EXPERIMENTAL BIOLOGY

Liver Resistance to Toxic Effects of CCl_4 under Conditions of Gadolinium Chloride Depression of Kupffer Cells

D. D. Cyrendorzhiev, S. N. Kutina, and A. A. Zubakhin

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Acute toxic hepatitis was modeled in (CBA×C57B1)F1 mice by single injection of 40% CCl_4 in oil. Pretreatment with gadolinium chloride, a selective blocker of Kupffer cells, considerably potentiated damage to hepatocytes leading to generalization of this process, delayed inflammatory infiltration, and inhibited reparative processes. Zymosan administered against the background of gadolinium chloride blockade improved liver resistance to CCl_4-induced damage, intensified mononuclear infiltration, and accelerated reparative processes.

Key words: *Kupffer cell; tetrachloromethane; liver*

Our previous experiments demonstrated that short-time blockade of Kupffer cells (KC) by colloidal carbon, observed 2 h after its injection, enhanced liver injury caused by tetrachloromethane (CCl_4) and delayed liver recovery [2]. It is known that gadolinium chloride ($GdCl_3$) selectively blocks KC, while repopulation of the liver with young macrophages starts not earlier than 4 days after intravenous injection of $GdCl_3$ [6,8]. The aim of this study was to evaluate the role of prolonged elimination of KC ($GdCl_3$ blockade) in the formation of hepatocyte resistance to CCl_4-induced injury and to elucidate the possibility of restoring the resistance of liver parenchyma to hepatotropic toxin in $GdCl_3$-treated mice by stimulation of macrophages by zymosan granules (ZG).

MATERIALS AND METHODS

Experiments were carried out on (CBA×C57B1)F1 mice weighing 20-22 g. Acute toxic liver injury was caused by intraperitoneal injection of 40% CCl_4 in olive oil (0.2 ml/100 g). Group 1 mice were treated with $GdCl_3$ 2 days before CCl_4 injection, group 2 mice were treated with $GdCl_3$ 2 days before and with ZG 1 day after CCl_4 injection, and group 3 mice received only ZG 1 day before the injection. Controls received 0.2 ml 0.85% NaCl according to the same scheme. $GdCl_3$ (Aldrich) and ZG were injected intravenously in doses of 10 mg/kg and 10 mg/100 g in 0.2 ml 0.85% NaCl. The animals were decapitated 24, 48, 72, and 96 h after CCl_4 poisoning. The severity of liver injury was estimated morphometrically on paraffin sections stained with hematoxylin and eosin by measuring the size of necrosis zones and zones of hydropic balloon degeneration [2] and biochemically by measuring serum alanine aminotransferase activity (ALT). The blood was drawn from the retroorbital sinus immediately before decapitation.

RESULTS

Inhibition of KC with $GdCl_3$ considerably potentiated the development of acute toxic CCl_4-hepatitis. Twenty-four hours after CCl_4 poisoning ALT activity in group 1 mice considerably surpassed the control (2.90±0.14 *vs.* 1.80±0.18 arb. units, $p<0.05$).

Histological examination revealed more pronounced and generalized damage to the liver in group 1 mice. Necrosis and hydropic degeneration were loca-

Research Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

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lized not only in the central, but also in the periportal zones of the hepatic lobule. Apart from merging areas of hepatocyte necrosis, cells with optically empty cytoplasm and signs of karyolysis were scattered over the whole parenchyma. Injury areas in group 1 mice were 1.8-fold more extensive than in the control ($p<0.05$, Fig. 1).

Injection of ZG to $GdCl_3$-treated mice improved hepatocyte resistance against CCl_4-induced damage. Twenty-four hours after administration of ZG, ALT activity decreased 1.8-fold (to 1.60±0.14 arb. units, $p<0.05$ compared to group 1) and approximated the control values, but remained above the corresponding parameter in group 3 (0.9±0.07 arb. units, $p<0.05$). The area of liver parenchyma containing hepatocytes with signs of cytolysis and degeneration decreased 1.7-fold compared to that in group 1 (Fig. 1) and did not differ significantly from the control, but 2.3-fold surpassed the corresponding value in group 3. In group 3 mice, zones of hepatocyte destruction were intensively infiltrated with mononuclears.

Forty-eight hours after CCl_4 poisoning, areas of liver injury in the control group decreased 6-fold in comparison with the previous term of observation and occupied about 8% cross-section area. Inflammatory infiltration of necrotic zones increased. In group 1 mice, large necrotic foci and zones of irreversible hepatocyte degeneration were 8-fold more extensive than in the control ($p<0.05$, Fig. 1). In group 2, the area of liver injury decreased more than twice ($p<0.01$) in comparison with the previous term of observation and was 3-fold ($p<0.01$) less extensive than in group 1. Mononuclear infiltration was clearly enhanced. At this period the structure of the liver tissue recovered, mononuclear infiltrates were seen in some preparations.

Seventy-two hours after CCl_4 poisoning, destructive and infiltration processes in the liver of control mice decayed and its structure became close to normal. In group 1 mice, destructive and necrotic changes were 1.3-fold less pronounced than before (Fig. 1). Inflammatory infiltration was less marked than in the control. In group 2 mice, morphological picture was in the whole close to the control: zones of hepatocyte degeneration the liver occupied 3.3% cross-section area and signs of inflammatory infiltration were observed.

Ninety-six hours after CCl_4 poisoning (6 days after administration of $GdCl_3$) liver injury noticeably decreased, which was confirmed by 2.4-fold reduction of the destruction zones in comparison with the previous term. Inflammatory mononuclear infiltration became more pronounced.

Thus, CCl_4-hepatitis after $GdCl_3$ pretreatment was characterized by more intensive and extensive injury persisting in the liver for longer time and delayed

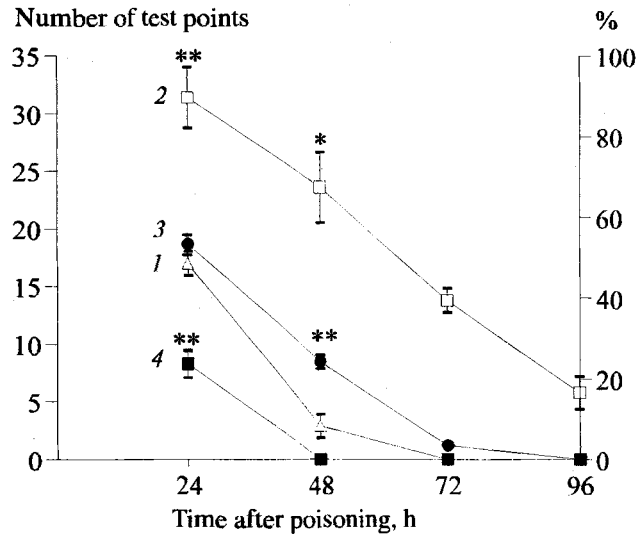

Fig. 1. Absolute and relative areas of liver injury in mice at different terms after CCl_4 poisoning. *1*) control, *2*) $GdCl_3$, *3*) $GdCl_3$+zymosan, *4*) zymosan. *$p<0.01$, **$p<0.05$ in comparison with the control.

mononuclear infiltration and regeneration of the liver parenchyma.

It was previously demonstrated that the rate of colloid carbon clearance from the blood, the number of KC absorbing colloid carbon particles, and activity of granulocyte-macrophage colony-stimulating factor from KC extract drastically decreased 1 day after $GdCl_3$ injection. These parameters remained at this level during days 2-3 and gradually returned to normal on day 4. ZG administered to $CdCl_3$-treated mice significantly increased the rate of colloid carbon clearance, but this parameter remained below the corresponding value in group 3. It is important to note that the dynamics of injury, inflammatory infiltration, and liver recovery after $GdCl_3$ blockade were similar to the time course of KC repopulation, *i.e.* with the increase in the number of macrophages and their clearing capacity [4]. Aggravation of liver damage can be attributed to a decrease in the number of functionally active KC and their hepatoprotective potential depending on secretion of interleukin-1, tumor necrosis factor, *etc.* by activated macrophages. These products inhibit cytochrome P-450-dependent monooxygenases [3] and effectively regulate LPO and oxidative balance in the liver [1]. There are also data on reduction of the regeneration potential of KC, *i.e.* inhibition of growth factors production [7]. Sharp suppression of inflammatory infiltration in mice treated with $GdCl_3$ can be due to reduced hemopoiesis-stimulating activity of KC [4]. At the same time, not only activated KC, but also macrophages potentiate hepatocyte regeneration [5]. The positive effect of ZG stimulation in $GdCl_3$-treated mice in our experiments probably results from repopulation of KC and potentiation of

inflammatory infiltration involving active macrophages in the liver.

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