Effect of Sodium Diclofenac on Microcirculation and Composition of the Lymph during Fever Reaction

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Therapeutic effect of sodium diclofenac during fever reaction is associated not only with specific modulation of thermoregulation, but also with the increase in the count of lymphocytes transported with the lymph into systemic circulation, stimulation of lymph microcirculation, and improvement of metabolism in the interstitial space. This preparation inhibited kininogenesis and stimulated degradation of kinins. Sodium diclofenac serves as a universal inhibitor of the kallikrein-kinin system.

Key Words: *fever reaction; lymphatic microvessels; sodium diclofenac; kallikrein-kinin system; lymph*

Taking into account the new data on the role of the lymphatic system in the pathogenesis of fever reaction (FR) [8], it is necessary to develop and realize the main principles of lymphocorrection of this pathological process (*i.e.*, therapeutic modulation of the structure and function in the lymphatic system). The search for new pharmacological preparations modulating changes in the lymphatic system during FR is of considerable importance in this respect. Possible effects of antipyretics on lymph components and lymphatic drainage in tissues are often underestimated. A complex study is required for evaluation of the effect of antipyretics on the lymphatic system.

Here we studied the effect of a nonsteroid anti-inflammatory preparation sodium diclofenac (SD) on lymph microcirculation, contractile activity of the wall and valves in lymphatic microvessels, content of components in the kallikrein-kinin system (KKS) and proteolytic system, concentration of proteinase inhibitors, and cell composition of the central lymph during FR.

MATERIALS AND METHODS

Experiments were performed on 92 male and female albino rats weighing 180-230 g. FR was produced by intramuscular injection of pyrogenal in a single dose of 100 μg/kg. SD (2.5%, 8 mg/kg) was administered intramuscularly 30 min after LPS injection. The animals were divided into groups 1 (physiological saline), 2 (FR+physiological saline), 3 (SD), and 4 (FR+SD). Acute experiments were performed on animals intramuscularly narcotized with 50 g/kg nembutal at during body temperature rise and drop (2.0-2.5 and 4.0-4.5 h after pyrogenal injection, respectively). The lymph was obtained from the thoracic duct. Lymph microcirculation and contractile activity of the wall and valves in lymphatic microvessels of the small intestine mesentery were studied by vital microscopy. The microscopic image was transferred to a personal computer. KKS components were assayed in the lymph and blood (concentrations of kininogen, prekallikrein, and kallikrein and activity of kininase). Total proteolytic activity and contents of α_2-macroglobulin and proteinase α_1-inhibitor were estimated. The total number of leukocytes in the lymph and count of individual cells in lymph smears were determined routinely.

RESULTS

SD increased the amplitude and frequency of spontaneous activity in the wall and valves of lymphatic microvessels in group 3 rats (by 1.3 times compared

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to group 1 animals, Table 1). The test preparation did not increase the number of vessels, but accelerated lymph flow (compared to group 1 animals).

During body temperature rise, activity of the wall and valves in lymphatic microvessels of group 4 rats was higher than in group 2 animals by 2.3 and 1.8 times, respectively. The number of microvessels with working valves and walls increased up to 65% (*vs.* 52% in group 2 animals). Contractile activity of lymphatic microvessels in group 4 rats remained unchanged 4.0-4.5 h after pyrogenal injection (Table 1). In animals receiving SD contractions of the wall and valves were more synchronous and had lower amplitude variability compared to animals not receiving the preparation.

Kallikrein concentration in the blood and lymph decreased by 1.5 times at various stages of FR, the concentrations of prekallikrein and kininogen and activity of kininase increased in both the lymph (by 2, 1.4, and 1.6 times, respectively) and blood (by 1.8, 1.2, and 1.5 times, respectively) compared to animals not receiving the preparation. However, the content of proteinase inhibitor and proteolytic activity in the blood and lymph remained unchanged under these conditions. Leukocyte number and percentage of lymphocytes in the lymph increased in various periods after administration of SD (particularly at the stage of a body temperature drop).

Antiinflammatory activity of SD is realized via inhibition of cyclooxygenase-2, while its side effects are mainly associated with its influence on cyclooxygenase-1 [3,4]. SD decreases cyclooxygenase activity in the microsomal fraction of brain cells, which prevents intracellular accumulation of cAMP (a mediator of FR) and Ca^{2+}, changes in the Na^{+}/Ca^{2+} ratio and activities of heat production and heat emission centers.

Endogenous pyrogen interleukin-1 (IL-1) formed during FR and fever mediator prostaglandin E_2 decrease the frequency of contractions, impair synchronous contractions, and reduce the tone of lymphatic microvessels. Therefore, these compounds serve as potent inhibitors of phasic activity in lymphatic microvessels. We believe that the effect of SD on microvascular motion is associated with inhibition of prostaglandin E_2 and IL-1 synthesis. Previous studies showed that contractile activity of myocytes in lymphatic vessels depends on blood flow. Sufficient blood circulation in capillaries surrounding mesenteric lymphatic microvessels is required for the maintenance of contractile activity of myocytes. SD significantly improves blood microcirculation under various pathological conditions and, therefore, maintains pacemaker activity of lymphangions in microvessels. SD abolishes the increase in intracellular Ca^{2+} concentration. Published data show that extracellular calcium is the main trigger of phasic contractions of microvessels [1]. Administration of SD during FR not only suppresses the acute-phase response (*e.g.*, fever), but also stimulates contractile activity of lymphatic microvessels. These changes improve lymph outflow and lymph circulation, which contributes to resorption and transport of metabolic cellular and tissue products from the interstitial space.

Most preparations used in the present time act as lymph flow stimulators of indirect action. They increase lymph flow via activation of lymph production. However, under conditions of lymph edema these changes can cause decompensation [2]. Our findings suggest that SD produces direct and indirect effects. This preparation has a complex effect on lymph circulation. On the one hand, SD stimulates contractile activity of the wall and valves in microvessels. On the other hand, SD increases lymph production.

SD is probably a universal inhibitor of KKS. This preparation inhibits kininogenesis and stimulates degradation of kinins. It was hypothesized that activation of KKS is an adaptive reaction during fever. However, administration of SD prevents the imbalance between KKS components and exhaustion or deficiency of this system.

The increase in the number of cells transported with the lymph was associated not only with accelera-

TABLE 1. Effect of SD on the Frequency of Wall Contractions and Valve Closure in Lymphatic Microvessels of the Small Intestine in Rats with FR (*M*±*m*)

Group	Time after pyrogenal administration, h	Frequency of wall contractions, min^{-1}	Frequency of valve closure, min^{-1}
1	—	8.10±1.03 (*n*=10)	5.70±0.76 (*n*=6)
2	2.0-2.5	12.30±1.74** (*n*=9)	11.10±1.88** (*n*=6)
	4.0-4.5	16.10±1.05*** (*n*=7)	13.50±1.48* (*n*=6)
3	—	10.10±1.23* (*n*=8)	7.50±0.95* (*n*=7)
4	2.0-2.5	25.20±2.91^{+} (*n*=10)	20.10±2.56^{+} (*n*=8)
	4.0-4.5	17.80±1.26 (*n*=9)	12.00±1.62 (*n*=8)

Note. *$p<0.001$, **$p<0.05$, and ***$p<0.01$ compared to group 1; $^{+}p<0.001$ compared to group 3.

tion of lymph flow, but also with activation of lymphopoiesis, washout of lymphocytes from lymph nodes, and decrease in blood cell aggregation under the influence of SD.

Our results suggest that the effect of SD during FR is related to stimulation of contractions of lymphatic microvessels, which improves resorption and transport of cell and tissue products from the interstitial space via lymphatic pathways. The preparation inhibits kininogenesis, stimulates degradation of active components in the kinin system, prevents inhibition and exhaustion of KKS, and increases transport function of lymphocytes in the lymphatic system.

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