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Use of the Innovative Morphological Research Methods for the Study of The Nanoparticles Impact.

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ABSTRACT

The common clinical usage of the nanostructured materials requires the substantive examination of the side effects and potential risks. The animals were introduced the iron nanoparticles applied both to the skin and nasally. The examination of the hair and skin as well as the brain, trachea, throat, lungs, heart, liver, kidneys with the use of the transmission and scanning electronic microscopy including the micro- and macro-element analysis and probe microscopy has been performed. By the nasal insertion of the iron nanoparticles the obstruction of the bronchial tree as well as of some vessels of liver and kidneys was observed which caused the massive death of animals.
Keywords: nanoparticles, nanomaterials.

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INTRODUCTION

The modern capabilities of medicine are based both an application of the new research and treatment methods and on searching for the innovative materials that are able to re-create the damaged organs and tissues. They include nanoparticles and the items consisting of them. The active implementation of nanomaterials in the clinical medicine requires the deep knowledge of the potential risks and side effects associated with the use of such materials [1, 2].

The nanoparticles may enter to a human body via inhalation, orally, percutaneously and parenterally (in case of introduction of the pharmaceutical and diagnostic agents conjugated with the nanoparticles). The contact of a human with nanostructured materials may take place at the stage of development, production, use and utilization [3, 4, 5]. The use of the nanoparticles as well as nanostructured powders, pastes finds the ever-growing application in medicine, chemistry, biology, in particular, as the sources of microelements. Thus, the nanoparticles of superparamagnetic iron-based oxide ensure the targeted delivery of drugs by the malignant hepatic tumors, presence of hemangiomas, cysts, nodular hyperplasias which may finds further application in the treatment of the specified nosological forms [6, 7]. However, there are works within the frameworks of which the use of nanoparticles provides not only apparent benefits but also potential adverse effect on the human health and natural ecosystems [8, 8, 10].

PROCEDURE

The experiment was performed on the 58 male Wistar rats weighing 200-250 grams. All the procedures of the animal management, carrying out manipulations and testing of data acquired have been performed in accordance with the standards ISO 10993-1-2003. During the experiment we used the nanoparticles of the ferrous oxide suspended in deionized water received from the "Fraunhofer Institute for Manufacturing Technology and Advanced Materials" (Bremen, Germany).

For the purpose of the further experiment the animals were divided into 3 groups:

- The animals to the skin of which the suspended nanoparticles of the ferrous oxide were applied once (24 species).
- The rats to whom the nanoparticles of the ferrous oxide were introduced intranasally in the form of suspension in the dose of 0,10±0,03 (24) after 1 and 4 weeks.
- 10 animals constituted the control group.

The content of carbon, oxygen, phosphor, calcium, nitrogen, sodium, magnesium, iron, aluminium and sulfur has been determined. The examination of the hair and skin as well as the brain (cortex), trachea, throat, lungs, heart, liver, kidneys has been performed. The sections stained with hematoxyline and eosine have been examined with the use of the optical microscope “Nikon Ti”. For the scanning electronic microscopy the samples were fixed in a standard glutaric fixative and then the photographs were taken and the morphometric evaluation was performed with the use of the microscope “FE1 Quanta 200 3D”. The atomic force microscope investigation was performed with the use of the scanning probe microscope “Ntegra-Aura”. For the transmission electronic microscopy the samples were charged into the epon-araldite solution. Then the ultra-thin sections for viewing and photographing in the electronic microscope Jeol “Jem 2100” were prepared.

MAIN PART

By examination of the animal hair coat one month after spraying of nanoparticles one could see that their structure was similar to the control group (Fig.1).

Fig. 1.

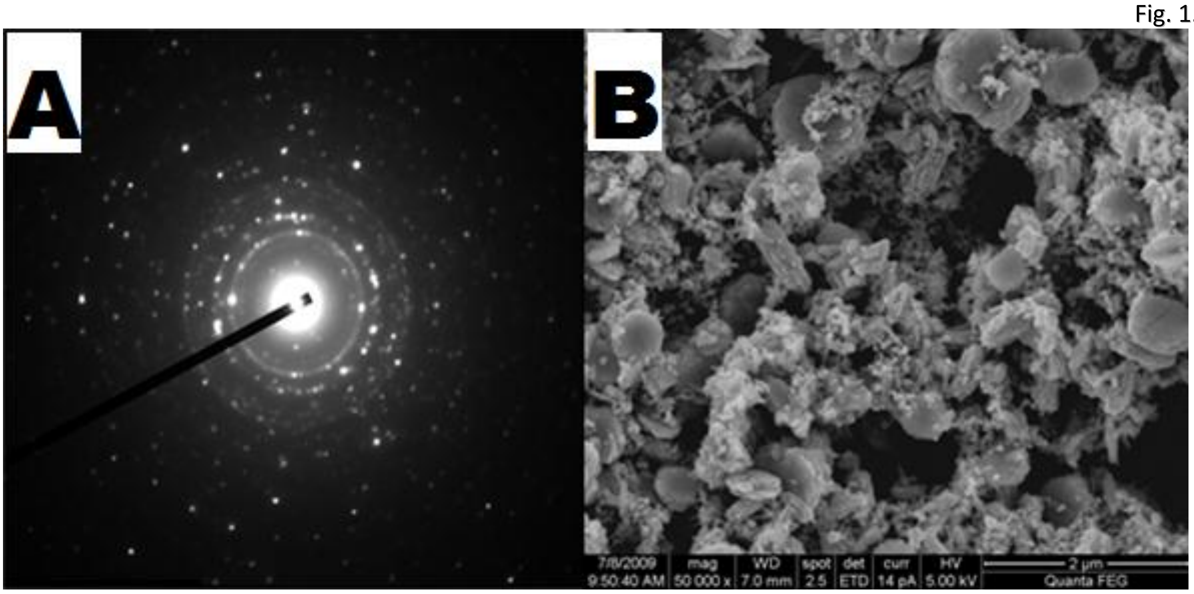

Figure1: Suspended iron nanoparticles.

Fig. A: TEM. Fe oxides - the particles have the shape of a needle and standard diameter of about 20 nm and the length/diameter ratio 1:05 - 1:10. The size of these particles is within the range of 5-100 nm.

Fig. B: (x50000), SEM.

With the use of the scanning electron microscopy it has been shown that the iron content in the 1st group at separate areas made from 0,10±0,01 to 8,48±0,46% [11, 12]. By the study of the hair coat of the laboratory animals kept in the standard cages three meters away from the laboratory animals that have not been sprayed with the ferrous oxides nanoparticles it was shown that the morphological pattern also did not differ from the control group. However, the iron content in particular hair coat fragments ranged from 0,08±0,01 to 2,36±0,30%. By the hair coat analysis performed before the experiment no ferrous oxide was detected.

During atomic force microscope investigation of the skin fragments foreign formations were observed in particular areas of the hair follicle bed (Fig. 2).

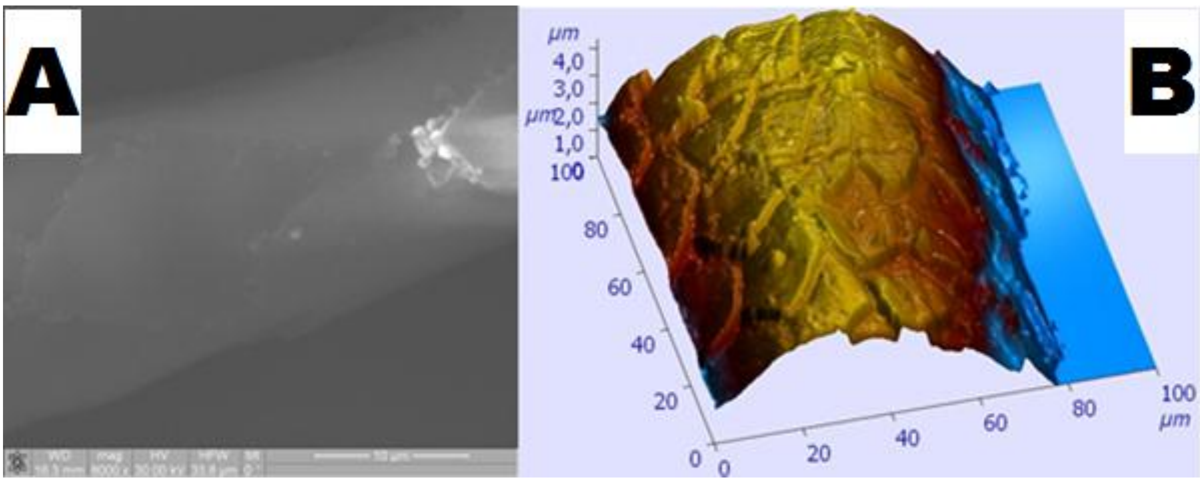

Figure 2: Hair coat of the animals one month after spraying of the iron nanoparticles.

Clusters of foreign formations seen on the surface.
Fig. A (x8000). SEM.
Fig. B. Atomic force microscope investigation. Three-dimensional histogram.

The second stage of the experiment was the single administration of the ferrous oxide nanoparticles solved in the distilled water through the nasal cavity of the laboratory animals. Within seven days eight animal subjects died. Another six – within the next three days. In the multi-row ciliated epithelium of the throat of the larynx of the surviving one week after the start of the experiment the destructive processes up to necrosis were observed. In the laryngeal lumen the aggregates of the mucus masses that partially or completely block its lumen (Fig. 3). With the use of the scanning microscopy we have shown that masses in the cavity appear tightly to the walls and at the depth that is exceeding the bronchiole diameter by several times. The micro- and macro-element analysis showed that the content of iron in them made 0,28±0,05%. The proper mucous plate represented by the loose areolar connecting tissue contained numerous yellow fibers without definite orientation. In the deep mucous layers the yellow fibers gradually graded into perichondrium and in the laryngeal midportion they penetrate through the cross-striped muscles of the vocal cords. The fibrocartilagenous laryngeal coat without pathological findings. Four weeks after obstruction of the laryngeal lumen was not observed. Destructive processes were pronounced to a lesser degree. At the animals death in the laryngeal lumen the aggregates of the mucus masses that completely blocked its lumen were found. The necrotic processes also prevailed. No differences were observed in the structure of the tracheal mucosa in different groups.

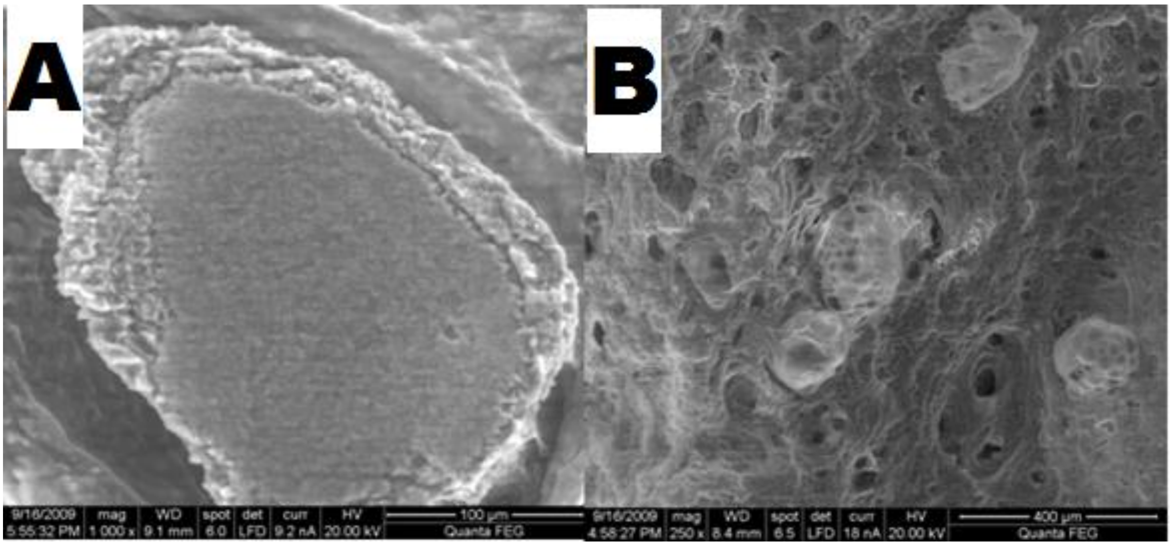

Figure 3: Organ fragments one week after the nasal administration of the iron nanoparticles.

A. Lumen of some central veins blocked. SEM (x1000).
B. Lumen of bronchioles and some alveoli blocked. SEM (x250).

Seven days after that the moderate congestion was observed in the lungs. Besides, in the interalveolar septums as well as around the bronchi and vessels the diapedetic hemorrhages were detected. The lumen of both the large bronchi an bronchioles was slightly narrowed. A small quantity of bronchioles had an obstructed lumen. The alveolar cavities were slightly enlarged. After a month the organs congestion changed to ischemia. The fragmentary atrophy of alveolocytes and emphysema were diagnosed. In the interalveolar septums some sclerosis areas were observed.

At the animals death the lungs were full-blooded. Besides, the stasis and thrombosis in the microvasculature vessels were diagnosed. The area of the blocked bronchioles was increased by a few times. In these areas the accumulation of the ferrous oxide nanoparticles was observed.

SUMMARY

The use of the nanoparticles, in particular iron ones, may induce certain risks.

CONCLUSIONS

As for the laboratory animals by the skin irrigation with the ferrous oxide nanoparticles in the form of a suspension the wide range of spreading thereof is to be observed which may constitute a hazard in the eco-systems in case of accumulation thereof. By the intranasal administration the high mortality rate was to be observed during the first ten days. It was caused by the obstruction of the respiratory bronchioles and central veins of liver with the aggregates of nanoparticles and mucus. This in its turn resulted in the development of the impairment of circulation and alterative processes in the parenchymal organs and as a result – the multi-organ dysfunction syndrome. The surviving animals also demonstrated the circulation failures as well as dystrophy and necrosis though pronounced to a lesser extent which changed by the end of the month to the pathological regeneration and destruction, however, without animal dying.

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