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THE STRUCTURAL AND METABOLIC DISORDERS OF CELLS' MEMBRANES IN EXPERIMENT*

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The effect of small subtoxical doses of sodium fluoride on the activity of hepatocytes' microsoms on 30 Wistar rats' populations was studied in subacute experiments. The results indicate the disorder of mitochondrial respiratory activity of rats' hepatocytes during prolonged fluoride intoxication. Identified changes can be explained by the initiation of sodium fluoride free radical reactions and lipid peroxidation, whose products are the factors damaging membranes, including mitochondrial, where localized respiratory chain. Increased activity of the mitochondrial NADH-coenzymeQ-oxidoreductase action in the case of sodium fluoride at a dose 1/100 LD_{50}, can probably be seen as a defensive response

Key words: the mitochondria's respiratory activity, rats.

На 30 щурах популяції Вістар досліджено в підгострому досліді дію малих субтоксичних доз фториду натрію на активність мікросом гепатоцитів. Одержані результати свідчать про порушення дихальної активності мітохондрій гепатоцитів щурів при тривалій фторидній інтоксикації. Виявлені зміни можна пояснити ініціюванням фторидом натрію вільнорадикальних реакцій та ПОЛ, продукти яких є факторами пошкодження мембран, у тому числі й мітохондріальних, де локалізований дихальний ланцюг. Підвищення активності мітохондріальної НАДН-коензимQ-оксидоредуктази у випадку дії фториду натрію у дозі 1/100 LD_{50}, ймовірно, можна розглядати як захисну реакцію.

Ключові слова: дихальна активність мітохондрій, щури.

The study was conducted at the Department of Clinical Pathophysiology, Topographical Anatomy and Operative Surgery at Kharkiv Medical Academy of Postgraduate Education according to the issue "Radiotoxins' pathochemical mechanisms on the body and methods of early diagnostics and correction" 0117U000589.

Introduction

Fluoride is involved in many biochemical processes as enzyme activator and inhibitor. It belongs to the elements of the first class of danger – especially hazardous chemicals. Long-term excessive intake of fluoride compounds in the composition of the body can cause pathological state – fluorosis. In addition, high concentrations of fluoride stimulate lipid peroxidation and inhibit antioxidant defense system. The process of free radical oxidation is an important component of nonspecific metabolic component of adaptation to the effects of stress factors, including chemical origin. Evidence of this position is confirmed by the nature radicals generative systems, especially electron transport chain of mitochondria and endoplasmic reticulum. In adaptive adjustment of the oxygen metabolism due to stress, for example, biotransformation of xenobiotics is usually accompanied by certain shifts in the mode of formation of free radical intermediates [1].

The aim of the research is to study the mitochondrial respiratory activity of hepatocytes during the chronic fluoride intoxication.

Materials and methods

The studies were conducted on mature Wistar rats weighing 180-220 line g, which were held in the hospital vivarium. Rats were subjected to oral probe using aqueous solutions of sodium fluoride (FS) once daily for 60 days at doses of 1/10, 1/100 and 1/1000 LD_{50}, which was respectively 20 mg/kg, 2 mg/kg and 0.2 mg/kg body weight (FS average lethal dose for rats received orally, is 200 mg/kg). The animals of the control group injected with the appropriate amounts of drinking water. Research conducted indicators 10, 20, 30, 50 and 60 days after the start of the experiment. Each group had 10 animals. Slaughter was performed by decapitation guillotine knife, pre-anesthetic thiopental sodium 50 mg/kg.

Rats' liver subcellular fractions were isolated by differential centrifugation. To separate the microsomal fraction was centrifuged supernatant hour at 18.000 g, the resulting precipitate was washed with suspended selection (protein in the microsomal suspension was 15-20 mg/ml).

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Hepatocytes' mitochondrial respiratory activity was assessed by polarographic [2]. Functional status I and II of the respiratory chain complexes were determined in the presence of specific substrates and inhibitors of the parameters:

1) state V4 – high content in the incubation medium substrates of complex I – 5 mM glutamate, 5 mM malate or substrate complex II – succinate in the absence of ADP;

2) state V3 – similar conditions as in the case of V4, but in the presence of 200 uM ADP (the factor that limits the rate of reaction is exactly the respiratory chain);

3) state Vd – similar conditions as in the case of V4, but in the presence of oxidation and phosphorylation uncoupler 30 mM 2,4-dinitrophenol.

Mitochondria were isolated by differential centrifugation [3]: hepatic tissue homogenized in an environment with 250 mM sucrose, 3 mm Tris-HCl buffer, 0.5 mM EDTA (pH 7.3), homogenates were centrifuged at 700 g, the resulting supernatant centrifuged among 250 mM sucrose, 3 mm tris-HCl buffer (pH 7.3) at 7.000 g.

Statistical analysis of the results was carried out using a computer application package for the processing of statistical information STATISTICA 6.1 (StatSoft, Inc., USA).

Results and discussion

A sensitive indicator of respiratory activity of cells are mitochondria, which can vary by the action of chemical agents [4-8]. Mitochondria are considered as energy system cell functional activity and determined by the work conjugated enzymes (e.g., NADH-coenzymeQ-oxidoreductase and succinate-coenzymeQ-oxidoreductase) respiratory chain, providing in the process of formation of oxidative phosphorizing macroergic ties.

Respiratory activity of rats' hepatocytes during prolonged fluoride toxicity was evaluated using specific substrates and inhibitors of NADH-coenzymeQ-oxidoreductase and succinate-coenzymeQ-oxidoreductase (as I and II of the respiratory chain complexes inner mitochondrial membrane of hepatocytes).

On the 30th day oral administration of FS to experimental animals in a dose of 1/10 LD_{50} determined significantly ($p<0.001$) when compared to control speed boost mitochondrial respiration of hepatocytes in the standings V3 and V4 on substrates of NADH-coenzymeQ-oxidoreductase 46 and 26% in accordance. FS action at this time of observation was accompanied by a statistically significant ($r\leq0.002$) decrease in respiratory activity of mitochondria in V3 and V4 states on the substrate succinate-coenzymeQ-oxidoreductase respectively 16 and 36%. If 2,4-dinitrophenol was present, the significant ($P<0.001$) dynamics of change relative to the control group of animals were recorded only on condition Vd succinate – a decline of 25%. On the 60th day of FS effect at a dose of 1/10 LD_{50} characterized decrease ($p<0.001$) respiratory activity of mitochondria in hepatocytes states V3 and V4, as the substrates of NADH-coenzymeQ-oxidoreductase (respectively 39 and 25%), and so the substrate succinate-coenzymeQ-oxidoreductase (respectively 38 and 53%). For the state to Vd succinate remained in the term fluoride intoxication in rats downward trend, which was more pronounced (average 45%).

On the 30th day of action FS 1/100 LD_{50} dose was determined statistically significant ($p<0.001$) relative to control speed boost oxygen consumption by mitochondria in rats' hepatocytes states V3 and V4, as the substrates of NADH-coenzymeQ-oxidoreductase (by 29 and 36%), and so on substrates succinate-coenzymeQ-oxidoreductase (respectively 27 and 32%). For state Vd substrates for both enzymes almost virtually no changes were found in this time of observation. On the 60th day of action FS 1/100 LD_{50} dose showed significantly ($r\leq0.001$) when compared to control increase (average 23-24%), respiratory activity of mitochondria in V3 and V4 states on substrates of NADH-coenzymeQ-oxidoreductase against the background of reduction (average 18-33%) on the substrate succinate-coenzymeQ-oxidoreductase. Adding 2,4-dinitrophenol resulted in a statistically significant ($p<0.001$) reduction 23% rate of mitochondrial respiration in rats in the presence of substrate succinate-coenzymeQ-oxidoreductase. Mitochondrial respiration in rats' hepatocytes Vd able to glutamate and malate when exposed in FS 1/100 LD_{50} dose virtually unchanged and equal to the value of control.

The results indicate disorders of mitochondrial respiratory activity of rats' hepatocytes during prolonged fluoride intoxication. On the 30th day of FS action in a dose of 1/10 LD_{50} is expressed by decreased activity of succinate-coenzymeQ-oxidoreductase, and on the 60th day decline as NADH-coenzymeQ-oxidoreductase well and NADH-coenzymeQ-oxidoreductase. If FS action in a dose 1/100 LD_{50} abuse mitochondrial respiratory activity occurring on the 60th day, as evidenced by decreased activity of succinate-coenzymeQ-oxidoreductase. Identified changes can be explained by FS initiation of free radical reactions and lipid peroxidation, products which are factors damaging membranes, including mitochondrial, where localized respiratory chain. The observed increased activity of mitochondrial NADH-coenzymeQ-oxidoreductase action in the case of FS in a dose 1/100 LD_{50}, can probably be seen as a defensive response. But, according to the literature, for the actions of mitochondrial respiratory chain enzyme may be one-electron reduction of oxygen to form the toxic superoxide anion radical [5]. Under physiological conditions this reaction occurs at a very low level through the work of mitochondrial antioxidant system. Perhaps with fluoride toxicity in rats' hepatocytes disrupted electron transport at complex I of the mitochondrial respiratory chain, which may result in the formation of a significant amount of superoxide anion radicals.

Conclusion

Analysis of the results indicates the disorder in separation of oxidation and phosphorylation of substrates to mitochondrial complex II of the respiratory chain on the 60th day of the FS action in a dose of 1/10 and in a dose 1/100 LD_{50}, the result of which can be energetic disbalance.

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