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ARTICLES

Rat Liver Mitochondria Impairments under Acute Carbon Tetrachloride-Induced Intoxication. Effects of Melatonin[1]

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Abstract—The aim of the present work was to investigate the mechanisms of oxidative damage of rat liver mitochondria in vitro, under hypochlorous acid (HOCl)-induced oxidative stress, and in vivo, under acute carbon tetrachloride-induced intoxication in rats. Hypochlorous acid (50–300 μM), the main inflammatory agent, inhibited liver mitochondria respiratory activity and caused uncoupling in the respiratory and phosporylation processes. The toxic damage of rat liver after 24 h of acute carbon tetrachloride-induced intoxication (4 g/kg, intragastrically) was accompanied by a significant reduction in succinate- and glutamate-dependent respiration rate in state 3 (by 65%, $p < 0.001$, and by 50%, $p < 0.01$, respectively). The respiration control ratio approached 1, reflecting the loss of respiration control. The phosphorylation coefficient significantly decreased due to uncoupling of the oxidation and phosphorylation processes. The mitochondrial alterations were associated with oxidation of intramitochondrial GSH by 25% ($p < 0.05$), the marked inhibition of succinate dehydrogenase (complex II) by 35% ($p < 0.05$), and the rise of blood plasma nitric oxide level by 45% ($p < 0.05$). The impairment of mitochondrial respiratory function may result from the inhibition of enzymatic activities in the respiratory chain and the damage of mitochondrial membrane during intoxication and plays a key role in the development of the CCl_4-induced hepatotoxicity. Melatonin administration under CCl_4-induced intoxication (three times at a dose of 10 mg/kg) increased the rate of succinate oxidation in state 3 by 30% ($p < 0.05$) and reversed the increase in glutathione peroxidase activity. Melatonin prevented an elevation of nitric oxide level in the blood plasma of intoxicated animals but did not protect mitochondrial functions under acute intoxication.

Key words: rat liver mitochondria, carbon tetrachloride, succinate dehydrogenase, melatonin, hypochlorous acid.

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Mitochondria play a key role in coordination of major cellular functions as a source of energy equivalents as well as a target, decoder, and commutator for intracellular signals and also as a generator of secondary messengers and proapoptotic factors [1]. Damage of mitochondria by oxidants and their subsequent dysfunction can cause cell death by either necrotic or apoptotic mechanisms [2, 3]. A concept has recently been put forward that mitochondria function as an intracellular peroxide sink controlled by Ca^{2+} ions, an increased Ca^{2+} intramitochondrial concentration favoring a prooxidant state [4]. Nohl et al. showed electron leakage and free radical generation under disturbing the redox conjugation at the ubiquinone/cytochromes bc1 site, which depends on the physical state of the inner mitochondrial membrane [5]. Activation of free radical processes in mitochondria brings about damages of the electron transport chain components and histone-unprotected mitochondrial DNA as well as disturbance in the mitochonrial membrane integrity and alterations in the transmembrane potential [2, 3].

The liver is an organ that is exceptionally sensitive to intoxication due to the central role in xenobiotic metabolism and the portal localization [6]. It is suggested that mitochondrial dysfunction is the initial step of hepatotoxicity manifestations, whereas mitochondria themselves are the primary target for hepatotoxins [7]. The molecular mechanisms of toxic effects of tetrachloromethane and other halogen alkanes widely used to simulate such hepatotoxic consequences as fibrosis, fatty dystrophy, and hepatocyte death, have been well documented [8, 9]. Impairment of mitochondrial functions under tetrachloromethane-induced intoxication underlay many hepatocyte disorders [8].

Taking into consideration the key role of mitochondrial dysfunctions in the pathogenesis of many

[1] The article was translated by the authors.

diseases [10], a pharmacological correction of mitochondrial respiratory activity should be regarded as a promising approach to drug treatment, while respiratory chain components, as targets for a specific pharmacological action. Of considerable interest is the search for mitochodrial antioxidants capable of preventing development of mitochondrial oxidative damage (so-called mitochondrial medicine) [10]. It has recently been found that the pineal hormone melatonin, an extensively studied antioxidant, protects mitochondria from intramitochondrial generation of free radicals [11]. The highest intracellular melatonin concentration was found in mitochodria [12], which suggests its direct involvement in functions of the organelles and modulation of their respiratory activity [13]. Moreover, melatonin oxidation by mitochondrial cytochrome *c* is considered as a pathway for melatonin biotransformation [12]. However, the exact molecular mechanisms of the regulation of mitochondrial homeostasis and electron transport chain functions by melatonin are still to be clarified.

The aim of this work was to study the changes in the functional activity of rat liver mitochondria in models of oxidative damages in vitro, while exposing mitochondria to HOCl, and in vivo, under toxic exposure to tetrachloromethane, and to assess the possibility of a pharmacological correction of the mitochondrial damages by melatonin.

EXPERIMENTAL

Chemicals. N-acetyl-5-methoxytriptamine (melatonin), succinic acid disodium salt hexahydrate, *L*-glutamic acid sodium salt, ADP, sodium hypochlorite (NaOCl), 5,5'-dithiobis (2 nitrobenzoic acid) (Ellman's reagent), trichloroacetic acid (TCA), NADH, tetrachloromethane (CCl_4), reduced glutathione (GSH), *tert*-butyl hydroperoxide were from Sigma-Aldrich (USA or Germany).

Animal models. The experiments were carried out on 40 male albino Wistar rats weighing 200–250 g. The animals were adapted to intermittent 12-h light (from 08.00 a.m.) and dark (from 20.00 p.m.) phases cycle for 1 week. Carbon tetrachloride at a dose of 4 g/kg (50% solution in olive oil, 2.5 ml/kg) was administered singly intragastrically (i.g) via a gastric tube at 9 a.m. Melatonin (10 mg/kg) as a 0.3% solution in 0.9% NaCl containing 5% ethanol was injected intraperitoneally (i.p.) three times: 30 min prior to CCl_4 treatment and 2 and 6 h following the treatment. The animals were subdivided into 4 groups: 1) control, rats treated with olive oil (i.g., 5 ml/kg b.w.) and a physiologic solution containing 5% ethanol (5%, i.p. in the same volume as the melatonin solution); 2) melatonin, rats treated with melatonin i.p. and olive oil i.g.; 3) CCl_4, rats treated with CCl_4 i.g. and physiological solution i.p.; and 4) melatonin + CCl_4, the animals treated with melatonin and carbon tetrachloride. Each experimental group included 10 animals. The rats were decapitated 24 h after the CCl_4 administration. They were sacrificed according to the rules defined by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Isolation of mitochondria and measurement of their respiratory activity. After decapitation, blood samples were taken. The liver was removed in cold (0–4°C), dried with filter paper, weighed and homogenized in the medium containing 0.25 M sucrose, 0.02 M Tris-HCl and 0.001 M EDTA, pH 7.2. Mitochondria were isolated by the method of differential centrifugation [14]. The homogenate was centrifuged at 600 g for 10 min (at 4°C) for nuclear precipitation. The supernatant was centrifuged at 8500 g for 10 min (at 4°C). The mitochondrial pellet was washed twice in the isolation medium and resuspended to a protein concentration of 35–40 mg/ml. The protein concentration was determined according to Lowry et al. [15].

The mitochondrial respiratory rate was measured polarographically at 26.5°C using a laboratory-made oxygen Clark-type electrode and a hermetic polarographic cell. The mitochondrial pellet was placed in a cell with the incubation medium containing 0.125 M sucrose, 0.02 M Tris-HCl, 0.05 M KCl, 0.02 M KH_2PO_4, 0.005 M $MgSO_4$, and 0.001 M EDTA, pH 7.5. Concentration of mitochondria in the probe was 1 mg/ml. The respiratory substrates, such as *L*-glutamate (4 mM) and succinate (5 mM), and ADP (180 μM; this corresponded to 210 nmol ADP in the polarographic cell) were introduced into the mitochondrial suspension. Mitochondrial respiratory rates in different metabolic states were determined: V_2, the substrate-dependent respiratory rate, and V_3, the rate of respiration coupled with phosphorylation (after addition of ADP). The parameters for coupling of mitochondrial oxidation and phosphorylation were also determined: the respiratory control ratio (V_3/V_2) and the coefficient of phosphorylation ADP/O (the ratio of the amount of ADP added to the amount of oxygen consumed throughout phosphorylation).

Biochemical measurements. The reduced glutathione content (GSH) and protein SH-groups level (PSH) in the hepatocyte mitochondrial fraction were measured according to Ellman [16], using the molar extinction coefficient $\varepsilon_{412} = 13600\ M^{-1}\ cm^{-1}$. Mixed disulfides formed by glutathione and accessible sulfhydryl groups of mitochondrial proteins (PSSG) were determined by the method described by Rossi et al. [17].

To assay for glutathione peroxidase activity, 0.1-ml mitochondrial pellet was resuspended in 0.1 ml H_2O, subjected to 3 cycles of freeze-thawing [4] and diluted with a 10-fold volume of isotonic phosphate buffer (150 mM NaCl, 10 mM Na_2HPO_4, pH 7.4). Twenty μl of the sample was used to assay for enzyme activity by the method of Martinez et al. [18]. The activity of mitochondrial succinate dehydrogenase was determined by the rate of 2,6-dichlorophenol-indophenol

Table 1. Amino transferase activity, blood plasma bilirubin and nitric oxide levels 24 h following acute tetrachloromethane intoxication. Effect of melatonin

Blood plasma biochemical parameters	Control	CCl_4	Melatonin + CCl_4	Melatonin
AlAT μcat/l)	0.78 ± 0.09	1.84 ± 0.04***	1.80 ± 0.04***	0.84 ± 0.10
AsAT (μcat /l)	0.74 ± 0.05	1.31 ± 0.08***	1.30 ± 0.05***	0.76 ± 0.04
Total bilirubin(μmol/l)	1.95 ± 0.41	15.15 ± 1.99***	15.87 ± 3.43**	2.87 ± 0.39
Conjugated bilirubin(μmol/l)	0.65 ± 0.43	9.01 ± 2.44*	11.90 ± 3.64*	0.54 ± 0.37
Total nitrites(μmol/l)	27.14 ± 1.49	42.07 ± 4.40**	27.50 ± 4.25$^{\#}$	37.14 ± 5.96

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared to control; $^{\#}$ $p < 0.05$ compared to tetrachloromethane-treated animals.

reduction, whereas that of α-ketoglutarate dehydrogenase, by the rate of NAD^+ reduction [19]. The activities of marker enzymes of hepatic cell membrane injury, alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT), as well the levels of free, conjugated, and total blood plasma bilirubin were determined using reagent kits from Pliva-Lachema a.s. (Czech Republic). The overall level of nitrites and nitrates reflecting nitrogen oxide generation was measured in blood plasma using the Griess reagent (N-(1-naphtyl) ethylenediamine dihydrochloride, sulfanilamide) and metallic cadmium as a reducer [20].

Statistical analysis. We used the standard Student *t*-test for the comparison of raw and transformed data showing no departures from normality. Data of 8–10 measurements were presented as a mean ± S.D.

RESULTS

Blood plasma parameters under rat liver toxic injury by tetrachloromethane. The toxic effect of tetrachloromethane was recorded as a marked increase in the levels of total (7.7-fold, $p < 0.001$) and conjugated (13.8-fold, $p < 0.01$) bilirubin in rat blood plasma 24 h after CCl_4 administration (Table 1). The considerable increase of blood plasma conjugated bilirubin level indicated maintenance of the liver conjugating function during intoxication. We simultaneously observed a significant elevation of the activities of the liver injury marker enzymes, AlAT (2.4-fold, $p < 0.001$) and AsAT (1.77-fold, $p < 0.001$), in rat blood plasma. The melatonin administration along with CCl_4 intoxication neither exerted a hepatoprotective effect nor changed the content of liver injury markers in rat blood plasma. The acute intoxication was accompanied by a considerable increase of blood plasma nitric oxide (by 45%, $p < 0.05$) (Table 1), which reflected a development of concomitant inflammatory processes. The melatonin treatment of intoxicated animals nearly brought the NO level to the control values, whereas in control animals, the melatonin injections did not change the blood plasma nitric oxide content significantly (Table 1). The data obtained suggest that melatonin, while modulating the activity of NO synthase system, decreases nitric oxide hyperproduction under intoxication.

Respiratory activity of rat liver mitochondria under toxic tetrachloromethane liver injury. Table 2 summarizes the parameters of oxidative, phosphorylating and coupling functions of liver mitochondria in intact animals and those exposed to tetrachloromethane and melatonin (as a possible hepatoprotector) using succinate or *L*-glutamate as oxidation substrate. Twenty-four hours following the acute tetrachloromethane intoxication of rats, an impairment of the mitochondrial respiratory function was observed. The rate of succinate-dependent oxygen consumption (V_2) was somewhat increased (by 25%), whereas the mitochondrial respiratory rate in state 3, coupled with ADP-consumption (V_3), was reduced significantly (by 65%, $p < 0.001$). At the same time, glutamate-dependent respiration rate (V_2) remained unchanged, while the rate of glutamate-dependent oxygen consumption coupled with phosphorylation (V_3) decreased markedly (by 50%, $p < 0.01$) and transition to the fourth metabolic state was entirely absent (Table 2). Accordingly, the respiration control ratio for exogenous substrates (succinate and glutamate) approached 1.0, whereas the phosphorylation coefficient drastically decreased, thus reflecting diminished efficiency of mitochondrial oxygen utilization during ATP production. Melatonin administration (three times at a dose of 10 mg/kg) in combination with CCl_4 did not cause a pronounced recovery of mitochondrial functional activity. However, during succinate-dependent mitochondrial respiration, the phosphorylation oxidation rate (V_3) in this animal group was higher (by 30%, $p < 0.05$) in comparison with rats treated only with CCl_4. It should be noted that in intact animals, melatonin increased the glutamate-dependent respiration rate (V_2) by 60% ($p < 0.05$) and V_3, by 15%, and diminished the respiration control ratio in comparison with the control group. However, during succinate-dependent respiration, the rate of oxygen consumption by mitochondria in state 3 was reduced insignificantly (by 10%), being accompanied by somewhat decreased respiration control ratio (by 15%) and phosphorylation coefficient (by 25%) as opposed to the control group (Table 2).

Table 2. Parameters of oxidative phosphorylation in rat liver mitochondria 24 h following acute tetrachloromethane intoxication. Effect of melatonin

Groups	Respiration rate, ng-at O/min per mg protein		Respiration control ratio (V_3/V_2)	Phosphorylation coefficient (ADP/O)
	in the presence of substrate, V_2	coupled with phosphorylation (in the presence of ADP), V_3		
Substrate: glutemate				
Control	18.7 ± 2.3	63.1 ± 4.7	3.41 ± 0.32	1.6 ± 0.1
CCl_4	20.8 ± 4.6	27.5 ± 4.2**	1.36 ± 0.51**	0.0
Melatonin + CCl_4	21.1 ± 3.5	29.5 ± 3.7**	1.39 ± 0.53*	0.0
Melatonin	33.0 ± 3.6*	73.4 ± 7.7	2.23 ± 0.22	1.7 ± 0.2
Substrate: succinate				
Control	47.8 ± 5.3	153.9 ± 11.2	3.20 ± 0.40	1.64 ± 0.1
CCl_4	61.6 ± 4.6	54.2 ± 3.6***	1.00 ± 0.12***	0.0
Melatonin + CCl_4	60.2 ± 4.8	71.9 ± 5.0***#	1.13 ± 0.13***	0.0
Melatonin	53.6 ± 6.7	141.4 ± 15.4	2.6 ± 0.3	1.48 ± 0.2

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, compared to control;
# $p < 0.05$, compared to tetrachloromethane-treated animals.

Table 3. Levels of reduced glutathione, glutathione peroxidase and succinate dehydrogenase activities in rat liver mitochondria 24 h following acute tetrachloromethane intoxication. Effect of melatonin

Parameter	Control	CCl_4	Melatonin + CCl_4	Melatonin
GSH, nmol/mg protein	10.22 ± 1.25	7.64 ± 2.27*	5.89 ± 2.32**	10.86 ± 2.41
Glutathione peroxidase, nmol GSH/min per 1 mg protein	32.62 ± 15.60	61.50 ± 12.98**	38.83 ± 15.12	51.68 ± 19.17
Succinate dehydrogenase, nmol succinate/min per 1 mg protein	27.88 ± 6.19	17.88 ± 2.24**	16.53 ± 4.20**	26.72 ± 6.28

* $p < 0.05$, ** $p < 0.01$ relative to control

Thus, the acute tetrachloromethane (4 g/kg body wt.) intoxication of rats caused a dramatic decrease of the rate of oxygen consumption coupled with phosphorylation as well as a complete uncoupling of respiration and phosphorylation. The administration of melatonin to intoxicated animals increased the rate of respiration coupled with phosphorylation but did not restore the phosphorylation mitochondrial function, even though melatonin enhanced the respiratory activity of intact animal mitochondria with glutamate as a substrate.

Enzyme activity and reduced glutathione content in rat liver mitochondria under toxic tetrachloromethane-induced liver injury. Tetrachloromethane intoxication provoked a development of oxidative stress in rat liver mitochondria. The GSH content in these organelles diminished by 25% 24 h after the CCl_4 treatment ($p < 0.05$) (Table 3), whereas the PSSG content of mixed glutathione disulfides with mitochondrial proteins increased (data not shown). The decreased GSH content in rat liver mitochondria under intoxication was related to the considerably elevated activity (by 50%, $p < 0.05$) of mitochondrial glutathione peroxidase (GSHPx) (Table 3). The administration of melatonin unaffected the GSH level in liver mitochondria from control and experimental animals. Simultaneously melatonin at pharmacologic doses decreased GSHPx activity by 30% in mitochondria of the intoxicated animals and increased it by 40% in the control group. We observed a pronounced decrease of mitochondrial succinate dehydrogenase activity (by 35%, $p < 0.01$) under the CCl_4 intoxication (Table 3), which agrees with the reduced mitochondrial respiratory activity in these animals. Melatonin administration to control and experimental animals had no effect on the activity of this enzyme (Table 3).

Oxidative modification of rat liver mitochondria by hypochlorous acid (HOCl) in vitro. The level of hypochlorous acid in the inflammation focus can be as high as 200 μM [21] and mitochondria can be a direct target for its action in a cell. When added to mitochondrial suspension at concentrations of 50–300 μM,

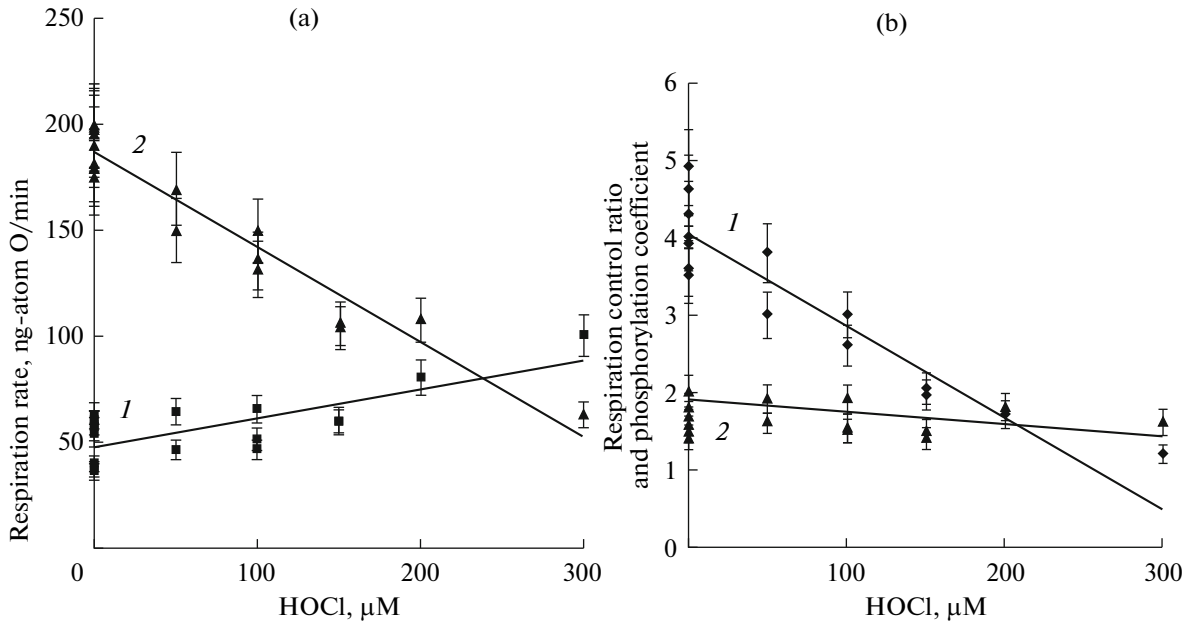

Fig.1 Effect of hypochlorous acid on rat liver mitochondria respiratory activity. (a) The rate of oxygen consumption in the presence of succinate as a substrate, V_2 (*1*), and the rate of phosphorylating oxidation, V_3 (*2*). (b) Respiration control ratio, V_3/V_2 (*1*) and phosphorylation coefficient, ADP/O (*2*). Varying concentrations of HOCl were added to the mitochondrial suspension at 26.5°C 5 min prior to recording of oxygen consumption.

HOCl impaired mitochondrial respiratory activity (Fig. 1). HOCl insignificantly elevated succinate-dependent respiration rate (V_2) and considerably lowered the rate of oxygen consumption coupled with phosphorylation (V_3) (Fig. 1a). This caused a decrease in the respiration control ratio, leaving the phosphorylation coefficient without appreciable changes (Fig. 1b). We also found similar regularities in using glutamate as respiratory substrate (data not shown). The decreased respiration rate in state 3 was related to reduced levels of mitochondrial protein sulfhydryl groups (PSH) and mitochondrial GSH (Fig. 2a, b) and it was also due to formation of mixed glutathione disulfides with mitochondrial proteins whose levels were raised (Fig. 2b). We did not find any significant changes in the activity of the key mitochondrial antioxidant protection enzyme, glutathione peroxidase. However, HOCl significantly inhibited a key enzyme in the Krebs cycle, α-ketoglutarate dehydrogenase (Fig. 2c). Since the respiration rate of HOCl-exposed mitochondria was considerably lowered in state 3, it can be suggested that HOCl in the concentration range used directly damaged the mitochondrial electron transport chain complexes.

DISCUSSION

Earlier studies showed that tetrachloromethane (CCl_4) intoxication is associated with an impaired mitochondrial structure and function. For instance, in CCl_4-induced liver cirrhosis the content of liver cellular mitochondria is lowered, mitochondrial metabolism is disturbed, and ATP production is considerably decreased [22]. It is known that in the liver CCl_4 is metabolized by the cytochrome P450-dependent system to form the trichloromethyl radical $^{\bullet}CCl_3$ (with participation of the cytochrome P2E1, P2B1 or P2B2 and, probably, of P3A isoforms), which is accompanied by a rapid cytochrome P450 destruction and generation of some radical products, including the dienyl radical $L^{\bullet}$ of membrane lipids, oxy $LO^{\bullet}$ and peroxy $LOO^{\bullet}$ lipid radicals [23]. Incubation of isolated hepatocytes with CCl_4 was accompanied by impaired mitochondrial calcium homeostasis, decreased intracellular ATP concentration [24] and changed mitochondrial permeability. The administration of carbon tetrachloride to rats was followed by raised levels of lipid peroxidation products in liver mitochondria, an impaired rate of oxygen consumption in the third and fourth metabolic states, as well as decreased mitochondrial heme concentration (by 60%) and cytochrome oxidase activity [25]. Chronic CCl_4 intoxication of rats causes a decrease in mitochondrial membrane potential by 30 mV, upsets the ATP synthesis and distribution of cholesterol and phospholipids in the mitochondrial membrane, and produces mitochondrial swelling [26].

In our experiment, 24 h after the CCl_4 intoxication, the rat blood plasma showed elevated contents of hepatic cell injury markers (bilirubin level, AlAT and AsAT activities), which is a consequence of the effects of the toxic tetrachloromethane metabolites and the produced free radicals on the cell organelles. In parallel with this, we observed a marked impairment of

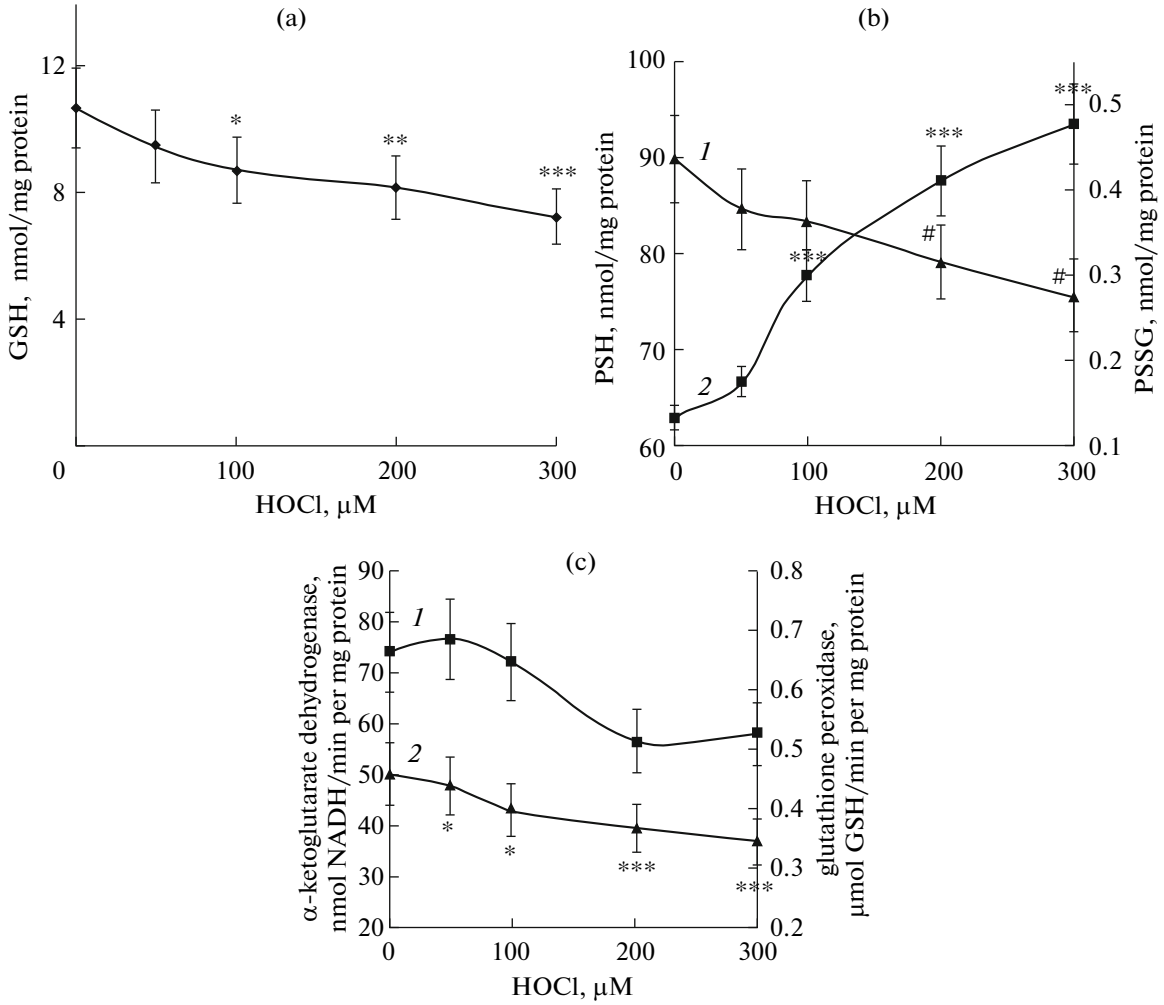

Fig.2. Effect of hypochlorous acid on: (a) the level of reduced glutathione, GSH; (b) the total content of protein sulfhydryl groups, PSH (*1*) and the level of mixed glutathione disulfides with proteins, PSSG (*2*); (c) activities of glutathione peroxidase (*1*) and α-ketoglutarate dehydrogenase (*2*) in rat liver mitochondria. Mitochondria (10–12 mg protein/ml) were exposed to varying concentrations of hypochlorous acid at 26.5°C for 10 min.
*, # $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

hepatic cell mitochondrial respiration accompanied by a complete uncoupling of oxidation and phosphorylation.

A glutathione level plays a decisive role in prevention of free radical impairment of mitochondrial electron transport chain components [27]. According to our measurements, the glutathione level in liver mitochondria was 10–12 nmol/mg protein, which agrees with earlier findings [28]. We found that under CCl_4 intoxication, the GSH content in rat liver mitochondria is considerably decreased, which was partially associated with the increased fraction of GSH incorporated into mixed disulfides with mitochondrial proteins and was a reflection of developing oxidative stress.

A decrease in succinate dehydrogenase activity during toxic liver injury observed in our study testifies to a direct injury of the components of the mitochondrial electron transport chain. The direct correlation between enzyme activity and GSH level ($R^2 = 0.36$, $p < 0.05$) in mitochondria suggests an oxidative damage of succinate dehydrogenase under intoxication. A linear dependence was earlier demonstrated between the level of intramitochondrial reduced glutathione and ATP-synthase activity and, finally, the rate of oxidative phosphorylation during liver regeneration after partial hepatectomy [29]. We believe that the raised activity of mitochondrial GSHPx, which we detected under intoxication, compensates for the reduced GSH availability for GSH-dependent antioxidant enzymes. The melatonin administration to intoxicated animals did not increase glutathione level and succinate dehydrogenase activity, which indicates a profound and largely irreversible mitochondrial damage.

A factor for liver injury and cytotoxicity mediator is hypochlorous acid whose level increases during excess

inflammatory response and leukocyte infiltration in liver tissue [30]. In our experiments, the exposure of mitochondria to HOCl in vitro impaired the mitochondrial functional activity, inhibiting respiration and considerably altering the respiration control ratio, which may be a consequence of a damage of the mitochondrial membrane (and, consequently, proton gradient dissipation) and the respiratory chain components. It is known that hypochlorous acid and chloramines induce oxidative damages of cellular proteins and membranes, including fatty acid and cholesterol chlorohydrine formation [30]. As has been recently found, hypochlorous acid induces swelling of mitochondria isolated from rat liver and of mitochondria in HepG2 cells, a decrease of mitochondrial membrane potential, leakage of cytochrome *c*, mitochondrial permeability transition, and as a result, cell death by an apoptotic mechanism [31].

Thus, the mitochondrial respiratory chain is very sensitive to the external damaging exposures (HOCl, CCl_4). The mechanism of rat liver mitochondria damage under CCl_4 intoxication can be associated with a direct damage of electron transport chain components by radicals, impairments of inner mitochondrial membrane integrity, depletion of intramitochondrial GSH, oxidation of mitochondrial protein sulfhydryl groups and a damaging effect of elevated nitric oxide concentrations. At the same time, under a toxic CCl_4 exposure in vivo, the phosphorylation coefficient ADP/O declined to zero, whereas under oxidative exposure to HOCl in vitro, it remained unchanged along with a drastically decreased substrate oxidation rate and respiration control ratio during oxidative exposures of both types. The effects of the agents on the phosphorylating mitochondrial function are qualitatively different.

It was proved earlier that the uncoupling effect of free fatty acids is linked to functioning of ADP/ATP and aspartate/glutamate antiporters in liver mitochondria [32–34]. Free fatty acids (and their oxidative degradation products) formed in the presence of HOCl due to mitochondrial membrane phospholipid hydrolysis are probably involved in the effects of this agent on mitochondrial functions.

Taking into account an important role of mitochondrial damage in the development of toxic liver injury and the mitochondrial ability to accumulate melatonin specifically, we considered the possibility of correction of disturbed mitochondrial functional activity by melatonin. Melatonin involvement in antioxidant protection of living cells and its role of a free radical scavenger is presently well known [35]. Melatonin beneficially influences the mitochondrial electron transport chain and oxidative phosphorylation [11–13]. It has recently been found that preliminary administration of melatonin (10 mg/kg) prevents the development of necrotic changes and oxidative liver tissue injury under methanol intoxication [36]. We showed earlier that melatonin administration (three times at a dose of 15 mg/kg body weight) prevented the development of CCl_4-induced structural and functional liver injuries [37]. The present study did not reveal a significant decrease of the levels of rat blood plasma liver injury markers after melatonin administration under severe tetrachloromethane intoxication. At the same time, the blood plasma nitric oxide level in intoxicated rats was significantly decreased after melatonin administration, reflecting its possible anti-inflammatory effect. Our earlier results showed the ability of melatonin to control the levels of nitrogen oxide in blood plasma and aorta tissue under experimental diabetes mellitus as well as to play a role of a direct NO scavenger [38].

Administration of melatonin (10 mg/kg, 3 times) in control animals was followed by a statistically significant elevation of the rate of non-phosphorylating oxygen consumption by mitochondria in state 2 (V_2) when using *L*-glutamate as a substrate. It should be noted that the effects of melatonin administration and intoxication detected in the presence of the substrate glutamate could be located both in the mitochondrial matrix and the respiratory chain. When succinate was used as a substrate, the rate of phosphorylating oxidation (V_3) was significantly elevated in CCl_4-exposed rats treated with melatonin compared to the intoxicated animals. Based on these results, we can suggest a specific interaction of melatonin with complex I and probably, a partial prevention by melatonin of dramatic injuries in mitochondrial complex II under intoxication. The treatment with melatonin of intoxicated animals also reduced mitochondrial GSHPx activity to the level characteristic of control animals. It should be noted that we observed a direct correlation between mitochondrial GSHPx activity and the nitric oxide level that changed under the intoxication and melatonin administration. Along with this, it was shown that nitric oxide was capable of inhibiting GSPHx in vitro [39].

Summarizing our findings, we can conclude that under severe tetrachloromethane intoxication, melatonin at a dose of 10 mg/kg does not exert a significant protective effect on the functions of the rat liver mitochondrial oxidative phosphorylation system but is capable of controlling mitochondrial GSHPx activity and NO level in blood plasma.

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