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Effects of nanoparticle size and gestational age on maternal biodistribution and toxicity of gold nanoparticles in pregnant mice

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HIGHLIGHTS

- Biodistribution of PEGylated GNPs in pregnant mice is size-dependent.
- GNPs exhibited distinct biodistribution profiles regardless of gestational ages.
- The main factor controlling GNPs clearance routes and rates was nanomaterial size.
- No adverse effect was found on pregnant mice by i.v. injection except 30 nm GNPs.
- This study laid a foundation for GNPs pregnancy application as drug vehicles.

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ABSTRACT

Gold nanoparticles (GNPs) have considerable applications in biomedicine, such as in bio-sensing, bio-imaging, drug delivery and photothermal therapeutics. However, currently there are limited information regarding the impact of pregnancy on their biodistribution, elimination and toxicity. In this study, we investigated the biodistribution and potential toxic effects of different-sized GNPs (1.5, 4.5, 13, 30 and 70 nm in diameter) in non-pregnant and pregnant mice at different gestational ages (E5.5, 7.5, 9.5, 11.5 and 13.5). 5 h after intravenous injection, GNPs exhibited size-dependent biodistribution profiles; however, regardless of size, no significant biodistribution changes were observed between non-pregnant and pregnant mice. Kinetic studies showed that 4.5 nm GNPs were primarily excreted through urine within 5 h, whereas 30 nm GNPs had a more prolonged blood circulation time. No apparent toxic effects (e.g., increased mortality, altered behavior, reduced animal weight, abnormal organ morphology or reduced pregnancy duration) were observed with different-sized GNPs in pregnant mice. However, treatment with 30 nm GNPs induced mild emphysema-like changes in lungs of pregnant mice. These results indicated that the maternal biodistribution patterns of GNPs in pregnant mice depended on particle size, but not gestational age; organ-specific adverse effects may arise with treatment with some GNPs according to their size.

1. Introduction

Gold nanoparticles (GNPs) hold great promise in biomedicine as carriers of pharmaceuticals or as novel diagnostic and therapeutic agents (Caruthers et al., 2007; Dreaden et al., 2012). At present, the likelihood of exposure to GNPs is increasing with the rapid development of nanotechnology (Cherukuri et al., 2010; Cobley et al., 2010; Keelan, 2011). Some of the beneficial characteristics of GNPs include their straight forward synthesis, high stability, low

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toxicity in vivo and ability to selectively incorporate recognition molecules such as peptides or proteins (Pissuwan et al., 2006). These properties make them well suited for biomedical and pharmaceutical applications, and ideal nanomaterials with which to evaluate the biological effects and safety of spherical nanoparticles in different settings. Such studies require the assessment of the biodistribution, pharmacokinetics and local or systemic toxicity of GNPs after systematic administration, and the influence of specific pathological and physiological states on these factors (Saunders, 2009; Holcberg et al., 2003; Menezes et al., 2011). Considerable research has been carried out on biodistribution, cellular uptake and toxicity of gold nanoparticles in recent years (Alkilany and Murphy, 2010; Khlebtsov and Dykman, 2011).

Nanoparticle size had proved to be one of the most important factors in influencing biodistribution, tissue uptake and applications in the biomedical fields (Arvizo et al., 2012; Yu et al., 2012; Wang et al., 2010). Particle size-dependent biodistribution and toxicity of GNPs has been widely studied in vivo (Hillyer and Albrecht, 2001; Sonavane et al., 2008; De Jong et al., 2008; Semmler-Behnke et al., 2008; Hainfeld et al., 2006). The in vivo biodistribution of the GNP influences their accumulation by secondary organs, which may eventually cause diverse health effects. In most studies, systemically administrated GNPs are primarily taken up by liver and spleen, with small amounts distributed in the lung, kidney, heart, and brain. Hyllier and Albertch showed that orally administered colloidal GNPs (58, 28, 10 and 4 nm in diameter) can be detected in various tissues in mice and that the amount of absorption and distribution in the body inversely correlated with particle size (Hillyer and Albrecht, 2001). In De Jong's study, after intravenous injection into rats, spherical GNPs ranging from 10 to 250 nm in diameter were shown to be taken up primarily by the liver and spleen, with the 10 nm nanoparticles more broadly distributed in various organs (De Jong et al., 2008). GNPs of 12.5 nm in diameter do not exert toxic effects of in the liver, lungs, kidneys, spleen or brain (Lasagna-Reeves et al., 2010; Schmid, 2008). Toxicity assessments of GNPs of different sizes (3, 10, 50, and 100 nm) in zebrafish embryos showed only minimal sub-lethal toxic effects with no size effect (Bar-Ilan et al., 2009). In contrast, size-dependent in vitro toxicity was found to occur with 1.4 nm GNP, but not 0.8 or 15 nm GNP (Pan et al., 2007).

In spite of a number of studies investigating the in vivo size effects of GNPs, little is known about the effects of size on biodistribution, pharmacokinetics and toxicity of GNPs in pregnant females and their fetuses after systematic administration. Pregnancy represents a unique physiological state with altered hemodynamics and pharmacokinetics in which the biodistribution and toxicity of nanoparticles is unpredictable (Abduljalil et al., 2012). Maternal physiological changes begin early in gestation and are most pronounced in the third trimester (Federiksen, 2001). Plasma volume increases by about 40–50% during pregnancy and the total plasma concentrations of albumin-bound drugs decrease due to hemodilution (Frederiksen, 2001; Dawes and Chowienczyk, 2001; Loebstein and Koren, 2002; Loebstein et al., 1997). Hepatic metabolic ability changes during pregnancy in response to the increase in estrogens and progesterone (Dawes and Chowienczyk, 2001; Loebstein and Koren, 2002; Loebstein et al., 1997). In pregnancy, renal blood flow and glomerular filtration rate increase, leading to enhanced elimination of some albumin-bound drugs (Jeyabalan and Conrad, 2007; Morgan, 1997). However, the renal clearance of nanoparticles in pregnancy has not been investigated, and it is still not clear whether the physiological changes exhibited by the main organs such as liver and kidney in pregnancy affect the biodistribution and in vivo metabolism dynamics of nanoparticles or how these adaptations change as pregnancy progresses.

To address these questions, we applied a series of PEGylated GNPs from 1.5 to 70 nm in diameter to pregnant mice (or non-pregnant female controls) by intravenous injection at different gestational ages, and assessed their biodistribution and in vivo maternal dynamics throughout pregnancy; we also assessed the acute and sub-acute toxicities of GNPs in pregnant mice compared to non-pregnant females using a variety of methods. Our aim was to provide fundamental information on maternal GNPs biodistribution and toxicity in pregnancy. Data on fetal and placental biodistribution and toxicity will be presented in a subsequent study.

2. Materials and methods

2.1. Synthesis of different-sized GNPs capped with PEG-5000

15 nm GNPs: triphenylphosphine (TPP) stabilized gold nanoparticles (5 nm Au-TPP) were synthesized as previously described (Hutchison et al., 2004). 20 mg of the thiolyated PEG-5000 was added to 5 mL solution including 20 mg of 5 nm Au-TPP in dichloromethane and stirred rapidly for 24 h to obtain the PEGylated particles.

4.5 nm GNPs: Au5-PEG was prepared according to reference Manna et al. (2003). The resulting crude GNPs were redissolved and purified by column chromatography using Sephadex LH-20

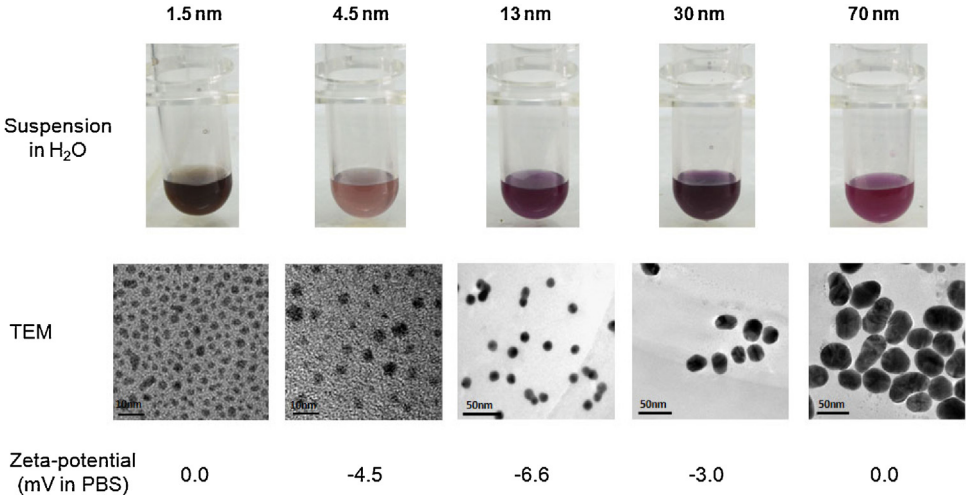

Fig. 1. Characterization of PEGylated GNPs with different sizes. PEGylated GNPs with diameters of 1.5, 4.5, 13, 30 and 70 nm were examined under TEM. Scale bars are 10 nm for images of 1.5 and 4.5 nm GNPs and 50 nm for images of 13, 30, and 70 nm GNPs. The lower panel shows the zeta-potential of GNPs.