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Antioxidant Properties of a Nanostructured Clathrate Complex Selenopyran with β -Cyclodextrin in Model Systems of Oxidative Stress

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We studied the effect of nanostructured clathrate complex 9-phenyl-symm-octahydroselenoxanthene (selenopyran) with β -cyclodextrin on the generation of OH^\cdot radicals in the Fenton system and parameters of oxidative stress in rat liver cells incubated at 37°C for 1 h. The complex inhibits the development of free-radical oxidative processes induced by ROS and the most toxic OH^\cdot radicals, reduces the increased level of ROS induced by prooxidants, and exhibits antioxidant activity.

Key Words: *selenium; Fenton system; malondialdehyde (MDA); 2',7'-dichlorofluorescein; liver cells*

A promising area of modern medicine is correction of disorders caused by changes in the content of vital microelements in the body. Selenium deficiency is a risk factor for the development of many diseases, including endemic, cardiovascular, oncological, cognitive disorders, infertility, and COVID-19 complications [14]. In the pathogenesis of diseases, the leading role belongs to oxidative stress resulting from imbalance between ROS generation and their detoxification by the antioxidant defense system. The biochemical functions of selenium are determined by selenium-containing proteins that perform various functions and are necessary for normal functioning of the immune and antioxidant systems [8]. The main enzymes of antioxidant defense are glutathione peroxidases containing selenium in the active center and catalyzing decomposition of hydrogen peroxide and organic hydroperoxides.

For the treatment and prevention of diseases, mineral and organic forms of selenium are used; their metabolism and mechanism of toxicity are associated with their biological activity and availability [6]. Heterocyclic compound 9-phenyl-symmetric octahydro-selenoxanthene (selenopyran) is a source of selenium with antioxidant properties and low toxicity. The efficiency of delivery of problematic drugs can be improved by using cyclodextrins, natural structures with a hydrophilic outer surface and a hydrophobic inner cavity capable of forming inclusion complexes with various groups of organic and inorganic compounds; the physicochemical and biological properties of the "guest molecule" change in this complex [4] Another way to increase the bioavailability of drugs is to reduce the size of their particles as much as possible [3].

The properties of atoms differ in the bulk of a substance and on its surface. The formation of nanoparticles with a size of 1-100 nm leads to the emergence of new properties of the substance associated with an increase in the number of atoms located on the surface,

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the peculiarity of the physical and chemical interactions of which becomes decisive. The prospects for using selenium-containing preparations are determined by their antioxidant properties [8].

The aim of this work was to study the antioxidant properties of the nanostructured clathrate complex of 9-phenyl-sym-octahydroselenoxanthene with β -cyclodextrin in model systems of oxidative stress.

MATERIALS AND METHODS

The nanostructured clathrate complex of 9-phenyl-sym-octahydroselenoxanthene with β -cyclodextrin, provided by the developer (Medbiofarm), is a crystalline light beige powder with a weak specific odor (molar ratio of components 1: 3, particle size 24.3 ± 0.4 nm). The solubility of 9-phenyl-sym-octahydroselenoxanthene in water is practically zero, while the solubility in the complex with β -cyclodextrin is 0.443 g/liter [5].

The antiradical properties of the drug were assessed on the model of OH^\bullet hydroxyl radical generation in the Fenton reaction system. The samples containing PBS (0.14 mol/liter NaCl, 20 mmol/liter phosphate buffer, pH 7.4), 3 mmol/liter deoxyribose, 0.1 mmol/liter EDTA, 0.1 mmol/liter $\text{FeSO}_4 \times 7\text{H}_2\text{O}$, and 2 mmol/liter H_2O_2 were incubated for 1 h in a water bath at 37°C in the presence of a clathrate complex in a concentration of 0.044% or without it. Then, 1 ml of 3% trichloroacetic acid and 1 ml of 1% TBA were added, and the mixture was incubated at 100°C for 20 min. 2-Deoxyribose is oxidized by hydroxyl radicals to form a number of products, in particular malondialdehyde (MDA), which reacts with TBA in an acidic medium to form a stably colored complex with an absorption maximum at 532 nm. Optical density was determined on a UV/Vis 2800 spectrophotometer (UNIKO-SIS) to determine the concentration of MDA (molar extinction coefficient 156,000 optical units) and to estimate the level of generation of OH^\bullet radicals proportional to this concentration.

The effect of clathrate complex on LPO intensity in primary cell cultures was evaluated using a suspension of liver cells from Wistar rats (males weighing 250-300 g); the cells were isolated as described elsewhere [7]. The suspension was incubated for 1 h at 37°C in a water thermostat without and in the presence of the complex at a concentration of 0.044%. To induce free radical oxidation, 0.2 mM $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ was used. The concentration of MDA was measured as described previously [7].

The effect of the clathrate complex on ROS content was determined by the fluorometric method using 2,7-dichlorodihydrofluorescein diacetate ($\text{H}_2\text{DCF-DA}$) deacetylated by alkaline hydrolysis with NaOH [9].

The use of deacetylated form of the probe avoids underestimating the level of ROS generation by cells due to low activity of cellular esterases and reduces the time of analysis. Suspension of hepatocytes was added to a 96-well microplate and incubated in a thermostat at 37°C for 60 min. The wells contained 10^6 cells in PBS (0.14 mol/liter NaCl, 20 mmol/liter phosphate buffer, pH 7.4). The analysis was carried out in the presence of the tested complex and without it in three variants: 1) suspension of liver cells; 2) suspension of liver cells with $20 \mu\text{M}$ $\text{FeSO}_4 \times 7\text{H}_2\text{O}$; 3) suspension of liver cells with $20 \mu\text{M}$ $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ and $200 \mu\text{M}$ ascorbic acid. After incubation, $50 \mu\text{M}$ fluorogenic probe was added to the wells. Fluorescence was recorded immediately (initial level) and after 20-min incubation in the dark at 25°C on a Panorama spectrofluorometer (LOMO) with $\lambda_{\text{ex}} = 485$ nm and $\lambda_{\text{em}} = 521$ nm. $\text{H}_2\text{DCF-DA}$ can penetrate into the cell, where it is hydrolyzed by cellular esterases to H_2DCF . The deacetylated form undergoes oxidation to form the fluorescent product DCF. The fluorescence intensity is an indicator of the intensity of ROS production. The results are presented as the difference between fluorescence of the samples determined after 20-min incubation and initially.

Statistical analysis was performed by standard methods using Statistica 6.0 software (StatSoft, Inc.). The significance of differences between the groups was assessed using Student's t test and Mann—Whitney U test. The data are presented as the arithmetic mean and mean square error of the mean ($M \pm m$).

RESULTS

To study the mechanisms of preventive and therapeutic action of the clathrate complex, we evaluated its effect on the generation of the most toxic OH^\bullet -radicals in the Fenton reaction system: $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\bullet$ [2]. The level of OH^\bullet -radical generation proportional to the concentration of MDA formed as a result of oxidation of the detector molecule of deoxyribose was $12.51 \pm 0.11 \mu\text{mol/liter}$ and decreased in the presence of the complex to $7.99 \pm 0.12 \mu\text{mol/liter}$ ($p < 0.05$), the protective effect was $36.1 \pm 0.3\%$. The obtained results are consistent with published data. It was reported that selenium plays an important role in increasing the antioxidant activity of polysaccharide extracts from *Ganoderma lucidum* mushrooms enriched with this element, because the percentage of OH^\bullet -radical inhibition in the Fenton reaction system increased with increasing selenium content [15].

The study of drugs in model systems with generation of OH^\bullet -radicals is considered one of the best indicators of the antioxidant potential of a compound [12]. In the body, the Fenton reaction occurs in tissues with

high iron content (e.g. in the liver) and further promotes initiation of LPO; therefore, it seemed appropriate to analyze changes in LPO in liver cells as an indicator of cell stability to the effects of damaging factors and to study the antioxidant properties of the tested drug.

Incubation of cells for 1 h at 37°C did not significantly increase the concentration of MDA, a biomarker of LPO (Table 1). A significant acceleration of LPO was observed in the presence of Fe²⁺ ions interacting with lipid hydroperoxides and promoting the branching of chain reactions of free radical oxidation. The complex exhibited antioxidant activity and reduced LPO intensity by 1.63 times.

Indirect methods for assessing oxidative stress, which include the determination of the content of LPO products in cells, make it possible to study the processes caused by reactive oxygen-containing radicals and molecules. Direct methods make it possible to determine the amount of ROS present in cells, for which fluorogenic probes are used. The use of the H₂DCF-DA probe has become a common method for determining the production of ROS by different types of cells. The results obtained (Fig. 1) indicate that the combination of ferrous iron ions and ascorbic acid is effective as an activator of ROS generation in liver cells. It is known that the reaction of H₂O₂ with Fe²⁺ ions (Fenton's reaction) leads to the formation of a highly active hydroxyl radical OH[•] and oxidation of Fe²⁺ to Fe³⁺ that does not react with H₂O₂. Ascorbic acid reduces Fe³⁺ to Fe²⁺ and returns active Fe²⁺ ion to the reaction; Fe²⁺ reacts again with hydrogen peroxide, which leads to the formation of new hydroxyl radicals OH[•] and accelerates ROS generation. The clathrate complex reduced the level of ROS generation in the samples under study by 1.92 times.

ROS play an important role in metabolic processes under normal conditions, but in excess con-

TABLE 1. Effect of Nanostructured Clathrate Complex of 9-Phenyl-Symm-Octahydroselenoxanthene with β -Cyclodextrin on LPO in Liver Cell Suspensions ($n=14$; $M\pm m$)

Experimental conditions	MDA content, μM
Initial level	0.94 \pm 0.03
Incubation at 37°C for 1 h	2.10 \pm 0.03
Incubation in the presence of the complex at 37°C for 1 h	1.83 \pm 0.09
Incubation in the presence 0.2 mM FeSO ₄ at 37°C for 1 h	28.91 \pm 0.27*
Incubation in the presence 0.2 mM FeSO ₄ and the complex at 37°C for 1 h	17.72 \pm 0.19**

Note. n is the number of animals. $p < 0.05$ in comparison with *initial level, **incubation in the presence of 0.2 mM FeSO₄.

Production of ROS

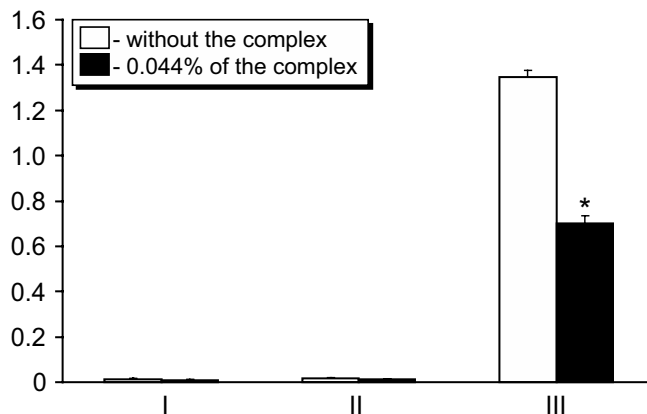


Fig. 1. Effect of prooxidants and nanostructured clathrate complex of 9-phenyl-symm-octahydroselenoxanthene with β -cyclodextrin on ROS generation in rat hepatocytes. I — without prooxidants; II — 20 μM FeSO₄·7H₂O; III — 20 μM FeSO₄·7H₂O and 200 μM ascorbic acid. * $p < 0.05$ in comparison with incubation without the complex.

centrations are capable of damaging DNA, lipids and proteins, changing their physicochemical and structural-functional properties. The results attest to antiradical and antioxidant properties of the clathrate complex and its ability to increase body's resistance to oxidative stress. In [13], it was found that food supplementation with sodium selenite and selenium-enriched yeast led to an increase in the concentration of antioxidant enzymes and a decrease in the content of MDA in the liver and serum of rats. The use of Selenaza drug in the postoperative period promoted earlier recovery of the detoxification and synthetic function of the liver in patients with liver tumors [1]. The protective effect of selenium-containing drugs in liver diseases is determined by transformation of toxins into inactive forms in its environment, preservation of the hepatocyte membranes structure as a result of stimulation of glutathione peroxidases reducing the level of free radicals and LPO, acceleration of ethanol catabolism, and the effect on both humoral and cellular immunity [10]. Selenium is also essential for the synthesis of liver enzymes [11]. Another important aspect of achieving the therapeutic effect is bioavailability of pharmaceuticals, for which surface-active water-soluble polymers of cyclodextrin were used in the development of the clathrate complex of 9-phenyl-symm-octahydroselenoxanthene [3]. Improved bioavailability allows reducing effective therapeutic doses and toxic load of selenium-containing preparations on the body.

Our findings allow us to conclude that the nanostructured clathrate complex of 9-phenyl-symm-octahydroselenoxanthene with β -cyclodextrin has antioxidant activity.

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