Shifts in the Lymph Flow and the Lymph Composition in Toxic Hepatitis and Their Correction by Protective Substances

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Disorders in the lymph biochemistry were detected in toxic hepatitis induced by injection of CCl_4. Activities of cytolytic enzymes and thymol test values were elevated, while total protein level was reduced. A method for correction of these disorders with natural mineral tagan adsorbent and α-tocopherol (antioxidant) has been developed.

Key Words: *tetrachloromethane; hepatitis; lymph; lymph flow; tagan adsorbent*

Of the chemical pollutants of the environment, industrial toxicants are most hazardous for humans. One of them is a volatile organic compound carbon tetrachloride (CCl_4) promoting the formation of free radicals in human body, stimulating LPO, selectively impairing liver cells, and in severe cases leading to fatty degeneration and necrosis of hepatocytes [5]. Exposure to CCl_4 leads to the development of disorders in protein and urea synthesis and to changes in enzyme activities [2,7].

The lymph system plays an important role in the maintenance of homeostasis and is involved in many pathological processes irrespective of their etiology and pathogenesis. However, the status of the lymph system in toxic hepatitis was never studied, despite its important role in tissue drainage and transport of substances [1].

We studied the lymphodynamics and biochemical composition of the lymph in toxic hepatitis induced by CCl_4 and the methods for correction of metabolic disorders.

MATERIALS AND METHODS

Experiments were carried out on 42 adult male Wistar rats divided into 3 groups. Group 1 (n=14) was control. In group 2 (n=13) animals, toxic hepatitis was induced by 4 intraperitoneal injections of CCl_4 in a dose of 0.3 mg/kg (every other day). Group 3 (n=15) animals received tagan adsorbent in a daily dose of 1 g/kg orally for 7 days and α-tocopherol in oil solution (1.5 μg/kg, intramuscularly), after which they were injected with CCl_4 similarly as in group 2, and then were treated with the enteroadsorbent and antioxidant for 21 days.

All animals were kept under standard vivarium conditions with free access to water and food. Lymph and blood samples were collected under ether narcosis. The lymph was collected from the enteric lymph vessel with a graduated microcannula. All experimental manipulations were carried out in accordance with biological ethics regulations approved by the European Convention for the Protection of Vertebrates Used in Experimental or Other purposes.

Total protein, urea, and creatinine concentrations in the lymph and plasma were measured using Bio-Lachema-Test kits. Plasma activities of ALT and AST and bilirubin content were measured and thymol test was carried out by the routine methods [4]. The homeostasis disorders caused by CCl_4 were corrected by tagan adsorbent (1 g/kg) and α-tocopherol (1.5 μg/kg). Tagan adsorbent is a natural substance, a kind of bentonite. It consists of montmorillonite of the aluminosilicate class (90-95%) and is characterized by high swelling capacity, dispersion, and exchange complex. This therapeutic and preventive preparation was registered as a drug in the Republican List approved

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by the Public Health Committee of the Republic of Kazakhstan (December 30, 1998) [6].

The results of experiments were processed by methods of variation statistics using Student's *t* test. The results were significant at $p<0.01$ and $p<0.05$.

RESULTS

In group 2, all biochemical parameters increased, particularly ALT activity (reached 310% of the control) and AST activity (257% of the control). This indicated activation of cytolytic processes in the liver. Plasma concentration of total bilirubin in group 2 rats increased by 23% compared to the control. These data indicated the development of toxic hepatitis in group 2 animals.

Injection of CCl_4 led to inhibition of the lymph flow from the enteric lymph duct (0.18±0.02 ml/h *vs.* 0.32±0.04 ml/h in the control). The lymph flow decreased by 44%. Mortality in group 2 reached 40%.

The content of total protein in the lymph and plasma decreased significantly in comparison with the control. Plasma level of total protein decreased by 27.5%, lymph level by 36% compared to the control (Fig. 1). The concentration of urea, creatinine, and residual nitrogen in this group of rats decreased by 60, 20, and 26%, respectively. In the lymph, the content of urea dropped by 63%, creatinine by 23%, and residual nitrogen by 20% of the control (Table 1).

After correction of these shifts with protective drugs (group 3), the total protein content in the plasma and lymph increased by 10.0±0.1 and 9.7±0.1 g/liter, respectively, in comparison with the corresponding parameters in group 2, but remained below the control values (Fig. 1). Parameters of nitrogen metabolism also increased in group 3 (Table 1).

These data suggest that CCl_4-induced toxic hepatitis was reproduced in group 2 rats: the values of thymol test and the level of total bilirubin increased and blood levels of ALT and AST were high. The slight shift of total bilirubin level in the blood can be explained by the fact that it usually increases at the late stages of hepatic dysfunction. Correction of the disorders led to improvement of blood biochemistry characterizing liver function (Fig. 2).

The lymph flow and the total protein content in the plasma and lymph decreased in group 2 rats. Presumably, the reduction of total protein levels in the plasma and lymph were caused by low synthesis of protein in the liver, and this led to inhibition of the lymph flow from the enteric lymph duct. According to the latest data, injection of CCl_4 to animals leads to disorders in protein synthesis in the liver and inhibits ammonium transformation into urea, because CCl_4 disturbs the hepatocyte functions and structure [7,8].

We detected a decrease in the levels of the final products of nitrogen metabolism and an increase in plasma ALT and AST activities in CCl_4 intoxication. High blood levels of ALT and AST in our experiments indicate intensification of destructive processes in the liver. A similar effect in hepatic dysfunction in rats with toxic hepatitis was described by other authors [7].

Treatment with tagan adsorbent and α-tocopherol before and after CCl_4 injection improved the resistance to destructive effects of CCl_4 and ameliorated the parameters of lymphodynamics and lymph composition. Antioxidants modulate activities of various regulatory systems. It is explained by their direct or indirect effects on the synthesis and transformations of many bioactive substances. It is known that α-tocopherol blocks LPO [3].

Oral administration of tagan adsorbent and α-tocopherol before CCl_4 intoxication and for 21 days after it improved the lymphodynamics and lymph biochemistry. Some biochemical parameters of the lymph

TABLE 1. Time Course of Nitrogen Metabolites in the Lymph and Plasma in Rats with Toxic Hepatitis

Parameter		Group		
		1	2	3
Plasma	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
	residual nitrogen, μg%	26.6±2.0	20.8±1.2*	23.5±1.2
Lymph	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*
	residual nitrogen, μg%	27.0±3.1	21.6±1.8*	24.5±1.5

Note. *$p<0.05$, **$p<0.01$ compared to the control.

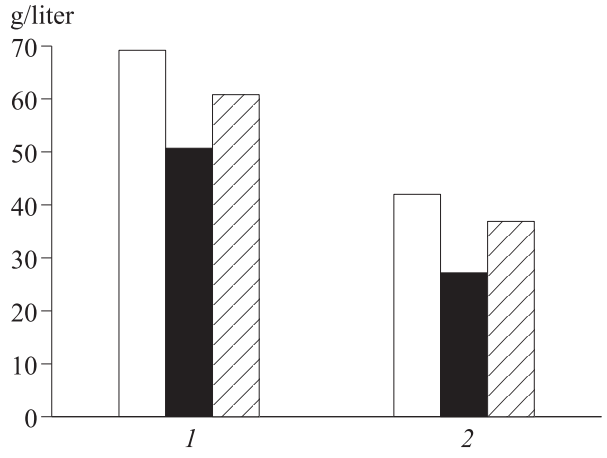

Fig. 1. Time course of total protein in the plasma (*1*) and lymph (*2*) in toxic hepatitis and its correction by protectors. Light bars: control group; dark bars: toxic hepatitis; cross-hatched bars: therapy by tagan adsorbent and antioxidant.

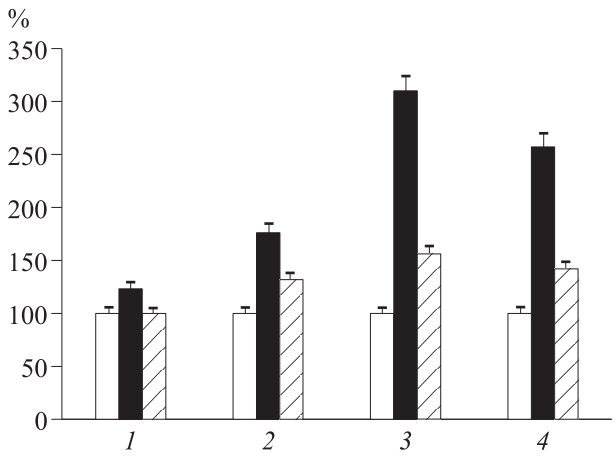

Fig. 2. Time course of plasma biochemistry in rats with toxic hepatitis and its correction by protective substances. *1*) total bilirubin; *2*) thymol test; *3*) ALT; *4*) AST. Light bars: basal values in controls, taken for 100%; dark bars: toxic hepatitis; cross-hatched bars: tagan adsorbent and antioxidant therapy.

and blood returned to normal, but the level of total protein remained below the control. ALT and AST activities tended to normal, though surpassed the control by 15-20%.

Hence, due to its adsorbtion properties, tagan adsorbent promoted detoxication, while α-tocopherol, blocking LPO, stimulated the defense forces of the organism. Increase in the lymph flow and normalization of the transporting function of the lymph nodes accelerate elimination of toxic products from the body.

REFERENCES

1. Yu. I. Borodin, *Trudy IK i EL Sib. Otdel. Rossiisk. Akad. Med. Nauk*, **8**, 5-9 (2000).
2. A. I. Vengerovskii, M. Yu. Kovalenko, V. S. Chuchalin, *et al.*, *Sib. Med. Zh.*, No. 2, 12-14 (2000).
3. V. V. Ivanov and A. M. Al'shanskii, *Byull. Eksp. Biol. Med.*, **94**, No. 7, 40-43 (1982).
4. V. S. Kamyshnikov, *Handbook of Clinical Biochemical Studies and Laboratory Diagnosis* [in Russian], Moscow (2004).
5. G. I. Oksengendler, *Toxins and Organisms* [in Russian], Moscow (1991).
6. E. M. Sapargaliev, L. A. Sapargalieva, K. D. Rakhimov, and G. M. Baturina, *Tagan Adsorbent: A Natural Mineral in Medicine* [in Russian], Ust-Kamenogorsk (2001).
7. M. Jwai, T. Morikowa, A. Muramatsu, *et al.*, *Acta Histochem. Cytochem.*, **33**, No. 1, 17-22 (2000).
8. A. Melin, A. Perromat, and G. Deleris, *Can. J. Physiol. Pharmacol.*, **79**, No. 9, 799-804 (2001).