BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
9 (2013) 257–263

Research Article

nanomedjournal.com

In vivo toxicity, biodistribution, and clearance of glutathione-coated gold nanoparticles

Carrie A. Simpson, PhD[a], Kenneth J. Salleng, DVM[b], David E. Cliffel, PhD[c], Daniel L. Feldheim, PhD[a,*]

[a]*Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado, USA*
[b]*Office of Animal Welfare and Assurance, Vanderbilt University, Nashville, Tennessee, USA*
[c]*Department of Chemistry, Vanderbilt University, Nashville, Tennessee, USA*

Received 6 January 2012; accepted 8 June 2012

Abstract

Gold nanoparticles are emerging as promising materials from which to construct nanoscale therapeutics and therapeutic delivery systems. However, animal studies have shown that gold nanoparticles modified with certain thiol monolayers such as tiopronin can cause renal complications and morbidity. Although these effects may be eliminated by coadsorbing small amounts of polyethylene glycol (PEG) onto the nanoparticle surface, PEG can also lower cellular internalization efficiency and binding interactions with protein disease targets, significantly reducing the potential for using gold nanoparticles as therapeutics. Using ICP-MS analysis of blood, urine, and several organs, we show in this article that glutathione-coated gold nanoparticles (1.2 nm ± 0.9 nm) cause no morbidity at any concentration up to and including 60 μM and target primary organs although providing gradual dissipation and clearance over time. This study suggests that glutathione may be an attractive alternative to PEG in the design of gold nanoparticle therapeutics.

From the Clinical Editor: This study describes the utility and toxicity of glutathione coated gold nanoparticles in comparison to PEGylated counterparts that are commonly used to increase "Stealth" properties and lower cytotoxicity. Too much PEG on the NPs can lead to lower cellular internalization efficiency and less efficient binding interactions with protein disease targets, significantly reducing the potential for using gold nanoparticles as therapeutics.

Key words: Nanoparticle; Glutathione; Mouse

The emergence of advanced metal nanoparticle synthesis methodologies has enabled control over the size, shape, composition, and surface chemistry of metal nanoparticles. Many types of metal nanoparticles may now be synthesized with perfect size monodispersity, tunable light absorption, and emission characteristics, and with one or more surface-bound organic ligands that can be designed to increase aqueous solubility and binding interactions with target biomolecules.[1,2] These advances are quickly leading to the creation of more sensitive disease diagnostics[3-9] and more targeted disease treatments.[10-16]

Many metal nanoparticle formulations have progressed recently from the design and synthesis phase to in vitro testing with encouraging results. The need to investigate toxicity, biodistribution, and clearance of nanoparticles in vivo is thus becoming increasingly important to their translation into clinical settings.[17] Pharmacologic profiles,[18] routes of administration, biodistribution patterns, and dosage are all important considerations that are only beginning to be addressed in detail.[19-22] Notable examples are the independent works of De Jong et al.[23] and Sonavane et al.[18] in which gold nanoparticles of various core diameters were administered intravenously into murine models. In both studies, the smaller particles (10 nm for the former, 15 nm for the latter) were found to infiltrate more organs than the larger diameter particles (250 nm for former, 200 nm for latter). In addition, Rotello's laboratory has investigated the role of nanoparticle charge on circulation half-life and biodistribution and found that both were charge dependent.[24] Finally, Cliffel's laboratory has recently examined the clearance of tiopronin monolayer protected clusters (TMPCs)[25] and various PEGylated MPCs in vivo.[26] Tiopronin is a widely used pharmaceutic for the treatment of cystinurea[27] and its use as a capping agent for gold

The authors have no competing interests in this work.

Funding for this research was provided by the Bill and Melinda Gates Foundation.

*Corresponding author: Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309.

E-mail address: daniel.feldheim@colorado.edu (D.L. Feldheim).

1549-9634/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nano.2012.06.002

nanoparticles has been studied extensively.[28,29] Therefore, the TMPC's risk in vivo was presumed to be minimal. However, deleterious renal effects leading to morbidity were noted with TMPCs at concentrations as low as 20 μM. This toxicity was alleviated by the addition of a long-chain PEG, which eliminated all deleterious effects and allowed for extended blood circulation times of up to 4 weeks post-injection.[25]

The addition of PEG, although alleviating toxicity and morbidity, caused both an immunologic response as well as complete avoidance of the reticuloendothelial system, likely obviating PEG as a ligand for alveolar targeting. PEG is also known to minimize binding interactions between surfaces and protein targets. As one of our primary interests is the treatment of *Mycobacterium tuberculosis* infections in the lungs with gold nanoparticle antibiotics discovered in our laboratory recently,[11] we began to consider other ligands that would afford biocompatibility and biomolecule binding without compromising lung targeting and macrophage internalization.

The objective of this study was to assess the in vivo biodistribution profile, toxicity, and clearance of glutathione-coated gold nanoparticles (1.2 nm ± 0.9 nm) and determine whether these nanoparticles could be used as a platform for the treatment of infectious diseases of the lungs. We chose glutathione because it is a highly abundant intracellular tripeptide, and because it has been used previously as a capping ligand for the synthesis of stable, water-soluble gold nanoparticles. Moreover, as zwitterions are known to reduce non-specific protein adsorption, we hypothesized that the amine and carboxylate moieties in glutathione might help minimize protein adsorption that could lead to opsonization.

Methods

Synthesis of glutathione-coated gold nanoparticles

Glutathione-coated gold nanoparticles were synthesized according to Bresee et al[11] with modifications. Briefly, a solution of 11.1 mM $HAuCl_4$ (Strem, Newburyport, Massachusetts), 37.8 mM glutathione (Sigma-Aldrich, St. Louis, Missouri), 178 mM NaOH in 55.6 % (v/v) aqueous methanol was prepared and allowed to equilibrate. Fifty milliliters of this solution (0.556 mmoles of Au^{3+}) were diluted to a final Au^{3+} concentration of 0.48 mM with the addition of 260 mL methanol and 740 mL water. The Au^{3+} was reduced with the addition of 10 mL of 0.25 M $NaBH_4$ (Sigma-Aldrich). The final methanol concentration was adjusted to 24.8 % with the addition of 100 mL of water. The reduction of gold was allowed to proceed for 48 hours at room temperature with constant stirring. Gold nanoparticles were precipitated with the addition of 68 mmol of NaCl and 500 mL of methanol (final methanol concentration of 47 % v/v) followed by centrifugation at 3200 RCF for 5 minutes. The precipitated nanoparticles were reconstituted in water. The concentration was measured by UV-Visible spectroscopy, using the ε_{510} nm of 400, 000 $M^{-1}cm^{-1}$. Further, it was noted that the source of reagents for this synthesis is important to the preparation. Ensuring that reagents were not stored with other chemicals that could react or contaminate them was also critical.

Animal models

Animals were housed in a Vanderbilt Division of Animal Care (DAC) facility, fully certified by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Animals were housed under supervision of full-time veterinarians and staff. All procedures performed were previously approved by the Vanderbilt University Institutional Animal Care and Use Committee. BALBc/cAnNHsd mice, 5–6 week, female, weighing 15–16 g, were purchased from Harlan Laboratory. All animals were allowed 1 week for acclimation before experimentation. Nanoparticles were prepared in sterile phosphate-buffered saline (n = 15 mice/concentration group) and injected subcutaneously. Dosage concentrations were 0 μM (saline only), 10 μM, 20 μM, 30 μM, 40 μM, and 60 μM in a 200 μL total volume of phosphate-buffered saline (PBS). Blood was drawn via submandibular bleeding techniques,[30] in compliance with our protocol and bleeding guidelines for mL/kg body weight per week.[31] Urine was collected on cellophane with special precaution to avoid fecal contamination.[32] Mice were euthanized (n = 5 per euthanasia point/concentration group) at 24 hours, 2 weeks, and 4 weeks for organ distribution data. Mice were euthanized via CO_2 asphyxiation, followed by cervical dislocation.

Inductively coupled plasma mass spectrometry (ICP-MS)

Fluid and tissue samples were prepared as described in Simpson et al[25] with modifications. A 10 μL standard blood/urine sample was used because of limitations in fluid collection. The fluid sample was then added to a 4.5 mL solution of distilled water. To this was added 0.5 mL of hydrochloric acid (10% HCl dilution, Optima grade, Fisher Scientific, Ottawa, Ontario, Canada) (total dilution 500:1 solution: blood/urine). Organ samples were excised, weighed, and digested in nitric acid (Optima grade, 70%). The samples were then heated to dryness, at which point the remnants were transferred to a solution of 4.5 mL distilled water. To this solution was added 0.5 mL hydrochloric acid (10% HCl dilution, Optima grade, Fisher Scientific) as described by Sadauskas et al.[33] All samples were then analyzed in duplicate by ICP-MS for gold content using a standard calibration as well as the addition of an internal standard (Indium, Chicago, Illinois). A blank and three standards were used for calibration. A 1000 ppm gold standard was made in house from 99.99% pure gold shot, diluted to 0.5 L. A standard check was made using a 10 ppm multi-element standard (SPEX CertiPrep, Metuchen, New Jersey). A 60-second rinse with 4% HCl was used between every standard and sample. Analyses of diluted fluid and tissue samples were performed on a Perkin Elmer SCIEX ICP-MS (Model # Elan DRC-e, Vernon Hills, Illinois) at the University of Colorado Laboratory for Environmental and Geological Sciences (LEGS).

Complete blood count (CBC)

Red and white cell counts were measured by Coulter counter as described in Simpson et al[26] with no exceptions.

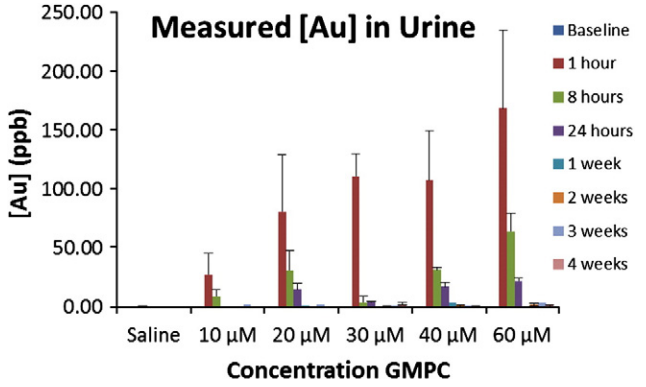

Figure 1. Urinalysis of glutathione-coated gold nanoparticless (n = 5 for each time point, 200 μL injections) shows a significant percentage of particles is cleared through renal filtration within 1 h, continues for up to 24 h and returns to baseline concentrations within 1 week. This indicates the glutathione-coated gold nanoparticles is capable of quick passage into the kidneys and bladder and does not cause a strain on the renal system as the injection concentration is increased. (ICP-MS detection limit = 0.4 ppb). Baseline time points (taken before injection) were essentially 0 ppb at all concentrations.

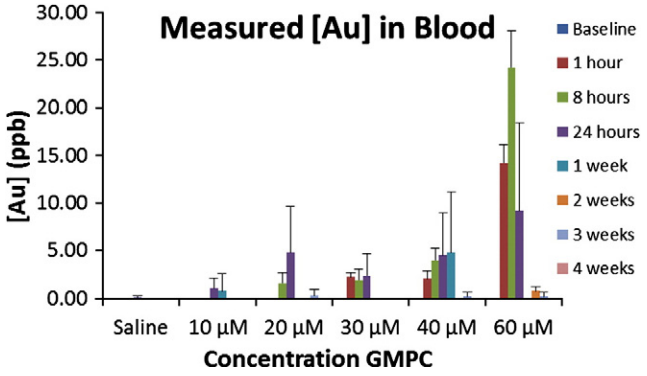

Figure 2. Gold content analysis of blood (n = 5 for each time point, 200 μL injections) shows relatively small amounts of circulating particles at low concentrations with an increase with increasing injection concentration. (ICP-MS detection limit = 0.4 ppb). Baseline time points (taken before injection) were essentially 0 ppb at all concentrations.

Histology

Histological staining and examination of renal tissue was performed as described by Simpson et al[25,26] with no exceptions.

Statistical analysis

Statistical analysis was performed for ICP-MS and Coulter counter samples. For ICP-MS data, individual samples were analyzed in duplicate and averaged to yield values for individual mice. These individual mouse values were then averaged to yield time point data, propagating error throughout. For Coulter counter data, a Student *t* test was performed to detect differences that were considered statistically significant when $P < 0.05$.

Results

Glutathione-coated gold nanoparticles are biocompatible and cleared via the kidneys

To determine whether glutathione-coated gold nanoparticles were compatible in vivo, a previously published method for the synthesis of p-mercaptobenzoic acid (pMBA) gold nanoparticles was used.[34] Replacing glutathione for p-mercaptobenzoic in the synthesis protocol yielded nanoparticles with a diameter of 1.2 nm ± 0.9 nm as determined by transmission electron microscopy (Supplementary information Figure S1, available online at http://www.nanomedjournal.com). These particles were similar in diameter to pMBA-coated gold nanoparticles[1] that were characterized by X-ray crystallography to have a molecular formula of $Au_{102}[pMBA_{44}]$.[1] Glutathione-coated gold nanoparticles were injected subcutaneously as described previously by Simpson et al.[25,26] Subcutaneous injection was chosen to enable comparisons between the biodistribution and clearance of glutathione-coated gold nanoparticles versus TMPCs and PEG/TMPCs. As biologic toxicity of nanomaterials has been correlated to retention and clearance,[25] whole blood, urine, and several organs (spleen, liver, heart, kidneys, and lungs) were collected at various time points and analyzed for gold by ICP-MS to determine the in vivo fate of the nanoparticles. In the data shown in Figures 1−3, each time point is the average gold concentration contained within whole blood, organ, and urine taken from five individual mice at each concentration (each individual sample was analyzed in duplicate then averaged for final value).

For glutathione-coated gold nanoparticles at all concentrations, a large percentage of particles (> 30%) were voided through the renal system within 1 hour and > 60% were excreted within 8 hours as determined by ICP-MS (Figure 1, Table S1). The gold concentration in the urine returned to baseline levels within 1 hour for nanoparticle injections of 10 μM and higher injection concentrations returned to baseline quantities within 1 week. This indicates that glutathione-coated gold nanoparticles were capable of rapid passage into the kidneys and bladder. Mice injected with glutathione-coated gold nanoparticles did not experience any clinical signs of illness, stress, or discomfort, nor did any expire over the course of the entire 6-week study. Therefore, glutathione-coated gold nanoparticles presumably did not cause any toxic effects in passing through the kidneys as were noted in the previous TMPC study at the same concentrations.[25]

Glutathione-coated gold nanoparticle residence time within the bloodstream

In addition to monitoring the excretion of gold in urine, whole blood was examined in time for total gold content (Figure 2). Parallel to the urinalysis, the average gold concentration in the blood increased with increasing injection concentration. Again, a time-dependent decrease in blood gold concentration was noted for all nanoparticle injection concentrations with complete clearance after 1 week. This was consistent with the primary clearance pathway of the particles being by excretion in urine. All concentrations injected showed particle circulation within the bloodstream up to and including 24 hours post-injection.

Glutathione-coated gold nanoparticle organ targeting and clearance with time

In addition to blood and urine, gold nanoparticles have been shown to target several major organs.[23,25] In this study, the heart,

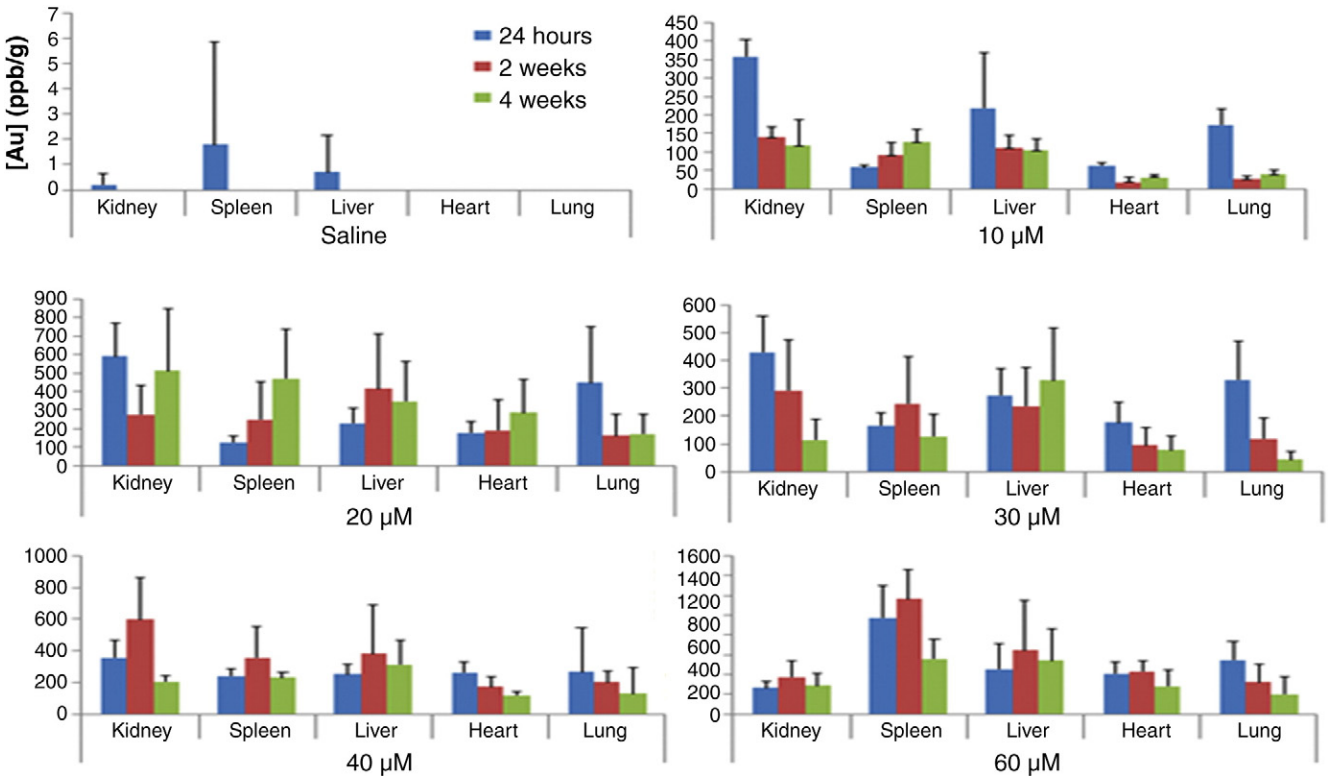

Figure 3. Organ distribution analysis at 24 h, 2 weeks, and 4 weeks (n = 5 for each time point, 200 μL injections) show an initial accumulation within the kidney and lungs at 24 h. This accumulation shifts to the liver and spleen at 2 and 4-week time points, presumably because of RES clearance.

liver, spleen, lungs, and kidneys were excised and analyzed for gold as described by Simpson et al.[25,26] Three time points were examined to determine an organ distribution and clearance pattern: 24 hours, 2 weeks, and 4 weeks (n = 5 at each point, each sample run in duplicate) (Figure 3). The analysis, which was corrected for differences in organ mass, showed gold accumulation within all tissues examined within 24 hours. At the 24-hour time point, nanoparticle targeting to the heart, lungs, and spleen trended upward with an increase in injection concentration (significantly different at the 95% CI). In contrast, the nanoparticle concentration in the kidneys appeared to increase from the 10 μM to 20 μM injection, and then decreased at higher injection concentrations. In addition, the highest concentration injected (60 μM) appears to have caused a shift in organ distribution in comparison to the lower concentration groups. Gold nanoparticles injected at this concentration were found in higher concentrations in the spleen compared with the other organs after 24 hours, whereas lower concentration injections led to relatively low concentrations of gold in the spleen. Finally, consideration of the data at all three time points revealed that the gold nanoparticles were largely cleared from the heart and lungs after 4 weeks (typically < 1% ID remaining, Table S1) but remained in the liver and spleen.

CBC analysis for possible immunogenic response

In addition to examining nanoparticle clearance and biodistribution, it was of interest to determine whether glutathione-coated gold nanoparticles would provoke an immune response. Previous accounts have shown an elevated white and red cell count in response to various ligand monolayers of MPCs, in particular PEG.[25,26] To ensure that glutathione-coated gold nanoparticles were not eliciting the same immune response as seen previously, red blood cell (RBC) count and white blood cell (WBC) count were monitored at 0, 2, and 4 weeks by Coulter counter to evaluate acquired immunity. The results are reported as a ratio of RBC count/WBC count for a better representation of the whole blood sample (Figure 4). The cell counts indicate that glutathione-coated gold nanoparticles did not cause a statistically relevant change ($P < 0.05$) in the RBC or WBC counts nor their ratio during the course of the study, with the exception of 60 μM at the 0−2 week time period. This statistically relevant change within the ratio parallels the initially high concentration of particles seen within the spleen at 24 hours and again at 2 weeks, presumably because of macrophage sequestration of particles. The cell count ratio returned to baseline levels at 4 weeks and no relevant change is noted between 0−4 weeks or 2−4 week time intervals.

Animals administered glutathione-coated gold nanoparticles showed no renal stress

TMPCs have been shown to produce toxic effects in the kidney, causing morbidity at high concentrations.[25] Although morbidity was not observed at lower concentrations, other deleterious effects (moderate tubular shedding, mild mineralization, and mild proteinaceous casts) were noted. Although glutathione-coated gold nanoparticles did not cause morbidity

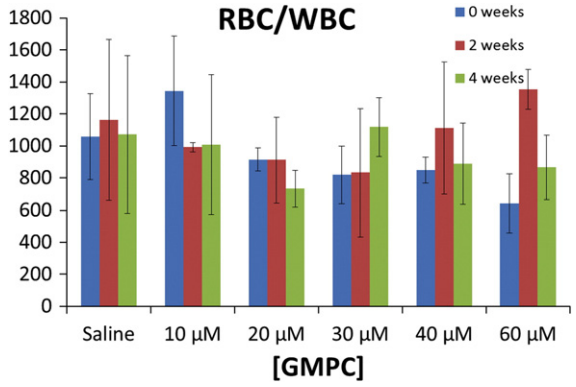

Figure 4. Coulter counter analysis of whole blood samples (n =5 for each concentration; 200 μL injections) shows no statistical change ($P < 0.05$) in ratio of RBC/WBC count at 0, 2, or 4 weeks for all injected concentrations, with the exception of 60 μM, 2 weeks, indicating the glutathione-coated gold nanoparticles do not induce an immunologic response up to and including 40 μM. At 60 μM, cell count ratio returns to baseline at 4 weeks, indicating sufficient RES clearance.

at any concentration, final confirmation that the glutathione-coated gold nanoparticles did not cause any undetectable stress to the mice was obtained through histological analysis. Representative renal sections of all concentrations after 4 weeks were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned to 5 μM, and submitted for hematoxylin and eosin (H&E) staining to the Vanderbilt ImmunoHistology Core. Blind analyses of the sections were performed by a veterinarian trained in veterinary pathology. For glutathione-coated gold nanoparticles at all concentrations, the only change noted was mild, multifocal, renal tubular epithelial hydropic change. The hydropic change consisted of increased vacuolation of the cytoplasm. This change was also noted within the saline group (no nanoparticle injected), indicating this change is most likely attributable to the saline itself but could also be indicative of fixative inconsistencies. Regardless of the source, it was concluded that the change was not a result of the glutathione-coated gold nanoparticles nor was it detrimental to the subjects as it was consistently present in all samples, the mice were physically healthy, and the changes were mild. Given these mild changes and the 100% survival rate at all concentrations, the glutathione-coated gold nanoparticles was deemed viable for in vivo applications.

The primary aim of this study was to determine whether glutathione is a suitable replacement for PEG as a ligand shell for gold nanoparticles that are (1) capable of targeting lung tissue, (2) cleared over time, (3) not immunogenic, and (4) non-toxic. ICP-MS analysis of urine and blood showed that glutathione-coated gold nanoparticles are primarily cleared via renal filtration within the first 8 hours. Whole blood analysis showed particle circulation within the bloodstream up to and including 24 hours post-injection. This was consistent with the primary clearance pathway of the particles being by excretion in urine. In comparison with previous experiments, which examined TMPCs[25] and short-chain PEG-TMPCs[26] by ICP-OES (inductively coupled plasma optical emission spectroscopy) at similar time points using similar protocols, glutathione-coated gold nanoparticles clearly exhibited a longer circulation time within the bloodstream. Both TMPCs and PEG-TMPCs were cleared in 8 hours.[25]

Biodistribution analysis of individual organs showed relatively efficient targeting of glutathione-coated gold nanoparticles to the lungs compared to other gold nanoparticles that have been studied. Approximately 2% of the glutathione-gold nanoparticles injected were found in the lungs after 24 hours, which is higher than for 5 nm diameter bis(sulfonatophenyl) phenylphosphine-coated gold nanoparticles (~0.07%), and 15 nm, 50 nm, and 100 nm diameter citrate-coated gold nanoparticles (0.244%, 0.073%, and 0.059%, respectively).[18] The accumulation within lung tissue is thought to be attributable to the uptake of macrophages and phagocytic engulfment of the particles.

The accumulation within the kidneys was expected as the urinalysis for gold content showed a large concentration of particles within the first 24 hours. However, the organ distribution at 24 hours shifted from primarily the lungs and kidneys to the liver and spleen as the nanoparticle injection concentration was increased. This shift is most likely indicative of RES clearance at higher injection concentrations, and has been noted previously for cationic quantum dots.[35]

Previous accounts have shown an elevated white and red cell count in response to various ligand monolayers of MPCs, in particular PEG.[25,26] RBC and WBC counts for glutathione-coated gold nanoparticles showed no statistically relevant change, with the exception of 60 μM. This change in ratio parallels the initially high concentration of particle seen within the spleen at 24 hours and again at 2 weeks, presumably because of macrophage sequestration of particles.

The ease with which gold nanoparticles may be synthesized and their monolayers modified by simple place-exchange reactions allows for effective tailoring for both in vitro and in vivo experiments. Recent reports have shown that varying ligand charge within the monolayer can affect cell entry as well as red blood cell counts and toxicity. Toxicity may be eliminated by a simple exchange in the monolayer with PEG. However, the incorporation of PEG severely limits the targeting efficiency of the particle, in particular RES targeting capabilities. For this reason, a particle capable of efficient targeting that does not require the incorporation of PEG within the monolayer was sought. Given the previous toxicity observed for the negatively charged ligand tiopronin, we chose to investigate the naturally occurring tripeptide ligand glutathione. With two carboxylates and an amine, glutathione possesses two anions and one cation at neutral pH. Despite the net negative charge that might lead one to anticipate a similar biodistribution profile and toxicity as the anionic tiopronin ligand, glutathione-coated gold nanoparticles clearly followed a different trajectory in vivo, displaying a longer circulation time and lack of acute toxicity. Whether this is due to the ammonium or more subtle chemical or nanoparticle size effects is currently being investigated.

Finally, it is interesting to compare lung targeting of our glutathione-coated gold nanoparticles with the small-molecule *M. tuberculosis* drug Isoniazid.[36] After subcutaneous injection

at a threefold higher dosage than our highest dose, it was found that Isoniazid reached baseline concentrations in the lungs at 4 hours post-injection. Our glutathione-coated gold nanoparticles, however, were present in steady percentages at 4 weeks post-injection within alveolar tissue, and slowly decreased with time. This could prove beneficial for targeting purposes as high concentrations are locally focused within 24 hours and slowly dissipate with time through urine and RES clearance mechanisms, potentially allowing for less frequent administrations. However, complete clearance from the spleen and liver remain a concern, even for the small diameter particles investigated here. The fate of nanoparticles in these organs at times in excess of 4 weeks is also under investigation.

In summary, we have shown the glutathione-coated gold nanoparticles are a promising candidate for in vivo applications given their low immunogenicity, clearance times, and biocompatibility. Our results showed efficient targeting to lung tissue that gradually dissipates over a 4-week time period. Organ distribution data showed initial clearance through the renal tissue with a subsequent shift to RES clearance at higher concentrations. Given these findings, the 100% survival rate at concentrations up to and including 60 μM injection concentrations, and the straightforward conversion of these nanoparticles into potent antibiotics in vitro via the addition of other thiol ligands,[11] glutathione-coated gold nanoparticles may be a useful platform for the synthesis of nanoscale therapeutics for the treatment of infectious disease.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nano.2012.06.002.

References

1. Jadzinsky PD, Calero G, Ackerson CJ, Bushnell DA, Kornberg RD. Structure of a thiol monolayer-protected gold nanoparticle at 1.1 Å resolution. *Science* 2007;**318**:430-3.
2. Verma A, Stellacci F. Effect of surface properties on nanoparticle-cell interactions. *Small* 2010;**6**:12-21.
3. Hainfeld JF, Slatkin DN, Focella TM, Smilowitz HM. Gold nanoparticles: a new X-ray contrast agent. *Br J Radiol* 2006;**79**:248-53.
4. Hainfeld JF, Slatkin DN, Smilowitz HM. The use of gold nanoparticles to enhance radiotherapy in mice. *Phys Med Biol* 2004; **49**:309-15.
5. Cheng MMC, Cuda G, Bunimovich YL, Gaspari M, Heath JR, Hill HD, et al. Nanotechnologies for biomolecular detection and medical diagnostics. *Curr Opin Chem Biol* 2006;**10**:11-9.
6. Giljohann DA, Mirkin CA. Drivers of biodiagnostic development. *Nature* 2009;**462**:461-4.
7. Storhoff JJ, Elghanian R, Mucic RC, Mirkin CA, Letsinger RL. One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold nanoparticle probes. *J Am Chem Soc* 1998;**120**: 1959-64.
8. Conde J, Doria G, Baptista P. Noble metal nanoparticles applications in cancer. *J Drug Deliv* 2012;**2012**:751075.
9. Larguinho M, Baptista P. Gold and silver nanoparticles for clinical diagnostics—from genomics to proteomics. *J Proteomics* 2012;**75**: 2811-23.
10. Tkachenko AG, Xie H, Coleman D, Glomm W, Ryan J, Anderson MF, et al. Multifunctional gold nanoparticle-peptide complexes for nuclear targeting. *J Am Chem Soc* 2003;**125**:4700-1.
11. Bresee J, Maier KE, Melander C, Feldheim DL. Identification of antibiotics using small molecule variable ligand display on gold nanocrystals. *Chem Comm* 2010;**46**:7516-8.
12. Bhattacharya R, Patra CR, Earl A, Wang S, Katarya A, Lu L, et al. Attaching folic acid on gold nanoparticles using noncovalent interaction via different polyethylene glycol backbones and targeting of cancer cells. *Nanomedicine* 2007;**3**:224-38.
13. Bhattacharya R, Patra CR, Verma R, Kumar S, Greipp PR, Mukherjee P. Gold nanoparticles inhibit the proliferation of multiple myeloma cells. *Adv Mater* 2007;**19**:711.
14. Patra CR, Bhattacharya R, Wang EF, Katarya A, Lau JS, Dutta S, et al. Targeted delivery of gemcitabine to pancreatic adenocarcinoma using cetuximab as a targeting agent. *Cancer Res* 2008;**68**: 1970-8.
15. Lal S, Clare SE, Halas NJ. Nanoshell-enabled photothermal cancer therapy: impending clinical impact. *Acc Chem Res* 2008;**41**: 1842-51.
16. Sperling RA, Parak WJ. Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. *Philos Transact A Math Phys Eng Sci* 2010;**368**:1333-83.
17. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI, et al. Renal clearance of quantum dots. *Nat Biotech* 2007;**25**:1165-70.
18. Sonavane G, Tomoda K, Makino K. Biodistribution of colloidal gold nanoparticles after intravenous administration: effect of particle size. *Colloids Surf B Biointerfaces* 2008;**66**:274-80.
19. Farrell N. *Transition metal complexes as drugs and chemotherapeutic agents*, vol. 11. Boston, MA: Kluwer Academic Publishing; 1989.
20. Herold DM, Das IJ, Stobbe CC, Iyer RV, Chapman JD. Gold microspheres: a selective technique for producing biologically effective dose enhancement. *Int J Radiat Biol* 2000;**76**:1357-64.
21. Bakri SJ, Pulido JS, Mukherjee P, Marler RJ, Mukhopadhyay D. Absence of histologic retinal toxicity of intravitreal nanogold in a rabbit model. *Retina* 2008;**28**:147-9.
22. Zhang LW, Yu WW, Colvin VL, Monteiro-Riviere NA. Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes. *Toxicol Appl Pharmacol* 2008; **228**:200-11.
23. De Jong WH, Hagens WI, Krystek P, Burger MC, Sips AJAM, Geertsma RE. Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. *Biomaterials* 2008;**29**:1912-9.
24. Arvizo RR, Miranda OR, Moyano DF, Walden CA, Giri K, Bhattacharya R, et al. Modulating pharmacokinetics, tumor uptake and biodistribution by engineered nanoparticles. *PLoS One* 2011;**6**: e24374.
25. Simpson CA, Huffman BJ, Gerdon AE, Cliffel DE. Unexpected toxicity of mono layer protected gold clusters eliminated by PEG-Thiol place exchange reactions. *Chem Res Toxicol* 2010;**23**: 1608-16.
26. Simpson CA, Agrawal AC, Balinski A, Harkness KM, Cliffel DE. Short-chain PEG mixed monolayer protected gold clusters increase clearance and red blood cell counts. *ACS Nano* 2011;**5**:3577-84.
27. Devi PU, Saharan BR. Chemical protection of mouse spermatocytes against gamma-rays with 2-mercaptopropionylglycine. *Experientia* 1978;**34**:91-2.
28. Templeton AC, Chen S, Gross SM, Murray RW. Water-soluble, isolable gold clusters protected by tiopronin and coenzyme A monolayers. *Langmuir* 1999;**15**:66-76.
29. Templeton AC, Wuelfing WP, Murray RW. Monolayer-protected cluster molecules. *Acc Chem Res* 2000;**33**:27-36.

30. Golde WT, Gollobin P, Rodriguez LL. A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. *Lab Anim* 2005;**34**:39-43.
31. National Institutes of Health. Available at: http://oacu.od.nih.gov/ARAC/Bleeding.pdf, 2005, Vol. 1. Accessed October 24, 2011.
32. Kurien BT, Scofield RH. Mouse urine collection using clear plastic wrap. *Lab Anim* 1999;**33**:83-6.
33. Sadauskas E, Danscher G, Stoltenberg M, Vogel U, Larsen A, Wallin H. Protracted elimination of gold nanoparticles from mouse liver. *Nanomedicine* 2009;**5**:162-9.
34. Bowman MC, Ballard TE, Ackerson CJ, Feldheim DL, Margolis DM, Melander CJ. Inhibition of HIV fusion with multivalent gold nanoparticles. *J Am Chem Soc* 2008;**130**:6896-7.
35. Al-Jamal WT, Al-Jamal KT, Cakebread A, Halket JM, Kostarelos K. Blood circulation and tissue biodistribution of lipid—quantum dot (L-QD) hybrid vesicles intravenously administered in mice. *Bioconjug Chem* 2009;**20**:1696-702.
36. Kaneo Y, Kubo H, Tabata T, Matsuyama K, Noda A, Iguchi S. Tissue distribution of isoniazid and its metabolites in rats. *J Pharmacobiodyn* 1981;**4**:590-5.