MORPHOLOGY AND PATHOMORPHOLOGY

Healing of Bone Fractures of Rat Shin and Some Immunological Indices during Magnetic Laser Therapy and Osteosynthesis by the Ilizarov Method

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The effect of magnetic and laser therapy on healing of bone fractures and blood levels of T and B lymphocytes was studied in rats during osteosynthesis by the Ilizarov method. Laser therapy induced changes in cells attesting to stimulation of reparative processes and normalization of immunological parameters.

Key Words: *osteosynthesis; laser; morphology; ultrastructure; lymphocyte*

Transosseous osteosynthesis by the Ilizarov method is widely used in orthopedics and traumatology. However, the morphogenesis of bone repair and ultrastructure of cells directly participating in bone healing after fracture are little studied [2,4,5].

Various types of laser radiation are widely used in traumatology and orthopedics [6,7]. However, the effect of magnetic and infrared laser therapy (MIRLT) on bone healing and, specifically, on ultrastructural changes in the corresponding cells, was not studied. Regenerative processes and the state of lymphoid organs determining the immunological status of an organism are interrelated [1], although changes of these indices during fracture healing under the effect osteosynthesis and MIRLT are poorly understood, which necessitated this work.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats (n=42) weighing 180-220 g. Skin on the shin was cut and fracture was inflicted under ether anesthesia. The fractures were fixed in a small-size Ilizarov device, and X-ray control was performed using an Arman apparatus (Fig. 1).

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Starting from postoperation day 2, the rats were daily subjected to MIRLT (3 min) using a Mustang apparatus working in a pulse mode (10 W, 0.89 μ wavelength, 3000 Hz frequency, 50 mT magnetic field intensity). The controls were not subjected to MIRLT.

The specimens were taken after immediate decapitation on postoperation days 3, 5, 7, and 14.

Specimens or light microscopy were fixed in 10% formalin by the method of Lilly and decalcified in ethylenediaminetetraacetic acid disodium salt dihydrate solution [5]. Paraffin sections were stained with hematoxylin and eosin.

For transmission electron microscopy (TEM), the decalcified specimens were postfixed in glutaraldehyde with phosphate buffer and then additionally fixed in 1% OsO_4 with the same buffer. After dehydration in alcohol and acetone, the specimens were embedded in epon and araldite. Ultrathin sections were cut on an Ultracut ultratome (Reichert—Jung) and contrasted in an Ultrastainer apparatus (LKB). The slides were analyzed and photographed in a Hitachi-H600 transmission electron microscope.

For evaluation of the state of the immune system, heparinized blood drawn immediately after decapitation was used.

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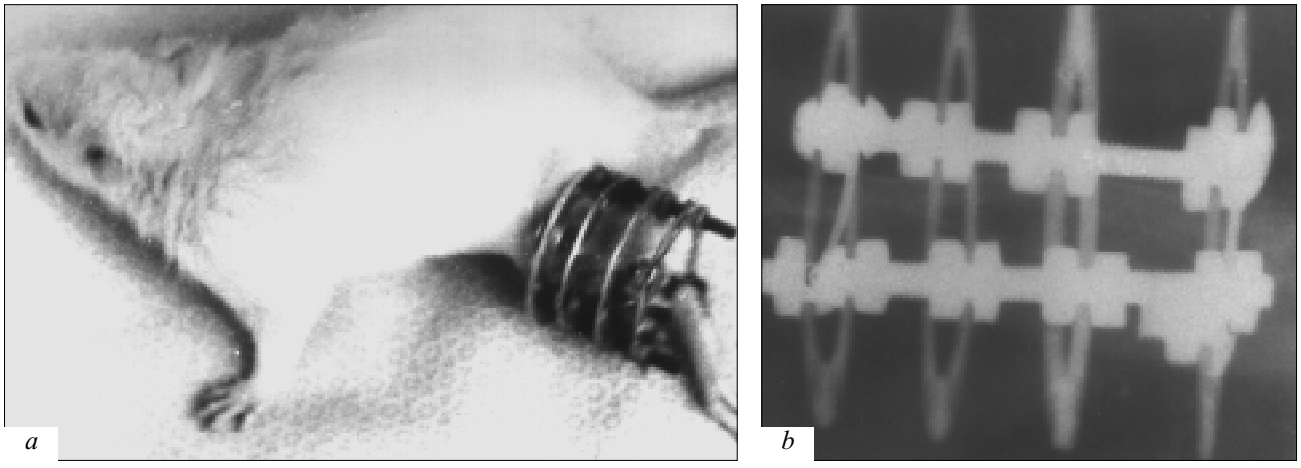

Fig. 1. Ilizarov miniapparatus applied to the shin immediately after fracture. *a*) general view; *b*) control X-ray image.

Lymphocytes were isolated by routine methods in a Ficoll-Verografin density gradient. The monoclonal antibodies (Institute of Immunology, Ministry of Health, Russia) were used to determine the following cell populations: $CD3^+$ (T lymphocytes), $CD4^+$ (T helpers), $CD8^+$ (T suppressors), $CD16^+$ (natural killers), and CD20 (B lymphocytes).

RESULTS

In the early period after fracture (on postoperation day 3) hyperplasia of osteoblast-like cells (round cells with hyperchromatic nuclei) and numerous mitoses were seen in the surface layers of bone fragments. Hemorrhages were detected in soft tissues near the bone. Osteocytes of the fibrous connective tissue had hyperchromatic nuclei, lacunas were poorly developed. The osteoblast proliferation area contained the microvessels of various diameters.

On postoperation days 5-7 the number of osteoblasts in the forming bone slightly decreased. The prevailing cells were osteocytes. Many osteocytes were surrounded by clearly formed lacunas (Fig. 2, *a*). Only solitary osteoclasts were seen. The number of osteoclasts increased on postoperation day 14, which corresponded to the formation of Haversian and Volkmann canals.

The pathomorphism of bone repair in Ilizarov device was pronouncedly affected by MIRLT. The

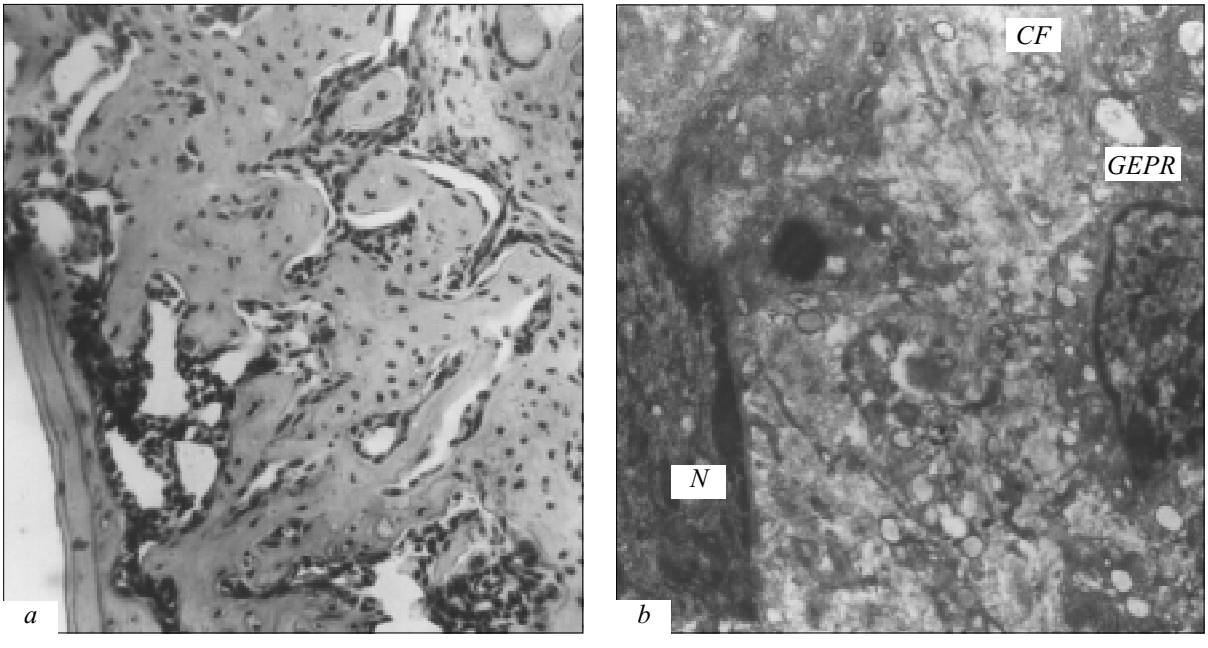

Fig. 2. Bone structure on day 5 after fracture and osteosynthesis. *a*) hematoxylin and eosin staining, ×200; *b*) osteoblast-like cells, ×7500. Here and in Fig. 3: *N*: nucleus, *GEPR*: granular endoplasmic reticulum, *CF*: collagen fibers.

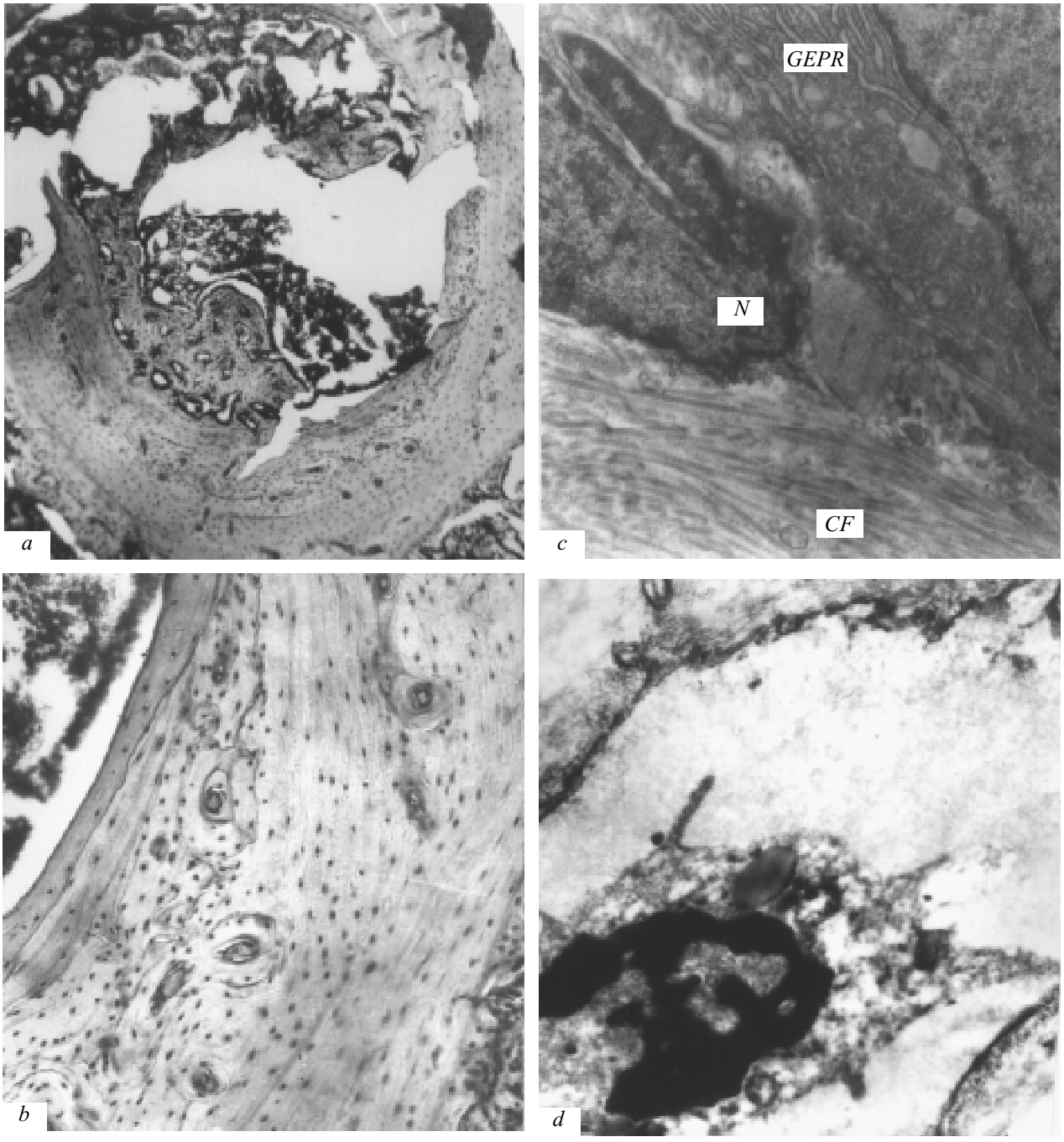

Fig. 3. Bone structure after osteosynthesis and magnetic infrared laser therapy. *a*) the development of central callus on postoperation day 7. Hematoxylin and eosin staining, ×100; *b*) Volkmann and Haversian canals in a newly formed bone. Hematoxylin and eosin staining, ×200; *c*) osteoblast-like cells surrounded by collagen fibers on day 5, ×15,000; *d*) an osteocyte in a lacuna on day 14, ×15,000.

early postoperation period (day 3) was characterized by hyperplasia of osteoblasts, which were seen as large oval cells with basophilic cytoplasm and hyperchromatic nuclei. The osteocyte-like cells were uniformly distributed among the fibers of the forming bone, they had round shape and light-basophilic cytoplasm. No lacunas were observed around osteocyte-like cells at this term.

On days 5-7 after closed fracture, endosteal callus was well formed and consisted of the primary bone enriched with osteoblasts (Fig. 3, *a*). Periosteal callus was also well developed.

On day 14, the osteocytes dominated in the newly formed bone. They were located in lacunas, and their processes formed osseous canals. Volkmann and Haversian canals with microvessels were clearly seen in the formed bone (Fig. 3, *b*).

In the control regenerating bone osteoblasts at various stages of structural and functional develop-

ment were also seen during the early observation period (on day 3 after fracture and osteosynthesis). Osteoblasts contained great number of free polysomes and few profiles of granular endoplasmic reticulum (GEPR) and Golgi complex and were relatively low-differentiated cells in the osteogenic cells—preosteoblast—osteoblast—osteocyte series.

The fibroblasts and osteoblasts have similar structure [2,4,5], but differ in location and environment.

As a rule, osteoblasts with developed heterosynthesis structures (GEPR and Golgi complex) surrounded by collagen fibers cannot be distinguished from fibroblasts. Such osteoblasts were observed on postoperation days 3 and 5 (Fig. 2, *b*).

MIRLT increased the number of fusiform cells with processes, great number of GERP profiles in the cytoplasm, large nuclei, and enlarged nucleoli. These cells were surrounded by collagen fibers (Fig. 3, *c*).

On day 7, small cells with processes of various lengths and low number of heterosynthesis structures (GERP and Golgi complex) representing transitory forms from osteoblasts to typical osteocytes were often seen in the experimental group. These cells were often embedded in collagen fibers.

On day 14, typical osteocytes predominated in newly formed bones in the experimental group. They were located in lacunas and had thin processes, which penetrated into basic osteal substance and formed numerous canals. These cells had pyknotic nuclei surrounded by thin cytoplasmic rim with low content of the membranous structures (Fig. 3, *d*).

Immunological studies revealed significant disturbances in basic immunological indices after fracture and osteosynthesis even on postoperation day 14. The differences in the content of various types of T lymphocytes were significant. Under the effect of MIRLT these indices approached the control values (Table 1).

TABLE 1. Parameters of Immune System (%) in Rats with Shin Fracture during Various Modes of Osteosynthesis and MIRLT ($M \pm m$)

Cell type	Control (n=5)	Ilizarov device (n=10)	Ilizarov device+ MIRLT (n=10)
T lymphocytes	35.4±0.6	30.6±0.5*	33.6±0.5
B lymphocytes	19.6±0.1	17.30±0.09	18.30±0.09
T suppressors	18.5±0.1	13.2±0.1*	15.2±0.1
T helpers	32.1±0.5	24.7±0.4*	26.7±0.4*
Natural killers	11.20±0.07	7.50±0.05*	9.40±0.05

Note. *p<0.05 compared to the control.

Thus, MIRLT induces structural changes in the bone, which activate its repair after fracture during osteosynthesis in Ilizarov device and promotes normalization of immunological indices. These observations confirm clinical efficiency of MIRLT during osteosynthesis in Ilizarov device.

REFERENCES

1. A. G. Babaeva, in: Ed. D. Sarkisov, *Structural Principles of Adaptation and Compensation of Disturbed Functions* [in Russian], Moscow (1987), pp. 185-193.
2. G. I. Lavrishcheva and L. N. Mikhailova, *Ibid.*, pp. 154-184.
3. Eds. D. S. Sarkisov and Yu. L. Perov, *Microscopic Technique (Practical Manual)* [in Russian], Moscow (1996).
4. D. S. Sarkisov, *Essays on General Pathology History* [in Russian], Moscow (1993).
5. D. S. Sarkisov, in: Ed. D. Sarkisov, *Structural Principles of Adaptation and Compensation of Disturbed Functions* [in Russian], Moscow (1987), pp. 9-83.
6. Ed. D. B. Apfelberg, *Evaluation and Installation of Surgical Laser Systems,* New York (1987).
7. T. Ohshiro and R. G. Calderhead, *Low Level Laser Therapy: a Practical Introduction*, New York (1988).