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MANIFESTATIONS OF ENDOGENIC INTOXICATION IN THE LIVER IN EXPERIMENT*

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The study was performed at the Department of Clinical Pathophysiology, Topographical Anatomy and Operative Surgery at Kharkiv Medical Academy of Postgraduate Education in the framework of the research project "Radiotoxins pathochemical mechanisms on the body and methods of early diagnostics and correction", state registration number 0117U000589.

We studied the effect of small doses of sub-toxic sodium fluoride on the activity of microsomal hepatocytes in 40 Wistar rats populations in the subacute experiment. Analysis of results suggests the disruption of reductase activity of hepatocyte microsomes in experimental animals subjected to seeding with aqueous solutions of sodium fluoride at doses of 1/10 and 1/100 LD_{50}, and as a result, the functioning of the electron-transport systems. Detected changes, especially on the 60th and in some cases also on the 50th day of oral administration of sodium fluoride, were associated with the restructuring of lipid environment of microsomal reductases as a result of formation of a significant amount of reactive oxygen species (ROS).The observed decrease in the overall pool of cytochrome P-450 on the 50th and especially on the 60th day of administration of sodium fluoride can be observed as an indicator of its inactivation process. The effect of inhibition of cytochrome P-450 in conditions of prolonged exposure to sodium fluoride is a rather significant fact in terms of structural integration membranes. The level of cytochrome b5 was characterized by an opposite change – an increase by 50% on the 50th day and a decrease by 47% on the 60th day of FS action. Increased activity of NAD (P) H-cytochrome-c-reductase in rats with administered sodium fluoride for one month can be considered on the one hand as a protective compensatory response, and on the other – as the cause of a more rapid flow of electrons from the reduced form of NAD (P) H in cytochrome P-450 and b5 and significant formation of ROS.

Keywords: active liver microsomes of hepatocytes, NAD (P) H-cytochrome-c-reductase, cytochrome P-450 and b5, sodium fluoride.

На 40 щурах популяції Вістар досліджено в підгострому досліді дію малих субтоксичних доз фториду натрію на активність мікросом гепатоцитів. Аналіз одержаних результатів дозволяє стверджувати про порушення редуктазної активності мікросом гепатоцитів експериментальних тварин, підвернених затравці водними розчинами фториду натрію у дозах 1/10 і 1/100 LD_{50}, і, як наслідок, функціонування електрон-транспортних систем. Виявлені зміни, особливо на 60-ту та у деяких випадках й на 50-ту добу перорального введення фториду натрію, пов'язані з перебудовою ліпідного оточення мікросомальних редуктаз внаслідок можливого, утворення значної кількості активних форм кисню (АФК). Виявлене зниження загального пулу цитохрому Р-450 на 50-ту та особливо на 60-ту добу введення фториду натрію можна розглядати як показник процесу його інактивації. Виявлений ефект інгібування цитохрому Р-450 за умов тривалої дії фториду натрію є досить суттєвим фактом з точки зору структурної інтеграції мембран. Для рівня цитохрому b5 була характерна різноспрямована динаміка змін – підвищення на 50 % на 50-ту добу і зниження на 47 % на 60-ту добу дії ФН. Підвищення активності НАД(Ф) Н-цитохром с редуктази у щурів при введенні фториду натрію протягом місяця можна розглядати з одного боку як захисно-компенсаторну реакцію, а з іншого – як причини більш швидкого потоку електронів від відновлених форм НАД(Ф)Н на цитохроми Р-450 і b5 та суттєвого утворення АФК.

Ключові слова: активність мікросом гепатоцитів печінки, активності НАД(Ф) Н-цитохром *с* редуктази, рівень цитохромів Р-450 і b5, щури популяції Вістар, фторид натрію.

Introduction

Fluoride is an essential trace mineral for living organisms, but obviously in limited quantities. The main biological role of fluoride and its compounds are bone formation, formation of dentin, enamel, preventing the development of senile osteoporosis. Fluoride is involved in many biochemical processes as enzyme activator and inhibitor. In addition, high concentrations of fluoride

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stimulate lipid peroxidation and inhibit antioxidant defense system. It belongs to the elements of the first class of danger – especially hazardous chemicals. Prolonged excessive intake of fluoride compounds can cause the pathological condition of fluorosis. Despite the significant content of fluoride in different tissues of the human body, its physiological role so far has not been clarified. Excessive fluoride in drinking water and food causes the destruction of tooth enamel, inhibits carbohydrate, phosphorous-calcium metabolism, the activity of certain enzymes. As an inhibitor of many enzymes, fluoride can inhibit the synthesis of intracellular processes that weaken the body's immune protection and physiological processes can accelerate aging.

The purpose of this study is the examination of enzymatic activity of microsomes of hepatocytes during chronic fluoride intoxication.

Materials and methods

Studies were conducted on mature rats of Wistar line weighing 180-220 g, which were kept in hospital vivarium. Rats were subjected to oral subjected seeding (n=30) using a probe 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 under 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 in the control group were injected with appropriate amounts of drinking water (n=10). Research registered indices on the 10th, 20th, 30th, 50th and 60 days after the beginning of the experiment. Each group included 10 animals (N=40). Rats were euthanized by decapitation with guillotine knife, pre-anesthetic with thiopental sodium 50 mg/kg. Subcellular fractions of rat liver were isolated by differential centrifugation. To separate the microsomal fraction, 18.000 g of supernatant were centrifuged for an hour, thus resulting precipitate was washed and suspended among the selection (protein in the microsomal suspension was 15-20 mg/ml).

The activity of NAD (P) H-cytochrome-c-reductase in the microsomal suspensions of rat liver was assessed in the presence of electron acceptor cytochrome-c, determining the change in absorption of electron acceptor in the transition from the oxidized form on the spectrophotometer «Specord UV VIS» at 30°S and wavelength 550 nm [1]. The enzyme activity was calculated using the molar extinction coefficient of cytochrome-c, equal to 18.5·103 cm-1M-1. The content of cytochrome P-450 in rat liver microsomal suspension was determined by spectrophotometer absorption values of the reduced form of the complex with carbon monoxide at 450 nm [1].

Results and discussion

The constancy of internal environment under physiological conditions is supported primarily by detoxification and excretion – the liver and kidneys, and vital functions are implemented through specific biochemical relationships under the control of central nervous system. In case of exposure to foreign chemicals, when homeostatic organs fail to ensure their detoxification and elimination, normal cytotoxic effects and apathy of the body develop. The main organ that is exposed to foreign chemicals is the liver [2, 3]. The study of pathochemical mechanisms of sodium fluoride (FS) is considered appropriate to begin assessing the structural and metabolic status of the body. Enzymatic rat liver microsomes condition was initially evaluated in terms of the activity of NAD (P) H-cytochrome-c-reductase in the dynamics of observation (the 10th, 20th, 30th, 50th, and 60th days) with administered FS to rats at doses of 1/10, 1/100 and 1/1000 LD_{50}.

The results showed that on the 10th and 20th day of oral dose of FS at 1/10 LD_{50} there was statistically significant ($r \leq 0.002$) gradual increase in activity of NADPH-cytochrome-c-reductase, when compared to the controls, by 24 and 36% respectively. On the 30th day of observation, we detected a tendency to decrease in enzyme activity as compared with the previous values, but relatively to the control activity it remained significantly ($p<0.001$) increased by 29%. Since this time, the experiment revealed a statistically significant ($p<0.001$) reduction when compared with the control group of animals in the activity of NADPH-cytochrome-c-reductase by 20 and 30% respectively on the 50th and 60th day of FS action at a dose of 1/10 LD_{50}.

FS oral administration at a dose 1/100 LD_{50} caused significant ($r \leq 0.004$), as when compared to control, increases in microsomal fraction of rat hepatocytes activity of NADPH-cytochrome-c-reductase by 6, 26 and 32%, respectively on the 10th, 20th and 30th day of the experiment. On the 50th day, there was a tendency to a significant reduction in enzyme activity (average 40%) as relative to the value on the 30th day, but as compared with controls there was a slight but statistically significant ($p<0.001$) increase rate by 19%. On the 60th day of action FS at 1/100 LD_{50} dose, it was accompanied a decreased activity of NADPH-cytochrome-c-reductase by 20% in comparison with the control group of animals.

The impact of UNFPA in 1/1000 LD_{50} dose did not cause statistically significant changes when compared with the control effect against NADPH-cytochrome-c-reductase on the 10th and 20th day of observation, but on the 30th, 50th and 60th day, there was a slight, but significant ($p \leq 0.005$) increase in enzyme activity by an average of 4-6%. Microsomes in rats hepatocytes with FS oral administration at a dose of 1/10 LD_{50} also led to an increase ($p<0.001$) as compared with the control group of animals in the activity of NADH-cytochrome-c-reductase by 10, 23, 31 and 20% respectively at the 10th, 20th, 30th and 50th day of the experiment. On the 60th day, it contributed to a statistically significant ($p<0.001$) decrease in enzyme activity by an average of 39%.

Action in FS 1/100 LD_{50} dose was accompanied by a gradual increase ($r \leq 0.038$) in the activity of microsomal NADH-cytochrome-c-reductase by 5, 11, 22 and 30%, respectively, in the 10th, 20th, 30th and 50th day of observation. On the 60th day, the indicator decreased relatively to the previous term experiment by an average of 38%, but in relation to the value of control – it remained increased ($p<0.001$) by 18%. UNFPA in action 1/1000 LD_{50} practically did not cause changes in the rat microsomal activity of NADH-cytochrome-c-reductase when compared with the control group of animals; only on the 30th and 50th day there was a slight (by 4.5%), but statistically significant ($p=0.013$ and $p=0.045$) increase in enzyme activity.

Overall analysis of the results suggests the disruption of reductase activity microsomes of hepatocytes in experimental animals at FS toxification doses 1/10 and 1/100 LD_{50}, and as a result, the functioning of the electron-transport systems. The detected changes, especially on the 60th and in some cases also on the 50th day of FS oral administration are probably associated with the restructuring of the lipid environment of microsomal

reductases, and as a result, according to the literature [4] initiating the formation of a significant amount of reactive oxygen species (ROS). Increased activity of NAD (P) H-cytochrome-c-reductase in rats with administered FS for a month can be considered on one hand as a protective compensatory response, and the other – as the cause of a more rapid flow of electrons from the reduced form of NAD (P) H in cytochrome P-450 and b5 and significant formation of ROS.

In connection with dynamic changes in the general pool of cytochrome P-450 and b5 in the microsomal fraction of hepatocytes, experimental animals were injected with doses of FS 1/10 and 1/100 LD_{50} (dose 1/1000 LD_{50} preliminary results are practically ineffective, allowing it not to consider) within 60 days. On 10th, 20th and 30th day of FS toxification of rats at a dose of 1/10 LD_{50}, statistically significant ($p<0.001$) changes were observed relative to controls, increase in the overall pool of cytochrome P-450 respectively by 44, 74 and 23.

A similar but more pronounced dynamic change characteristic of the general pool of cytochrome b5 under the action of UNFPA in the doses of 60, 113 and 78% on the 50th and 60th day of observation was the level of cytochrome P-450 microsomes in rat hepatocytes when compared with the decreased control ($p<0.001$), respectively by 48 and 52%.

The level of cytochrome b5 in this observation period was characterized by an opposite change – an increase by 50% on the 50th day and a decrease by 47% on the 60th day of FS action.

Oral administration to rats in doses of FS 1/100 LD_{50} contributed to a statistically significant ($r\leq0.001$) increase in the content of cytochrome P-450 on the 50th day of the experiment (most pronounced on the 20th day – 40%) and cytochrome b5 and (the most pronounced on the 30th day – 112%). On the 60th day of observation in the level of cytochrome microsomal fraction of rat hepatocytes under conditions in steps UNFPA 1/100 LD_{50} dose was significantly reduced by an average of 35-39% ($p<0.001$) when compared with controls.

The observed (mostly within 30 days) increase in the overall pool of cytochrome hepatocyte microsomes in experimental animals with administered FS can be seen as an adaptive response. However, it should be kept in mind that the oxidation of xenobiotics and endogenous substrates in hepatocytes monooxygenase system is an essential source for the formation of free radicals – lipid peroxidation initiators whose products increases with the activation system [5]. In turn, the deployment of lipid peroxidation process inevitably leads to the destruction of membrane endoplasmic reticulum [6], which may be accompanied by inactivation of cytochrome P-450 because of its conversion to an inactive form – cytochrome P-420 [7-12]. With this in mind, a reduction in the overall pool of cytochrome P-450 on the 50th and especially on the 60th day administration FN can be seen as an indicator of its inactivation process.

The effect of inhibition of cytochrome P-450 in conditions of prolonged exposure FS is a rather significant fact in terms of structural integration membranes. It should be noted that the activity of microsomal enzymes, including cytochrome P-450, essentially depends on the membrane conformation and physicochemical properties of the lipid bilayer. Identified changes in their activity for UNFPA action may affect the detoxification capabilities of hepatocytes, the key role of which belongs to the cytochrome P-450.

Reduction of cytochrome b5 as well, according to the literature [8-12], is often associated with the initiation of lipid peroxidation in microsomal membranes of hepatocytes as a result of cytotoxic xenobiotics.

Analysis of the results can also assume the occurrence of disruption in different layers microsomal membranes due to inhibition of FN (especially on the 60th day of the experiment) cytochrome P-450, localized mainly in the hydrophobic zone, and cytochrome b5, localized in the outer hydrophilic zone [9-12].

Conclusion. In general, long-term oral administration of FN, especially at a dose of 1/10LD50, leads to disruption in detoxification function of microsomal membranes in rats' hepatocytes due to the gradual suppression of enzymes activity and reduction in biotransformation of xenobiotics as well as endogenous substrates.

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