Advances in Natural Sciences:
Nanoscience and Nanotechnology

PAPERS • OPEN ACCESS

Biodistribution of gold nanoparticles synthesized by *γ*-irradiation after intravenous administration in mice

To cite this article: Quang Luan Le *et al* 2014 *Adv. Nat. Sci: Nanosci. Nanotechnol.* **5** 025009

OPEN ACCESS
IOP Publishing | Vietnam Academy of Science and Technology Advances in Natural Sciences: Nanoscience and Nanotechnology
Adv. Nat. Sci.: Nanosci. Nanotechnol. **5** (2014) 025009 (5pp) doi:10.1088/2043-6262/5/2/025009

Biodistribution of gold nanoparticles synthesized by γ-irradiation after intravenous administration in mice

Quang Luan Le[1], Thi Phuong Linh Do[2], Huynh Phuong Uyen Nguyen[1], Van Phu Dang[3] and Quoc Hien Nguyen[3]

[1] Center for Nuclear Techniques, 217 Nguyen Trai Street, 1 District, Ho Chi Minh City, Vietnam
[2] Institute of Malarialogy—Parasitology—Entomology in Ho Chi Minh City, 699 Tran Hung Dao Street, 5 District, Ho Chi Minh City, Vietnam
[3] Research and Development Center for Radiation Technology, 202A, 11 Street, Linh Xuan Ward, Thu Duc District, Ho Chi Minh City, Vietnam

E-mail: hien7240238@yahoo.com

Received 7 February 2014
Accepted for publication 12 March 2014
Published 7 May 2014

Abstract

In the present research work we evaluate the *in vivo* distribution of gold nanoparticles (AuNPs) at different time durations after intravenous administration in mice. AuNPs with size of about 20 nm and concentration of 1 mM were synthesized by gamma irradiation method using 0.5% alginate as a stabilizer. AuNPs were characterized by UV-Vis spectrum and transmission electron microscope (TEM) image. The as-synthesized AuNPs solution was centrifuged to concentrate to 2 mg AuNPs/1 ml solution. Intravenous administration of AuNPs in mice was done at the tail with 1 mg AuNPs (0.5 ml). After 1, 3, 6 and 12 h of injection, blood was collected, mice were sacrificed and various tissues/organs were removed. The blood haematology and serum clinical chemistry indexes of mice intravenously injected with AuNPs were not significantly different compared to those of the control ones. In addition, gold content in the samples was quantitatively determined by k_0-neutron activation analysis (k_0-NAA) at nuclear research reactor, Da Lat Vietnam. Results showed that after 1 h of administration, AuNPs were mainly accumulated in blood (41.56%), in liver (51.60.%), in lung (6.16%) and in kidney (0.53%). After that the content of AuNPs in blood was decreased to nearly normal at 6 h while the content of AuNPs in liver, lung and kidney was accumulatively increased. After 6 h of administration AuNPs were mainly accumulated in organs like liver (76.33%), lung (11.86%) and kidney (2.23%). Thus, the obtained results are practically useful for using AuNPs as x-ray contrast agent, especially for blood and liver.

Keywords: gold nanoparticles, γ-irradiation, *in vivo* distribution, mice

1. Introduction

Colloidal gold nanoparticles (AuNPs) are emerging as a lead candidate of the nanocarriers in the field of nanopharmceuticals, as AuNPs have good biocompatibility and can be conjugated to protein and other molecular species without altering their biological activity [1]. AuNPs are used for many applications especially in bioscience and medical fields [2, 3] such as cancer diagnose and therapy [4–8], carriers for transmucosal delivery of insulin [9], x-ray contrast agent [10] etc. AuNPs can also be used in the cosmetics field particularly for facial mask or cream because AuNPs help to improve blood circulation, skin elasticity, and can rejuvenate the skin and reduce the formation of wrinkles [11]. Different methods can

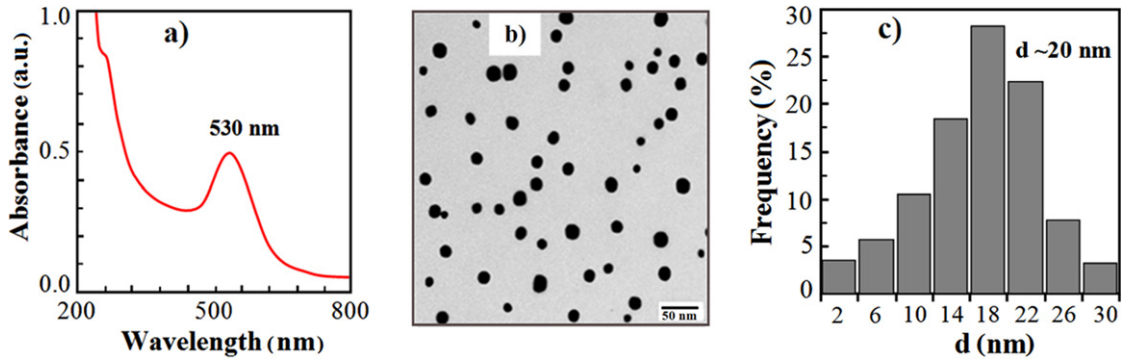

Figure 1. UV-Vis spectrum (a), TEM image (b) and size distribution (c) of 1 mM AuNPs/0.5% alginate.

be used for synthesis of AuNPs and compared to other methods, gamma Co-60 ray irradiation is considered as an effective method due to several advantages such as: (1) the reaction is carried out at room temperature; (2) yield of AuNPs is high; (3) AuNPs can be purely prepared without contamination of excessive chemical reductant and Au^{3+} ions residue; (4) the size of AuNPs is easily controlled by varying Au^{3+} ions or seed enlargement approach [12, 13]; (5) mass production can be carried out and (6) processing is satisfied to requirement of clean production [14]. According to Lasagna-Reeves *et al* [15], for applications of AuNPs in therapy and drug delivery it is necessary to study the bioaccumulation and toxic effect of different doses as well as to choose the appropriate size of AuNPs.

In the present study, AuNPs of 20 nm and concentration of 1 mM were synthesized by gamma Co-60 irradiation method using alginate as stabilizer, as reported in our previous paper [12]. Intravenous administration of AuNPs in mice was done and blood haematology and serum clinical chemistry indexes were investigated. In addition, AuNPs accumulation in blood, liver, lung and kidney after intravenous administration was also determined.

2. Experimental

2.1. Materials

Hydrogen tetrachloroauratetrihydrate ($HAuCl_4 \cdot 3\,H_2O$), pure water from Merck, Germany and sodium alginate ($M_n = 4.35 \times 10^5$ g mol^{-1}) from Hayashi Pure Chemical Industry Ltd, Japan were used as received. Glassware was treated with regia solution (1 V HNO_3 : 3 V HCl), washed with pure water and dried.

2.2. Preparation of Au^{3+}/alginate solution and irradiation

Two stock solutions, particularly 10 mM Au^{3+} and 2% alginate were prepared by dissolving $HAuCl_4 \cdot 3H_2O$ and alginate into water. Au^{3+} 1 mM/alginate 0.5% solution was prepared by pouring Au^{3+} solution into alginate solution with desired concentration while stirring for about 10 min. Then the prepared Au^{3+}/alginate solution of 200 ml was put into a glass bottle with plastic cap. Irradiation was carried out on the gamma Co-60 irradiator STSVCo-60/B at a dose of 8 kGy with dose rate of 1.3 kGy h^{-1} at VINAGAMMA Center in Ho Chi Minh City.

2.3. Characterization of AuNPs/alginate

The absorption spectrum of AuNPs solution, which was diluted by water to 0.1 mM calculated as Au^{3+} concentration, was taken on a UV-Vis spectrophotometer model UV-2401PC, Shimadzu. The size and size distribution of the AuNPs were characterized by TEM images on transmission electron microscope (TEM) model JEM1010 (JEOL, Japan) and statistically calculated from about 300 particles.

2.4. Intravenous administration in mice

Mice of the Swiss line were supplied by Pasteur Institute of Ho Chi Minh City and fed at Institute of Malarialogy-Parasitology-Entomology in Ho Chi Minh City for 8 weeks and their average body weight was about 30 g. The mice were then injected with 0.5 ml AuNPs solution containing 1 mg gold via a tail vein. The control mice were also intravenously injected by 0.5 ml of 0.5% alginate solution without AuNPs.

2.5. Haematology analysis

The blood of mice after intravenous AuNPs injection at 0, 1, 3, 6 and 12 h was collected and put into tubes containing heparin for analysis. Haematology indexes including haematocrit (Htc), haemoglobin (Hb), total white (WBC) and red (RBC) blood cell counts, lymphocytes (Lympho), monocytes (Mono), and granulocytes (GR) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were analyzed by the 18 parameters celltac α (Nihon Kohden, Japan) automatic haematology analyzer.

2.6. Serum clinical chemistry analysis

The blood of injected mice was collected in the same way as haematology analysis. The blood was then centrifuged at 5000 rpm for 10 min to separate the serum for analysis. Serum clinical chemistry indexes including glucose (Glu), creatine

Table 1. Blood haematology of mice intravenously injected with 1 mg AuNPs.

Treatment	Hct (%)	Hgb (g/dL)	RBC ($10^6/\mu$l)	MCV (fL)	MCH (Pg)	MCHC (g/dL)	WBC ($10^3/\mu$l)	Mono (%)	Lympho (%)	GR (%)
1 h control	40.03	13.9	4.59	87.56	30.30	34.73	6.39	0.25	2.29	3.86
1 h injected	40.66	14.7	4.76	87.16	33.23	34.00	6.43	0.30	2.30	3.83
Statistically	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
3 h control	43.40	14.1	5.38	86.00	26.96	33.50	6.59	0.26	2.43	3.90
3 h injected	46.90	16.5	5.50	85.33	30.20	35.10	7.11	0.31	2.93	3.86
Statistically	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
6 h control	39.53	14.1	4.13	95.00	33.33	35.40	6.30	0.44	2.16	3.69
6 h injected	40.86	14.9	4.40	92.96	33.86	36.56	6.23	0.43	2.13	3.66
Statistically	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
12 h control	40.93	13.6	5.32	77.00	25.66	33.23	6.40	0.45	2.23	3.93
12 h injected	37.53	13.4	5.43	69.30	26.70	35.70	6.62	0.46	2.30	3.90
Statistically	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

NS: No significant difference by T-test with probability level >0.95.

Table 2. Serum clinical chemistry of mice intravenously injected with 1 mg AuNPs.

Treatment	GLU (mg/dl)	AST (unit/l)	ALT (unit/l)	Ure (mg/dl)	CR (mg/dl)	ALB (g/dl)	TP (g/dl)	TBLI (mg/dl)
1 h control	160.00	41.33	47.13	27.93	0.18	3.53	6.23	0.17
1 h injected	208.67	42.00	54.67	32.67	0.32	3.47	4.97	0.21
Statistically	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
3 h control	222.00	38.66	45.67	24.93	0.20	2.63	5.73	0.29
3 h injected	243.33	42.00	55.67	20.06	0.92	2.83	5.93	0.25
Statistically	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
6 h control	182.00	31.33	46.30	28.13	0.17	2.50	5.73	0.31
6 h injected	210.00	32.67	37.73	29.73	0.19	2.53	5.23	0.28
Statistically	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
12 h control	119.67	29.00	46.67	28.33	0.17	2.59	4.96	0.29
12 h injected	125.33	30.33	47.00	32.30	0.15	2.91	5.80	0.30
Statistically	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

NS: No significant difference by T-test with probability level >0.95.

(CR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), globin (GLB) and albumin (ALB) were analyzed by BioSystem A17 analyzer (Belgium).

2.7. Gold analysis

The mice after intravenous AuNPs injection were killed and the liver, kidney, lung and blood at 0, 1, 3, 6 and 12 h were collected. The tissues were then dried at 180 °C for 5 h and the content of gold in each tissue was quantitatively analyzed at Da Lat Nuclear Research Institute by k_0-neutron activation analysis (k_0-NAA) method as described by Abd *et al* [17].

3. Results and discussion

3.1. Synthesis and characterization of AuNPs

The UV-Vis spectrum of AuNPs solution in figure 1(a) showed the maximum absorption wavelength (λ_{max}) at 530 nm for Au^{3+} concentration of 1 mM. The TEM image and particles size distribution are shown in figures 1(b) and (c). The average diameter of AuNPs was determined to be about 20 nm with a fairly narrow Gaussian distribution.

Different AuNPs sizes can be pre-selected by varying Au^{3+} concentration and/or seed enlarge-ment approach. [12–14, 16] According to Anh *et al* [12] gamma Co-60 irradiation method is suitable for production of AuNPs with controllable size and high purity.

3.2. Toxicity test

Since the intravenous administration was proposed as the best way for application of AuNPs in medical fields such as cancer diagnostics and therapy [4–8], carriers for transmucosal delivery of insulin [9], x-ray contrast agent [10] etc. The toxicity test of AuNPs after intravenous administration is very important. In this study, 10 blood haematology indexes (Htc, Hb, WBC, RBC, Lympho, Mono, GR, MCV, MCH and MCHC) of intravenous AuNPs injected mice and control mice were analyzed at 1, 3, 6 and 12 h after injection. The results in table 1 show that there was no significant difference in blood haematology indexes between control and AuNPs injected mice.

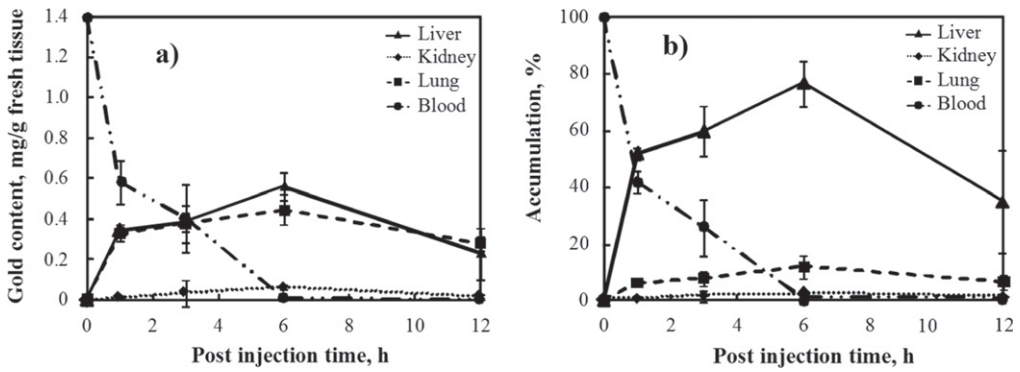

Figure 2. The *in vivo* distribution content (a) and accumulation ratio (b) of AuNPs (d ~ 20 nm) in different tissue of mice after intravenous injection with a dose of 1 mg AuNPs per mouse (30 g).

Table 2 records the serum clinical chemistry indexes of AuNPs injected and control mice. The results also showed that the contents of glucose, creatine, aspartate aminotransferase, alanine aminotransferase, total protein, total bilirubin and albumin of AuNPs injected mice were not significantly different from those of control mice. The analyzed results on haematology and serum clinical chemistry of tested mice proved that AuNPs were not toxic by the intravenous administration at a dose of 1 mg/mouse (30 g). The results are in good agreement with those reported by Hainfeld *et al* [10].

3.3. In vivo distribution of AuNPs

The distribution of AuNPs in different tissues of mice is displayed in figure 2. The results indicated that the concentration of AuNPs in blood decreased with time, the gold content almost disappeared after 6 h injection (0.01 mg per 1 gram fresh tissue) and the blood became normal at 12 h after injection. The results in figure 2 also indicated that at 1 h after injection, AuNPs penetrated into liver, kidney and lung. At 6 h after post injection, AuNPs were mainly distributed in liver and lung tissue, in particular, the gold content in liver tissue was found to be about 0.56 mg per 1 gram fresh tissue and attained 76.33% of injected dose, while at the same time the gold content in lung tissue was found to be about 0.45 mg per 1 gram fresh tissue and attained 11.86% of injected dose. The gold content in kidney was lower than that in liver and lung (0.06 mg per 1 gram fresh tissue and only 2.23% of injected dose). At 12 h after injection, the gold content decreased in all tissues. The results in figure 2 also indicated that after injection, AuNPs transferred from blood into other tissues and mostly accumulated in liver and lung with the maximal concentration at 6 h after injection.

Thus, the AuNPs/alginate product with particle diameter of about 20 nm synthesized by gamma Co-60 irradiation using alginate (0.5%) as stabilizer is not toxic at a dose of about 33.3 mg kg^{-1} body weight of mice. Thus, it can be potentially applied as x-ray contrast agent, especially for blood and liver. In addition, Nghiem *et al* also studied *in vivo* toxicity of 15 nm AuNPs capped by bovine serum albumin (BSA) by tail vein injection for mice with a dose from 1.16 to 5.84 mg kg^{-1} body weight [18]. They concluded that the AuNPs did not produce any mortality or gross behavioral changes in mice at the doses studied; all animals experimented on were healthy and had normal weight development. Porkharkar *et al* reported that LD_{50} (dose required to kill half of the population) value of AuNPs/chitosan was found to be greater than 2000 mg kg^{-1} body weight of rats by oral administration [19]. They concluded that AuNPs/chitosan produced no toxicity in rats that can be exploited for potential therapeutic applications. Furthermore, spherical AuNPs of different sizes (5–70 nm) were reported not to be toxic to human skin cells [20]. Thus, AuNPs/alginate product is promising to apply in biomedicine, cosmetics and in other fields as well.

4. Conclusion

AuNPs with size of about 20 nm were successfully synthesized by gamma Co-60 irradiation method using alginate as a stabilizer. The AuNPs product was not toxic for mice by intravenous injection at a dose of 33.3 mg AuNPs kg^{-1}. The accumulation of AuNPs was mainly concentrated in lung and liver tissue after intravenous injection. The maximal gold content was about 0.56 mg (76.33% of injected dose) and 0.45 mg (11.86% of injected dose) in 1 g fresh lung and liver tissue, respectively. Thus, gamma Co-60 irradiation is suitable tool for production of AuNPs with high purity and AuNPs/alginate can be favorably used in biomedicine and in other fields as well.

Acknowledgement

This research work was supported by the Ministry of Science and Technology of Vietnam (Project No. DTDL.2011-G/80).

References

[1] Sonavane G, Tomoda K and Makino K 2008 *Coll. Surf. B: Biointerfaces* **66** 274
[2] Baptista P *et al* 2008 *Anal. Bioanal. Chem.* **391** 943

[3] Boisselier E and Astruc D 2009 *Chem. Soc. Rev.* **38** 1759
[4] Lee H *et al* 2008 *Biomaterials* **29** 4709
[5] Huang X and El-Sayed M A 2010 *J. Adv. Res.* **1** 13
[6] Mukherjee S *et al* 2012 *Nanotechnology* **23** 455103
[7] Joshi P *et al* 2012 *Coll. Surf. B: Biointerfaces* **95** 195
[8] Geetha R *et al* 2013 *Cancer Nano* **4** 91
[9] Bhumkar D R *et al* 2007 *Pharma. Res.* **24** 1415
[10] Hainfeld J F *et al* 2006 *Brit. J. Radiol.* **79** 248
[11] Fathi-Azarbayjani A *et al* 2010 *AAPS PharSciTech.* **11** 1164
[12] Anh N T *et al* 2010 *Rad. Phys. Chem.* **79** 405
[13] Duy N N *et al* 2013 *Coll. Surf. A: Physico. Chem. Eng. Asp.* **436** 633
[14] Hien N Q *et al* 2012 *Carbohydr. Polym.* **89** 537
[15] Lasagna-Reeves C *et al* 2010 *Biochem. Biophys. Res. Commun.* **392** 649
[16] Li T, Park H G and Choi S H 2007 *Mater. Chem. Phys.* **105** 325
[17] Abd A E *et al* 2009 *J. Nucl. Radiat. Phys.* **4** 21
[18] Nghiem T H L *et al* 2012 *Adv. Nat. Sci.: Nanosci. Nanotechnol.* **3** 015002
[19] Pokharkar V *et al* 2009 *J. Biomed. Nanotechnol.* **5** 233
[20] Wang S *et al* 2008 *Chem. Phys. Lett.* **463** 145