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Acute liver damage and interaction of cisplatin with silver nanoparticles depend on particle size

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Nanomaterials are innovative materials that have novel properties that differ from those of macroscale materials in terms of response to stimuli such as heat, light, and voltage. However, the potential unknown effects of nanomaterials on living organisms have raised concerns. There are few reports describing the effects of silver nanoparticles on living organisms and the effects of nanoparticle interactions with chemical substances such as pharmaceuticals. Previously, we investigated the effects of silver nanoparticles on living organisms and their interactions with drugs. In that study, silver nanoparticles with a particle size of 10 nm induced acute liver injury, and silver nanoparticles with a particle size of 10, 50, or 200 nm interacted with drugs when administered to mice *via* the tail vein. Therefore, to investigate the relationship between the particle size of silver nanoparticles and degree of injury, we examined silver nanoparticles of 5, 10, 20, 30, 40, and 50 nm and the extent of acute liver injury and liver injury due to interactions with drugs. We found that silver nanoparticles ≤30 nm in size induced acute liver injury. Silver nanoparticles with a 5-nm particle size induced the most severe liver injury.

1. Introduction

The use of nanomaterials such as carbon black, silica nanoparticles, and silver nanoparticles in nanotechnology applications is increasing worldwide (Pirela et al. 2015; Marin et al. 2015). Compared with conventional submicron-sized materials, nanomaterials have unique properties, such as high conductivity, tensile strength, and chemical reactivity (Kumar and Raza 2017; Tee et al. 2016). Nanosize-scale materials used in nanotechnology are employed practically in microelectronics, cosmetics, and sunscreens, and their potential use in drug delivery systems is being evaluated (Farokhzad and Langer 2009; McIntyre 2012). However, the rapid expansion of nanomaterial production has raised concerns about health effects on the human body and other organisms. For example, carbon nanotubes reportedly induce mesothelioma-like lesions similar to those induced by crocidolite asbestos (Numano et al. 2019; Sakamoto et al. 2009). Nanomaterials can exhibit toxicities not associated with micromaterials; thus, it is essential to increase understanding of the biological activity and potential toxicity of nanomaterials.

Silver is chemically stable and exhibits high electrical and thermal conductivity and visible light reflectance (You et al. 2012; Reznickova et al. 2015). Silver is used in many products, including chemical analytical instruments, coins, accessories, and medical instruments (Souza et al. 2018). The synthesis of silver nanoparticles has been the subject of extensive research. Silver nanoparticles reportedly offer excellent optical and electrical properties and strong antimicrobial activity (Reznickova et al. 2015; Iravani et al. 2014). Given these characteristics, silver nanoparticles are being used in an increasingly wide variety of products, such as solar cells, sensors, antibacterial materials, and medical products (You et al. 2012; Zhao et al. 2017). Silver nanoparticles are also potentially beneficial for living organisms due to their antibacterial properties. However, silver nanoparticles can have detrimental effects as well. In our previous study, we reported that silver nanoparticles with a particle size of 10 nm induce acute liver injury in mice following administration via the tail vein and also induce liver injury due to interactions with the anticancer drug cisplatin (CDDP) (Isoda et al. 2019).

Many reports have described the toxicity of silver nanoparticles. It was reported that silver nanoparticles with a diameter of ≤100 nm are neurotoxic *in vivo* and that particles ≤10 nm can pass through the blood-brain barrier (Báez et al. 2021). Furthermore, silver nanoparticles are both cytotoxic and genetically toxic, and it has been reported that genetic toxicity increases as particle size decreases (Zhang et al. 2014). In our previous study, we reported that silver nanoparticles with a particle size of 10 nm induce liver injury, but to date there have been no reports examining the effect of silver nanoparticle size on liver injury. Therefore, in this study, we investigated the effect of silver nanoparticles with a particle size (in nm) of 5, 10, 20, 30, 40, and 50 (SnP5, SnP10, SnP20, SnP30, SnP40, and SnP50, respectively) on liver injury due to interaction with the anticancer drug CDDP. We found that silver nanoparticles induce liver injury at a particle size of ≤30 nm and that SnP5 particles induce the most severe liver injury.

Abbreviations:
ALT, alanine aminotransferase; AST, aspartate aminotransferase; SnP5, 50-nm silver nanoparticles; SnP10, 10-nm silver nanoparticles; SnP20, 20-nm silver nanoparticles; SnP30, 30-nm silver nanoparticles; SnP40, 40-nm silver nanoparticles; SnP50, 50-nm silver nanoparticles

2. Investigations, results, and discussion

In a previous study, we reported that silver nanoparticles with a particle size of 10 nm induced acute liver injury in mice following administration *via* the tail vein (Isoda et al. 2019). Furthermore, it was reported that silver nanoparticles with a particle size of 10 nm induced liver injury *via* a strong interaction with the anticancer drug CDDP.

In the present study, we examined liver injury in mice administered silver nanoparticles ranging in size from 10 to 50 nm. Mice were

administered SnP10, SnP20, SnP30, SnP40, and SnP50 particles *via* the tail vein at a dose of 4 mg/kg. Serum alanine aminotransferase (ALT) and aspartate transaminase (AST) activity was measured 24 h after administration of silver nanoparticles (Fig. 1). The SnP10 group exhibited the greatest increases in ALT and AST activity, and the SnP20 and SnP30 groups also exhibited increased ALT and AST activity. Liver injury was observed in both groups. No increases in ALT or AST activity were observed in the SnP40 and SnP50 groups, and no liver injury was observed in these mice. These data indicate that silver nanoparticles ≤30 nm in size induce acute liver injury.

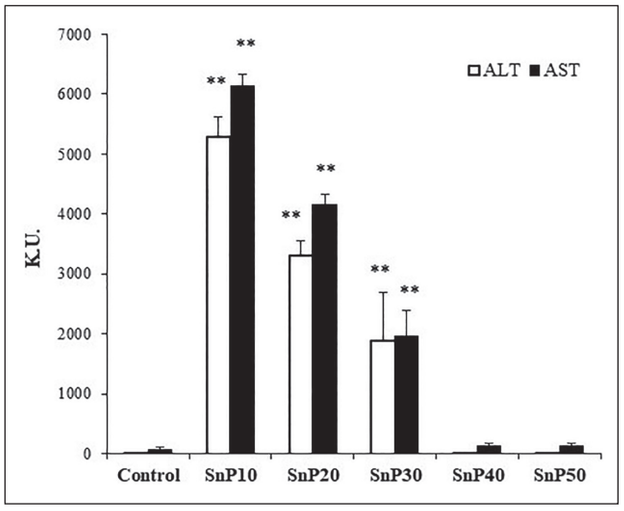

Fig. 1: Comparison of acute liver toxicity of silver nanoparticles. Serum activity of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined using commercially available kits (see 'Experimental' section) 24 h after intravenous administration of nanoparticles of the indicated size at 4 mg/kg. Data are presented as mean±standard error of the mean (n=4), except for the control. **Significant difference (P<0.01) compared with the control group.

Figure 2 shows the results of analyses of liver injury caused by the SnP10, SnP20, and SnP30 nanoparticles at various doses. In the SnP10 and SnP20 groups, an increase in ALT and AST activity was observed at a dose of 2 mg/kg, but the highest ALT and AST activity was observed at the maximum dose of 4 mg/kg (Fig. 2A, B). In the SnP30 group, ALT and AST activity increased only at a dose of 4 mg/kg (Fig. 2C). Thus, SnP10, SnP20, and SnP30 nanoparticles induce liver injury in a dose-dependent manner.

SnP5 nanoparticles were used to investigate the hepatotoxicity of silver nanoparticles ≤10 nm in size. Figure 3A shows the results of administration of SnP5, SnP10, SnP20, and SnP30 particles to mice *via* the tail vein at a dose of 1 mg/kg followed by measurement of serum ALT and AST activity after 24 h. Elevations in ALT and AST activity were observed only in the SnP5 group, demonstrating that the degree of liver injury increases with smaller size of silver nanoparticles, even at a low dose. Figure 3B shows the dose dependence of liver injury caused by SnP5 particles. Liver injury was dose dependent in the SnP5 group, with the most extensive injury observed at a dose of 3 mg/kg. These data indicate that the smallest silver nanoparticles induce liver injury at lower doses than larger particles.

We previously reported that co-administration of SnP10 with the anticancer drug CDDP in mice induces liver injury (Isoda et al. 2019). In the present study, we therefore co-administered SnP5 or SnP10 particles with CDDP in mice and assessed the extent of liver injury due to the interaction between silver nanoparticles and CDDP. Mice were administered SnP5 or SnP10 at 0.5 mg/kg *via* the tail vein, and CDDP (100 μM) was administered intraperitoneally at the same time. Figure 4 shows the results of ALT and AST analyses in serum collected 24 h after administration. Liver injury resulting from interaction between the silver nanoparticles and CDDP was greater in the SnP5 group than the SnP10 group.

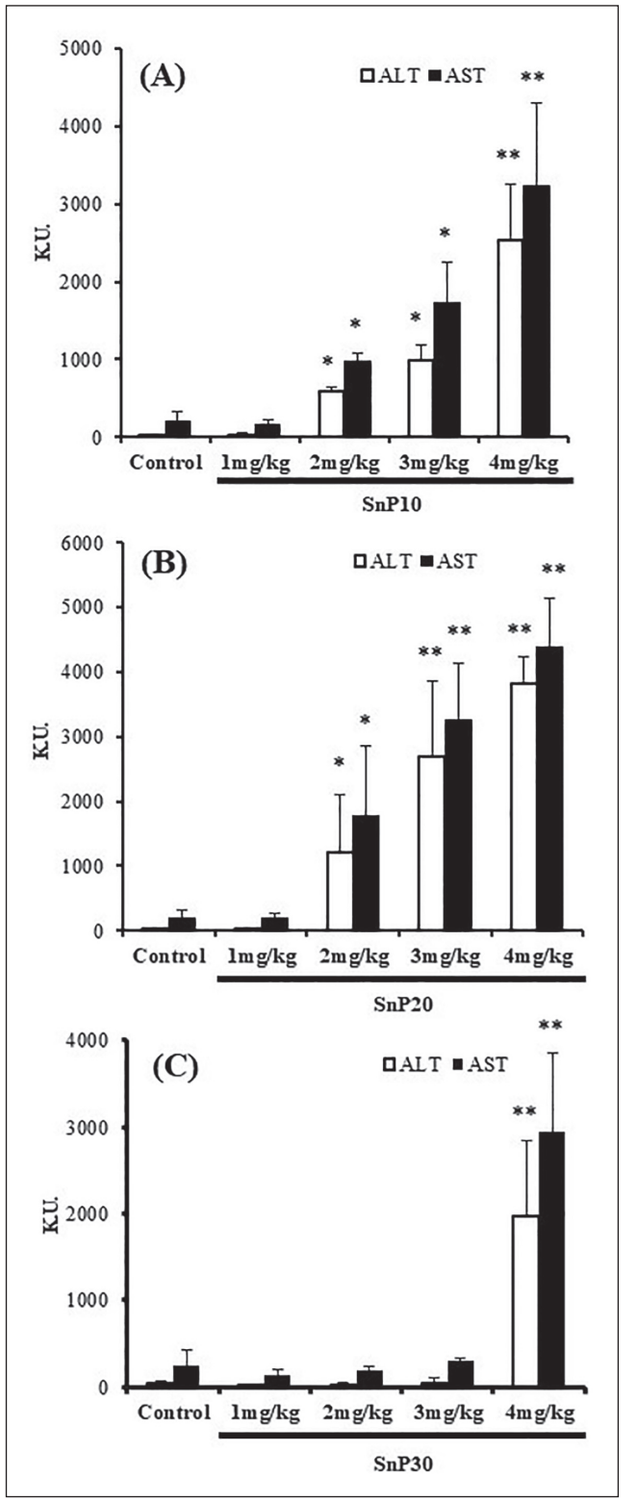

Fig. 2: Dose dependence of SnP10-, SnP20-, and SnP30-induced liver injury. Serum activity of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined using commercially available kits (see 'Experimental' section) 24 h after intravenous administration of SnP10 (A), SnP20 (B), and SnP30 (C) nanoparticles at the indicated doses. Data are presented as mean ± standard error of the mean (n=4). Significant difference (*P<0.05, **P<0.01) compared with the control group.

This result suggests that the severity of liver injury resulting from interactions between pharmaceuticals and silver nanoparticles increases with decreasing particle size.

Our data demonstrated that silver nanoparticles with a particle size of ≤30 nm induce acute liver damage and particle size–dependent toxicity. Kim et al. (2012) also reported that silver nanoparticles exhibit particle

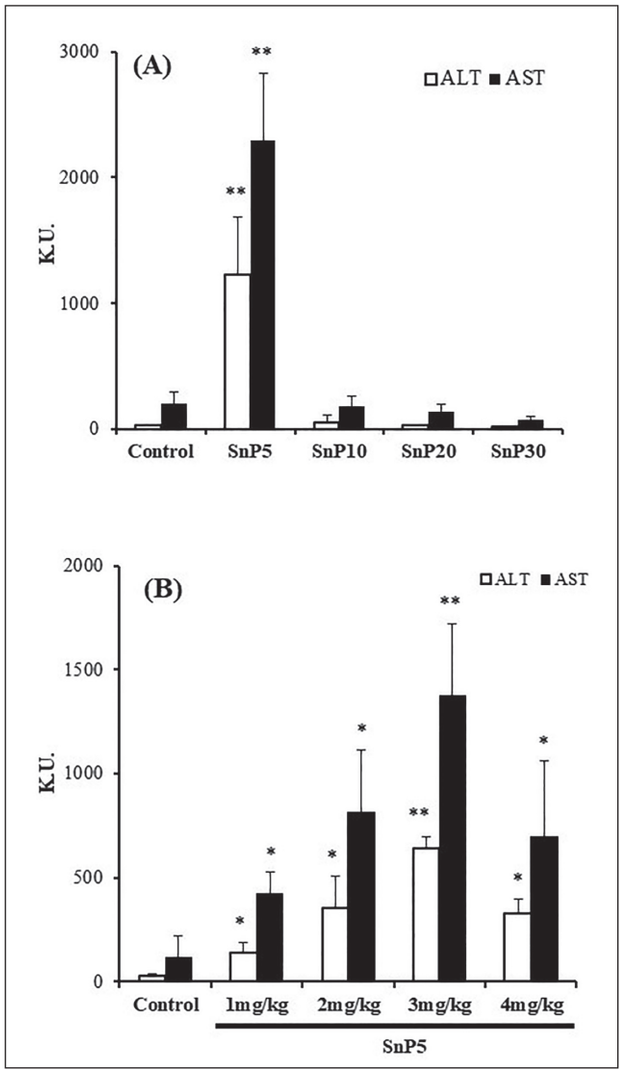

Fig. 3: Evaluation of acute liver toxicity induced by SnP5 silver nanoparticles. (A) Serum activity of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined using commercially available kits (see 'Experimental' section) 24 h after intravenous administration of nanoparticles of the indicated size at 1 mg / kg. Data are presented as mean ± standard error of the mean (n=4), except for the control group. (B) ALT and AST activity was determined using commercially available kits (see 'Experimental' section) 24 h after intravenous administration of SnP5 nanoparticles at the indicated doses. Data are presented as mean ± standard error of the mean (n=4). Significant difference (*P<0.05, **P<0.01) compared with the control group.

size–dependent cytotoxicity. Furthermore, it was reported that silver nanoparticles accumulate in the liver (Liao et al. 2019). Our study also showed that silver nanoparticles exhibit increased hepatotoxicity as the particle size decreases. Particle size thus appears to be an important safety consideration when using silver nanoparticles.

Liver injury associated with SnP5 particles was found to be dose dependent, with the most severe injury observed at a dose of 3 mg/kg (Fig. 3B). This result suggests that the acute liver injury of SnP5 should not worsen at doses above 3 mg/kg. However, it was reported that SnP5 particles exhibit dose-dependent cytotoxicity, with greater toxicity as the dose increases (Mei et al. 2012). As the silver nanoparticles used in this study were purchased standard products, the maximum dose possible was only 4 mg/kg. In the future, it could be informative to investigate SnP5-induced toxicity at higher doses. The results of the present study demonstrated that silver nanoparticles ≤30 nm in size induce acute liver injury in a dose-dependent manner. Furthermore, SnP5 particles exhibited the most severe liver injury. In the future, we would like to further study the safety of nanoparticles with the aim of advancing nanotechnology and nanotoxicology research.

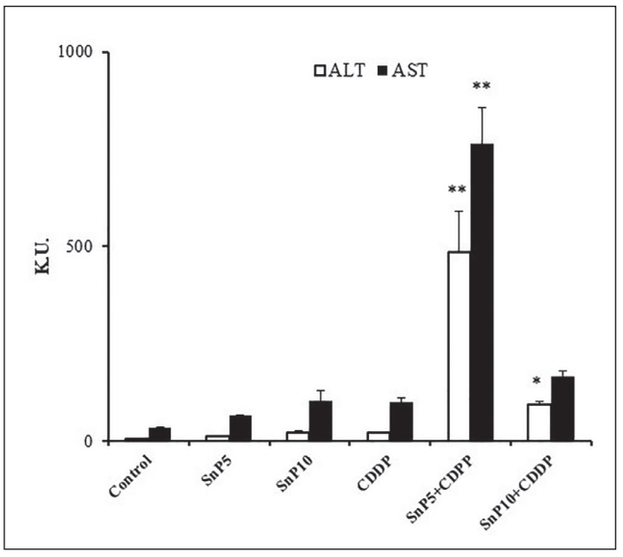

Fig. 4: Effect of SnP5 and SnP10 nanoparticles on cisplatin-induced toxicity. Mice were injected intraperitoneally with cisplatin (CDDP) at 100 mmol/kg together with intravenous injection of silver nanoparticles (0.5 mg/kg) of the indicated size. At 24 h post-injection, serum activity of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined using commercially available kits (see 'Experimental' section). Data are presented as mean±standard error of the mean (n=4). Significant difference (*P<0.05, **P<0.01) between control and CDDP-treated groups.

3. Experimental

3.1. Materials

Silver nanoparticles with a diameter of 5, 10, 20, 30, 40, or 50 nm were obtained from Nanocomposix, Inc. (San Diego, CA). The size distribution of the SnP5, SnP10, SnP20, SnP30, SnP40, and SnP50 silver nanoparticles as analyzed using a Zetasizer (Sysmex Co., Kobe, Japan) was as follows: mean diameter of 5.31±1.3 nm, 12.0±.2.9 nm, 18.7±3.2 nm., 30.5±5.2 nm, 41.8±6.2 nm, and 55.7±6.3 nm, respectively. Aqueous suspensions of nanoparticles were prepared at 1 mg/mL and thoroughly dispersed by sonication and diluted appropriately with water before use. The absence of ionized silver in the suspensions of silver nanoparticles was confirmed using inductively coupled plasma–mass spectrometry. An identical volume of suspension was injected in each experiment. CDDP (Wako Pure Chemical Industries) was dissolved in saline and stored at -20 °C until use. All reagents were research grade.

3.2. Animals

Eight-week-old BALB/c male mice were purchased from Funabashi Farm Co., Ltd. (Chiba, Japan) and maintained in a controlled environment (temperature: 23±1.5 °C; light: 12-h light/dark cycle) with free access to standard rodent chow and water. The mice were given 1 week to adapt before commencing the experiments. The experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences, Teikyo Heisei University, Japan.

3.3. Biochemical analysis

Serum ALT and AST activity was measured using commercially available kits (Wako Pure Chemical Industry, Osaka, Japan) according to the manufacturer's protocols.

3.4. Statistical analysis

Statistical analyses were performed using Microsoft Excel with the Statcel add-in (EMS Publication Co., Ltd., Saitama, Japan). All data are presented as means ± SEMs. The significance of differences between the control and experimental groups was assessed using Dunnett's test. A value of $P < 0.05$ was considered indicative of statistical significance.

Authors' contributions: K.I., Y.S. and I.I. designed the study and wrote the Discussion. K.I. and Y.A. performed the experiments. K.I. and I.I. wrote the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest: None declared.

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