ELECTRON-MICROSCOPIC AND MORPHOMETRIC STUDY OF REGENERATING BONE DURING COMBINED MAGNETIC AND LASER STIMULATION

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The theoretical and practical aspects of the stimulating action of a constant magnetic field (CMF) and of laser irradiation (LI), used separately or in combination, on reparative regeneration of bone tissue have been studied intensively in recent years [1-4, 7]. Nevertheless, changes in the structural organization of bone during combined stimulation by a magnetic field and laser irradiation have not yet been adequately studied.

This paper describes an electron-microscopic and morphometric study of the dynamics of the cellular and noncellular components of regenerating bone under the influence of CMF, low-intensity LI, and a combination of both (CMF + LI).

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats in which a standard perforation defect of the tibia was produced under hexobarbital anesthesia (0.1 ml of a 10% solution/100 g body weight) by the method described in [8]. The animals were divided into four groups: 1) control; 2 and 3) subjected to isolated stimulation by CMF and LI, respectively; 4) subjected to CMF + LI. A local CMF was applied by means of ferrite magnets, with induction of 31-33 mT and with an exposure of 15 min. LI was applied by means of an LG-52 helium-neon laser with wavelengths of λ = 632.8 nm, a power of 8 mW, and exposure of 15 min. The duration of the local action of CMF + LI was 30 mim (15 min CMF + 15 min LI). Regenerating tissue was taken for electron-microscopic investigation on the 3rd, 7th, and 14th days after the operation by the method in [5], fixed in 2.5% glutaraldehyde solution in phosphate buffer (pH 7.4), and postfixed in 1% osmic acid solution by the method in [6]. Ultrathin sections of regenerating bone were studied in the EMV-100AK electron microscope. The morphometric analysis was carried out on negatives obtained under a magnification of 3000 by means of a dot test system (R_d = 384). The time course of the following parameters was studied: the fraction of cells by volume (V_{cell}), the fraction of collagen fibers by volume (V_{coll}), and the fraction of intercellular edema by volume (V_{edema}). A stabilized value of the parameters and error of the mean were determined by graphic analysis.

TABLE 1. Relative Fractions by Volume of Cellular and Noncellular Components of Regenerating Bone during Stimulation by Physical Factors (M ± m, %)

Stimulation	Parameter	Time of investigation, days		
		3	7	14
Control	V_{edema}	31,80±0,60	—	—
	V_{cell}	27,20±0,60	34,20±0,50	62,80±0,40
	V_{coll}	41,00±0,50	65,80±0,50	37,20±0,50
CMF	V_{edema}	0,70±0,50	—	—
	V_{cell}	34,90±0,50	64,20±0,50	68,40±0,50
	V_{coll}	64,40±0,50	35,80±0,50	31,60±0,40
LI	V_{edema}	1,60±0,50	—	—
	V_{cell}	62,40±0,50	70,40±0,50	72,00±0,50
	V_{coll}	36,00±0,50	29,60±0,30	28,00±0,80
CMF + LI	V_{edema}	—	—	—
	V_{cell}	39,70±0,30	50,20±0,60	51,70±0,40
	V_{coll}	60,30±0,30	49,80±0,40	48,30±0,30

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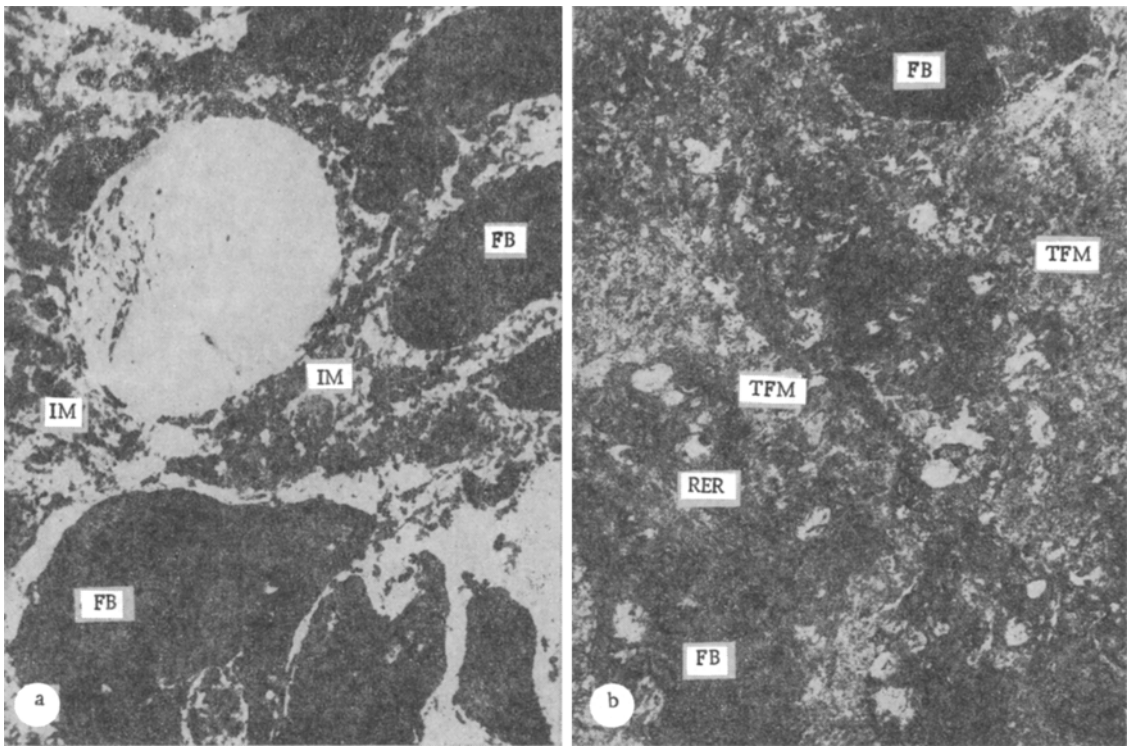

Fig. 1. Ultrastructure of regenerating bone on 3rd day of experiment. a) Control: loose intercellular matrix (IM) and fibroblasts (FB) in different stages of differentiation. b) Experiment (CMF + LI): well-developed RER, intercellular matrix composed of thin fibers (TFM).

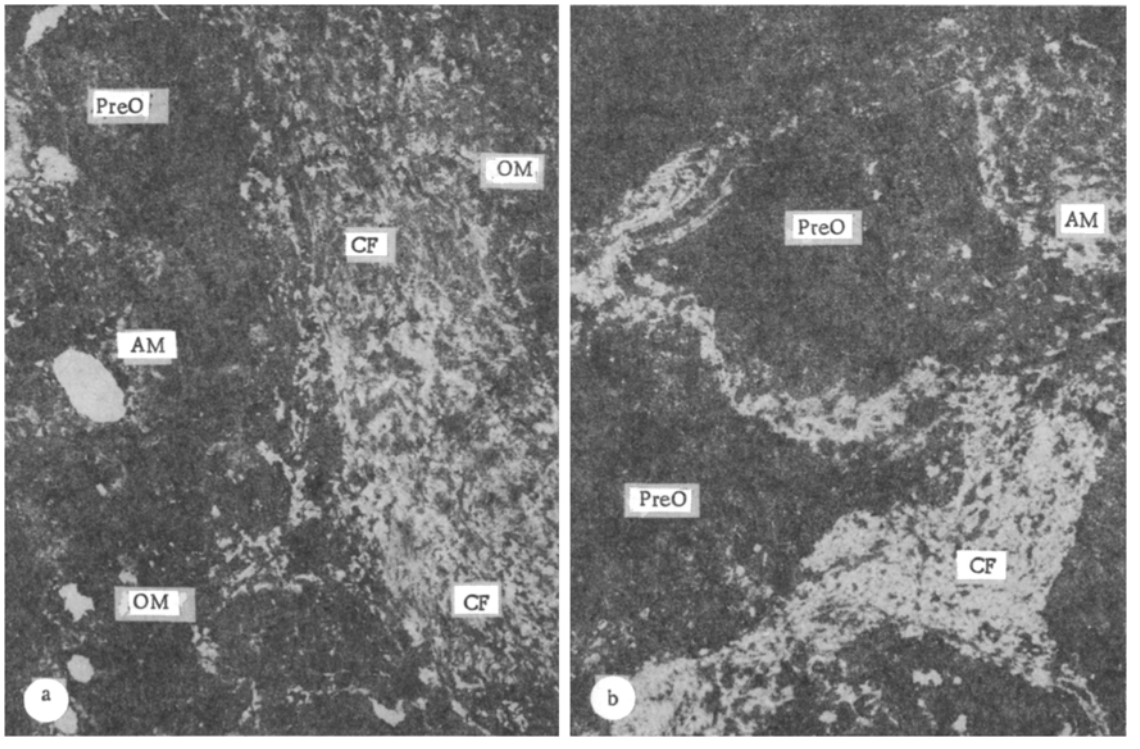

Fig. 2. Ultrastructure of regenerating bone on 7th day of experiment: a) control; b) experiment (CMF + LI). OM) Osteoid matrix; preo) preosteoblasts; CF) bundles of collagen fibers; AM) areas of mineralization. 5000 ×.

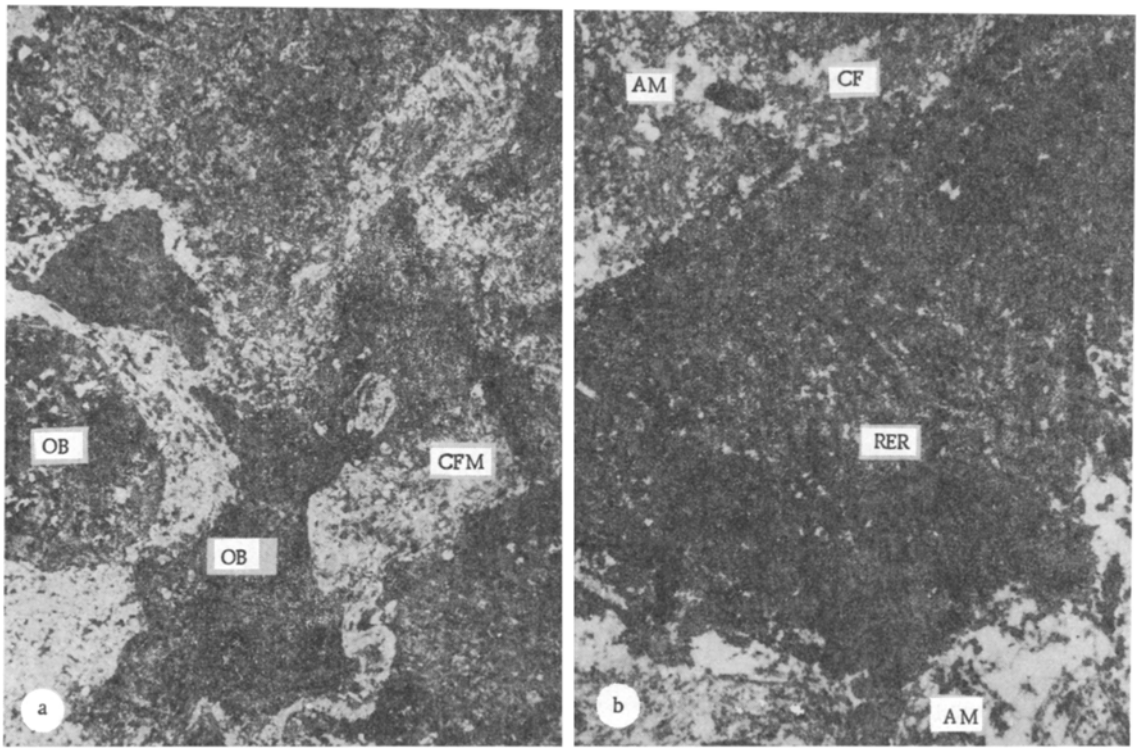

Fig. 3. Ultrastructure of regenerating bone on 14th day of experiment: a) control: osteoblasts (OB), matrix of collagen fibers (CFM). 3000×; b) experiment (CMF + LI): strongly developed RER. 7000×. Remainder of legend as to Fig. 2.

EXPERIMENTAL RESULTS

The defect in the control animals on the 3rd day after trauma was filled mainly by concentrations of blood cells, loosely arranged fibrin threads, and individual collagen fibers. In some parts of the defect fibroblasts were present among the collagen fibers, in various stages of differentiation (Fig. 1a). A particular feature of the structural organization of regenerating bone in the control animals was the marked intracellular edema. The isolated action of CMF and LI caused considerable reduction of the edema and an increase in the fraction of the cellular component by volume, mainly on account of fibroblasts, together with a significant reduction in the relative volume of collagen fibers. After exposure to CMF + LI the defect was filled with polyblasts and thin collagen fibers, among which were distributed fibroblasts, with numerous flattened tubules of the rough endoplasmic reticulum (RER) and preosteoblasts with a well-developed tubular system (TS) and a well-marked lamellar complex (Fig. 1b).

The mitochondria varied in shape, with a translucent matrix and clearly outlined cristae. The nucleus was mainly ellipsoidal in shape, with well-marked nucleoli. Numerical values of the morphometric parameters are given in Table 1.

On the 7th day after trauma intercellular edema could not be seen in the control animals. The cellular component consisted mainly of fibroblasts, near which there were many collagen fibers (Fig. 2a). After stimulation by CMF or LI alone the cellular components of the regenerating bone consisted not only of fibroblasts, but also of osteoblasts. The fibroblasts contained a fairly well developed RER. After combined exposure to CMF and LI, many osteoblasts were seen in the regenerating bone; intensive development of the RER and lamellar complex was taking place in their cytoplasm (Fig. 2b).

On the 14th day of repair the fraction of osteoblastic cells was increased in volume in the control. In the experimental animals subjected to the action of CMF or LI alone the defect was filled with newly formed osteoid trabeculae (Fig. 3a). The ultrastructure of the osteoblasts in the regenerating bone 7 days after isolated exposure to CMF or LI was almost the same as that in the control on the 14th day. In animals subjected to the combined action of CMF and LI the regenerating bone consisted of osteoid and coarsely fibrous trabeculae, surrounded by osteoblasts; mineralization of the nature collagen fibers could be detected. Many

osteoblasts, located on the trabeculae, were surrounded on all sides by mineralized osteoid (Fig. 3b), reflecting their differentiation into osteocytes.

During exposure to the isolated and combined action of CMF and LI a considerable reduction of intercellular edema thus takes place in the regenerating bone, accompanied by activation of differentiation of the cells forming the fibrogenic and osteoid components of the regenerating bone in the early stages. The strongest action on differentiation of the cellular components of the regenerating bone is given by a combination of low-energy magnetic and laser stimulation (CMF + LI), which induces filling of the effect with newly formed bone within a shorter time.

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