Pro- and Antioxidants in the Central Lymph in Experimental Chronic Toxic Hepatitis

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Using rat model of chronic toxic hepatitis we showed the involvement of the lymph system in the formation of the response to toxic liver damage consisting in deposition of LPO products in the central lymph and active involvement of antioxidants in their neutralization with redistribution of ceruloplasmin into lymph vessels against the background of reduced α-tocopherol content in the central lymph.

Key Words: *lymph system; lipid peroxidation; chronic toxic hepatitis*

Liver diseases lead to significant changes in cell structure and function, disturb the endoecological balance, and eventuate in failure of the draining and detoxication processes with the development of generalized endotoxicosis. It is assumed that the release of tissue degradation products through the lymph into the blood is the leading mechanism in the development of endotoxicosis [1]. LPO processes play the key role in the pathogenesis of chronic hepatitis [10], and hence, study of the pro- and antioxidant status of the lymph and evaluation of the plasma and lymph pro- and antioxidant proportions is essential for subsequent development of approaches to correction of oxidative stress.

We studied LPO processes in the central lymph and detected regularities in the plasma and lymph redistribution of LPO products and antioxidants in intact rats and animals with chronic toxic hepatitis.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (220 g). Chronic toxic hepatitis was induced by administration of tetrachloromethane in mineral oil and ethanol (through a tube) for 4 weeks [8]; mortality at the stage of modeling was 40%. Animals receiving mineral oil without tetrachloromethane served as controls. LPO parameters and the levels of antioxidants were studied immediately after toxic treatment and on days 14 and 28 after it was discontinued (on days 42 and 56 of the experiment, 10-12 animals per term). We previously showed that laboratory manifestations of the cytolytic and cholestatic syndromes, inhibition of the microsomal oxidation enzyme systems, and activation of LPO processes in the microsomal fraction of the liver peaked on day 28 of intoxication [6].

The lymph was collected from the thoracic duct cistern under thiopental narcosis [3]. The levels of LPO products, MDA and conjugated dienes (CD), in the plasma and central lymph were measured. The antioxidant status was evaluated by the content of α-tocopherol and ceruloplasmin. The lipid oxidation index (OI) was expressed in arbitrary units [7]. The redistribution of the studied parameters in the plasma-central lymph system was evaluated by the plasma/lymph index (PLI) [2] expressed in arbitrary units. A 4-week treatment of controls with mineral oil caused virtually no changes in the studied parameters.

The results were processed using Student's *t* test.

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RESULTS

In controls, plasma concentration of CD was 2.97 times higher than in the lymph, while plasma lipid OI was by 16% higher than in the central lymph (Table 1). No differences between the plasma and lymph concentrations of MDA were detected in controls, but a trend to an increase in plasma MDA level in comparison with the central lymph was noted. The concentration of α-tocopherol in the lymph of these animals was 1.45 times higher than in the plasma (Table 2). This was presumably due to absorption of α-tocopherol from the intestine directly into the small intestinal lymph vessels [11]. Plasma concentration of ceruloplasmin in controls 1.9-fold surpassed its concentration in the central lymph. Hence, accumulation of LPO products and ceruloplasmin in the plasma were observed in these animals, with predominant accumulation of α-tocopherol in the central lymph (Fig. 1).

Immediately after intoxication, plasma CD concentration decreased by 21%, presumably because of active utilization of intermediate LPO products in peroxidation reactions [4]. This was paralleled by a 2.6-fold increase in CD concentration in the central lymph in comparison with the control group. Plasma CD concentration remained low 14 and 28 days after intoxication in comparison with intact animals, while CD concentration in the lymph decreased and after 14 days surpassed the control by 1.4 times and after 28 days virtually did not differ from the control. Plasma and lymph MDA concentrations increased directly after intoxication (1.66 times in the plasma and 3.17 times in the lymph) and decreased 14 days after intoxication, but remained high in comparison with intact animals. After 28 days, plasma MDA concentration was by 29% higher than in the control, while the concentration in the lymph virtually did not differ from the control.

Directly after intoxication, plasma OI increased by 28% and lymph OI increased by 92% in comparison with the control. After 14 days, plasma OI did not differ from the control, while lymph OI increased by 52%. Plasma and lymph OI values 28 days after intoxication virtually did not differ from the control.

Measurement of α-tocopherol over the course of intoxication showed no appreciable shifts in its plasma concentration in rats (Table 2). Its concentration in the central lymph decreased by 48% in comparison with the control immediately after intoxication. After intoxication was over, in α-tocopherol concentration the lymph gradually increased, but did not reach the level in observed intact rats, and 28 days after intoxication remained below the control by 27%. Plasma ceruloplasmin level directly after intoxication did not change in comparison with the control. On the other hand, the con-

TABLE 1. Content of LPO Products and Lipid OI in the Plasma and Central Lymph of Rats with Chronic Toxic Hepatitis (*M*±*m*; *n*=10-12)

Object of study	Parameter	Control	Day after intoxication		
			0	14	28
Plasma	CD, E233/mg lipids	0.98±0.10	0.77±0.09*	0.68±0.04*	0.62±0.06*
	MDA, nmol/ml	1.89±0.27	3.14±0.30*	2.47±0.21*	2.44±0.27*
	OI	0.29±0.01	0.37±0.02*	0.27±0.01	0.26±0.03
Lymph	CD, E233/mg lipids	0.33±0.04+	0.87±0.07*	0.45±0.03*+	0.38±0.03+
	MDA, nmol/ml	1.44±0.21	4.57±0.46*+	2.85±0.27*	1.80±0.15+
	OI	0.25±0.01*	0.48±0.03*+	0.38±0.02*+	0.28±0.02

Note. Here and in Table 2: $p<0.05$ compared to: *control, +blood plasma.

TABLE 2. Content of Antioxidants in the Plasma and Central Lymph of Rats with Chronic Toxic Hepatitis (*M*±*m*; *n*=10-12)

Object of study	Parameter	Control	Day after intoxication		
			0	14	28
Plasma	α-Tocopherol, μg/ml	4.20±0.45	4.27±0.43	4.14±0.47	4.23±0.40
	Ceruloplasmin, mg %	33.90±1.83	38.72±3.07	42.68±2.04*	35.97±1.71
Lymph	α-Tocopherol, μg/ml	6.09±0.69+	3.16±0.39*+	3.95±0.35*	4.47±0.45*
	Ceruloplasmin, mg %	17.85±1.13+	24.06±2.16*+	29.54±2.43*+	27.57±1.98*+

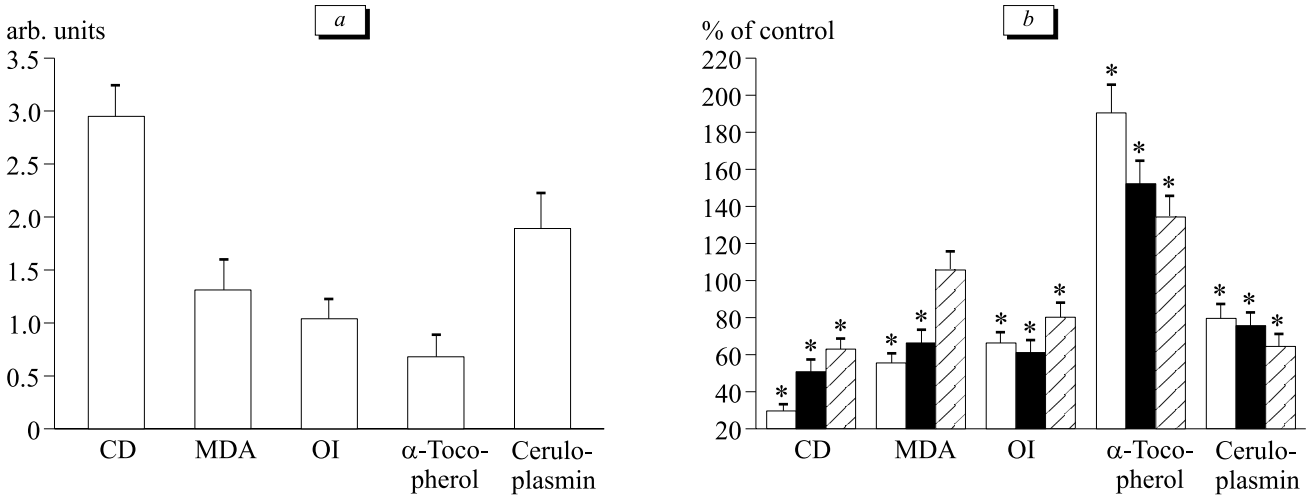

Fig. 1. The LPO products and antioxidant PLI values in control (*a*) and experimental animals (*b*). *b*: light bars: day 28; dark bars: day 42; cross-hatched bars: day 56. **p*<0.05 compared to the control.

centration of ceruloplasmin in the central lymph of these animals was 1.35 times elevated in comparison with the control. Ceruloplasmin concentration in the lymph was elevated throughout the entire recovery period in comparison with the control (by 66% on day 14 and by 55% on day 28).

The development of chronic toxic hepatitis was associated with significant reduction of the CD, MDA, OI, and ceruloplasmin PLI and an increase of α-tocopherol PLI (Fig. 1). This confirmed the lymph attraction phenomenon [3] for LPO products and for such a high molecular weight antioxidant as ceruloplasmin, the main plasma antioxidant [9]. A reciprocal relationship between ceruloplasmin and α-tocopherol concentrations in the central lymph (control: r=-0.597, p<0.05; directly after intoxication: r=-0.773, p<0.01; after 14 days: r=-0.690, p<0.01; after 28 days: r=-0.721, p<0.01) and a positive correlation between ceruloplasmin concentrations and lipid OI in the central lymph were noted (control: r=0.372, p>0.05; directly after intoxication: r=0.714, p<0.01; after 14 days: r=0.549, p<0.05; after 28 days: r=0.735, p<0.01).

Hence, the lymph system serves as a sort of a "depot" for LPO products under conditions of chronic intoxication of the liver. This is paralleled by development of lymphotoxicosis [1,5] with absolute and percent accumulation of LPO products in the central lymph. Increased content of LPO products in the central lymph is paralleled by a reduction of α-tocopherol concentration and an increase in ceruloplasmin concentration. Hence, the lymph system, as a regulator of oxidative homeostasis, can be regarded as a target for correction during the development of chronic toxic hepatitis.

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