Pharmacological Regulation with Antioxidants of Hepatocyte Cytolysis in Acute D-Galactosamine-Induced Intoxication

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A rat model of D-galactosamine-induced (0.3 g/kg) liver damage is used to study antioxidant and membrane-stabilizing activity of sodium selenite, vitamin E, and their combination (administered *per os*, daily, for 3 days before intoxication). It is shown that sodium selenite is 300-fold more effective than vitamin E, while their combination exhibits synergism and potentiation of the antioxidant and membrane-stabilizing activities, underlying inhibition of hepatocyte cytolysis.

Key Words: *sodium selenite; vitamin E; D-galactosanime hydrochloride; liver; cytolysis*

Modern pharmacotherapy of various acute and chronic diseases includes both etiotropic and pathogenetic agents. The latter are directed toward elimination of the main cause of liver damage — destabilization of hepatocyte membranes, which depends on activation of lipid peroxidation (LPO). Acceleration of LPO leads to structural disturbances of lipid bilayer due to predominant oxidation of unsaturated fatty acids. This results in dissociation and deactivation of membrane-bound enzymes, oxidation of SH-groups of transmembrane proteins, and complexation between proteins and oxidized lipids, which promotes polymerization of protein molecules [1,3-5]. These pathological processes destroy subcellular and plasma membranes of hepatocytes [10]. Membrane-stabilizing agents with antioxidant activity prevent or restrict these pathological processes. In the present study we explored the possibility of using the antioxidants sodium selenite, vitamin E, and their combination for correction of hepatocyte membrane permeability in animals treated with the selective hepatotoxin D-galactosamine hydrochloride (D-GA).

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Morphological and biochemical alterations in the liver induced by D-GA are similar to those observed in hepatitis B [6]; therefore, this is the most suitable model for screening and pharmacological studies of hepatoprotective drugs [9].

MATERIALS AND METHODS

Experiments were carried out on 160 random-bred albino rats of both sexes (150-230 g). Acute hepatic failure was modeled with a single intraperitoneal injection of D-GA in a dose of 0.3 g/kg. The animals were treated with antioxidants (vitamin E, sodium selenite, and their combination) for 3 days before D-GA injection. Vitamin E and sodium selenite were administered in therapeutic doses as oil and aqueous solution, respectively.

The intensity of LPO was evaluated by measuring the content of primary (conjugated dienes, CD) and secondary (malonic dialdehyde, MDA) LPO products in liver homogenates [7,8] 30 min, 4, 14, 20, 24, 72 h, and 5-7 days after D-GA injection.

At the same times, the disturbances of hepatocyte membrane permeability were assessed by serum activity of alanine and aspartate aminotransferase

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[11] and histidine ammonialyases [2], a specific marker of the hepatocyte cytolysis enzymes. Hepatoprotective effect of antioxidants was evaluated 24-72 h after D-GA injection, which corresponded to the maximum liver damage. Control animals received distilled water and vegetable oil. Each group comprised 7-8 rats, and all biochemical parameters were measured in each animal. Significance of differences was evaluated using the Student *t* test.

RESULTS

D-GA acts as a selective hepatotoxin. Single injection of 0.3 g/kg G-HA induced LPO in hepatocyte membranes, which manifested itself in a rise of primary (CD) and secondary (MDA) LPO products in the liver. A significant accumulation of CD in the liver was noted 20 h after D-GA injection, while the content of MDA in the liver attained its maximum on the 3rd day postintoxication (Fig. 1) and on the 7th day returned to normal. Different dynamics of the liver content of CD and MDA can be attributed to the fact that LPO is a sequential process.

Damage to hepatocyte plasma membrane associated with LPO activation and destructive action of CD and MDA results in release of cytoplasmic content into circulation. This indicates a cytolytic damage to liver parenchyma caused by D-GA. Maximum cytolysis was accompanied by a 2-4-fold increase in alanine and aspartate transaminase activity and a 17-fold increase in histidine ammonialyase activity in comparison with the control (Fig. 2). It should be noted that in control animals plasma activity of histidine ammonialyase was not detected.

In control rats, vitamin E had practically no effect on the content of CD in liver homogenate, while sodium selenite decreased this parameter 2.7-fold in comparison with the control. The decrease in the CD content produced by combination of these antioxidant surpassed that of individual agents, i.e., we observed potentiation of their antioxidant effects. Vitamin E and sodium selenite had no effect on the content of MDA in intact animals, while their combination decreased this parameter 1.7-fold in comparison with the untreated control ($p<0.05$).

Twenty-four and 72 h after injection of D-GA (the time corresponding to maximum liver damage), the content of CD and MDA in the liver of animals treated with sodium selenite and vitamin E decreased 2.2-3.0- and 1.4-1.8-fold, respectively, in comparison with untreated rats. Sodium selenite and vitamin E administered in different doses exerted similar effects on the content of CD and MDA, i.e., the doses were isoeffective (Fig. 1). Hence, sodium selenite was 300-fold more effective than vitamin E. Compared to individual antioxidants, their combination exhibited maximum antioxidant activity as a result of synergism.

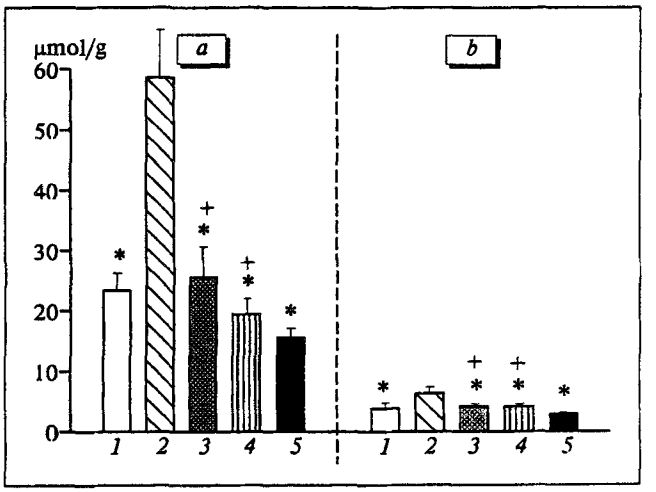

Fig. 1. Effect of antioxidants on the content of conjugated dienes (24 h, *a*) and malonic dialdehyde (72 h, *b*) in rat liver after D-galactosamine hydrochloride (D-GA) intoxication. Here and in Fig. 2: *1*) control animals; *2*), D-GA; *3*) D-GA+sodium selenite; *4*) D-GA+ vitamin E; *5*) D-GA+sodium selenite+vitamin E; $p<0.05$: *compared with *2*; +compared with *5*; °compared with *4*.

Sodium selenite, vitamin E, and their combination due to antioxidant potential of these drugs reduce plasma membrane permeability, thus preventing the release of cytoplasmic enzymes into the blood. Twenty-four hours after D-GAA injection (peak of their activity in D-GA treated animals), plasma activity of alanine and aspartate transaminases and histidine ammonialyase in animals treated with antioxidants, returned to normal. In D-GA intoxication, vitamin E exhibited a moderate membrane-stabilizing effect, while sodium selenite (administered in isoeffective dose) was 300-fold more effective (Fig. 2).

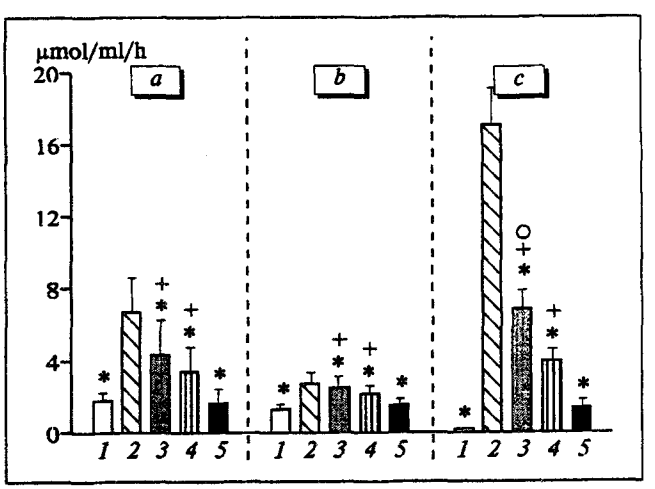

Fig. 2. Effect of antioxidants on plasma alanine (*a*) and aspartate (*b*) transaminases and histidine ammonia-lyase (*c*) activities 24 h after D-galactosamine hydrochloride (D-GA) intoxication.

Combination of these preparations produced maximum antioxidant effect due to synergism and potentiation of their effects.

Thus, our experiments demonstrate antioxidant and membrane-stabilizing activity of sodium selenite, vitamin E, and their combination. Inhibition of hepatocyte cytolysis in acute intoxication with D-GA is accompanied by normalization of chemical markers of this processes: activities of alanine and aspartate transaminase and histidine ammonialyase. Consequently, the antioxidants vitamin E and selenium in form of sodium selenite can be considered as essential preparations in the pharmacological restriction of cytolysis in the liver.

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