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Synthesis of Nanostructured Erbium Oxides and Investigation of Influence Oxides on the Immune System and of Some Animal Tissues

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Abstract: Nanostructured erbium oxides (Er_2O_3) with coherent scattering length 26, 31, 62 and 65 nm were obtained using as a precursor of erbium chloride and erbium oleate. The influences of Er_2O_3 on the immune system and some animal tissues were carried out. The experiments have been made on white mouse's and outbred rats. Complex pharmaco-toxicological research presented erbium oxide nanostructure size of coherent scattering regions 26, 31, 56 and 65 nm showed that when administered orally no acute toxicity, no effect on the immune system of the body, has no effect on blood cells. But, long-term (30 day) intragastric administration shows toxicities on the internal organs of experimental animals, which lead to structural changes and functional impairment due to tissue accumulation of nanoparticles.

Key words: Nanostructured, erbium oxides, pharmaco-toxicological.

1. Introduction

At present, there is intensive research which aimed at the application of nanotechnology in various fields of medicine, that promote actively expanding range of nanoparticles as well as approaches to their use in diagnosis and therapy, in particular for the development of drug delivery systems to organs and tissues, targets [1]. Use of different nanoparticles is increasingly used in the treatment of patients with oncological diseases [2]. Along with this, high penetration of nanoparticles causes a number of concerns in relation to their possible impact on the various cells and systems of a healthy body, including the immune system. You cannot exclude the possibility of malfunction under the influence of nanoparticles that define optionally to study the impact of such a functional activity of various immune cells. This question has been the subject of research only in recent years and therefore the amounts of published data are relatively small. Particular attention was attracted to study authors, benefits of nanotechnology, refers to the ability of nanoparticles to cause some negative effects. In studies in vitro and in vivo have shown that the nanoparticles activate the production of proinflammatory cytokines, chemokine, adhesion molecule expression, stimulate cells of inflammation including basophils, neutrophils and eosinophils, macrophages, and dendritic cells, T-lymphocytes [3]. These changes may affect the balance Th1/Th2-lymphocytes, leading to the creation of conditions for the development of allergies, autoimmune and neoplastic diseases. Studying of the influence on the

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human immune system titania nanoparticles also observed increased levels of proinflammatory cytokines, increased dendritic cell maturation and activation of proliferation of CD4 + T-cells [4]. There are reports of influence of colloidal gold nanoparticles on mast cells depending on the exposure time: a longer interaction of nanoparticles to these cells leads to an increase in their primary granules and reduced serotonin secretion without affecting the living. Several authors noted the negative effect of carbon nanotubes on cells of immunity. Thus, study of the effects of nanotubes to the respiratory system of mice revealed small lesions in the lung, combined with inhibition of T-lymphocytes, without changing of amount. It is also shown that the multi-layer carbon fiber tubing cause of allergic response in mice with activation of B cells and increased production of IgE; also a dose-dependent increase in the production of pro-inflammatory cytokines.

Despite increased research of influence of various nanoparticles on the immune system of a healthy body, there are still many uncertainties. There is an unknown data about the influence of erbium oxide nanoparticles (OE) on the function of tissues and organs of the immunity.

In this regard, the purpose of this study was the impact of the doctrine of nanoparticles on functional activity of tissues and organs of the immune system intact rats at different time steps.

2. Experiments

2.1 Synthesis Nanostructured Oxides Erbium (Er_2O_3)

These particles were obtained by using as precursors of erbium chloride (method I) and erbium oleinate (method II). In method I the corresponding metal amount was dissolved in hydrochloric acid and chloride was obtained. Distilled water + NaOH + NaCl were added to obtained chloride. It was heated at pH = 3.6-3.8 and evaporated. This mixture was roasted at 440 °C, washed and filtered using Bruchner filter, the sediment was dried. The second sample was roasted at 540 °C, the third one at 640 °C. Roasting time for all samples was similar 1 h.

Synthesis of Er_2O_3 in a frame of method II consisted of several steps: (1) synthesis of sodium oleate; (2) synthesis of erbium chloride; (3) synthesis of erbium oleate; (4) Er_2O_3 nanocrystal preparation.

2.2 Analysis Methods Oxides

X-ray powder patterns were obtained using DRON ADP—1 diffract meter with Cu Kα monochromatic radiation.

IR spectrums were measured using Fourier—spectrometer Perkin Elmer Spectrum 100 with UATR device in diapason 4,000-675 cm^{-1}.

2.3 Object and Methods of Experiments Toxic Investigation

The influence of nanoparticles MA (erbium oxide Er_2O_3 size of coherent scattering 29, 56 and 65 nm) were studied at different times when exposed to cells and immunity (after the introduction of nanoparticles in the body) [5, 6].

Work carried out on material obtained from intact white rats. The general effect of the acute toxicity of nano-powder and erbium oxide (Er_2O_3) began with a study in white mice. The acute toxicity LD50 (lethal dose 50% of the animals) and LD100 (lethal dose 100% of the animals) was calculated according to Kerber. The study was conducted with an intragastric administration of 5% and 10% of starch paste in doses of 2.5 mg of the suspension per 20 g body weight of animal [7]. Picture of peripheral blood was determined by gemoanalizatore (NemaLite 1270). The immune status was determined by ELISA (HEMA-MED Ltd., Moscow, Russian) when the totalIgM, as well as cell-mediated immunity was determined Method E-rosette (E-ROCK) (NPL “Granum” Kharkiv. Ukraine). A histological examination of the liver and kidneys was done.

3. Results and Discussion

3.1 X-ray Powder Patterns

All obtained samples were crystallized in VCC (volume-centered cubic) lattice (Fig. 1). Crystal sizes were defined according to Sherrer's formula:

$$d = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

where, d—average size of crystallites, λ—wave length of applied radiation $\lambda(\mathrm{Cu}K_\alpha)$ = 1,54051 Å, (β—peak width on the half of height, θ—diffraction angle, k = 0.9. Sample obtained using organic had the least value of d (26 nm). Samples with d = 31, 56 and 65 nm were obtained using chloride. The value was increased when increasing roasting temperature [8].

3.2 IR Spectrum

IR spectrum of the studied samples can be divided into two types. For spectra of the first type, the most intensive adsorption band is AB at 1,428 cm^{-1} (Fig. 2a). The second intensive sample is AB at 879 cm^{-1}. In the spectrum of the second type, we can observe two wide, quite intensive and partially overlapped ABs at approximately 1,505 cm^{-1} and 1,422 cm^{-1} (Fig. 2b). First type spectra are particular for Er_2O_3 samples but not completely washed from sodium chloride. Second type spectra demonstrate a good washing from NaCl. We can note here that AB peculiar for spectra of both types are sometimes present in IR spectrum.

3.3 Investigation of Toxicological Properties of Nanostructured Oxide of Erbium (Er_2O_3)

During the experiment, the toxic dose has not been established, as the volume of doses higher than critical. However, the further observations of the animals were observed lethal outcomes in animals of the two cases only group at day 7 at a dose of 2.5 mg of a 5% suspension of nanoparticles size 31 NM.

Late mortality of the animals of groups can be explained by small particle size and their ability to penetrate the blood brain barrier and act in the later stages.

It is known that the indicator of the antitoxic and excretory functions of the liver is the elimination rate bromsulfalein. Bromsulfalein sample is positive for any parenchymal organ (acute and chronic hepatitis), even in the earliest stages of intoxication and liver disease.

The experiments showed that the studied compound in intragastric administration for 10 days at doses of 1

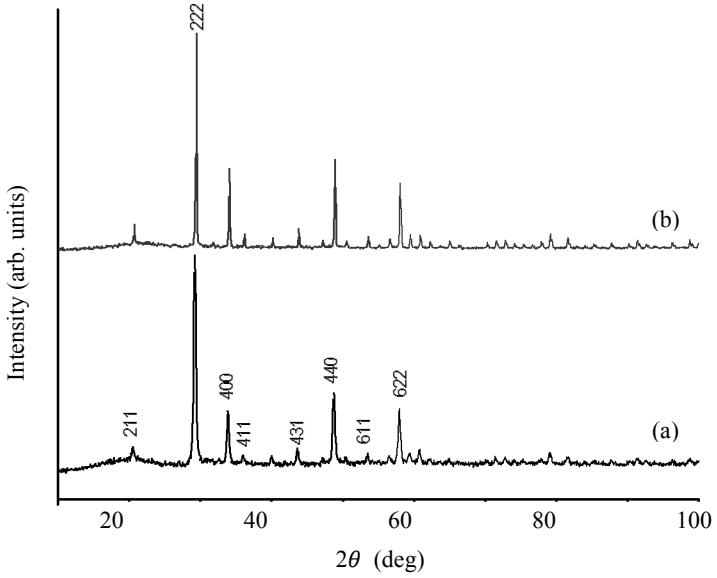


Fig. 1 Powder diffraction patterns of the samples of erbium oxides with CSR (a) 26 and (b) 56 nm.

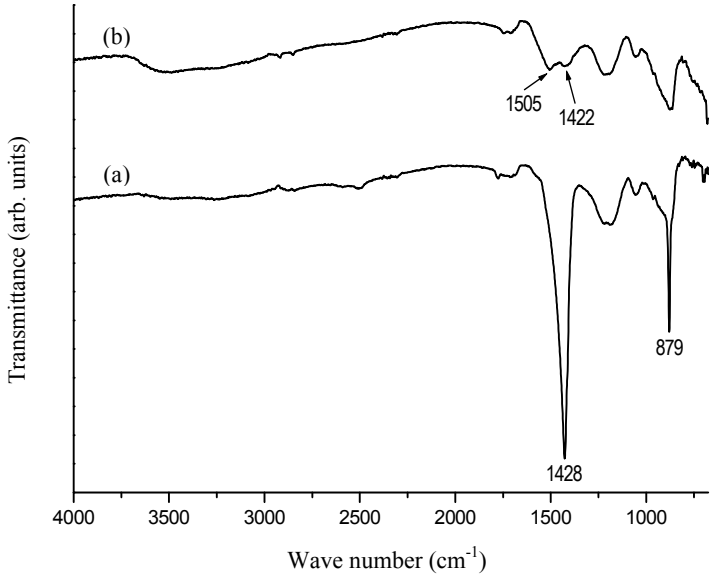


Fig. 2 Peculiar IR spectra of the studied erbium oxides samples.

Table 1 The morphological structure of the peripheral blood of white rats.

Indicators	Unit of measure	Before	After
Erythrocytes	10^{12} in 1 mL	7.94 ± 0.17	8.12 ± 0.11
Hemoglobin	mmol/L	7.76 ± 0.35	7.52 ± 0.062
Platelets	10^{9} in 1 mL	675 ± 44	925 ± 27
Leukocytes	10^{9} in 1 mL	12.06 ± 0.33	14.3 ± 0.77
Lymphocytes	%	67.5 ± 2.0	66.0 ± 1.7
Monocytes	%	2.56 ± 0.4	0.82 ± 0.12

-2-3 mg/kg body weight of the animals had no effect on the functional capacity of hepatocytes; liver cells' ability to excrete the bile dye is not compromised.

Further study of the blood picture was conducted on the following parameters: the number of red blood cells, white blood cells, platelets in 1 mL of blood (1 mL 10^{12} - 10^{9} in 1 mL). During the work, it was found that the test substance in the doses tested did not have a noticeable effect on the overall picture of red and white blood cells.

It is known that high concentrations of serum IgM are the major feature of the primary immune response. Therefore, an experiment to determine the impact on the immune system of animals nanostructured oxide erbium determined total IgM (immunoglobulins), because the antibodies are synthesized first in response to antigenic stimulation.

Surveys have shown that the application of erbium oxide nanoparticles increases the amount of IgM on day 7, an increase of 4.2 g/L at a rate of 3.5 g/L at day 14 relative to 7-days there was a slight decrease of 4.3 g/L that is connected with the adaptation of the organism. At 21 and 28, this figure gradually decreases and approaches the normal controls IgM (3.8 g/L) (Table 2).

To obtain a complete picture of the impact of nanoparticles, we performed determination of cellular immunity (T and B lymphocytes).

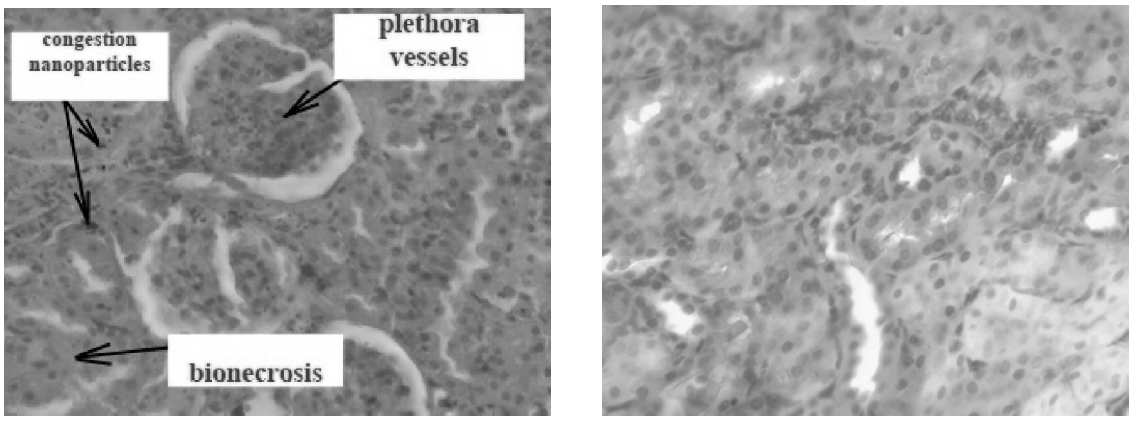
These data showed that after administration of the nanoparticles of the experiment on day 1 significantly increased the number of B-lymphocytes by 48% at a rate of 31%, and T-lymphocytes activated later by one day, they were 69% at a rate of 66%. On day 7, these figures were leveled and B lymphocytes 61%, T lymphocytes were 78%. On day 21 the results of the study showed that the number of both B- and

Table 2 Indicators of IgM (g/L) in rats at various time exposure (Er_2O_3).

Sample number	Control	7th day	14th day	21st day	28th day
1	3.5	4.4	4.3	4.1	3.8
2	3.5	4.4	4.4	4.2	3.9
3	3.5	4.5	4.3	4.2	3.8

Table 3 Indicators of B- and T- lymphocytes% in rats at various times the impact of nanoparticles.

Sample number	Control (lymphocyte count in%)		B-lymphocytes	T-lymphocytes
	B-lymphocytes	T-lymphocytes		
1st day	31	66	48	69
7th day	31	66	61	78
21th day	31	66	45	68
28th day	31	66	34	67



(a) (b)

Fig. 3 Pattern morphological change of renal tissue under influence of erbium oxide nanoparticles (Er_2O_3) at a concentration of 2.5 mg/kg (a) in comparison with (b) the control group.

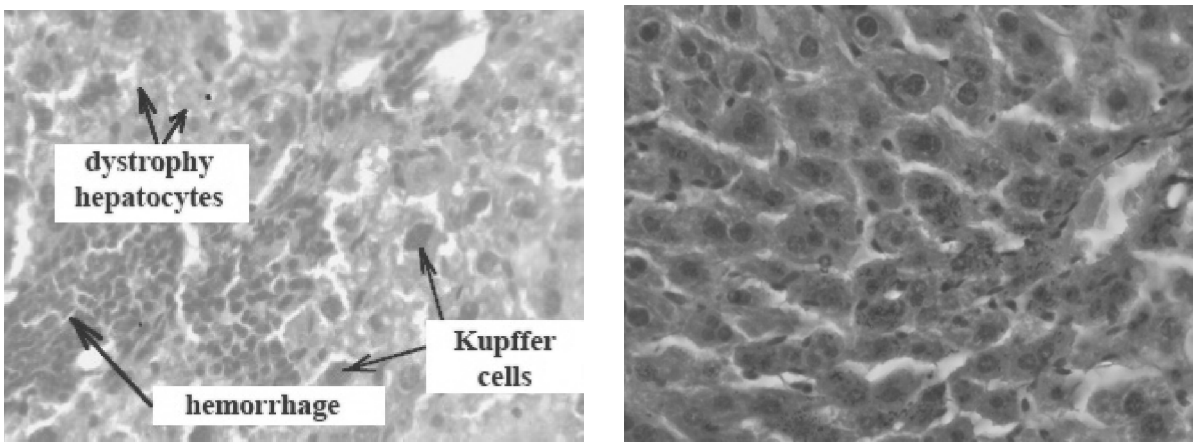
T-lymphocytes decreased significantly, and got close to the norm, making the B-lymphocytes 45%, T-lymphocytes 68%. On day 28 indicators did not differ from normal B-lymphocytes 34%, T-lymphocytes 67%.

Painting change lymphocyte given in Table 3 and Fig. 3.

To obtain a complete picture of the toxic effects of erbium oxide nanostructured histological studies were conducted, in order to identify morphological changes in the structure of animal tissues. The objects were selected histology thyroid gland, liver, spleen and kidneys as these organs perform a barrier function.

The nature of the changes in the kidney under the influence of the studied nanoparticles was presented epithelial dystrophy and severe plethora, and the intensity of changes detected increased in direct proportion to the concentrations of the nanoparticles, which is probably due to excessive accumulation of the particles in the studied organ. Pronounced damaging effect occurred for the introduction of nanoparticles in the kidneys of experimental animals, caused the death of proximal tubule cells, glomerulonephrosis, massive bionecrosis.

The conclusion that the damaging effect of nanoparticles studied in respect of the liver, also showed that in the liver of these animals showed hepatic loss of relationship (discomplexatio) beams, separate foci of hemorrhage and lysis of the nuclei of hepatocytes. The slices were detected many Kupffer cells belonging to the reticulo-endothelial system, suggesting activation of the immune response to effect nanoparticles. A significant number of rats after administration of various amounts of nanoparticles is



(a) (b)

Fig. 4 The abnormality of liver under the influence of nanoparticles Er_2O_3 concentration 2.5 mg/kg (a) compared to the control (b).

determined by the solution of individual necrosis of hepatocytes, cells are found in the nucleus pyknosis, the phenomena of karyopyknosis, karyorhexis and the plethora of blood vessels.

4. Conclusions

Complex pharmaco-toxicological research presented erbium oxide nanostructure size of coherent scattering regions 29, 31, 56 and 65 nm showed that when administered orally no acute toxicity, no effect on the immune system of the body, has no effect on blood cells. But long-term (30 days) intragastric administration shows toxicities on the internal organs of experimental animals.

Acknowledgments

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References

[1] Dobrovolskaia, M. A.; McNeil, S. E. Immunological Properties of Engineered Nanomaterials. *Nat Nanotechnol* **2007,** *2(8),* 469-78.

[2] Zolnik, B. S.; Gonzalez-Fernandez, A.; Sadrieh, N.; Dobrovolskaia, M. A. Nanoparticles and the Immune System. *Endocrinology* **2010,** *151(2),* 458-65.

[3] Chang, C. The Immune Effects of Naturally Occurring and Synthetic Nanoparticles. *J. Autoimmun* **2010,** *34(3),* J234-46.

[4] Finkelman, F. D.; Yang, M.; Orekhova, T. Diesel Exhaust Particles Suppress in Vivo IFN-gamma Production by Inhibiting Cytokine Effects on NK and NKT Cells. *J. Immunol* **2004,** *172(6),* 3808-13.

[5] Marquis, B. J.; Love, S. A.; Braun, K. L.; Haynes, C. L. Analytical Methods to Assess Nanoparticle Toxicity. *Analyst* **2009,** *134(3),* 425-39.

[6] Mitchell, L. A.; Lauer, F. T.; Burchiel, S. W.; McDonald, J. D. Mechanisms for How Inhaled Multiwalled Carbon Nanotubes Suppress Systemic Immune Function in Mice. *Nat Nanotechnol* **2009,** *4,* 451-6.

[7] Park, E. J.; Kim, H.; Kim, Y. Inflammatory Responces May be Induced by a Single Intratracheal Instillation of Iron Nanparti-cles in Mice. *Toxicology* **2010,** *275(1-3),* 65-71.

[8] Abdusalyamova, M. N.; Kabgov, K.; Sharopov, F.; Shulga, Y. M. Composition and Magnetic Properties Nanostructured Erbium Oxides. *ISJAEE* **2012,** *8(112),* 66-71.