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LIPID PEROXIDATION IS A PREREQUISITE FOR GALACTOSAMINE-INDUCED DAMAGE

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The aim of the study was to investigate some mechanisms of D-galactosamine hepatotoxicity as well as the hepatoprotective effect of cresacin. Rats received 400 mg/kg D-galactosamine via intraperitoneal injection. Cresacin at a dose 20 mg/kg was administered intraperitoneally 10 min after D-galactosamine. The following parameters were measured: hepatic activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and ceruloplasmin, intensity of liver lipid peroxidation and of mitochondrial and microsomal oxidative processes.

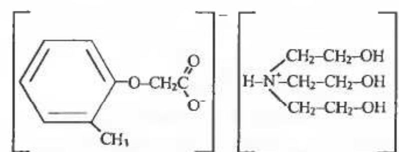
After intoxication the activation of free radical oxidation in the liver was observed along with attenuation of the antioxidant system, inhibition of oxygen absorption rate in mitochondria, uncoupling of oxidative phosphorylation and tissue respiration and depression of microsomal oxidation. Treatment of rats with cresacin prevented partially the toxic effects of D-galactosamine.

Chemiluminescence intensity was increased 1 h after intoxication while all other changes became apparent from 6 h after treatment. This fact suggests that lipid peroxidation may be a prerequisite for galactosamine-induced damage. The protection offered by cresacin was related to its capacity to inhibit lipid peroxidation.

INTRODUCTION

Hepatotoxicity induced by D-galactosamine is a suitable animal model of human acute hepatic failure (Sielaff, Hu & Rollins, 1995). According to current theories the activation of free radical oxidation may play an important role in the pathogenesis of toxic hepatitis (Seckin, Kocak-Toker, Uysal & Oz, 1993). Increased lipid peroxidation is responsible for the loss of membrane integrity and inhibition of both oxidation and phosphorylation in mitochondria as well as for the inhibition of detoxicative processes in liver microsomes.

The search of new medicinal preparations that may inhibit lipid peroxidation is an area of considerable interest. Recently, *in vitro* experiments using the peroxidase/H_2O_2 system and Cu^{2+}-stimulated LDL oxidation we have shown that tris(2-oxyethyl)ammonium *ortho*-cresoxyacetate (cresacin) may be a potent scavenger of the free radicals. Similarly, cresacin was found to be a strong suppresant of luminol-dependent chemiluminescence induced by phagocyting leucocytes (Korda, 1998a, b). It is known that the biological activity of antioxidants is determined by features of their chemical structure, in particular, availability of hydroxy- or aminogroups containing mobile hydrogen atom. The structure of cresacin suggests that it may act as a free radical scavenger and thereby potentially inhibit free radical oxidation reactions.



Cresacin

The aim of the study was to investigate the role of lipid peroxidation in D-galactosamine hepatotoxicity as well as the hepatoprotective effect of cresacin.

MATERIALS AND METHODS

Male Vistar rats received the aqueous solution of D-galactosamine at a dose of 400 mg/kg via intraperitoneal injection. Cresacin at a dose of 20 mg/kg was administered intraperitoneally 10 min

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after intoxication. Intact rats were used as a control. At 1, 6 and 24 h after D-galactosamine injection the animals were decapitated. The liver tissue was homogenized in isotonic sodium chloride solution and the following parameters were measured: hepatic activities of superoxide dismutase (Beachamp & Fridovich, 1974), catalase (Koroliuk, Ivanova, Maiorova & Tokarev, 1988), glutathione peroxidase and glutathione reductase (Kruglikova & Shtutman, 1976), ceruloplasmin (Kolb & Kamyshnikov, 1982), and liver lipid peroxidation intensity (by a chemiluminescence method) (Juravliov & Sherstniov, 1985). Mitochondria and microsomes of hepatocytes were obtained by the differential centrifugation method. The intensity of mitochondrial and microsomal oxidative processes were measured by a polarographic method (Frank, Kondrasheva & Mokhova, 1973; Riabinin & Lifshic, 1986).

Student's "t" test was used. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

After D-galactosamine intoxication activation of free radical oxidation in liver was observed. Fig. 1 shows the chemiluminescence intensity dynamics of liver homogenate of rats after D-galactosamine poisoning. Even at one hour after the intoxication the activity of free radical processes in liver was increased comparing with control animals. The maximum indexes of both spontaneous and initiated chemiluminescence were found at 24 hours after D-galactosamine injection.

It is clearly evident that cresacin shows an antioxidant effect. Cresacin was found to lower the chemiluminescence intensity (spontaneous and initiated) approximately 1.8 times 24 hours after D-galactosamine injection.

Fig. 2 represents the effect of D-galactosamine on the functional state of mitochondrial respiratory chain. The rate of oxygen uptake during succinate oxidation was decreased in metabolic states I and II. The respiratory control index was decreased significantly 6 and 24 hours after D-galactosamine administration. Cresacin partially reduced the metabolic disorders in liver mitochondria of rats with acute D-galactosamine hepatitis.

We have investigated the effect of D-galactosamine on the rate of oxygen absorption by hepatocyte microsomes by the polarographic method. 1 and 6 h after intoxication the negative effect of D-galactosamine was not observed (Fig. 3). 24 h after intoxication the intensity of microsomal oxidation was lower comparing with the control group on 22% ($P < 0.05$). The treatment of intoxicated rats by cresacin did not effect the intensity of oxygen absorption by hepatocyte microsomes.

As the processes of free radical oxidation in an organism are determined by the functional state of the antioxidant system we have studied the D-galactosamine effect on some antioxidant enzyme activities. Fig. 4A shows the changes of liver superoxide dismutase activity after D-galactosamine injection. Superoxide dismutase activity was decreased after intoxication but the degree of this decrease was the most significant at 24 h after D-galactosamine administration. Cresacin was found to partially prevent the superoxide dismutase oppression.

Another important antioxidant enzyme blocking the initial stage of lipid peroxidative process is catalase. We have observed the decrease of catalase activity in liver of rats at 6 and 24 h after intoxication (Fig. 4B). Cresacin has exhibited a positive effect only at 24 h from the beginning of experiment.

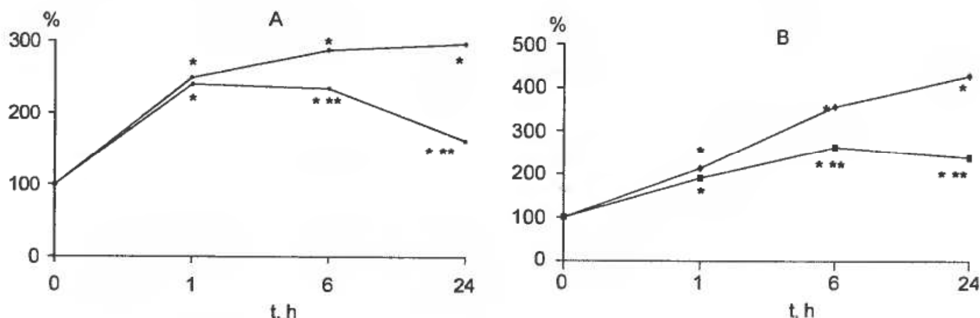

Fig. 1. Intensity of spontaneous (A) and initiated (B) chemiluminescence of liver homogenates of rats intoxicated with D-galactosamine (rats not treated with cresacin, closed diamonds; rats treated with cresacin, closed squares). *$P < 0.05$ compared with control (100 %), **$P < 0.05$ compared with rats not treated with by cresacin

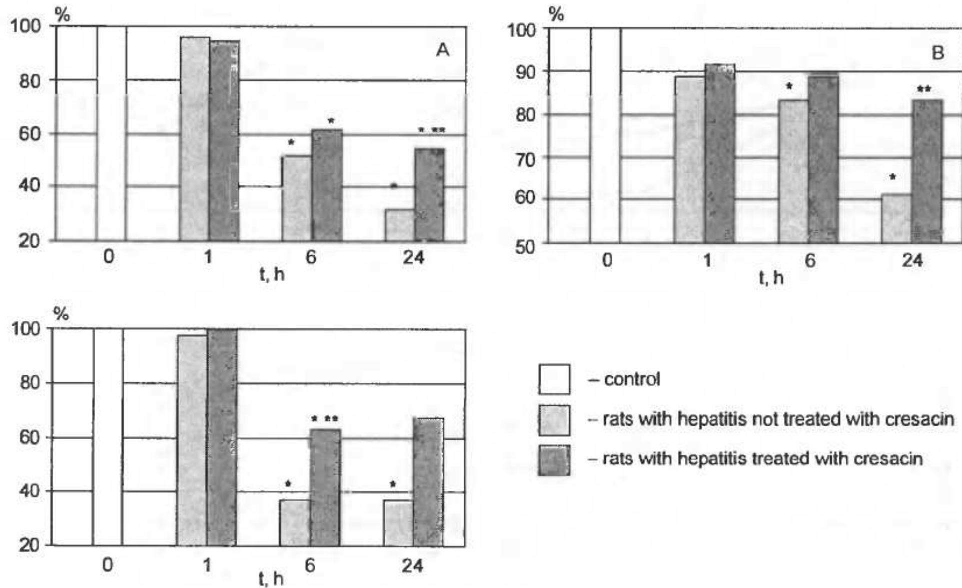

Fig. 2. The effect of cresacin on the rate of oxygen absorption by mitochondria (A – metabolic state I; B – metabolic state II) and respiratory control index (C) of rats with D-galactosamine hepatitis. *P < 0.05 compared with control (100%), **P < 0.05 compared with rats not treated with cresacin

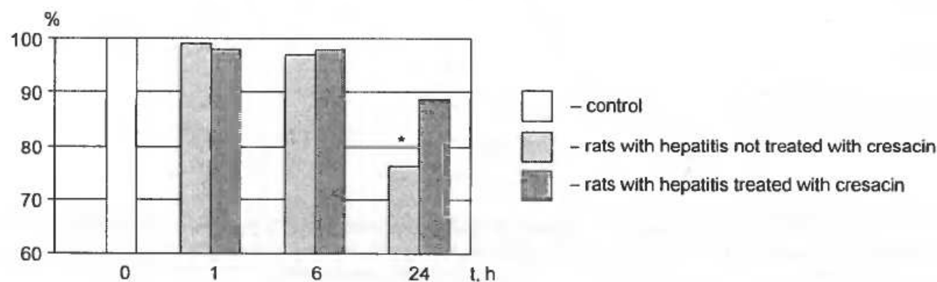

Fig. 3. The effect of cresacin on the rate of oxygen absorption by microsomes of rats with D-galactosamine hepatitis. *P < 0,05 compared with control (100%), **P < 0,05 compared with rats not treated with cresacin

During hepatitis we also observed a decrease of glutathione peroxidase activity in liver of rats (Fig. 4C). The activity decreased by 16% after 1 h, by 45% after 6 hs, and by 40% after 24 hrs. Cresacin prevented the glutathione peroxidase inactivation in late stages (6 and 24 h). The activity of glutathione reductase showed similar changes.

In contrast to above mentioned enzymes the ceruloplasmin activity increased during the late stages of hepatitis (Fig. 4E). The treatment by cresacin did not modity the changes of ceruloplasmin activity.

D-galactosamine was found to inhibit the processes of protein synthesis. It has been shown that protein synthesis is decreased 30 min after D-galactosamine injection (Hayashi, Yamazoe, Yamaguchi & Kunitomo, 1992). Therefore one can suspect that the inhibition of antioxidant enzymes activity could be due to inhibition of enzyme synthesis in hepatocytes by D-galactosamine. Another possibility is an interaction of D-galactosamine with the enzyme molecules.

These findings suggest that the decrease of antioxidant enzymes activities conditioned by the inhibition of protein synthesis plays an important role in the pathogenesis of toxic hepatitis induced by D-galactosamine. The protection offered by cresacin was related to its capacity to inhibit lipid peroxidation.

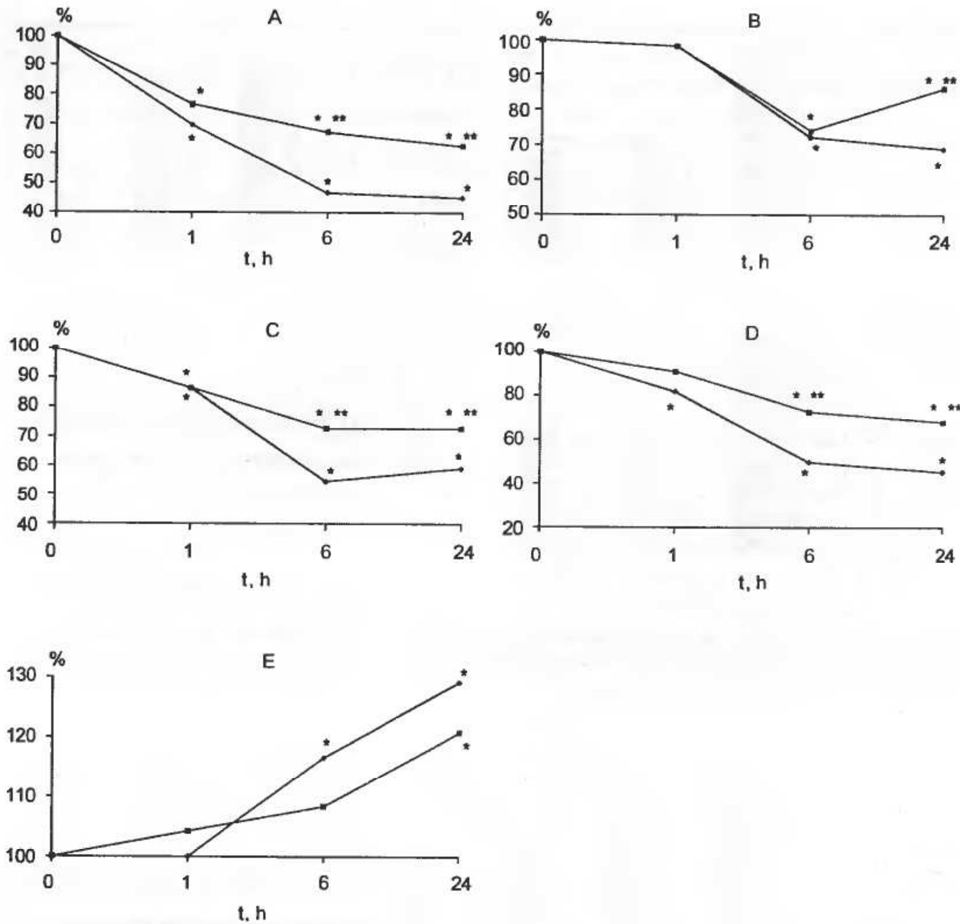

Fig. 4. Dynamics of superoxide dismutase (A), catalase (B), glutathione peroxidase (C), glutathione reductase (D) and ceruloplasmin (E) activities in the liver of rats with D-galactosamine hepatitis (rats not treated with cresacin, closed diamonds; rats treated with cresacin, closed squares). *P < 0.05 compared with control (100%), **P < 0,05 compared with rats not treated with cresacin

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