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Accumulation of silver nanoparticles in mice tissues studied by neutron activation analysis

Inga Zinicovscaia[1,2] · Sergey S. Pavlov[1] · Marina V. Frontasyeva[1] · Alexandra L. Ivlieva[3] · Elena N. Petritskaya[3] · Dmitriy A. Rogatkin[3] · Vyacheslav A. Demin[4]

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Abstract
For estimation of toxicity of silver nanoparticles under long-term exposure for mammals and humans, the accumulation of silver in mice tissues (blood, liver, brain) during 2 and 4 months experiments was examined. Neutron activation analysis revealed silver in all examined tissues with its highest concentrations in liver followed by brain (including silver in blood vessels). The lowest concentration of silver was observed in blood samples. The mean specific mass content of silver which crossed the blood–brain barrier was 225 ± 99 ng (for male) and 395 ± 150 ng (for female) of the brain sample after 2 months of administration, 860 ± 200 ng (for male) and 880 ± 200 ng (for female) of brain sample after 4 months of administration. The obtained results are of great importance for nanotoxicological studies.

Keywords Silver nanoparticles · Brain · Liver · Blood · Accumulation · Neutron activation analysis

Introduction

Currently, inorganic nanoparticles have attracted increasing attention, as they are used in various fields of industry and medicine [1]. Silver nanoparticles (AgNPs), due to their antimicrobial and anti-inflammatory properties, are ones of the most widely used nanoparticles in commercial and medical products [1–3]. Approximately 24% of the products currently registered in nanoproduct databases claim to contain AgNPs, and their use is foreseen to increase in the future [4].

The extended use of AgNPs leads to human exposure via oral administration, skin penetration, and inhalation during manufacture, use and disposal. Furthermore, nanoparticles are characterized by high surface area to volume ratio, particle reactivity, and can undergo surface modifications, which could result in greater side effects once these are absorbed into the body. Therefore, for the application of nanoparticles it is important to know in which tissues they are mainly accumulated and to understand the potential biological responses to silver nanoparticles in vivo [1, 3, 5].

To quantify nanoparticles bioaccumulation in tissues several analytical techniques were used in different studies. Lasagna-Reeves et al. [5] used ICP-MS to study gold nanoparticles bioaccumulation in mice tissues, including brain, liver, kidney, spleen and lungs. Yang et al. [1] applied AAS and ICP-MS techniques to determine silver and gold concentration in mice tissues. Total silver concentrations in mice were determined in organs and whole blood by means of a triple quadrupole ICP-MS [6].

Wide application of ICP-MS and ICP-AES techniques is explained by possibility of multi-elemental analysis, high selectivity, sensitivity and low detection limits for most of the elements [7]. However, the above-mentioned techniques require sample dissolution in acids and other type of solvents that may induce errors in elements determination. In case of AAS, the increase in the number of determined elements lead to the increase in the quantity of solution.

✉ Inga Zinicovscaia
zinikovskaia@mail.ru

[1] Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russian Federation 141980

[2] Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, Bucharest, Magurele, Romania

[3] Moscow Regional Research and Clinical Institute named after M. F. Vladimirskiy, Moscow, Russia 129110

[4] National Research Centre «Kurchatov Institute», Ploshchad' Akademika Kurchatova, Moscow, Russian Federation

For some organs (e.g. lymph nodes, vena cava and aorta) the amount of tissue material is not sufficient for the tissue dissolution. In the very low detection levels ICP-MS/AAS may not be the most sensitive methods for detection of metal nanoparticles [8]. Neutron activation analysis is a high-sensitive analytical technique which allows determination of a wide range of elements with high precision, using small samples and without any chemical pre-treatment [9].

The aim of the present study was to examine distribution of silver nanoparticles in mice tissues after a 2- and 4-month oral exposure. Neutron activation analysis was used to determine total silver content in liver, brain (including silver in blood vessels) and blood samples.

Experimental

Silver nanoparticles

The concentrated (13 mg/ml) colloid solution of silver nanoparticles "Argovit-C" (average diameter 8.7 ± 1.4 nm) dispersed in distilled water and coated with a stabilizing coating of polyvinylpyrrolidone was purchased from SPC Vector-Vita company. Relatively small AgNPs were selected, since accumulation of smaller particles is usually higher and more widespread than that of larger particles [10]. The solution for consumption by mice was prepared by dilution of purchased concentrated solution mixed with pure water (in a ratio of 1:500).

Animals

Three and a half to four month old mice were purchased from two different farms of Moscow region in Russia: the Andreevka Farm used in the first experiment and the Stolbovaya Farm in the second experiment. Thereafter the animals were maintained in the vivarium of Moscow Regional Research and Clinical Institute named after M.F. Vladimirskiy. The animals were kept in steel cages with sizes of 31.5 × 23 × 15.7 cm, and each cage contained 3–4 mice, with a natural cycle of illumination and the temperature of 22–24 °C; each cage was cleaned once a day. The methodology of the experiments and the maintenance of the animals at the vivarium of the institute were performed according to the principles of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Experimental design

Two type of experiments were performed: the only difference between them was the period of exposure to nanoparticles—2 months in the first experiment and 4 months in the second experiment. In each experiment, the experimental mice were given the solution with concentration of 26 μg of silver nanoparticles per 1 ml of drinking water. The total daily intake was 100 μg per animal—the daily norm of liquid per mouse was 4 ml [11]. The control mice (five for each experiment) were given drinking water. The number of mice used in experiment varied from 10 to 12 individuals.

After each experiment, mice were euthanized. The euthanasia was performed by intraperitoneal injection of urethane solution. The concentration of the water solution based on body weight and dosage of 1.2 g of dry urethane per kg of body weight, proposed by a veterinarian. Brain (whole), liver and blood were extracted from each mouse: a freshly cut brain and liver were weighed (in g), and the volume of blood was measured (in ml). The samples were packed in aluminum foil and dried for 24 h at a temperature of 75 °C until constant weight.

Neutron activation analysis

The elemental content of the isolated tissues was determined using neutron activation analysis at the IBR-2 reactor in Dubna, Russia. The description of irradiation channels and pneumatic transport system of the REGATA installation can be found elsewhere [9]. The temperature in the irradiation channels of the IBR-2 reactor does not exceed 60–70 °C, which allows the analysis of biological samples without damaging them.

The analyzed samples were divided into 3 groups: blood, liver and brain (63 samples in each group) and packed in identical aluminium foil cups (16 mm in diameter). Due to the high flux of fast neutrons and gamma rays in the irradiation channels, it was not possible to use polyethylene as a packaging material for irradiation lasting longer than 30 min because of its low radiation resistance. The weight of the samples varied in the following range: 0.05–0.2 g for blood and brain samples and 0.2–0.4 g for liver samples.

The samples of each group packed in an aluminium foil were placed in 6 containers and irradiated simultaneously with two reference materials: SRM 2710 (Montana Soil, Highly Elevated Trace Element Concentrations (NIST, USA) and 2891 [Cupric Sandstones, polymetallic ores, Karaganda, Kazakhstan (LLP «Centrgeolanalit»)] as well as with samples of packaging foil (blanks). Reference materials and blanks were placed in each container. All

samples were irradiated with epithermal neutrons in the irradiation channel with a cadmium shield during 24–48 h at a neutron flux of 1.8×10^{11} cm^{-2} s^{-1}. The neutron flux gradient along the length of the container was insignificant. The use of reference materials NIST SRM 2710 and SRM 2891 with the matrix different from the analyzed samples is explained by the fact that the silver content (certified value) in the above-mentioned reference materials is significantly higher than in NIST SRM 1566b (Oyster Tissue). It is assumed that under the above-mentioned experimental conditions (neutron flux and irradiation time) the area of the total-absorption peak for Ag^{110m} in NIST SRM 1566b would have low statistics and could be poorly processed. The matrix effect in case of small samples (size and weight) is insignificant [12].

Gamma spectra of induced activity were obtained using three spectrometers based on HPGe detectors with an efficiency of 40–55% and resolution of 1.8–2.0 keV for total-absorption peak 1332 keV of the isotope ^{60}Co and Canberra spectrometric electronics after 7 days for 1.5–5 h.

The analysis of the spectra was performed using the Genie2000 software from Canberra, with the verification of the peak fit in an interactive mode. The calculation was carried out for ^{110m}Ag 657.8 (94.6%), 763.9 (22.2%), and 937.5 (34.3%) keV peaks (gamma-ray emission probability is indicated in parentheses). In this case, a correction was made for possible interference with the isotope ^{76}As ($T_{1/2}$ = 1.1 days, 657.1 keV). In the blank spectra the silver isotope was not detected, therefore blank correction was not performed.

The calculation of concentration was carried out using software "Concentration" developed in FLNP [13]. The software automatically proposes to create the so-called group standard sample (calibrator) from all reference materials irradiated in one container for calculation of concentrations in the analyzed samples. This group standard includes the values of radionuclide activities determined with the lowest uncertainty. Then, using the obtained group standard, the program allows one to check the quality of analysis by calculating the concentrations in each of irradiated reference materials and automatically comparing the obtained values with the certified ones.

In the present study with lower net inaccuracy the isotope ^{110m}Ag from reference material SRM 2710 was used as a calibrator, and SRM 2891 was used for quality control. The obtained values for concentrations for SRM 2891 in all irradiated containers differed from the certified values in the range (0.2–5.5%).

Results and discussion

Distribution of nanoparticles in the body occurs via the blood [8]. After prolonged oral administration, the content of silver in the blood was slightly higher in administered mice than in control samples (Table 1). Low content of silver in blood can be connected with the translocation of nanoparticles from the bloodstream to the organs that contain large numbers of phagocytic cells (liver, spleen, lung) which are devoted to clearing foreign body particles from the circulating blood [6]. The clearing of blood from nanoparticles is a fast process. In Yang et al. [1] study the content of gold in blood was undetectable; indicating that injected gold nanoparticles was rapidly extracted from the blood and effectively distributed into other tissues.

The highest content of silver was detected in the liver (Table 2). It could be explained by both the higher presence of sulfur in this organ and the consequent formation of silver–thiol complexes [14] and the detoxification function of the liver. After 2 and 4 months of administration, the difference in silver nanoparticles accumulation between genders was not so evident. At the same time, it can be noted that after 4 months of admission the silver content for both males and females were approximately two times lower in comparison with the corresponding amounts in the 2-months experiment. Lower silver content in liver after 4 months can be explained by its excretion into the bile [6].

The results obtained from the brain samples (including blood vessels) of experimental and control mice are presented in Table 3. After 4 months of admission an increase of silver content in brain in comparison with the corresponding values after 2 months of administration, for both genders was observed. The increased accumulation of nanoparticles in the brain suggest on their non-saturable uptake from the blood to the brain. There were no significant differences in the silver accumulated in the brain (the samples included the silver in blood vessels) between males and females. Table 3 also shows results of the total silver mass in the mice brain that are derived using an information about dry weight of mice brain (Table 4).

Brain is an organ with a high blood content, consequently residual blood in the blood vessels and capillaries could influence the measurements of silver content. The specific mass content of silver, which crossed the blood–brain barrier (excluding silver in blood vessels) was calculated according to the methodology described in details in Antsiferova et al. [15], using an additional information about specific activity of the blood with respect to ^{110m}Ag and ^{59}Fe (not shown here). Obtained values are presented in Table 5.

The significant difference in the content of silver, in both type of experiments, between experimental and

Table 1 Silver content in mice blood

Gender	Content, μg/g dry weight			
	Male		Female	
	Range	Mean ± SD	Range	Mean ± SD
Control (2 months)	0.09–0.73	0.3 ± 0.2	0.2–0.6	0.4 ± 0.2
2 months of administration	0.9–4.5	2.5 ± 1.2	0.5–8.5	2.4 ± 2.1
Control (4 months)	0.05–1.5	0.6 ± 0.4	0.04–0.2	0.1 ± 0.09
4 months of administration	0.9–2.7	1.7 ± 0.6	0.6–1.5	1.2 ± 0.3

Table 2 Silver content in mice liver

Gender	Content, μg/g dry weight			
	Male		Female	
	Range	Mean ± SD	Range	Mean ± SD
Control (2 months)	0.8–1.6	1.2 ± 0.6	0.1–0.2	0.13 ± 0.05
2 months of administration	37–293	140 ± 90	2.3–186	95 ± 65
Control (4 months)	0.3–0.5	0.4 ± 0.1	0.18–0.22	0.2 ± 0.03
4 months of administration	9.6–104	65 ± 40	4.7–81	50 ± 34

Table 3 Silver content in mice brain (including silver in blood vessels)

Gender	Content, μg/g dry weight				Total Ag content, ng	
	Male		Female		Male	Female
	Range	Mean ± SD	Range	Mean ± SD	Mean ± SD	Mean ± SD
Control (2 months)	0.6–0.9	0.7 ± 0.1	0.6–2.0	1.1 ± 0.5	50 ± 9	91 ± 46
2 months of administration	5.2–10.6	6.8 ± 1.8	3.1–11.7	7.0 ± 2.3	449 ± 131	532 ± 186
Control (4 months)	2.4–2.5	2.4 ± 0.01	2.4–2.5	2.4 ± 0.1	194 ± 22	194 ± 21
4 months of administration	8.9–18.7	12.9 ± 2.9	8.7–16.6	12.0 ± 2.4	1019 ± 251	731 ± 231

Table 4 Dry weight of the mice brain

Gender	Brain's weight, mg	
	Male Mean ± SD	Female Mean ± SD
Control (2 months)	72 ± 8.4	83 ± 18
2 months of administration	66 ± 8.4	76 ± 8.6
Control (4 months)	81 ± 8.9	81 ± 8.1
4 months of administration	79 ± 8.4	84 ± 13

control animals indicated on successful crossing of AgNPs through blood–brain barrier (BBB). In the literature, there are suggested two mechanisms for the entry of nanoparticles into the brain through the BBB: (1) vascular endothelial cell transcytosis and (2) BBB destruction by loosening of the endothelial junction or by dissolving of the endothelial membrane [16]. It can been seen that after 2 months of administration the amount of silver accumulated in females was higher compared to that in males. However, after 4 months of admission the accumulated amount of silver, both for males and females was almost the same.

Data obtained in the present study are in agreement with results reported previously by De Jong et al. [8] who found the highest levels of gold in liver and lower levels in brain, lung, heart, kidney, small intestine and stomach of rats. Lasagna-Reeves et al. [5] studying the bioaccumulation of gold nanoparticles in mice have showed that gold levels in blood did not increase with the dose administered. However, in all the organs examined (spleen, liver, kidney, lungs and brain) there was a proportional increase on gold, indicating efficient tissue uptake. It should also be mentioned that in the control groups (Tables 1, 2, 3, 5), silver was present at background levels as shown by NAA data for the examined organs.

Table 5 Specific mass of silver accumulated in mice brain, ng

Gender	Male		Female	
	Range	Mean ± SD	Range	Mean ± SD
Control (2 months)	12–52	32 ± 15	14–81	46 ± 35
2 months of administration	112–397	225 ± 99	163–650	395 ± 150
Control (4 months)	171–190	180 ± 13	177–201	189 ± 16
4 months of administration	630–1280	860 ± 200	600–1245	880 ± 200

Conclusions

Neutron activation analysis proved to be an efficient technique to trace silver accumulation in mice tissues after prolonged consumption of small daily doses of AgNPs. Obtained results showed that silver was present in all examined organs with the highest levels in the liver, followed by brain (including silver in blood vessels) and blood. The content of silver in brain samples after 2 and 4 months of administration increased significantly in comparison with control samples, indicating on crossing of AgNPs through blood–brain barrier. Significant silver accumulation in brain of mice should be considered in the risk assessment of AgNPs in living organisms.

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