Influence of intramuscular introduction of nanosized copper particles on element status of rats

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Abstract: The paper presents the results of studies on influence of copper nanoparticles on mineral content of body tissues of rats with parenteral way of their introduction in the body. It was established that during manifold introduction of copper nanoparticles in biotic dosages no critical change in common pool concentration of microelemenents, toxic and essential elements as well as in content of administered copper in tissues of animals occur. This fact attests to the positive stability of common pool of macro- and microelements and to the absence of irregularities in the system of metal homeostatic regulation in organism during nanoparticles introduction in organism.

[Sizova Elena, Miroshnikov Sergey. **Influence of intramuscular introduction of nanosized copper particles on element status of rats.** *Life Sci J* 2014;11(9):606-609]. (ISSN:1097-8135). http://www.lifesciencesite.com. 97

Key words: element status, nanoparticles, copper, chemical elements.

Introduction.

Among the developed methods of targeted drug delivery use of essential metals in the form of nanoparticles as a source of elements in the body and as a possible drug carrier is a promising one. However, in modern literature there is no shared vision on the safety of introduction of metal nanoparticles in the body: some data indicate low toxicity [1, 2, 3], others demonstrate the negative consequences of their use [4, 5, 6]. The study of common patterns of manifestation of biological and toxic effects of nanoparticles depending on their shape, size, source material, surface area, charge, and other physical and chemical characteristics of the structure, as well as dose, route of administration, concentration in target organs and duration of exposure are defined as the most important issues of nanoparticles toxicology that require detailed examination [7, 8, 9].

Among the variety of nanosized forms, copper nanoparticles obtained under controlled synthesis with subsequent modification by oxygen and materials certification by electron scanning and transmission microscopy, X-ray analysis [10, 11, 12] deserve attention. The need to study the impacts of nanoparticles and nanomaterials on living systems and man is connected with the ever-increasing level of nanomaterials production for various needs [13, 14], which becomes a source of environmental pollution and factor of increasing load on living systems. In this regard, the definition of element status, identification of violations in the regulation system of elements homeostasis in various organs and tissues in terms of increasing loading of metal nanoparticles on body is an important issue. Such study allows us to identify toxical thresholds of metal nanoparticles in order to solve the issue of safety of nanoparticles introduction in organism.

Considerable success was achieved during the study of mineral metabolism [15, 16]. Meanwhile, the literature contains no information on the nature and influence patterns of increasing load of metal nanoparticles on total mineral status of organism.

Objective: To study the element status of tissues in experimental animals after repeated intramuscular injection of copper nanoparticles and deciding on the possibility of using metal nanoparticles as a substance for biologic drug.

Materials and Methods.

The research was conducted in experimental biological clinic (vivarium) of Orenburg State University using 60 white male Wistar rats 150-180 g, their thigh muscles were injected once a week within a period of 12 weeks with suspension of copper nanoparticles obtained by dispergating of precisely weighed amount of nanopowder dissolved in water, in dose of 2.0 mg/kg. Copper nanoparticles, obtained by the method of high temperature condensation, are spherical particles with size of 103.0 ± 2.0 nm and oxide film thickness is 6 nm, were obtained at the Institute of Energy Problems of Chemical Physics RAS (Moscow). Phase composition was determined by X-ray phase analysis: 96% crystalline copper, copper oxide 4%. Control animals were injected with water for injections. The slaughter of animals was carried out by decapitation under nembutal anesthesia as follows: I and II groups - after 1 and 7 days after the first injectionrespectively, III group - 7 days after the second injection, IV group – 7 days after the third injection, V group - 7 days after the 12^{th} injection.

The experimental research on animals was conducted according to instructions for works with

experimental animals (Order #755 as of 12.08.1977, USSR Ministry of Health).

Mineral content of tissue samples of experimental animals of control and tested groups was analyzed in test laboratory of ANO "Centre for Biotic Medicine", Moscow (accreditation certificate GSEN.RU.TSOA.311, registration number in State Register ROSS RU. 0001.513118 as of May 29, 2003).

Statistical processing of received data was performed using software package Excel, Statistica 5.

Results and Discussion.

It is well-known that microelements play a significant role in life of organisms, providing normal flow of biochemical and physiological functions [17]. The study of mineral concentration is one of the most informative methods for the study of mineral metabolism, being the most sensitive indicator of state of living systems it allows to estimate the element status of organism. Study of changes in the index of total mineral content in experimental animals tissues, conducted within 84 days (1-5 points) attests to the presence of natural fluctuations in the level of elements in tissues of rats in the range of 1.51 to 1.88 mmol/g of tissue. Introduction of exogenous nanoscale copper to organism of animal leads to a change in total mineral content of rat tissues ranging from 1.4 mmol/g of tissue in the 4th point corresponding to the 21st day of experiment and maximum up to 1.88 mmol/g in the 2nd point, that corresponds to the 7th day of experiment (Figure 1). Significant reduction and increase in index of total mineral content of rat tissues occur on the first day after 1st injection - by 7.6 % ($p < 0.05$) and 7.5 % ($p < 0.001$) (group I) and on the 7th day after the 2nd injection (group III) during the introduction of copper nanoparticles in comparison with the control group. On week 12 of the experiment values of total mineralization in experimental and control groups are the same with a slight increase of index to 1.18 % (0.02 mmol/g) in the experimental group compared with the control.

Therefore, the nanosized copper injections in dose of 2 mg/kg of animal weight do not disturb the natural fluctuations in index of total mineral content of rat tissues, and the amplitude of these fluctuations in experimental and control groups is within the range from 1.18% to 7.6%.

According to the modern ideas, all elements in the organism are divided into groups according to the nature of their influence on the organism and role in ensuring normal metabolism [16]. Figure 2 shows data on the change in the content of essential elements (As, B, Co, Cr, Cu, Fe, I, Li, Mn, Ni, Se, Si, V, Zn) in tissues of control and experimental animals. It is obvious that the character of changes of this indicator for control and experimental groups is the same; the amplitude of fluctuations is comparable and is 2.5-3.0 μm/g of tissue. However, throughout the experiment the total number of essential elements in tissues of experimental animals exceeds the level of control and best expressed in the fourth test group - 7 days after the III injection and significantly (at $p \leq 0,05$) exceeds the value of control by 8.3%. The nature of such changes in concentration of essential elements in experimental groups is associated with specific changes in the content of some elements. Thus, in the first test group fluctuation of essential elements level by 1.82% occurs due to increasing concentration of Cu by 29.26%, Mn – 8.26%, Se – 33.96% and decrease of Li by 18.2%, As – 17.5%, Co – 13.23%, B – 8.2%, Zn – 2.7%, Fe - 2%.

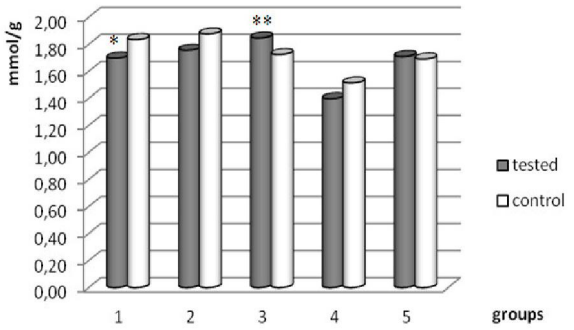

Figure 1. Changing concentration of total mineral content of rat tissues of control (◦) and tested (•) groups in different time of copper nanoparticles introduction, mmol/g

Data are presented as: mean (X) ± standard error of mean (SE) (n=5).

* – comparison according to Student t-test with control group, * – $p < 0.05$; ** - $p<0.001$

Simultaneous reduction in total concentration of essential elements in experimental and control groups is 5.26 % seven days after the first injection (group II), this occurs due to reduction of Cu content by 4.5% (at $p \leq 0,05$), values of concentrations of Cr, Fe, Mn, Ni still exceed control indices. Seven days after the second injection the total number of essential elements significantly ($p \leq 0.05$) increases by 2.7 % in the third experimental group as compared with the control. In this case simultaneous increase of the following elements occurs: B, Cu, Mn, Si, Zn and copper level reduces by 8.2 % in comparison with the initial period of the experiment. In the fourth experimental group (seven days after the third injection) difference of index in comparison with the control group is 8.3 %. At the same time concentration of Cu, Fe, Mn, Si, Zn increases. At the final stage of experiment (seven days after 12th injection) indices of experimental group are close to indices of the control group.

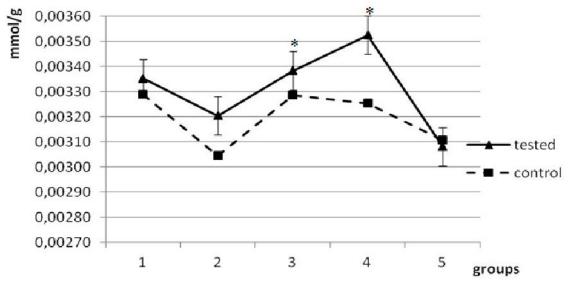

Figure 2. Changing concentration of essential microelement amount rat tissues of control and tested groups in different time of copper nanoparticles introduction, mmol/g

Data are presented as: mean (X) ± standard error of mean (SE) (n=5).

* – comparison according to Student t-test with control group, * – $p < 0.05$.

Analyzing correlation of macroelements Ca, K, Mg, Na, P it has been revealed that the total pool of macroelements is more stable in comparison with the total pool of essential elements during introduction of copper nanoparticles, and range is 5.2 - 6.6 % (Figure 3). Higher indices of total number of macroelements in control groups have been registered as compared to the tested group at all stages of the experiment, except for the fourth group, where a significant decrease by 17.1 % ($p \leq 0,05$) of the total number of macroelements in experimental and control groups in comparison with the initial stage of experiment (the first group) is observed. Comparing the changes in the first and the fourth experimental groups the established decrease in concentration of macroelements occurs due to reduction in the level of elements: K, Ca, P, Mg, Na, and after comparison with the first and fourth control groups - by decrease in concentration of Na, Ca, P, Mg.

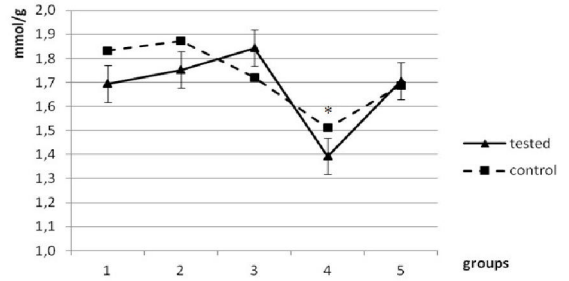

Figure 3. Changing concentration of macroelements amount in tissues of rats in control and tested groups in different time of copper nanoparticles introduction, mmol/g

Data are presented as: mean (X) ± standard error of mean (SE) (n=5).

* – comparison according to Student t-test with control group, * – $p < 0.05$.

It is worth noting that in the fourth experimental group content of K decreases by 56.6%, Ca - by 20.6%, P - by 12.69% Mg, Na – by 4.3% in comparison with the first test group. In the fourth control group a significant decrease of Na by 22.8%, Ca – 22.6%, P - by 16.55%, Mg - 6.65 % ($p \leq 0.05$) in comparison with the first control group is observed. The obtained data indicate that the reduction in the total pool of macroelements in control groups is expressed with greater intensity than in the test group. Apparently, the introduction of exogenous nanosized copper leads to inhibition of natural elimination of some macroelements.

Studying the ratio of the total pool of toxic elements (Al, Cd, Hg, Pb, Sn, Sr) it should be noted that major changes occur in the beginning of the experiment and at the final stage of the experiment (Figure 4).

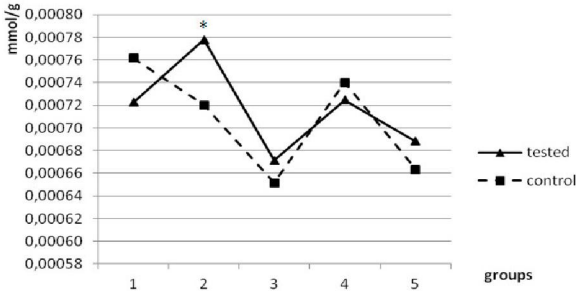

Figure 4. Changing concentration in amount of toxic elements in tissues of rats in control and tested groups in different time of copper nanoparticles introduction, mmol/g

Data are presented as: mean (X) ± standard error of mean (SE) (n=5).

* – comparison according to Student t-test with control group, * – $p < 0.05$.

Thus, one day after the first injection twofold decrease of total amount of toxic components was registered in tissues of experimental animals in comparison with the control group. Decrease in levels of elements such as Cd, Sr, Al had the best expression. Seven days after the first injection (second experimental group) indices of the total concentration of toxic elements in experimental group are significantly beyond the same of the control group by 17.6 % ($p \leq 0,05$). Subsequent injections do not affect the total amount of toxic elements, and indicators of the experimental and control groups are similar. However, 12-fold introduction of copper (the fifth point) leads to an increase in the total amount of toxic components in the experimental group in comparison with the control. Increase is due to the accumulation of such elements as: Al, Sn, Sr, Pb.

Thus, the conducted research allow us to conclude that during repeated introduction of copper

nanoparticles in biotic doses in organism no critical changes in concentrations of common pool of elements, toxic and essential elements occur and the content of introduced copper in animal tissues do not change.

This fact attests to the absence of disorders in homeostatic regulation of metal level in organism during introduction of nanoparticles. However, histological researches and the study of apoptosis in cells confirmed the presence of significant structural changes in tissues and a gradual increase of risk of apoptosis in cells by the 12th week of introduction of metal [8]. This indicates that in formation of biological response to a manifold introduction of nanoparticles a significant contribution is made by separate elements or group of elements, while the common pool of macro and microelements is sufficiently stable.

Research was done with financial support of the Russian Science Foundation №14-16-00060

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8/8/2014