMENTAL METHOD

(NZB × NZW)F_1 mice of different age groups were used in the experiments. The number of hematopoietic colonies in the spleen was counted by the method in [10]. Migration of stem cells was assessed by the method described previously [3]. Suspensions of bone marrow cells were prepared in the usual way and injected intravenously into lethally irradiated syngeneic recipients aged 2-2.5 months in a dose of $1 \cdot 10^5$ karyocytes. For the histological study of the character and number of colonies, spleens fixed in Bouin's fluid were embedded in paraffin wax and used for cutting series of sections 5-7 μ thick. The sections were stained with hematoxylin-eosin. The numerical results were subjected to statistical analysis in the usual way.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 224-226, February, 1980. Original article submitted February 8, 1979.

0007-4888/80/8902-0215$07.50