Effect of Ketorolac Tromethamine on Lymph Circulation, Contractile Activity of the Lymphangion and Lymphatic Microvessels, and Cellular Composition and Toxicity of the Lymph during Fever

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Single parenteral administration of ketorolac tromethamine produced a lymphotropic effect, which was manifested in acceleration of lymph flow in the thoracic duct and increase in contractile activity of the wall and valves in mesenteric lymphatic microvessels of rats with fever. These changes improved lymph circulation.

Key Words: *fever response; lymphatic microvessels; ketorolac tromethamine; lymph circulation*

Fever response (FR) is one of the first signs of disease that has considerable importance. Until now, treatment with drugs alleviating fever did not take into account their effect on lymph circulation in tissues and lymph components.

Nonsteroid antiinflammatory drugs are extensively used for the correction of FR. Here we studied the influence of ketorolac tromethamine (KT) on lymph circulation, contractile activity of the wall and valve cusps in mesenteric lymphatic microvessels (LM), and cellular composition and toxicity of central lymph during FR.

MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 200-230 g. The animals were divided into 4 groups. Group 1 rats received apyrogenic solution. Pyrogenal in a dose of 100 μg/kg was injected intraperitoneally to group 2 rats. Group 3 rats were treated parenterally with KT (batch R 3018, Dr. Reddy's Laboratories Ltd.) in a therapeutic dose of 0.5 mg/kg. Group 4 rats received the test drug in an equivalent dose 30 min after pyrogenal administration.

The study was conducted on sodium ethaminal-anesthetized rats (50 mg/kg intramuscularly) at stages of temperature rise and fall (2-2.5 and 4-4.5 h after pyrogenal administration, respectively). Lymph flow rate was estimated by the amount of lymph outflowing from the thoracic duct during puncture. Contractile activity of the wall and valve cusps in mesenteric LM was studied by means of vital microscopy. The image was transferred from the digital video camera to a personal computer. Cellular composition of the central lymph was evaluated routinely. Toxicity of the central lymph was determined by calculating the lymphocyte index of intoxication [6]. The animals were euthanized by narcotic overdose. The results were analyzed by Student's *t* test.

RESULTS

Administration of KT accelerated lymph flow in the thoracic duct of group 3 rats by 1.5 times (Table 1). The test drug had no effect on contractile activity of the wall and valve cusps in LM. No differences were revealed in amplitude of vasomotion. Therefore, the

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process of contraction involves functionally similar myocytes that are simultaneously excited by a pacemaker. Administration of KT to intact rats increased the number of LM with simultaneously functioning cusps and wall (45 *vs*. 22% in group 1 animals).

The test drug produced significant changes in the test parameters only at the initial stage of FR. The amount of lymph outflowing from the thoracic duct increased by 45%. The frequency of contractions of the wall and valve cusps in LM increased by 76 and 61%, respectively. After treatment with the test drug during FR, qualitative changes were more significant than quantitative changes. The number of functioning LM and valves increased. Contractions of the lymphangion became more synchronous; the amplitude of contractions increased; group vasomotion disappeared. Lymph flow rate in the lumen of LM remained high. The lymph was transparent. It should be emphasized that during FR the lymph included a considerable number of erythrocytes (Fig. 1). KT had no effect on cellular composition and toxicity of the lymph in intact rats and animals with FR.

KT is a pyrrolysine derivative (α-substituted arylacetic acid, pyrrolysine carboxylic acid) belonging to the group of nonnarcotic nonsteroid antiinflammatory drugs. Antipyretic activity of KT 20-fold surpassed that of acetylsalicylic acid [12]. The therapeutic effect of KT is realized via cyclooxygenase-2 blockade. The side effects of this drug are associated with inhibition of cyclooxygenase-1 [2,7]. Nonsteroid antiinflammatory drugs decrease cyclooxygenase activity in the microsomal fraction of brain cells, which prevents the increase in intracellular cAMP concentration (FR mediator), intracellular Ca^{2+} accumulation, change in the Na^{+}/Ca^{2+} ratio, and reconstruction of centers for heat production and heat loss. Moreover, nonsteroid antiinflammatory drugs suppress free radical reactions and production of macroergic phosphates and cyclic nucleotides during oxidative and glycolytic phosphorylation, inactivate lysosomal hydrolases, inhibit platelet aggregation, impair the synthesis of biogenic amines and kinins, decrease functional activity (at the level of guanosine triphosphate-binding protein) and phagocytic properties of neutrophils, and modulate lymphocyte function. Due to their anionic properties, these drugs permeate the phospholipid bilayer in immunocompetent cell membranes and directly modulate the protein-protein interaction. These changes are followed by cell inhibition in the early stage of a pathological process [11-13,15].

FR is accompanied by the formation of an endogenous pyrogenic substance interleukin-1 (IL-1) and fever mediator prostaglandin E_2. These compounds decrease the frequency of contractions, impair the mechanism for synchronous contractions, and reduce the tone of LM [14]. The effect of KT on LM contraction is probably associated with inhibition of prostaglandin E_2 and IL-1 synthesis. It should be emphasized that sufficient blood circulation in capillaries surrounding mesenteric LM is required to maintain contractile activity of myocytes [10]. Published data show that test drug significantly improves blood microcirculation under pathological conditions [1,3,4,9]. We conclude that this drug maintains pacemaker activity of lymphangions in LM. Our results indicate that high therapeutic effectiveness of KT after endolymphatic administration is associated with the lymphotropic effect [4,5].

Drugs act as lymph stimulators of indirect action, because they accelerate lymph flow due to stimulation of lymph production. However, these changes can cause decompensation under conditions of lymphatic edema [8]. We showed that KT directly modulates lymph production and stimulates contractile activity of the wall and valve cusps in LM. It cannot be excluded that KT produces also an indirect effect on lymph circulation during FR. This effect is associated with an increase in the area of functioning capillaries, rise in colloid osmotic pressure in terminal lymphatic vessels,

TABLE 1. Effect of KT on Lymph Flow Rate in the Thoracic Lymph Duct and Contractile Activity of the Wall and Valve Cusps in Lymphatic Microvessels of Intact Rats and Animals with FR (*M*±*m*)

Parameter	Group 1	Group 2		Group 3	Group 4	
		2-2.5 h	4-4.5 h		2-2.5 h	4-4.5 h
Lymph flow rate, 10^{-2} ml/100 g/sec	0.45±0.04 (*n*=8)	0.75±0.07* (*n*=8)	0.83±0.09* (*n*=8)	0.69±0.06* (*n*=7)	1.08±0.13^{+} (*n*=8)	0.73±0.10 (*n*=7)
Frequency of wall contractions, min^{-1}	8.10±1.03 (*n*=10)	12.30±1.74* (*n*=9)	16.10±1.05* (*n*=9)	7.78±1.27 (*n*=8)	21.66±3.53^{+} (*n*=7)	18.37±1.87 (*n*=6)
Frequency of closure of valve cusps, min^{-1}	5.70±0.76 (*n*=6)	11.10±1.88* (*n*=6)	13.50±1.48* (*n*=6)	6.12±0.84 (*n*=7)	17.90±2.11^{+} (*n*=7)	11.82±1.79 (*n*=6)

Note. $p<0.05$: *compared to group 1; $^{+}$compared to group 2.

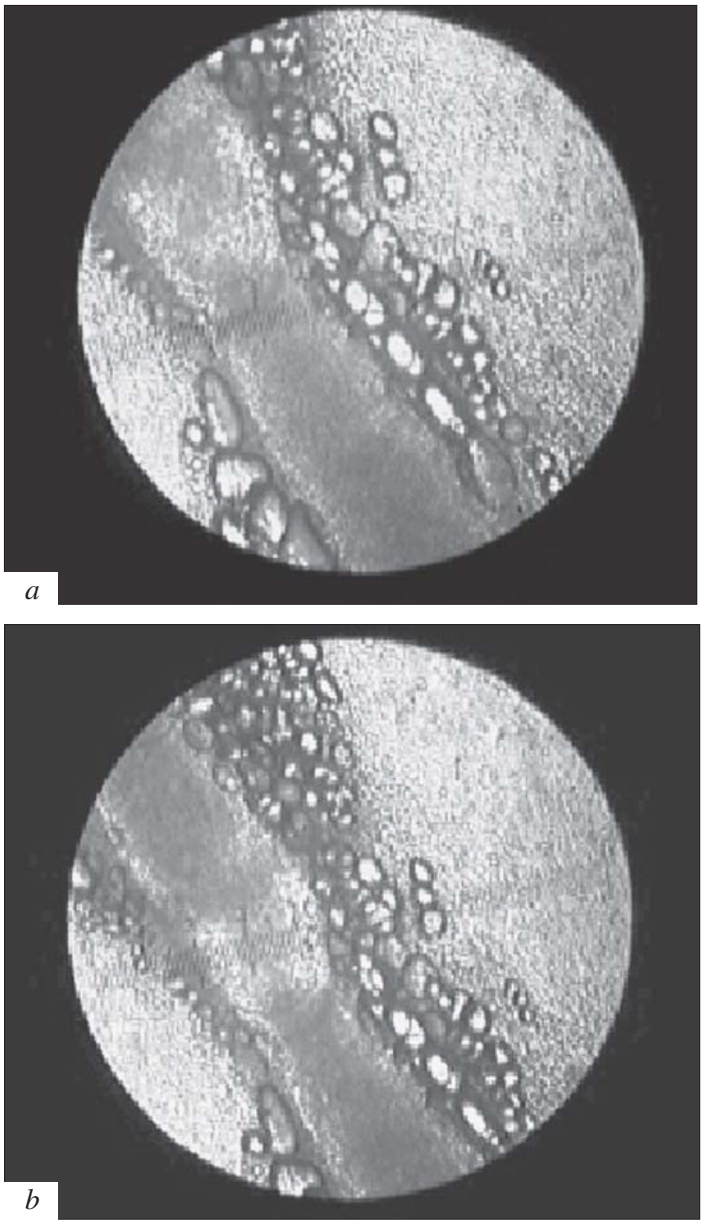

Fig. 1. Contractions of the wall in rat mesenteric lymphatic microvessels during fever: (*a*) relaxation and (*b*) contraction of the wall. Lymph in the lumen of vessel contains a considerable number of erythrocytes. Biomicroscopy, ×125.

and improvement of filtration in LM. These changes contribute to the increase in lymph production.

Our findings indicate that KT produces a lymphotropic effect, increases contractile activity of lymphangions in LM, improves lymph outflow, and stimulates lymph circulation.

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