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Effect of N-[Imino(4-morpholyl)methyl]guanidine on the Oxidative Status in Rats with Toxic Hepatitis

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The hepatoprotective and antioxidant properties of a synthetic biguanide N-[imino(4-morpholyl)methyl]guanidine (IMMG) were prognosticated by the method of computer prediction. Administration of IMMG was accompanied by a decrease in serum transaminase activity in rats with toxic hepatitis, which reflects inhibition of hepatocyte cytolysis. IMMG treatment was followed by a decrease in biochemiluminescence parameters reflecting the intensity of free radical oxidation. We revealed an increase in activity of aconitase, which was reduced during toxic hepatitis. The content of citrate in the liver and serum was returned to normal under these conditions. IMMG also increased activities of superoxide dismutase and catalase and total antioxidant activity in rat liver. Our results suggest that the hepatoprotective effect of IMMG is associated with its antioxidant activity.

Key Words: *toxic hepatitis; guanidines; free radical oxidation*

Liver diseases caused by toxic agents are accompanied by severe homeostasis disturbances. Activation of free radical oxidation is followed by the development of oxidative stress, which plays an important role in the pathogenesis of toxic hepatitis [2,9]. The search for hepatoprotective agents with antioxidant activity is an urgent problem. The PASS program (Prediction and Activity Spectra for Substances) provides selection of structures with specified biological activity. Among a variety of synthetic structures, guanidine derivatives attract much attention because of a wide range of biological properties and strong clinical effect. For example, biguanide metformin is used for the therapy of type 2 diabetes mellitus and exhibits a unique combination of properties [12].

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Here we studied the effect of synthetic guanidine derivative with predicted hepatoprotective and antioxidant properties, N-[imino(4-morpholyl)methyl]guanidine (IMMG), on free radical oxidation and activity of some antioxidant enzymes in the liver and blood serum of rats with experimental toxic hepatitis.

MATERIALS AND METHODS

Experiments were performed on male albino rats (*Rattus rattus L.*) weighing 150-200 g. The animals were divided into groups. Group 1 rats (control, n=19) were maintained under standard vivarium conditions. In group 2 animals (n=20), toxic hepatitis was induced by single treatment with a hepatotropic toxin CCl_4 after 24-h food deprivation. Group 3 rats (n=8) intraperitoneally received IMMG (50 mg/kg) in 1 ml 0.9% NaCl (twice a day). IMMG was synthesized at the Department of Organic Chemistry (Voronezh State

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University) [10]. The serum was obtained from the venous blood. A weighted sample of rat liver was homogenized in a 4-fold volume of cold isolation medium (0.1 M Tris-HCl buffer, pH 7.8) containing 1 mM EDTA and 1% β-mercaptoethanol and centrifuged at 10,000*g* for 15 min. The intensity of free radical oxidation and total antioxidant activity were estimated by the method of biochemiluminescence on a BKhL-07M chemiluminometer with special software [7]. Enzyme activity was measured on a SF-56 spectrophotometer. The amount of the enzyme catalyzing conversion of 1 μmol substrate at 25°C over 1 min was taken as a unit of enzyme activity (U). Enzyme activity was expressed in U per 1 g wet liver or 1 ml serum. Aconitase activity was measured in the medium of 50 mM Tris-HCl buffer (pH 7.8) and 4 mM citrate at 235 nm. Catalase activity was measured at 410 nm [3]. The concentration of citrate was estimated by the method of Natelson [1]. Superoxide dismutase (SOD) activity was evaluated by inhibition of nitroblue tetrazolium (NBT) reduction [5]. Transaminase activity was measured with Bio-La-Tes kits. The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Serum transaminase activity increased on day 4 of toxic hepatitis, which reflected cytolysis of rat hepatocytes. Activities of alanine transaminase and aspartate transaminase in group 3 animals were lower than in group 2 rats (by 3.6 and 3.1 times, respectively). These data suggest that IMMG has the hepatoprotective properties.

The development of toxic hepatitis was accompanied by an increase in the total luminescence (biochemiluminescence signal, S) and flash intensity of biochemiluminescence (I_{max}) in the liver and serum [7,8]. These changes reflect activation of free radical oxidation (Table 1). Aconitase activity was reduced in animals with toxic hepatitis (Fig. 1) [6,7]. The active site of this enzyme contains an iron-sulfur cluster, which is easily destructed under the influence of free radical oxygen species. Cysteine residues can be modified in the active site of aconitase [4]. The inhibition of aconitase is followed by accumulation of citrate (substrate) [6,7]. The increase in citrate concentration probably plays an adaptive role, which is associated with chelating properties of the citric acid anion in relation to divalent iron ions. These ions have prooxidant activity [2]. Moreover, citrate protects the active site of aconitase from injury [4]. Administration of IMMG was followed by the decrease in S, I_{max} (Table 1) and citrate content (Fig. 1) and increased aconitase activity in the liver and blood serum (Fig. 1). Aconitase activity in the liver of these animals surpassed the control level. The increase in aconitase activity is probably related to activation of glucose utilization in the liver. The observed changes are accompanied by an increase in functional activity of the tricarboxylic acid cycle. Our results are consistent with published data that biguanide derivatives, phenformin and metformin, contribute to glucose accumulation in liver cells, activate glycolysis, and inhibit gluconeogenesis (similarly to insulin) [11]. At the same time, enzyme activity in blood serum decreased the control level (similarly to transaminase activity), probably due to reduction of hepatocytes cytolysis.

Previous studies showed that activation of free radical oxidation is followed by intensification of antioxidant processes [15]. Toxic hepatitis was accompanied by an increase in activities of SOD and catalase in rat liver and serum [9]. These enzymes are induced under the influence of redox-sensitive transcription

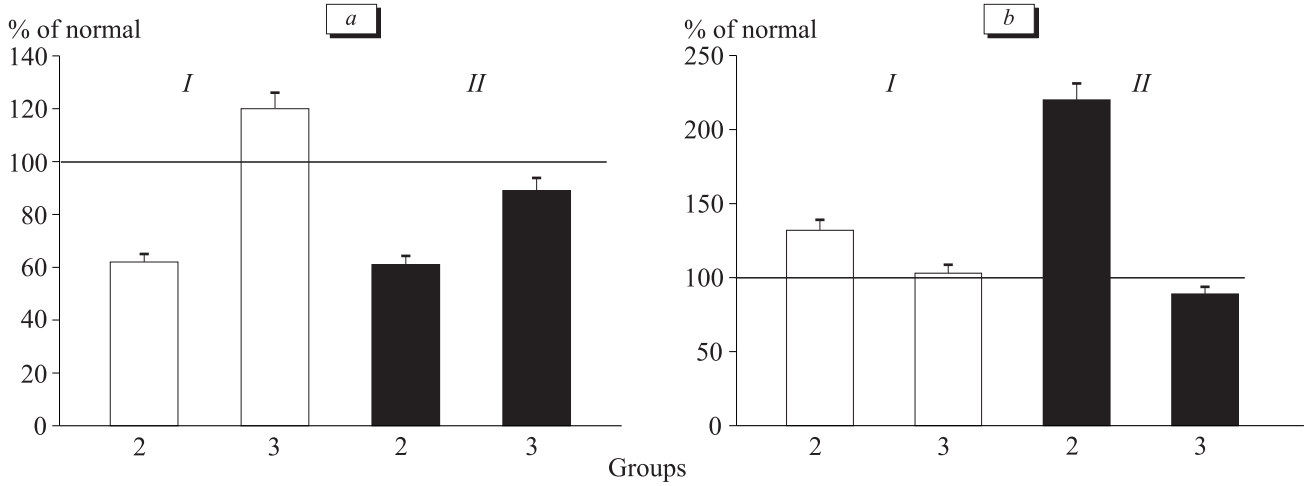

Fig. 1. Aconitase activity (*a*) and citrate content (*b*) in the liver (*I*) and blood serum (*II*) from rats with toxic hepatitis (group 2) and IMMG-receiving animals (group 3) with the pathological process. Here and in Fig. 2: normal, 100%.

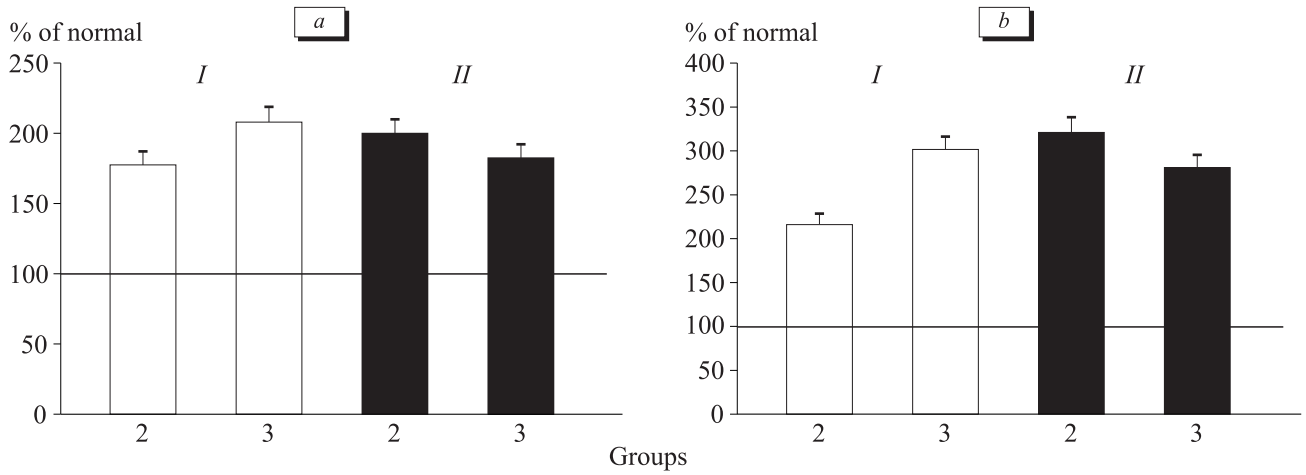

Fig. 2. Activities of SOD (*a*) and catalase (*b*) in the liver (*I*) and blood serum (*II*) from rats with toxic hepatitis (group 2) and IMMG-receiving animals (group 3) with the pathological process.

TABLE 1. Biochemiluminescence in Rat Tissues under Control Conditions, Experimental Toxic Hepatitis, and Treatment with IMMG during the Pathological Process (*M*±*m*)

Group	Total luminescence of the chemiluminescence flash (S), mV×sec		Maximum flash intensity (I_{max}), mV		Slope of the kinetic curve ($tg\alpha_2$)	
	liver	serum	liver	serum	liver	serum
1 (control)	92±4	305±15	42±2	35±2	8±1	13±1
2	250±10	555±26	82±4	128±6	18±1	36±2
3	212±8*	490±23*	66±3*	102±5*	30±2*	44±2*

Note. *$p<0.05$ compared to group 2.

factor NF-kB [14]. The tangent of the biochemiluminescence kinetic curve slope ($tg\alpha_2$) reflecting total antioxidant activity was shown to decrease during toxic hepatitis [7,8].

Administration of IMMG was followed by an increase in SOD and catalase activities in rat liver (by 1.2 and 1.4 times, respectively, compared to animals with toxic hepatitis; Fig. 2). The decrease in activities of these enzymes in blood serum was probably associated with inhibition of hepatocyte cytolysis. Moreover, IMMG induced an increase in tga_2 in the liver and blood serum of rats (Table 1). The ability of IMMG to increase antioxidant enzyme activity probably underlies the hepatoprotective effect of this substance during toxic hepatitis. The same effect is typical of strong antioxidants, including melatonin [15] and thioctic acid [13]. They not only interact with free radicals, but also induce transcription of antioxidant enzymes.

Our results suggest that the hepatoprotective effect of IMMG is associated with its antioxidant activity.

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REFERENCES

1. V. G. Afanas'ev, V. S. Zaitsev, and T. I. Vol'fson, *Lab. Delo*, No. 4, 115-116 (1973).
2. N. K. Zenkov, V. Z. Lankin, and E. B. Men'shikova, *Oxidative Stress: Biochemical and Pathophysiological Aspects* [in Russian], Moscow (2001).
3. M. A. Korolyuk, L. I. Ivanova, I. G. Maiorova, *et al*., *Lab. Delo*, No. 1, 16-19 (1988).
4. L. V. Matasova and T. N. Popova, *Biokhimiya*, **73**, No. 9, 1189-1198 (2008).
5. B. N. Matyushin, *Lab. Delo*, No. 7, 16-19 (1991).
6. A. N. Pashkov, S. S. Popov, V. I. Zoloedov, *et al*., *Sistemnyi Analiz Upravlenie Biomeditsinskikh Sistemakh*, **5**, No. 1, 120-122 (2006).

7. A. N. Pashkov, S. S. Popov, A. V. Seminikhina, *et al*., *Probl. Endokrinol*., **51**, No. 6, 41-43 (2005).
8. A. N. Pashkov, S. S. Popov, A. V. Seminikhina, and T. I. Rakhmanova, *Zh. Teoretich. Praktich. Med*., **4**, No. 1, 93-96 (2006).
9. S. S. Popov, A. N. Pashkov, T. N. Popova, *et al*., *Eksper. Klin. Farmakol.*, **70**, No. 1, 48-51 (2007).
10. Kh. S. Shikhaliev, A. V. Falaleev, and D. V. Kryl'skiy, *Selected Methods for the Synthesis and Modification of Heterocyclic Compounds* [in Russian], Vol. 1, pp. 450-477 (2003).
11. K. Cusi and R. A. DeFronzo, *Diabetes Rev*., **6**, 89-131 (1998).
12. D. Kirpichnikov, S. I. McFarlane, and J. R. Sowers, *Ann. Intern. Med*., **13**, 25-33 (2002).
13. K. Petersen Shay, R. F. Moreau, E. J. Smith, and T. M. Hagen, *IUBMB Life*, **60**, No. 6, 362-367 (2008).
14. Y. Sun and L. W. Oberley, *Free Radic. Biol. Med*., **21**, No. 3, 335-348 (1996).
15. C. Tomas-Zapico and A. Coto-Montes, *J. Pineal Res*., **39**, No. 2, 99-104 (2005).