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Synthesis and Safety Evaluation of Gold Nanoparticles in the *in Vivo* Experiment

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Abstract

In continuation of works on toxicology of nanocarbon materials, investigations of safety of colloidal gold nanoparticles in the 10 to 40 nm size range in two lines of mice (outbred and line C57BL/6j) were performed. It was shown that in 24 h and 30 days after a single parenteral injection of colloidal gold nanoparticles in mice, the toxic action on indicators of peripheral blood, behaviour of animals, as well as internal organs was not revealed.

Key words: gold nanoparticles, toxicity, peripheral blood, behaviour, microscopic examination, outbred mouse, C57BL/6j mice

INTRODUCTION

Safety of nanomaterials and nanotechnologies is an important factor regulating the industrial production. Given the peculiarities of the ingress of nanoparticles into the human body and animals, the main target organs are the tissues of the respiratory, interstitial, immune and skin systems. More severe on consequences is a hit to the brain, bone marrow, heart, reproductive and excretory organs. However, for the absolute majority of nanomaterials, the mechanisms of ingress into the organism, biocompatibility, cumulation in organs and tissues, elimination, and the degree of their toxicity are unknown.

Earlier, investigations on safety of such nanomaterials and nanosubstances as carbon nanofibers and nanotubes, titan, nickel, zinc, copper, aluminum, silver nanopowders were carried out and their harmlessness at the intragastric method of introduction to animals in experiments *in vivo* was shown [1–4]. In works by Ismagilov and Zarytova published in the last decade, the successful use of titan dioxide nanoparticles for the delivery therapeutic oligonucleotides into cells was shown. In experiments *in vitro*, a low toxicity of nanoparticles and high antiviral activity in relation to strains of influenza viruses was established [5–15].

Considering a high scientific interest to nanoobjects and their perspectiveness for nanobiomedicine, the goal of the present study was determined – gaining new information about the safety of colloidal gold nanoparticles. The given task was posed on one hand, as the study of a more methodically deterministic object in the continuation of the works on nanocarbon materials, as well as in connection with a great concernment of the application of gold nano-

particles in various manufacture areas. For gold nanoparticles, along with positive effects, negative effects are known, as the quantitative accumulation in the internal organs (liver, spleen, lungs, kidneys) that lead to the disruption of organ and tissue microcirculation and development in them of dystrophic changes [16, 17], as well as toxic effects of nanoparticles on the whole organism at various introduction ways. It is known that the majority of researchers give preference to parenteral introducing of gold nanoparticles [18].

In this regard, investigations on the safety study of colloidal gold nanoparticles were carried out in mice of two different lines at the parenteral method of introducing.

EXPERIMENTAL

Gold particles were prepared by the Frens method [19] – citrate reduction of chloroauric acid (CAA). To a boiling aqueous solution of 0.01 % of CAA, an aqueous solution of 1 % sodium citrate (Cit) was added using molar ratios of Cit/Au, equal to 4 : 1, 3 : 1, 2.3 : 1, 1.5 : 1 for the preparation of particle with an average diameter (nm): 12 (Au-1); 16 (Au-2), 22 (Au-3) and 40 (Au-4), respectively.

Research methods of gold nanoparticles

The concentration of gold in solutions was determined by the method of inductively coupled plasma atomic emission spectroscopy on a PerkinElmer OPTIMA 4300DV ICP spectrometer.

Dispersion characteristics of gold were studied by the methods of transmission electron microscopy (TEM), atomic force microscopy (AFM) and UV-Vis spectroscopy. TEM studies of preparations applied on a grid with a formvar substrate, stabilized with carbon, were carried out on an electron microscope JEOL JEM 1400 (Japan). For AFM, a drop of a preparation of 20 μL was deposited on freshly cleaved mica of the area of 25–30 mm^2, was adsorbed for 1 h at room temperature and examined in an atomic force microscope SolverP47Bio (NT-MDT, Zelenograd, Russia) in the tapping mode. Electronic transmission spectra of the solutions were collected on a spectrophotometer Shimadzu UV-2501PC. The spectra were recorded with a compensation of the absorption relatively to water in the range of 11 000–54 000 cm^{-1} in a quartz cell with an optical path length of 10 mm and presented as optical density (D), after computer processing.

Research methods in vivo

The determination of toxicological parameters was conducted on white outbred mice and C57Bl/6j mice (20–25 g). The animals were provided by the laboratory of experimental animals of the Institute of Cytology and Genetics, SB RAS (Novosibirsk). The maintenance of animals was implemented in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). The mice were kept in standard plastic cages of the firm VELAZ on a small wood chips bedding, the cages size for mice amounted to 42 × 25 × 14 cm. The air temperature in the vivarium is 20–23 °C, humidity – at most 50 %, volume of air exchange (exhaust/influx) – 8 : 10, light regime (day/night) – 1 : 1. Animals were fed with standard granulated combined feed with mineral and vitamin supplements and porridge from cereals. Experimental groups were formed of 8–10 individuals with the same body mass.

Solutions of colloidal gold were injected at the concentrations indicated in Table 1 by 0.2 mg/mL per mouse. The condition of the animals was assessed by appearance, behaviour, and with the help of integral indices: of mice – by the change of the body weight (balances OHAUS, the USA), its temperature (electrophysiological complex LabLinc, Couloubrn Instruments, the USA), spontaneous motor activity and research reaction (automatic registration of motor acts in a chamber TruScan, Couloubrn Instruments, the USA); hematological indices were recorded on a hemo-analyzer Medonic (the USA), physiological – through one day and 30 days after injection. At the end of the experiment, heart, lungs, liver, kidneys, spleen, brain were taken for morphological studies. The organs were fixed in a solution of 4 % paraformaldehyde, subjected to standard histological processing on a complex

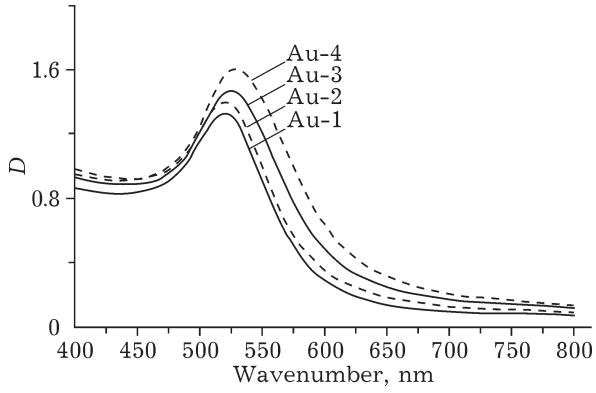

Fig. 1. UV-Vis spectra of the colloidal gold samples.

TABLE 1

Au nanoparticles (AuNPs) description

Samples	Adsorption bands, nm	Number of AuNPs per mL	Particle size, nm
Au-1	519	$3.4 \cdot 10^{12}$	12±1.8
Au-2	520	$1.4 \cdot 10^{12}$	16±2.3
Au-3	524	$5.6 \cdot 10^{11}$	22±3.2
Au-4	530	$1.4 \cdot 10^{11}$	40±6.0

Note. Concentraion of AuNPs is 60 mg/mL.

MICROM (Carl Zeiss Jena, Germany). Sections of 4 μm thickness are collared with hematoxylin and eosin, investigated by the method of light microscopy in transmitted light. Statistical data processing was performed using software Statistica 7.0, the results are presented as mean (M)±the standard error of the mean (SE). The reliability was determined by Student's t-test. The results were considered dependable at $p < 0.05$.

RESULTS AND DISCUSSION

For gold nanoparticles with the size from 10–40 nm in the form of colloidal solutions, the study of their disperse properties was carried out by a set of physicochemical methods and the characteristics were established. The results are presented in Fig. 1 and Table 1.

When using this method only in cases of the excessive or stoichiometric amount of the reducing agent, relatively uniformly-disperse sols with the size of spherical particles from 12 to 22 nm are obtained. At the shortage of the reducing agent [Au]/[Cit] = [1] : [0.9], a polydisperse mixture of particles in a wide size range of predominantly 6–15 and 30–40 nm is obtained.

By virtue of the conducted studies on biosafety, it was found that a single-shot intraperitoneal administration in mice of two lines of colloidal solutions of gold with a particle size of from 10 to 40 nm (samples Au-1, Au-2, Au-3, Au-4) did not render toxic effects generally on the body, did not change indexes of the weights and body temperature; did not exert toxic effects on parameters of the spontaneous motor activity and research reaction of white outbred mice during a monthly experiment. However, after the single-shot intraperitoneal injection of the same colloidal solutions of gold in C57BL/6j mice after 14 days did not exert an effect (Table 2), and after 30 days contributed to the increase of the stimulating activity of the CNS, which was manifested in the increase of the travelling speed, traversed distance, number of vertical racks, time and number of studies of openings (Table 3).

TABLE 2

Effect of gold nanoparticles on the CNS activity of mice (C57BL/6j mice) after 14 days (after a single-shot intraperitoneal injection)

Groups	A	B	C	D	E	F	G	H
Control	12.9±0.6	102.6±0.9	299.6±32.8	2.5±0.3	0	17.4±0.9	2.4±0.6	3.0±0.9
Au-1	13.0±0.9	102.3±1.8	273.4±24.4	2.3±0.2	0	17.7±1.8	3.4±0.8	4.6±1.1
Au-2	13.1±1.7	101.7±2.6	248.1±28.0	2.0±0.3	0	18.2±2.6	3.4±0.6	4.4±0.8
Au-3	9.6±1.2	107.4±1.6	298.6±35.0	2.4±0.2	0	12.6±1.6	4.7±0.9	6.9±1.5
Au-4	12.2±0.6	103.6±1.4	272.4±12.6	2.2±0.2	0	16.2±1.0	7.0±0.8	9.4±1.1

Note. A – total motor activity; B – motor activity (cal); C – distance (cm); D – speed of motion (cm/s); E – nonmotile moment; F – vertical struts; G – number of the studied holes; H – time of research reactions (C).

TABLE 3

Effect of gold nanoparticles on the CNS activity of mice (C57BL/6j mice) after 30 days after a single-shot intraperitoneal injection

Groups	A	B	C	D	E	F	G	H
Control	16.5±1.4	57.5±6.5	109.0±19.8	0.9±0.15	0	2.2±0.4	0.5±0.2	0.7±0.3
Au-1	16.3±1.3	79.4±7.2	169.3±26.0	1.3±0.2	0	4.0±0.8	1.1±0.3	1.1± 0.5
Au-2	16.5±1.1	123.1±3.8	169.1±20.0	1.4±0.2	0	5.1±0.9	3.0±0.7	3.6±0.8
			$P < 0.02$	$P < 0.03$		$P < 0.03$	$P < 0.01$	$P < 0.01$
Au-3	19.1±1.3	82.5±4.4	189.5±24.3	1.6±0.25	0	5.5±1.6	4.2±0.75	5.1±1.0
		$P < 0.003$	$P < 0.02$	$P < 0.02$		$P < 0.04$	$P < 0.01$	$P < 0.005$
Au-4	15.9±1.2	86.9±6.6	190.4±34.5	1.5±0.3	0	6.0±1.3	2.4±0.6	6.8±3.7
		$P < 0.02$					$P < 0.03$	

Note. *P* is the reliability in relation to the intact control.

The injection of colloidal gold nanoparticles in mice of both lines did not exert toxic effects on peripheral blood indexes.

When analysing the data of the pathomorphological investigation it was concluded that when intraperitoneal introducing to animals of colloidal gold nanoparticles in the organs under study (heart, lungs, liver, kidneys, spleen, brain) signs of toxicity were not identified. However, at the microscopic and macroscopic studies, it was found that after 30 min and 24 h after the injection of Au-3 (22 nm) and Au-4 (30–40 nm), the main distinction were detected in animals in the spleen.

Thus, in the intact animals, significant changes in the structure of the red and white pulp of spleen tissue are not observed (Fig. 2), whereas after the introduction of colloidal gold nanoparticles, after 30 min and 24 h pronounced hyperplasia of the white pulp is observed. Lymphoid couplings are predominantly formed by small lymphocytes. In the sinuses of the red pulp, a large number of macrophages, leukocytes and erythrocytes are observed (Fig. 3). Such a short-time, during a day, disturbance in the cell structures of the spleen can be attributed to a stimulation of the immune response to the injection of gold nanoparticles. After 30 days after the introduction of colloidal gold nanoparticles, the ratio of structural elements of the red and white pulp is returned to the original (control) level (Fig. 4).

In the liver 24 h after the injection of gold particles, adaptive changes in the form of agitation of the nuclei (observed differences in size of nuclei), the emergence of a large num-

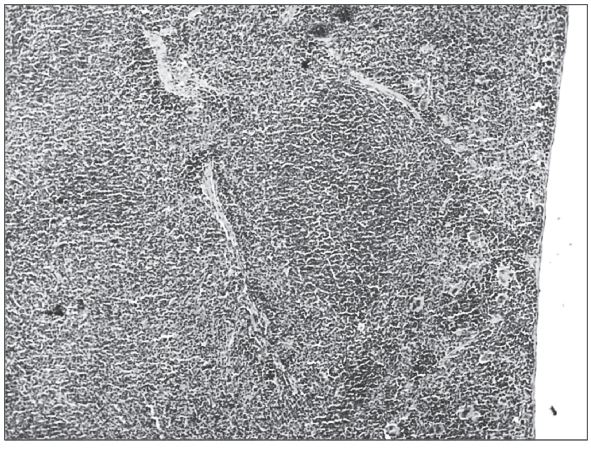

Fig. 2. Spleen of the control mice. Ratio of the white and red pulp is not changed. Hematoxylin and eosin staining. Magn. of 100×.

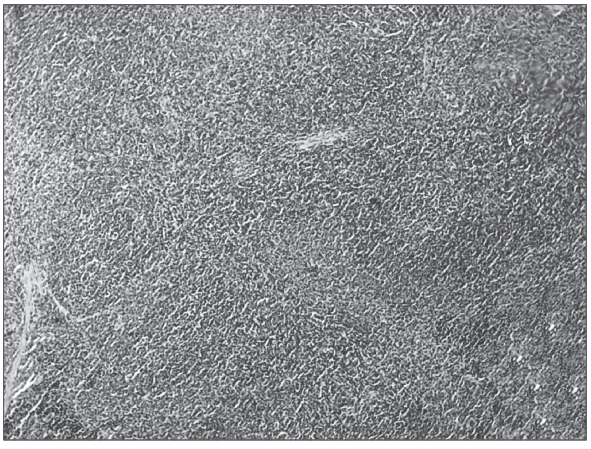

Fig. 3. Spleen of the mouse in 30 min after the injection of gold. Hyperplasia of the white pulp. Hematoxylin and eosin staining. Magn. of 100×.

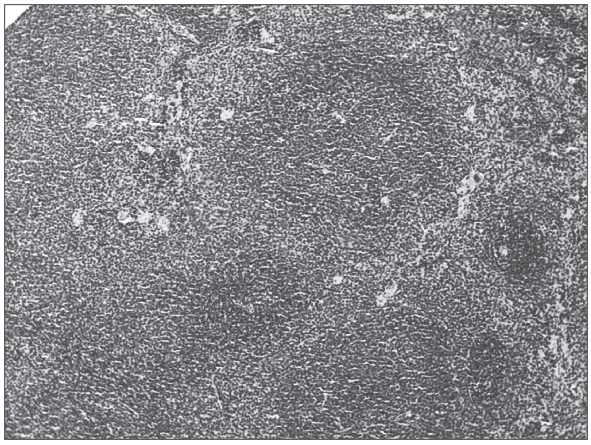

Fig. 4. Spleen of the mouse after 1 month after the injection of gold. Ratio of the white and red pulp is not changed. Hematoxylin and eosin staining. Magn. of 100×.

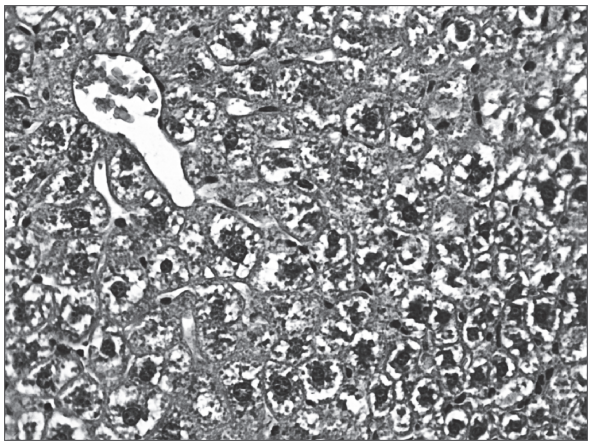

Fig. 5. Liver of the mouse after 24 h after the injection of gold. Hyaline-drop and hydropic dystrophy of hepatocytes. Hematoxylin and eosin staining. Magn. of 400×.

ber of two-nuclear hepatocytes, cells with giant nuclei, and also the increase of the mitotic activity are revealed (Fig. 5). This can be explained by the ability of the preparations to bind with nuclear and cytoplasmic molecules that are accompanied by growth of the smooth cytoplasmic network [20]. After 30 days, everything completely comes to normal. The indicated changes do not lead to the structural reconstruction of the organs and, as a rule, are cut short after discontinuation of the preparation. As a whole, gold nanoparticles do not have a toxic effect on the condition of the studied organs.

CONCLUSION

1. It has been established that a single-shot intraperitoneal injection of colloidal gold solutions with a various particle size from 10 to 40 nm (samples Au-1, Au-2, Au-3, Au-4) during a month does not exhibit the toxic impact generally on the organism, does not change indicators of the mass and temperature of the body; does not exert the toxic effect on the parameters of the spontaneous motor activity and research reaction of white outbred mice, but for C57BL/6j mice contributes to increasing the stimulating activity of CNS, which indicates the species-specify of the impact of these particles on the organism, and besides, to a greater extent, of size particles the of 22 nm (Au-3).

2. The nanoparticles injection of colloidal gold solutions in mice of both lines did not exert the toxic impact on the indices of the peripheral blood.

3. The injection of nanoparticles of colloidal gold solutions leads to a short-time stimulation of the immune response in the tissues of the spleen, but does not exert the toxic effect on the condition of the kidney, liver, heart, and brain.

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Синтез и оценка безопасности наночастиц золота в эксперименте *in vivo*

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Аннотация

В продолжение работ по токсикологии нано углеродных материалов выполнены исследования безопасности наночастиц коллоидного золота размером от 10 до 40 нм на двух линиях мышей (беспородные и линия C57Bl/6j). Показано, что через 24 ч и 30 сут после однократного парентерального введения наночастиц коллоидного золота мышам токсического воздействия на показатели периферической крови, поведение животных, а также на внутренние органы не было выявлено.

Ключевые слова: наночастицы золота, токсичность, периферическая кровь, поведение, микроскопическое исследование, мыши беспородные, мыши линии C57Bl/6j