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Biokinetic Study of Selenium Nanoparticles and Salt Forms in Living Organisms

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Abstract—An experimental study of absorption, distribution, metabolism, and excretion from the body of radioisotope-labeled nanoparticles (NPs) of elemental Se in comparison with the traditional form of this trace element (sodium selenite) has been performed, being administered in the gastrointestinal tract of rats that were normally supplied with Se or experiencing its nutritional deficiency. Nuclear activation analysis is used for the detection of Se in the biological tissue, which is based on the measurement of a gamma-emitting label [$^{75}Se_{34}$] that is introduced in the Se NPs or its salt by thermal neutron irradiation in a nuclear reactor. It is shown that Se NPs administered into the gastrointestinal tract have bioavailability comparable to the salt form of this element. According to some experimental data, the metabolic assimilation of both forms of Se is greater in animals that suffer from a deficiency in this element when compared to those that are normally supplied with it. There are some differences in the kinetics of accumulation of Se NPs and its salts in the blood and liver, which can be explained by the presence of limiting steps in the absorption and biotransformation of Se-containing NPs. The retention of Se that is administered in the form of NPs or salts to Se-deficient animals differs significantly. These results demonstrate the capacity of the Se NPs to be a source of Se in a new generation of biologically active food supplements.

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INTRODUCTION

Selenium (Se) refers to essential trace elements that play an important biological role in the animal and human body. Its main function is the composition of active centers of a number of proteins, including enzymes, that are involved in the antioxidant defense of the body, the maintenance of redox homeostasis, and thyroid hormone metabolism [1]. The appropriate consumption level of Se with food for an adult is 70 μg/day, with the maximal permissible level being 150 μg/day.

When Se consumption with food is less than 30–40 μg/day, its alimentary deprivation may develop, and its deficiency can be observed when its supply is less than 16–21 μg/day [2]. The clinical manifestation of Se deficiency is cardiomyopathy (Keshan disease) and damage to the connective tissue of joints, or Kashin–Beck disease. At less severe forms of Se deprivation, the suppression of functions of cellular immunity with an increase in the risk of development of a number of infectious and oncological diseases is noted [3]. Se enters the body with food in the form of Se-containing proteins. To enrich specialized food products and make dietary supplements, inorganic Se forms, selenite and sodium selenite; a synthetic derivative—selenopyran; enriched baker's yeast biomass; or spirulina, which contains selenium-containing amino acids, can be used [4, 5]. Out of the selenium forms mentioned, inorganic Se salts are the most toxic, and an overdose of 400–800 μg/day with respect to Se could lead to intoxication.

Since the 2000s there have been a number of reports in the literature on the possibility to use nanoparticles (NPs) of elemental Se, which are bioavailable upon introduction into the gastrointestinal tract, as a Se source in food [6–9].

The advantage of the use of nanoscale Se for food is believed to be its small toxicity, stability, and biocompatibility with other components of the product. However, its biokinetic characteristics and the effectiveness of its assimilation from NPs have been poorly studied.

The goals of this study include an experimental investigation of the kinetics of absorption, biodistribution, and excretion from the body of radioisotope-labeled nanoparticles (NPs) of elemental Se when compared with the traditional form of this trace element (sodium selenite), with the Se forms being administered in the gastrointestinal tract of rats that are normally supplied with Se or experience its nutritional deprivation.

MATERIAL AND METHODS DESIGN OF THE BIOLOGICAL EXPERIMENT

The study employed 88 male Wistar rats in total that were obtained from the Stolbovaya rat-breeding facility; they had an average weight of 80 ± 5 g in the beginning of the experiment. The animals were housed in groups of three to four in transparent polycarbonate plastic cages with sawdust bedding at a temperature of the environment of 20–22°C and 12 : 12 h light dark cycle. The animals were handled according to the international [10] and Russian guidelines [11]. Throughout the entire experiment, the animals had unrestricted access to food and water that was purified by reverse osmosis. All the animals in the beginning of the experiment were divided into four groups (1–4) that contained 24, 24, 20, and 20 rats, respectively. The animals from groups 1 and 3 had a selenium-deficient diet (MP Biomedicals, LLC, United States) that was based on Torula yeasts, which contained less than 0.03 mg Se/kg according to the data of microfluorimetric analysis. The animals from groups 2 and 4 had a standard balanced diet for rodents (OOO Laboratorkorm, Russia) that contained no less than 0.55 ± 0.03 mg/kg Se.

To prevent coprophagy, the bedding (sawdust litter) in cages was changed every day for the first 7 days of feeding and after that once every 3 days. The total duration of feeding with the experimental and control diets was 22 days. After that, six rats from groups 1 and 2 were removed from the experiment by decapitation under diethyl ether anesthesia, with their blood and liver being sampled. Se was determined in the blood serum and liver according to the method in [12]. The other animals from groups 1 and 2 and all the animals from groups 3 and 4 were used in the biokinetic experiment on the 22nd day of the feeding.

OBTAINING RADIOISOTOPE-LABELED PREPARATIONS

A preparation of NPs of elemental Se that was obtained by the laser ablation of a massive selenium target was used in the study [13]. NPs that enter the composition of the preparation were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS). The preparation consisted of free, almost nonaggregated, NPs that had an average diameter of 97 ± 5 nm according to the data of DLS (Fig. 1a) or 92 ± 26 nm according to the results of the processing of TEM images (Fig. 1b) [7] and a predominantly spherical shape. A traditional source of Se was chemically pure sodium selenite pentahydrate $Na_2SeO_3 \cdot 5H_2O$, CAS No. 10102-18-8 (Sigma-Aldrich, Germany). These Se preparations in the form of an aqueous suspension of NPs or sodium selenite solution were sealed in several ampoules of extremely pure quartz. Radioactive labels were introduced into the initial selenium-containing preparations by irradiating the ampoules in the IR-8 research reactor of the National Research Centre "Kurchatov Institute" for 8 h by a homogeneous flux of thermal neutrons with the flux density $\approx 1.5 \times 10^{12}$ cm^{-2} s^{-1}. The reaction

$$^{74}Se_{34} + {}^{1}n_0 \rightarrow {}^{75}Se_{34} + \gamma$$

led to the preparation of NPs and salts of a natural mixture of Se isotopes being enriched in the radioactive nuclide ^{75}Se with a half-life of 120 days and energy of gamma quants in the two main peaks of the spectrum of 0.2647 and 0.1360 MeV. This isotope was detected in the initial preparations and biological samples obtained from the animals by low-background gamma ray spectrometry using a GS3018 spectrometric complex (Canberra) [14].

BIOKINETIC EXPERIMENT

In the experiment, 16 animals from each experimental group were used, which in the end of the feeding with a selenium-deficient or balanced diet were subjected to 16 h starvation with free access to water and then were gavaged at the same time (9:00–9:30 a.m.) with preparations that contained 15 μg of Se in the form of [^{75}Se]-labeled Se NPs (groups 1, 2) or sodium [^{75}Se]-selenite (groups 3, 4). The activity of the labeled preparations of selenium NPs and its salts was 106 Bq per one animal.

After having been gavaged, the animals were immediately placed in metabolic cages and their feces and urine were sampled separately during fixed periods of time (12, 24, and 48 h after the introduction of the preparations). The animals were removed from the experiment after 3, 12, 24, and 48 h in subgroups of four specimens by draining blood from the inferior vena cava under deep ester anesthesia, with the blood, liver, and brain being sampled. The organs and urine and feces samples that had been taken were put into polyethylene vials and sent to gamma ray spectrometric study.

By comparing the activity of biological samples that were introduced into the animals with that of the

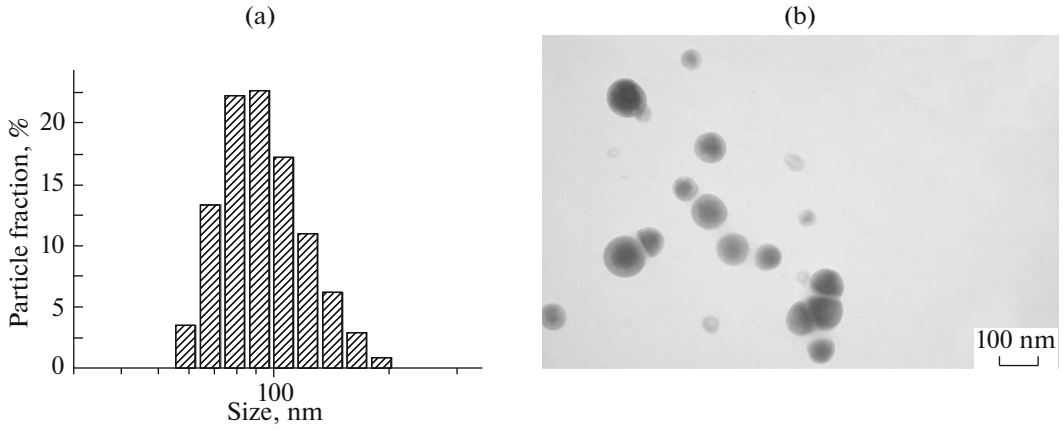

Fig. 1. Results of the characterization of Se NPs by dynamic light scattering (a) and their image obtained by transmission electron microscopy (b) (reproduced from [7] with consent of the authors).

reference specimens of the preparations, Se mass was calculated per a whole organ or biomaterial under study [14, 15]. The total mass of the blood was calculated as 6% of the body mass of the animal. The remaining animals were used as an active control, with pure drinking water being introduced instead of the labeled preparation.

Statistical processing of the results was carried out using an SPSS 16.0 program. A sample mean (M) and standard deviation (s.d.) were calculated. The significance of the differences between groups was determined using ANOVA, Student's *t*-test, and Mann–Whitney nonparametric rank test.

RESULTS AND DISCUSSION CHARACTERISTICS OF THE BIOLOGICAL MODEL OF SELENIUM DEFICIENCY

Throughout the entire period of feeding the animals with the experimental diets, the animals from all the groups were active and had normal stool and state of mucous membranes. Morbidity and mortality were absent. Beginning from the 8th day of the experiment, the animals from groups 1 and 3 (selenium deficiency) gained weight significantly more slowly than the animals from control groups 2 and 4 ($p < 0.001$; ANOVA); on the 22nd day of the experiment their fur was also less thick in appearance when compared to the rats from groups 2 and 4. No other pathological changes were observed. The results of sample analysis of the content of Se in the liver and blood serum (Fig. 2) of animals with a selenium-deficient diet revealed a significant decrease (~3.5-fold; $p < 0.001$) in the Se content in the both biological substrates when compared to the control group.

It is known that the development of the clinical manifestation of selenium deficiency in people corresponds to a decrease in the Se concentration in the blood serum by a factor of 2.3–2.6 when compared to the norm [2, 3]. The results make it possible to conclude about the development of selenium deficiency in groups 1 and 3 of animals with a low-selenium diet.

RESULTS OF BIOKINETICS STUDY

The results of detecting labeled Se in the organs, tissues, and feces of animals with normal and insufficient supplies of Se at different time intervals after gavage administration of [^{75}Se]-labeled NPs and sodium selenite are given in the table. There was no elevation of a gamma ray spectroscopic signal over the background in the region of analyzed [^{75}Se] lines for

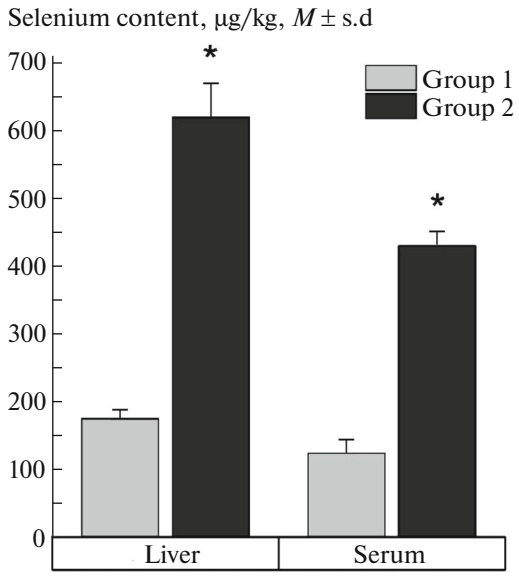

Fig. 2. Selenium content in the blood serum and liver of rats of groups 1 and 2. *The difference with group 1 is significant, $p < 0.001$. The Student's *t*-test and Mann–Whitney nonparametric rank test. The number of rats $N = 6$ in each of the groups.

Selenium mass, μg ($M \pm$ s.d.), in the organs, tissues, and feces of rats with normal and selenium-deficient diets after gavage administration of 15 μg Se in the form of [^{75}Se]-labeled nanoparticles or sodium selenite

Se form	Biological substrate	Rat group	Time after introduction of selenium substrate, h			
			3	12	24	48
Sodium selenite	Blood	Norm	0.662 ± 0.030	0.656 ± 0.054	0.506 ± 0.030	0.356 ± 0.036
		Deficiency	0.924 ± 0.100*	0.967 ± 0.078*	0.771 ± 0.056*	0.623 ± 0.023*
	Liver	Norm	4.450 ± 0.630	1.790 ± 0.185	1.113 ± 0.066	1.058 ± 0.095
		Deficiency	4.275 ± 0.419	2.033 ± 0.422	1.249 ± 0.061	1.329 ± 0.085*
	Brain	Norm	0.013 ± 0.001	0.017 ± 0.002	0.021 ± 0.003	0.020 ± 0.002
		Deficiency	0.025 ± 0.008	0.035 ± 0.004*	0.048 ± 0.014	0.033 ± 0.001*
	Urine	Norm	–	1.807 ± 0.587	2.224 ± 0.157	2.541 ± 0.175
		Deficiency	–	1.018 ± 0.090	1.072 ± 0.047*	1.329 ± 0.126*
	Feces	Norm	–	0.740 ± 0.184	2.101 ± 0.310	2.422 ± 0.277
		Deficiency	–	0.304 ± 0.138	1.355 ± 0.240	2.009 ± 0.302
Selenium nanoparticles	Blood	Norm	0.804 ± 0.034#	0.890 ± 0.031#	0.689 ± 0.059#	0.582 ± 0.057#
		Deficiency	0.601 ± 0.043*#	0.713 ± 0.027#	0.520 ± 0.061#	0.517 ± 0.015#
	Liver	Norm	6.455 ± 0.478	2.351 ± 0.143#	2.255 ± 0.140#	1.862 ± 0.101#
		Deficiency	3.426 ± 0.780*	2.007 ± 0.085	1.686 ± 0.047*#	1.622 ± 0.124
	Brain	Norm	0.027 ± 0.003#	0.026 ± 0.002	0.027 ± 0.003	0.025 ± 0.008
		Deficiency	0.021 ± 0.002	0.026 ± 0.002	0.044 ± 0.008*	0.032 ± 0.004
	Urine	Norm	–	2.325 ± 0.144	3.465 ± 0.267#	3.984 ± 0.393#
		Deficiency	–	0.939 ± 0.217*	1.618 ± 0.392*	1.888 ± 0.232*
	Feces	Norm	–	1.596 ± 0.607	2.490 ± 0.442	2.601 ± 0.230
		Deficiency	–	1.154 ± 0.457	2.028 ± 0.166	2.218 ± 0.468

*Difference between animals with normal supply of Se and its deficiency is significant, $p < 0.05$.
#Difference for the Se salt form and NPs is significant, $p < 0.05$; Mann–Whitney nonparametric rank test.

any of the samples from the organs, tissues, or feces (data are not given) of the animals from the active control group.

As follows form the data given, fecal excretion of [^{75}Se] differs insignificantly in animals that had a normal and selenium-deficient diet in the case of the introduction both of salt and NPs. At this, the values of excretion for these two forms of the label also differ insignificantly at all periods of observation within the error of determination ($p > 0.1$). This, in particular, indicates that the bioavailability of Se (determined as the difference between the amounts of Se that is introduced and excreted with feces and expressed in % from the introduced dose, which is 15 μg in the both cases) from its NPs is sufficiently high and comparable with the bioavailability of Se from sodium selenite, which is from 84 to 87% at an observation period of 48 h and coincides with the literature data [16]. The data of the Chinese authors state that the availability of nanoscale elemental Se and that of sodium selenite are comparable according to their ability to recover the activity of glutathione peroxidase during selenium deficiency [17]. On the other hand, it is known that the bioavailability of red elemental Se with micron particles, which is obtained by microbiological synthesis or nonenzymatic reduction by ascorbic acid, is quite low and does not exceed 2–4% [18].

The urine excretion of a [^{75}Se] label is found to be significantly reduced at Se deficiency, when compared to its normal supply, at 24 and 48 h of the experiment both in the cases of Se NPs and its salt form; however, at 12 h of the experiment, this difference is significant only for the nanoscale form. This indicates the enhanced retention of Se at a selenium-deficient diet when compared to the normal supply, which is known from literature and reflects a significantly greater number of sites for the potential inclusion of Se in specific selenoproteins at its deficiency [19]. It should be noted that, at the duration of the experiment exceeding 24 h, the urine excretion of Se in animals that are supplied with it is significantly greater in the case of NPs when compared to its salt form ($p < 0.05$), whereas upon its deficiency this difference is not observed. This demonstrates that Se assimilation from its NPs in the body is reduced when compared to its

salt form only upon the normal supply of Se, but not upon its deficiency.

The number of [^{75}Se] labels that circulate in the blood is significantly increased for animals with selenium deficiency when compared to those with normal supply, in the case of administration of its salt form at all periods of the experiment. This is in accordance with the fact that the main part of Se circulating in the blood enters the composition of several specific selenoproteins, glutathione peroxidases of red blood cells, and plasma and selenoprotein P of the plasma. During their biosynthesis, Se from the selenite anion is used quickly and efficiently and to the greatest extent upon selenium deficiency.

In the case of the NPs, the difference in the Se content in the blood between animals with normal and selenium-deficient diets is absent at 12–48 h, and at 3 h even a significant decrease in its content is observed in the case of selenium deficiency. At this, for the case of Se deficiency, the content of the label in the blood for all periods of the experiment is significantly smaller when compared to its salt form, and for the normal supply it is increased, though the differences in the mean levels are small with respect to the absolute value. Together these results make it possible to suggest that the use of Se from NPs is restricted even to a greater extent during selenium deficiency than upon normal supply.

One of the rate-limiting steps during early stages of Se assimilation from the diet could be Se absorption in the intestine. As is known from the literature [20], selenite anion is transported in the small intestine by an energy-independent mechanism of passive diffusion. As for Se NPs, the mechanism of their absorption has not been studied to date, but it can be suggested that like for other NPs it is active pinocytosis (persorption); i.e., it is, on the contrary, energy-dependent. Upon selenium deficiency, which is accompanied by oxidative stress and a disturbance of trophic processes in the intestinal mucosa, this transport seems to be suppressed more than passive diffusion, which is typical for a salt form. This results in the reduced accumulation of a ^{75}Se label in the blood (and liver as well) of animals that have been introduced with the NPs and have had selenium deficiency at the early stage of the experiment (3 h) when compared to rats with a normal selenium supply. At later stages of the assimilation (12–48 h), the inclusion of Se that has entered the body in the composition of NPs into selenoproteins appears to be restricted by other stages, in particular, by the transition of elemental Se from the NPs into a soluble form, which can occur at the tissue level, including nonenzymatically under the effect of intracellular oxidants. Due to the presence of these stages that do not directly depend on the Se status in the body, the difference between animals with a normal selenium supply and its deficiency in the case of administration of the NPs is found to be leveled.

The Se level in the animal liver upon the administration of its salt form (which is mainly determined by the kinetics of passive diffusion from the intestinal lumen) does not differ significantly between the animals with normal and selenium-deficient diets after 3 h of the experiment, but there is a tendency toward its greater accumulation in the liver at selenium deficiency during greater periods of time (12 h and more), which becomes significant at 48 h and seems to reflect a greater number of available selenium-binding sites in liver selenoproteins at its deficiency. In the case of the NPs, Se accumulation in liver, on the other hand, is reduced at all periods of the experiment in rats with selenium deficiency when compared with the animals with normal supply, with the decrease being significant at 3 and 12 h. As in the case of the Se content in the blood, the latter seems to be connected with the presence of rate-limiting steps in Se accumulation, which are specific for NPs and the rate of which is decreased at selenium deficiency, in particular, absorption by the endocytosis mechanism. At the same time, accumulation of the label in the liver is significantly greater for Se NPs than its salt form at 12–48 h of the experiment in animals with normal supply and at 24 h in rats with selenium deficiency, which could be due to liver deposition of part of Se in the form of NPs that have not been metabolized during the first day after the introduction. However, an additional study is required to prove this assumption.

As can be seen from the experimental data, [^{75}Se] labels from the both preparations penetrate the blood–brain barrier and are accumulated in the brain in amounts that are insignificant in absolute value, but reliably measured. Both in the salt and nanoscale forms this trace element tends to be accumulated more upon selenium deficiency when compared to the animals with normal supply at observation periods that exceed 12 h. This difference is significant in the case of the salt form at 48 h and in the case of NPs at 24 h of observation. A twofold increase in the penetration of the label into the brain is noted for the NPs when compared to the salt form after 3 h of the experiment in animals with normal supply. The absence of significant differences in other cases could be due to the great spread of the experimental data.

CONCLUSIONS

Thus, the results of the experiment on the biokinetics of the two forms of Se in the rat body are in agreement with the literature data (in regards to the salt form), demonstrate the comparable bioavailability of Se in the form of salt and NPs, and indicate the presence of a significant difference in the kinetics of the metabolism of these two forms of Se in the blood and liver. These differences could be demonstrated most clearly by a biokinetic compartmental model of the metabolism of Se in the composition of its salt form and NPs. This model will be given in our next paper.

The data demonstrate that, in the case of selenium deficiency, its retention (assimilation) in the body (which is assessed by its urine excretion) is greater than at the normal supply after the administration of both forms of the trace element. Se from the NPs is excreted faster, when compared to its salt form, at a normal supply of the trace element, whereas upon selenium deficiency the differences in the retention of the both forms of selenium are insignificant. The data obtained indicate that Se in the composition of NPs is bioavailable, actively accumulated in the body, and involved in metabolic processes; therefore, these NPs could be used during the development of a new generation of food supplements—selenium sources.

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