Lymphotropic Effect of Dimephosphon, Mexidol, and Ketorolac is Realized via Activation of the Lymphangion and Stimulation of Lymph Formation

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Single parenteral administration of dimephosphon, mexidol, and ketorolac produces a lymphotropic effect. Dimephosphon directly affects the lymph system by increasing the number of functioning lymph capillaries and contractile activity of the walls and valves of small intestinal mesenteric lymphangions in rats, which leads to stimulation of lymph circulation. Mexidol and ketorolac increased only lymph flow rate in the thoracic lymph duct, which attested to their indirect effect of the lymph system (presumably associated with stimulation of lymph formation). Despite these differences in the mechanisms of lymphotropic effect, all these drugs activate lymph drainage and, hence, transporting function of the lymph system.

Key Words: *lymph capillaries; lymph flow; dimephosphon; mexidol; ketorolac*

The lymph system, one of the key components in the homeostasis and humoral transport, is the most important element of the complex physiological system with defense functions in the body. Disorders in the lymph drainage are essential for the development and outcome of various diseases, while correction of dysfunction of the lymph system is an important aspect of clinical lymphology. On the other hand, the lymphotropic characteristics in the mechanism of action of the majority of drugs are little studied. Elucidation of the mechanisms of pharmacological tropism of drugs to the lymph system components will help to substantiate approaches to target drug correction of abnormalities in the lymph system and to the treatment of pathologies involving the lymph system [5].

The aim of our study was to detect and compare the lymphatic component in the realization of the therapeutic effects of dimephosphon, mexidol, and ketorolac.

MATERIALS AND METHODS

Experiments were carried out on 48 albino rats (200-230 g). Control animals were injected with apyrogenic solution, experimental rats received an injection of one of the studied drugs in a therapeutic dose: 50 mg/kg dimephosphon, 5 mg/kg mexidol, or 0.5 mg/kg ketorolac. Control rats were taken into acute experiment 30 min after injection and experimental rats at the peak of the drug effect. The animals were narcotized with pentobarbital (50 mg/kg).

Flow rate of the lymph released during time unit from the thoracic lymph duct (TLD) punctured at the site of its connection to the left venous angle was evaluated.

Contractile activity of myocytes in the walls and valvular leaflets of small intestinal mesenteric lymph capillaries (LC) was studied by vital micro-

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scopy. The microscopic picture was input into PC using a digital camera. Standard Adobe Premier 6.0 software was used for the analysis of video images. Lymph capillary wall contraction and closure of the valves were recorded using pickups fixed to the videomonitor device. The contraction registration is based on detection of difference in the optical density of the wall or valve in comparison with optical density of the adjacent tissue and capillary lumen.

The percents of lymphangions with spontaneous contractile activity of the wall, with a working valve, with simultaneous functioning of the valves and wall, and without valve and wall contractions were evaluated. The LC diameter was measured during the diastole for 3 sections in the central (free from valves) part of the lymphangion. The arithmetic mean of the capillary diameter was then estimated. The amplitude of LC wall contractions in the part between the valves was estimated in percent of the initial diameter.

The animals were sacrificed by a lethal dose of pentobarbital.

The data were statistically processed using parametrical Student's *t* test.

RESULTS

In control animals, the lymph flow was recorded in 85% lymphangions of 50-250 μ in diameter. Visual inspection showed clearly discernible individual cells in the lymph. The rate of the lymph flow did not depend on LC diameter. This fact is a principal difference between the lymph and blood flow. Noncontracting LC with relaxed walls and valves were seen in the visual field. At the same time, constant spontaneous rhythmic contractions of the wall of the same amplitude were observed in 48% LC, which attested to the involvement of a functionally uniform group of myocytes simultaneously stimulated by the pacemaker in the contractile process [14]. The greater was the lymphangion diameter, the lower was the amplitude of spontaneous contractions. The mean interval between individual contractions was 6 sec. Lymph flow was registered in all LC with phasic contractility. The valvular leaflets were open during the greater part of time, allowing free flow of the lymph. On the other hand, there were cases of successive involvement of the lymphangions in the contractile wave. The walls and valves functioned synchronously and asynchronously; in some LC the walls contracted or valve leaflets closed alone. Wall contractions later caused valve functioning and, vice versa, the work of valves stimulated LC wall contractions.

The percent of lymphangions with spontaneous contractile activity of the wall was 26%, with working valves 5%, with simultaneously working valves and wall 22%. No wall contractions and valvular leaflet closure were detected in 47% cases.

Injections of all the studied drugs to animals significantly increased lymph flow rate in the TLD: dimephosphon by 1.4 times. mexidol by 1.6 times, and ketorolac by 1.5 times (Table 1). Dimephosphon increased in the frequency of spontaneous vasomotions of mesenteric LC walls and valvular leaflets and increased the number of functioning capillaries by 1.5 times. Injections of mexidol and ketorolac did not change contractile activity of the wall and valvular leaflets of LC lymphangion, but the number of functioning capillaries increased by 35% ($p<0.05$) after mexidol injection, while the ratio of lymphangions with different functional activity (presence of phase contractions of wall myocytes and valvular work) did not differ from that in the control. By contrast, ketorolac significantly increased the number of LC with simultaneously functioning wall and valves (45% *vs.* 22% in the control). None of the drugs modified LC diameter and amplitude of contractions of their walls.

The increase in contractility of lymphangion components in our experiments attests to a direct lymphotropic effect of dimephosphon on the lymph circulation. Due to its antioxidant effect, the drug

TABLE 1. Effects of Dimephosphon, Mexidol, and Ketorolac on Lymph Flow Rate in the TLD and on Lymphangion Parameters in the Rat Small Intestinal LC (*M*±*m*)

Parameters	Control	Dimephosphon	Mexidol	Ketorolac
Lymph flow rate, 10^{-2} ml/100 g/sec	0.45±0.04 (*n*=8)	0.62±0.07* (*n*=7)	0.76±0.08* (*n*=8)	0.69±0.06* (*n*=7)
Wall contraction frequency, min^{-1}	8.1±1.03 (*n*=10)	12.10±1.56* (*n*=7)	8.43±1.67 (*n*=7)	7.78±1.27 (*n*=8)
Valve contraction frequency, min^{-1}	5.7±0.76 (*n*=6)	8.50±0.72* (*n*=7)	6.17±0.81 (*n*=7)	6.12±0.84 (*n*=7)
Lymph capillary diameter, μ	138±4 (*n*=8)	147±3 (*n*=7)	144±7 (*n*=7)	135±8 (*n*=7)
Wall contraction amplitude, %	23.5±6.4 (*n*=8)	25.2±5.3 (*n*=7)	24.1±6.7 (*n*=7)	24.8±4.9 (*n*=7)

Note. *$p<0.05$ compared to the control.

activates oxidative synthesis of ATP in mitochondria [6]. Hence, the pacemaker activity of smooth-muscle cells in the LC wall and their sensitivity to transmitters and bioactive substances increase. By stimulating activity of the key enzyme of the pentose-phosphate cycle (glucose-6-phosphate dehydrogenase) responsible for transmembrane K^+ current into cells, dimephosphon provides sufficient content of K^+ in smooth-muscle cells of LC. The presence of K^+ provides an adequate response of these cells to contractile stimuli [12]. The membrane-stabilizing effect of the drug is worthy to note; it is realized at the expense of Ca^{2+} binding, which is paralleled by reduction of its level in the cells [7]. On the other hand, some scientists claim that intracellular Ca^{2+} is the main source of phasic contractions of LC. Pacemaker activity is impossible without available Ca^{2+} in extracellular space and potential-dependent L-type Ca^{2+} channels. These ions trigger the next cycle of contractions and represent a rapidly exchanged Ca^{2+} fraction [4,9,15]. The content of Ca^{2+} determines secretion of NO activating guanylate cyclase in vascular smooth-muscle cells, and increasing the concentration of cyclic guanosine monophosphate, which promoting a reduction of the vascular wall myocyte tone [3]. The function of the lymph flow stimulation can be realized by the lymphangions only on condition of complete relaxation of myocytes [13]. We cannot rule out the indirect effect of dimephosphon on the lymph circulation, which can be due to increased area of functioning capillaries, increased colloid osmotic pressure in the lymph system terminals, and increased filtration capacity of LC, which stimulate the lymph formation processes. This effect of the drug can lead to rapid filling of the lymph bed by new portions of the lymph, which increases the mechanical pressure on LC walls; they are stretched and their motoricity is additionally activated. Acceleration of the lymph flow in the TLD under the effect of ketorolac is presumably due to the capacity of nonsteroid antiinflammatory drugs to improve blood microcirculation and interfere in the cell metabolism processes [8,10,11].

The membrane-stabilizing and antioxidant effects of mexidol [1,2] suggest that the lymphotropic mechanism of its action is due to modification of the microenvironment of membrane receptors on cell surface. This treatment can modify the protein conformation and capacity to bind bioactive substances. As a result of diffuse distribution of the antioxidant in the cell, it modulates the membrane structures, leading to inhibition of LPO processes and inhibiting release of its metabolites into the extracellular space. Our findings indicate that this drug stimulates the release of metabolic products from the interstitium into lymph flow, this initiating stimulation of the lymph formation and lymph outflow. Presumably, activation of blood microcirculation, increase in the hydraulic conduction of the interstitium, and improvement of tissue liquid rheology are essential for the mechanisms of lymph drainage acceleration under the effect of this drug.

Hence, due to their lymphotropic effect, dimephosphon, mexidol, and ketorolac protect the lymph circulation. The mechanism of realization of this effect is different in these drugs: dimephosphon directly stimulates contractile activity of myocytes in LC walls and valvular leaflets, while the effects of mexidol and ketorolac stimulating lymph formation are indirect.

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