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Hepatotoxicity of sub-nanosized platinum particles in mice

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Nano-sized materials are widely used in consumer products, medical devices and engineered pharmaceuticals. Advances in nanotechnology have resulted in materials smaller than the nanoscale, but the biologic safety of the sub-nanosized materials has not been fully assessed. In this study, we evaluated the toxic effects of sub-nanosized platinum particles (snPt) in the mouse liver. After intravenous administration of snPt (15 mg/kg body weight) into mice, histological analysis revealed acute hepatic injury, and biochemical analysis showed increased levels of serum markers of liver injury and inflammatory cytokines. In contrast, administration of nano-sized platinum particles did not produce these abnormalities. Furthermore, snPt induced cytotoxicity when directly applied to primary hepatocytes. These data suggest that snPt have the potential to induce hepatotoxicity. These findings provide useful information on the further development of sub-nanosized materials.

1. Introduction

Nanotechnology involves manipulation of matter on the scale of the nanometer and has the potential to improve quality of life via functional products. Nanomaterials are commonly defined as objects with dimensions of 1 to 100 nm and are now widely used in electronics, catalysts, clothing, drugs, diagnostic devices, and cosmetics (Baughman et al. 2002; Patra et al. 2010; Service et al. 2007; Ariga et al. 2010). Recent progress in the field has allowed the creation of sub-nanosized materials that have different physicochemical properties, including improved conductivity, durability and strength. Although these materials may be useful for industrial and scientific purposes, the biologic safety of these materials has not been fully evaluated (Nel et al. 2006; Oberdorster et al. 2005).

Nano-sized platinum particles (nPt) are used for industrial applications and in consumer products, such as cosmetics, supplements and food additives (Gehrke et al. 2011; Horie et al. 2011). The biological influence of exposure to nPt has been previously investigated. For example, nPt has anti-oxidative activity (Watanabe et al. 2009; Onizawa et al. 2009; Kajita et al. 2007), and may be useful for the medical treatment of diseases related to oxidative stress and aging. However, some reports suggest that these substances can induce inflammation in mice or impair DNA integrity (Pelka et al. 2009; Park et al. 2010). Thus, the understanding of the biological influences of nPt has still not been definitively established, and our knowledge regarding the biological effects of sub-nanosized platinum particles (snPt) is severely lacking.

Nano-sized particles can enter and penetrate the lungs, intestines and skin. The degree of penetration depends on the size and surface features of the nano-sized particle. Furthermore, nanoparticles can enter the circulatory system and migrate to various organs, such as the brain, spleen, liver, kidney and muscles (Zhu et al. 2008; Furuyama et al. 2009; Oberdorster et al. 2004; Ai et al. 2011). The liver is a vital organ that is involved in the uptake of nutrients and the elimination of waste products and pathogens from the blood; it is also an important organ for the clearance of nanoparticles. However, some nanoparticles are hepatotoxic (Nishimori et al. 2009a, b; Ji et al. 2009; Cho et al. 2009; Folkmann et al. 2009). In the present study, we investigated the influence of sub-nanosized platinum particles (snPt) on the liver.

2. Investigations and results

To investigate the acute liver toxicity of snPt, we administered snPt (15 mg/kg body weight) into mice by intravenous injection. Histological analysis revealed acute hepatic injury, including vacuole degeneration (Fig. 1). Furthermore, administration of snPt at doses over 15 mg/kg resulted in significant elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (Fig. 2A and B) and of interleukin-6 (IL-6) levels (Fig. 2C). ALT and AST levels were increased at 3 h to 24 h after intravenous administration at 20 mg/kg snPt (Fig. 3A and B). Cell viability assessment by WST assay demonstrated that direct treatment of isolated hepatocytes with snPt at concentrations of 0.1, 1, 10, 50 and 100 μg/ml resulted in a dose-dependent decrease in hepatocyte viability when compared with vehicle-treated cells (Fig. 4). These observations suggest that snPt induced inflammation and hepatocyte death.

Previous reports showed that biological influences of nanomaterials vary according to material size (Nishimori et al. 2009a, b; Jiang et al. 2008; Oberdorster et al. 2010). Therefore, we examined whether nPt, with a diameter of approximately 15 nm, leads

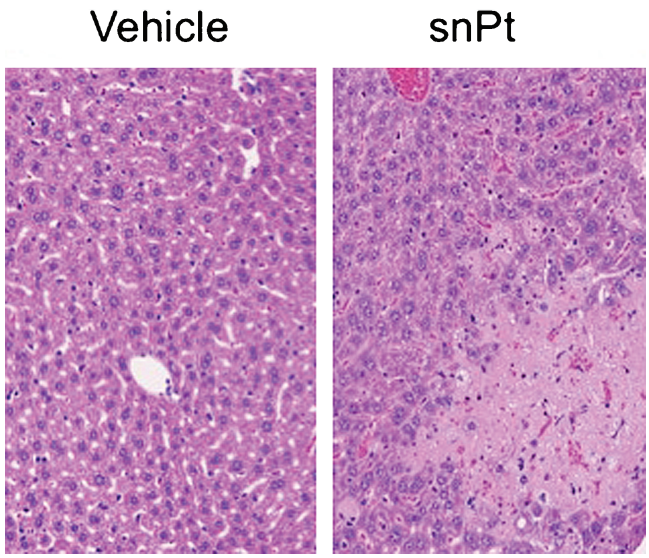

Fig. 1: Histological analysis of liver tissues in snPt-treated mice. snPt was intravenously administered to mice at 15 mg/kg. At 24 h after administration, livers were collected and fixed with 4% paraformaldehyde. Tissue sections were stained with hematoxylin and eosin and observed under a microscope. The pictures show representative data from at least four mice

to a different biologic effect than snPt. As shown in Fig. 5, snPt administration resulted in dose-dependent increases in serum ALT and AST levels, whereas nPt did not. Furthermore, IL-6 levels did not change in response to administration of nPt. These results suggest that the biological effects of platinum particles are dependent on their size.

3. Discussion

The influence of size and of physiochemical properties of nanoparticles on their biologic safety is an important issue. Animal experiments have demonstrated rapid translocation of nanoparticles from the entry site to various organs (Almeida et al. 2011). In particular, nanoparticles tend to concentrate in the liver and are cleared from the body in the feces and urine after intravenous infusion (Ai et al. 2011). While the liver plays a pivotal role in the clearance of nanoparticles, some nanomaterials can induce liver injury. Therefore, we assessed the influence of snPt on the liver and demonstrated that snPt induced liver toxicity *in vitro* and *in vivo*.

Some studies have reported that nPt exert anti-oxidant and anti-inflammatory effects (Watanabe et al. 2009; Onizawa et al. 2009; Kajita et al. 2007), while other studies reported that nPt have negative biological effects. For example, treatment of a human colon carcinoma cell line with nPt resulted in a decrease in cellular glutathione level and impairment in DNA integrity (Pelka et al. 2009). Furthermore, Park et al. (2010) found that nPt prepared from K_2PtCl_6 may induce an inflammatory response in mice. In this study, we found that snPt damaged liver tissues and induced inflammatory cytokines. Kupffer cells present in liver sinusoids may mediate this process via phagocytosis of the particles and subsequent release of inflammatory cytokines. However, when we added snPt to primary hepatocytes, the viability of the cells was significantly reduced, suggesting that snPt may also exert a direct hepatotoxic effect. Thus, the cellular influences of Pt nano- and sub-nano particles may be dependent on the target cells as well as on the size and physical and chemical properties of the particles.

snPt may damage other tissues as well. Cisplatin, a first-line chemotherapy for most cancers, is a platinating agent that can cause kidney damage (Daugaard et al. 1990; Brabec et al. 2005). Furthermore, snPt-induced increases in systemic IL-6 may cause damage to various organs. Further analysis of the distribution and toxic effects of snPt is necessary.

Widespread application of sub-nanosized materials comes with an increased risk of human exposure and environmental release, and the future of nanotechnology will depend on the public acceptance of the risk-benefit ratio. The present study demonstrated that snPt induces hepatotoxicity *in vitro* and *in vivo*. However, our research also indicates that the toxicity of platinum particles could be reduced by altering their size. Additionally, biocompatible coatings can reduce the negative effects of nanoparticles on cells (Oberdorster et al. 2010; Nabeshi et al. 2011; Singh et al. 2007; Clift et al. 2008). Therefore, future studies will contribute to the development of sub-nanosized materials and will also help produce safer products.

4. Experimental

4.1. Materials

Platinum particles with a diameter of 15 nm (nPt) and less than 1 nm (snPt) were purchased from Polytech & Net GmbH (Rostock, Germany). The

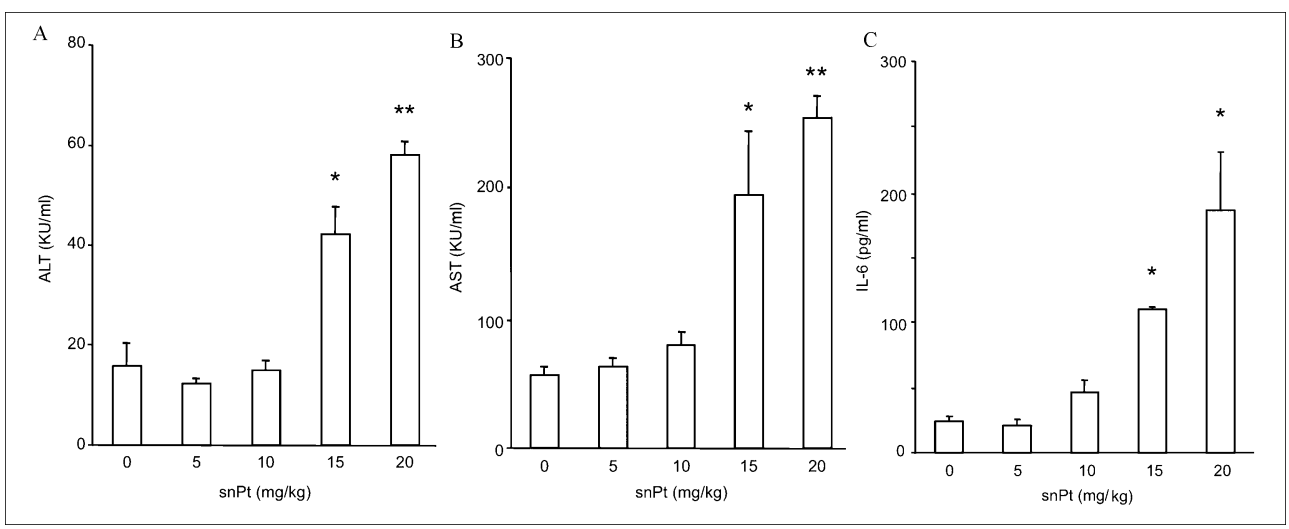

Fig. 2: Dose dependency of snPt-induced liver injury. snPt was intravenously administrated at 5, 10, 15 and 20 mg/kg. At 24 h after administration, blood was recovered, and the resultant serum was used for measurement of ALT (A), AST (B) and IL-6 (C), as described in the "Experimental" section. Data are means ± SEM (n = 3). *Significant difference when compared with the vehicle-treated group (*, $p < 0.05$, **, $p < 0.01$)

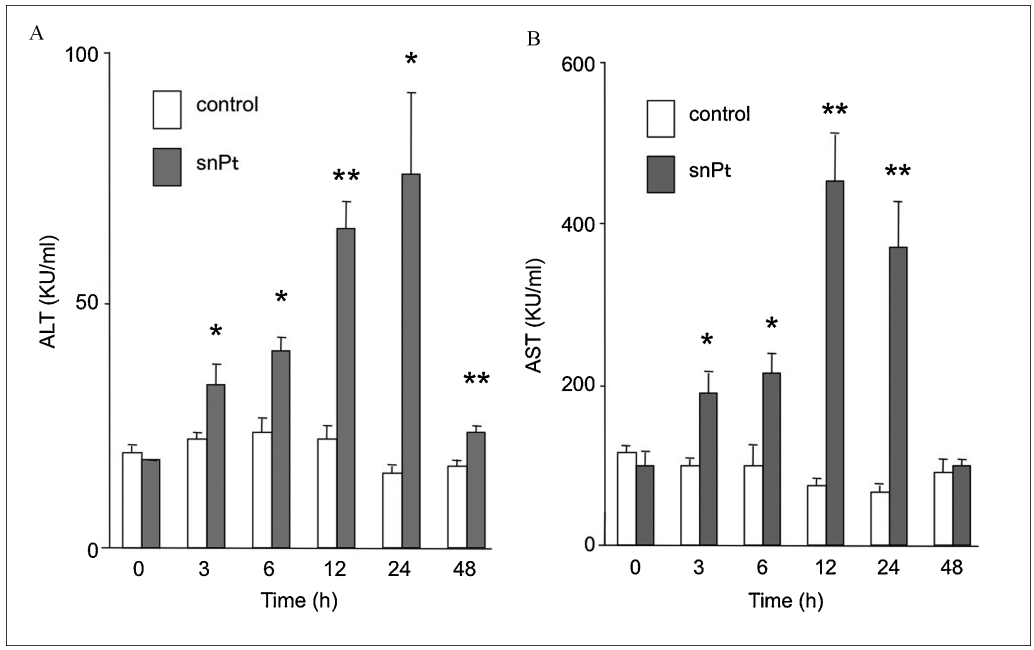

Fig. 3: Time-dependent changes of a biological marker of liver injury. snPt was intravenously administered to mice at 15 mg/kg. Blood was recovered at 3, 6, 12, 24 and 48 h after administration. The serum was used for measurement of ALT (A) and AST (B), as described in the "Experimental" section. Data are means ± SEM (n = 3). *Significant difference when compared with the vehicle-treated group (*, $p < 0.05$, **, $p < 0.01$)

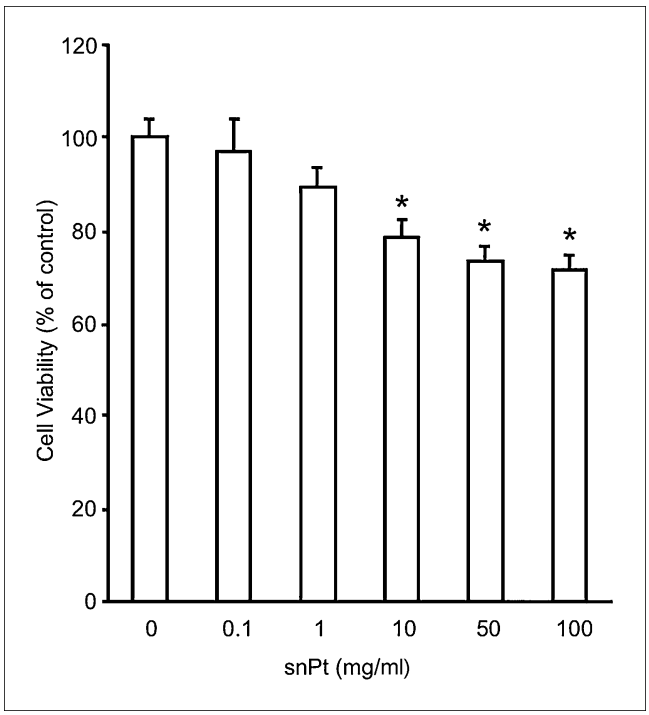

Fig. 4: Cytotoxicity of snPt in hepatic cells. Primary hepatocytes were treated with snPt at 0.1, 1, 10, 50 or 100 μg/ml. After 24 h of culture, cell viability was evaluated with the WST assay, as described in the "Experimental" section. Data are means ± SEM (n = 3). *Significant difference when compared with the vehicle-treated group ($P < 0.05$)

particles were stocked in a 5 mg/ml aqueous suspension. The stock solutions were suspended using a vortex mixer before use. Reagents used in this study were of research grade.

4.2. Animals

BALB/c male mice (8 weeks old) were obtained from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan), and were housed in an environmentally controlled room at 23 ± 1.5 °C with a 12 h light/12 h dark cycle. Mice had access to water and commercial chow (Type MF, Oriental Yeast, Tokyo, Japan). Mice were intravenously injected with nPt or snPt at 5 to 20 mg/kg body weight. The experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences, Osaka University.

4.3. Cells

Mouse primary hepatocytes were isolated from BALB/c mice (Shimizu Laboratory Supplies Co.) by the collagenase-perfusion method (Seglen 1976). Isolated hepatocytes were suspended in Williams' E medium containing 10% fetal calf serum, 1 nM insulin, and 1 nM dexamethasone. Next, cell viability was assessed by Trypan blue dye exclusion. Cells that were at least 90% viable were used in this study. Cells were cultured in a humidified 5% CO_2 incubator at 37 °C.

4.4. Histological analysis

After intravenous administration of snPt, mouse livers were removed and fixed with 4% paraformaldehyde. Thin tissue sections were stained with hematoxylin and eosin for histological observation.

4.5. Biochemical assay

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using commercially available kits (WAKO Pure Chemical, Osaka, Japan), respectively. Interleukin-6 (IL-6) levels were measured with an ELISA kit (BioSource International, Camarillo, CA, USA). These assays were performed according to the manufacturer's protocols.

4.6. Cell viability assay

Cell viability was determined using WST-8 (Nacalai Tesque, Osaka, Japan), according to the manufacturer's protocol. Briefly, 1×10^4 cells/well were seeded on a 96 well plate at 37 °C overnight. After 24 h of treatment with snPt, WST-8 reagent was added to each well. The plate was incubated for 1 h at 37 °C and assessed at an absorbance of 450 nm by a plate reader. Obtained data were normalized to the control group, which was designated as 100%.

4.7. Statistical analysis

Data are presented as means ± SD. Statistical analysis was performed by student's t-test. $P < 0.05$ was considered statistically significant.

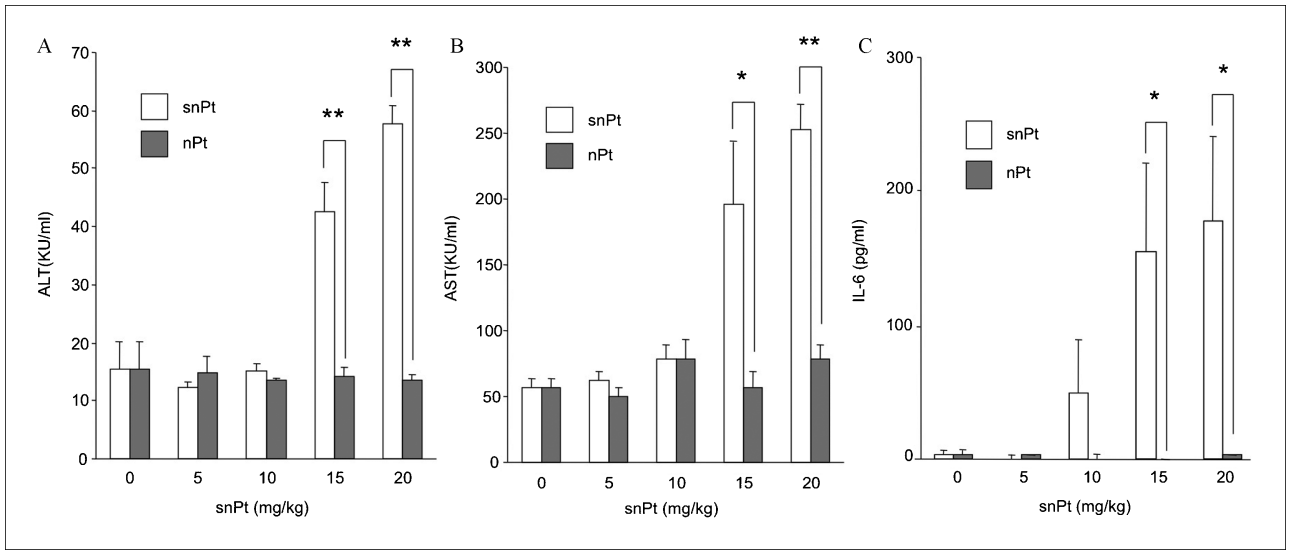

Fig. 5: Effect of particle size of platinum on liver injury. snPt or nPt was intravenously injected into mice at the indicated doses. Blood was recovered at 24 h after injection. Serum ALT (A), AST (B) and IL-6 (C) levels were measured. Data are means ± SEM (n = 3). *Significant difference between the snPt- and nPt-treated groups (*, $p < 0.05$, **, $p < 0.01$)

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