THE DAMAGE OF MEMBRANES STRUCTURE OF HEPATOCYTES IN RATS DURING FLUORIDE INTOXICATION*

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We studied the effect of small subtoxic doses of sodium fluoride on the phospholipid composition of hepatocyte membranes of 30 rats of the Wistar line in the subacute experiment. The analysis of the obtained results allows us to confirm the disrution of the quantitative content of general and individual phospholipids in lipid extracts of hepatocyte membranes, depending on the dose and duration of xenobiotic activity. The calculation of the ratio of the amount of rapidly oxidizing phospholipid fractions (phosphatidylserine, phosphatidyl ethanolamine, phosphatidylinositol) to the sum of severely oxidizing phospholipid fractions (sphingomyelin, phosphatidylcholine, lysophosphatidylcholine) with sodium fluoride at a dose of 1/10 LD50 indicates a decrease by 1,4 and 2,3 times respectively on the 30th and 60th day of the experiment, reflecting the depletion of the adaptive capacity of the liver cells. In case of sodium fluoride at a dose of 1/100 LD50, this factor increases by 1.2 times on the 30th day of the experiment, and on the 60th day it decreases by 1.6 times, which reflects the stresses of the adaptive potential with its subsequent breakdown.

Key words: sodium fluoride, phospholipid composition of hepatocyte membranes, phospholipid fractions, rats of Wistar line.

На 30 щурах популяції Вістар досліджено в підгострому досліді дію малих субтоксичних доз фториду натрію на фосфоліпідний склад мембран гепатоцитів. Аналіз одержаних результатів дозволяє стверджувати про порушення кількісного вмісту загальних та індивідуальних фосфоліпідів у ліпідних екстрактах мембран гепатоцитів в залежності від дози та тривалості дії ксенобіотика. Обчислення коефіцієнта співвідношення суми швидко окислювальних фосфоліпідних фракцій (фосфатидилсерину, фосфатидилетаноламіну, фосфатидилінозитолу) до суми важко окислювальних фосфоліпідних фракцій (сфінгомієліну, фосфатидилхоліну, лізофосфатидилхоліну) при дії фториду натрію у дозі 1/10 LD_{50} свідчить про його зниження в 1,4 та 2,3 рази відповідно на 30 – ту та 60 – ту добу експерименту, що відображує виснаження адаптаційного потенціалу клітин печінки. У випадку дії фториду натрію у дозі 1/100 LD_{50} цей коефіцієнт на 30 – ту добу експерименту збільшується в 1,2 раза, а на 60 – ту добу знижується в 1,6 раза, що відображує напруження адаптаційного потенціалу з наступним його зривом.

Ключові слова: фторид натрію, фосфоліпідний склад мембран гепатоцитів, фосфоліпідні фракції, щури популяції Вістар.

Introduction

Relevance of the study of fluorine and its derivatives is due, above all, to the lack of in – depth evaluation of their biological activity. In particular, research papers are mainly devoted to the study of some state organs and body systems that determine the formation of pathological processes. For a better understanding of the pathogenic mechanisms of action of fluoride and its derivatives, it is appropriate to conduct a comprehensive approach to the study of the functional state of the organism as a single self – regulating system.

As a mechanism that destabilizes cell membrane, lipid bilayer may render an excessive activity of LPO against the background of antioxidant reserves. The result is usually a change of physical and chemical properties of biomembranes and membrane – active enzymes and receptors that provide metabolic, transport, regulatory cell function. Given the preliminary results of studies on intensification of free radical processes and lipid peroxidation against the background of decreased activity of antioxidant system in the liver of experimental animals under long – term sodium fluoride (SF), especially at a dose of 1/10 LD_{50}, the assessed phospholipid composition of hepatocytes' membranes has been represented.

Material and methods

Studies were conducted on mature Wistar rats weighing 18 –220 line g, which were held in the hospital vivarium. Rats were subjected to oral exposure using a probe and aqueous solutions of sodium fluoride (SF) 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 (SF average lethal dose for rats received orally, is 200 mg/kg). The animals of the control group were injected with the appropriate amounts of drinking water (n=10). Research conducted indicators on 10, 20, 30, 50, 60 days after launching the experiment (n=20). Each group included 10 animals (N=30). Animals were euthanized by decapitation with guillotine knife and pre – anesthetic thiopental sodium 50 mg/kg.

To study of phospholipid composition in extraction of hepatocytes lipids was performed with a mixture of chloroform – methanol at a ratio of 1:2 followed by evaporation in a stream of dry nitrogen separation of individual phospholipid fractions was performed by micro – thin layer chromatography in a solvent system: hexane: diethyl ether: acetic acid (73:25:2 by volume) [1]. Identification of phospholipids were conducted by standard solutions and by specific reactions. Quantitative overall and individual content in the lipid extracts were evaluated [2]. Value phospholipid fractions were calculated as a percentage of

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phosphorus phospholipid fractions of each phosphorus to total lipids taken as 100%.

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

On 30th day of SF oral administration to rats at a dose of 1 SF/10LD_{50} there was a significant ($p<0.001$) reduction in regard to easy oxidation phospholipid fractions by 36% when contrasted to the comparison level of phosphatidylethanolamine (PEA) against the background of improbable reduction ($p=0.059$) by 22% of content of phosphatidylinositol (PI) and increase ($p=0.762$) by 7% of phosphatidylserin (PS). As to the hard oxidation fractions of phospholipids, in this time of observation we recorded statistically significant ($p<0.001$) as contrasted to comparisons enhance of phosphatidylcholine (PH) 35% and lysophosphatidylcholine (LPH) by 92% while reducing sphingomyelin (SM) by 41%.

On the 60th day of the experiment, the effect of SF in a dose of 1/10 LD50 resulted in significant when contrasted to the comparison group decrease ($p<0.001$) in rats' hepatocytes content of PEA and PI respectively by 60 and 57%. SF level tended to false ($p=0.059$) reduction of 19%. At the time of observation, we also found a significant increase ($p<0.001$) of LPH and PH by 122% and 52%. Content of SM under the action of SF in a dose of 1/10 LD_{50} on the 60th day of exposure, statistically significant when contrasted to the comparison, was reduced by 62%.

SF oral administration to rats at a dose 1/100 LD_{50} was accompanied by other changes in the dynamics of phospholipid composition of hepatocytes. On the 30th day of observation of easy oxidation factions, there was a statistically significant ($p=0.004$) increase as contrasted to the comparison found only in PHI – 42%, while improving PEA by 21% was unlikely ($p=0.104$). Content of SF was practically unchanged during this term. For severe oxidative phospholipid fractions on the 30th day of dose administration SF 1/100 LD_{50} we discovered a statistically significant ($p<0.001$) increase of only LPH (43%). For CM and PH, we observed probable changes as relative to the comparison group.

On the 60th day, the action SF in 1/100 LD_{50} dose resulted in a statistically significant ($p<0.001$) reduction of PEA and PI by 40–48%. SF level was practically unchanged and equal to the value of comparison. SF in a dose 1/100 LD_{50} caused this increase in observation period ($p<0.001$) and content of SF LPH respectively by 26 and 47%, and reduction ($p<0.001$) of SM on average by 40%.

Overall analysis of the results indicates a reduction of easy oxidation (PEA, PI) phospholipid membrane fractions of rats' hepatocytes with an increase of heavy oxidation (PH, LPH) in case of prolonged oral administration of SF in a dose of 1/10 LD_{50}. On the 60th day of SF toxification of rats in this dose, we determined the opposite dynamics change: reducing oxidative easy fractions (PEA, PI) against the background of increasing oxidative hard fractions (PH, LPH). These changes are probably the result of the detected increase in free radical processes and lipid peroxidation by prolonged exposure SF. The results coincide with literature data. Thus, it is proved that under current active free radical processes, the number of phospholipids, which are composed of polyunsaturated fatty acids – SF, PI, PEA [3] are most dramatically reduced. Intensification of lipid peroxidation is usually accompanied by significant changes in the composition and degree of oxidation of membrane phospholipids, thereby reducing the activity of enzyme systems and phospholipide–dependend leads to disruption of the integrity of the cell membrane lipid bilayer [4, 5.8]. We should emphasize the significant increase in the long – term LPH rats' toxification with SF, which has a strong cytolytic activity. Particular attention should be paid to the changing content of CM – one of the most resistant to peroxidation phospholipide fractions [6 – 9]. For SF conditions of prolonged exposure, especially at a dose 1/10 DL50 in rats we determined a gradual reduction of SM in the membranes of hepatocytes that under the literature suggests chronic process of free radical oxidation and lipid peroxidation [10].

Lipid molecules are important structural and functional components of cell membranes; they regulate mobility and activity of membrane proteins, identifying potential adaptation of cells [11].

Conclusion

Calculating coefficient ratio of easily oxidizing phospholipid fractions (SF, PEA, PI) on the amount of oxidative hard PL fractions (SM PH, LPH) by the action of SF in a dose of 1/10 LD_{50} provides evidence of its decrease by 1.4 and 2, 3 times respectively on the 30th and 60th day, reflecting the depletion of adaptive capacity of liver cells. In case of action SF 1/100 LD_{50} dose of this factor on the 30th day of the experiment, the reflecting tensions of adaptive capacity with subsequent breakdown increased by 1.2 times, and on the 60th day it reduced by 1.6 times.

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