Effects of Homeopathic Preparations on the Liver in Rats with Acute and Chronic Toxic Hepatitis

T. V. Vetoshkina and T. I. Fomina

Ultralow doses of antibodies to phenobarbital and their mixture (1:1) with ultralow doses of antibodies to cholecystokinin reduced the severity of structural and metabolic disturbances in the liver of rats with acute CCl_4-induced hepatitis. The mixture of antibodies had no effect on the course of CCl_4-induced hepatitis.

Key Words: *ultralow doses; antibodies to phenobarbital; antibodies to cholecystokinin; acute and chronic CCl_4-induced hepatitis*

Preparations used for the therapy of patients with acute and chronic hepatitides exhibit low therapeutic activity and cause various side effects [8]. The search for new highly efficient and low toxic hepatoprotectors is an urgent problem. We studied hepatoprotective activity of homeopathic preparations containing antibodies to cholecystokinin and phenobarbital ("Materia Medica Holding" Research-and-Production Company).

MATERIALS AND METHODS

The effects of antibodies to phenobarbital (C12+C30+C200, AP) and their mixture with antibodies to cholecystokinin (C12+C30+C200, APC) were studied on 40 Wistar rats weighing 170-200 g (Laboratory of Biological Modeling, Institute of Pharmacology). The animals were kept according to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). Before and during the experiments the rats were maintained in a vivarium under standard conditions.

Acute hepatitis (AH) was induced by intragastric administration of 50% CCl_4 in olive oil (1.25 ml/kg) for 4 days. Chronic hepatitis (CH) was produced by intragastric administration of 50% CCl_4 in olive oil (2 ml/kg) 2 times a week for 3 weeks. AP or APC in a dose of 0.5 ml were introduced intragastrically 1-2 h after hepatotoxin administration for 10 (AH) and 30 days (CH). Control animals received an equivalent volume of potentiated distilled water (C12+C30+C200) according to the same schemes. The rats were decapitated 1 day after administration of the last dose of preparations or water.

Activities of plasma alkaline phosphatase, aspartate transaminase (AST), and alanine transaminase (ALT) were measured using Lachema and Cormay kits. The liver mass index was calculated as the ratio between the weight of the liver and body weight. This parameter was used for evaluation of hepatoprotective activity of xenobiotics [7]. Antitoxic functions of the liver and activity of the microsomal system were evaluated by the duration of hexenal-induced sleep. Hexenal was injected intraperitoneally in dose of 80 mg/kg (1% solution). Sleep time was determined by the period, when rats lay on the side without overturning [9].

Liver samples were fixed in Carnoy's fluid and embedded in paraffin. Deparaffinized sections were stained with hematoxylin and eosin and with picrofuchsin by the Van Gieson technique (specific staining of the connective tissue) [5]. Necrotized hepatocytes were counted in preparations stained with hematoxylin and eosin (per 1000 cells). The relative area of infiltration of the liver parenchyma with macrophages and leukocytes was measured using an Avtandilov ocular grid [1]. The relative area of collagen fibers was determined in preparations stained with picrofuchsin. Cryostat sections (10 m) were prepared from nonfixed liver samples to reveal lipids. Sections were fixed with 4% calcium formol and stained with Sudan black B. The degree of fatty degeneration was expressed in points [2].

The results were analyzed by Student's *t* test.

RESULTS

Hyperemia, discomplexation, moderate fatty degeneration, and hepatocyte necrosis were revealed in histo-

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; "Materia Medica Holding" Research-and-Production Company, Moscow

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TABLE 1. Morphometric Parameters of the Liver in Rats with AH and CH Receiving Homeopathic Preparations ($\bar{X}\pm m$)

Parameter	AH+AP		AH+APC		CH+APC	
	control	experiment	control	experiment	control	experiment
Fatty degeneration, points	2.7	2.0	2.7	1.8	1.5	1.4
Number of necrotized hepatocytes, %	1.00±0.13	0.68±0.14	1.00±0.13	0.65±0.09*	0.32±0.08	0.23±0.03
Relative area, % infiltration	8.6±0.6	7.7±0.5	8.6±0.6	8.04±0.24	16.6±1.50	12.80±1.13
connective tissue	—	—	—	—	8.04±1.67	4.90±0.81

Note. *$p<0.05$ compared to the control.

TABLE 2. Effects of Homeopathic Preparations on the Liver Mass Index and Biochemical Parameters of the Plasma in Rats with AH ($\bar{X}\pm m$)

Parameter	Intact	AH		
		Control	+AP	+APC
Liver mass index	42.91±1.93	41.00±0.78	43.85±0.46	41.59±0.78
AST activity, μcat/liter	0.67±0.02	0.66±0.02	0.62±0.01	0.60±0.03
ALT activity, μcat/liter	0.48±0.07	0.44±0.03	0.43±0.03	0.39±0.03
AST/ALT	1.34±0.25	1.55±0.09	1.50±0.09	1.50±0.09
Alkaline phosphatase activity, units/liter	66.0±3.6	87.0±6.08*	63.0±3.0	62.4±2.4

Note. Here and in Table 3: *$p<0.05$ compared to intact rats.

logical preparations of the liver from rats with AH. Focal agglomerates of macrophages and lymphocytes were found in lobules and around the hepatic triads. The degree of fatty degeneration and parenchymal infiltration did not differ in animals receiving homeopathic preparations (AP or APC) and water. In rats receiving APC the count of necrotized hepatocytes was much lower than in the control (Table 1).

Morphological examination of the liver in rats with CH receiving APC and distilled water revealed degeneration and necrosis of individual hepatocytes and formation of collagen fibers in portal and periportal zones. Sclerosis of portal tracts was accompanied by intralobular with macrophage and lymphocyte infiltration. These morphological signs correspond to chronic active hepatitis [6]. In rats receiving APC the relative area of collagen fibers tended to decrease (insignificantly, Table 1).

Biochemical assay showed that plasma alkaline phosphatase activity in rats with AH was higher than

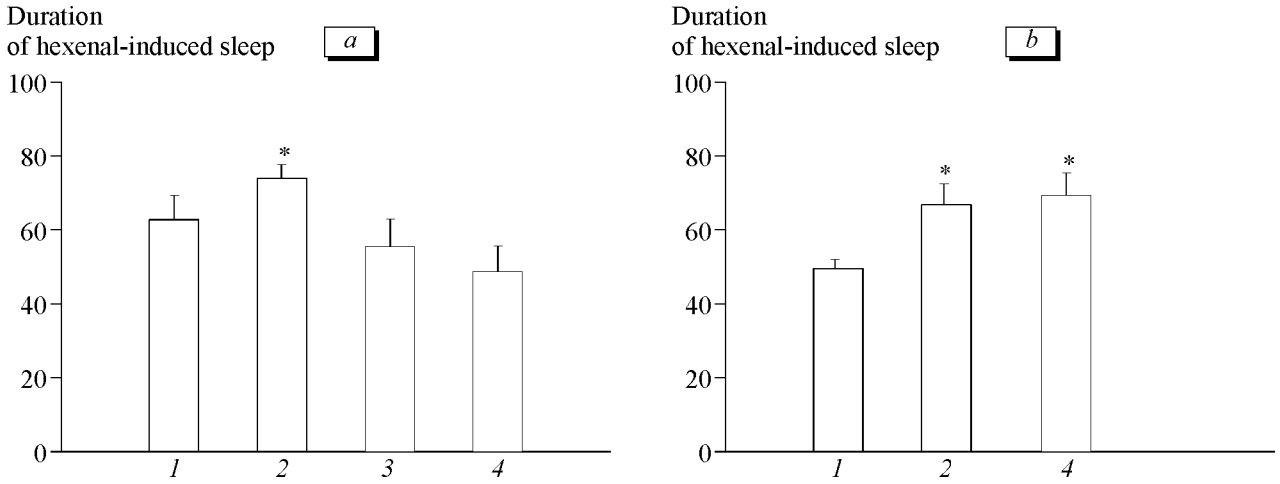

Fig. 1. Effects of homeopathic preparations on the time of hexenal-induced sleep in rats with acute (*a*) and chronic toxic hepatitis (*b*): intact animals (*1*) and rats with hepatitis receiving H_2O (*2*), antibodies to phenobarbital (*3*), and mixture of antibodies to phenobarbital and cholecystokinin (*4*). *$p<0.05$ compared to intact animals.

TABLE 3. Effects of Homeopathic Preparations on the Liver Mass Index and Biochemical Parameters of the Plasma in Rats with CH ($\bar{X}\pm m$)

Parameter	Intact	CH	
		Control	+APC
Liver mass index	35.89±1.74	45.22±0.81*	45.66±1.76*
AST activity, μcat/liter	0.51±0.03	0.59±0.02	0.58±0.03
ALT activity, μcat/liter	0.46±0.06	0.56±0.07	0.56±0.06
AST/ALT	1.19±0.14	1.09±0.11	0.94±0.10
Alkaline phosphatase activity, U/liter	66.9±16.6	374.85±10.7*	316.7±31.2*

in intact animals (Table 2). These changes were probably associated with cholestasis and intensive diffusion of the enzyme through the sinusoidal membrane of hepatocytes [4]. It should be emphasized that the duration of hexenal-induced sleep in rats treated with hepatotoxin increased, which was related to inhibition of enzymes responsible for antitoxic activity of the liver (Fig. 1, *a*). Administration of APC to rats with AC normalized activity of alkaline phosphatase and stimulated hexenal biotransformation in the hepatic monooxygenase system.

CH was accompanied by activation of plasma alkaline phosphatase, prolongation of hexenal-induced sleep, and increase in liver mass index (Fig. 1, *b*; Table 3). APC did not abolish the toxic effects of repeated treatment with CCl_4 (Table 3; Fig. 1, *b*).

Our results suggest that homeopathic preparations containing AP and APC prevented the development of structural and metabolic disturbances in the liver of rats with CCl_4-produced hepatitis. These preparations reduced the severity of hepatocyte necrosis, intensified antitoxic functions of the liver, decreased the duration of hexenal-produced sleep, and normalized activity of alkaline phosphatase (enzyme of cholestasis).

The preparation containing APC had no effect on the course of chronic CCl_4-produced hepatitis.

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry* [in Russian], Moscow (1990).
2. A. I. Vengerovskii, I. V. Markova, and A. S. Saratikov, *Manual on Experimental (Preclinical) Studies of New Pharmacological Preparations* [in Russian], Moscow (2000), pp. 228-231.
3. T. B. Dmitrieva, *Ros. Med. Vestn*., No. 3, 15-26 (1997).
4. L. L. Gromashevskaya, L. V. Tat'yanko, I. M. Radzevich, *et al*., *Progress in Hepatology* [in Russian], Riga (1980), Vyp. 8, pp. 145-163.
5. G. A. Merkulov, *Pathohistological Technique* [in Russian], Leningrad (1969).
6. *Morphological Diagnosis of Liver Diseases* [in Russian], Eds, V. V. Serov and K. Lapish, Moscow [1989].
7. *Characteristics of Normal in Laboratory Animals during Toxicological Studies* [in Russian], Eds. I. M. Trakhtenberg *et al*., Moscow (1978).
8. N. P. Skakun, V. V. Shman'ko, and L. L. Okhrimovich, *Clinical Pharmacology of Hepatoprotectors* [in Russian], Ternopol (1995).
9. I. P. Ulanova, G. G. Avilova, *et al*., *Methods for Estimating Toxicity and Dangerousness of Chemicals* [in Russian], Moscow (1970), pp. 189-199.