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Experimental Research of Zinc Oxide-labeled Nanoparticles Biokinetics in Rats' Organism after Single Oral Administration by Labeled Atoms Technology

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Экспериментальное исследование биокинетики наночастиц оксида цинка в организме крыс после однократного перорального введения с использованием технологии меченых атомов

ABSTRACT

Purpose: To make quantitative assessment of zinc oxide-labeled nanoparticles' biokinetics in laboratory animals' (rats') organism after single oral administration.

Material and methods: Suspension of zinc oxide nanoparticles with 30 nm median diameter was used as source material. Before the injection nanoparticles were irradiated to thermal neutron beam, so that part of the atoms was activated with formation of radioactive nucleus of ^{65}Zn with half-life of 234.8 days. Four groups of animals were used in the experiment (3 male Wistar rats with average body weight of ~ 170 g in each group). According to number of animals used in the experiment, the suspension of nanoparticles was divided into 12 parts of 0.5 mL each. Animals were sacrificed at hours 4, 24, 72 and 120 after administration; then organs of the sacrificed animals including brain, heart, lung, liver, spleen, pancreas, kidneys, testicle, also remained carcass, blood, excrement and urine were analyzed. Changes of organs' activity were recorded on low-background gamma-ray spectrometer.

Results: Biokinetic parameters of Vistar rat-males are evaluated after single oral administration of zinc oxide nanoparticles. Maximum content of nanoparticles was observed in 24–72 hours after oral administration. ^{65}Zn mass distribution in rats' organs at maximum point decreases according to the following sequence: liver → kidneys → spleen → pancreas → heart → blood → brain.

Conclusion: 1. Technology of producing new type of product – labeled nanoparticles by means of irradiating them with thermal neutrons of nuclear reactor is elaborated. 2. It is proved that there is a possibility to do research of nanoparticles' biokinetics in the laboratory animal organisms using labeled atoms technology. 3. ^{65}Zn activity records in the rats' brain (near 0.06 % of administered activity) proves that nanoparticles can mount blood brain barrier.

Key words: *nanoparticles, zinc oxide, radioactive label, experiment, rats, biokinetics, blood brain barrier*

РЕФЕРАТ

Цель: Количественная оценка биокинетики наночастиц окиси цинка в организме лабораторных животных (крыс) после однократного перорального введения с использованием технологии меченых атомов.

Материал и методы: Исходным материалом являлась суспензия наночастиц окиси цинка с медианным диаметром около 30 нм. Перед введением животным наночастицы облучались потоком тепловых нейтронов, в результате чего часть атомов активировалась с образованием радиоактивных ядер ^{65}Zn с периодом полураспада 243,8 дня.

Исследование проводилось на четырех группах животных (в каждой группе по 3 белые крысы-самцы линии Вистар со средней массой тела ~ 170 г). Суспензия наночастиц была разделена на 12 частей по 0,5 мл. Забой животных производился через 4, 24, 72 и 120 часов после введения суспензии наночастиц с последующим взятием на исследование органов животных, включая головной мозг, сердце, легкое, печень, селезенку, поджелудочную железу, почки и семенники, а также оставшуюся тушку, кровь, кал и мочу. Измерения активности органов и тканей осуществлялось на низкофоновом гамма-спектрометре.

Результаты: Оценены биокинетические параметры транспорта наночастиц окиси цинка для крыс-самцов линии Вистар после однократного перорального введения. Максимальное содержание по массе наночастиц в органах крыс наблюдается в период 24–72 часа после перорального введения. В точке максимума распределение содержания ^{65}Zn по массе в органах уменьшается в соответствии со следующей последовательностью: печень → почки → селезенка → поджелудочная железа → сердце → кровь → головной мозг.

Выводы: 1. Отработана технология получения нового вида продукции – меченых металлосодержащих наночастиц путем облучения тепловыми нейтронами ядерного реактора. 2. Доказана возможность проведения исследований биокинетики наночастиц в организме лабораторных животных с использованием технологии меченых атомов. 3. Регистрация активности ^{65}Zn в головном мозге лабораторных животных (крыс) в количестве около 0,06 % от введенной активности доказывает, что наночастицы (или продукты их модификации в организме) способны преодолеть гематоэнцефалический барьер.

Ключевые слова: *наночастицы, окись цинка, радиоактивная метка, эксперимент, крысы, биокинетика, гематоэнцефалический барьер*

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Introduction

Accelerated development of nanotechnology in Russia positively creates new source of potential menace and raises the issues of nanotechnology safety, human health products produced by means of nanotechnology and safety of life environment [1]. The addressing this issue presupposes founding specialized laboratories in Russia and fitting them out with modern equipment to conduct thorough research dedicated to providing safety of human health in nanotechnology.

One of the issues to be solved at the first stage of research of effects of nanoproducts' influence upon bio-objects is researching biokinetics of nanoparticles in laboratory animal organisms after different types of administration. By now certain experience in this area is accumulated. There are some reviews containing analysis of this data [2, 3]. The records of experiment results obtained after analysis of silver and gold nanoparticles' biokinetics are cited in the paper [4, 5]. But, information available in literary sources does not make it possible to reconstruct a broad picture of biokinetics of researched nanoproducts, therefore, this area should be hereafter subject to more detailed study. It also stands to mention that common methods of quantitative estimation of nanoparticles in bio-objects need rather expensive equipment, moreover they are very effortful in practical application.

Radiotracer method (or method of radioactive indicators) makes possible to overcome the difficulties inherent in other methods [1]. Radionuclide tracer virtually does not influence chemical and surface properties of particles and does not change its biological behavior (in particular, ability to penetrate into cells of different organs). Not very complicated equipment is needed to reveal radionuclide tracer which allows to fix the tracer's isotope activity and, thus, integral quantity of nanoparticles (by weight) in biological samples (internals, blood and excrements). High sensitivity, accuracy and selectivity of modern gamma spectrometric equipment makes possible to use in experiments small amount of radioactive materials which is below the minimum significant activity (MSA) magnitude, in accordance with radiation safety standards (Radiation Safety Standards 99/2009).

Experiments on laboratory animals with nanoparticles labeled with radioactive isotopes were performed by a number of researchers and contained particular questions concerning biokinetics, e.g. $^{59}Fe_2O_3$ [6].

Choosing zinc oxide as subject of research is caused by the fact that zinc is one of the most important essential trace elements that are necessary for organism activity and participate both in process of metabolism and in the form of saved resources. Radiotracer method when *in vivo* research of biokinetics of essential elements is held has substantial advantage as it allows to distinguish the administrated element from the one that has been in the organism before administration. Zinc in the form of its oxide is chosen as nanoparticles of zinc oxide are rather stable and do not have strongly marked tendency to agglomerate and form clusters as opposed to zinc its pure form.

The aim of present experiment is to study biokinetics of zinc oxide nanoparticles in organism of laboratory rats after single intragastric administration.

Part of experimental data is published in paper [7], where the question of zinc availability in form of zinc oxide nanoparticles concerning estimating necessary essential trace element delivery possibility is considered. Present work foremost considers methodological component of experiment, some questions of biokinetics particularly overcoming of brain-blood barrier and the problem of translating the results from animals to humans.

Material and methods

Dry ultrafine powder of zinc oxide obtained from «Sigma-Aldrich» had been taken as initial material and its water suspension of nanoparticles was prepared. Figure (Fig.1) demonstrates distribution of typical geometric dimensions of initial dry powder's nanoparticles which was measured by X-ray diffraction (small-angle X-ray scattering). This measuring method provides dimensions of nanoparticles' crystalline fraction, but at the same time it does not allow to estimate the size of potential clusters integrating several crystals. Measured median particle size in initial dry ultrafine powder of zinc oxide was about 30 nm [7], it coincides with specification of manufacturer. It is shown that crystals have hexagonal structure with following parameters: a = 3.253Å and c = 5.209Å.

When preparing water suspention of these nanoparticles measures against their aggreration and clusters' composing were taken. For this purpose according to standard practice surface-active substance – biologically subactive bovine albumin with concentration about 0.1 % – was added to utilized deionized water. Distribution of

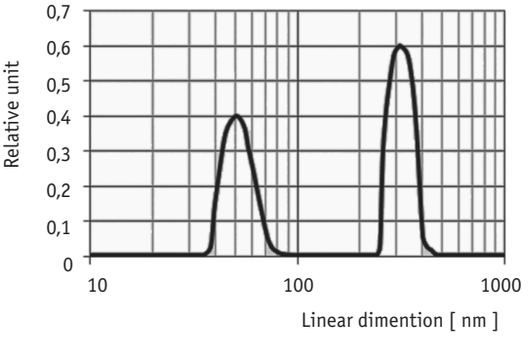

Fig. 1. Median size distribution in zinc oxide nanoparticles water suspension measured by photon correlation spectroscopy

dimesions of revealed nanoparticles clusters has bimodal form with median diameter about 50–300 nm relatevely. As regards fraction with diameter about 50 nm, it corresponds to initial crystal nanoparticles. The difference between mesuarments by diffraction and photon correlation spectrometry is that photon correlation spectrometry provides the size of nanoparticles in their hydrodynamic diameter (round sphere) approximation, its deflection causes an increase of magnitude derived from measurements. It is worthwhile to say that used photon correlation spectrometry equipment did not allow to estimate quantatively the part of fraction with different size, thus, Fig.1 can only demonstrate that the suspention contains clusters with size about 300 nm. In order to diminish fraction of these clusters, the suspention was exposed to the ultasound with frequency 20 kHz by emitter with power 6 W.

The most common method of radiolabel formation in zinc oxide nanoparticles is neutron activation of isotope ^{64}Zn that made up 46 % of total natural mix of zinc stable isotopes. Due to thermal neutrons flow exposure, some part of ^{64}Zn atoms took part in nuclear reaction where radioactive isotope ^{65}Zn with half-life period equal to 243.8 days is released: $^{64}Zn + n_{th} \rightarrow {}^{65}Zn$. This isotope has two energy lines of gamma radiation of which the line 1115.5 keV with quantum yield about 51 % is more useful in gamma spectral measurements. The value of specific activity of suspension produced for experimental research is foremost estimated by radiation safety of personnel and then by resource of measurement technology. The resources of modern gamma spectral instrumentation make it possible to measure with satisfactory accuracy specific for ^{65}Zn energy line while sample activity is about several Bq, i.e. activity is dramatically below the natural background radiation. In this connection there is a possibility to use level of activity that is below the minimum significant value (in accordance with Radiation Safety Standards 99/2009).

After theoretical examination, the questioned optimum time of neutron flow exposure of nanoparticles suspension in channel of IR-8 research reactor was found to be 48 hours. Water suspension of nanoparticles was sealed in ampoule by volume of 6.2 mL made from ultra-pure (99.99 %) quartz that was then placed into container made from pure aluminum which was put into vertical neutron channel of IR-8 research nuclear reactor (reactor construction allows to put into and take the samples out in vertical channels "on-the-run", without reactor shutdown).

Irradiated nanoparticles had enrichment with radioactive isotopes (activity by ^{65}Zn: 48.8 Bq/mg expressed as zinc or 97.6 kBq/mL of suspension) and could be used in technology of studying biokinetics of radioactive products in laboratory animals' organism.

Experiment was performed in 12 Wistar male rats with mean body weight of 170 g. The ampoule with suspension was opened before primer, suspension was filtered to cut off micron fractions and treated with ultrasound to decrease conglomeration of nanoparticles. Individual dosage was equal to 0.5 mL of suspension contained 1.24 mg of zinc oxide nanoparticles that conforms to zinc with weight equal to 1 mg and activity equal to 48.8 kBq. Water suspension was consequently administered into stomach of animals with a help of steel probe (Fig. 2).

Fig. 2. General view of intragastric administration to rat of zinc oxide nanoparticles labeled with ^{65}Zn by thermal neutron radioactivation

Then groups of 3 animals were sacrificed 4, 24, 72, 120 hours after agent administration. Brain, heart, lungs, liver, spleen, pancreas, kidneys, testes, blood, tibia and remaining carcass were taken for gamma counting. Urine and feces samples were also collected and gamma counted 24 and 72 hours after tracer administration. All organs of sacrificed animals were weighed carefully and then changes of activity in organs were recorded on low-background gamma-ray spectrometer. In order to enhance accuracy measurements of biosample, the activity was taken for several times with successive turn of measured sample and following averaging.

Results and discussion

Table 1 demonstrates results of zinc mass fraction estimate in organs of sacrificed animals according to radioactive isotope ^{65}Zn activity corrected to its half-life. Value of this quantity was obtained by dividing mass zinc content in animal organism by value of this organ's mass and then averaged in group.

Figures 3–4 demonstrate kinetic characteristics of ^{65}Zn labeled nanoparticles radioactivity in rat's internals [7]. Distribution has funnel shaped form that to a large extent corresponds to theoretical solution of simultaneous

Table 1

Mass fraction of ^{65}Zn, 10^{-7}, in organs and tissues of Wistar male rats after single intragastric administration of zinc oxide labeled nanoparticles suspension estimated with radiotracer method

№№	Organ	Organ's mass (average in group), g	Hours after administration			
			4	24	72	120
1	Brain	1,66	1,26±0,27	2,16±0,49	3,85±0,89	3,19±0,66
2	Heart	0,87	7,76±1,83	12,9±3,8	19,1±5,09	5,84±1,60
3	Spleen	1,27	15,6±0,85	20,16±3,48	27,9±6,48	8,26±1,43
4	Pancreas	0,39	45,38±7,43	54,6±15,87	58,34±9,88	7,50±2,09
5	Kidney	1,54	24,42±5,58	27,75±5,89	34,81±4,1	7,55±0,56
6	Liver	8,36	30,1±5,92	46,5±5,96	47,70±6,79	15,38±1,40
7	Lungs	1,66	4,64±0,96	8,83±1,12	11,93±2,2	4,28±0,95
8	Testicle	2,40	3,24±0,56	7,53±0,93	8,24±1,23	2,93±0,52
9	Blood	10,78	1,42±0,35	3,27±0,67	5,23±0,92	1,64±0,26
10	Tighbone	1,77	1,57	2,85	3,22	1,78

equations when multi-compartment model of biokinetics is described. The peak content of nanoparticles in different organs of animals varied from 24 to 72 hours after intragastric infusion. The highest obtainable amount of ^{65}Zn tracer decreased in following order of organs and tissues: liver → kidney →spleen → pancreas → heart → blood → brain.

^{65}Zn content analysis in brain allowed to assume nanoparticles or their biotransformation products ability to penetrate blood – brain barrier. The largest fraction of brain ^{65}Zn content makes up 0.06 % total radioactivity administered that corresponds to 1 % of ^{65}Zn activity remaining in rat's body at corresponding time.

In analysis of measurements at the point of 24 and 72 hours ^{65}Zn activity in carcass, all animals' organs and discharge was summarized. The activity value and intragastric administration activity agreed within 10 % [7].

Considering the present technology advances for biokinetics, study of other kinds of other metal nanoparticles turns to Table 2. The table shows that biokinetics in laboratory animal organism at least biokinetics of four types of metal-containing nanoparticles may be successfully researched using the method suggested. Properly, this list may be enlarged. On the other hand, such results can be obtained by another method – neutron activation analysis. In this method, non-activated nanoparticles are injected in animals' body, biological samples are analyzed (according to order considered in the experiment, for example), and radionuclide tracer is activated when these samples are exposed to neutrons. This design of experiment has a number of advantages, especially in the case of short-lived radionuclide tracer (^{198}Au for example). In case of element

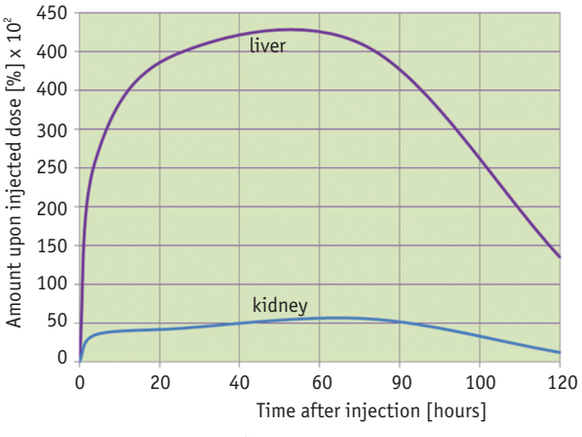

Fig. 3. Kinetic curves of ^{65}Zn tracer accumulation and excretion in liver and kidney of laboratory rats after single intragastric administration of labeled zinc oxide nanoparticles water suspension

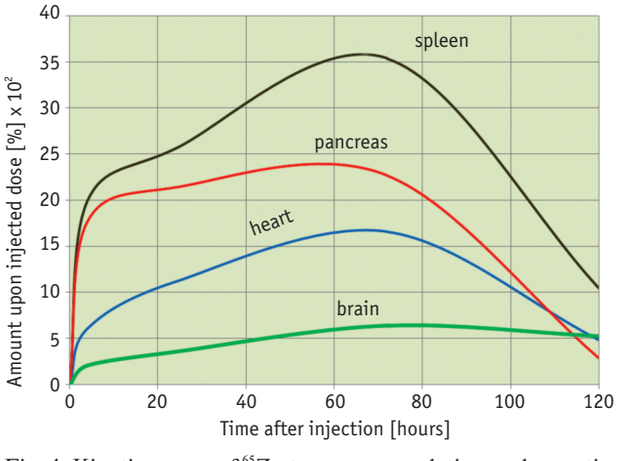

Fig. 4. Kinetic curves of ^{65}Zn tracer accumulation and excretion in some internals of laboratory rats after single intragastric administration of labeled zinc oxide nanoparticles water suspension

Table 2

Elements and isotopes that can be used in technology of labeled atoms derived from thermal neutron-capture reaction

Nanoparticles	Nuclide of the target			Radioactive nuclide			
	Nuclide	Concentration in total natural mix, %	σ_n, barn	Radioactive nuclide (tracer) from n_{th}-capture	$T_{1/2}$, day	Type of radiation	E_γ, MeV
Zinc oxide	^{64}Zn	48.6	0.76	^{65}Zn	243.8	γ	1.1
Ferric oxide	^{58}Fe	0.28	1.3	^{59}Fe	44.5	β^-, γ	1.1; 1.3
Silver	^{109}Ag	48.2	87	^{110m}Ag	249.8	β^-, γ	0.66; 0.88
Gold	^{197}Au	100	98.7	^{198}Au	2.70	β^-, γ	0.41

Symbols:
σ_n – thermal-neutron capture cross-section, $T_{1/2}$ – radio isotope half-life, E_g – radio isotope gamma-ray energy

in nanoparticle substance chosen as radionuclide tracer is biogenic and contained in normal biological tissue, there is a possibility to derive results by differential method using control group of laboratory animals.

Considering the advances of present method, future research is supposed to get answers to following questions:

- how different ways of nanoparticles' administration (intravenous, peroral, inhalation) influence on its toxicity and biokinetics in laboratory animals' organism;
- to what extent nanoparticles' dispersion influences on biokinetic characteristics;
- how much biokinetic characteristics of nanoparticles with different by chemical composition differs from another;
- does nanoparticles form influence on their biokinetic characteristics (it's particularly actual for zinc oxide nanoparticles that can in different conditions assume various forms) [8];
- is it possible to use biokinetic model with parameters derived during single primer of animals to forecast biokinetics of nanoparticles if administrated permanently;
- do nanoparticles cumulate in organs of laboratory animals (and in human body) if administrated continuously;
- what are the physicochemical characteristics and microdistribution of nanoparticles in animals' organs particularly in brain.

A number of theoretical computation issues related to present issue are to be solved after the experiment has been conducted successfully:

- development of mathematical formulation of multicompartmental model in order to analyze and forecast biokinetics of nanoparticles (or its derivatives) in animals' organism after different ways of administration;
- biokinetic characteristics estimation using multi-compartmental model;
- adapting developed model to make it possible to use in human body and estimation of its biokinetic characteristics in order to translate appropriately the results of experiments from animals to humans.

It is also necessarily to solve a number of methodological questions especially the question of standardization of this method in order to match the results of research conducted in different laboratories.

As gold nanoparticles to a large extend are not going to have chemical changes during the process of biokinetics in laboratory animals' organism (and human body), research of biokinetic properties of these particles is more preferable to solve questions posed.

Conclusion

A technology has been worked out of metal containing nanoparticles biokinetic study in Wistar male rats applying radioactive tracers. Said technology merit to be approved as a standard.

Experiment in adult rats demonstrated that if only ^{65}Zn-labeled zinc oxide nanoparticles reaching blood after intragastric administration do not have any substantial chemical transformations then said nanoparticles may be supported to penetrate blood-brain barrier (in amount about 0.06 % of administered dose that is equal to 1 % activity of ^{65}Zn contained in animal's organism at that moment).

The peak content of ^{65}Zn tracer from labeled nanoparticles was observed in organs and tissues of animals 24–72 hours after labeled nanoparticles administration.

The highest obtainable amount of ^{65}Zn tracer decreased in following order of organs and tissues: liver → kidney → spleen → pancreas → heart → blood → brain.

Present technology is considered to be applicable in production of new kind of nanotechnology material – labeled nanoparticle tracers for scientific and medical purposes.

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