MECHANISM OF THE HEPATOPROTECTIVE ACTION OF NEW NICOTINIC ACID DERIVATIVES IN EXPERIMENTAL CCl_4-INDUCED LIVER DAMAGE

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An important place in the combined treatment of liver diseases is occupied by preparations of metabolite origin. Among them an important role is played by nicotinic acid (NA), nicotinamide, and their derivatives, whose hepatoprotective properties are generally known [1, 3]. New biologically active substances (BAS) effective against liver disease induced by hydrazine and alcohol have been created on their basis [2, 9]. The aim of this investigation was to study the possible mechanisms of the hepatotropic action of NA derivatives, lithium nicotinate (litonit) and nicogamol, in toxic liver damage.

EXPERIMENTAL METHOD

Experiments were carried out on 120 male Wistar rats weighing 180-220 g. Experimental liver damage was caused by a single intragastric injection of carbon tetrachloride (CCl_4), in the form of a 50% oily solution and in a dose of 5 ml/kg of CCl_4 [15]. The animals were divided into groups: 1) intact animals serving as the control; 2) animals with experimental hepatitis, not receiving medication; 3, 4, and 5) animals with experimental hepatitis receiving one of the therapeutic agents (NA, lithium nicotinate – LN, nicogamol). All the preparations were administered in the course of 2 weeks (at the beginning – 7 days before injection of CCl_4) subcutaneously in mean therapeutic doses (10 mg/kg). Activity of marker enzymes of cytolysis – alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [14], and of cholestasis – alkaline phosphatase (AlP) [12], and gamma-glutsmyl transferase (GGT) [13], in the blood serum was determined with the aid of the "Bio-La-Test" kit of chemical reagents from "Lachema" (Czechoslovakia). In parallel tests, the content of lipid peroxidation (LPO) products, namely conjugated dienes (CD) [7] and malonic dialdehyde (MDA) [11], and also activity of the key enzymes of antiradical protection of the cells, namely superoxide dismutase (SOD) [8] and catalase [6], was determined in liver homogenates from the experimental animals. The degree of the biochemical changes was estimated at intervals of 1, 3, 5, and 7 days after administration of CCl_4. The morphological changes were evaluated at the same time intervals after administration of CCl_4 in pieces of liver fixed in 10% neutral formalin. From one batch of pieces, dehydrated in alcohols of increasing concentration and embedded in celloidin, histological sections stained with hematoxylin and eosin were prepared. In frozen sections stained with Sudan black B by Lyson's method lipids were demonstrated [5]. Glycogen was determined by the PAS reaction after McManus [5]. The numerical results were subjected to statistical analysis by Student's t test.

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TABLE 1. State of Antiradical Protection System, Content of LPO Products in the Liver, and Activity of Blood Serum Enzymes of Experimental Animals in a Model of Hepatic Pathology and Its Correction by the Products Studied (M ± m)

Parameter studied	Experimental conditions				
	control	CCl_4	lithium nicotinate + CCl_4	nicogamol + CCl_4	Nicotinic acid + CCl_4
ALT, μmoles/ml·h	2,30±0,27	4,58±0,33*	2,69±0,13**	2,61±0,35**	4,36±0,32*
AST, μmoles/ml·h	2,18±0,16	4,16±0,13*	1,81±0,09**	1,44±0,25*,**	2,90±0,25*,**
GGT, nmoles/sec·liter	45,07±8,77	71,82±4,21*	49,43±4,90**	60,53±8,50	74,39±6,51*
AlP, mmoles/liter	2,80±0,14	3,87±0,20*	3,96±0,42*	6,31±0,21*,**	5,18±0,29*,**
CD, optical density units/mg	0,031±0,002	0,038±0,002*	0,023±0,001*,**	0,028±0,002**	0,028±0,002**
MDA, μmoles/g	0,793±0,046	3,841±0,070*	0,799±0,069**	1,131±0,078*,**	1,706±0,343*,**
SOD, conventional units/mg pro	20,89±1,74	8,96±1,05*	18,81±1,72**	27,70±1,92*,**	5,12±1,14*,**
Catalase, mmoles H_2O_2 /min·g	60,21±4,01	9,88±0,79*	42,07±5,76*,**	58,39±5,09**	37,82±2,71*,**

Legend. *$p < 0.05$ Compared with control, **$p < 0.05$ compared with CCl_4. Tests were carried out on 3rd day after administration of CCl_4.

EXPERIMENTAL RESULTS

The dynamic study of toxic liver damage by CCl_4 showed that it was accompanied by severe disturbances of hepatocyte function and structure. Activity of SOD and catalase in the liver tissue was depressed. Activity of SOD, which controls enzymic dismutation of the superoxide anion-radical into the less reactive molecules of H_2O_2 and triplet oxygen, fell, to reach a minimum after 3 days (a fall of 57.1%) after injection of CCl_4. Activity of catalase, which is responsible for further detoxication by removal of the damaging action of hydrogen peroxide, formed in the course of the SOD reaction, fell during this time period by 83.6%. The disturbance of activity of these enzymes was accompanied by accumulation of active forms of oxygen, lipoperoxides, and free fatty acids, and by marked activation of LPO processes, exerting a membrane-destructive action. The content of CD in the liver tissue was increased by 80.6% only 24 h after injection of CCl_4 and was held at high levels during the next 5 days of observation. There was a parallel increase in the MDA content, which reached 3.841 ± 0.070 μmoles/g 3 h after injection of CCl_4, which is 4.8 times higher than in intact animals (Table 1). Our results are in full agreement with those of previous investigations [4]. The membrane-damaging action of these products was manifested as a single increase in serum activity of enzymes of cytolysis and cholestasis. This increase began 24 h after injection of CCl_4 and reached a maximum, but after 5 days ALT and AST activity was restored; activity of GGT and ALT remained high during subsequent times of observation. Meanwhile, on the 3rd day, ALT activity rose by 99.1%, ACT by 90.8%, GGT by 59.4%, and AlP by 38.2%. Simultaneously with changes in the biochemical parameters marked morphological changes were observed in the liver tissue, beginning as early as 24 h after injection of the hepatotoxin, reaching a maximum after 5 days of the investigation, and showing a tendency toward recovery on the 7th day. These changes were manifested as hydropic and fatty degeneration of the hepatocytes, disappearance of glycogen, congestion of blood vessels, edema of the stroma, focal necrotic changes and hemorrhages, and dilatation and inflammatory infiltration of the portal zones by lymphocytes and histiocytes. These features characterize and confirm the phenomena of nonspecific reactive hepatitis [10]. The biochemical and morphological data this indicate that as early as during the first day toxic hepatitis develops, and its clinical picture continues to worsen, so that disturbances of the biochemical parameters reach a maximum on the 3rd day and morphological changes on the 5th day, to be followed by gradual restoration of the original values.

A prophylactic course of the therapeutic agents chosen for study had a marked hepatoprotective action. For instance, the survival rate of the animals was increased after administration of LN and nicogamol by 36.8%, and of NA by 26.8%, to 89, 89, and 79% respectively, whereas in the untreated animals it was 52.2%. Besides the increase in the animals' survival rate, the basic integral parameter of the protective action of the agents – the biochemical parameters studied returned to normal sooner. Activity of enzymes of the antiradical system, which was strongly inhibited in experimental hepatitis, rose significantly in conjunction with administration of the therapeutic substances, was significantly increased after 24 h, and returned to the normal level by the 3rd day: SOD activity after administration of LN was 18.81 ± 1.72, and of nicogamol 27.70 ± 1.92 compared with 20.89 ± 1.74 in intact animals, and 8.96 ±

1.05 conventional units/mg protein in animals with experimental hepatitis. NA caused no significant change in activity of this enzyme. Catalase activity after 3 days under the influence of LN was 42.07 ± 5.76, of nicogamol 58.39 ± 5.09, and of NA 37.82 ± 2.71 compared with 60.21 ± 4.01 in the control and 9.88 ± 0.79 mmoles H_2O_2/min · g protein in hepatitis. In parallel tests in liver homogenates these preparations delayed accumulation of LPO products, and by the 3rd day their content did not differ significantly from that in intact animals. Under the influence of LN, for instance, the content of CD was 0.023 ± 0.001, of nicogamol 0.028 ± 0.002, and of NA 0.028 ± 0.002 (compared with 0.031 ± 0.002 in the control and 0.038 ± 0.02 optical density unit/mg tissue in hepatitis). MDA was correspondingly 0.799 ± 0.069, 1.131 ± 0.078, and 1.706 ± 0.343 μmole/g (0.793 ± 0.046 in the control, 3.841 ± 0.070 in hepatitis). Activity of ALT, the principal enzyme characterizing the state of the liver function in pathology, was significantly lower than its level of activity in untreated animals 24 h after administration of CCl_4 against the background of treatment with the test preparations. Restoration of activity of this enzyme took place twice as quickly, and by the 3rd day it was indistinguishable from the control, under the influence of LN and nicogamol, whereas NA did not affect ALT activity. Similar changes were observed in the activity of the other enzyme of cytolysis, namely AST, the only difference being that NA enabled normal AST activity to be restored on the 3rd day of the investigation. Dissimilar results were obtained in a study of activity of the enzymes of cholestasis, namely GGT and AlP. Only LN significantly prevented the rise of GGT activity, which was 49.43 ± 4.90 nmoles/sec · liter (45.07 ± 8.77 in the control, 71.82 ± 4.21 nmoles/sec · liter in hepatitis), whereas nicogamol and NA itself did not change its activity. None of the preparations studied had any stabilizing action on AlP activity. The morphological picture of the liver in animals receiving the test preparations differed significantly from the picture of experimental hepatitis. The use of LN prevented damage to the liver structure. It was virtually identical with that in intact animals; the only feature which remained was congestion of the vessels, which may have been due to the effect of NA on processes in the microcirculation Nicogamol also reduced the severity of the morphological changes accompanying toxic liver damage, but its hepatoprotective action was weaker than that of LN. Vacuolar degeneration was still found in individual hepatocytes and congestion of the vessels still remained; the glycogen content rose but did not reach the level in intact animals. Hemorrhages were solitary and petechial in character. Against the background of NA the morphological picture of the liver differed only a little from that observed in experimental hepatitis.

The results of this investigation thus demonstrate that new biologically active substances created on the basis of nicotinic acid possess a hepatoprotective action. This action is evidently based on stimulation of activity of the principal enzymes of the antioxidative system of the cell and regulation of free-radical oxidation of lipids, which ultimately lead to a membrane-stabilizing effect. The facts described above confirm the value of a search for and creation of new hepatoprotective agents based on nicotinic acid.

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