EXPERIMENTAL BIOLOGY

Use of Osteogenic Bone-Marrow Precursor Cells for Reparative Osteogenesis in the Mandible of Experimental Animals

I. N. Ivasenko, D. A. Ivasenko, and V. A. Almazov

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Using a model of "bony tissue tunnel defect" produced by the removal of a mandibular incisor in rats, it was found that closing the defect with a bioprosthesis prevented the washing out of osteogenic bone marrow precursor cells, which serve as a substrate for reparative osteogenesis, from the mandibular spongy bone. The reparative process was strongly stimulated if the bioprosthesis contained estrone; in this case, the time required for the tooth socket to be filled with osteogenic tissue was shortened by half. When no bone marrow elements were present in the socket, it was filled with fibrotic connective tissue, the number of bone marrow elements in spongy bone cavities was small, and the mandibular osteogenic tissue underwent atrophy.

Key Words: *bone marrow; osteogenesis; bioprosthesis; estrone; mandible*

Reparative osteogenesis is a major consideration for dental surgeons deciding how to cope with the alveolar bone atrophy that arises after the extraction of teeth [7,11]. Inducers of osteogenesis have found wide application in dental surgery in the treatment of periodontal disease and dental cysts and in the reconstruction of maxillary or mandibular alveolar limbi [6,8,10].

Unfortunately, the reported information about tissues that can be used as a substrate for reparative osteogenesis in scant [5]. Using an animal model of myelofibrosis, we have been able to show that abundant proliferation of osteogenic tissue can be achieved through stimulation of osteogenic precursor cells isolated from the bone marrow.

The purpose of the present study on rats was to evaluate prospects for the use of such cells in the reparative osteogenesis of mandibular spongy bone.

Group for Isotopic Methods of Investigation, Department of Internal Medicine, I. P. Pavlov First Medical Institute, St. Petersburg

MATERIALS AND METHODS

A total of 36 male Wistar rats weighing 150 g on average were used. A tunnel defect was created in the mandibular bone by removing the left lower incisor (which extended over two-thirds of the mandible) under Hexenal anesthesia. As a result, the bone marrow elements present in cavities of the mandibular spongy bone could freely enter the tunnel that had formed.

The rats were divided into three groups, 12 in each. In group 1, a bioprosthesis with estrone (5 mg/kg) diluted in olive oil was placed at the site of the removed incisor; group 2 received a bioprosthesis with olive oil alone. In group 3 rats, which served as controls, no bioprosthesis was placed and marrow elements were allowed to freely enter the oral cavity.

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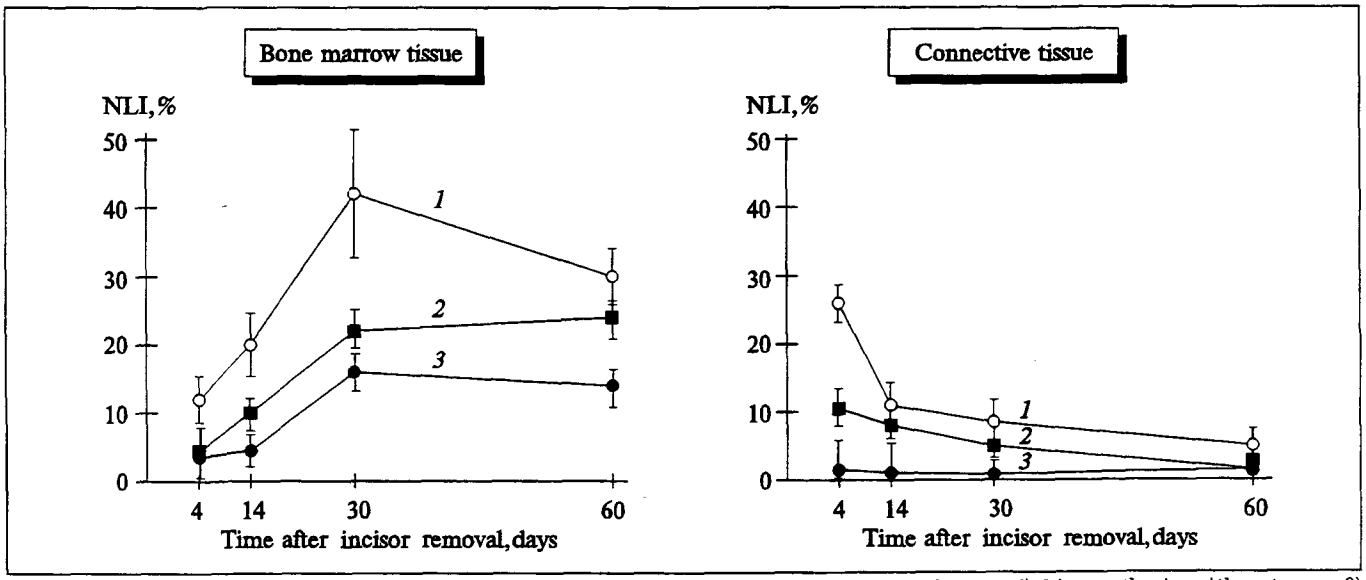

Fig. 1. Temporal variation in the NLI in mandibular bone marrow and connective tissues. *1*) bioprosthesis with estrone; *2*) bioprosthesis without estrone; *3*) control.

On days 4, 14, 30, or 60 after the operation, three rats from each group were sacrificed 1 h after being injected intraperitoneally with ^{3}H-thymidine in a dose of 1 μCi/g. The lower half of the mandible was fixed in 10% neutral formalin, decalcified in a 6.5% nitric acid solution, washed, taken through graded alcohols and celloidin-castor oil, and routinely embedded in paraffin; 5-μ sections were then prepared, covered with type-M emulsion, exposed, developed, and stained with hematoxylin-eosin.

For the evaluation of the proliferative activity of the tissues, a nuclear labeling index (NLI) was calculated (in percent) both for connective tissue cells in the socket of the removed tooth and for marrow elements of the mandibular spongy bone.

At all times during the study (i.e., on days 4, 14, 30, and 60), reparative osteogenesis in tissues of the mandibular spongy bone was evaluated morphologically.

The results were treated statistically using the Student-Fisher test [3].

RESULTS

Both tissues under study + namely, the connective tissue which had been contiguous to the removed tooth's socket before filling it, and the bone marrow tissue which was present in cavities of the mandibular spongy bone - were found to have responded by enhanced proliferative activity to the bioprosthesis introduced into the tooth socket. The highest activity, as measured by the NLI, was recorded as early as on day 4 for the connective tissue and on day 60 for the marrow tissue (Fig. 1).

The bioprosthesis itself did not produce visible changes in the mandibular tissues surrounding the

Fig. 2. Osteogenic tissue filling the socket of the mandibular incisor 30 days after its removal and application of the bioprosthesis without estrone. ×70. Hematoxylin–eosin staining here and in Figs. 3 and 4.

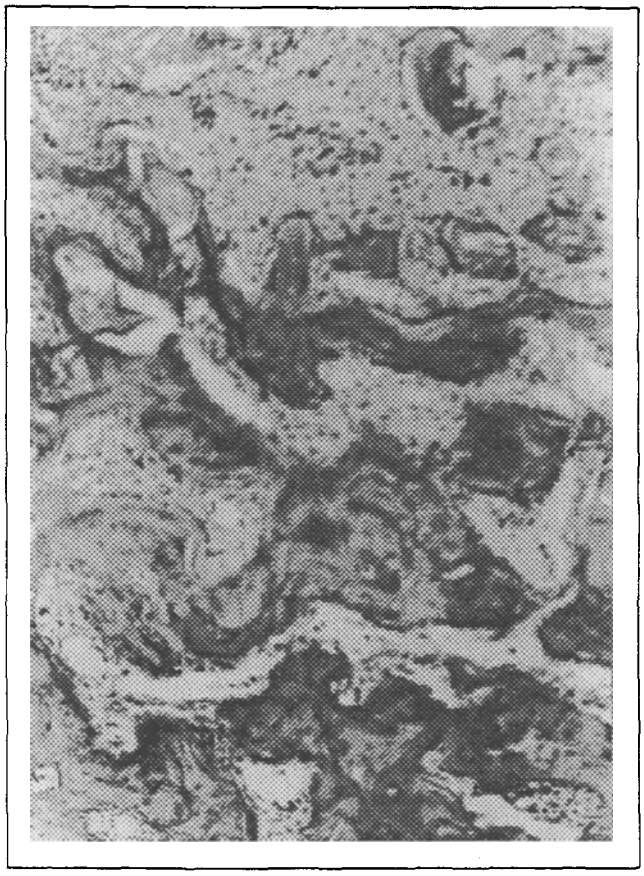

Fig. 3. Osteogenic tissue filling the socket of the mandibular incisor 30 days after its removal and application of the bioprosthesis with estrone. ×70.

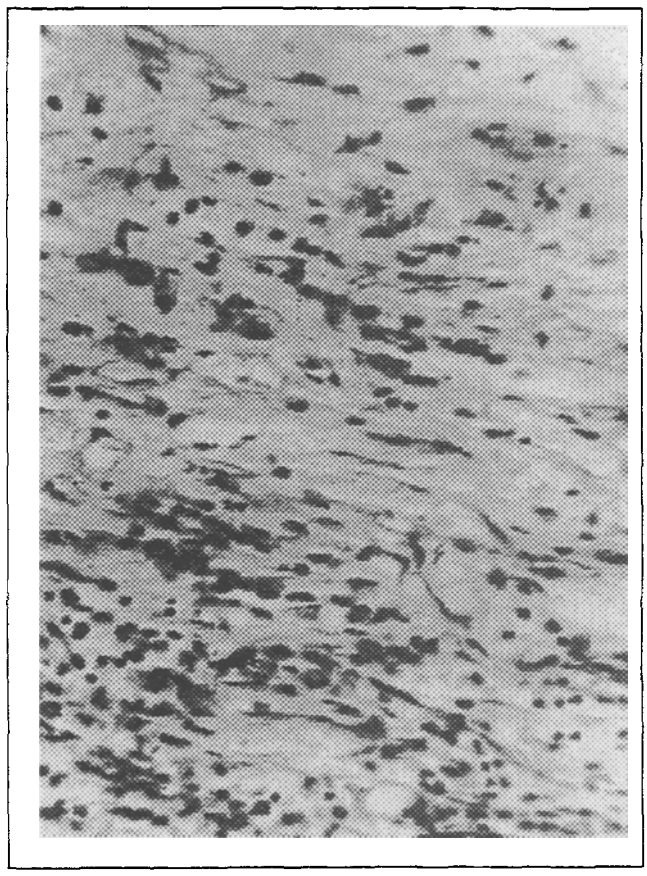

Fig. 4. Connective tissue filling the socket of the mandibular incisor 30 days after its removal in control rats. ×30.

tooth socket. The observed changes in the NLI appear to reflect the physiological nature of shifts in the proliferative activity of these tissues in the process of reparative osteogenesis.

The bioprosthesis, which had become absorbed by day 14 and had no effects on the surrounding mandibular tissues, allowed osteogenic precursor cells to proliferate and differentiate by preventing their being washed out from the marrow cavities that opened into the tooth socket (Fig. 2). As a result, the socket was filled with osteogenic tissue by day 60.

In addition to being powerful stimulators of cell proliferation in tissues of target organs, estrogens have also been shown to exert similar stimulatory, though less marked, effects on virtually all tissues [4]. That estrone is capable of stimulating osteogenic marrow precursor cells has been demonstrated in our earlier studies [2,5].

TABLE 1. Variation in Proliferative Activity of Mandibular Spongy Bone Tissues in Rats after Incisor Removal and Application of a Bioprosthesis with or without Estrone

Group	NLI, %			
	Time after incisor removal, days			
	4	14	30	60
	Connective tissue			
Control	1.26±0.19	0.83±0.48	1.0±0.0	0.90±0.04
Bioprothesis	10.50±0.50	8.56±2.43	4.96±0.04	0.90±0.04
Bioprothesis with estrone	26.5±0.5	10.96±0.77	7.96±0.78	4.16±0.10
	Bone marrow tissue			
Control	3.4±0.2	4.9±0.4	16.2±0.1	13.6±1.9
Bioprothesis	4.9±0.4	9.46±0.24	21.4±0.9	23.6±0.6
Bioprothesis with estrone	4.16±0.10	19.1±0.2	41.6±8.4	28.9±3.2

Indeed, the use of a bioprosthesis with estrone led to still higher values of the NLI in the test tissues, particularly the marrow tissue (Table 1), which is in accord with the reported stimulatory effects of this estrogen on bone marrow elements [9].

Moreover, the present study lends support to our previous hypothesis that estrone, which is capable of greatly shortening the mitotic cycle, probably induces to cycle once those hematopoietic cells that were previously in the G_0 phase or had a very long mitotic cycle. These cells then leave the S phase and begin to differentiate without undergoing mitosis [5]. This is confirmed by the considerable growth of osteogenic tissue observed in spite of greatly reduced hematopoiesis in cavities of the mandibular spongy bone in rats.

In rats with an estrone-containing bioprosthesis, the tooth socket was already filled with osteogenic tissue on day 30 after incisor removal as a result of the periosteal osteogenesis and proliferation of osteogenic marrow precursor cells (Fig. 3).

The morphological examination of tooth sockets from the control rats showed that, after a thrombus had formed in the socket of the removed tooth, the socket became progressively filled with connective tissue which had undergone fibrosis by day 60 (Fig. 4). In addition, as the socket was being filled with connective tissue, a marked reduction of hematopoiesis occurred in the marrow cavities of the mandibular spongy bone, and this was accompanied by a decrease in the thickness of its trabeculae, which was probably associated with a free exit of monocytes/macrophages capable of inhibiting proliferation of connective tissue cells [1].

The present study led us to conclude that:

- if reparative osteogenesis in the mandibular spongy bone of experimental animals is to be ensured, the bone marrow elements occurring in cavities of this bone need to be prevented from being washed out;
- the bioprosthesis filling the socket of a removed tooth retains bone marrow elements, thereby promoting the proliferation and differentiation of osteogenic precursor cells without affecting the tissues surrounding the socket;
- estrone strongly stimulates both the osteogenic precursor cells and periosteal cells of the mandible; thus, it cuts by half the time taken for the socket to become filled with osteogenic tissue.

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