Role of Peritoneal Macrophages in the Effect of Natural Metabolites in Toxic Hepatitis

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It is demonstrated that under the action of CCl_4 cytosol of rat pup liver activates peritoneal macrophages with various enzyme activity. The immunomodulating effect of liver cytosol manifests itself in normalization of cellular and humoral parameters of natural resistance.

Key Words: *inflammation; macrophages; liver cytosol*

It is generally recognized that mononuclear phagocytes are involved in the expression of surface structural antigens and receptors [1-4]. Macrophages are polypotent cells performing regulatory and effector functions in immunogenesis, inflammation, infection, and cell proliferation. The course of dystrophic processes in the liver and the protection of hepatic tissues from damage are largely dependent on macrophages [4,5]. Secretions of liver macrophages modify the organism's resistance to infection (via lysozyme and phagocytic activity). Under the toxic influence of CCl_4 liver macrophages cooperate with lymphocytes and fibroblasts. Not only the activity of the phlogogenic agent but also that of the mediators of cell-cell interactions depend on the secretion and reactivity of macrophages [2-7].

Our objective was to study the response of mononuclear phagocytes to natural metabolites (liver cytosol, LC) stimulating proliferation in the liver and producing an immunomodulating effect.

MATERIALS AND METHODS

The macrophagal response to intraperitoneal injection of LC was studied in 48 male Wistar rats of similar body weight. The animals were assigned to four equal groups (n=12). Group 1 consisted of intact animals and served as the control, group 2 animals were injected with 0.5 ml LC, group 3 animals were injected with 40% CCl_4 in olive oil in a dose of 0.1 ml/100 g body weight, and group 4 rats were injected with LC and CCl_4 in the above-mentioned doses. Liver cytosol was prepared from homogenized livers of 4-7-day-old rat pups. The homogenate was centrifuged in normal saline at 6000 rpm, and the supernatant was used in the experiments. The LC preparation contained 3 volumes of normal saline and 1 volume of supernatant.

The enzyme activity of peritoneal macrophages was assayed in a KFK-2MP spectrophotometer 48 and 72 h after CCl_4 and LC injection. The activities of lactate dehydrogenase (LDH) [8], cytochrome oxidase (CCO) [10], and glucose-6-phosphate dehydrogenase (G-6-PD) [9] were measured. The enzyme activities were expressed in minute enzyme units (MEU) per 10^6 cells. An MEU was defined as the amount of enzyme that converts 1 nM substrate in 1 min under optimal conditions.

Phagocytic activity of macrophages and serum lysozyme activity were used as the parameters of natural resistance. Phagocytic activity was evaluated in a 24-h culture of *Staphylococcus alba* at 39°C. Blood smears were fixed in methanol and stained with azure II-eosin, and the phagocytosis index was determined. Serum lysozyme activity was determined nephelometrically in a KFK-2MP photoelectric colorimeter with the use of a 24-h culture of *Bacterium lysodeiticus*. The enzyme activity was calculated from the following formula: $(D_1\text{-}D_2)/D_1 \times 100\%$, where D_1 and D_2 are the pa-

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TABLE 1. Activity of Macrophage Enzymes (MEU/10^6 Cells) after Intraperitoneal Administration of LC of Newborn Rat Pups in CCl_4 Intoxication ($M \pm m$)

Experimental group	Observation period, h	Enzyme activity		
		LDH	CCO	G-6-PD
Intact rats		42.96±8.48	0.78±0.04	5.64±1.03
Administration of LC	48	149.01±12.3**	3.96±0.6*	10.28±1.87*
	72	68.72±6.02**	1.44±0.7***	6.21±0.91***
CCl_4 intoxication	48	206.40±15.26***	0.42±0.09**	1.26±0.06*
	72	190.10±12.06***	0.31±0.10**	1.20±0.03**
Administration of LC in CCl_4 intoxication	48	166.80±12.5**	2.06±0.30*	4.29±1.19*
	72	88.40±9.7*	1.04±0.20**	6.01±2.03***

Note. *$p<0.001$; **$p<0.01$; ***$p<0.05$ compared with intact rats.

rameters of the experimental and control cuvettes, respectively, after a 1-h incubation at 39°C.

Statistical processing included determination of the mean, error of the mean, comparison of the means using Student's t test, and correlation analysis.

RESULTS

A marked reduction in oxidative phosphorylation and a noticeable increase in glycolysis were observed in rat peritoneal macrophages 48 h after the administration of CCl_4 (Table 1). The activity of the hexose monophosphate shunt enzymes simultaneously decreased. A single administration of LC against the background of CCl_4 inhibited glycolysis after 48 h, the effect being more pronounced after 72 h. Administration of LC stimulated the oxidative phosphorylation enzymes and abolished the blockade of the hexose monophosphate shunt. Meanwhile, the activity of G-6-PD remained virtually the same as that in the control.

The boost in the activity of the studied enzymes 48 h after the administration of LC may be due to the fact that new populations of mononuclear cells with markedly decreased enzyme activity appeared in the peritoneal exudate. Presumably, under the action of LC activated macrophages migrated primarily from abdominal lymphatic structures but could also have been activated by LC at the site.

Administration of LC against the background of CCl_4 normalized lysozyme and phagocytic activity (Table 2). A strong positive correlation ($r=0.8$) between lysozyme and phagocytic activity was established in intact rats (group 1). In groups 2 and 4, the correlation between phagocytic and lysozyme activity was negative ($r=-0.5$ and $r=-0.4$, respectively) compared with group 3. Administration of LC against the background of CCl_4 normalized phagocytic activity and markedly suppressed the lysozyme reaction, which is very pronounced in acute intoxication.

Administration of LC against the background of CCl_4 also markedly reduced serum lysozyme activity. Table 2 illustrates the immunomodulating effect of LC on natural resistance in acute CCl_4 intoxication.

Thus, LC of newborn rat pups activates peritoneal macrophages in CCl_4 intoxication. The different activities of LDH, CCO and G-6-PD in the peritoneal macrophage population imply a decrease in the activity of glycolytic enzymes, an increase in oxidative phosphorylation with normalization of the enzymes of the hexose monophosphate shunt, and a redistribution of the intact and activated macrophage populations. Presumably, LC induces monocytopoiesis, which is markedly inhibited in CCl_4 intoxication. The immunomodulating effect of LC in toxic hepatitis manifests itself in the normalization of cellular and humoral parameters of natural resistance.

TABLE 2. Changes of Cellular and Humoral Factors of Natural Resistance upon Correction of Toxic Hepatitis with LC (72 h after Administration)

Experimental group	Parameter	Mean value, %	Mean square deviation	Coefficient of variation
Intact rats	Lysozyme	9.37	15.23	1.625
	Phagocytic activity	2.48	1.56	0.629
Administration of LC	Lysozyme	3.97	6.87	1.730
	Phagocytic activity	3.28	1.29	0.393
CCl_4 intoxication	Lysozyme	25.70	32.53	1.265
	Phagocytic activity	1.94	1.06	0.547
Administration of LC in CCl_4 intoxication	Lysozyme	10.87	16.85	1.549
	Phagocytic activity	3.58	3.74	1.043

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