Energy Metabolism in Lymphocytes During Bone Tissue Regeneration

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Phasic changes in the activity of aerobic oxidative processes in blood lymphocytes during distraction of a limb are demonstrated on a model of distraction osteogenesis. The rate of cell respiration during a favorable course of regeneration increases at the end of the postoperative period, at the end of distraction, and during the 9th month after its completion. Unsuccessful regeneration is associated with more marked shifts of lymphocyte respiration. The described changes are related to activation of the function of lymphocytes participating in the regulation of regenerative processes.

Key Words: *lymphocytes; bioenergetics; distraction osteogenesis*

A topic of current interest is the regulation of regenerative processes, in which blood cells play an important role. A concept of lymphocyte-macrophage regulation of repair during distraction has been formulated in recent years [5]. Blood monocytes have been shown to contribute directly and indirectly to bone regeneration [5,8-11]. Specific changes in the lymphocyte count, population composition, and enzymatic status have been detected during bone distraction [3,4,7]. However, the function of lymphocytes during distraction osteogenesis, specifically, the energy metabolism of these cells, is still little known. This prompted us to study some parameters of energy metabolism of lymphocytes during bone regeneration over the course of limb distraction.

MATERIALS AND METHODS

The study was carried out on 34 dogs in which bone tissue regeneration was modeled during monolocal distraction osteosynthesis after transverse osteotomy of the tibia and application of Ilizarov's device. The animals were divided into 2 groups on the basis of clinical and x-ray data: with a favorable (n=23) and unfavorable (n=5) course of regeneration.

The parameters of lymphocyte bioenergetics were studied over the course of distraction osteogenesis (before the operation, before the beginning of distraction, on days 10, 20, and 30 of distraction, and during months 1, 3, 6, 9, and 12 after it). Lymphocytes were isolated from the peripheral blood routinely in a Verographin gradient. Lymphocyte respiration parameters were assessed by polarography using a closed Clarke's electrode in a cell placed in an incubator at 37°C. The lymphocyte respiration rate was assessed in endogenous substrates and in the presence of 10 mM sodium succinate, as well as after the addition of 2,4-dinitrophenol (40 μM), sodium amytal (15 mM), and sodium malonate (10 mM). The cell respiration rate was expressed as the amount of the loss of O_2/min/10^6 cells. The results were processed using variational statistics.

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RESULTS

During a favorable course of osteogenesis the time course of lymphocyte respiration was characterized by a phase pattern with two peaks. The first peak was observed at the end of the postoperative pe-

TABLE 1. Cell Respiration of Lymphocytes During Bone Tissue Regeneration

Time of examination	Respiration rate on endogenous substrates, nmol O_2/min/10^6 cells		Stimulating effect of exogenous succinic acid, %		Stimulating effect of DNP, %	
	normal regeneration	impaired regeneration	normal regeneration	impaired regeneration	normal regeneration	impaired regeneration
Outcome	1.6±0.1 (19)		114.4±3.3 (9)		108.8±3.9 (9)	
Beginning of distraction	2.6±0.1* (5)	1.0±0.1* (3)	132.3±10.3 (3)		119.7±11.4 (3)	
Distraction, day 20	1.4±0.5 (3)	1.1±0.7 (4)	140.8±11.3* (3)	160.0±17.5* (3)	120.1±10.1 (3)	138.5±14.0* (3)
End of distraction	1.9±0.2 (4)	1.3±0.3 (7)	126.0±25.3 (4)	175.0±31.0 (5)	124.5±17.8 (4)	123.0±2.3* (3)
Regeneration: month 1	1.3±0.2 (8)	1.7±0.4 (6)	112.7±11.2 (3)	110.5±7.6 (4)	116.3±2.1 (3)	112.0±10.9 (4)
month 6	1.9±0.3 (3)	3.2±0.6* (4)		141.3±14.7 (3)		
month 9	2.6±0.5* (4)		128.3±9.5 (3)		105.0±5.9 (3)	
month 12	1.6±0.3 (8)	1.3±0.3 (3)	112.6±5.7 (5)	125.6±10.0 (5)	120.2±6.7 (5)	

Note. Number of experiments shown in parentheses. Asterisk shows $p<0.05$ in comparison with initial values.

riod (162%, p=0.001) and was followed by a decline by day 10 of distraction. The second peak occurred during the 9th month after distraction (162%, p=0.05), followed by a final normalization of the parameter at the end of the experiment (Table 1).

In the case of impaired regeneration the time course of this parameter was different. The respiration rate dropped to 63% (p=0.001) at the beginning of distraction and remained low over virtually the entire period of distraction. The sole rise of this parameter developed earlier than the one during a favorable course of regeneration, with the peak observed during the 6th month after distraction (200%, p=0.01).

The addition of exogenous sodium succinate to lymphocytes of animals with different courses of bone regeneration brought about different responses. During a favorable course the index of the stimulating action of succinic acid rose at the beginning of distraction. Its maximum was observed on day 20 of distraction, and by the end of the period it returned to the initial level and remained more or less the same till the end of the experiment. The increase found in this parameter might be due to increased permeability of the lymphocyte membranes or to a deficit of endogenous succinate. During impaired regeneration the stimulating action of succinic acid was more pronounced. Two rises of this paramater were detected, with the peaks at the end of distraction and during the third month after it (153 and 160%, p=0.05). In view of the important role of succinate, which possesses a high energy and plastic potential, in adaptive changes of tissue energy metabolism [1,2], it should be noted that the increased capacity of lymphocytes for its oxidation is indicative of more pronounced shifts of energy metabolism in these cells, particularly during an impaired regenerative process.

Experiments with the addition of 2,4-dinitrophenol (DNP), a respiration and phosphorylation disconnector, showed the extent of DNP stimulating action in intact lymphocytes to be 108%, this pointing to a solid conjugation of oxidative phosphorylation in them. During a favorable course of bone regeneratiopn this parameter changed only negligibly. During unfavorable regeneration the picture was different: the stimulating effect of DNP increased on day 20 of distraction and during the third month after it was over (127 and 137%, p=0.05). This can be regarded as an overall activation of the metabolic oxygen-dependent processes, which is more pronounced in lymphocytes during impaired osteogenesis.

The addition of sodium amytal (inhibiting oxidation of the first segment of the respiratory chain) and sodium malonate (a succinate dehydrogenase inhibitor) did not cause appreciable differences in the rate of cell respiration over the course of the bone repair process. The lack of reliable changes in these parameters may indicate that the optimal ratio of the oxidizing endogenous substrates in the cells examined was unchanged.

Hence, the results point to the development of phase increases in the activities of aerobic oxidative processes in the lymphocytes, which occur in the mitochondria and endoplasmic reticulum. Their stimulation is associated with an increase of lymphocyte functional activity and shifts of population composition observed during activation which occur at the end of the postoperative period, at the end of distraction, and during the 9th month after distraction. A high correlation between the rate of endogenous respiration of lymphocytes, the incorporation of ^{3}H-thymidine, and the T lymphocyte count is observed at the early stage of the repair process [4]. In the case of impaired regeneration the pattern of these relationships is different. That the mechanisms of immunological regulation in the whole body are linked with those of metabolic regulation is self-evident. A study of such a relationship is not only theoretically valuable, extending our knowledge of the reactions of the whole organism to repair processes, but will also help in the development of prognostic and diagnostic tests. For example, it has been demonstrated that disturbed bone regeneration is associated with changes in the cytochemical profile of leukocytes [6]. The metabolic processes provide the structural basis for tissue regeneration. Specifically, they meet the energy and plastic requirements of cells involved in the production of DNA and protein, including bioactive substances (interleukin-1, tumor necrosis factor, etc.) contributing both to the regulation of regenerative processes in somatic tissues and to the stimulation of hemopoiesis in newly formed bone. The more pronounced shifts in lymphocyte respiration on endogenous substrates in the presence of succinic acid and DNP during impaired bone regeneration result from more significant changes in their bioenergetics and seem to be linked to an unfavorable course of repair in tissues. Since the differences in lymphocyte metabolism during a favorable and unfavorable course of regeneration manifest themselves as early as during the postoperative period, this opens up new vistas for devising prognostic tests to assess the course of regeneration in bone tissue.

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