Glutathione System and Activity of NADPH-Generating Enzymes in the Liver of Intact Rats and Animals with Toxic Hepatitis Receiving Melatonin

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Melatonin administered to intact animals increased glutathione concentration and activities of glutathione peroxidase, glutathione reductase, NADP-isocitrate dehydrogenase, and glucose-6-phosphate dehydrogenase in the liver. In animals with toxic hepatitis melatonin treatment decreased glutathione concentration and enzyme activities, which was probably associated with inhibition of free radical oxidation under the influence of this hormone.

Key Words: *toxic hepatitis; melatonin, antioxidant system*

Progression of various liver diseases, including toxic hepatitis, is accompanied by overproduction of reactive oxygen species (ROS) and depletion of the antioxidant system. These changes are associated with activation of free radical (FR) reactions, impairment of biomembranes, and cell dysfunction, which results in oxidative stress [1,12]. The glutathione system plays an important role in the regulation of FR oxidation. Reduced glutathione is an important antitoxic compounds in the liver. This agent reacts with free radicals, quenches singlet oxygen and hydroxyl radicals, and inhibits lipid peroxidation [6]. The enzyme system of glutathione reductase (GR, EC 1.6.4.2) and glutathione peroxidase (GPX, EC 1.11.1.9) is responsible for H_2O_2 detoxification. The reaction involves reduced glutathione and is catalyzed by GPX [7]. The rate of reduced glutathione formation in the coupled reaction catalyzed by GR mainly depends on NADPH concentration [9]. The pentose phosphate pathway is the major source of NADPH for the GR/GPX system. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) is a key enzyme of this pathways that catalyzes glucose-6-phosphate conversion into 6-phosphogluconolactone [5]. The reaction catalyzed by NADP-isocitrate dehydrogenase (EC 1.1.1.42) is an alternative source of NADPH. This reaction is accompanied by oxidative decarboxylation of isocitrate into 2-oxoglutarate [8]. Antioxidant activity of some hormones, including melatonin, was studied in previous experiments [3,13]. It was hypothesized that generation of ROS and intracellular or intracellular signal processes induce the synthesis of protective cell components, regulate activity of antioxidant enzymes, and modulate nonspecific organism's resistance. However, this problem is poorly understood. Melatonin is a neurohormone produced in the pineal gland and extrapineal tissues and involved in synchronization of diurnal and seasonal biorhythms, neuroendocrine regulation of the reproductive system, inhibition of hypophyseal function, and antistress protection [15]. Melatonin can trap hydroxyl radicals, singlet oxygen, and nitric oxide [2]. This work was designed to study the mechanisms of interaction between the antioxidant system of glutathione, NADPH-generating enzymes, and melatonin. We measured glutathione concentration and activities of GR, GPX, glucose-6-phosphate dehydrogenase, and NADP-isocitrate dehydrogenase in the liver of intact rats and animals with toxic hepatitis receiving melatonin.

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MATERIALS AND METHODS

Experiments were performed on male albino rats (*Rattus rattus* L.) weighing 150-200 g. The animals were divided into 4 groups. Group 1 animals were maintained in a vivarium under standard conditions (control, *n*=8). Group 2 rats were deprived of food for 1 day and received hepatotropic toxin CCl_4 in a dose of 0.064 ml per 100 g body weight to produce toxic hepatitis (*n*=9). Published data show that the intensity of hepatocyte cytolysis is maximum after single administration of this toxin [10]. Intact animals of group 3 intraperitoneally received melatonin in a daily dose of 2 mg/kg for 3 days (*n*=8). Group 4 rats received melatonin in the same dose over 3 days starting from the 1st day after induction of toxic hepatitis (*n*=8). The liver was removed from narcotized animals after perfusion with physiological saline. Weighted liver samples were homogenized in a 4-fold volume of cold isolation medium containing 0.1 M Tris-HCl buffer (pH 7.8) with 1 mM ethylenediaminetetraacetic acid (EDTA) and 1% β-mercaptoethanol. The homogenate was centrifuged at 10,000*g* for 12 min. Enzyme activities were measured spectrophotometrically at 340 nm. 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. GR activity was measured in a medium containing 50 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, 0.16 mM NADPH, and 0.8 mM oxidized glutathione. GPX activity was measured in 50 mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA, 0.12 mM NADPH, 0.85 mM reduced glutathione, 0.37 mM H_2O_2, and 1 U/ml GR. The medium for spectrophotometric assay of glucose-6-phosphate dehydrogenase included 0.05 mM Tris-HCl buffer (pH 7.8), 3.2 mM glucose-6-phosphate, and 0.25 mM NADP. NADP-isocitrate dehydrogenase activity was assayed in a medium containing 50 mM Tris-HCl buffer (pH 7.8) with 1.5 mM isocitrate and 0.25 mM NADP. Reduced glutathione concentration was determined in the reaction with 5,5-dithio-bis-(2-nitrobenzoic) acid [4]. Activities of alanine transaminase (ALT) and aspartate transaminase (AST) were measured using standard Bio-La-Test kits. The total protein content was estimated by the method of Lowry. The concentration of conjugated dienes was determined spectrophotometrically [11]. Experiments were performed in 3-4 repetitions. Each sample was analyzed 2 times. The results were processed by means of Student's *t* test. The differences were significant at $p<0.05$. We used melatonin, isocitrate (Sigma), NADP, NADPH, Tris-HCl buffer, EDTA (Reanal), oxidized and reduced glutathione, glucose-6-phosphate (ICN), and other reagents (Russia, chemically pure and pure for analysis).

RESULTS

Activities of ALT and AST in the plasma and concentration of conjugated dienes increased by 7.4, 4.3, and 1.8 times, respectively, 4 days after administration of CCl_4 (Fig. 1, *a*, *b*). It reflected the development of pathological changes in the liver and activation of FR oxidation. Specific activities of GPX and GR increased by more than 3 and 2 times, respectively (Table 1). We also revealed a significant increase in enzyme activities expressed in U/g wet tissue. Functional activity of the GR/GPX system probably increases in re-

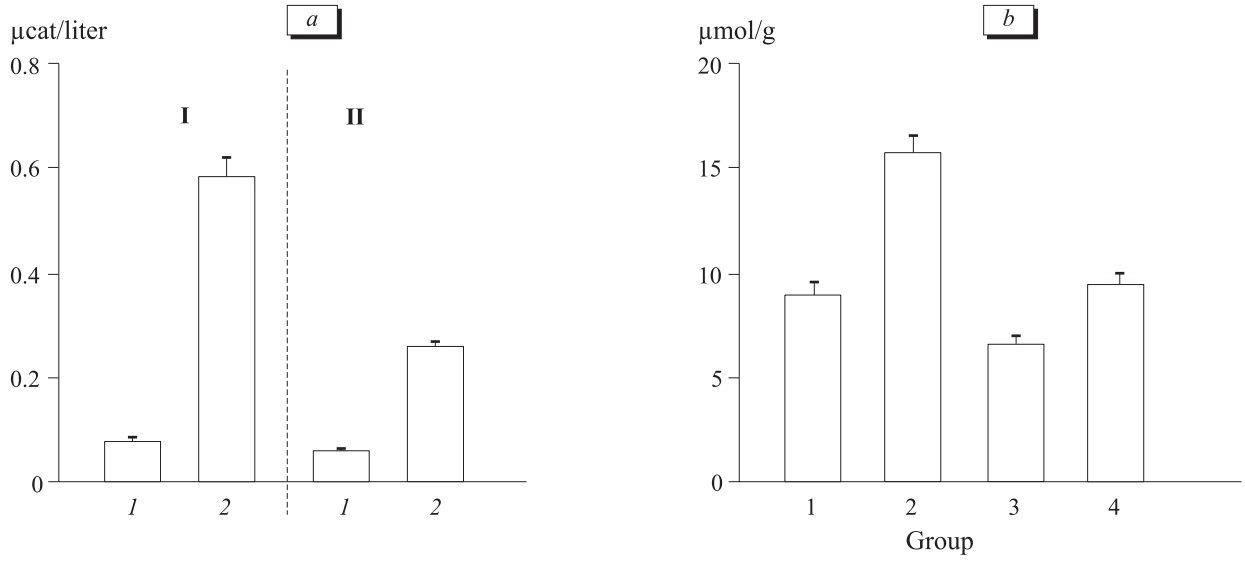

Fig. 1. Activities of transaminases (*a*): alanine transaminase (*I*) and aspartate transaminase (*II*). Concentration of conjugated dienes (*b*). Intact rats (*1*) and animals with toxic hepatitis (*2*). Effect of melatonin on intact rats (*3*) and animals with toxic hepatitis (*4*).

TABLE 1. Activities of Glutathione Peroxidase and Glutathione Reductase and Concentration of Glutathione in the Liver of Intact Rats and Animals with Toxic Hepatitis. Effects of Melatonin under Normal Conditions and during Toxic Hepatitis (*M*±*m*)

Group	Glutathione peroxidase activity		Glutathione reductase activity		Glutathione concentration
	U/mg protein	U/g wet tissue	U/mg protein	U/g wet tissue	mM
1	0.012±0.001	0.135±0.006	0.068±0.002	0.780±0.031	0.210±0.010
2	0.040±0.002*	0.620±0.028*	0.128±0.003*	1.193±0.044*	0.580±0.010*
3	0.013±0.001	0.309±0.012*	0.079±0.002*	0.881±0.036*	0.440±0.010*
4	0.036±0.001+	0.575±0.019+	0.089±0.003+	1.070±0.042+	0.370±0.010+

Note. $p<0.05$: *compared to group 1; +compared to group 2.

sponse to oxidative stress under pathological conditions. Glutathione concentration increased by 2.8 times compared to normal. Melatonin increased specific activity of GR (by 1.2 times). Similar changes were observed in enzyme activities (Table 1).

Specific activity of GPX little varied. However, activity of GPX expressed in U/g wet tissue increased more than twice. Glutathione concentration in melatonin-treated animals increased by 2.1 times compared to the control (Table 1). Our results are consistent with the hypothesis that antioxidant activity of melatonin can be associated with modulation of antioxidant enzymes (activation or stimulation of synthesis) [14]. Administration of melatonin to rats with toxic hepatitis decreased activities of GPX and GR and concentration of glutathione by 1.2, 1.5, and 1.6 times, respectively, compared to group 2 animals. Inactivation of the glutathione system probably results from inhibition of FR oxidation and decrease in activity of the total antioxidant system. This assumption is supported by a 40% decrease in the content of conjugated dienes in group 4 rats (Fig. 1, *b*).

Specific activities of NADP-isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase in rats with toxic hepatitis increased by 1.4 and 1.5 times, respectively (Figs. 2 and 3). Enzyme activities expressed in U/g wet tissue increased to a greater extent. The total protein content increased in the liver of rats with toxic hepatitis, which is consistent with published data. Oxidative stress is accompanied by activation of chaperone synthesis, which contributes to the increase in intracellular glutathione concentration. These changes determine the ability of proteins to protect cells from reactive oxygen [15]. The increase in activities of glucose-6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase is related to the role of these enzymes in the maintenance of NADPH level. This compound determines activation of the GR/GPX system during oxidative stress. Administration of melatonin to intact animals was followed by an increase in

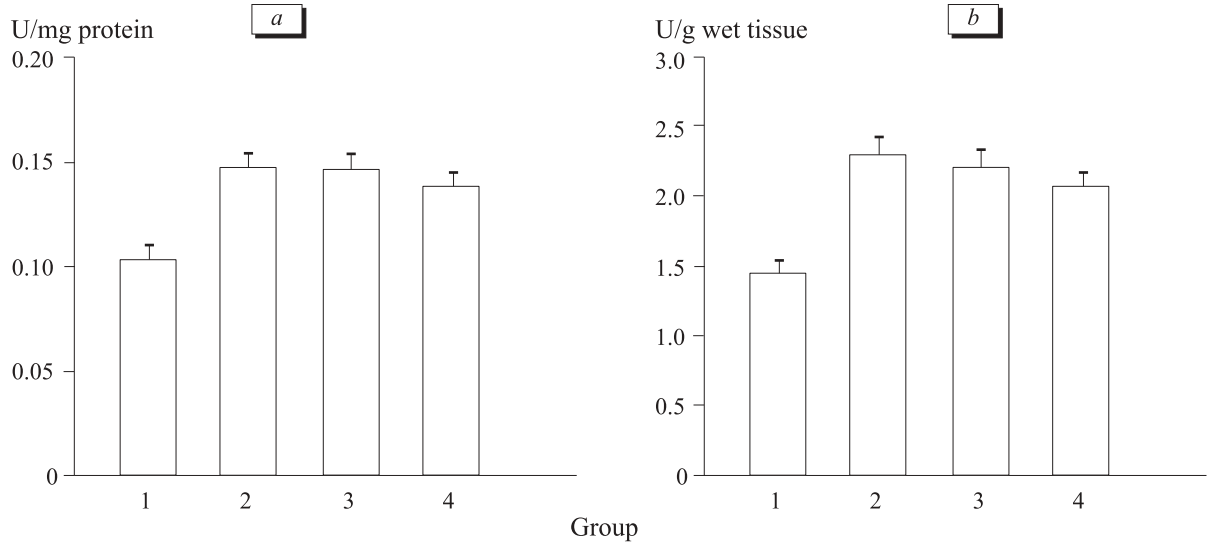

Fig. 2. NADP-isocitrate dehydrogenase activity in the liver of intact rats (group 1) and animals with toxic hepatitis (group 2). Effect melatonin on intact rats (group 3) and animals with toxic hepatitis (group 4). Here and in Fig. 3: specific activity (*a*); activity in U/g wet tissue (*b*).

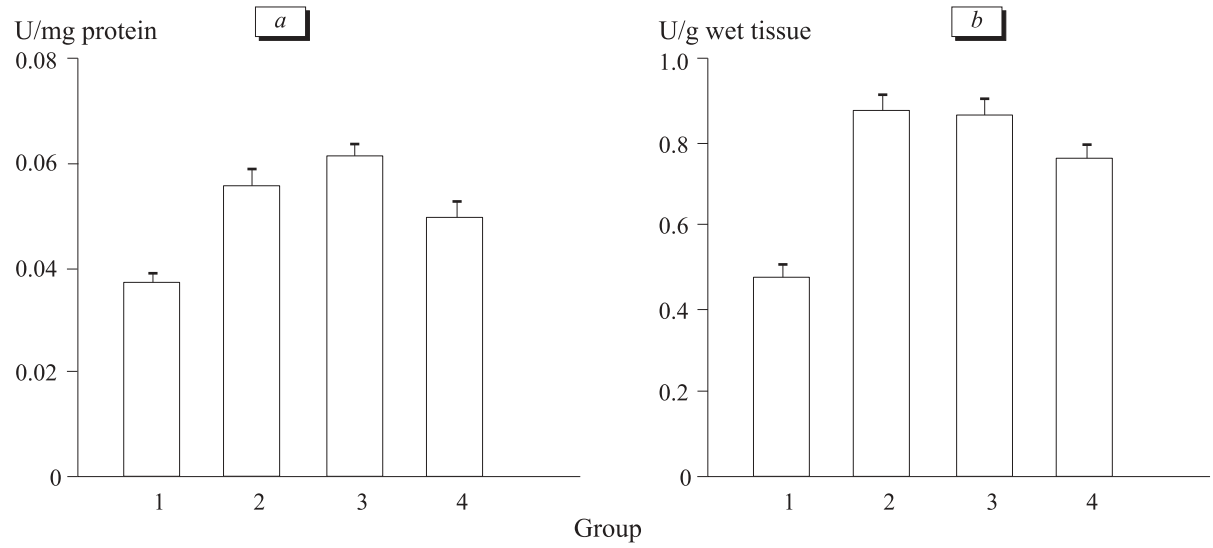

Fig. 3. Glucose-6-phosphate dehydrogenase activity in the liver of intact rats (group 1) and animals with toxic hepatitis (group 2). Effect melatonin on intact rats (group 3) and animals with toxic hepatitis (group 4).

specific activities of NADP-isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase by 1.4 and 1.6 times, respectively (Figs. 2 and 3). Probably, melatonin activates the GR/GPX system and NADPH-generating enzymes. Activities of NADP-isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase in group 2 rats were lower than in group 2 animals (Figs. 2 and 3). It can be hypothesized that melatonin acts as a free radical trap and inhibiting FR oxidation. These changes result in a decrease in activity of the GR/GPX system and NADPH-generating enzymes.

Our results suggest that the regulatory effect of melatonin on cell homeostasis depends on the action of pathogenic factors.

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