Liver Resistance to CCl_4-Induced Injury after Stimulation of Macrophages with Various Preparations

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 6, pp. 620-622, June, 2000
Original article submitted April 5, 2000

Acute toxic hepatitis in male Wistar rats was produced by single injection of 40% CCl_4 (0.2 ml per 100 g body weight in oil). Pretreatment with various immunostimulators (bacterial polysaccharides prodigiozan and salmozan; yeast polysaccharides zymosan, peptidoglycan, and mannan; and hydrolytic enzyme egg lysozyme) produced a hepatoprotective effect correlating which the stimulatory influence on macrophages and increasing in the following order: mannan<peptidoglycan<zymosan<lysozyme<salmozan<prodigiozan.

Key Words: *macrophages; Kupffer cells; liver; CCl_4*

Our previous studies showed that substances stimulating mononuclear phagocytes protect hepatocytes from damage induced by tetrachloromethane (CCl_4) in experimental animals. Hepatoprotective effects are primarily related to activation of liver macrophages (MP), *i.e.*, Kupffer cells (KC) [2,3].

There are many MP-stimulating agents, including natural high-molecular-weight compounds (polysaccharides of pathogenic and nonpathogenic bacteria, yeasts, and actinomycetes) and purified and recombinant products of MP used for experimental modeling and prevention of stress-induced damages. These compounds produce diverse effects on MP, which probably explains their opposite effects on organism's resistance. Here we studied the effects of MP-stimulating substances on hepatocytes resistance to CCl_4-induced injury.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-220 g fed standard laboratory diet. Acute toxic hepatitis was produced by single subcutaneous injection of 40% CCl_4 (oil solution, 0.2 ml per 100 g body weight). Experimental animals received the following MP-stimulating substances: bacterial polysaccharides prodigiozan from gram-negative bacteria *S. marcescens* (Central Institute of Postgraduate Medical Education, 250 μg/kg) and salmozan from *S. typhy* (N. F. Gamaleya Institute of Epidemiology and Microbiology, 1 mg/kg) (intraperitoneally 24 h before CCl_4 administration); yeast polysaccharides zymosan from *Candida albicans* (Biokhimreaktiv, 5 mg/kg intravenously 4 days before CCl_4 administration), peptidoglycan from *Saccharomyces cerevisiae* (Fluka, 100 mg/kg), and mannan (major *Candida albicans* polysaccharide, Fluka, 2 mg/kg); and egg lysozyme (Sigma, 5 mg/kg). Control rats received an equivalent volume of 0.85% NaCl. The animals were decapitated 24, 48, and 72 h after administration of CCl_4. The degree of liver damages was estimated by morphometry of necrotic zones and hydropic balloon and acidophilic degeneration on slices stained with hematoxylin and eosin and by plasma activity of alanine transaminase (ALT) [11]. Functional activity of KC was estimated by the rate of colloidal carbon clearance (Gunter Wagner) from the blood [5]. The content of prostaglandins in whole liver homogenates was estimated using a ^3H Prostaglandin E RIA kit (Travel. Diagnost.).

RESULTS

Injection of MP-stimulating substances considerably enhanced absorbing properties of mononuclear phagocytes (primarily KC), which was confirmed by accele-

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0007-4888/00/0006-524$25.00

rated clearance of colloidal carbon. Prodigiozan, salmozan, zymosan, and lysozyme decreased the half-time of colloidal carbon removal ($T_{1/2}$) by 2.6, 2.4, 3.0, and 2.2 times, respectively, compared to that in intact rats ($p<0.001$, Table 1).

Serum ALT activity 24 h after CCl_4 administration 6-fold surpassed the control value ($p<0.001$, Table 2). Foci of centrolobular necroses and balloon hepatocyte degeneration (morphological signs of acute toxic hepatitis) were revealed in the liver at this period. The lesions occupied 57.5%cross-section area. Forty-eight hours after CCl_4 administration, destructive changes in the liver were less pronounced, and damaged regions were infiltrated with inflammatory cells. Seventy-two hours postinjection, we found signs of hepatocyte degeneration and inflammatory infiltration. Pretreatment with bacterial polysaccharides produced a hepatoprotective effect. Preliminary injection of prodigiozan and salmozan 3.3- and 2.7-fold decreased serum ALT activity, respectively, 24 h after CCl_4 administration ($p<0.01$ compared to the control, Table 2). Only solitary necroses of hepatocytes and moderate degenerative changes (granular acidophilic and fatty degeneration) were detected in the liver. The area of lesions decreased by 1.9 times ($p<0.01$). Forty-eight hours after CCl_4 administration, we found only insignificant signs of fatty degeneration of hepatocytes and mononuclear infiltration. The ultrastructure of hepatocytes was restored 72 h postinjection.

Yeast polysaccharide zymosan produced similar effects. Serum ALT activity was reduced by 3 times ($p<0.01$), and the area of CCl_4-induced damages to the parenchyma decreased by 1.8 times compared to the control ($p<0.01$). Stimulation of KC with peptidoglycan and mannan produced less pronounced hepatoprotective effects: serum ALT activity decreased by 2.5 and 2.0 times ($p<0.05$), and the area of CCl_4-induced damages to hepatocytes decreased by 1.5 and 1.3 times ($p<0.05$), respectively. Forty-eight hours after CCl_4 administration, only residual acidophilic and fatty degeneration and inflammatory infiltration were observed. Minor signs of inflammatory infiltration persisted also 72 h postinjection; the ultrastructure of hepatocytes was practically restored.

Lysozyme attenuated CCl_4-induced damages to the liver tissue and reduced serum ALT activity by 2.6 times. Hepatocytes with moderately vacuolated cytoplasm predominated in the central part of hepatic lobules. Solitary cells with signs of hydropic balloon degeneration were revealed. The area of lesions decreased by 2.1 times compared to the control ($p<0.01$). Zones of destructive lesions were infiltrated with inflammatory mononuclear cells. Forty-eight hours after CCl_4 administration, we revealed only insignificant signs of inflammatory mononuclear infiltration and proliferation of hepatocytes; 72 h postinjection, hepatocyte ultrastructure was completely restored.

Hence, pretreatment of KC with various stimulating substances enhances MP-dependent hepatoprotective mechanisms, prevents structural and metabolic damages to the liver tissue and, therefore, activates reparative processes. Bacterial polysaccharides, lysozyme, and yeast polysaccharide zymosan were most effective in protecting the liver, while purified yeast polysaccharides mannan and peptidoglycan were less potent. Hepatoprotective effects decreased in the following order: prodigiozan>salmozan>lysozyme>zymosan>peptidoglycan>mannan.

There are data that activation of MP is accompanied by inhibition of cytochrome P-450 in the liver [4]. In this case, interferons [10], interleukin-1 [13], and tumor necrosis factor [9] secreted by activated MP act as inhibitory monokines. The amount and type of synthesized products depend on the nature, dose, molecular weight, physical properties, chemical composition, and structure of the stimulating substance, as well as on the reciprocal regulation by MP mediators. Synthesis and cytoprotective effects of the majority of

TABLE 1. Blood Clearance of Colloidal Carbon and Content of Prostaglandin E in Rat Liver Homogenates after Stimulation of MP with Various Preparations ($M\pm m$, $n=6$)

Group	$T_{1/2}$, min	Clearance indexes	Prostaglandin E, pg/500 mg liver tissue
Intact	9.5±1.5	0.033±0.002	413.4±78.8
Prodigiozan	3.61±0.24*	0.079±0.015*	2691.0±321.3*
Salmozan	3.91±0.28*	0.077±0.009*	—
Zymosan	3.17±0.18*	0.095±0.017*	3020.2±110.1*
Lysozyme	4.30±0.48*	0.070±0.012*	1951.0±66.2*
Peptidoglycan	5.10±0.32**	0.059±0.005**	—
Mannan	6.64±0.20***	0.044±0.007***	1221.0±245.7*

Note. *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to intact rats.

TABLE 2. Effects of MP-Stimulating Substances on CCl_4-Induced Damage to the Liver ($M\pm m$, n=6)

Group	ALT, U	Area of damage	
		arb. Units	%
0.9% NaCl+CCl_4 (control)	1.8±0.1	20.70±0.09	57.5
Prodigiozan+CCl_4	0.54±0.07*	10.90±0.54*	30.3*
Salmozan+CCl_4	0.68±0.12*	11.1±0.9*	30.8*
Zymosan+CCl_4	0.60±0.14*	11.8±1.3**	32.7**
Peptidoglycan+CCl_4	0.72±0.12**	13.9±0.7*	38.6*
Mannan+CCl_4	0.90±0.09**	16.0±0.6**	44.4**
Lysozyme+CCl_4	0.70±0.13*	9.8±1.2*	31.2*

Note. *p<0.01 and **p<0.05 compared to the control.

macrophagic factors depend on prostaglandin E [12] produced (for the most part) by activated KC [6]. Five hours after single stimulation of KC with prodigiozan, zymosan, lysozyme, and mannan, the content of prostaglandin E in liver homogenates increased by 6.5, 6.7, 4.72, and 3.0 times, respectively (p<0.01, Table 1). It can not be excluded that high resistance to CCl_4 after stimulation of KC is associated with increased production of prostaglandin E in the liver. These results are consistent with previous data showing hepatoprotective [1] and inhibitory [7,8] effects of prostaglandin E on microsomal monooxygenase activity in hepatocytes.

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