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PROTECTIVE EFFECT OF UKRAIN AGAINST ACUTE ACETAMINOPHEN-INDUCED HEPATITIS IN RATS

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Summary: *A high dose of acetaminophen (AAP; 1000 mg/kg, single, p.o) administered to rats induced acute liver failure with centrolobular degeneration and necrosis. We studied the role of hepatic macrophages (Kupffer cells) in the modulation of the acetaminophen-induced damage to the liver. Ukrain, which is known to possess immunomodulatory characteristics, has been shown to exhibit a positive hepatoprotective effect in human viral hepatitis C, significantly preventing liver cell injury. The selective inhibitor of Kupffer cells, gadolinium chloride ($GdCl_3$, 7.5 mg/kg i.v., administered 24 h before AAP), and the macrophage stimulator carboxymethylated (1→ 3)-beta-D-glucan (CMG; 25 mg/kg i.p., administered 48 h before AAP), were used to modulate the activity of liver macrophages. It was shown that preliminary administration of Ukrain, CMG or $GdCl_3$ to rats produced in each case a protective effect, normalizing liver functional tests and reducing damage to the liver. The role of tumor necrosis factor (TNF)-alpha secretion in the protective effect of these agents in AAP-induced hepatitis is discussed.*

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Introduction

Ukrain has been shown to exhibit a significant hepatoprotective effect in human viral hepatitis C, preventing liver cell injury (1-3). We suggested that Ukrain could prevent liver damage in other types of

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hepatitis, in particular those induced by drugs that have side effects on liver structure and function.

Acetaminophen (AAP) is a well known medical drug, widely used in the therapy of numerous inflammatory diseases (4-6). It has been shown that high doses of AAP can induce toxic hepatitis and liver failure in humans and experimental animals, accompanied by the development of centrolobular necrosis (6-8). It has been suggested that AAP toxicity is the result of the interaction of its metabolite *N*-acetyl-*p*-benzoquinone with liver proteins and of the depletion of glutathione in liver cells (8). In this study, we tested the hypothesis that the functional activity of liver nonparenchymal cells (macrophages, endothelial cells) can play an important role in the development of toxic hepatitis induced by high doses of AAP (4, 7, 9-11).

Materials and methods

Study design. Male Wistar rats weighing 200-250 g (Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, Russia) were used. Rats were pretreated with Ukrain (Nowicky Pharma, Austria) at a dose of 2 mg/kg i.v. administered 48 h before administration of AAP. As positive and negative controls of macrophage stimulation, water-soluble carboxymethylated (13)-beta-D-glucan (CMG; Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia) at a dose of 25 mg/kg i.p. 48 h before administration of AAP, and a macrophage activity suppressing agent, gadolinium chloride ($GdCl_3$; kind gift of Prof. Hardonk, Department of Pharmacokinetics and Drug Delivery, University of Groningen, The Netherlands) at a dose of 7.5 mg/kg i.v. 24 h before administration of AAP, were used.

As control, intact rats were used (group 1). Toxic hepatitis was induced by AAP (ICN Biomedicals, Irvine, CA, USA) administered to the rats in a single dose of 1,000 mg/kg p.o. (7); animals were sacrificed 24 h after AAP administration (group 2). Ukrain was injected at a dose of 2 mg/kg i.v. to intact rats (group 3), or 48 h prior to administration of AAP (group 6). CMG was administered at a dose of 25 mg/kg i.p. to intact rats (group 4), or 48 h prior to administration of AAP (group 7); $GdCl_3$ was administered in the tail vein at a dose of 7.5 mg/kg to intact rats (group 5), or 24 h prior to administration of AAP (group 8).

Liver functional tests. Functional assays of serum transaminase activity (alanine transaminase [ALT], aspartate transaminase [AST]) were carried out using a Cobas Mira biochemical analyzer (Cobas Mira La Roche Diagnostic System, Basel, Switzerland) and Biocon Diagnostik kits (Biocon Diagnostik GmbH, Vöhl-Marienhagen, Germany). The activity of enzymes was expressed in U/L.

TNF-alpha. TNF-alpha level was measured in the supernatant of peritoneal macrophages by the biological method according to Flick (12), using the TNF-alpha sensitive cell line L-929 purchased from the Russian Ministry of Public Health State Research Center for Virology and Biotechnology 'Vector' (Koltsovo, Russia). For the standard curve, murine recombinant TNF-alpha was used, the results being expressed in pg/ml.

Light microscopy study of the liver. For the preparation of histological samples, livers were removed and fixed in 10% buffered formalin (ALTEY Laboratory, Moscow, Russia) and paraffin-embedded sections were stained with hematoxylin-eosin (ALTEY Laboratory). Statistical analysis of the results was carried out on Statistica for Windows (release 5.1 A, 1984-1996, Tulsa, OK, USA), using Student's *t*-test; values were considered as significant at $p < 0.05$.

Results

TNF-alpha production by isolated peritoneal macrophages. In the first series of experiments it was re-

vealed that CMG (25 mg/kg, single dose) increased TNF-alpha production by isolated peritoneal macrophages. The TNF-alpha level in the macrophage supernatant of intact animals was 333 ± 30 pg/ml. Following CMG administration this increased to 831 ± 80 pg/ml. The value in the positive control group in this part of study after treatment with zymosan (Sigma, St. Louis, MO, USA), a known macrophage stimulator, at a dose of 100 mg/kg i.p. 6 h after administration of CMG, was 1,560 ± 200 pg/ml. This showed that CMG-induced macrophage stimulation was followed by increased TNF-alpha production, but the effect was less than that of the macrophage stimulator zymosan.

Liver weight. Liver weight increased in the AAP hepatitis group and in the AAP groups receiving preliminary administration of Ukrain or CMG. There were no changes in spleen weight in any group studied (Table I).

Light microscopic study of the liver. AAP administration to rats was followed by the development of acute toxic hepatitis with centrolobular necroses. Vacuolar dystrophy occurred in some hepatocytes, and proliferation of sinusoidal cells was observed. In animals with AAP hepatitis, preliminary administration of the immunomodulator Ukrain or the macrophage stimulator CMG prevented the development of centrolobular necrosis and the dystrophy of hepatocytes.

Serum ALT and AST activity. Serum ALT and AST activity was studied in rats with AAP-induced hepatitis and in the groups receiving a combined administration of AAP and the macrophage function modulator. The treatment of the rats with AAP resulted in a 10- to 13-fold increase in serum ALT and AST activity, indicating the development of cytolytic syndrome and damage to liver cells (Table II). The elevation of ALT and AST activity was significantly reduced by pretreatment with Ukrain and CMG (Table II). Administration of Ukrain or CMG alone to intact rats had no effect on serum transaminase activity. Positive, but less significant, effects were observed in rats with AAP hepatitis pretreated with $GdCl_3$.

White blood cells. It was shown that AAP decreased the total number of white blood cells compared with the control animals (Table III). CMG administration to intact animals was followed by relative neutropenia and a slight tendency to lymphocytosis.

Table I *Effect of Ukrain, carboxymethylated (13)-beta-D-glucan (CMG) and gadolinium chloride ($GdCl_3$) treatment on rat liver and spleen weight during acetaminophen (AAP)-induced hepatitis*

Group	Treatment	Liver weight (g)	Relative liver weight (g)	Spleen weight (g)
1	Control (n = 8)	8.5 ± 0.33	3.1 ± 0.03	1.01 ± 0.03
2	AAP (n = 10)	9.9 ± 0.30*	4.2 ± 0.18*	0.94 ± 0.07
3	Ukrain (n = 5)	8.6 ± 0.36	3.7 ± 0.13	1.16 ± 0.07
4	CMG (n = 5)	8.5 ± 0.42	4.5 ± 0.06*	1.3 ± 0.12
5	$GdCl_3$ (n = 5)	8.8 ± 0.55	3.4 ± 0.23	1.38 ± 0.17
6	AAP + Ukrain (n = 7)	9.9 ± 0.34*	3.9 ± 0.37	1.11 ± 0.07
7	AAP + CMG (n = 7)	9.9 ± 0.15*	4.34 ± 0.04	1.15 ± 0.18
8	AAP + $GdCl_3$ (n = 8)	9.5 ± 0.34*	4.05 ± 0.11	0.97 ± 0.09

*$p < 0.05$, compared to control.

Table II *Effect of Ukrain, carboxymethylated (13)-beta-D-glucan (CMG) and gadolinium chloride ($GdCl_3$) treatment on the serum alanine transaminase (ALT) and aspartate transaminase (AST) activity of rats with acetaminophen (AAP)-induced hepatitis*

Group	Treatment	ALT (U/l)	AST (U/l)
1	Control ($n = 8$)	104 ± 6.5	298.6 ± 28.53
2	AAP ($n = 10$)	1,367 ± 532*	4,411 ± 2,100*
3	Ukrain ($n = 5$)	108.5 ± 11.3	264.8 ± 23.25
5	CMG ($n = 5$)	113.3 ± 10.1	238.3 ± 12.93
4	$GdCl_3$ ($n = 5$)	98.75 ± 6.24	254.3 ± 21.63
6	Ukrain + AAP ($n = 7$)	217 ± 18.3*	412 ± 44.43*
7	CMG + AAP($n = 7$)	136.2 ± 7.86*	224.7 ± 14.12*
8	$GdCl_3$ + AAP ($n = 8$)	458.5 ± 141.9*	896.5 ± 327.9*

*$p < 0.05$, compared to control.

There was also a tendency to neutropenia in the case of administration of Ukrain to intact rats (Table III). Combined administration of Ukrain and AAP produced no changes in white blood cell count or differential count as compared with intact rats, nor were there any changes in these indexes in the groups receiving CMG with AAP and $GdCl_3$ with AAP (Table III).

Discussion

It is known that inflammation is followed by the secretion of primary inflammatory mediators (cytokines), among which the most important are TNF-alpha, interleukin (IL)-6 and IL-8 (9, 10, 13). Cytokines induce the neutrophils to release the biologically active substances—H_2O_2, free oxygen radicals and polymorphonuclear cell elastase—with a simultaneous decrease in peroxisomal catalase activity in liver cells (5, 14). This is probably why preliminary application of the known peroxisome activator clofibrate, resulting in a decrease in H_2O_2 synthesis, considerably reduced toxic damage to the liver by AAP (15).

At 24 h after its administration, AAP administered to rats at a dose of 1,000 mg/kg leads to acute liver failure accompanied by centrolobular degeneration

Table III *Effects of gadolinium chloride ($GdCl_3$), carboxymethylated (13)-beta-D-glucan (CMG) and Ukrain on the white blood cell (WBC) and differential count in rats with acetaminophen (AAP)-induced hepatitis*

Group	Treatment	WBC (10^9/l)	PMN (%)*	Lymphocytes (%)*	Monocytes (%)*
1	Control ($n = 8$)	11.6 ± 2.46	30.0 ± 3 .72	63.3 ± 5.06	6.8 ± 2.49
2	AAP ($n = 10$)	6.8 ± 1.29**	36.8 ± 2.8	59.17 ± 2.41	4.0 ± 0.82
3	$GdCl_3$ ($n = 5$)	8.4 ± 1.62	26.8 ± 3.99	66.0 ± 4.02	7.3 ± 2.02
4	CMG ($n = 5$)	13.6 ± 0.63	14.5 ± 1.19**	76.5 ± 2.65	9.0 ± 1.63
5	Ukrain ($n = 5$)	13.7 ± 3.34	20.8 ± 4.5	73.5 ± 6.25	5.8 ± 1.11
6	$GdCl_3$ + AAP ($n = 8$)	10.9 ± 2.05	29.0 ± 3.1	65.0 ± 3.43	6.0 ± 1.39
7	CMG + AAP ($n = 7$)	10.9 ± 2.57	21.2 ± 5.56	72.2 ± 2.21	6.7 ± 1.12
8	Ukrain + AAP ($n = 7$)	9.1 ± 1.27	31.6 ± 4.39	63.1 ± 4.92	5.3 ± 1.14

PMN: polymorphonuclear leucocyte; * percent of total leucocytes; **$p < 0.05$, compared to control.

and necrosis, with a considerable increase in serum transaminase activity (ALT and AST). The pretreatment of rats with the immunomodulator Ukrain, the macrophage stimulator CMG and the selective inhibitor of liver macrophages $GdCl_3$, prevented in each case the development of AAP-induced hepatitis, normalizing functional and structural changes to liver cells. Changes in TNF-alpha secretion by macrophages due to CMG can modify the degree of liver injury caused by hepatotropic agents. Ukrain was shown to exhibit a hepatoprotective effect in experimental hepatitis, and may be useful in the treatment of drug-addictive disorders.

We found that in acute toxic hepatitis induced by AAP, Ukrain and CMG each had a protective effect *via* the stimulation of liver macrophages (with increased TNF-alpha production in isolated macrophages in the case of CMG), while the protective effect of $GdCl_3$ was *via* the suppression of liver macrophages. According to previous results, preliminary stimulation of liver macrophages by lipopolysaccharide (LPS) has a protective effect in toxic liver injury (**7**). A positive effect from macrophage stimulation was also found in models of LPS-induced sepsis and during acute blood loss. It is probable that previously primed macrophages are not able to secrete significant amounts of cytokines, and as a result such macrophages are less involved in the development of toxic liver injury.

The protective effect of Ukrain in experimental AAP hepatitis opens up the possibility of its use in the treatment of hepatitis induced as a side effect by some medical drugs. There is a significant possibility that Ukrain may exhibit a hepatoprotective effect in human viral hepatitis C, especially in cases of drug-addictive disorders (16).

We also observed a protective effect of $GdCl_3$ against experimental AAP hepatitis, depressing liver macrophages *in vivo* (17). It is known that $GdCl_3$ administration to rats decreases secretion of TNF-alpha and IL-6 by macrophages, reducing their participation in the inflammatory process (6). $GdCl_3$ both selectively depletes the population of large macrophages in the liver, and decreases nitric oxide production and the formation of peroxide oxidation products (4). It appears that the mechanism of protective action in AAP hepatitis of the various compounds studied is connected with the secretory activity of macrophages and requires further analysis.

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