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Ф Ц ВТИЧЕСКИЕ И ПИЩЕВЫЕ БИОТЕХНОЛОГИИ И СИСТЕМА ОБРАЗОВАНИЯ: ПРАВОВЫЕ АСПЕКТЫ

PHARMACEUTICAL AND FOOD TECHNOLOGIES AND THE SYSTEM OF EDUCATION: LEGAL ASPECTS

СБОРНИК

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RAT LIVER MITOCHONDRIAL PROTECTION BY MELATONIN AND FLAVONOIDS UNDER CARBON TETRACHLORIDE-INDUCED INTOXICATION

We investigated the oxidative disorders of rat liver mitochondria during and intoxication and evaluated the possibility of corrections of mitochondrial disorders. After 30-day chronic rat intoxication we observed an increase (by 25 %, $p < 0.01$) of oxygen consumption rate V_2 of liver mitochondria in the case of succinate (not glutamate) as a substrate. The ACR and ADP/O did not change. The level of mitochondrial glutathione as well as cytoplasmic mixed protein-glutathione disulfides enhanced considerably (by 60 %, $p < 0.01$ and by 30 %, $p < 0.01$, respectively) in the liver cells of chronically intoxicated animals. The activities of mitochondrial enzymes succinate dehydrogenase and glutathione peroxidase in liver cells inhibited markedly (by 15 %, $p < 0.05$, 45 %, $p < 0.001$, respectively) Long-term melatonin administration (10 mg/kg, 30 days, daily) to chronically intoxicated rats diminished the toxic effects of CCl_4, reducing elevated plasma activities of alanine aminotransferase and aspartate aminotransferase and bilirubin concentration, prevented the enhancement of cellular and mitochondrial GSH contents during intoxication and accumulation of membrane lipid peroxidation products in rat liver. The treatment of the animals by the complex of melatonin (10 mg/kg) plus succinate (50 mg/kg) plus cranberry flavonoids (7 mg/kg) was even more effective in prevention of toxic liver injury and liver mitochondria damage.

Introduction

Mitochondria play a key role in coordination of the main cellular functions. They provide energy required for all cellular processes as well as pa-

ticipate in cell signaling and in necrotic and apoptotic cell death. There is evidence that defective mitochondrial oxidative phosphorylation plays an important role in the pathogenesis of many diseases: Alzheimer's disease, diabetes, and ageing [1]. The mitochondrial function is particularly susceptible to oxidative stress, leading to decreased mitochondrial ATP synthesis, induction of mitochondrial permeability transition, all of which predispose cells to necrosis or apoptosis [2].

The risk of toxic liver damage has markedly increased in recent years due to the exposure to environmental toxins, pesticides and chemotherapeutics. Many compounds, including useful drugs, can cause liver cell damage through their metabolic conversion to highly reactive substances and the generation of free radicals [3]. It was suggested that reactive metabolite formation, gluthatione depletion, outer mitochondrial membrane pore formation, intramembrane protein release, and the diminished capacity to ATP synthesis, are critical events in hepatotoxicity [4]. Mitochondrial dysfunction is an early manifestation of hepatotoxic agent effects. Prevention of the mitochondrial oxidative damage is a therapeutic strategy in diabetes and intoxication and mitochondria-targeted antioxidants are to have a therapeutic potential under toxic liver damage. Correction of the mitochondrial functions is the basis of «mitochondrial medicine» [1].

The aim of the present work was to investigate the possible role of a specific functional damage in rat liver mitochondria under intoxication as well as evaluate the possibility of mitochondrial impairment corrections by antioxidants (melatonin, flavonoids) and succinate.

Materials and methods

Chemicals

Melatonin, carbon tetrachloride (CCl_4), 5, 5'-dithiobis (2-nitrobenzoic acid) (Ellman's reagent), reduced glutathione (GSH), trichloroacetic acid (TCA) were from Sigma-Aldrich, St. Louis, USA. All other reagents were of analytical grade and were purchased from Reakhim, Moscow, Russia. All the solutions were made with water purified in the Milli-Q system.

Animal models

The investigations were performed using 80 male albino Wistar rats (150–180 g). A standard balanced diet and tap water were provided *ad libitum*. The animals were adapted to an intermittent 12-h light and dark phases cycle for 1 week.

Chronic rat intoxication with carbon tetrachloride. The rats were subdivided into 5 groups. The first group served as controls and received a subcutaneous injection (s.c.) of olive oil (2.0 ml/kg b.w., 30 days, twice a week) and a physiologic solution containing 5 % ethanol (i.g. in the same volume as the melatonin solution). The second group received an injection of CCl_4 (1.6 g/kg b.w., 30 days, twice a week, as a 50 % solution in olive oil, s.c.) at 9 h and a physiologic solution (i.g., 30 days, every day) at 20 h. The third, fourth and fifth groups were given melatonin (10 mg/kg b.w., 30 days, i.g.); melatonin and succinate (50 mg/kg b.w., 30 days, i.g.); melatonin, succinate and cranberry flavonoids (7 mg/kg b.w., 30 days, i.g.), respectively. The animals were sacrificed by decapitation 24 hours after last CCl_4 injection. Blood was collected and the activity of serum aminotransferases [(alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] as well as total, free and conjugated bilirubin levels in blood plasma were measured. The animals were killed according to the rules defined by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Isolation of rat liver mitochondria

Mitochondria were isolated by differential centrifugation from the liver [5]. The protein concentration was determined by the method of Lowry et al. [6].

The respiration of mitochondria was measured using a laboratory-made oxygen Clark-type electrode and a hermetic polarographic cell (volume 1.25 ml) with constant gentle stirring [7]. The incubation medium contained 125 mM sucrose, 20 mM Tris-HCl, 50 mM KCl, 20 mM KH_2PO_4, 5 mM $MgSO_4$, 1 mM EDTA, pH 7.5. The experiments were performed at 26.5°C using 5 mM succinate or 4 mM L-glutamate as substrates. Mitochondrial protein concentration in the probe was 1.0 mg/ml. The functional state of mitochondria was determined by the acceptor control ratio (ACR, V_3/V_2), and the coefficient of phosphorylation (ADP/O). The rate of mitochondrial respiration corresponding to State 3 (V_3) was registered after addition of 180 μM ADP. State 1 corresponded to the respiration in the presence of endogenous substrates (V_1), State 2 corresponded to the respiration in the presence of substrate (glutamate, or succinate) added (V_2), and State 4 corresponded to the respiration when the ADP added was exhausted (V_4).

Biochemical measurements

The concentration of non-protein thiols (predominantly, of reduced glutathione, GSH) in mitochondria was determined spectrophotometrically by the method of Ellman [8].

Statistical analysis

Data for 8–10 rats in each group are presented as a mean ± SD for the normally distributed parameters or as a median and interquartile range for the data showing departures from normality. We used the standard Student *t* test for the comparison of the raw and transformed data showing no departures from normality (according to Shapiro-Wilk's test), and the non-parametric Mann-Whitney U test for the remaining variables. $P<0.05$ was taken to indicate statistical significance.

Results

Earlier we have shown that the experimental diabetes mellitus resulted in a considerable damage of respiratory activity in rat liver mitochondria [9]. The effects of diabetes and melatonin administration on mitochondrial functional activity we compared with those of CCl_4 – induced rat intoxication and melatonin treatment. In the present work, we evaluated the parameters of mitochondrial physiology, metabolic state after chronic intoxication by carbon tetrachloride and melatonin and cranberry flavonoids treatment in rats.

Under chronic rat intoxication we observed some increase in the rate of endogenous oxygen consumption V_1 (by 20 %, $p < 0.05$), substrate-dependent respiration rate V_2 (by 25 %, $p < 0.05$) and ADP-stimulated oxygen consumption rate V_3 (by 15 %, $p < 0.05$) of succinate, but not glutamate, as respiratory substrate [10]. There was significant change in the redox state of mitochondria: the level of mitochondrial reduced glutathione (GSH) was enhanced considerably (by 60 %, $p < 0.01$) after 30 days of intoxication. Combined administration of succinate and melatonin or the treatment by the combination of melatonin plus succinate plus crude cranberry flavonoid extract for 30 days did not influence the GSH levels in mitochondria. Increased level of mixed protein – glutathione disulfides (GSSP) in mitochondria under intoxication was also noted (by 30 %, $p < 0.01$). We observed some changes of the activities of the mitochondrial enzymes under chronic intoxication: succinate dehydrogenase (complex II) activity and glutathione peroxidase activity decreased (by 15 %, $P < 0.05$, and by 45 %, $p < 0.001$, respectively). At the same time the activities of the main enzyme of the Krebs cycle, 2-oxoglutarate dehydrogenase, and the main detoxifying

enzyme, glutathione transferase, in mitochondria did not change. Long-term melatonin administration (10 mg/kg, 30 days, daily) diminished many signs of liver damage (aminotransferase activities and bilirubin content in the blood plasma), prevented the enhancement of cellular and mitochondrial GSH contents and cellular lipid peroxidation products levels under chronic intoxication [11]. Much more effective in the prevention of the toxic liver injury and liver mitochondria impairments was the animal treatment by the complex melatonin (10 mg/kg) plus succinate (50 mg/kg) plus cranberry flavonoids (7 mg/kg) [11].

Discussion

Melatonin (N-acetyl-5-methoxy-tryptamine) has been shown to be an effective antioxidant in a number of experimental models both *in vitro* and *in vivo* [12–13]. In our experimental animal models, the intoxication as well as diabetes resulted in impairment of mitochondrial state and respiration activity [9–11]. The elevated content of GSH in mitochondria may serve as a factor of mitochondrial adaptation to constant effect of the toxic agent. Mitochondrial dysfunction may be the first sign of toxic and diabetic liver tissue damage.

Under intoxication melatonin administration decreased the level of oxidative stress and, correspondingly, prevented a considerable elevation of cellular and mitochondrial glutathione content. It has been shown that melatonin may recycle NADH by electron donation and by this way may improve the efficiency of NADH as an energy carrier and antioxidant [14]. The effects of melatonin might be due to both its radical scavenging properties, its metabolic effects and specific interaction with complexes of the respiratory chain.

The complex of melatonin plus succinate plus cranberry flavonoids was much more effective in preventing chronic liver damage.

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