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Influence of 50-nm polystyrene particles in inducing cytotoxicity in mice co-injected with carbon tetrachloride, cisplatin, or paraquat

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The toxicity of nanomaterials has yet to be fully investigated. In particular, the interactions between nanomaterials and therapeutic drugs require further study. We investigated whether nano-sized polystyrene particles affect drug-induced toxicity. The particles, which are widely used industrially, had diameters of 50 (NPP50), 200 (NPP200) or 1000 (NPP1000) nm. The toxic chemicals tested were carbon tetrachloride, cisplatin (a popular anti-tumor agent), and a widely used herbicide, paraquat. Mice were treated intraperitoneally with either carbon tetrachloride (0.01 ml/kg), cisplatin (100 μmol/kg) or paraquat (50 mg/kg), with or without intravenous administration of polystyrene particles. All treatments in the absence of the nanoparticles were non-lethal and did not result in severe toxicity. However, when mice were injected with paraquat or cisplatin together with polystyrene particles, synergistic, enhanced toxicity was observed in mice injected with NPP50. These synergic effects were not observed in mice co-injected with NPP200 or NPP1000. These findings suggest that further evaluation of the interactions between polystyrene nano-particles and drugs is a critical prerequisite to the pharmaceutical application of nanotechnology.

1. Introduction

Nanomaterials are frequently used in microelectronics, cosmetics and sunscreens, and their potential use in drug-delivery systems is being investigated (Caputo et al. 2008; Dobson 2006; Nohynek et al. 2008). Nanomaterials are typically defined as engineered structures having at least one dimension of 100 nm or less. Nano-sized materials have a larger surface area than micro-sized materials, and may have unique physicochemical properties due to their small size, chemical composition, surface structure, solubility, and shape. Although the increased surface area of nanomaterials may be advantageous in some applications, the large surface area can result in increased interactions with biological tissues, cells, proteins, and nucleic acids, leading to toxic effects in humans (Nel et al. 2006; Oberdorster et al. 2005; Fischer et al. 2007). Human exposure to nanomaterials is generally accompanied by exposure to other potentially toxic substances such as dust, food additives, and pharmaceutical agents.

Polystyrene nanoparticles have been used in diagnostic products, cosmetics, and electronic industry materials. The intravenous administration of polystyrene nanoparticles results in biodistribution of the nanoparticles to diverse organs such as the liver, spleen and lungs (Sarlo et al. 2009). Both micro- and nano-sized polystyrene particles are commercially available, and a variety of nano-materials are now present in the environment. Thus, the synergistic effects of nano-sized materials with other toxic substances should be evaluated.

This study investigates the synergistic effects of 50-nm polystyrene nanoparticles with three chemicals: carbon tetrachloride, a well-known reagent with strong toxicity to liver (Weber et al. 2003), cisplatin, a widely used anti-tumor agent (Ozols et al. 1991; Hartmann et al. 1999; Witjes 1997), and paraquat, a widely used and highly toxic herbicide (Vandenbogaerde et al. 1984). The results provide evidence for synergistic enhanced toxicity resulting from interactions between the polystyrene nanoparticles and these chemicals.

2. Investigations, results and discussion

We first investigated the acute toxicity of polystyrene particles with diameters of 50 (NPP50), 200 (NPP200) or 1000 nm (NPP1000) at a maximal dose of 100 mg/kg, and found that polystyrene nanoparticles alone do not cause acute toxicity (Fig. 1A, B). We then investigated whether there are interactions between chemicals and the polystyrene particles. To avoid interactions between the chemicals and polystyrene particles prior to administration and absorption, the chemicals were injected intraperitoneally and the polystyrene particles were injected intravenously. Carbon tetrachloride induces hepatic injury following intraperitoneal administration (Weber et al. 2003). We administered carbon tetrachloride (0.01 ml/kg) to mice at a dose that does not induce hepatic injury (Fig. 1A, B). Co-treatment with NPP50/200/1000 caused severe toxicity, with NPP50 causing the strongest toxicity. Co-administration of carbon tetrachloride and NPP50 resulted in raised ALT and AST levels (Fig. 1A, B).

Abbreviations: NPP50, 50-nm polystyrene particles; NPP200, 200-nm polystyrene particles; NPP1000, 1000-nm polystyrene particles; CDDP, cisplatin; PQ, paraquat; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

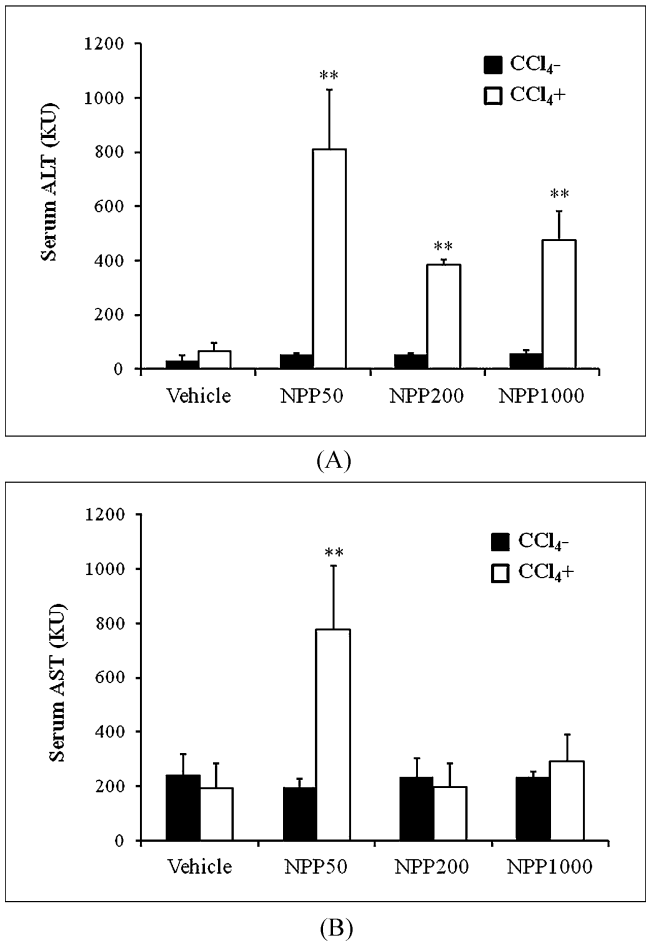


Fig. 1: Effect of NPP50 on carbon tetrachloride-induced toxicity. Mice were injected intraperitoneally with carbon tetrachloride at 0 (solid column) or 0.01 ml/kg (open column) together with one size of polystyrene particle (NPP50, 50-nm particles; NPP200, 200-nm particles; NPP1000, 1000-nm particles) injected intravenously at a dose of 100 mg/kg. At 24 h post-injection, serum was recovered. ALT (A) and AST (B) levels were assayed as described in the Experimental part. Data are representative of three independent experiments. Data are mean ± SEM (n = 4). **Significant difference between vehicle and carbon tetrachloride-treated group ($p < 0.01$)

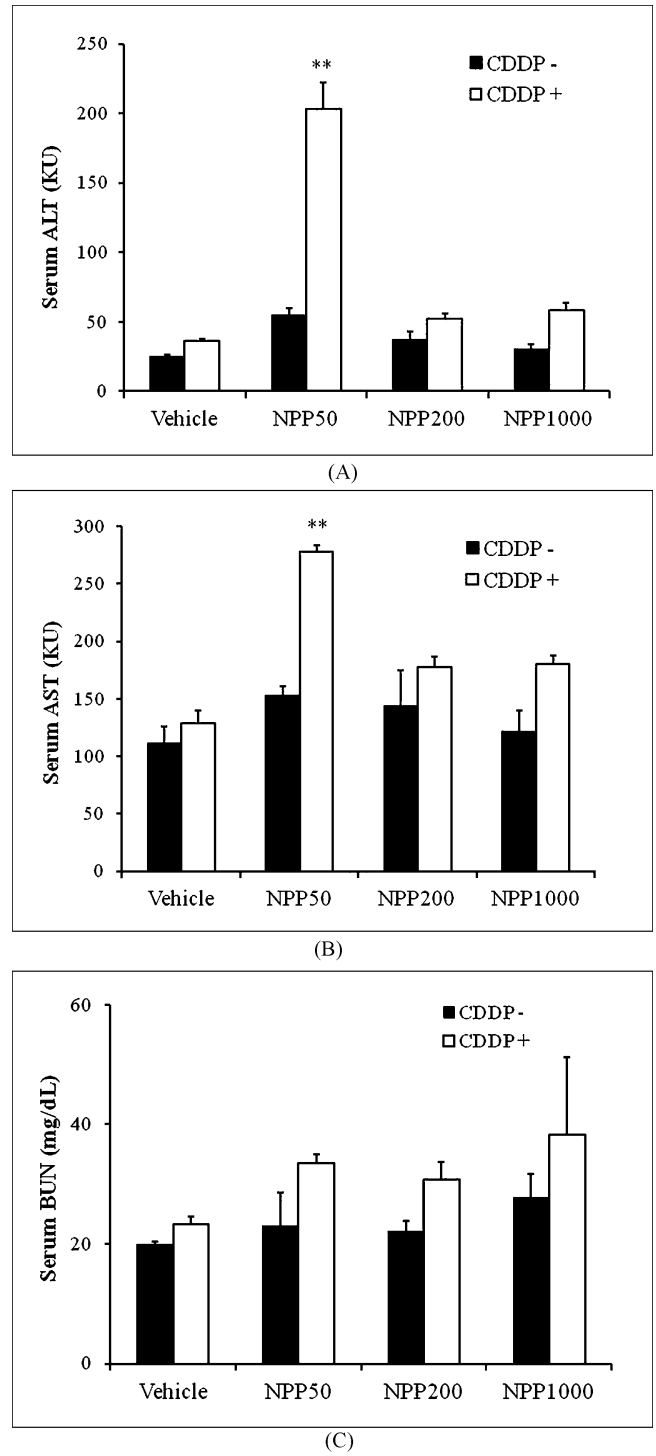


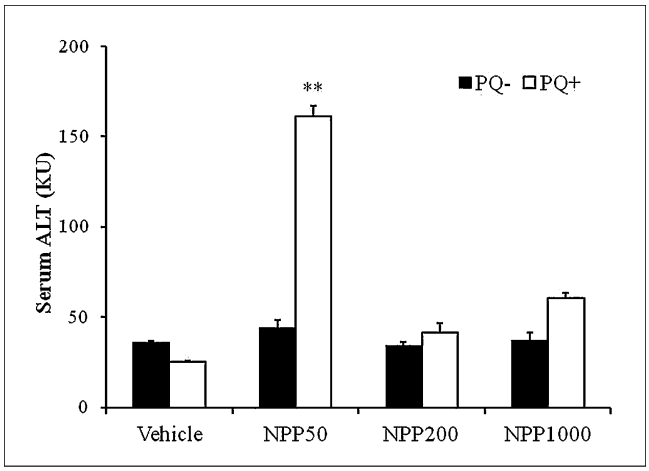
Fig. 2: Effect of NPP50 on cisplatin-induced toxicity. Mice were injected intraperitoneally with cisplatin at 0 (solid column) or μmol/kg (open column) together with one size of polystyrene particle (NPP50, 50-nm particles; NPP200, 200-nm particles; NPP1000, 1000-nm particles) injected intravenously at a dose of 100 mg/kg. ALT (A), AST (B) and BUN (C) levels were assayed as described in the Experimental part. Data are mean ± SEM (n = 4). **Significant difference between vehicle and cisplatin-treated group ($p < 0.01$)

We next investigated the interaction between cisplatin and polystyrene particles. Administration of cisplatin causes adverse effects such as renal and hepatic failure (Lu et al. 2006; Ramesh et al. 2007). Co-administration of cisplatin (80 μmol/kg) and NPP200 or 1000 did not result in elevated serum ALT and AST levels. However, NPP50 produced synergistic elevation of serum ALT levels from 54.7 to 203.3 KU, and serum AST levels from 153.4 to 278 KU (Fig. 2A, B). No increase in serum BUN levels was observed (Fig. 2C).

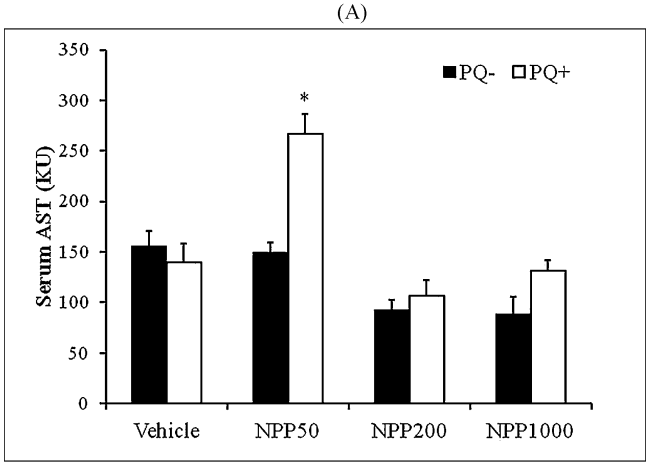
We also investigated the interaction between paraquat and polystyrene particles. Co-administration of paraquat (50 mg/kg) and NPP200 or 1000 did not elevate serum ALT and AST, whereas NPP50 produced synergistic elevation of serum ALT levels, from 44.0 to 161.4 KU (Fig. 3).

In this study, we investigated the toxicity induced by chemicals combined with nano-sized particles, and found that carbon tetrachloride, cisplatin and paraquat produce synergistic toxic effects when combined with 50-nm polystyrene particles.

Liver injury induced by carbon tetrachloride, paraquat and cisplatin is caused by oxidative stress (Weber et al. 2003; Lu et al. 2006; Liu et al. 2006). Fernandez-Urrusuno et al. (1997) reported that polystyrene nanoparticles cause oxidative stress in the liver. To further investigate this, we added polystyrene nanoparticles to cultured hepatocytes and examined cell survival rate, but observed no evidence of cellular cytotoxicity (data not shown). Moreover, in mice, no liver or kidney damage was caused by the administration of polystyrene nanoparticles alone (Fig. 1, 2), indicating that the safety of polystyrene nanoparticles is high. Although Sarlo et al. (2009) reported that polystyrene nanoparticles were deposited in the liver, lungs and spleen, resulting in acute lung injury, our results indicate that no hepatic toxicity is caused by polystyrene nanoparticles accumulating in the liver, although polystyrene nanoparticles do induce



(A)



(B)

Fig. 3: Effect of NPP50 on paraquat-induced toxicity. Mice were injected intraperitoneally with paraquat at 0 (solid column) or 50 mgl/kg (open column) together with one size of polystyrene particle (NPP50, 50-nm particles; NPP200, 200-nm particles; NPP1000, 1000-nm particles) injected intravenously at a dose of 100 mg/kg. At 24 h post-injection, serum was recovered. ALT (A) and AST (B) levels were assayed as described in the Experimental part. Data are representative of three independent experiments. Data are the mean ± SEM (n = 4). *Significant difference between vehicle and paraquat-treated group (*$p < 0.05$, ** $p < 0.01$)

synergistic toxicity with chemicals known to cause oxidative stress. Further biochemical and other analyses, such as proteome and genome assays, will be performed in our laboratory to determine the mechanism of these synergistic effects.

This report is the first to indicate toxicity due to synergistic effects between nano-sized polystyrene particles and chemical agents. Clearly, further evaluation of interactions between nano-sized materials and pharmaceutical agents is required prior to the pharmaceutical application of nanotechnology.

3. Experimental

3.1. Materials

Polystyrene particles with diameters of 50, 200 or 1000 nm were obtained from Micromod Partikeltechnologie GmnH (Rostock, Germany). The size distribution of the particles was analyzed using a Zetasizer (Sysmex Co., Kobe, Japan); the mean diameters were 50.2, 249, and 1030 nm. The particles were spherical and nonporous. Aqueous suspensions of 10 mg/ml (50 nm), 25 mg/ml (300 nm) and 50 mg/ml (1000 nm) were prepared. The suspensions were thoroughly dispersed using sonication before use and were diluted with water. Identical volumes of solution were injected in each experiment. Carbon tetrachloride was dissolved in olive oil. Paraquat and cisplatin were dissolved in saline and stored at −20 °C until use. All reagents used were research grade.

3.2. Animals

Eight-week-old BALB/c male mice were purchased from Funabashi Farm Co., Ltd. (Chiba, Japan). They were 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 alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and blood urea nitrogen (BUN) were measured using commercially available kits according to the manufacturer's protocols (WAKO Pure Chemical, Osaka, Japan).

3.4. Statistical analysis

Statistical analyses were performed using two-way ANOVA, followed by Student's t-test. $P < 0.05$ was considered statistically significant.

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