Differential lethality of gold nanoparticles is associated with immunogenecity

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ABSTRACT

Animal and cell culture experiments were performed to investigate the lethality of gold nanoparticles (GNP). GNPs of size larger than 8 nm induced lethality in BALB/C mice within 2-week period. Incubation of GNPs with cell culture revealed LC_{50} in the millimolar rage. GNPs were almost non-toxic to cultured cells. The lethality in animal was irrelevant to GNPs' ability to induce cytotoxicity. Surface conjugation of 17 nm GNP with IsdA peptide, BSA, MDA-BSA, and Lysozyme was performed. The antigen-coated 17 nm GNP induced differential lethality in mice. The ability of coated antigen to stimulate immune response is proportional to survival rate of mice. Pathological study indicated that heart and spleen tissues were damaged while lung, brain, and kidney remained normal. Damage in myocardium might be the major cause of acute death in mice.

Keywords: gold nanoparticles,

1 INTRODUCTION

Manufactured nanoparticle has been applied in imaging [1, 2, 3], biosensing [4, 5, 6] gene transfer [7, 8] and drug delivery [9, 10, 11]. Toxicity of nanoparticles has been recognized in recent years. Particles include carbon nanotubes, zinc oxide, titanium oxide, fullerene, etc. However, gold nanoparticles (GNPs) are categorized as non-toxic in many reports.

We have accidentally observed lethality by GNP injection in the previous investigation. The current study is based on the previous observation in the hope to reveal careful handling of this seemingly safe material.

2 MATERIAL AND METHODS

2.1 Preparation gold nanoparticles

The seed colloids were prepared by adding 1 mL of 0.25mM $HAuCl_4$ to 90 mL of H_2O and stirred for 1 min at 25 °C. Two milliliters of 38.8 mM sodium citrate was added to the solution and stirred for 1 min followed by addition of 0.6 mL freshly prepared 0.1 M $NaBH_4$ in 38.8 mM sodium citrate. Different diameters of gold GNPs ranging from 3.5 nm to 12 nm were generated by changing the volume of seed colloid added. The solution was stirred for an additional 5 to 10 min at 0 to 4 °C (Brown, Walter, Natan, 2000, Chem Mater, 12, 306-313). Fifty microlitters of 0.1M ascorbic acid, 9mL growth solution (0.25mMHAuCl4, 0.08M cetyltrimethylamonium bromide, CTAB), and 1.0mL Seed colloid (8.0±0.8nm in diameter) was combined in a glass beaker, followed by continuous stirring for 10 to 20 min at room temperature until the solution turned reddish brown (approximately 17 nm) or brown (37 nm).

2.2 Enzyme-Linked Immuno-sorbent Assay

Each microwell of the 96-well Corning plate was pre-treated with 100μL 2% Glutaraldehyde for 20 min at room temperature. One hundred and 50 microliters of 15 mM gold nanoparticles were added to the microwells and incubated for 2 hour at room temperature followed by Milli Q water wash for three times and then washed with 0.5% Triton X-100 in PBS for three-times. Blocking for non-specific binding was performed by adding 100uL of 3% BSA and incubated for 60 min at room temperature followed by PBS wash for three times. Binding was performed by adding 100uL properly diluted antiserum into microwells and incubated for 1 hr at room temperature followed by thoroughly washes. HRP-conjugated anti-mouse IgG, ABTS, and H2O2 was incorporated in sequence to the wells according to manufacture's protocol and the binding efficiency was monitored by absorbance at 405 nm.

3 RESULT AND DISSUSION

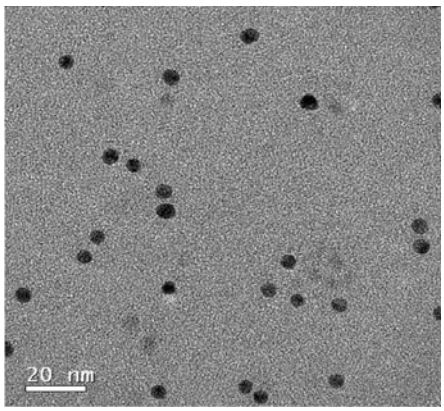

Fig. 1. TEM image of 5 nm GNP conjugated with immunoglobulin. Conjugation of immunoglobulin improved dispersion of GNP. GNP exhibits solid circular spots while immunoglobulin shows blurred image accompanying each GNP.

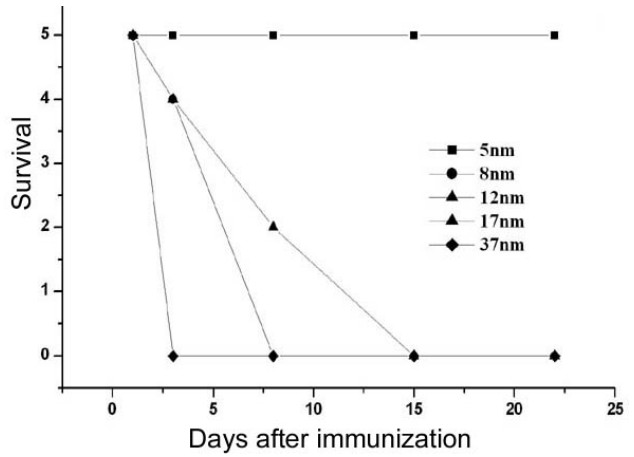

Fig. 2. Survival of mice injected with 5-, 8-, 12-, 17- and 37-nm GNP.

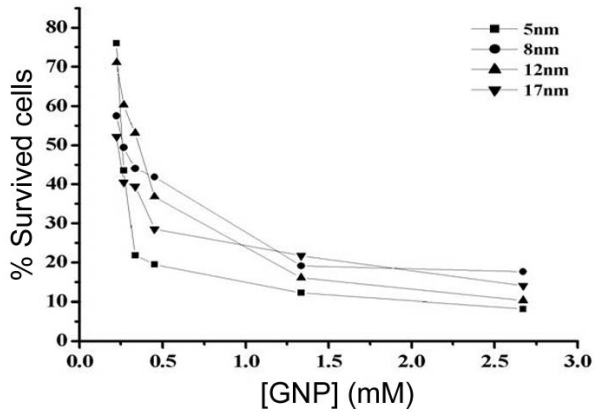

Fig. 3. MTT assay for the toxicity of GNPs to NIH 3T3 cells.

Gold nanoparticles (GNPs) were synthesized and examined under electraon microscope (Fig. 1). To obtain antibodies specifically recognizing GNPs BALB/C mice were immunized by weekly intra-peritoneal injection of adjuvant-emulsified 5 nm, 8 nm, 12 nm, 17 nm, and 37 nm GNPs. Mice injected with 8 nm, 12 nm, 17 nm, or 37 nm GNP died within 2 weeks, while mice injected with 5 nm GNP were feeble but survived at the end of the fourth week (Fig. 2).

It is unexpected that GNPs of larger size were lethal to mice. The differential toxicity might due to the difference in cytotoxicity. To obtain this information GNPs were incorporated into the growth media of NIH 3T3 cell culture. Colorimetric methyl-thiazol-tetrazolium (MTT) assay was performed to measure cytotoxicity for GNPs. LC_{50} ranging from 0.3 to 0.4 mM indicated that all GNPs exhibited essentially the same and low cytotoxicity (Fig. 3) [12-14]. The uncoupling of lethality and cytotoxicity indicated that factors other than cell toxicity are involved in the lethal effect of larger sized GNPs.

The differential ability of mice to produce antibodies against different size of GNP indicated that the immunogenecity, not cytotoxicity of GNPs might play central role regarding the differential lethality in animal. To test this hypothesis surface modification of GNP was performed to display a spectrum of different antigens on the surface of GNP. The immunogenic peptide IsdA peptide was designed from membrane protein of *Staphylococcus aurcus* as a highly immunogenic candidate. Bovine serum albumin (BSA) and Lysozyme were selected to represent moderately immunogenic antigens. Malonedialdehyde-modified BSA (MDA-BSA) was selected to shown the effect of lipid peroxidation. Conjugation of antigen with 17 nm GNP was performed following routine titration and monitored by UV absorption to reach saturation. The conjugated complexes were purified by centrifugation followed by peritoneal injection into mice. Unmodified 17 nm GNP was also injected into mice and served as positive control. Mice received PBS (phosphate-buffered saline) injection served as negative control. Although each group included only 3 mice, injection of modified GNP induced distinct severity in acute response (Fig. 4.). The severity and occurrence in sequence was: MDA-BSA > Positive control > BSA = Lysozyme > IsdA peptide > Negative control.

Fig. 4. Picture of control mouse (left) and GNP-dosed mouse (right).

This sequence was also consistent with the survival days for each group (Fig. 5). We noticed that mice developed rough skin, thin hair, and camel-like outlook (Fig. 4.).

We collected sera from injected mice and performed Enzyme-linked immunosorbant assay (ELISA) to obtain the binding activity against injected antigen. As expected, serum against IsdA peptide exhibited the highest titer. The ability of coated-GNP to stimulate immune response was consistent with the survival rate.

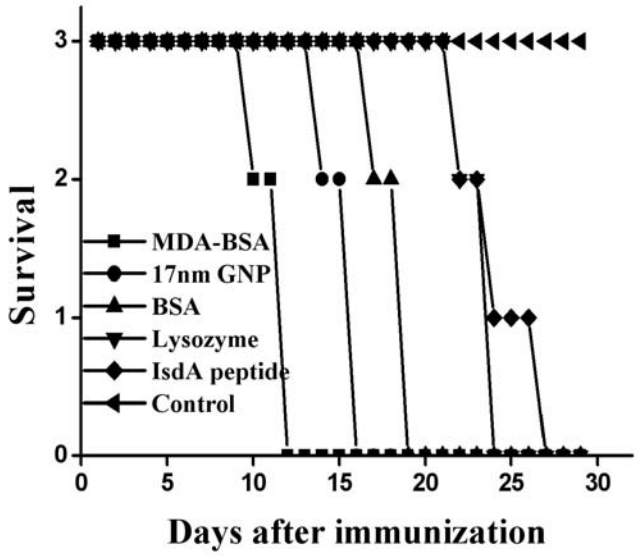

Fig. 5. Survival of mice injected with 17-nm GNP. Each experimental group received GNP conjugated with BSA, MDA modified BSA (MDA-BSA), Lysozyme, or IsdA peptide. Unmodified GNP was injected to positive control group (17 nm GNP). Mice received PBS (phosphate-buffered saline) injection served as negative control (control).

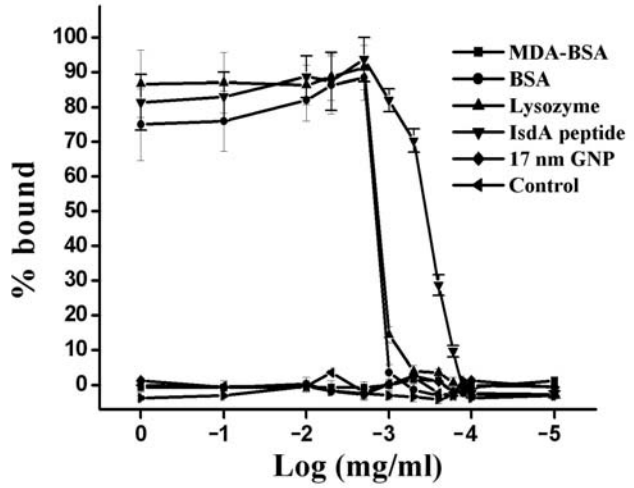

Fig. 6. Binding of antiserum withdrawn from GNP-injected mice against corresponding antigens. IsdA peptide-coated GNP induced highest titer in mouse serum; BSA- and Lysozyme-coated GNP induced moderate titer; while unmodified GNP and MDA-BSA coated GNP did not induce immuno-response in mice.

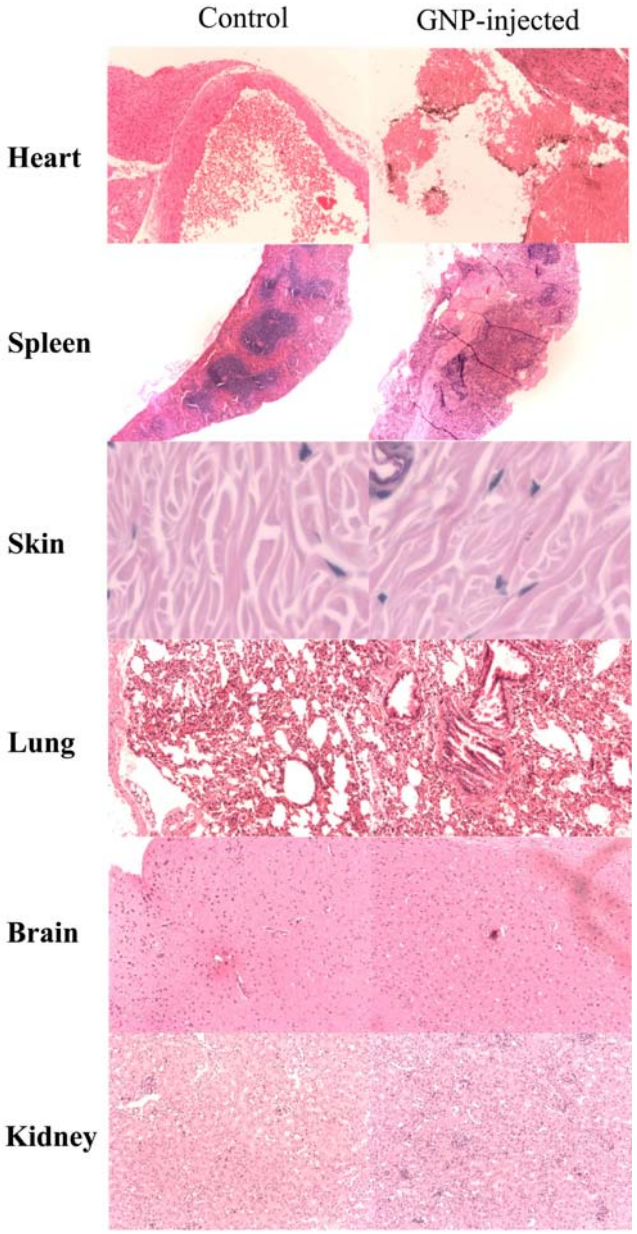

Fig. 7. H&E stain showing GNP-induced abnormality in major organs.

The acute sickness and eventually death of mice receiving GNP indicated that the injected GNP might damage major organ. Heart tissue presented major difference between experimental and control group. Microscopic picture showed that dark spots distributed at the myocardium. We suspected that GNP might invade into vascular system and cause necrosis in heart tissue that lead to sudden death.

Spleen also showed significant abnormality in the GNP-treated group. White pulps consisting of splenic nodules appeared diffused in the experimental group. Although spleen is not an essential organ, abnormality indicated damaging power of GNP in the blood stream.

Skin appeared abnormal with less hair and darker color from naked eyes. However, we could not distinguish the abnormality from microscopic picture.

Other organs: lung, brain, and kidney, appeared indistinguishable from normal tissue (Table 1). However, microscopic investigation might reveal detail damages.

In summary, injected GNP induced damages in myocardium and spleen. The former probably caused acute symptom in mice.

Table 1 Summary for the organ damages by antigen-coated GNP injection.

	NC[b]	PC	MDA	BSA	Lys	IsdA
H[a]	—[c]	+++	++	++	++	++
Sp	—	+++	+++	+	+	+
Sk	—	+++	++	++	++	+
Lu	—	—	—	—	—	—
Br	—	—	—	—	—	—
Kid	—	—	—	—	—	—

[a]abbreviations for organs are: H, heart; Sp, spleen; Sk, skin; Lu, lung; Br, brain; and Kid, kidney.

[b]abbreviations for coated antigens are: NC, negative control; PC, positive control (untreated 17 nm GNP); MDA, MDA-modified BSA; BSA, bovine serum albumin; Lys, lysozyme; and IsdA, IsdA peptide from *Staphylococcus aurcus*.

[c]—, normal; +, abnormal.

4 ACKNOWLEDGMENTS

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