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CROWN-ETHER INFLUENCE ON RAT BLOOD PLASMA CYTOKINES AND IMMUNOGLOBULINES CONCENTRATIONS AND PROTEIN PEROXIDATION

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The present paper illustrates the experimental results of investigation of immunoglobulins, cytokines, Schiff's bases and 2.4-dinitrophenylhydrazones blood plasma and serum concentrations in rats organism subjected to intoxication by crown-ethers. The research program used sub-acute toxicological experiment on sexually mature white male rats of WAG population (body mass – 180-220 g). The animals were administered with water emulsion of investigated crown-ethers (12-crown-4, 15-crown-5, 18-crown-6, and aza-12-crown-4) in 1/100 daily (0.0117, 0.0135, 0.0127, 0.022 g/kg respectively), within 30 days per orally. The animals of the control group were given water at the same conditions. On the 30th day of the experiment the rats of all groups were anesthetized by sodium thiopental (50 mg/kg) and slaughtered by decapitation with the Guillotine knife. Cytokine (interleukins-4, -8 (ILs-4, -8), tumor necrosis factor-alpha (TNF-α)) and immunoglobulins (IGA, IGG, IGE, IGM) blood plasma concentrations were determined by immunoenzyme method with application of correspondent standard reagents «Protein Contour» (Saint-Petersburg, Russia) and an immunoenzyme analyzer Stat Fax 303 Plus. Blood serum Schiff's bases were extracted by Falch mixture (chloroform-methanol) with the following spectrofluorometric analysis in chloroform extract using initiating wave length of 360 nm and emission wave length of 430 nm. Evaluation of blood serum protein oxidative modification intensity was performed by Dubinina's modified spectrofluorometric method, which is based on an interaction reaction between oxidized protein amino acids residues and 2.4-dinitrophenylhydrazine with the formation of 2.4-dinitrophenylhydrazones (DNPHs). The latter were registered using wave length of 356 (for neutral aldehyde-DNPHs), 370 (for neutral ketone-DNPHs) and 430 (for basic aldehyde-DNPHs) nm.

The action of crown-ethers resulted in the significant reduction (25% on average) of IGA concentration in rat blood plasma compared to the control magnitudes, diminishing the very first link of organism immune defense, and potentially being a cause of virus and bacteria growth enhance in the organism. Crown-ethers effects were also displayed in significant decrease in IGG blood plasma concentration (in 45, 40, 38, 30 % for 12-crown-2, 15-crown-5, 18-crown-6 and aza-12-crown-4, respectively), non-specifically inhibiting the producing function of B-cells and which may signify the exhaustion of this class antibodies defensive role. These results very well correspond to the decrease in IGE rat blood plasma concentrations at the influence of the investigated chemical agents. The less decrease in this index in percentage compared to the percentage decrease in IGG concentration (30, 25, 28, 20% for 12-crown-2, 15-crown-5, 18-crown-6 and aza-12-crown-4, respectively) may signify mostly the disturbance of B-cells immune link in the action of the investigated substances.

Crown-ethers action upon the rat organism resulted in significant decrease in IL-4 blood plasma concentration (in 38, 33, 30, 25% for 12-crown-2, 15-crown-5, 18-crown-6, and aza-12-crown-4, respectively). Simultaneously, crown-ethers influence upon IL-8 blood plasma concentration was displayed in the significant raise of the index in 34, 30, 27, and 22% for 12-crown-2, 15-crown-5, 18-crown-6 and aza-12-crown-4, respectively. The experiments for blood serum Schiff's bases contents determination revealed the significant increase in this index in the organism of all experimental groups animals. Obviously, Schiff's bases are intermediate products of amino acids decarboxilation and transamination reactions. Therefore, the increase in their blood serum concentration may suggest the excessive proteolysis in tissues and involvement of amino acids in different metabolic pathways. The action of crown-ethers 1.5-2 fold increased the blood serum neutral aldehydedinitrophenylhydrazones, and 1.3-1.5 fold increased neutral ketone-DNHPs. These alterations were accompanied by the 2-2.3 raise in basic aldehyde-DNHPs blood serum concentration in the organism of experimental group animals. Significant changes in the levels of basic aldehyde-DNHPs may be connected with the enhanced process of protein glycozylation at conditions of oxidative stress.

Key words: *crown-ethers, immunoglobulins, cytokines, Schiff's bases, protein oxidative modifications.*

INTRODUCTION

One of the actual scientific problems nowadays should be the investigation of newly obtained xenobiotics with the goal of establishment of their biological mechanism and potential danger on the environment. Crown-ethers could be regarded as quite widely-spread industrial chemical polluters of biosphere, especially of water ecosystems. These compounds are derivatives of polyesters and characterized by high volume of production, for they are applied in different fields of electrochemistry, pharmacy, medicine, because of their unique properties of solubility in many non-aqueous solvents, high stability, selectivity in oxido-reductive reactions, ability to form complexes with metals, etc [5]. Presently, the possibility of crown-ethers entering the human organism with drinkable water is proven [6]. Therefore, their contents in environmental objects must be strictly limited below harmful hazardous concentrations. At the same time, crown-ethers biological action mechanism is investigated insufficiently, particularly with the connection of their environmental concentrations measurements and elaboration of medicine-biological and prophylactic ways of population protection from the compounds influence.

One of the human organism main links which can be impaired by xenobiotics action is immune system. It recognizes alien large molecules, viruses, bacteria, microorganisms – antigens, having invaded the organism, and forms reactions directed to their binding and elimination. The most elaborated and tangible to the harmful influence of toxic compounds is humoral immunity with the number of special glycoproteins – immunoglobulins (antibodies), which are responsible for selective antigen binding and depriving the latter of its biological activity. Another link of immunity is cytokines – large and diverse group of small (8-80 kDa) protein messengers participating in immune system signal transmission. With the usage of cytokines particularly, T-helpers coordinate the synthesis of specific antibodies.

Previously, we showed the negative effect of crown-ethers on protein biosynthesis [6], therefore, it was quite interesting for us to investigate, whether this effect would be non-specific and encompassing immune system proteins as well, and could be associated with protein peroxidation.

Objective. Investigation of immunoglobulins, cytokines, Schiff's bases and 2.4-dinitrophenylhydrazones blood plasma and serum concentrations in rats organism subjected to intoxication by crown-ethers.

MATERIALS AND METHODS

The investigation involved the usage of 40 male rats of WAG population (body mass 200-220 g). The animals were divided into four experimental and one control groups. The experimental groups of rats were administered water emulsion of investigated crown-ethers – 12-crown-4.15-crown-5.18-crown-6 and aza-12-crown-4 in 1/100 LD_{50} (0.0117, 0.0135, 0.0127, 0.022 g/kg respectively [6]) daily within 30 days per orally. The animals of the control group 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/kg [3]) and slaughtered by decapitation with the Guillotine knife.

Cytokine (interleukins-4, -8 (ILs-4, -8), tumor necrosis factor-alpha (TNF-α)) and immunoglobulins (IGA, IGG, IGE, IGM) blood plasma concentrations were determined by immunoenzyme method with application of correspondent standard reagents «Protein Contour» (Saint-Petersburg, Russia) and an immunoenzyme analyzer Stat Fax 303 Plus. Blood serum Schiff's bases were extracted by Falch mixture (chloroform-methanol) with the following spectrofluorometric analysis in chloroform extract using initiating wave length of 360 nm and emission wave length of 430 nm [7]. Evaluation of blood serum protein oxidative modification intensity was performed by Dubinina's modified spectrofluorometric method [1], which is based on an interaction reaction between oxidized protein amino acids residues and 2.4-dinitrophenylhydrazine with the formation of 2.4-dinitrophenylhydrazones (DNPHs). The latter were registered using wave length of 356 (for neutral aldehyde-DNPHs), 370 (for neutral ketone-DNPHs) and 430 (for basic aldehyde-DNPHs) nm.

Statistical analysis of digital material was performed with the usage of computer software instrumentation package for processing and analysis of statistical information – Statistica 6.1 (StatSoft Inc., USA). The primary statistical processing the digital data was started with the preliminary screening the assumption at the conformity of the samples to Gaussian distribution law. Quantitative traits, which had normal distribution, were described by parametric characteristics – arithmetic mean of variations number, i.e. Mean Values (M) with Standard Deviation (S). In the case of normal distribution absence, the quantitative traits were processed by non-parametric characteristics – median (Me) with interquartile swing. If, at the least, one of the distributions was not normal, then the comparison of independent samples was performed by Mann Whitney criterion. The differences between two samples were considered as significant if the probability of random difference was not higher then 0.05 ($p < 0.05$).

RESULTS AND THEIR DISCUSSION

The action of all the investigated crown-ethers resulted in the significant reduction (25% on average) of IGA concentration in rat blood plasma compared to the control magnitudes. To be noted, IGA is the antibody of mucous membranes, the structures, which are the first ones to contact with viral and bacterial antigens and to respond to their invasion. Obviously, the action of crown-ethers diminished the very first link of organism immune defense may potentially be a cause of virus and bacteria growth enhance in the organism (Table 1).

Crown-ethers action was also displayed in significant decrease in IGG blood plasma concentration (in 45, 40, 38, 30% for 12-crown-2.15-crown-5.18-crown-6 and aza-12-crown-4, respectively, Table 1). IGG is produced exclusively by plasma B-cells and is the main antibody in blood and extracellular fluids. It protects the organism from the wide range of viruses, bacteria and fungi, this way composing the main humoral immune response. Undoubtedly, all the investigated crown-compounds as well as the products of their destruction [6], being present in blood, non-specifically inhibited the producing function of B-cells and which may signify the exhaustion of this class antibodies defensive role.

Table 1

Influence of crown-ethers on rat blood plasma immunoglobulins concentra tion (ng/ml)

Crown-ether	IGA	IGG	IGE	IGM
12-crown-4	43.1±5.3*	37.4±4.8*	18.4±1.9*	45.3±6.3
15-crown-5	45.3±4.5*	41.3±3.9*	19.7±1.9*	46.5±4.3
18-crown-6	46.8±5.3*	42.5±4.4*	19.3±2.1*	47.3±5.4
Aza-12-crown-4	50.3±4.8*	48.1±4.5*	21.1±2.1*	50.1±4.4
Control	63.4±4.5	68.5±5.7	26.3±2.0	51.3±4.4

Notes: *n=8, * – p<0.05*

These results very well correspond to the decrease in IGE rat blood plasma concentrations at the influence of the investigated chemical agents (Table 1). IGE is known to be synthesized by all plasma cells, which are capable of antibody production at all, and to be responsible for mostly unicellular parasites immunity. The less decrease in this index in percentage compared to the percentage decrease in IGG concentration (30, 25, 28, 20% for 12-crown-2., 15-crown-5. 18-crown-6 and aza-12-crown-4, respectively, Table 1) may signify mostly the disturbance of B-cells immune link in the action of the investigated substances.

Crown-ether action insignificantly decreased blood plasma concentration of IGM (Table 1). This may be explained by the facts that IGM is the largest amongst antibodies and is involved in the initial exposure to an antigen and primary contact and humoral immune response. Thus, IGM is in the charge of the most vital immune reaction with the highest protection against xenobiotics action [2].

Production of antibodies in the organism is controlled by certain cytokines. Crown-ethers action upon the rat organism resulted in significant decrease in IL-4 blood plasma concentration (in 38, 33, 30, 25% for 12-crown-2.15-crown-5.18-crown-6 and aza-12-crown-4, respectively, Table 2). Simultaneously, crown-ethers influence upon IL-8 blood plasma concentration was displayed in the significant raise of the index in 34, 30, 27, and 22% for 12-crown-2.15-crown-5.18-crown-6 and aza-12-crown-4, respectively (Table 2).

Table 2

Influence of crown-ethers on rat blood plasmacytokines concentra tion (ng/ml)

Crown-ether	IL-4	IL-8	TNF
12-crown-4	31.2±3.5*	77.0±3.5*	152.4±11.3*
15-crown-5	33.6±4.3*	79.3±3.3*	148.5±10.8*
18-crown-6	35.1±3.7*	71.1±3.1*	150.8±12.2*
Aza-12-crown-4	37.7±3.8*	73.8±4.4*	155.1±13.3*
Control	50.2±4.6	56.2±4.8	131.6±9.8

Notes: *n=8, * – p<0.05*

IL-4 is known to induce differentiation of helper T-cells, to stimulate activated B- and T-cells proliferation. It also enhances B-cell class switching to IGE and reduces macrophages activity, thus encouraging the reactions of humoral specific immunity [4]. On the contrary, produced by mostly monocytes and macrophages, IL-8 is responsible for recruitment of neutrophils to the site of the damage or infection (chemotaxis) inducing acute inflammation reactions and nonspecific cellular immunity [4]. Crown-ethers negative influence upon IL-4 production may result in total inhibition of humoral immunity. Previously [6] we showed crown-compounds at certain concentrations to induce the development of pathogenic bacteria and microorganisms, so that might be why cellular defensive phagocytosis in the rat organism, subjected to 30 day crown-ether intoxication may get enhanced as a compensatory reaction on the xenobiotics action by stimulating IL-8 synthesis and release [2]. TNF is released in the organism in acute phase of inflammation by macrophages, neutrophils, mast cells, eosinophils. It also induces fever, apoptosis, inhibits viral replication. The enhance of TNF production by crown-compounds signifies the induction of the above-mentioned processes.

The experiments for blood serum Schiff's bases contents determination revealed the significant increase in this index in the organism of all experimental groups animals (Table 3).

Table 3

Influence of crown-ethers on concentration of Schiff's bases (conditional units / ml), aldehyde- and ketonedinitrophenylhydrazones (optical density units / protein g) in rat blood serum

Crown-ether	Schiff's bases	Neutral aldehyde-DNPHs (356 nm)	Neutral ketone-DNPHs (370 nm)	Basic aldehyde-DNPHs (430 nm)
12-crown-4	4.22±0.51*	57.2±4.4*	58.2±6.9*	7.33±0.73*
15-crown-5	3.93±0.42*	42.3±4.9*	53.7±6.9*	7.15±0.63*
18-crown-6	4.71±0.53*	42.8±5.1*	51.4±6.1*	6.93±0.74*
Aza-12-crown-4	3.83±0.46*	48.3±5.3*	55.1±5.6*	7.11±0.77*
Control	1.71±0.19	28.3±5.1	36.6±4.3	3.32±0.41

Notes: *n=8, * - p < 0.05*

Obviously, Schiff's bases are intermediate products of amino acids decarboxilation and transamination reactions. Therefore, the increase in their blood serum concentration may suggest the excessive proteolysis in tissues and involvement of amino acids in different metabolic pathways.

At the end of the sub-acute experiment, the experimental groups of animals were observed to activate the processes of proteins oxidative modification which was verified by statistically significant increase in neutral aldehyde- (356 nm), neutral ketone- (370 nm), and basic aldehydedinitrophenylhydrazones (430 nm) in animals blood serum (Table 3). It is worth mentioning, that, the control group of rats also had DNHPs in their blood serum, which signifies the minor occurrence of the process at normal physiological conditions. Besides, the control animals group had the level of neutral-DNPHs total sum 11 times as high as the basic-DNPHs level, which is supported by the paper [1]. The action of crown-ethers 1.5-2 fold increased the blood serum neutral aldehydedinitrophenylhydrazones, and 1.3-1.5 fold increased neutral ketone-DNHPs. These alterations were accompanied by the 2-2.3 raise in basic aldehyde-DNHPs blood serum concentration in the organism of experimental group animals. Significant changes in the levels of basic aldehyde-DNHPs may be connected with the enhanced process of protein glycozylation at conditions of oxidative stress. Although, in general, the increased blood serum level of protein oxidative modifications in the organism of experimental animals could signify the exhaustion of reserve-adaptive capacities of the organism at conditions of crown-ethers action.

CONCLUSIONS

1. The action of crown-ethers results in the changes in humoral components of non-specific resistance and specific immunologic reactivity of the rat organism. This is verified by the decrease in blood plasma concentrations of immunoglobulins A, E, G, and interleukin-4, on the background of increase in interleukin-8 and tumor necrosis factor-alpha concentrations.
2. The consequences of changes in the indexes of non-specific and specific immune resistance of rat organism at conditions of crown-ether intoxication could be the reduction of anti-infectious, and anti-tumor immunity; formation of inflammatory, allergic and autoimmune reactions.
3. The crown-ether intoxication of the rat organism leads to the initiation of oxidative stress processes with the destruction of protein molecules, which is displayed by the increase in blood serum levels of Schiff's bases and dinitrophenylhydrazones.
4. The products of protein oxidative modifications due to their high reactivity and biological action selectivity may present the negative basic chain, which could limit the resistance of the organism to crown-ethers or other xenobiotics invasion via alterations of cellular membrane physico-chemical characteristics, membrane-bound enzymes activity, and reactivity of immune system.
5. Inhibition of immune system on the background of enhance of protein oxidative modifications presents one of pathogenic links in the action mechanism of crown-ethers.

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ВПЛИВ КРАУН-ЕТЕРІВ НА КОНЦЕНТРАЦІЮ ЦИТОКІНІВ ТА ІМУНОГЛОБУЛІНІВ В ПЛАЗМІ КРОВІ ЩУРІВ І ПЕРЕКИСНЕ ОКИСЛЕННЯ БІЛКІВ

Кратенко Р.І.

В статті розміщені експериментальні дані щодо змін концентрацій цитокінів, імуноглобулінів, Шифових основ та 2.4-динітрофенилгідразонів у плазмі та сироватці крові щурів, інтоксикованих краун-етерами. Програма дослідження базувалася на використанні підгострого токсикологічного експерименту на статевозрілих білих щурах-самцях популяції WAG масою тіла 180-220г. Тваринам вводили водну емульсію досліджуваних речовин (12-краун-4, 15-краун-5, 18-краун-6 та аза-12-краун-4) у 1/100 LD_{50}, щоденно, перорально на протязі 30-ти днів. Тварини контрольної групи одержували воду при тих же умовах. На 30-й день експерименту тварин усіх груп анестезували тиопенталом натрію (50 мг/кг) і забивали декапітацією гільйотинним ножем.

Вплив краун-етерів на організм тварин експериментальних груп призвів до достовірного зниження концентрацій імуноглобулінів А, G та Е в плазмі крові. Дія досліджуваних речовин також призвела до достовірного зниження концентрації інтерлейкіну 4, на тлі підвищення концентрацій інтерлейкіну 8 та фактору некрозу пухлин. Формування запальних, алергічних та аутоімунних реакцій може виникнути внаслідок змін показників імунітету щурів інтоксикованих краун-етерами.

Експерименти з вмісту Шифових основ в сироватці крові виявили підвищення цього показника в організмі експериментальних тварин, що свідчило про активацію процесів протеолізу та переамінування амінокислот під впливом краун-етерів. Дія досліджуваних речовин також призводила до підвищення перебігу процесу окисних модифікацій білків, що виявлялося зростанням концентрацій 2.4-динітрофенилгідразонів у сироватці крові щурів.

Таким чином, інгібування функцій імунної системи на тлі прискорення процесів протеолізу та окисних модифікацій білків може бути однією з патогенетичних ланок механізму дії краун-сполук.

Ключові слова: *крану-етери, імуноглобуліни, цитокіни, Шифові основи, окисні модифікації білків.*

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