

# Effect of Panangin on Tissue Repair in Limb Distraction

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The effect of panangin on osteogenesis and on the state of the lymphoid and hemopoietic tissues is studied by distraction osteosynthesis with limb elongation in dogs. The drug eliminates the postoperative depression of immunity and erythropoiesis. Panangin is shown to stimulate repair in the bone tissue.

**Key Words:** *panangin; regeneration; osteogenesis; hemopoiesis; immunity*

Previously we studied the effects of aspartic acid on the lymphoid and hemopoietic cells, particularly its capacity to stimulate the bone marrow stroma, which possesses osteogenic properties [3]. This served as a pathogenetic basis for the use of preparations containing asparagin and its derivatives to optimize the repair processes, including those in the bone tissue. The aim of the present study was to assess the effect of panangin on bone tissue repair and on the state of the hemopoietic and immune systems in the case of elongation of a limb segment by the method of monolocal distraction osteosynthesis after Ilizarov.

## MATERIALS AND METHODS

The experiment was carried out on 16 mongrel dogs aged 1-2 years and weighing 10-12 kg. Osteotomy of the right tibia was performed in 12 control animals (first group) under thiopental anesthesia, after which Ilizarov's apparatus was mounted and shank elongation was started 7 days postoperation at a rate of 1 mm per day. The animals of the second group (4 dogs) underwent the same operation, but they were injected with 0.5 ml/kg panangin intravenously one day prior to the operation as well as 1, 3, and 7 days after it and on the 10th day of distraction.

After sacrifice, distraction osteogenesis was assessed by isolating samples of shinbone, which

were fixed in 10% formalin solution. The regenerated region with bone spicules was then cut out, decalcified in 7% nitric acid solution, dehydrated in alcohols, and embedded in celloidin. Histological sections were stained with hematoxylin-eosin and picrofuchsin. The preparations were inspected visually and analyzed morphologically by counting the number of osteoblasts and osteoblastlike cells in three paired zones of the regenerate along the growth region on 40 areas of an ocular measuring grid, which contained 1000 test points [1].

Parameters of the peripheral blood and bone marrow were determined routinely. The number of rosette-forming cells with either human erythrocytes (E-RFC) or mouse erythrocytes (Em-RFC) was counted to determine the level of T and B lymphocytes, respectively [6].

The results were processed statistically using variational statistics with the Student *t* test and the Wilcoxon-Mann-Whitney *U* test.

## RESULTS

The dogs of the first group developed a moderate depression of the lymphoid tissue, manifested by a fall of the lymphocyte count in the bone marrow and of E-RFC in the peripheral blood, as well as by a tendency toward erythropoiesis inhibition. During the distraction the principal indexes of hemopoiesis and cell immunity normalized (Table 1). The monocytic reaction described previously and typical of the period of activation of osteogenesis in distraction osteosynthesis [5] was

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TABLE 1. Main Parameters of Hemopoiesis and Cell Immunity in Distraction Osteosynthesis

Parameter	Group of animals	Observation period			
		preoperative	beginning	10 days	40 days
			distraction		
E-RFC, $10^9$ /liter	Control	$0.48 \pm 0.06$	$0.19 \pm 0.02^*$	$0.43 \pm 0.08$	$0.57 \pm 0.14$
	Panangin	$0.35 \pm 0.07$	$0.99 \pm 0.11^{***}$	$0.60 \pm 0.05^*$	$0.67 \pm 0.15^*$
Em-RFC, $10^9$ /liter	Control	$0.12 \pm 0.03$	$0.09 \pm 0.02$	$0.11 \pm 0.02$	$0.20 \pm 0.05$
	Panangin	$0.10 \pm 0.02$	$0.33 \pm 0.10^{**}$	$0.31 \pm 0.09^{***}$	$0.38 \pm 0.10^*$
Bone marrow, % erythroid cells	Control	$22.6 \pm 1.9$	$16.7 \pm 2.7$	$29.3 \pm 4.1$	$20.7 \pm 1.8$
	Panangin	$19.1 \pm 1.7$	$18.0 \pm 1.7$	$19.4 \pm 0.8$	$15.0 \pm 0.9^{**}$
granulocytes	Control	$51.4 \pm 1.6$	$65.0 \pm 2.7^*$	$45.4 \pm 5.8$	$53.1 \pm 1.7$
	Panangin	$57.4 \pm 2.8$	$59.6 \pm 1.9$	$55.1 \pm 3.8$	$55.3 \pm 5.1$
lymphocytes	Control	$22.1 \pm 1.1$	$14.3 \pm 0.5^*$	$20.1 \pm 3.9$	$20.9 \pm 1.3$
	Panangin	$20.2 \pm 1.0$	$18.9 \pm 1.8^{**}$	$22.8 \pm 3.8$	$26.0 \pm 3.7$
monocytes	Control	$2.6 \pm 0.7$	$3.0 \pm 0.7$	$3.8 \pm 0.6$	$4.8 \pm 0.2^*$
	Panangin	$2.3 \pm 0.3$	$2.9 \pm 0.3$	$3.1 \pm 0.8$	$4.5 \pm 0.7^*$

Note:  $p < 0.05$ : \* as compared to the preoperative level, \*\* as compared to the control group.

noted at the end of the period of distraction. Panangin abolished the depression of the lymphoid tissue postoperation and, in fact, the E-RFC even increased. Stimulation of the erythroid stem of the bone marrow was not found. An elevated T-cell count in the blood persisted and monocytosis developed during distraction. Therefore, panangin abolished the immunodeficiency and erythroid reaction characteristic of the postoperative period.

Assessment of osteogenesis in the control animals revealed the following. Histotopograms show that bony parts of the regenerate are tightly fused with the ends of the spicules. Bone marrow cavities filled with hemopoietic marrow are found at the base of the regenerate. Longitudinal trabeculae occur in the zone of diastasis. A cortical plate is noted in the peripheral zones at the boundaries of soft tissues. The width of the fibrous tissue preserved in diastasis does not exceed 1/3 of the height of the regenerate. Numerous osteoblasts and sometimes fibroblasts are found at the apex of trabeculae, facing toward the connective tissue zone. The regenerate's contour is twisted due to the interpenetration of new trabeculae of the distal and proximal zones. Resorption of trabeculae with gradual replacement of reticular tissue with hemopoietic and fatty bone marrow occurs in parallel with osteogenesis in the regenerate. This process prevailed in the central parts of the regenerate, whereas signs of the formation of cortical plates appeared on the periphery. The distraction regenerate in dogs treated with panangin seemed analogous, but markedly twisted and with a signifi-

cantly greater number of osteoblasts of varying degrees of differentiation situated on both sides in several layers along the connective tissue layer near collagen fibers. These features attest to more active osteogenic processes in distraction osteosynthesis against the background of panangin. The morphometric data on osteogenic cells (their number was  $105.5 \pm 1.8$  per 1000 test points versus  $86.4 \pm 4.6$  in the control,  $p < 0.05$ ) also confirm this assumption.

Thus, panangin stimulates repair in distraction osteosynthesis, a phenomenon which is probably connected with the activation of the metabolic and proliferative activity of hemopoietic and stromal bone marrow cells [3,4]. Stimulation of the immune system is also important for optimization of the reparative process [2], particularly under conditions of distraction of a limb segment [5].

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