Mutagenic Effect of Gold Nanoparticles in the Micronucleus Assay

D. S. Jumagazieva, G. N. Maslyakova, L. V. Suleymanova, A. B. Bucharskaya, S. S. Firsova, B. N. Khlebtsov*, G. S. Terentyuk, S. M. Kong, and N. G. Khlebtsov*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 6, pp. 677-680, June, 2011
Original article submitted April 14, 2010

Mutagenic activity of gold nanoparticles of different sizes were studied by micronucleus assay. Karyological analysis was performed and the count of micronucleoli in interphase bone marrow polychromatic erythrocytes in white outbred rats was determined. The animals orally received gold nanoparticles 16 and 55 nm in diameter and gold nanoshells 160 nm in diameter once a day for 7 days in a dose of 0.25 mg gold/kg. To ensure stability and biocompatibility, the surface of nanoparticles was functionalized with polyethylene glycol molecules. There were no significant differences in the number of micronucleoli in comparison with the control group, which suggests that gold nanoparticles of the specified size administered orally in the specified doses do not exhibit mutagenic activity.

Key Words: *gold nanoparticles; micronucleus assay; bone marrow; mutagenic activity*

Gold nanoparticles (GNP) are now intensively used in various fields of nanomedicine in diagnostic and therapeutic purposes [1,2]. Simultaneously with the beginning of the medical use of GNP, questions arose on their distribution in the body, circulation, pharmacokinetics, and excretion, as well as on their possible toxicity for the whole organism or at the cellular and gene levels. Discrepancies in the opinions and conclusions on these issues can be explained by short duration of the experiments, considerable variation in their designs (animal models, doses applied, administration routes), the use of different size and types of GNP and different methods of their functionalization, different tests for toxicity, *etc*.

Most studies are devoted to evaluation of GNP toxicity in experiments with cell cultures *in vitro*. MTT-test based on the ability of cell enzymes to reduce NBT to formazan depending on respiratory activity of cells is most often used for evaluation of toxicity [14].

The data obtained for GNP in *in vivo* animal experiments are scanty. Toxicity of 13-nm GNP coated with polyethylene glycol (PEG) was studied in mice receiving single intravenous injection of GNP in doses of 0.17, 0.85, and 4.26 mg/kg [3]. GNP were shown to cause symptoms of acute inflammation and apoptosis in liver cells. The toxic effects of GNP (3-100 nm) intraperitoneally injected to mice in a dose of 8 mg/kg/week were assessed. It was found that GNP with diameters of 3, 5, 50, and 100 nm produced no toxic effects, while GNP with diameters of 8, 12, 17, and 37 nm induced increased fatigue, loss of appetite, change of fur color, and weight loss [2]. These findings are not consistent with the previously reported data [8] on bioaccumulation and cytotoxicity of 12.5-nm GNP in mice receiving daily intraperitoneal injections of these particles in doses of 0.04, 0.2, and 0.4 mg/kg/day for 8 days. The authors found no evidence of toxicity of GNP including survival, behavior, animal weight,

V. I. Razumovsky Saratov State Medical University; *Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia. ***Address for correspondence:*** gmaslyakova@mail.ru. G. N. Maslyakova; khlebtsov@ibppm.sgu.ru. N. G. Khlebtsov

0007-4888/11/1516-0731

organ morphology, blood biochemistry, and tissue histology. This example is typical for a small array of currently available data on experiments *in vivo*.

Here we studied the mutagenic effect of GNP of various sizes on polychromatic erythrocytes (PCE) of the bone marrow in white outbred rats using the micronucleus assay based on the count of micronuclei in bone marrow PCE. The use of this test for evaluation of the mutagenic effect of drugs can be explained by its simplicity and stability of the obtained results in comparison with other methods of chromosome analysis. PCE can be easily recognized, have a short life cycle, and any micronucleus they contain is a consequence of chromosomal aberrations in the blast cells (spontaneous or induced by the test agents) [15].

MATERIALS AND METHODS

GNP were synthesized at the Laboratory of Nanobiotechnology, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Medical Sciences: particles of colloidal gold (16 and 55 nm in diameter, concentration of 1.3×10^{12} and 3.5×10^{10} particles/ml, respectively; gold concentration 57 μg/ml) and gold nanoshells (GNS, 160 nm in diameter) consisting of a 120-nm silica core and a 20-nm-thick gold shell (concentration 2×10^{9} particles/ml or 47 μg/ml). The mean size of GNP was determined by electron microscopy on a Libra-120 Electron Microscope (Carl Zeiss; Fig. 1).

GNP were prepared by the citrate method after Frans [1] by reduction of $HAuCl_4$ (Aldrich) with sodium citrate. GNS were obtained using a two-step protocol [7]; first, silica cores of the required size and concentration were synthesized and then, gold shells of the required thickness were formed on surfaces the by reduction of $HAuCl_4$ with formaldehyde on 2-4-nm gold nucleation sites previously adsorbed on the surface of aminated silica cores.

For improving bioavailability and biocompatibility of the nanoparticles, they were conjugated with PEG-SH (Nektar). To this end, 45 μl 0.2 M potassium carbonate and 500 μl 5 mM methyl-poly(ethylene glycol)-thiol were added to 50 ml GNP suspension (nanospheres or GNS). Covalent binding of thiol groups to the surface of the gold shell yields conjugates. The reaction time is approximately 10 hours. The conjugates were twice washed from the excess of reaction products with 0.9% NaCl by centrifugation and resuspension.

White outbred male rats (n=24) weighing 180-200 g (6 animals in each experimental and control group) were used for the experiment. The experimental rats orally received GNP of various sizes (1 ml) once a day for 7 days: GNS with diameters of 16, 55, and 160 nm were administered to rats of groups 1, 2, and 3, respectively. Controls daily received 1 ml saline. The cells were fixed 24 h after the last dose.

Bone marrow smears were stained with 5% Giemsa stain and then poststained with diluted May–Grünwald stain. Micronuclei were counted in 1000 PCE.

RESULTS

In bone marrow smears of the control rats, single PCE with 1-2 micronuclei were found, on average 2.5±0.7 per 1000 cells (Fig. 1, *4-6*). In the bone marrow of experimental rats, the frequency of cells containing two or more micronuclei was slightly higher. In rats receiving 160-nm GNS, cells with 3 micronuclei were detected (Fig. 1, *6*). The number of cells with micronuclei in groups 1, 2, and 3 was 3.3±0.3, 3.7±0.7, and 3.8±0.6, respectively, per 1000 bone marrow PCE. There were no significant differences between the control group (2.5±0.4) and any experimental group, which indicates the absence of mutagenic activity of investigated GNP in this assay.

Our results are consistent with *in vitro* experiments [2,9,10] demonstrating that toxic effects are observed for particles of about 1.4 nm in diameter corresponding to Au_{55} atomic clusters, whereas particles of typical colloidal size 15-50 nm and larger (up to 150 nm) can be considered as non-toxic up to micromolar concentrations. Cytotoxicity of small particles can be associated with similar diameter (1.4 nm) of Au_{55} cluster and B-form DNA helix [12]. Because of this spatial matching, 1.4 nm GNP can bind to DNA and influence transcription and replication.

Size distribution of GNP after intravenous injections to animals was studied in many papers [4], including biodistribution of 160-nm GNS [13] used in this study. Although in our study [13] morphological changes in rat organs after intravenous administration of GNP depended on particle size, no appreciable toxic effects were detected. The liver and the spleen are the primary sites for accumulation of intravenously injected 10-100-nm GNP after [4,13]; slow kinetics of GNP elimination (up to 3 months) can cause inflammation in the target organs. However, it is also important to consider the dose, type of administration, and surface functionalization of GNP with stabilizers.

Our experimental conditions were similar to those of experiments [6], in which biodistribution of 4-, 10-, 28-, and 58-nm non-functionalized GNP was studied by neutron activation after administration to mice as drinking in suspension *ad libitum* for 7 days with a concentration of GNP 200 μg/ml. It was found that the smallest 4-nm GNP can be absorbed in the gastrointestinal tract and redistributed to 9 studied organs in

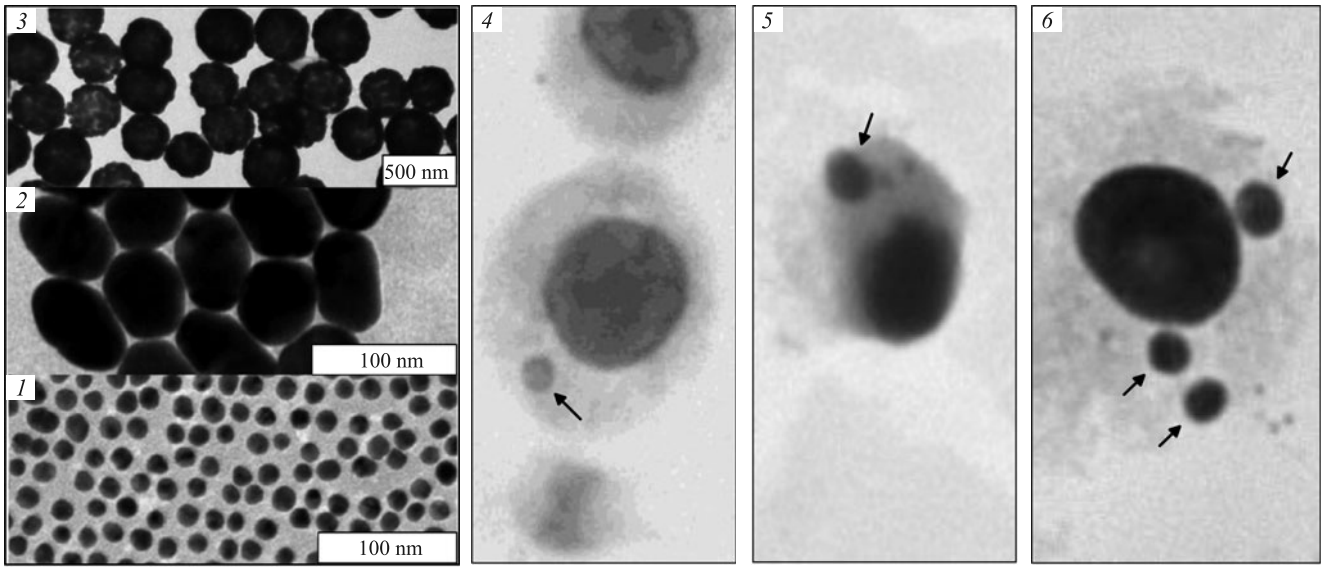

Fig. 1. Electron microscopic images of 16-nm (*1*) and 55-nm (*2*) GNP and 160 nm GNS on 120 nm silica spheres (*3*). Images of bone marrow PCE from a control group rat (*4*) and rats receiving 16-nm GNP (*5*) and 160-nm GNS (*6*). May–Grünwald–Giemsa staining, ×1000. The arrows show micronuclei.

significant quantities from 15 to 75 ng/g. The effect of penetration from the gastrointestinal tract to other organs decreased with increasing particle size, and the level of gold for 10 and 58 nm GNP (which is compatible with our 16 and 55 nm GNP) was about 15-20 ng/g of organ weight in the stomach and small intestine. GNP with a diameter of 10 nm in significant quantities were found only in the spleen (17 ng/g). These small amounts of GNP cannot cause significant toxic effects. This may explain the fact that under our experimental conditions (oral administration in a daily dose 0.25 mg gold/kg) we revealed no significant changes in the amount of micronuclei in bone marrow PCE of rats treated with GNP compared to the control. Detection of significant toxicity in previous studies [2,3] is probably associated with substantially higher doses (0.8-8.0 mg/kg) and intravenous [3] or intraperitoneal [2] administration.

Micronucleus assay can be used to assess toxicity of GNP as an additional informative and easy method for evaluating mutagenic activity. The obtained preliminary results attest to the absence of mutagenic activity of 16-, 55-, and 160-nm GNP administered orally in the specified doses. For more information on size-dependent cytotoxicity of GNP *in vivo,* further studies in animal models with a wider range of nanoparticle sizes are necessary.

This work was partially supported by Russian Foundation for Basic Research (grants No. 08-02-00399a and 09-02-00496-a), Federal Target Programs Development of scientific potential of higher education (contract № 2.2.1.1/2950), Scientific and Pedagogical Cadres of Innovative Russia (contract No. 02.740.11.0484) and grant of the President of the Russian Federation (MK-684.2009.2).

REFERENCES

1. L. A. Dykman, V. A. Bogatyrev, S. Y. Shchegolev, and N. G. Khlebtsov, *Gold Nanoparticles: Synthesis, Properties and Biomedical Applications* [in Russian], Moscow (2008).
2. Y. S. Chen, Y. C. Hung, I. Liau, and G. S. Huang, *Nanoscale Res. Lett.*, **4**, No. 8, 858-864 (2009).
3. W. S. Cho, M. Cho, J. Jeong, *et al*., *Toxicol. Appl. Pharmacol*., **236**, No. 1, 16-24 (2009).
4. W. H. De Jong, W. I. Hagens, P. Krystek, *et al*., *Biomaterials*, **29,** No. 12, 1912-1919 (2008).
5. J. A. Edgar and M. B. Cortie, *Gold: Science and Applications*, Eds. C. Cortie and R. Holliday, Boca Raton (2010), pp. 369-397.
6. J. F. Hillyer and R. M. Albrecht, *J. Pharm. Sci*., **90**, No. 12, 1927-1936 (2001).
7. B. N. Khlebtsov and N. G. Khlebtsov, *Nanotechnology*, **19**, 435703 (2008).
8. C. Lasagna-Reeves, D. Gonzalez-Romero, M. A. Barria, *et al*., Biochem. Biophys. Res. Commun., **393**, No. 4, 649-655 (2010).
9. N. Lewinski, V. Colvin, and R. Drezek, *Small*, **4**, No. 1, 26-49 (2008).
10. Y. Pan, S. Neuss, A. Leifert, *et al*., *Small*, **3**, No. 11, 1941-1949 (2007).
11. N. R. Panyala, E. M. Peña-Méndez, and J. Havel, *J. Appl. Biomed*., **7**, 75-91 (2009).
12. M. Semmler-Behnke, W. G. Kreyling, J. Lipka, *et al*., *Small*, **4**, No. 12, 2108-2111 (2008).
13. G. S. Terentyuk, G. N. Maslyakova, L. V. Suleymanova, *et al*., *J. Biophotonics*, **2**, No. 5, 292-302 (2009).
14. S. Wang, W. Lu, O. Tovmachenko, *et al*., *Chem. Phys. Lett*., **463**, 145-149 (2008).
15. K. L. Witt, E. Livanos, G. E. Kissling, *et al.*, *Mutat. Res*., **649**, Nos. 1-2, 101-113 (2008).