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INFLUENCE OF CROWN-ETHERS ON MICORSOMAL OXIDATION SYSTEM COMPONENTS OF WHITE RATS LIVER

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The article represents laboratory experimental investigations of microsomal system components (NADP-dependent dehydrogenases activities, cytochromes b_5 and P_{450} contents) in white rats liver at the conditions of long-term administrations of 12-crown-4, aza-12-crown-4 and thia-12-crown-4 (within 30 days, perorally, in 1/100 LD_{50}). Crown-ethers significantly decrease contents of microsomal cytochrome P_{450}, do not influence cytochrome b_5 contents and increase activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP-dependent-decarboxylizing malate dehydrogenase in rat hepatocytes. These alterations are connected with activation of lipid peroxidation and development of oxidative stress reaction.

Key words: crown-ethers, microsomal oxidation, cytochromes, NADP-dependent dehydrogenases, oxidative stress.

Вплив краун-ефірів на компоненти системи мікросомального окислення у печінці білих щурів. Кратенко Р.І. – Стаття представляє експериментальні дослідження компонентів системи мікросомального окислення (активності НАДФ-залежних дегідрогеназ, вміст цитохромів P_{450} та b_5) у печінці білих щурів за умов тривалого надходження 12-краун-4, аза-12-краун-4, тіа-12-краун-4 (30 діб, перорально у 1/100 $ДЛ_{50}$). Краун-ефіри достовірно знижують вміст мікросомального цитохрому P_{450}, не впливають на вміст цитохрому b_5 і підвищують активності глюкозо-6-фосфат-дегідрогенази, 6-фосфоглюконат-дегідрогенази та НАДФ-залежної декарбоксилюючої малат-дегідрогенази у гепатоцитах щурів. Ці зміни пов'язані з активацією перекисного окислення ліпідів і розвитком оксидативної стрес-реакції.

Ключові слова: краун-ефіри, мікросомальне окислення, цитохроми, НАДФ-залежні дегідрогенази, оксидативний стрес.

Investigations of various origin xenobiotics influence on prooxidative-antioxidative system have been confidently gaining their place amongst the global recent tendencies of experimental biology and medicine. If a xenobiotic invades the organism for a long time in quantities, which are much lower than lethal doses, its action or the action of its metabolites, in most cases, would be connected with accumulation of free radicals, and lipid and/or protein peroxidation activation [7]. The organism responds to the negative influence with the formation of a number of defensive compensatory mechanisms directed towards inhibition of free-radical oxidation and homeostasis maintenance [22]. One of the main protectory reactions ought to be generation of cellular reduced NADP, which is a necessary component of glutathione reduction, fatty acids and nucleotides biosynthesis, functioning monooxigenase system of poisons detoxification [6; 9].

Earlier we showed some macroheterocyclic crown-ethers representatives to have belonged to moderately toxic and supremely cumulative substances [2]. In 1/100 LD_{50} crown-ethers evoked alterations in oxido-reductive processes, activated lipid peroxidation and antioxidant system [3], and in higher doses inhibited the latter [3; 4]. Although, what remains unclear and insufficiently investigated, is the work of liver monooxigenase system itself, as a whole, and the behavior of its functional components facing the invasion of various crown-ethers doses.

Objective. Investigation of alterations in NADP-dependent dehydrogenase activities and cytochromes b_5 and P_{450} contents in white rats liver at the conditions of prolonged administrations of 12-crown-4, aza-12-crown-4 and thia-12-crown-4.

MATERIALS AND METHODS OF RESEARCH

The research used white male rats (body mass 180-210 g) kept at the standard conditions of vivarium. The animals of experimental groups (10 rats in each group) were administered emulsion of the investigated crown-ethers in 1/100 LD_{50} (0.0117; 0.022; 0.0365 g/body mass kg, for 12-crown-4, aza-12-crown-4 and thia-12-crown-4 respectively [2]) daily within 30 days perorally. The animals of the control group (n=10) were given water at the same conditions. On the 30^{th} day of the experiment the rats of all groups were anesthetized by sodium thiopental (50 mg/body mass kg

[8]) and slaughtered by decapitation with the Guillotine knife. The liver was perfused by cold isotonic NaCl solution directly after the slaughter, and was homogenized on ice. Microsomal and cytosolic factions were obtained with the method of differentiated centrifuging the liver homogenate by aid of centrifuge CRV-1. Activities of glucose-6-phosphate dehydrogenase (G-6-PDG; EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-PGDG; EC 1.1.1.44) were determined by Glock and McLeanne method in Bottomley modification [14]; of NADP-decarboxylizing malate dehydrogenase (dMDG; EC 1.1.1.40) - by Ochoa method in Usatenko modification [12]. The rate of reduced NADP formation was registered with spectrophotometer CP-46 using 340 nm wave length, and the activities of the enzymes was expressed in nmol of NADP for 1 min per 1mg of protein. The contents of microsomal cytochromes was determined by differentiated spectrophotometry with Omura method [20], using coefficient of molar extinction: $164 \cdot 10^{-3}$ $M^{-1} \cdot cm^{-1}$ for cytochrome b_5, and $91 \cdot 10^{-3}$ $M^{-1} \cdot cm^{-1}$ for cytochrome P450 and expressed in nmol/protein mg. Protein concentration was determined by Lowry [18]. Experimental results were calculated by traditional methods of parametrical statistics.

RESULTS OF RESEARCH AND THEY DISCUSSION

The action of crown-ethers resulted in significant decrease in contents of microsomal cytochrome P_{450} and did not influence the contents of cytochrome b_5 in hepatocytes of the experimental animals groups compared to the control (tab. 1).

The animals, toxified by various representatives of crown-ethers, had the similar tendencies of the indexes alterations with somewhat stronger effect for 12-crown-4.

Table 1

Contents of microsomal cytochromes P_{450} and b_5 in hepatocytes of rats toxified by crown-ethers, (M±m) nmol/ protein mg

Index	Control	12-crown-4	Aza-12-crown-4	Thia-12-crown-4
P_{450}	0,68±0.04	0,43±0.04*	0,47±0.05*	0,49±0.04*
B_5	0,57±0.05	0,55±0.06	0,55±0.07	0,56±0.04

*Notes: 1. n-10; 2. *p<0.05*

The powerful oxidative stress is known to evoke changes in some enzymes of heme and hemoproteins metabolism, particularly induction of hemoxigenase which is the key enzyme of heme degradation [17], as well as the decrease in contents of cytochrome P_{450} [21] and concentrations of reduced glutathione [15]. The cytochrome P_{450} contents reduction after crown-ethers administrations may be connected with inhancing lipid peroxidation of microsomal membrane. Cytochrome P_{450} is shown to be a membrane-linked hemoprotein, molecules of which are practically completely sunk in membrane lipid bilayer [11]. At the conditions of oxidative stress

development after crown-ethers action there has been proved the occurrence of alterations in phospholipid composition of membranes [5], phospholipid microsurrounding membrane-linked receptors [3], and, therefore, most probable, there may be the changes in phospholipid microsurrounding cytochrome P_{450} in membranes of hepatocyte smooth endoplasmic reticulum. This may result in disturbance of native conformation of the hemoprotein, and, in many cases, in its conversion to its inactive form. At the same time the experiments in vitro showed hepatic cytochrome P_{450} to have got reduced by 50 % when incubated together with high concentrations of lipid peroxidation products [13]. Apart from that, one of lipid peroxidation products – trans-4-hydroxy-2-nonenal induced inactivation different isoforms of cytochrome P_{450} by forming Schiff's bases with aminogroups of apocytochrome lysine residues although heme with its sulfhydrilic ligands remained intact [13].

The decrease in microsomal cytochrome P_{450} contents in the liver of rats toxified by crown-ethers may be also explained by impairment of incorporation of Fe^{3+} atom in heme synthesis. Admittedly, crown-ethers have high affinity to mono- and bivalent metal ions involving the ion in their molecular hole and binding it with their oxygen, nitrogen or sulfur atoms (for crowns, aza-crowns and thia-crowns respectively) by coordinational bonds [10], depriving the ion of its metabolic activity.

Earlier we showed the disturbance of concentrational gradient of metal ions and metal-dependent enzymes activity at the action of similar crown-ethers doses [3]. Besides, the experiments of American scientists revealed inhibition of cytochrome P_{450} human 1A1 gene with the participation of NF1 transcription factor at the conditions of oxidative stress and its consequences as active oxygen forms accumulation and cellular thioldisulfide metabolism impairment [19].

The second part of our experiment was devoted to investigation of crown-ethers action at the level of activities of two NADP-dependent pentose phosphate pathway dehydrogenases (G-6-PDG and 6-PGDG) and the enzyme of NADP-reduction alternative route (dMDG). The activities of all the three enzymes mainly increased significantly at the influence of experimental substances (tab.2).

Table 2

Activities of glucose-6-phosphate dehydrogenase (G-6-PDG), 6-phosphogluconate dehydrogenase (6-PGDG), NADP-malate dehydrogenase (dMDG) in hepatocytes of rats toxified by crown-ethers (M±m) NADP per for 1 min per 1mg of protein

Index	Control	12-crown-4	Aza-12-crown-4	Thia-12-crown-4
G-6-PDG	27.3±3.1	36.6±3.2*	34.4±3.3*	31.5±4.0
6-PGDG	30.4 ±3.3	39.7±3.8*	35.5±3.3	32.7±3.3
dMDG	23.5±2.1	28.6±2.0*	26.8±2.0	27.4±2.5

*Notes: 1. n-10; 2. *p<0.05*

The work [16] reflects the direct correlation between NADPH-generating enzymes activities enhance and maintenance of reduced glutathione adequate level for prevention of cellular DNA and proteins oxidative impairments. Induction of NADP-dependent dehydrogenases synthesis and activation of NADP-reduced generation for resynthesis of antioxidants and xenobiotics detoxification might be an important link in realization of cells and organism protective mechanisms at the conditions of oxidative stress development and lipid peroxidation enhance. There is the reverse dependence between the rate of lipid peroxidation, its products accumulation and contents of reduced NADP – the increase in quantity of NADPH and antioxidants reacting with free radicals reduces lipid peroxidation rate; acceleration of lipid peroxidation leading to free radicals increase reduces NADPH and antioxidants contents. Our present and previous experiments proved crown-ethers not to be exhausting agents for antioxidant system and NADPH-generating processes in 1/100 LD_{50} , although action of higher doses of these xenobiotics leads to significant accumulation of toxic products of lipid peroxidation, which gives the following disbalance in metabolic processes and may result in disturbance of enzymes systems regulation, conformational changes of membrane lipiproteinic complex, appearance of hydrophilic incorporations in the whole hydrophobic layer of membrane [1].

CONCLUSIONS

1. Crown-ethers (12-crown-4, aza-12-crown-4 and thia-12-crown-4) in 1/100 LD_{50} at the condition of 30-days peroral administration to the rats organism significantly decrease microsomal cytochrome P_{450} contents and do not influence cytochrome b_5 contents, and also induce activities of NADP-generating enzymes (glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, NADP-decarboxylizing malate dehydrogenase) in rats hepatocytes.
2. The alterations of cytochrome P_{450} contents and NADP-generating enzymes activities are connected with activation of lipid peroxidation and development of oxidative stress reaction.

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Влияние краун-эфиров на компоненты системы микросомального окисления в печени белых крыс. Кратенко Р.И. – Статья представляет экспериментальные исследования компонентов системы микросомального окисления (активности НАДФ-зависимых дегидрогеназ, содержание цитохромов P_{450} и b_5) в печени белых крыс в условиях длительного поступления 12-краун-4, аза-12-краун-4, тиа-12-краун-4 (30 суток, перроральноо в 1/100 $ДЛ_{50}$). Краун-эфиры достоверно снижают содержание микросомального цитохрома P_{450}, не влияют на содержание цитохрома b_5 и повышают активности глюкозо-6-фосфат-дегидрогеназы, 6-фосфоглюконат-дегидрогеназы и НАДФ-зависимой декарбоксилирующей малат-дегидрогеназы в гепатоцитах крыс. Эти изменения связаны с активацией перекисного окисления липидов и развитием оксидативной стресс-реакции.

Ключевые слова: краун-эфиры, микросомальное окисление, цитохромы, НАДФ-зависимые дегидрогеназы, оксидативний стресс.