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In vivo toxic studies and biodistribution of near infrared sensitive Au–Au_2S nanoparticles as potential drug delivery carriers

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Abstract Near infrared (NIR) sensitive Au–Au_2S nanoparticles are intensively being developed for biomedical applications including drug and gene delivery. Although all possible clinical applications will require compatibility of Au–Au_2S nanoparticles with the biological milieu, their in vivo capabilities and limitations have not yet been explored. Au–Au_2S nanoparticles and cisplatin-loaded Au–Au_2S nanoparticles were successfully synthesized by the reduction of tetrachloroauric acid ($HAuCl_4$) using sodium sulfide (Na_2S), and cisplatin was loaded onto NIR sensitive Au–Au_2S nanoparticles via an MUA (11-mercaptoundecanoic acid) layer. In this work, acute systemic toxicity in vivo, blood biochemistry assay, and tissue distribution in mice were carried out to further investigate the biocompatibility and biodistribution of these nanoparticles. The results from these studies demonstrated that both of nanoparticles (<200 μg/mL) might have a great advantage in biocompatibility and good biological safety.

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Introduction

Nanostructured materials have long been explored as carriers for delivery of therapeutic and diagnostic agents [1]. This had been attributed to the increased permeability through the tumor vasculature where the cell gap junctions are between 100 nm and 600 nm. Besides the localization at tumors and increased surface-to-volume ratio, nanoparticles exhibited other attractive properties unique to its size. Near infrared (NIR) sensitive nanoparticles [2] with size and shell thickness dependent properties, are being investigated for applications in drug delivery systems [3] and immunoassays [4]. NIR light (λ = 650–1000 nm) with its superior propagation in living tissues and signal to background ratio, had been exploited for biomedical imaging [5, 6], photoablation [7] and photodynamic therapy [8]. NIR light had been reported to travel through 10 cm of breast tissue and 4 cm of skull tissue using microwatt sources [9]. West and co-workers reported photothermal ablation of tumor tissues using silica-gold nanoshells [10]. The accumulated heat due to absorption of continuous NIR laser radiation resulted in selective cell destruction.

Following literatures [11, 12], we have successfully synthesized Au–Au_2S nanoparticles (NPs) with NIR sensitivity. These as-synthesized Au–Au_2S NPs were chemically stable and exhibited two absorption bands at ~520 nm and in the NIR region of 650–1100 nm [13]. Cisplatin was loaded onto Au–Au_2S NPs through an 11-mercaptoundecanoic acid (MUA) layer to develop a targeted drug delivery system by using tissue penetrative NIR light to trigger drug release. Those cisplatin-loaded Au–Au_2S NPs were stable in physiological conditions where pH is about 7.2–7.4, however, when NIR light is applied in therapy, ~90% cisplatin could be released from the nanoparticles [13]. Therefore, we suggested that Au–Au_2S NPs with NIR

sensitivity might show a great promise as drug delivery carriers because the loaded drug might only delivered at tumor which was exposed to NIR light, but not healthy tissue without irradiation. Moreover, our previous in vitro short and long-term data provided preliminary evidence suggesting that cisplatin-loaded Au–Au_2S nanoparticles are non-toxic below the maximum recommended dosage (L. Ren et al. accepted in J Biomed Mat Res A). However, recent toxicological investigations of manufactured nanoparticles revealed such a nature that compared with the larger particles of the same chemical composition (on the identical mass basis), nanoparticles tends to exhibit quite different toxicological effects in vivo [14, 15]. Thus, the present studies were undertaken in order to further investigate the acute cytotoxicity, as well as the biodistribution in vivo of NIR sensitive Au–Au_2S NPs.

Experimental

Synthesis

The growth of Au–Au_2S NPs occurred when the aqueous solutions of $HAuCl_4$ and Na_2S were mixed. Briefly, 20 mL of 2 mM $HAuCl_4$ was respectively mixed with 20 mL of 1 mM Na_2S, and stored at 25 °C for 1 day. The reaction was monitored using a UV-visible spectrophotometer at a range of 400–1100 nm. After centrifuging at 15,000 rpm (Beckman, Avanti J-25), Au–Au_2S NPs were re-dispersed in a 100 mM 11-mercaptoundecanoic acid (MUA) solution in ethanol for 3 days at 40 °C. Excess MUA was removed from solution by at least three repeated cycles of centrifugation at 15,000 rpm, and subsequent re-dispersing in water. Finally, 10 mg of cisplatin was mixed with 10 mL MUA-modified Au–Au_2S NPs by sonication. Afterwards the flask containing the drug carriers was capped and left to stand for 2 days. Determination of the loading degree of cisplatin on Au–Au_2S NPs was carried out by high performance liquid chromatography (HPLC) method [13]. The morphological examination of the as-synthesized nanoparticles was performed by transmission electron microscope (TEM, JEM 2100) at 120 kV.

Toxicity in vivo

KM mice (provided by Anti-Cancer Research Center, Xiamen University), aged 7 weeks and weighting 18–22 g, were used in in vivo experiments. All animal experiments were performed in compliance with the local ethics committee. Following the method provided by the Organization for Economic Cooperation and Development (OECD, guideline 425) [16], a series of doses were set (Table 1) to process the toxicological studies in vivo of Au–Au_2S NPs. The first mouse received a dose one step below the assumed estimate of the LD_{50}. If the animal survived, the second animal received a higher dose. If the first animal dies, the second animal received a lower dose. The signs of toxicity or anaphylactic response after injection of nanoparticles were recorded following US Pharmacopeia [17].

Table 1 Acute systemic toxicity in vivo of Au–Au_2S NPs and cisplatin-loaded Au–Au_2S NPs

Normalized Au–Au_2S NPs (mg/kg)	Absorbed cisplatin (mg/kg)	Status	Passive behavior	Hypopnea	Tremor	Arching of back	Loss of appetite	Diarrhea and vomiting
Au–Au_2S NPs								
1	0	Good	No signs	None	None	None	None	None
2.5	0	Good	No signs	None	None	None	None	None
5	0	Good	No signs	None	None	None	None	None
10	0	Good	No signs	None	None	None	None	None
100	0	Good	No signs	None	None	None	None	None
200	0	Common	Faintness	None	None	None	None	None
300	0	Common	Median	Faintness	Faintness	None	None	None
Cisplatin-loaded Au–Au_2S NPs								
1	30	Good	None	None	None	None	None	None
2.5	75	Good	None	None	None	None	None	None
5	150	Good	None	None	None	None	None	None
10	300	Good	None	None	None	None	None	None
100	3000	Good	Faintness	Faintness	None	None	None	None
200	6000	Common	Faintness	Faintness	Faintness	None	Faintness	None
300	9000	Common	Median	Faintness	Faintness	Faintness	Faintness	None

To examine the changes of biochemical parameters, healthy KM mice were exposed by the intravenous injection with the same dose (10 mg/kg) of Au–Au_2S NPs, cisplatin-loaded Au–Au_2S NPs, respectively. Blood samples were collected via the ocular vein after exposure up to 14 days, and were then centrifuged twice at 3000 rpm for 10 min in order to separate serum. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), alkaline phosphatase (ALP), cholinesterase (CHE), albumin (ALB), total protein (TP), creatinine (Cr), and blood urea nitrogen (BUN) levels of serum were measured by a biochemistry analyzer (Hitachi 7080). Each sample was repeated three times. The differences between the results were analyzed statistically using the two-sample *t*-test.

To examine the pathological changes due to Au–Au_2S NPs injected, the organs of mice such as liver, spleen, lung, and kidney were immediately fixed in 10% formalin and subject to further pathological examinations by dehydrating in a sequence of 50, 70, 95, and 100% ethanol. After clearing in xylene, the tissue was finally embedded in paraffin wax, then 2–3 μm sections were sliced using a microtome (Leica RM2235). After histological H-E staining, the slides were observed and the photos were taken using a fluorescent microscope (Olympus BX41).

Biodistribution in vivo

After filtration through 0.22 μm filter membrane, 200 μL Au–Au_2S or cisplatin-loaded NPs solution (10 mg/kg) was injected into a healthy KM mouse via the tail vein, respectively. In order to make a comparison between biodistribution of Au–Au_2S NPs in a healthy mouse and tumor-bearing one, 0.1 mL mouse sarcoma180 (S180) cell suspensions, which were harvested from the peritoneal cavity of tumor-bearing KM mouse after inoculation for 10 days, at a concentration of 1×10^8/mL was injected subcutaneously in the armpit of healthy KM mice. After tumor initiation for 6 days, 200 μL Au–Au_2S NPs (10 mg/kg) were injected via the intra tumor or the tail vein. Up to injection for 7 days, blood, muscle (left thigh), bone (left femur with marrow), heart, liver, spleen, lung, kidney, brain of KM mice were collected, respectively. Except the blood, each sample was washed thoroughly with deionized water and dried for 4 h at 120 °C [18]. In order to prepare ICP-MS solution, the samples were digested by a microwave accelerated reaction system (CEM MARS 240/50), following the preset protocols. The digested solutions were then qualitative analyzed for Au by using an inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, SCIEX ELAN DRC-e). 10 μg/L Au aqueous solution (purchased from National Research Center for CRM'S) was used as the standard control. Each sample was repeated five times.

To understand the status of Au–Au_2S NPs injected in vivo, the organs of the mice were collected and subsequently fixed with glutaraldyhyde at 4 °C for 24 h. After removing the fixatives by 0.1 M PBS, samples were subsequently post-fixed and stained with 1% osmium tetraoxide in buffer and then dehydrated in an alcohol series, embedded in Epon, and sliced to a thickness of 50–70 nm by using a ultramicrotome (LKB Nova). Images of the slices were finally taken with a transmission electron microscope (TEM, JEM 2100) at 120 kV.

Results and discussion

Synthesis

The NIR-sensitive Au–Au_2S NPs employed for the drug delivery carriers in our work were synthesized by reduction of tetrachloroauric acid ($HAuCl_4$) using sodium sulfide (Na_2S), following earlier methods [11–13]. Typical size distribution of the polygonal particles was estimated to be 20–50 nm (Fig. 1a). Those as-synthesized Au–Au_2S NPs exhibited two absorption bands as illustrated in Fig. 1b. The band I at 527 nm was assigned to the surface plasmon resonance of the Au nanoparticles, whereas the band II at 768 nm (NIR region) was attributed to multiply-twinned Au-rich particles containing S [19]. We also loaded 30 mg cisplatin onto per mg Au–Au_2S NPs via a MUA layer, and the spectrophotometric analysis revealed that there was only <10% reduction for NIR absorbing ability after loading with cisplatin [13].

Toxicity in vivo

Acute systemic toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. The signs of toxicity or anaphylactic response were presented in Table 1. It is seen that as the injected Au–Au_2S NPs concentration varied from 1 mg/kg to 100 mg/kg which dissolved in 0.4 mL PBS, no mice showed any passive behavior, hypopnea, tremor, and arching of back or any symptoms of poising such as loss of appetite, diarrhea, and vomiting to these dosages up to 14 days' of observation; whereas the mice showed a tendency of toxicity that exposed to the concentration ranged from 200 mg/kg to 300 mg/kg. In the cisplatin-loaded Au–Au_2S NPs group, as the dosage was below 200 mg/kg, the mice exhibited the similar tendency as Au–Au_2S NPs group; as the dosage reached 200 mg/kg, the treated mice showed median severity.

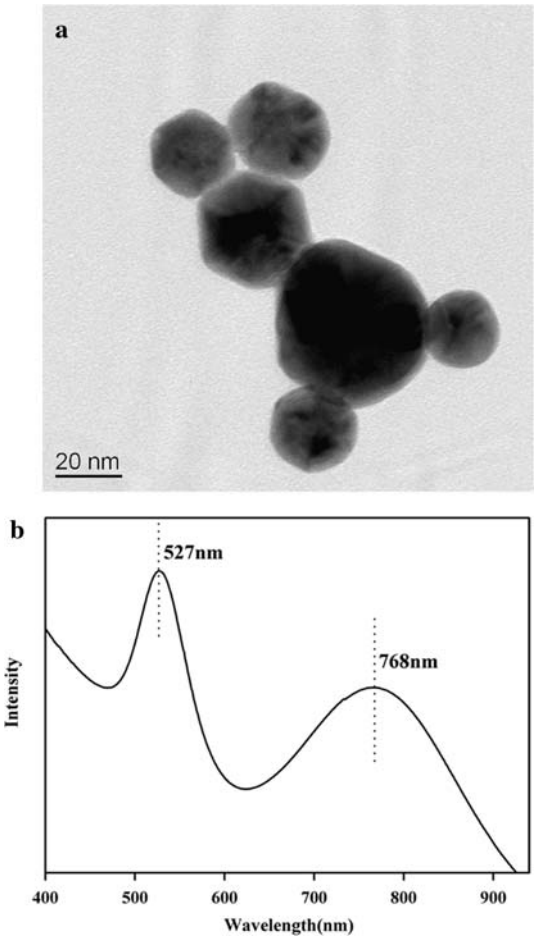

Fig. 1 (**a**) Transmission electron microscopy image, and (**b**) UV–vis spectrum of Au–Au_2S NPs

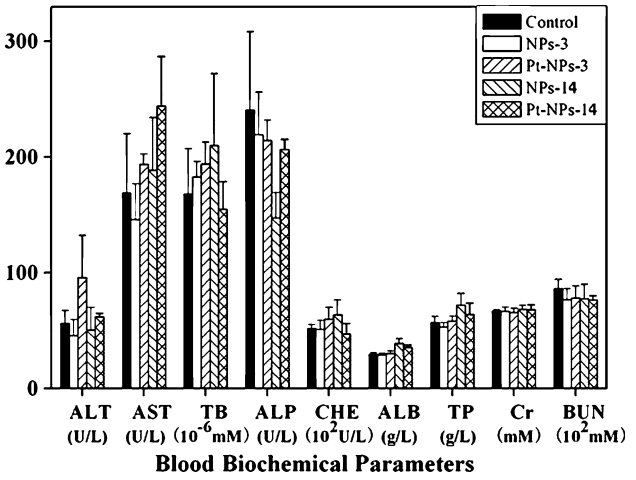

Fig. 2 Statistic results of blood biochemical parameters of KM mice exposed to Au–Au_2S NPs (NPs) and cisplatin-loaded Au–Au_2S·NPs (Pt-NPs) for 3 days and 14 days, respectively. Control: mice without any treatment

Blood biochemical testing is used extensively both in diseases that have an obvious metabolic basis and those in which biochemical changes are consequence of the diseases [20]. To date most biochemical studies in mice have been used for toxicological testing [21]. In our experiments, blood biochemical parameters which reflect the hepatic (ALT, AST, TB, ALP, CHE, ALB, TP) and renal (Cr and BUN) functions were further investigated. No statistically significant difference between the control and nanoparticles treated mice groups was observed in Fig. 2 for 3rd day and 14th day, respectively. Thus, both of Au–Au_2S NPs and cisplatin-loaded Au–Au_2S NPs do not cause any abnormality (relevant to dysfunction of liver and kidney) in the blood biochemical parameters.

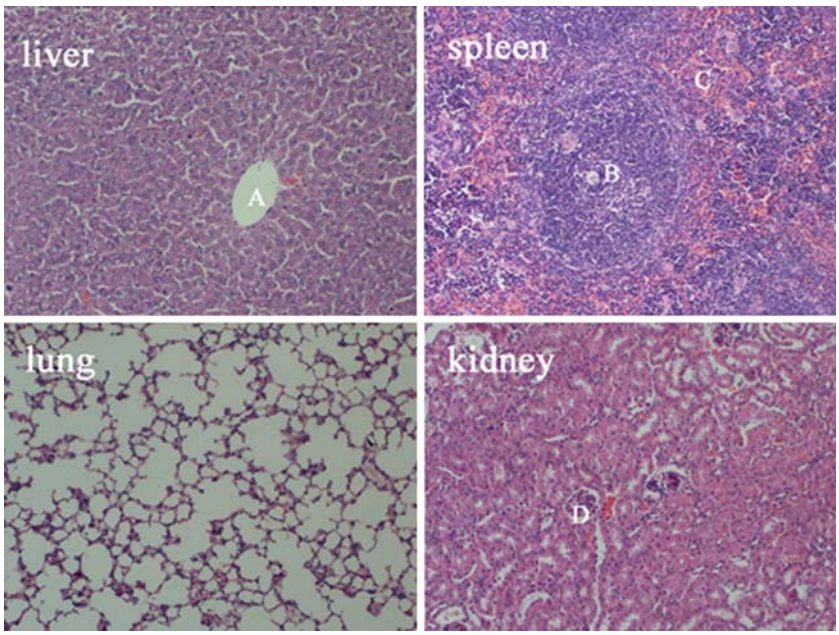

Fig. 3 The microscopic pictures (×100) of liver, spleen, lung, and kidney after injection of Au–Au_2S NPs for 14 days. A: central vein; B: white pulp; C: red pulp; D: glomeruli

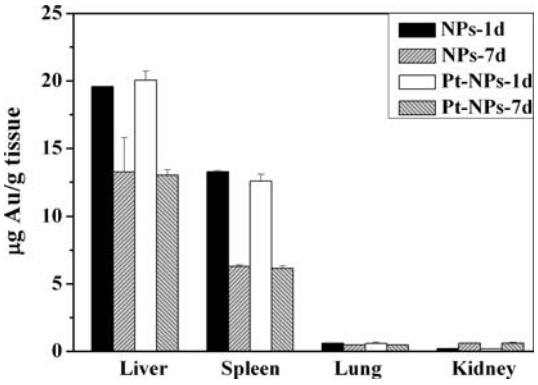

Fig. 4 In vivo biodistribution of Au–Au_2S NPs (NPs) and cisplatin-loaded Au–Au_2S NPs (Pt-NPs) in healthy KM mice after injection for 1 day (1d) and 7 days (7d), respectively

Figure 3 showed the microscopic pictures of liver, spleen, lung, and kidney at 14th day of the injection. There was no inflammation or active immunocyte congregating in these organs. In the case of the liver, the polygonal cells are hepatocytes joined to one another, and the lobule is the structural unit with a central vein (A) in the middle. In terms of spleen, white pulp (B) is equivalent to the lymphocyte population, in the form of the periarteriolar lymphocyte sheath. Red pulp (C) is everything else, which means the splenic cords and the sinuses between them. There are bronchioles, alveolar ducts and alveoli in the lung section, with alveolar cells and capillaries in the alveolar walls. On the kidney section, the renal cortex contains glomeruli (D), other vessels, tubules and interstitium. Thus, no pathological changes were found in each organ such as liver, spleen, lung, and kidney, which showed similar to the control group.

Biodistribution in vivo

The results of the biodistribution in vivo for Au–Au_2S NPs and cisplatin-loaded Au–Au_2S NPs in healthy mice are presented in Fig. 4. In general, within the whole time post injection, the Au–Au_2S NPs exhibited the highest uptake in liver and spleen followed by lung and kidney. At the 1st day of injection of Au–Au_2S NPs, the mass of Au in liver was 19.57 μg/g, in spleen was 13.27 μg/g, in lung was 0.61 μg/g, in kidney was 0.21 μg/g, respectively. After injection for 7 days, the deposited Au–Au_2S NPs decreased significantly in liver and spleen, whereas decreased slightly in lung. However, there was 3-fold higher deposition of Au–Au_2S NPs in kidney as compared to the 1st day of injection. No deposition of Au–Au_2S NPs was measured not only in heart and brain but also in muscle and intestines after injection up to 7 days. The in vivo biodistribution at 1st and 7th day for cisplatin-loaded Au–Au_2S NPs indicated a tendency similar to the results obtained for the bare Au–Au_2S NPs, as shown in Fig. 4. Thus, the loaded cisplatin might not influence in vivo distribution of Au–Au_2S NPs.

The presence of Au–Au_2S NPs in organs was also confirmed by TEM. No visible difference could be observed between Au–Au_2S NPs and cisplatin-loaded Au–Au_2S NPs. Figure 5a showed a typical TEM image of the ultrastructural features of liver cells exposed to cisplatin-loaded Au–Au_2S NPs. The status of cells was completely normal as usual. It is found that nanoparticles were aggregated in the endosome, which was close to but not in the mitochondria or nucleus. These observations were consistent with the results in spleen, as shown in Fig. 5b.

Considering that the biodistribution in tumor bearing animals may be different from that in normal animals due to some physiological changes brought about by tumor development, S180 bearing mice instead of normal mice were again employed in the biodistribution investigation. Both the tail vein and the intra-tumor injection were adopted in the experiments, and the results are represented in Fig. 6, respectively. In comparison with the healthy mice group, Au–Au_2S NPs exhibited a similar tendency of biodistribution in S180 bearing mice via the tail vein injection. Namely, the nanoparticles were mainly deposited in liver and spleen, some in lung and kidney at the 1st day. When the time passed at 7th day, the amount of the deposited

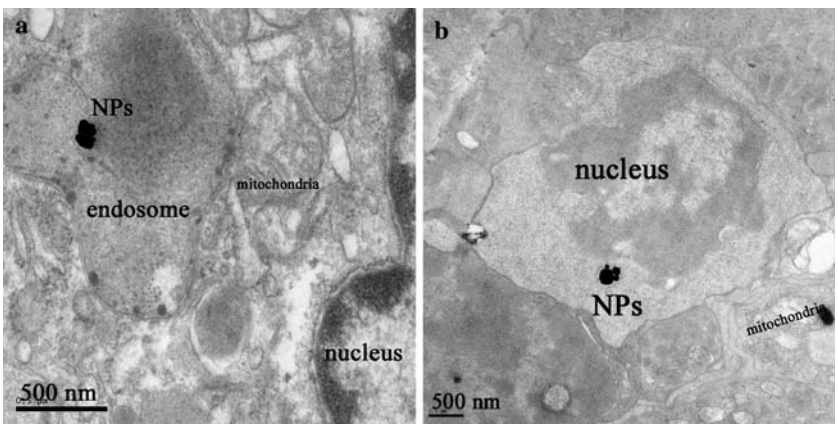

Fig. 5 TEM image of cisplatin-loaded Au–Au_2S·NPs in (**a**) liver, and (**b**) spleen cell after i.v. injection for 14 days

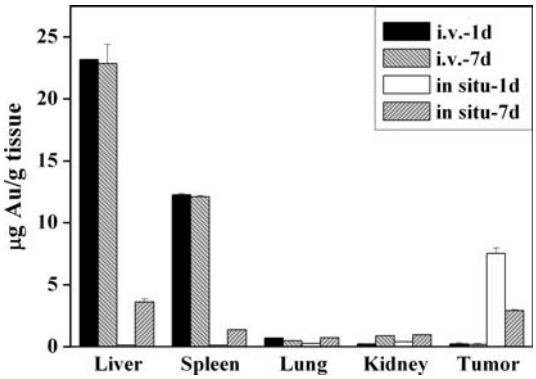

Fig. 6 In vivo biodistribution of Au–Au_2S NPs in S180-bearing mice via the tail vein injection (i.v.) and the intra-tumor injection (in-situ), respectively

Au–Au_2S NPs did not change obviously in liver and spleen, but decreased in lung and increased to 4-fold in kidney. It is noted that little Au–Au_2S NPs in tumor was measured via the tail vein injection. In contrast, most of the injected Au–Au_2S NPs deposited in tumor and retained for a long time via intra-tumor injection, though it varied from 83% to 55% within 7 days of injection, as shown in Fig. 6. Comparing with the tail vein injection, a same tendency but dramatically lower deposition of Au–Au_2S NPs in liver and spleen could be found for the intra tumor group. At the 1st day of injection, the mass of Au in liver was 0.13 μg/g, in spleen was 0.11 μg/g, while the mass of Au–Au_2S NPs increased and presented sharp differences in these organs at 7th day. The top two accumulating organs were liver (3.59 μg Au/g) and spleen (1.35 μg Au/g), respectively. It is noted that the deposited Au–Au_2S NPs via intra-tumor injection in lung and kidney exhibited a similar tendency as via the tail vein. Thus, the administration route might affect in vivo biodistribution of the Au–Au_2S NPs.

Discussion

It is clear that Au-based materials rarely trigger toxicity or immune response when used as implants [21, 22]. The high surface reactivity of Au nanoparticles, coupled with their biocompatible properties, has spawned major interest in the utility of Au-based nanoparticles for in vivo molecular imaging and therapeutic applications [23]. The purpose of the present study was to investigate the bio-safety of Au–Au_2S nanoparticles. Since the male mice exhibit more severe toxic symptoms for nanoparticles than the females [23], we chose male KM mice in our assay. Acute systemic toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. After several years of debate, the LD50 test was finally deleted by the end of 2002 [24]. The Up and Down Procedure [16] has been developed which give rise to significant improvements in animal welfare, which now can be used within a strategy for acute toxicity testing for all types of test substances. Based on this opinion, the experiments we carried out were designed in accordance with the method provided by the OECD guideline 425. Unlike other nanoparticles, such as quantum dot [25] and carbon nanotube [26], Au–Au_2S NPs which were based on bio-inert gold, had a good exhibition in toxicity assays in vivo, as shown in Table 1. Both Au–Au_2S NPs and cisplatin-loaded Au–Au_2S NPs showed no acute systemic toxicity in vivo, when the dosage was below 100 mg/kg. Our previous work indicated that per mg Au–Au_2S NPs could load 30 mg cisplatin, and after conversion the suggested cisplatin dosage instructed by US Pharmacopoeia was 1.38–3.30 mg/kg, which was far less than our tested concentrations of toxicity. Hence, the side effects of cisplatin could be minimized by use of the current Au–Au_2S NPs delivery system, and cisplatin-loaded Au–Au_2S NPs might be non-cytotoxic below the maximum recommended dosage.

Besides toxicity above, more microcosmic and detailed assays were carried out to further examine the safety of Au–Au_2S NPs. Tissue section was taken and the results showed that there were no pathological changes caused by Au–Au_2S NPs. On the other hand, the blood biochemical tests are frequently used in diagnosis diseases of heart, liver, kidney, and cardiovascular system, etc [27]. They are also widely used in monitoring the response to the exogenous toxic exposure. The ALT is often tested along with AST, ALP, and LDH to evaluate whether the liver is damaged or diseased. When the liver is in dysfunction, the levels of the above enzymes will rise. The blood BUN and CR are good indicators for renal function. If kidney function falls, the BUN and CR levels will rise. Therefore, our current results indicated that the both Au–Au_2S NPs and cisplatin-loaded Au–Au_2S NPs may not induce any functional change of important organs. We also noticed that although no in vivo relevant toxicity has yet been found, it is too early to draw meaningful conclusions about the inherent dangers of Au–Au_2S NPs. It remains to be determined whether the unique physicochemical properties of Au–Au_2S NPs will introduce new mechanisms of injury and whether these nanoparticles will result in new pathology.

The applications of nanoscale materials in therapeutic systems have been well documented, and various systems have been designed for intelligent modulated delivery. One of the greatest challenges is to selectively and successfully transport nanoparticle systems to cancerous tissue. So far, selective targeting to the specific organs has rarely been achieved. TEM images in Fig. 5 indicated that those Au–Au_2S NPs were likely to aggregate in the endosome of liver or spleen cells. However, this finding is not in good agreement with the presence of Au–Au_2S NPs in vitro

cellular uptake analysis, which not only aggregated Au–Au_2S NPs but also individual Au–Au_2S NPs were observed in TEM (L. Ren et al. accepted in J Biomed Mat Res A). It is thus suggested that the blood, serum protein or other factors might play a crucial role in making all the Au–Au_2S NPs aggregating in vivo.

In the present study, both the bare Au–Au_2S NPs and cisplatin-loaded Au–Au_2S NPs showed the similar in vivo biodistribution results which were mainly lodged in the liver and spleen, which are two major organs of reticulo-endothelial system (RES) [28]. A little of Au–Au_2S NPs was found in lung might be caused by that those nanoparticles aggregated and embolized the capillaries in the lung [29]. This organ-specific targeting behavior could be defined as passive targeting, and liver and spleen would be fully explored as the therapeutic sites when Au–Au_2S NPs was loading cisplatin.

It is well known that normal tissues have tight, continuous vessel walls with pores that are approximately 9–50 nm. However, tumor tissues which have discontinuous capillary walls, allow particles less than 100 nm to penetrate easily [30]. Moreover, tumor tissues lack a lymphatic system for eliminating materials from them; therefore, once the particles penetrate the tumor tissues, they cannot be eliminated easily. Thus, tumors exhibit enhanced penetration and retention effect (EPR effect) for 50–100 nm particles [31]. This was in accordance with our data that most of the injected Au–Au_2S NPs deposited in tumor and retained for a long time via intra-tumor injection, though it varied from 83% to 55% within 7 days of injection, as shown in Fig. 6. In contrast, in the vein injection group, the mass of Au–Au_2S NPs in tumor was very small (~14%) comparing to the intra-tumor injection group in S180-bearing mice not only in the 1st day but also after injection for 7 days. Thus, our current studies highlighted that the administration route could give a sharp effect on in vivo biodistribution of the Au–Au_2S NPs. Namely, the intra-tumor injection might be a promise administration route in cancer therapy by improving the local tumor response and minimizing the systemic side effects.

Conclusions

In the present study, NIR sensitive Au–Au_2S nanoparticles were synthesized by the reduction of tetrachloroauric acid ($HAuCl_4$) using sodium sulfide (Na_2S), and cisplatin was loaded onto NIR sensitive Au–Au_2S nanoparticles via a MUA layer. Our in vivo acute toxic data provided preliminary evidence suggesting that cisplatin-loaded Au–Au_2S Nps were non-toxic below the maximum recommended dosage. The in vivo biodistribution examinations revealed that liver, spleen, kidney and lung are the target organs for Au–Au_2S NPs. It is also suggested that intra-tumor injection may improve local tumor response and minimize systemic side effects. Therefore, the demonstrated biocompatibility could offer the potentials of cisplatin-loaded Au–Au_2S NPa with NIR sensitivity for cancer therapy.

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References

1. S. M. MOGHIMI, A. C. HUNTER and J. C. MURRAY, *FASEB J.* **19** (2005) 311
2. S. J. OLDENBURG, R. D. AVERITT, S. L. WESTCOTT and N. J. HALAS, *Chem. Phys. Lett.* **288** (1998) 243
3. S. SERSHEN and J. WEST, *Adv. Drug Del. Rev.* **54** (2002) 1225
4. L. R. HIRSCH, J. B. JACKSON, A. LEE, N. J. HALAS and J. L. WEST, *Anal. Chem.* **75** (2003) 2377
5. C. LOO, A. LOWERY, N. J. HALAS, J. WEST and R. DREZEK, *Nanolett.* **5** (2005) 709
6. J. V. FRANGIONI, *Curr. Opin. Chem. Biol.* **7** (2003) 626
7. S. SATO, M. OGURA, M. ISHIHARA, S. KAWAUCHI, T. ARAI, T. MATSUI, A. KURITA, M. OBARA, M. KIKUCHI and H. ASHIDA, *Lasers Surg. Med.* **29** (2001) 464
8. D. E. G. J. DOLMANS, D. FUKUMURA and R. K. JAIN, *Nat. Rev. Cancer* **3** (2003) 380
9. R. A. WEISSLEDER, *Nat. Biotechnol.* **19** (2001) 327
10. L. R. HIRSCH, R. J. STAFFORD, J. A. BANKSON, S. R. SERSHEN, B. RIVERA, R. E. PRICE, J. D. HAZLE, N. J. HALAS and J. L. WEST, *Proc. Natl. Acad. Sci. USA* **100** (2003) 13549
11. R. D. AVERITT, D. SARKAR and N. J. HALAS, *Phys. Rev. Lett.* **78** (1997) 4217
12. H. S. ZHOU, I. HONMA, J. W. HAUS, H. SASABE and H. KOMIYAMA, *J. Lumin.* **70** (1996) 21–34
13. L. REN and G. M. CHOW, *Mater. Sci. Eng. C-Bio.* **23** (2003) 113
14. G. OBERDORSTER, E. OBERDORSTER and J. OBERDORSTER, *Environ. Health Perspect.* **113** (2005) 823
15. P. H. HOET, I. BRUSKE-HOHLFELD and O. V. SALATA, *J. Nanobiotech.* **2** (2004) 12
16. A. WHITEHEAD and N. STALLARD, *ATLA* **S2** (2004) 73
17. M. D. ROCKVILLE, U.S. Pharmacopeia, USA (2000)
18. H. KAMEL, D. H. BROWN, J. M. OTTAWAY and W. E. SMITH, *Talanta* **24** (1977) 309
19. M. C. TAN, PhD assay, National University of Singapore, Singapore (2006)
20. B. ZHANG, X. L. HUANG, L. REN, Q. Q. ZHANG, M. C. TAN and G. M. CHOW, *Key. Eng. Mater.* **334–335** (2007) 1177
21. M. THOMAS, A. M. KLIBANOV, *Proc. Natl. Acad. Sci.* **100** (2003) 9138
22. A. G. TKACHENKO, H. XIE, D. COLEMAN, W. GLOMM, J. RYAN, M. F. ANDERSON, S. FRANZEN and D. L. FELDHEIM, *J. Am. Chem. Soc.* **125** (2003) 4700
23. A. G. TKACHENKO, H. XIE, Y. LIU, D. COLEMAN, J. RYAN, W. GLOMM, M. K. SHIPTON, S. FRANZEN and D. L. FELDHEIM, *Bioconjugate Chem.* **14** (2004) 482

24. P. A. BOTHAM, *ILAR J.* 43 Suppl. (2002) S27
25. R. HARDMAN, *Environ. Health Perspect.* **114** (2006) 165
26. L. LACERDA, A. BIANCO, M. PRATO and K. KOSTARELOS, *Adv. Drug Deliv. Rev.* **58** (2006) 1460
27. W. J. MARSHALL, *"Clinical Chemistry"*, (Mosby: London, 1997)
28. J. H. SENIOR, *Crit. Rev. Ther. Drug Carr. Syst.* **3** (1987) 123
29. L. ILLUM, S. S. DAVIS, C. G. WILSON, N. W. THOMAS, M. FRIER and J. G. HARDY, *Int. J. Pharm.* **12** (1982) 135
30. A. E. TAYLOR and D. N. GRANGER, *Fed. Proc.* **42** (1983) 440
31. X. QI, Y. MAITANI, T. NAGAI and S. WEI, *Int. J. Pharm.* **146** (1997) 31