Lymph Flow and Contractile Activity of Mesenteric Lymph Nodes in Rats with Toxic Hepatitis Effects of Antioxidants

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We studied contractile function of isolated mesenteric lymph nodes in rats with toxic hepatitis. We observed suppression of spontaneous and stimulated contractile activity of mesenteric lymph nodes and changes in biochemical composition of the lymph. We propose a method of correction of these dysfunctions with antioxidant α-tocopherol and Selen-Active.

Key Words: *hepatitis; lymph; lymph flow; Selen-Active; contractile activity of lymph nodes*

Exposure to antropogenic factors increases the prevalence of not only hepatic pathology, but also disturbances in other functional systems of the organism [6]. Wastes from oil and chemical industries containing toxic volatile organics pose a particular hazard to human health. It was established that CCl_4 has a negative effect on blood composition, visceral functions, CNS and peripheral nervous system, impairs hepatic functions [4], enhances lipid peroxidation and free radical generation in the body.

Lymph nodes participate in various processes: transport, drainage, detoxification, barrier, and metabolism [3]. The role of metabolic function of lymph nodes in the development of some pathological processes attracts interest, *e.g.* in the development of toxic hepatitis. However, we found no published data on contractile activity of lymphatic vessels and nodes upon toxic hepatitis in accessible literature. Our previous studies showed suppression of lymph flow and contractile activity of lymph nodes under conditions of acute CCl_4 intoxication [1].

We studied contractile responses of lymph nodes in animals with toxic hepatitis induced by administration of CCl_4 and during correction of the revealed dysfunctions with protective compounds.

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MATERIALS AND METHODS

The study was conducted on 70 white mature male Wistar rats weighing 220-250 g. Group 1 (n=12) consisted of control animals. In group 2 (n=25), experimental toxic hepatitis was modeled; to this end, the animals intraperitoneally received 50% oil solution of CCl_4 (0.3 ml per 100 g body weight 4 times every other day). Correction of homeostasis disturbances produced by $CC1_4$ was performed using Selen-Active and α-tocopherol. Group 3 rats (n=30) received Selen-Active *per os* with water (1.25 g/liter) for 7 days and α-tocopherol intramuscularly in oil solution (1.5 μg/kg) in therapeutic doses [7], then $CC1_4$ solution similar to group 2, followed by Selen-Active and antioxidant for 21 days. Animals of groups 2 and 3 were taken into experiment 30 days later. All experimental groups were housed in a vivarium on a standard diet with free access to food and water.

Selenium is an essential microelement necessary for normal human and animal vital activities, but it can be toxic in large doses. Selenium is an oxidizer with low bioavailability, it acts as antioxidant and immunomodulator (selenoproteins) and is used in medical practice and animal breeding [11].

Lymph flow from intestinal lymph vessel was measured intravitally under ether anesthesia, blood and lymph were sampled for biochemical analysis. Lymph was obtained from the intestinal lymph vessel using graduated microcannula. Contractile activity of isolated lymph nodes was studied routinely [2]. Mes-

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enterial lymph nodes were fixed with wire loops: one end was fixed to the bottom of a horizontal temperature-controlled camera and the other was fixed to a mechanotron 6MX1C sensor. Krebs solution (pH 7.4, 37°C) was used as nutrient solution for isolated rat lymph nodes. The nutrient solution was oxygenated with gas mixture (95% O_2 and 5% CO_2). Vasoactive substances epinephrine hydrochloride, acetylcholine chloride, and histamine dihydrochloride in concentrations 10^{-8}-10^{-3} M were for stimulation of contractile activity of lymph nodes. Node contractions were recorded on H339 and H3012 tape recorders. Total protein content in the lymph and blood plasma was determined using biuret method, ALT and AST activity was measured using Reitman–Frankel method, bilirubin was detected by Jendrassik–Cleghorn–Grof method, thymol test was performed using Huerga and Popper method with thymol-veronal buffer, creatinine was assayed using Jaffe reaction with picric acid, urea was detected using unified method basing on color reaction with diacetylmonoxime using Bio-Lachema-Test reagents according to standard procedure [5]. All experiments were carried out with accordance to bioethic rules, approved by European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Obtained data were processed using variation statistics and Student *t* test. The differences were considered significant at $p<0.01$ and $p<0.05$.

RESULTS

Twenty-one days after CCl_4 administration, lymph flow in group 2 was below the control by 44%, in group 3 it was higher than in group 2. Group 2 rats demonstrated significant decrease of levels of total protein, urea, and creatinine in the lymph and blood plasma. Total protein content in the lymph decreased to 44%, in blood plasma to 30% of the control. In group 3, total protein, urea, and creatinine in blood plasma and lymph were higher than in group 2, but below the control values (Table 1).

In rats from group 2 lymph and plasma ALT level increased by 450-445% and AST increased by 250-252% as compared to control. Total bilirubin level in lymph and plasma was increased by 16-20% and thymol test level was higher by 66-65% than in control (Table 1). These data indicate the development of toxic hepatitis in group 2 rats. It is believed that total blood bilirubin level in case of toxic hepatitis usually increases at later stages of hepatic function impairment [10].

TABLE 1. Lympho- and Hemodynamics and Biochemical Parameters of Rat Lymph and Blood Plasma in Toxic Hepatitis and after Correction with Antioxidants (*M*±*m*)

Index	Group 1	Group 2	Group 3
Lymph flow, ml/h	0.32±0.04	0.17±0.02*	0.27±0.03
Lymph			
Total protein, g/liter	42.3±0.3	23.7±0.2**	33.7±2.1*
Urea, mmol/liter	8.5±0.4	5.4±0.6**	6.8±0.6
Creatinine, μmol /liter	90.0±1.7	70.2±2.3**	82.7±2.2*
ALT, mmol/liter	0.35±0.10	1.57±0.30**	0.53±0.10*
AST, mmol/liter	0.33±0.10	0.82±0.10**	0.46±0.20*
Bilirubin total, μmol/liter	10.4±0.7	12.1±0.9	10.9±0.2
Thymol probe, U	0.30±0.02	0.50±0.06*	0.40±0.04
Blood Plasma			
Total protein, g/liter	68.1±0.5	47.3±1.2**	57.5±2.2*
Urea, mmol/liter	8.3±0.4	5.2±0.3**	7.5±0.5*
Creatinine, μmol/liter	64.2±2.1	52.0±1.3**	57.0±1.4
ALT, mmol/liter	0.60±0.10	2.67+0.20**	0.93±0.20*
AST, mmol/liter	0.44±0.10	1.11±0.20**	0.93±0.30*
Bilirubin total, μmol/liter	9.8±0.7	11.7±0.9	8.7±0.2
Thymol probe, U	0.4±0.1	0.66±0.02*	0.50±0.04

Note. *$p<0.05$, **$p<0.01$ in comparison with the control.

In group 3, the lymph and plasma bilirubin levels after correction remained within the control range. Thymol test decreased after correction, but exceeded the control by 25%. ALT and AST in lymph and blood plasma decreased after correction, but exceeded the control values by 51-54% and 39-43%, respectively (Table 1).

In experiments on mesenteric lymph nodes preparations, phase rhythmic contractions were recorded. In control rats, spontaneous contractions of isolated mesenteric lymph nodes with a frequency of 5.0±0.2 per minute and amplitude of 7.2±0.7 mg were recorded. In case of toxic hepatitis (group 2), the frequency of contractions in mesenteric nodes was 1.3±0.2 per minute and amplitude was 1.2±0.3 mg. Contractile reactions were noted after exposure to vasoactive substances. After correction of homeostasis impairment (group 3), the frequency of contractions in mesenteric isolated lymph nodes was 3.3±0.2 per minute, amplitude was 3.9±0.3 mg.

We performed 90 linear measurements of lymph nodes and 340 physiological observations. In control rats, epinephrine solution in concentrations 10^{-8}-10^{-3} M produced contractile responses of mesenteric lymph nodes in the form of contractions with frequency, increased by 47.0±1.4% and amplitude, increased by 29±1%. Similar responses were produced by acetylcholine (10^{-8}-10^{-3} M). Exposure of lymph nodes to histamine increase frequency and amplitude of contractions by 32.0±1.2 and 27.0±0.9%, respectively.

In group 2 (toxic hepatitis), motor function of lymph nodes and their sensitivity to vasoactive substances sharply decreased. Weaker contractile responses were noted against the background of slow tonic waves (the decrease was 60-65% of the control values). In the majority of experiments, contractile responses of nodes to vasoactive substances against the background of slow tonic waves did not contain rhythmic contractions. In 2-5% experiments, the contractile responses were followed by small rhythmic oscillations. Contractile responses of the mesenteric lymph nodes were induced by epinephrine (10^{-8}-10^{-3} M) in 33% experiments, acetylcholine (10^{-8}-10^{-3} M) in 28% experiments, and histamine (10^{-8}-10^{-3} M) in 30% experiments. In other experiments, no responses were recorded (Fig. 1).

In group 3, spontaneous and stimulated contractile activity of lymph nodes was close to the normal level after correction with protective compounds. Higher level of node responsiveness was noted (70-75%). Contractile responses of lymph nodes were recorded in 125 observations and were induced by epinephrine (10^{-8}-10^{-4} M) in 55 observations, acetylcholine (10^{-8}-10^{-4} M) in 40 observations, and histamine (10^{-8}-10^{-4} M) in 30 observations. Stimulation threshold for vasoactive compounds grew up to 10^{-6} M as compared to the threshold in toxic hepatitis (10^{-4} M). After correction, the percent of responsive nodes was higher and the contractile reactions were more pronounced than in rats not receiving antioxidants (Fig. 1).

These data suggest that lymph flow and total protein content in the plasma and lymph in group 2 rats were reduced, which was associated with suppression of protein synthesis in the liver, and this was the reason for reduced lymph production. Elevated thymol test results and insignificant increase of bilirubin were found, which is typical of toxic hepatitis. Correction of the revealed abnormalities in biochemical composition of blood plasma and lymph in toxic hepatitis with Selen-Active and α-tocopherol for 28 days (group 3) reduced the negative effect of toxic hepatitis on lymph flow dynamics and blood plasma and lymph biochemical composition.

In group 3, the majority of biochemical indices was within the limits of physiological standards with minor deviations. However, ALT and AST in the lymph and plasma remained sufficiently higher than

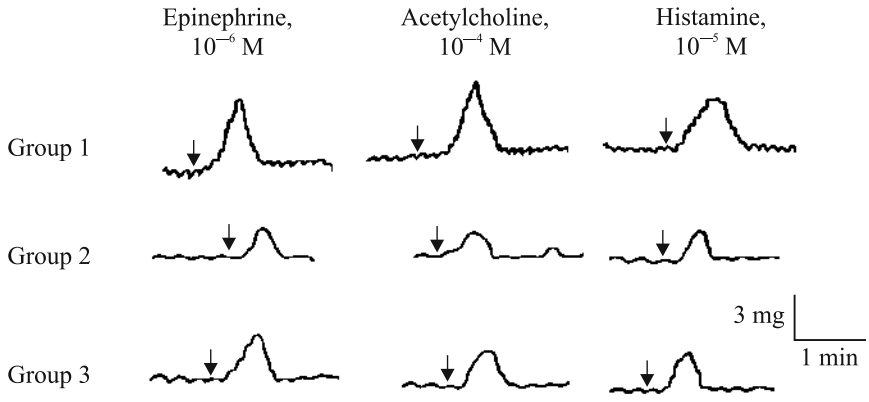

Fig. 1. Contractile responses of mesenteric lymph nodes in case of toxic hepatitis (group 2) and upon simultaneous administration of Selen-Active and α-tocopherol (group 3). Arrow indicates the moment of substance administration.

in the control group. These parameters indicate that hepatic cytolytic processes were still active.

According to published data [14,15], CCl_4 poisoning is followed by protein synthesis disturbances in the liver and ammonium transformation to urea is inhibited, because CCl_4 affects hepatocytes structure and function. In can be hypothesized that the decrease in total protein in rat blood plasma and lymph is associated with inhibition of protein synthesis in the liver and the decrease of lymph flow from the intestinal lymphatic duct is linked to these processes. Apart from biochemical shifts in the blood and lymph, the development of toxic hepatitis was followed by lymph flow deceleration, suppression of lymph node contraction, and decrease in lymph node sensitivity to vasoactive compounds, which is indicative of impaired drainage and transport function of the lymphatic system and aggravates the course of toxic hepatitis.

Treatment with α-tocopherol and Selen-Active before and after CCl_4 administration had a positive effect on organism's resistance to the damaging action of CCl_4, lymphodynamics indices, and lymph composition. Antioxidants can modulate activity of various regulatory systems due to their direct or indirect influence on the synthesis and transformation of various biologically active substances. It has been established that α-tocopherol is one of powerful natural antioxidants and plays an important role in the mechanisms of cell defense from free radicals. Being lipophilic, α-tocopherol acts mainly inside the cells and organelles and also as part of blood plasma lipoproteins, preventing propagation of free radical reactions, associated mainly with lipid radicals [8,13]. It has been established that selenium is a part of enzymes catalyzing complex chemical and metabolic processes and thus participating in the maintenance of cell peroxide homeostasis [9,12]. As part of selenoproteins, selenium acts as antioxidant and immunomodulator.

Thus, due to its high antioxidant activity, Selen-Active improved functional activity of the organism and α-tocopherol inhibited LPO thus improving body defense. Our findings suggest that preliminary prophylactic administration of α-tocopherol and Selen-Active improved organism's resistance to the damaging action of CCl_4. Correction of the observed abnormalities with Selen-Active and α-tocopherol producing a protective effect on cellular membranes and significantly attenuated the development of toxic hepatitis and its suppressing effect on lymph flow, lymphopoesis, and biochemical composition of the lymph.

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