Hepatoprotective Properties of Soybean Proteins and Their Use for Dietary Therapy of Chronic Toxic Hepatitis

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Hepatoprotective effect of a diet containing soybean proteins most pronounced in soybean milk was demonstrated on the model of chronic toxic hepatitis. Results of biochemical studies correlate with morphological data indicating that soybean proteins hold much promise for supportive therapy of patients with chronic liver diseases.

Key Words: *chronic toxic hepatitis; soybean protein; dietary therapy; glycogen; bilirubin*

The liver plays an important role in the biosynthesis of blood plasma proteins and deamination and transamination of amino acids. The intensity of these processes depends on dietary proteins [9]. Inflammation, intensive lipid peroxidation (LPO), impairment of hepatocyte membrane permeability, and inhibition of protein synthesis contribute to the pathogenesis of acute and chronic hepatitis and liver cirrhosis [4,5,10]. Protein and amino acid deficiency adversely affects the immune status of the organism [6,12]. Therefore, the therapy of patients with toxic hepatitis must include dietary treatment.

Our previous studies showed that diets including soybean milk and isolated soybean protein Supro-760 produces strong therapeutic effect in patients with alimentary dystrophy and chronic gastric ulcer [2,3]. In addition, a diet containing soybean milk improved the immune status in immunocompromised patients [6].

Here we studied hepatoprotective properties of soybean proteins and evaluated the possibility of their use for dietary treatment of toxic hepatitis.

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MATERIALS AND METHODS

Experiments were performed on 180 male outbred albino rats weighing 180-200 g (Rappolovo nursery, Russian Academy of Medical Sciences). Toxic hepatitis was induced with tetrachloromethane (CCl_4), a specific hepatotropic poison causing acute and chronic hepatitis and liver cirrhosis. CCl_4-induced damages to liver cell membranes are associated with LPO induced by free radicals, which are intensively generated due to the activation of CCl_4 with microsomal enzymes [1,10,14].

CCl_4 (50% mineral oil solution) was subcutaneously injected in a dose of 0.4 ml/kg for 4 days. Morphological and functional changes typical of chronic hepatitis were observed in the liver on day 7 after the start of CCl_4 poisoning [5]. Soybean milk and isolated soybean protein Supro-2725 (PTI) were administered through an intragastric tube in a dose of 2 g/kg body weight. The hepatoprotector Carsil obtained from milk thistle (*Silybum*) was used as a reference drug. Hepatoprotective effects of Carsil are associated with its antioxidant properties, stimulation of protein synthesis, and normalization of phospholipid metabolism [7]. Carsil in a dose of 20 mg/kg was administered intragastrically through a tube 2 times a day. Bio-

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chemical and histological assays were performed on days 7, 14, and 21 of therapy.

The contents of malonic dialdehyde (MDA) [11] and glycogen [13] in the liver and bilirubin concentration in the plasma [8] were measured. The material was fixed in 10% formaldehyde and embedded in paraffin for histological examination. Liver slices were stained with hematoxylin and eosin. The results were analyzed by Student's *t* test.

RESULTS

In animals with toxic hepatitis, MDA content increased by 59% and glycogen decreased by 71%; plasma bilirubin increased by 134% (Table 1). These data indicate intensification of LPO and impairment of carbohydrate metabolism and pigment formation in the liver. Morphological signs were typical of active chronic hepatitis: loss of normal cytoarchitectonics of liver cells, small-drop fatty degeneration and eosinophilic degeneration of the majority of hepatic lobules, moderate portal sclerosis, and lymphatic infiltration of the connective tissue; adaptive changes were not found (Fig. 1, *a*).

Plasma bilirubin concentration and the content of glycogen and MDA in the liver of animals fed a standard diet tended to normal on days 7 and 14 of chronic hepatitis, respectively, but did not reach the initial levels even on day 21 of observations. Morphological examination showed the persistence of pathological processes on day 7 of chronic hepatitis. The severity and distribution of degenerative and necrotic changes were reduced on day 14 of observations. On day 21, morphological signs were typical of moderate chronic hepatitis. This period was characterized by a considerable variability in the intensity and regularity of pathological processes: propagation of sclerotic processes in the portal system on the one hand, and nuclear polymorphism of hepatocytes in some hepatic lobules on the other hand (Fig. 1, *b*).

In rats with experimental toxic hepatitis fed soybean milk, the contents of MDA and glycogen in the liver and plasma bilirubin concentration returned to normal on days 7 and 14, respectively.

Morphological examination of liver slices revealed intensive adaptive processes in hepatic lobules of rats fed soybean milk for 7 days. These processes were more pronounced on day 14 of dietary therapy. On day 21 of treatment with soybean milk, morphological signs were typical of latent persistent hepatitis with adaptive processes and hepatocyte mitoses (Fig. 1, *c*).

Similar changes were observed in rats fed a standard diet and receiving isolated soybean protein Supro-2725 or Carsil (intragastrically). It should be noted that biochemical signs of liver damage (contents of glycogen, MDA, and bilirubin) returned to normal on day 14 of therapy.

Morphological examination revealed chronic hepatitis; damages and necrosis of hepatocytes were reduced from day 7 to 14 day of therapy. In rats treated with isolated soybean protein Supro-2725 or Carsil for 21 days, chronic hepatitis was characterized by transient signs of damage and adaptive polymorphism of hepatocytes (Figs. 1, *d*, *e*).

Thus, biochemical and morphological studies in rats with chronic toxic hepatitis showed that a diet containing soybean proteins (in particular, soybean milk) possesses hepatoprotective properties, which are

TABLE 1. Contents of MDA, Glycogen, and Bilirubin in Rats with CCl_4-Induced Chronic Hepatitis Treated with Soybean Proteins (*M*±*m*)

Indexes	Intact animals (control)	CCl_4-induced hepatitis	Therapy, days	Standard diet			
				without dietary therapy	+ soybean milk	+ supro 2725	+ Carsil
MDA, nmol/g	4.7±0.4	7.5±0.4*	7	7.2±0.4	4.4±0.22+	4.3±0.28+	5.0±0.27+
			14	6.1±0.43	—	—	—
			21	5.8±0.4	—	—	—
Glycogen, g%	4.8±0.25	1.4±0.09*	7	1.2±0.07*	4.1±0.32+	3.2±0.19+	2.4±0.12+
			14	2.3±0.19*	4.8±0.22+	4.4±0.27+	3.5±0.20+
			21	2.6±0.24*	5.2±0.23+	4.6±0.28+	3.7±0.25+
Bilirubin, μmol/liter	22.4±0.84	52.4±4.2*	7	36.4±2.25*	29.1±3.9	30.8±1.7	26.8±3.2
			14	32.5±2.25*	23.4±0.84+	24.7±0.86+	20.8±1.7+
			21	28.0±0.84*	21.6±1.4+	20.2±0.84+	20.0±3.2+

Note. Significant differences: *compared to the control; +compared to animals with CCl_4-induced hepatitis fed a standard diet.

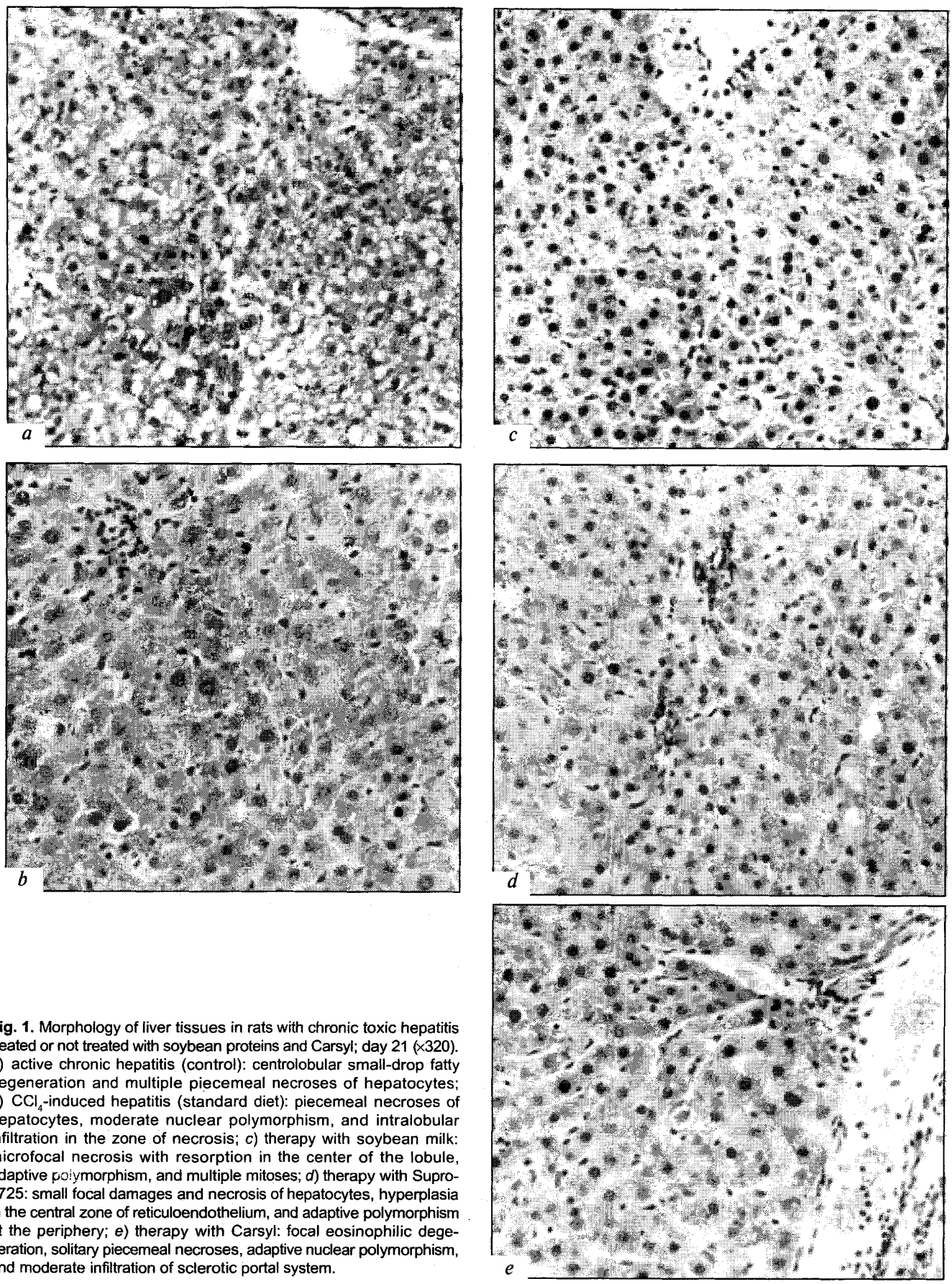

Fig. 1. Morphology of liver tissues in rats with chronic toxic hepatitis treated or not treated with soybean proteins and Carsyl; day 21 (×320). *a*) active chronic hepatitis (control): centrolobular small-drop fatty degeneration and multiple piecemeal necroses of hepatocytes; *b*) CCl_4-induced hepatitis (standard diet): piecemeal necroses of hepatocytes, moderate nuclear polymorphism, and intralobular infiltration in the zone of necrosis; *c*) therapy with soybean milk: microfocal necrosis with resorption in the center of the lobule, adaptive polymorphism, and multiple mitoses; *d*) therapy with Supro-2725: small focal damages and necrosis of hepatocytes, hyperplasia in the central zone of reticuloendothelium, and adaptive polymorphism at the periphery; *e*) therapy with Carsyl: focal eosinophilic degeneration, solitary piecemeal necroses, adaptive nuclear polymorphism, and moderate infiltration of sclerotic portal system.

related not only to antioxidant activity and normalization of protein synthesis, but also to immunomodulatory effect [2,6]. Our findings indicate that soybean milk holds much promise for supportive therapy in patients with chronic liver diseases.

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