EFFECT OF UBIQUINONE-10 ON DEVELOPMENT OF D-GALACTOSAMINE-INDUCED HEPATITIS IN RATS

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Experimental hepatitis induced by administration of D-(+)-galactosamine (GA) is regarded because of its morphological, histological, and biolochemical characteristics as an adequate model of virus hepatitis B in man, and for that reason it is very useful for screening compounds diminishing pathological processes in the liver. The toxic action of GA is based on its ability to block nucleic acid and protein synthesis and also on its membranotropic action [1, 10]. An experimental and clinical study of ubiquinone-10 demonstrated the good prospects for its use in the treatment of several diseases associated with a disturbance of energy metabolism, and in particular, in the treatment of diseases of the cardiovascular system [8]. Other experiments have shown that ubiquinone-10 protects the liver against toxic damage, for example, by chloroform [9].

The aim of this investigation was to study the hepatoprotective action of ubiquinone-10 on an experimental model of hepatitis induced by GA.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 200-250 g. The animals were divided into three groups, with eight rats in each group: one control group and two experimental groups with hepatitis, induced by a single intraperitoneal injection of GA in a dose of 500 mg/kg body weight. The animals of one of the experimental groups received ubiquinone-10 in a dose of 10 mg/kg perorally in starch mucilage, dally for 14 days before poisoning with GA. Ubiquinone-10 was obtained at the "Vitaminy" Research and Production Combine from biomass of the microorganism *Gluconobacter oxidans* [6]. The control animals received starch mucilage alone. The rats were killed 24 h after receiving the injection of GA. Blood serum levels of activity of alanine- and aspartate-transaminase were determined as in [11], lactate dehydrogenase as in [12], the bilirubin level as in [3], cholesterol as in [5], and triglycerides as in [10] The initial concentration of malonic dialdehyde (MDA) and of MDA formed in the course of enzymic and nonenzymic induction of lipid peroxidation (LPO) was determined by the method in [2, 13]. The protein content in the liver was determined by Lowry's method. The results were subjected to statistical analysis by Student's method. The liver was investigated by methods of light microscopy ("Januval") and electron microscopy ("Philips EM-420" microscope). Morphometry of the hepatocytes was carried out on the MOP-Videplan instrument with computer on histologic sections through the liver. The information obtained was processed by statistical software.

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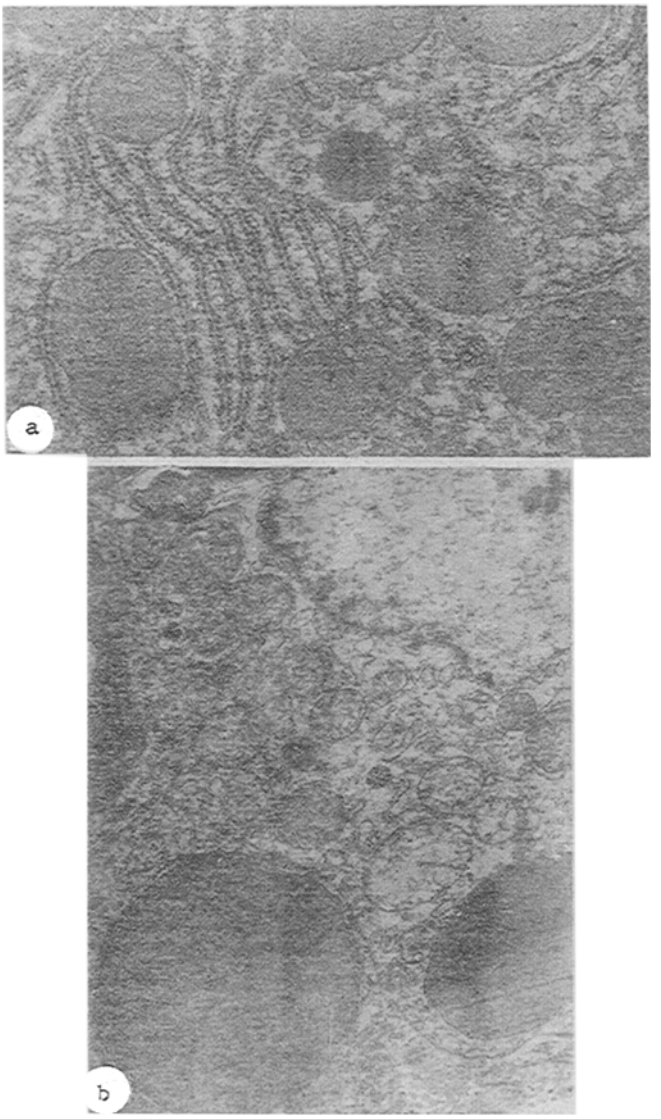

Fig. 1 Ultrastructure of a hepatocyte: a) normal state, b) in hepatitis. 30,000×.

EXPERIMENTAL RESULTS

The structural changes observed during light-optical investigation 24 h after injection of GA were characterized by the development of cloudy swelling degeneration and fine-droplet vacuolar degeneration. Morphometry of the cells revealed an increase in area of the hepatocyte nuclei sometimes to 100 μ^2 (60-65 μ^2 normally). A marked decrease in area of the hepatocyte nucleoli compared with normal also was found. The results of karyometry suggest a disturbance of protein synthesis in the hepatocytes. A study of the ultrastructure of the hepatocytes revealed changes in protein-synthesizing and energy-forming organelles. Swelling of the karyoplasm, segregation of the nucleoli, and fragmentation of the endoplasmic reticulum were noted. Meanwhile swelling of the mitochondrial matrix and destruction of the mitochondrial membrane were observed (Fig. 1b). It can be concluded from analysis of the results that 24 h after injection of GA marked reactive changes characteristic of the early stage of hepatitis developed in the liver. Similar morphologic changes in hepatocytes caused by administration of GA were described in [10].

TABLE 1. Effect of Preliminary Injection of Ubiquinone-10 on Some Biochemical Parameters of Rat Blood Serum and Liver during Development of Galactosamine Hepatitis (M ± m)

Parameter	Groups of animals		
	control	hepatitis	treatment with ubiquinone + hepatitis
Relative mass of liver, %	3,22±0,07	4,0±0,1***	3,46±0,11°°°
Protein content, mg/g liver	123±2	85±4***	121±4°°°
Aspartate-aminotransferase activity, μmoles/ml·h	1,91±0,09	3,0±0,35**	2,0±0,06°°
Alanine-aminotransferase activity, μmoles/ml·h	0,95±0,04	3,0±0,1***	2,33±0,18°
Lactate dehydrogenase activity, μmoles/ml·min	0,54±0,03	1,9±0,5*	1,17±0,22°
Bilirubin, μmoles/liter	14,1±0,8	25±4**	18±2
Cholesterol, mg %	153±4	226±24**	198±16
Triglycerides, mg %	1,6±0,06	1,97±0,07**	1,77±0,13
MDA, mmoles/mg protein	0,38±0,04	0,68±0,1*	0,28±0,01°°
MDA, induction by NADPH, mmoles/mg protein	0,28±0,17	0,93±0,06**	0,49±0,04°°°
MDA, induction by ascorbate, mmoles/mg protein	0,16±0,10	0,48±0,13	0,24±0,09

Legend. Significance of differences compared with control: *p < 0.05, **p < 0.01, ***p < 0.001. Significance of differences of data compared with hepatitis group: °p < 0.05, °°p < 0.01, °°°p < 0.001.

Table 1 gives biochemical parameters of the blood serum and liver of the control animals and of rats with experimental GA-induced hepatitis and animals receiving ubiquinone before being poisoned with GA. The development of hepatitis is accompanied by a significant increase in relative mass of the liver and a decrease in its protein content, which is characteristic of galactosamine hepatitis [10]. Liver damage by the poison led to distinctly raised blood enzyme levels: alanine-aminotransferase activity in the blood serum of rats with hepatitis was increased threefold, activity of aspartate-amino transferase by 1.6 times, and of lactate dehydrogenase almost fourfold compared with the control. The free serum bilirubin level of the affected animals was significantly raised, evidence of a disturbance of the detoxicating function of the liver in this disease. Poisoning the animals with GA caused disturbances of lipid metabolism, expressed as a significant rise in the serum concentration of total cholesterol on triglycerides. We know that in liver diseases of varied etiology LPO processes in biomembranes are enhanced, and this is typical pathogenetic mechanism of hepatitis. It will be clear from Table 1 that 24 h after injection of GA, rat liver homogenates contained 1.8 times more MDA than in the control. Meanwhile, in the affected liver, the reaction of enzymic LPO induced by NADP was significantly activated. Ascorbate-dependent induction of LPO also took place, but was less marked. These results are evidence of a pro-oxidant action of GA and they confirm the results of previous experiments both in vivo and in vitro [1, 4].

The study of the liver of rats treated with ubiquinone for 2 weeks before poisoning with GA revealed moderate swelling of the mitochondrial matrix, evidence of a functional strain on individual liver cells. The results of morphometry of the liver cells agreed with data for the control animals (Fig. 1a).

Preliminary treatment of the rats with ubiquinone before injection of GA (Table 1) prevented changes in the relative mass of the liver and the decrease in its protein content, which was observed in hepatitis; these parameters in rats treated with ubiquinone did not differ from the control. Injection of ubiquinone significantly inhibited the development of high blood enzyme levels: activity of all three serum enzymes in these animals was significantly lower than in those not treated with ubiquinone. Ubiquinone protected the detoxicating function of the liver, for the bilirubin content in animals receiving ubiquinone did not differ significantly from that in intact animals. As regards serum levels of cholesterol and triglycerides, no significant difference could be found between the affected rats, treated or not treated with ubiquinone, but a tendency could be noted for the hyperlipidemia to be less marked in animals treated with ubiquinone. The possibility cannot be ruled out that in this experimental setup the protective action of ubiquinone on lipid metabolism could be manifested more sharply in hepatitis. Preliminary injection of ubiquinone abruptly depressed LPO in the poisoned liver. For instance, in rats with hepatitis and treated with ubiquinone the MDA concentration in the liver was sharply reduced compared with animals not receiving ubiquinone, and moreover, it was significantly lower than in the control. It is interesting to note that in additional experiments, which we conducted on intact animals receiving ubiquinone for 14 days but not thereafter poisoned with GA, it was shown that the MDA concentration in the liver of these animals was significantly lower than in the control (0.22 ± 0.04 compared with 0.38 ± 0.04 nmoles/mg protein). Hence it follows that ubiquinone-10 exhibits its action

not only under pathological conditions, i.e., when LPO has been initiated, but also under normal conditions. As regards NADPH- and ascorbate-dependent LPO, preliminary injection of ubiquinone depressed the activation of these processes induced by GA, as could be seen particularly clearly in the case of the NADP-dependent reaction.

Thus ubiquinone-10 significantly weakens the membranotropic action of GA. The results as a whole, indicating a protective action of ubiquinone-10 against the damaging action of GA, raises the question of whether ubiquinone-10 can be used with advantage in the prevention of virus hepatitis, especially under conditions of increased risk for this disease.

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