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The State of Antioxidant Systems and Lipids' Peroxidation Under the Action of Complex Substances

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The article presents the prospects for nanomaterials' application in medicine, biology, and pharmacology. The possibility of nanoparticles' usage as contrast agents for tomography, luminophores, and biologically active substances is shown. At the same time, the potential toxic effect on living organisms of the concerned substances is indicated, in particular, the possibility of free-radicals' formation. These substances are capable of triggering the chain reaction of free-radicals' transformations, which can result in the development of pathological processes. To clarify this issue, effects of the complex substance composed of lanthanides' nanoparticles on the biochemical parameters of free-radical processes have been studied in experiment. The results show that infusion of the preparation induces an increase in the content of diene conjugates and TBA-reactive products. Moreover, the content of sulfhydryl groups reduces significantly as compared with the intact group. Catalase activity also decreases, while superoxide dismutase activity increases. Thus, the results show that the substance concerned affects the processes of free-radical oxidation. However, the effect depends on a number of factors, and additional studies are needed to determine the contribution of each of them.

У статті зазначено перспективи застосування наноматеріялів у медицині, біології та фармакології. Показано можливість використання наночастинок як контрастних реаґентів для томографії, люмінофорів і біологічно активних речовин. При цьому вказується на можливість потенційного токсичного впливу цих матеріялів на живі організми, зокрема ініціювання вільних радикалів або інтермедіятів радикальної природи, які здатні запускати ланцюговий механізм вільнорадикальних перетворень, що врешті-решт може призводити до розвитку патологічних процесів. Для з'ясування цього питання проведено експериментальні

дослідження стосовно впливу розчину комплексного препарату, складеного з наночастинок лантаноїдів на біохемічні показники вільнорадикальних процесів. Результати показали, що після інфузійного введення даного препарату спостерігалося підвищення вмісту дієнових коньюґатів і ТБК-активних продуктів. При цьому вміст сульфгідрильних груп достовірно понижувався в порівнянні з даними інтактної групи. Активність каталази також залишалася пониженою, тоді як активність супероксиддисмутази підвищувалася. Таким чином, результати показали, що даний препарат впливає на процеси вільнорадикального окиснення, однак цей вплив залежить від ряду чинників, і для з'ясування внеску кожного з них потрібні додаткові дослідження.

Kew words: nanoparticles, lanthanides, toxicity, radicals, enzymes.

Ключові слова: наночастинки, лантаноїди, токсичність, радикали, ферменти.

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1. INTRODUCTION

Recently, inorganic nanomaterials based on rare-earth metals are of particular scientific interest [1, 2].

Low toxicity and unique optical properties allow them to be intensively used in biology, pharmacology, and medicine [3].

Colloidal solutions containing lanthanide ions are most commonly used [4]. They are used in medicine both in the treatment of cancer and as contrast agents in tomography. The use of these solutions allows luminescence analysis of cells due to autoluminescence of biological material [5]. Metals' orthovanadates activated by rare-earth elements are considered particularly effective luminophores [6].

Terbium and europium ions with filled $4f$ energy level are of greatest interest for luminescent-labels' creating [7].

Luminescent systems based on lanthanide ions show great solubility, biocompatibility and low toxicity, which contribute to their application [8]. Therefore, studies in this field are of considerable interest.

It is known that lanthanides are able to regulate effectively biochemical processes in cells [9]. Since the ionic radius of lanthanum is almost the same as that of calcium, it can sometimes replace calcium and directly affect calcium-dependent processes in cells. Interaction of nanoparticles with blood components is of particular interest because they are the first substances that injected nanoparticles meet [10]. Being introduced into the bloodstream, nanoparticles adsorb plasma proteins on their surface. These interactions make

significant changes in the properties of nanoparticles, such as aggregation state, surface chemistry, and surface charge, which affect their functionality [11].

The above-mentioned ability of lanthanides to influence the structure of a protein is widely used in medicine. For example, lanthanides can be used as anticoagulants (inhibit prothrombin synthesis).

It is also known that, in the human body, lanthanides are able to accumulate in cancerous tumours in large quantities and disrupt calcium, magnesium and phosphorus metabolism in them. This property determines the use of radioactive isotopes of lanthanides in oncology. Lanthanum compounds reduce accumulation of calcium in the skin that positively affects its elasticity and prevents the appearance of wrinkles. In addition, lanthanide ions have an affinity for phospholipids that helps to stabilize the cell membrane by blocking of ion channels [12].

Biological properties of lanthanides are mainly due to the presence of *f*-electrons in the electronic shells, which create the effect of magnetic microfields. This effect explains the ability of lanthanides to increase the phagocytic activity of leukocytes. In addition, the organic complexes of lanthanum in biological systems contribute to the removal of heavy metals, have osmotic activity, bactericidal properties, as well as accelerate wound healing and skin regeneration.

Nanoparticles have specific physicochemical properties and biological effects on living organisms, which make them possible to be potentially used in biological analysis and medicine. Therefore, the study of the potential risk of effects of nanomaterials on health and environment is very actual [13]. The risk of pathologies caused by nanomaterials is not yet completely clear; therefore, investigation of toxic effects of nanoparticles today is becoming the new direction in experimental medicine [14].

According to the literature data [15], toxicity of nanoparticles directly depends on their size and, therefore, on the specific surface area, which causes high chemical activity. The smaller the particle size, the larger its specific surface and the higher the degree of substance toxicity [17].

Thus, nanoparticles entering the body can cause formation of highly reactive intermediates or free radicals participating in many biochemical reactions. These products can catalyse free-radical oxidation and can be harmful because they trigger the chain reaction of new free-radicals' formation [18]. Free-radical products can interact with lipid components of membranes leading to the formation of lipid hydroperoxides. As a rule, their excessive accumulation leads to change of membranes' structure, alteration of their physi-

cochemical properties, disturbance of ion transport, *etc*.

Modification of the components of cell membranes caused by free-radical oxidation affects all its functions. This results in disruption of the functioning of cells, imbalance in regulatory processes, decline in tissue repair potential, and, as a result, the development of irreversible pathological processes, which form the base of various diseases in individual organs and systems of the body, such as inflammation, immune status disorders, endocrine disorders, and cardiovascular diseases, *etc*. [19]. The intensity of free radical processes in cells and tissues is controlled by physiological antioxidant (AO) systems. They inhibit the processes, which contribute to the destruction of cell membranes inhibiting free-radical oxidation of lipids and other biological compounds [20].

Metal ions with variable valence are also an equally important factor of the regulation of free-radical oxidation in cells and tissues. They are in the composition of the active centres of some enzymes including antioxidant ones. They catalyse many biochemical reactions being in a free state or in the form of complex compounds with proteins and other substances [21].

Some enzymes involved in the inactivation of certain products of lipid peroxidation can also be attributed to substances exhibiting an antioxidant effect. These include: superoxide dismutase that limits formation of reactive oxygen species and inactivates the superoxide anion radical; catalase that decomposes hydrogen peroxide H_2O_2 into water and molecular oxygen; glutathione-related enzymes involved in the utilization of lipid hydroperoxides, which help to reduce their toxic effects on membranes and prevent the initiation of secondary reactions of free-radical oxidation. Without direct participation in non-enzymatic reactions, AO enzymes affect their intensity by inactivation of either initiators or products of chain reaction and, thereby, reducing the possibility of chain propagation [22].

Nitric oxide (NO) is of great interest in the fields of biology and medicine. It is a highly reactive short-lived free radical, which can control many functions and biochemical processes in cells. Nitric oxide regulates the intracellular concentration of Ca^{2+} ions, transport and consumption of O_2 in tissues, affects activity of number of enzymes, and acts as an important neuromediator that transmits a signal between the central and peripheral nervous systems [23].

Nitric oxide causes intensification of vascular permeability, formation of edema, and subsequent development of inflammatory response. In this case, nitric oxide combines with superoxide radical and forms a peroxynitrite anion ($ONOO^-$), which contributes to DNA damage and mutations. Thus, peroxynitrite anion is involved in the oxidative stress developing. Nitric oxide is irreversibly inac-

tivated by superoxide radical in the wall of a blood vessel or by free oxygen in a solution. Reaction of nitric oxide with oxygen is accompanied by formation of stable end products—nitrites and nitrates, which are indirect markers of the nitric-oxide concentration in the body.

Thus, it can be concluded that lipid peroxidation is not only a universal modifying of the properties, structure and function of biological membranes, but it is also an important physiological regulator of normal cell activity, and nitric oxide is one of the key links in the oxidative stress pathophysiology.

Studying the mechanisms of oxidative stress development and preventing its detrimental effects open up new prospects for medical science.

The aim of this work is to study the effect of $CdVO_4Eu^{3+}$ preparation on a number of biochemical parameters of free-radical processes.

2. MATERIALS AND METHODS

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008 (5), as well as the national law. All institutional and national guidelines for the care and use of laboratory animals were followed.

The experiments were carried out using 25 sexually mature WAG line female rats with weight of 180–200 g. Rats were divided into 2 groups:

— group 1 included 15 rats, which received intramuscular injections of the $CdVO_4Eu^{3+}$ ($C = 0.2$ g/l) preparation once a day at a dose of 1 mg/kg;

— group 2 included 10 intact rats.

The blood was taken from the caudal vein in test tubes with EDTA solution after the 1st, 3rd, and 5th injections. The following lipid peroxidation (LPO) parameters were determined in the blood plasma: the content of diene conjugates (DCs), thiobarbituric acid reactive substances (TBARS), sulfhydryl groups (SH-groups), total nitric oxide metabolites (mNO), nitrates and nitrites. The activity of antioxidant enzymes' catalase and superoxide dismutase (SOD) was determined in erythrocyte haemolysates. Determination of the DCs level was done in accordance with Ref. [24]. The method is based on lipid peroxidation-products' determination in the blood using absorption of ultraviolet monochromatic light flux by lipid extract. Determination of the malondialdehyde (MDA) concentration was carried out according to Uchiyma M. & Michara M. using reaction with thiobarbituric acid (TBA) [25]. The method is based on the

interaction of MDA with two molecules of thiobarbituric acid (TBA) at a temperature of 90–100°C. Coloured trimethine complex with maximum absorption is formed. Catalase activity was determined by the rate of hydrogen peroxide H_2O_2 utilization. The method is based on the ability of H_2O_2 to form a stable coloured complex with molybdenum salts [26]. The activity of SOD was determined by the quercetin-oxidation method [27]. The method is based on the ability of SOD to inhibit the quercetin-autooxidation reaction at pH = 10 in the presence of tetramethylethylenediamine. The content of SH-groups was determined spectrophotometrically using Ellman's reagent [28]. The content of total nitrogen-oxides' metabolites was determined according to Ref. [29]. The method is based on the ability of primary aromatic amines to form intensely coloured diazo compounds in the presence of nitrous acid. Determination of nitrite content was carried out according to Ref. [30]. The method is based on photometric measurement of colour intensity of a pink-raspberry-coloured azocompound, which is formed by the reaction of nitrites with alpha-naphthylamine and sulfanilic acid (Griss reagent) in acidic medium. The reaction is specific for nitrites. Statistical processing was carried out according to the standard method by means of the Student's *t*-test.

3. RESULTS AND DISCUSSION

The data obtained (Table 1) show that the first administration of the studied substance did not provoke significant changes in the LPO parameters in rats of group 1. The levels of peroxide products—DCs and TBARS coincide with intact values.

Intensification of peroxidation processes was observed after the

TABLE 1. Influence of the $CdVO_4Eu^{3+}$ on the LPO parameters.

Indexes	Intact, $n = 10$	Terms		
		after 1 administration, $n = 15$	after 3 administrations, $n = 15$	after 5 administrations, $n = 15$
Diene conjugates, mmol/L	19.26 ± 1.83	20.68 ± 2.36	23.74 ± 2.23 $^*P_1 < 0.05$	30,16 ± 3,16 $^*P_1 < 0.05$
TBA reactive substances, mcmol/L	3.83 ± 0.33	3.79 ± 0.33	5.11 ± 0.36 $^*P_1 < 0.05$	5.53 ± 0.45 $^*P_1 < 0.05$

Note: $^*P_1$—statistically significant *versus* intact.

TABLE 2. Influence of the $CdVO_4Eu^{3+}$ on the AO systems.

Indexes	Intact, $n = 10$	Terms		
		after 1 administration, $n = 15$	after 3 administrations, $n = 15$	after 5 administrations, $n = 15$
SH-groups, mmol/L	5.65 ± 0.63	5.40 ± 0.57	6.35 ± 0.61	2.91 ± 0.27 $^{*}P_1 < 0.05$
Catalase activity, U/L	3.25 ± 0.38	3.05 ± 0.33	2.82 ± 0.32	2.76 ± 0.32
Superoxide dismutase activity, U/L	4.96 ± 0.48	4.80 ± 0.31	5.57 ± 0.56	5.90 ± 0.56 $^{*}P_1 < 0.05$

Note: $^{*}P_1$—statistically significant *versus* intact.

3rd injection of the studied preparation as evidenced by the increased plasma content of lipids peroxidation products—DCs and TBARS. The level of DCs in rats of group 1 was elevated on average by 23%; the concentration of TBARS was increased by 33–34%.

Peroxidation activity continued to increase after the 5th injection in rats of group 1. The content of peroxide-oxidation products was significantly higher than both intact values and results after the 3rd injection.

Introduction of the substance also influenced the work of protective antioxidant systems (AO) in a certain way (Table 2). The single injection of the preparation did not cause any changes in the studied parameters: the level of SH-groups in the blood plasma remained within the intact values; the activity of AO enzymes was not affected.

There was a tendency to increase the concentration of SH-groups in plasma after the 3rd administration, although the differences with the intact values were not significant. At this stage of the study, a slight decrease in the activity of catalase was noted, and the activity of another AO enzyme, SOD, on the contrary, tended to increase (Table 2).

The content of SH-groups was changed significantly after the 5th injection: it decreased almost 2 times compared to the intact group. Catalase activity remained decreased, while SOD activity continued to increase and was 20–30% higher than intact values (the differences are significant, $P_1 < 0.05$).

$CdVO_4Eu^{3+}$ administration influenced the nitric-oxide system most significantly (Table 3).

After the 1st administration, plasma contents of *m*NO, nitrates and nitrites were tended to increase, however, the differences with

TABLE 3. Influence of the $CdVO_4Eu^{3+}$ on the level of nitric-oxide metabolites.

Indexes	Intact, $n = 10$	Terms		
		after 1 administration, $n = 15$	after 3 administrations, $n = 15$	after 5 administrations, $n = 15$
Total metabolites of nitric oxides, mcmol/L	42.04 ± 3.77	47.57 ± 3.65	55.74 ± 6,27 $^*P_1 < 0.05$	45.95 ± 4.98
Nitrates, mcmol/L	36.37 ± 3.26	40.91 ± 3.63	47,53 ± 5,28 $^*P_1 < 0.05$	40.04 ± 5.11
Nitrites, mcmol/L	5.67 ± 0.49	6.65 ± 0.40	8.21 ± 0.73 $^*P_1 < 0.05$	5.91 ± 0.50

Note: $^*P_1$—statistically significant *versus* intact.

the intact values were not significant.

Later, after the 3rd injection, accumulation of the metabolites in rats of group 1 continued to increase, and experimental values exceeded the intact ones: for *m*NO content, by 32%; nitrates, by 30%; nitrites, by 44% ($P_1 < 0.05$).

By the end of the experiment, the content of nitric-oxide metabolites as well as nitrates and nitrites in the blood plasma decreased to intact values and completely corresponded to the normal level.

Thus, it can be concluded that the experimental substance $CdVO_4Eu^{3+}$ influences free-radical oxidation processes, in particular, lipid peroxidation and AO systems, and response of the studied systems was ambiguous. A single administration of the substance caused changes only in the NO metabolism system that was manifested in increase in *m*NO, nitrates' and nitrites' contents in the blood plasma of rats. Intensification of nitric-oxide formation after the 1st injection can be explained as a nonspecific response of the body to any external effect. NO radical is able to trigger a cascade of free-radical reactions in the cells, which leads to the activation of LPO.

Experimental results show that the 3rd injection induced intensive formation of *m*NO, nitrates and nitrites as well as peroxide products—DCs and TBARS. Such activation of free-radical reactions caused significant shifts in the function of protective AO systems. After the one injection, the level of SH-groups, activity of catalase and SOD did not change, but then, after the 3rd injection, activity of catalase decreased and activity of SOD increased that, obviously, was a compensatory reaction of the protective AO systems.

After the 5th injection, levels of *m*NO, nitrates and nitrites corresponded to intact values, but the concentration of SH-groups was significantly reduced that indicated the depletion of AO resources of the glutathione system. Intensity of LPO continued to increase that was expressed in high concentrations of DCs and TBARS (differences with the intact group were significant). The data obtained indicate that the administration of $CdVO_4Eu^{3+}$ has destructive effect on cell membranes.

The substance concerned did not cause acute intoxication during the entire experimental period. Rats were mobile, active; they did not show a decrease in appetite, the stool was normal, the coat was smooth, clean, and the skin was without lesions.

4. CONCLUSIONS

Administration of $CdVO_4Eu^{3+}$ nanocrystals did not cause acute intoxication in experimental animals, but affected free-radical oxidation processes depending on the number of injections of the substance.

— The first administration of nanocrystals did not cause any significant changes but minor changes in the NO metabolism were observed. This fact can be attributed to a non-specific response of the body to any external influence.

– After the 3rd injection, peroxide processes as well as activity of protective antioxidant systems were intensified. Further administration of the substance caused increasing of peroxidation activity and depletion of protective antioxidant systems.
– After the 5th injection, serum levels of NO metabolites returned to normal intact values.
– Reducing concentrations of NO metabolites in the liver, kidneys, and brain after the 5th injection can be explained by decrease in the prooxidative effect of nanocrystals in the body due to the compensatory action of antioxidant systems.
– The ambiguity of the data obtained shows selective accumulation of nanocrystals in different organs and requires additional studies to identify specific effects of the substance on target organs.

It is still hard to evaluate degree of toxicity of $CdVO_4Eu^{3+}$ due to the short time of experiment.

Most of the biochemical parameters returned to normal values by the end of the study. Increased contents of DCs and TBARS in the blood plasma of rats are probably due to the presence of Cd (cadmium) in the preparation, which can cause metabolic disturbances, or due to the presence of V (vanadium), which enhances tissue oxygen uptake and, thus, can activate LPO.

Additional experiments are needed to determine the degree of

toxicity of $CdVO_4Eu^{3+}$ and its effect on different types of metabolism.

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