Influence of Compound of Osteal Growth Factors on Maturation of Distraction Regenerative Substrate

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The effects of Stimbone-1, a complex of growth factors deposited by osteal tissue, on maturation of distraction regenerative substrate are studied in dogs. At the end of distraction, the preparation is injected into the regenerative connective tissue interlayer. Roentgenological, biochemical, and quantitative morphological data show that Stimbone-1 stimulates reparative osteogenesis.

Key Words: *growth factors; distraction osteosynthesis; reparative osteogenesis*

Shortening of medical treatment of orthopedic patients is an important problem. Elongation of extremities and repair of the tubular bone defects by perosseal osteosynthesis according to Ilizarov requires more than half of the treatment period for maturation and mineralization of the osteal regenerative substrate obtained by distraction [7]. A new preparation was developed in our Center, which is a complex of growth-regulating noncollagenous proteins of mature osteal tissue [2]. Previously, we reported that systemic administration of this preparation modifies reparative osteogenesis [3]. In this work we examined the effects of this preparation named Stimbone-1 on maturation of the distraction osteal regenerative substrate (DORS) after local administration into the connective tissue interlayer that separates the ossified subdivisions of the regenerative substance.

MATERIALS AND METHODS

Experiments were carried out on 24 adult mongrel dogs subjected to distraction of the tibia using the Ilizarov apparatus. "Severe" conditions for distraction osteosynthesis were created to reduce osteosynthetic activity and to provide the background against which preparation could exhibit the highest activity. To this end transverse osteotomy was performed in the median third of the tibia with cutting the bone marrow and intraosteal vessels. Distraction was started 3 days after operation at a rate of 1 mm/day (4 times by 0.25 mm) and stopped after 28 days. During the first 3-4 days of fixation, the preparation (2 ml) was injected into the median interlayer of regenerative substrate. Control dogs received 0.15 M NaCl (2 ml). During the 28-day distraction period and 6- and 12-week fixation periods, the activity of osteogenesis was estimated by roentgenological data: size of DORS cross-section, height of the connective tissue interlayer, its crossing and replacement by trabecular ghosts, formation of cortical plate (CP) and bone marrow lumen (BML), by optical density, and using a 5-point scale.

The animals were euthanized 6 or 12 weeks after fixation. DORS were divided into two parts in the sagittal plane. The lateral half was studied by biochemical methods [4] with determination of collagen, noncollagen protein, calcium, and nonorganic phos-

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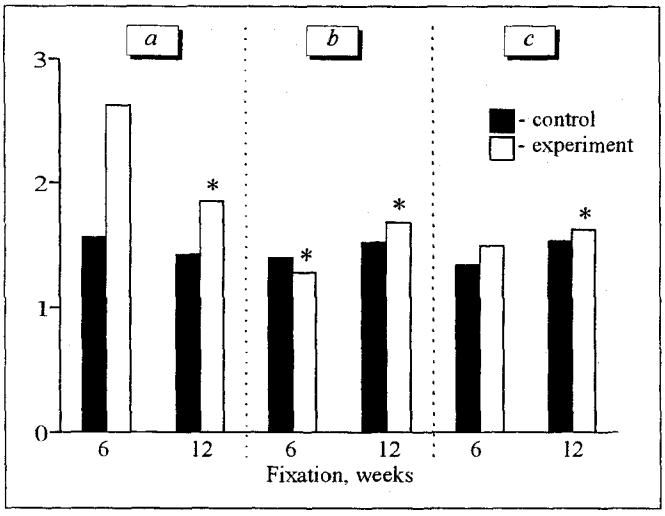

Fig. 1. Biochemical data characterizing maturation of regenerative substrate. *a*) noncollagenous protein/collagen, ×10; *b*) mineral phase crystallization index; c) mineralization index. Here and in Figs. 2 and 3: asterisk indicates $p<0.05$ compared with the control values.

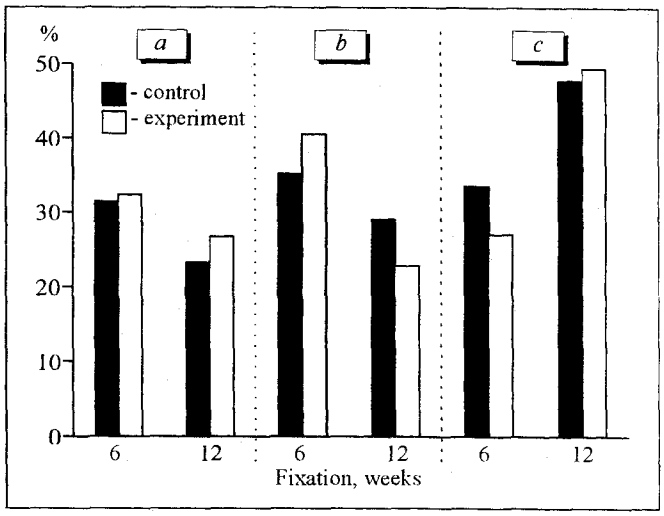

Fig. 2. Distribution of distraction regenerative substrate volume between osteal and nonmineralized tissues according to roentgen electron probe microanalysis. *a*) nonmineralized tissue volume; *b*) interosteal space volume; c) osteal tissue volume.

phate contents; low-mineralized (density <1.65 g/cm³) fractions in the regenerate substrate and in intact bone were isolated. The following indices were calculated: noncollagen protein/collagen; mineral phase crystallization index [Ca]/[PO_4], and mineralization index [[Ca]+(PO_4)]/collagen. The median half of DORS was studied by morphological methods. Stereological analysis [5] was performed on transversal paraffin DORS slices stained by the method of Masson [1]. Stereometric data were used to calculate the relative volumes of osteal tissue and interosteal space, and their ratio, i.e., the compact index, which reflects the degree of maturation of osteal regenerative substrate. The roentgen electron probe microanalysis of DORS was performed. For this purpose the central longitudinal cuts were fixed in glutaraldehyde and OsO_4 in anhydrous acetone and embedded in Epon. The samples were studied in a LINK-860-500 roentgen microanalyzer, mounted on a JLM-840 scanning electron microscope. Histograms of DORS test-volume distribution relative to nonmineralized structures and to osteal tissue at various degree of calcification were analyzed.

RESULTS

The roentgenograms showed that diastasis between fractured fragments was 27-31 mm long by the end

TABLE 1. Roentgenological Estimation of Osteal Formation Activity (*M*±*m*)

Index	Dogs	Observation period		
		28 days of distraction	6 weeks of fixation	12 weeks of fixation
Activity index, points	Control	2.7±0.3	2.3±0.2	2.3±0.4
	Experiment	2.3±0.2	2.3±0.2	3.4±0.2*
Changes in comparison with values on day 28 of distraction (% of number of dogs in group):				
without changes	Control		0	16.7
	Experiment		25	0
worse	Control		83.3	16.7
	Experiment		41.7	0
better	Control		16.7	16.7
	Experiment		33.3	100

Note. Here and in Table 2: the asterisk indicates $p<0.05$ compared with the control values.

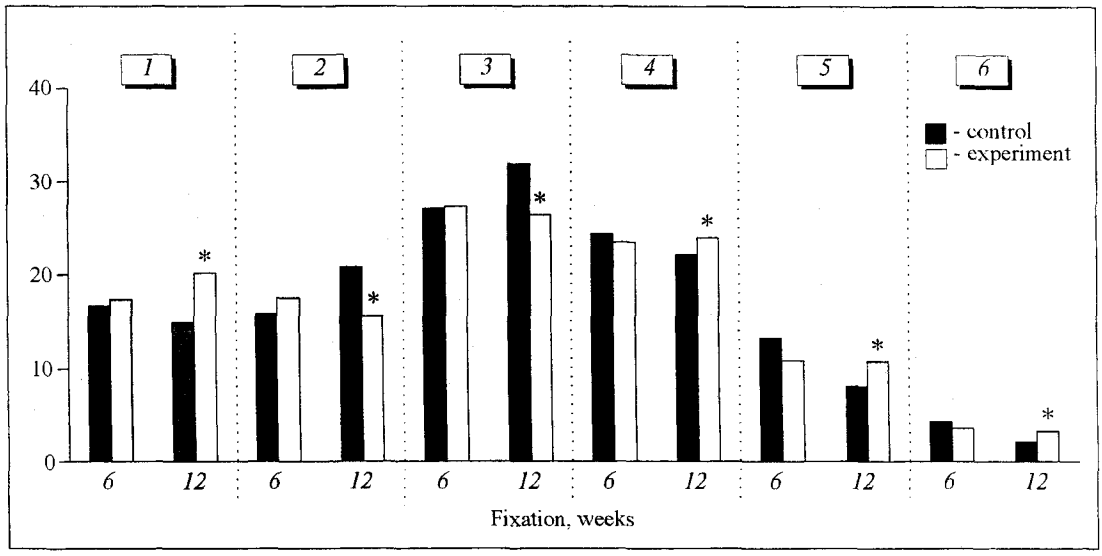

Fig. 3. Distribution of the volume of distraction regenerate occupied by bone tissue between the matrix in the initial stages of mineralization (*1*), degree of bone mineralization: low (*2*), medium (*3*), intermedium (*4*), high (*5*), and maximum (*6*).

of day 28, being filled with ghost DORS of longitudinal striated structure. The regenerative substrate consisted of contacting fractured fragments and criss-cross translucent zone 1-12 mm in height, located distal to the median part of diastasis. There were additional translucent zones in the osteal subdivisions, indicating BML formation. Compactification of the external parts of the osteal subdivisions of DORS, i.e., formation of CP occurred at the ends of the fragments. During fixation the translucent zone was replaced by osteal tissue. At the same time, the size of BML increased. A continuous CP and single BML were then formed. Table 1 shows the roentgenogram-based quantitative estimates of the osteogenic activity and its individual dynamics during fixation, which demonstrate the stimulating effect of the preparation.

Biochemical and morphological data on this effect are listed in Tables 2 and 3 and illustrated by Figs. 1-3. These and previous data suggest that the complex of growth factors deposited by osteal tissue [3,7], i.e., Stimbone-1, stimulates proliferation and differentiation of cambial elements of the connective tissue interlayer (DORS regenerative zone) into osteogenic cells, thus stimulating biosynthetic activity of these cells. Enhanced biosynthesis of osteal organospecific extracellular matrix, which initiates mineralization, acceleration of DORS formation. Six weeks after administration of Stimbone-1, this effect manifests itself as "rejuvenation" of the newly formed bone: an increase in the noncollagenous protein content and a decrease in the crystallization index. However, a tendency toward an increase in the mineralization index was observed during this period.

TABLE 2. Biochemical Parameters of DORS Maturation (*M±m*)

Index, g/100 g tissue	Dogs	Observation period	
		6 weeks of fixation	12 weeks of fixation
Collagen	Control	19.9±3.6	18.2±4.6
	Experiment	19.0±1.5	19.5±1.9
Noncollagenous protein	Control	3.7±0.9	4.8±1.4
	Experiment	2.7±0.9	3.6±1.1
Calcium	Control	16.9±1.2	17.1±0.9
	Experiment	18.6±2.2	21.4±2.4
Phosphorus	Control	9.7±0.7	10.7±0.9
	Experiment	9.8±1.2	10.2±1.4
Low-mineralized fraction	Control	32.7±2.4	15.8±0.9*
	Experiment	15.5±1.5	6.8±3.3*

TABLE 3. Compactification Index of Forming CP of DORS ($M \pm m$)

Observation period, week of fixation	Control	Experiment
6	0.92±0.04	1.34±0.04
12	1.71±0.05	2.25±0.08

Note. Asterisk indicates $p<0.001$ compared with the control values.

The compactification index of growing CP increased considerably. Both biochemical and morphological indices characterizing DORS maturation and roentgenological data indicate that Stimbone-1 has a positive effect on reparative osteogenesis.

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