Regeneration of Nerve Fibers by Laser Irradiation of Spinal Nerve Projections

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The effect of short infrared laser irradiation on regeneration of nerve fibers in rat sciatic nerve was studied. Starting from the first day after nerve crushing, the projection of proximal portion of the nerve of the test group rats was transcutaneously stimulated by laser irradiation with the wavelength of 890 nm and total dose of 42 mJ/cm^2. The test rats demonstrated higher rate of restoration of cutaneous sensitivity in comparison with control rats, which were operated but not irradiated. The local vestibular reaction occurred 2 days earlier in the test group than in the control. On day 30 after crushing there were no significant differences in the number of myelinated fibers and mass of regenerating soleus muscle in the test and control groups.

Key Words: *regeneration; sciatic nerve; myelinated fibers; infrared laser irradiation*

Recent studies show that laser irradiation (890 nm, total dose 0.33 J/cm^2) in the intersection region of a peripheral nerve does not affect regeneration of myelinated fibers [4]. By contrast, an increase in total dose to 9.33 J/cm^2 as well as irradiation at the wavelength of 1220 nm and dose 0.98 J/cm^2 decrease the number of regenerating myelinated fibers [4], which is presumably related to increase in fibroblast proliferation in the damaged region and to scar formation. This hypothesis agrees with the data on activation of fibroblast proliferation *in vitro* under the action of laser irradiation [3,5]. By contrast to intersection, the post crushing nerve regeneration is followed by a lesser scar and is generally more effective. In this work we studied postcrushing regeneration of nerve fibers under the action of irradiation with a low-intensity short infrared laser light directed to proximal (in respect to the damaged part) portion of sciatic nerve. Selective irradiation of this portion of peripheral nerve makes it possible to avoid the direct effect of radiation on fibroblasts in the region of nerve trauma and thus attenuate cicatrization.

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MATERIALS AND METHODS

Experiments were performed on 3.5-month-old male albino rats weighing 150-250 g. The left sciatic nerve was preparated under ketamine hydrochloride anesthesia (175 mg/kg intraperitoneally). A portion of nerve 3 mm in length located at the middle part of the femur was crushed with a microsurgery occlusion clamp applied for 30 sec [7]. The wound was sutured in layers. In the experimental group (15 rats), the projections of proximal subdivisions of the spinal nerves were irradiated transcutaneously with a semiconductor laser during 14 days in the region of their emergence from the spinal cord vents (at the level of L_4-S_1 segments). The radiation parameters were: wavelength 890 nm, power 3.5 W, pulse frequency 1 kHz, exposure time 21 sec, total dose 42 mJ/cm^2. The control group (n=7) consisted of rats operated under the same conditions but not irradiated. From the postoperation day 7, cutaneous sensitivity was determined in the paw every other day [1]. If the response to the standard nociceptive stimulation applied to dorsal and plantar surface of the paw did not differ in the operated and contralateral (intact) hind limb, it was assessed as complete restoration of

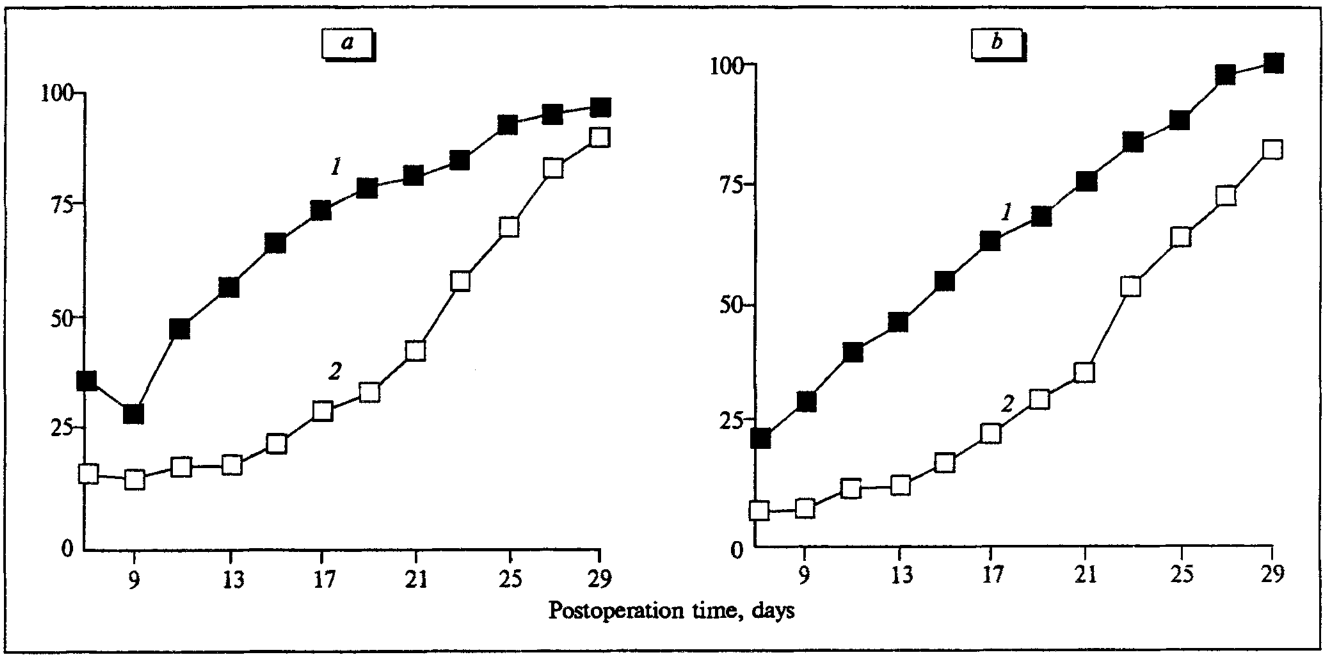

Fig. 1. Dynamics of restoration of cutaneous sensitivity at the dorsal (*a*) and plantar (*b*) surfaces of the foot under the action of laser irradiation after crushing of sciatic nerve (*1*) and in control (2). Ordinate: restored sensitivity area in percentage.

sensitivity. The area of regions of cutaneous sensitivity was calculated using a Caddy 900 software. Motor function was evaluated daily with the help of local vestibular reaction beginning from the day 7. On the postoperation day 30, the soleus muscle was isolated from rats of both groups and weighted [6]. Regeneration efficiency was assessed by comparing the mass of the muscle at the operated side in control and experimental rats. At the same time, a portion of sciatic nerve 6-mm long was isolated from the rats of both groups at the middle part of the femur both in operated and intact (contralateral) hind limb. The isolated nerve fragment included crushed region (3 mm) and a more distal portion of the nerve (3 mm). The preparations were fixed in glutaraldehyde and osmium tetroxide and after dehydration embedded in Epon-Araldite mixture. The count of myelinated fibers was performed on semithin transverse sections of sciatic nerve stained with methylene blue [4]. At the same period (post operation day 30) sciatic nerves were isolated from control and experimental rats under ketamine hydrochloride anesthesia (175 mg/kg intraperitoneally). The amplitude, duration, threshold, and latency of the compound action potential (CAP) were measured with the help of bipolar electrodes. The proximal part of the nerve was stimulated with a pair of electrodes. The results were statistically analyzed using Student's *t* test.

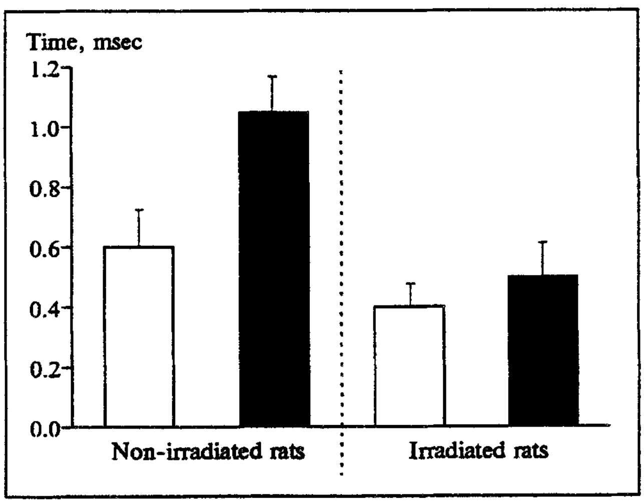

Fig. 2. Latency of CAP of sciatic nerve (*M±m*) at the operated (solid bars) and intact contralateral (light bars) hind limbs at day 30 after nerve crashing.

RESULTS

Significant intergroup differences in cutaneous sensitivity were revealed at all periods of observation both for dorsal and plantar sides of the paw ($p<0.05$) with exception of the dorsal surface on day 29 ($p>0.05$, Fig. 1).

Local vestibular reaction was completely restored in irradiated rats 17±1.6 days after the nerve trauma, while in control group it was restored after 19±1.6 days.

Comparison of the soleus muscle mass in operated limbs and the number of myelinated fibers in the distal portion of traumatized nerve did not reveal any significant differences between control and experimental groups.

Laser irradiation decreased the latency of CAP by 52% ($p<0.05$) in the regenerating nerve (Fig. 2). However, there were no significant difference in control and experimental groups in threshold, amplitude, and duration of CAP ($p>0.05$).

Thus, we found a stimulating effect of low-intensity short infrared laser irradiation on restoration of sensitivity of traumatic nerve. The most pronounced differences between control and experimental groups were observed in the first half of the regeneration period (Fig. 1). Since the greater part of fibers (80%) in the sciatic nerve are afferent [8], the rate of restoration of nervous sensitivity is the most adequate parameter to describe the rate and effectiveness of regeneration.

The dynamics of local vestibular reaction shows that restoration of motor function during regeneration after laser irradiation occurs 2 days earlier in comparison with control. Nevertheless, laser irradiation did not affect the mass of denervated soleus muscle as well as the number of myelinated fibers on day 30 of regeneration. This can be related to the absence of functional differences between rats by the moment of the muscle and nerve isolation.

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