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Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles

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ABSTRACT

In general, gold nanoparticles are recognized as being as nontoxic. Still, there have been some reports on their toxicity, which has been shown to depend on the physical dimension, surface chemistry, and shape of the nanoparticles. In this study, we carry out an *in vivo* toxicity study using 13 nm-sized gold nanoparticles coated with PEG (MW 5000). In our findings the 13 nm sized PEG-coated gold nanoparticles were seen to induce acute inflammation and apoptosis in the liver. These nanoparticles were found to accumulate in the liver and spleen for up to 7 days after injection and to have long blood circulation times. In addition, transmission electron microscopy showed that numerous cytoplasmic vesicles and lysosomes of liver Kupffer cells and spleen macrophages contained the PEG-coated gold nanoparticles. These findings of toxicity and kinetics of PEG-coated gold nanoparticles may have important clinical implications regarding the safety issue as PEG-coated gold nanoparticles are widely used in biomedical applications.

Introduction

Advances in nanotechnology have identified promising candidates for many biological and biomedical applications, and because of their small particle size, novel physicochemical properties, and easy surface modification, nanoparticles have been widely used. In particular, gold nanoparticles (AuNPs) have been used as Raman sensors (Qian et al., 2008), photocatalysts (Costi et al., 2008), and photoelectrochemical materials (Kerman and Kraatz, 2007) for their unique optical properties arising from the surface plasmon oscillation of free electrons (Huang et al., 2007). In addition, AuNPs are used as localized photothermal agents mediating tumor cell necrosis from hyperthermia after irradiation with laser light (O'Neal et al., 2004) and have also been useful as biosensors (Nam et al., 2003) and carriers for the delivery of drugs and genes (Gibson et al., 2007).

Despite the huge potential benefit of AuNPs in the field of biomedical and industrial applications, very little is known about their toxicity and tissue bioavailability of AuNPs on animals. Although AuNPs are recognized as being as nontoxic, (Merchant, 1998; Connor et al., 2005; Shukla et al., 2005) there have been still some reports on their toxicity (Goodman et al., 2004; Pernodet et al., 2006; Chithrani and Chan, 2007; Pan et al., 2007), which has been shown to depend on the physical dimension, surface chemistry, and shape of the AuNPs. Nanoparticles could also be identified as foreign by the immune cells, causing the cells to react against either surface or core components to mount an inflammatory response, which involves secretion of signaling molecules (known as cytokines) to attract more cells to destroy the foreign substances (Dobrovolskaia and McNeil, 2007; Stern and McNeil, 2008). However, many of these molecular events are still poorly understood, partly because most papers addressing AuNPs toxicity have only used *in vitro* models. Here we carry out an *in vivo* toxicity study using PEG-coated AuNPs 13 nm in size.

Materials and methods

Synthesis of PEG-coated AuNPs. AuNPs of 13 nm in size were used in this work. AuNPs of sized 13 nm were synthesized as described by Frans (1973). The gold ion solution (120 mg of $HAuCl_4$ dissolved in 250 mL of water) was prepared and boiled. Then, the 1% citric acid solution (50 mL) as a reducing agent was added and the solution was further boiled for 10 min. The AuNP solution was cooled down to room temperature. The average diameter of as-prepared AuNP was determined to be 13±2 nm using transmission electron microscopy CM20 (Philips, Eindhoven, Netherlands) at an acceleration voltage of

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12 kV. AuNPs were modified using thiol-terminated poly (ethylene) glycol (HS-PEG, of MW 5000; SunBio Inc, Anyang, Korea). After PEGylation, solutions were exchanged for PBS buffer by centrifugation. Transmission electron microscopy images of PEG-coated AuNPs were obtained using a CM20 (Philips, Eindhoven, Netherlands) at an acceleration voltage of 120 kV. Hydrodynamic diameter of PEG-coated AuNPs was measured by dynamic light scattering (ELS-Z, Otsuka, Japan) at 653 nm laser source. The hydrodynamic diameter of PEG-coated AuNPs determined in this work was calculated by the cumulant method provided in software of DLS instrument. The absence of endotoxin contamination of AuNPs suspensions was confirmed using the Limulus Amebocyte Lysate assay (Cambrex, Walkersville, MD).

Animals. We maintained 6-week old male BALB/c mice (Charles River Laboratories) in accordance with the AAALAC International Animal Care Policies as approved by the Animal Care and Use Committee of the National Institute of Toxicological Research, Korea Food and Drug Administration, Korea. The mice were housed individually. Water and feed were available *ad libitum*. The environmental conditions (temperature, 23±1 °C; relative humidity, 55±5%; 12-h light/dark cycle) were monitored at ~4-h cycles for 24-h/day and maintained within the acceptable ranges through the study. One hundred μL amounts of PEG-coated AuNPs suspensions were administered intravenously (via a tail vein) to the BALB/c mice at 0, 0.17, 0.85 or 4.26 mg/kg of body weight (nine animals/group). The average injected numbers of particles per mice were 1.76×10^{11}, 8.8×10^{11}, and 4.4×10^{12} for low, middle, and high dose, respectively. The animals were euthanized at 5 min, 30 min, 4 h, 24 h, or 7 days post-injection. At each time-point, the animals were anesthetized with ether and weighed. Blood was collected from an abdominal vein for blood clearance assays. All organs were fixed in 10% (v/v) neutral buffered formalin and processed using routine histological techniques. After paraffin embedding, 3 μm sections were cut and stained with hematoxylin and eosin (H&E) for histopathological evaluations. Histopathological diagnosis was performed according to Standardized System of Nomenclature and Diagnostic Criteria (SSNDC).

TUNEL assay. To detect apoptosis, paraffin-embedded sections were stained with terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) kits (Roche Diagnostics GmbH, Mannheim, Germany). Numbers of positively stained cells were measured in five randomly selected high-power (×200) fields per slide and the significances of differences between treatment and control groups were determined using the Dunnett *t* test after ANOVA analysis.

Tissue distribution. The inductively coupled plasma technique was used to determine gold concentrations in blood, liver, spleen, kidneys, lung, brain, and testis after administering 0.85 or 4.26 mg/kg of 13 nm-sized PEG-coated AuNPs. Samples were taken at 5 min, 30 min, 4 h, 24 h, or 7 days post-injection. Samples were prepared by digestion in a microwave oven after addition of 5 mL of 55% (12 M) HNO_3. Gold concentrations were determined using an ICP MS X7 unit (ThermoElemental, UK). Transmission electron microscopy (TEM) was used to determine the cellular localization of PEG-coated AuNPs in BALB/c mice. Liver, spleen, kidneys, lung, brain, and testes were obtained at 24 h and 7 days after treatment with 4.26 mg/kg of PEG-coated AuNPs, stained *en bloc* with uranyl acetate, and embedded in epoxy resin. Ultra-thin (60 nm) sections were cut, stained with uranyl acetate and lead citrate, and examined with a TEM (JEM-1200EX II, JEOL).

Pharmacokinetic analysis. Pharmacokinetic parameters for PEG-coated AuNPs were determined by compartmental analysis. Maximum plasma concentrations (C_{max} values) were obtained by visually inspecting concentration–time profiles. Areas under the plasma concentration curves versus time, from zero to infinity ($AUC_{0-\infty}$ values), and mean residence times (*MRTs*), were determined using the linear trapezoidal rule and by extrapolation to infinity using standard techniques (Gibaldi and Perrier, 1982). The total clearance (*CL* value) was calculated from the quotient of dose (*D*) and $AUC_{0-\infty}$ for the intravenous route. The fitting of compartmental equations to experimental data was performed using WinNonlin software (Version 2.1; Pharsight, Mountain View, CA). To select the best pharmacokinetic model, a number of criteria, including Akaike's information criterion (AIC), the Schwartz criterion, and correlation coefficients were used. In addition, residual plots were visually inspected and calculated coefficients of variation (CVs, % values) of the pharmacokinetic parameter estimates were used to select the best model. Coefficients of variation lower than 50% were considered acceptable.

RNA extraction, cDNA construction, and quantitative real-time PCR. The liver tissues were prepared to determine mRNA expression of adhesion molecules, chemokines, and cytokines. Total RNA extraction, cDNA synthesis were performed previously described methods (Cho et al., 2007). Quantitative real-time PCR was performed by adding Universal PCR buffer and Taqman primer/probe assay reagents specific for adhesion molecule mRNAs (encoding ICAM-1, VCAM-1, PECAM-1, VEGF, and E-selectin), chemokines (MCP-1/CCL-2, MIP-1α/CCL-3, MIP-1β/CCL-4, RANTES/CCL-5, and MCP-5/CCL-12), cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12β, IFN-γ, TNF-α, and GM-CSF), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2), according to the manufacturer's instructions. Dye-labeled (VIC) GAPDH primers/probes were used to amplify control GAPDH. All primers/probes were purchased from Applied Biosystems as Assay-on-Demand™ Gene Expression Products (Taqman MGB probes, FAM dye-labeled). PCR was performed using an ABI Prism 7500 unit (Applied Biosystems) under the following conditions: 50 °C for 2 min, 95 °C for 10 min, and 40 amplification cycles (95 °C for 15 s and 60 °C for 1 min). The C_T (threshold cycle) values obtained for genes of interest were normalized to GAPDH (a housekeeping gene) levels, and -fold increases were compared with controls.

Immunohistochemistry. Adhesion molecules (ICAM-1 and VCAM-1), chemokines (MCP-1/CCL-2, MIP-1α/CCL-3, MIP-1β/CCL-4, and

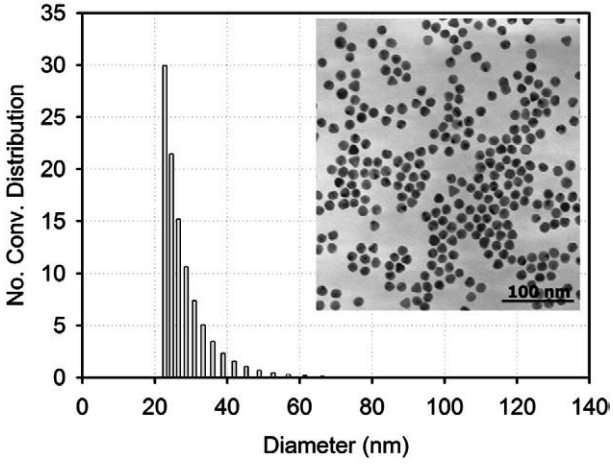

Fig. 1. Synthesis and characterization of PEG-coated AuNPs. Transmission electron microscopy (TEM) and dynamic light scattering measurements of 13 nm PEG-coated AuNPs.