Hepatoprotective Properties of Potentiated Antibodies to Cholecystokinin

T. V. Vetoshkina and T. I. Fomina

Ultralow doses of antibodies to cholecystokinin prevented hepatocyte death, delayed the formation of the connective tissue, and normalized plasma of liver enzyme in rats with experimental acute and chronic toxic hepatitis.

Key Words: *antibodies to cholecystokinin; experimental toxic hepatitis*

In recent years the incidence of liver diseases markedly increased in various countries [6]. This is associated with environmental pollution, urbanization and industrialization, and consumption of considerable amounts of xenobiotics that enter the composition of water and food products and impair liver functions in humans [3]. High prevalence of acute and chronic hepatitides and low efficiency of pharmacotherapy of these diseases dictate the need for new medicinal preparations prevent progression of inflammatory and dystrophic changes and liver fibrosis without producing serious side effect during long-term treatment. It was hypothesized that the preparation containing antibodies to cholecystokinin and synthesized at the "Materia Medica Holding" Research-and-Production Company (Moscow) possess these properties.

MATERIALS AND METHODS

Hepatoprotective activity of antibodies to cholecystokinin (ACK, C12+C30+C200) was studied on 35 Wistar rats weighing 170-200 g and obtained from the Laboratory of Biological Models (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 was induced by administration of 50% CCl_4 in olive oil (1.25 ml/kg intragastrically) or D-galactosamine (300 mg/kg intraperitoneally) for 4 or 3 days, respectively. Chronic hepatitis was induced by intragastric administration of 50% CCl_4 in olive oil (2 ml/kg) 2 times a week for 3 weeks. ACK were introduced intragastrically in a daily dose of 0.5 ml 1-2 h after administration of hepatotoxins. The rats with acute hepatitis induced by CCl_4 and D-galactosamine received ACK for 10 and 3 days, respectively. In animals with chronic hepatitis ACK were administered for 30 days. Control animals received an equivalent volume of activated distilled water (C12+C30+C200) according to the same scheme. 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 biochemically using Lachema and Cormay kits. The liver mass index was calculated as the ratio of weight of the liver to body weight. This parameter was used to estimate hepatoprotective activity of xenobiotics [7].

Liver samples were fixed in Carnoy's fluid and embedded in paraffin. Deparaffinized sections were stained with hematoxylin and eosin. Staining with picrofuchsin by the Van Gieson technique was specific for the connective tissue [4]. 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 determined 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, fixed with 4% calcium formol, and stained with Sudan black B to reveal lipids. The degree of fatty degeneration was expressed in points [2].

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

RESULTS

Morphological examination of the liver in rats with acute CCl_4-induced hepatitis revealed hyperemia, dis-

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

0007-4888/03/supp-0088$25.00

TABLE 1. Effect of ACK on Morphometric Parameters of the Liver in Rats with Acute and Chronic Hepatitides ($\bar{X}\pm m$)

Parameter	Acute hepatitis				Chronic hepatitis	
	CCl_4		D-galactosamine			
	control	experiment	control	experiment	control	experiment
Fatty degeneration, points	3.0	2.3	1.4	1.1	1.5	1.4
Count of necrotized hepatocytes, %	1.1±0.2	0.60±0.06*	10.54±3.08	2.92±0.87*	0.73±0.13	0.37±0.06*
Relative area, %						
infiltration	7.1±0.8	8.6±0.7	13.70±0.75	16.9±1.9	18.2±1.32	16.5±1.07
connective tissue	—	—	—	—	6.73±0.55	3.95±1.09*

Note. *p<0.05 compared to the control.

complexation, moderate fatty degeneration, and hepatocyte necrosis. Focal agglomerates of macrophages and lymphocytes were found in hepatic lobules and portal tracts. The animals with D-galactosamine-induced hepatitis we observed hydropic degeneration of hepatocytes; considerable number of hepatocytes in the state of apoptosis, whose degree varied from degradation of DNA to the formation of Councilman's bodies (indirect markers of viral hepatitis in humans) were found [5]. The portal and intralobular stroma was diffusely infiltrated with lymphocytes, macrophages, and neutrophilic leukocytes.

Morphological examination of the liver from rats receiving CCl_4 for a long time revealed fatty degeneration and necrosis of individual hepatocytes. Periportal infiltration with macrophages and lymphocytes destroyed the terminal plate and involved the intraportal region. Infiltration of portal tracts with lymphocytes and macrophages was accompanied by the formation of collagen fibers. This morphological picture corresponded to chronic active hepatitis [5].

The degree of fatty degeneration and relative area of liver infiltration did not differ in rats with acute and chronic hepatitis receiving ACK and water. In animals receiving ACK the count of necrotized hepatocytes was below the control. It should be emphasized that the relative area of collagen fibers in rats with chronic hepatitis treated with ACK was lower than in control animals (Table 1).

Plasma alkaline phosphatase activity in rats with acute CCl_4-induced hepatitis was higher than in intact animals (Table 2). Previous studies showed demonstrated massive release of hepatic alkaline phosphatases into the blood after cell death or during cholestasis (intensive diffusion through the sinusoidal membrane in hepatocytes and reduced excretion with the bile). D-Galactosamine produced severe damage to hepatocyte membranes. In these rats we found increased ALT activity, AST/ALT ratio, and liver mass index (Table 3). Plasma AST activity increased in rats with chronic CCl_4-induced hepatitis (Table 4). ACK normalized activities of alkaline phosphatase and ALT in rats with acute and chronic CCl_4-induced hepatitis, respectively (Tables 2 and 4). This preparation produced a protective effect in rats with acute D-galactosamine-induced hepatitis. In these rats the liver mass index and activities of ALT and AST did not differ from those in intact animals (Table 3).

Our results show that ACK decreased the severity of morphological and biochemical changes in the liver, prevented hepatocyte death, and normalized plasma levels of liver enzymes in rats with experimental toxic

TABLE 2. Effect of ACK (C12+C30+C200) on Biochemical Parameters of the Plasma from Rats with Acute and Chronic Hepatitides ($\bar{X}\pm m$)

Parameter	Intact	Acute CCl_4-induced hepatitis	
		control	+ACK
Liver mass index	40.09±1.72	40.32±1.66	40.63±1.56
AST activity, μcat/liter	0.66±0.04	0.71±0.02	0.55±0.08
ALT activity, μcat/liter	0.68±0.04	0.76±0.04	0.66±0.05
AST/ALT	0.98±0.07	0.93±0.05	0.83±0.04
Alkaline phosphatase activity, units/liter	81.6±6.6	109.8±4.8*	82.2±5.4

Note. Here and in Tables 3 and 4: *p<0.05 compared to intact rats.

TABLE 3. Effect of ACK on the Liver Mass Index and Biochemical Parameters of the Plasma from Rats with Acute Hepatitis Produced by D-Galactosamine ($\bar{X}\pm m$)

Parameter	Intact	Acute hepatitis	
		control	+ACK
Liver mass index	45.31±2.34	55.40±0.89*	48.85±2.98
AST activity, μcat/liter	0.50±0.04	0.62±0.05	0.50±0.03
ALT activity, μcat/liter	0.51±0.06	0.73±0.08*	0.55±0.01
AST/ALT	0.98±0.04	0.86±0.03*	0.92±0.06
Alkaline phosphatase activity, units/liter	192.5±21.8	297.9±54.9	215.5±12.7

TABLE 4. Effect of ACK on the Liver Mass Index and Biochemical Parameters of the Plasma from Rats with Chronic CCl_4-Induced Hepatitis ($\bar{X}\pm m$)

Parameter	Intact	Chronic hepatitis	
		control	+ACK
Liver mass index	39.91±1.25	42.65±2.03	38.46±3.71
AST activity, μcat/liter	0.57±0.04	0.64±0.02	0.58±0.03
ALT activity, μcat/liter	0.68±0.06	0.88±0.08*	0.69±0.04
AST/ALT	0.93±0.09	0.73±0.03	0.86±0.08
Alkaline phosphatase activity, units/liter	321.60±6.51	329.80±2.35	448.0±37.40

hepatitis. ACK suppressed the development of sclerotic changes in the liver in rats with chronic CCl_4-induced 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. N. F. Gerasimenko, *Ros. Med. Vestn.*, No. 3, 5-14 (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. F. I. Komarov and V. T. Ivashkin, *Vestn. Ros. Akad. Med. Nauk*, No. 11, 3-6 (1997).
8. *Parameters of Normal in Laboratory Animals during Toxicological Studies* [in Russian], Eds. I. M. Trakhtenberg *et al.*, Moscow (1978).