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Toxicity of 50-nm polystyrene particles co-administered to mice with acetaminophen, 5-aminosalicylic acid or tetracycline

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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 acetaminophen (APAP), 5-aminosalicylic acid (5-ASA), tetracycline (TC), and sodium valproate (VPA). All treatments in the absence of the nanoparticles were non-lethal and did not result in severe toxicity. However, when mice were injected with APAP, 5-ASA or TC 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. On the other hand, co-administration of VPA and NPP50, NPP200 or NPP1000 did not elevate toxicity. The results show that NPP50 differs in hepatotoxicity depending on the drug co-administered. These findings suggest that further evaluation of the interactions between polystyrene nanoparticles and drugs is a critical prerequisite to the pharmaceutical application of nanotechnology.

1. Introduction

Recently, there have been explosive developments in nanotechnology, leading to nanomaterials which are finding use in many fields such as medical supplies, cosmetics, and electronics (Bisquert 2008; Nohynek et al. 2008). Nanomaterials show new characteristics not found in micro-materials, including reaction to exogenous stimuli such as heat, light, and voltage (Buesser and Pratsinis 2011; Patnaik 2007). The diameter of a nanoparticle is 100 nm or less (Yah et al. 2012). Nanoparticles are artificial materials which have unprecedented effects on the living body. 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 (Fischer and Chan 2007; Nel et al. 2006; Oberdorster et al. 2005). Human exposure to nanomaterials is generally accompanied by exposure to other potentially toxic substances such foods, food additives, cosmetics, and pharmaceutical agents.

Polystyrene is a familiar everyday material used, for example, to make food transport containers or food trays, as it does not have a smell or impart a taste to the food, and it is resistant to damage by external shocks (Peralta-Videa et al. 2011). Polystyrene nanoparticles have been used in diagnostic products, cosmetics, and as 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 nanomaterials are now present in the environment. Thus, the synergistic effects of nano-sized materials with other toxic substances should be evaluated.

We previously reported hepatotoxicity resulting from the synergistic effect of 50-nm polystyrene nanoparticles with three chemicals: carbon tetrachloride, cisplatin, and paraquat (Shimizu et al. 2012). The current study investigates the synergistic effects of 50-nm polystyrene nanoparticles with four pharmaceutical products: acetaminophen (APAP), a widely used antipyretic agent and well-known reagent that exhibits toxicity to the liver (Ameer and Greenblatt 1977; Jaeschke et al. 2011); 5-aminosalicylic acid (5-ASA), a widely used anti-inflammatory agent and antibacterial drug (Hirschfeld and Clearfield 1995); tetracycline (TC), a widely used antibiotic (Nelson and Levy 2011); and sodium valproate (VPA), an anti-convulsant and anti-epileptic drug widely used in psychiatry (Roullet et al. 2013). The results provide evidence for synergistic, enhanced toxicity resulting from interactions between the polystyrene nanoparticles and these pharmaceutical products.

2. Investigations, results and discussion

We previously investigated the acute toxicity of polystyrene particles with diameters of 50, 200 or 1000 nm at a maximal dose of 100 mg/kg, and found that polystyrene nanoparticles alone do not cause acute toxicity (Shimizu et al. 2012). However,

Abbreviations: NPP50, 50-nm polystyrene particles; NPP200, 200-nm polystyrene particles; NPP1000, 1000-nm polystyrene particles; APAP, acetoaminophen; 5-ASA, 5-aminosalicylic acid; TC, tetracycline; VPA, sodium valproate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

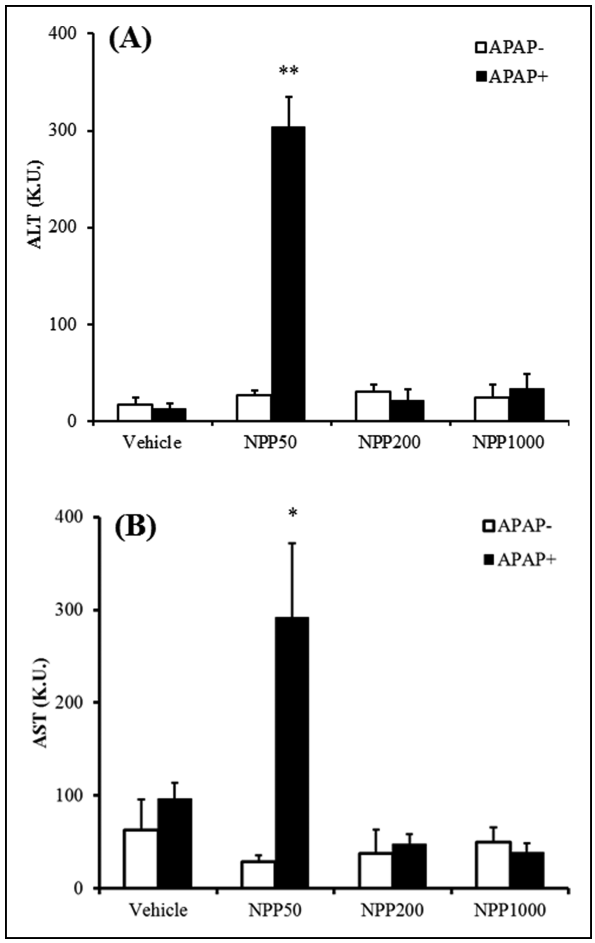

Fig. 1: Effect of NPP50 on APAP-induced toxicity. Mice were injected with APAP at 0 (open column) or 200 mg/kg (solid column) and with polystyrene particles (NPP50, 50-nm particles; NPP200, 200-nm particles; NPP1000, 1000-nm particles) at a dose of 100 mg/kg, intraperitoneally and intravenously, respectively. At 24 h post-injection, serum was recovered. ALT (A) and AST (B) levels were assayed as described in the Materials and Methods. Data are representative of three independent experiments. Data are mean ± SEM (n = 4). Significant difference between the vehicle and APAP-treated groups were observed (*$p < 0.05$, ** $p < 0.01$).

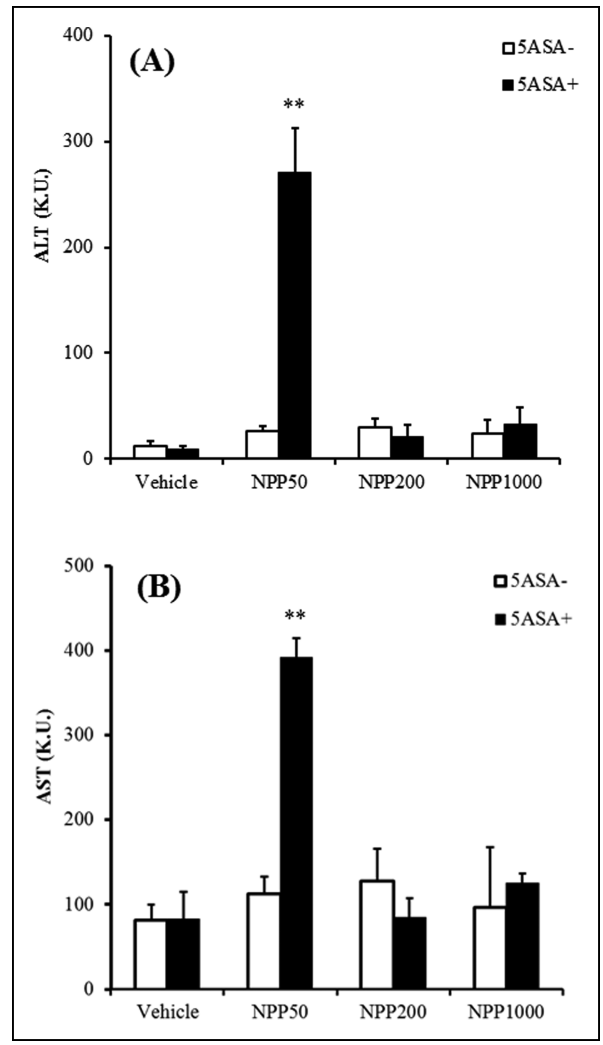

Fig. 2: Effect of NPP50 on 5-ASA-induced toxicity. Mice were injected with 5-ASA at 0 (open column) or 500 mg/kg (solid column) and with polystyrene particles (NPP50 or NPP200 or NPP1000) at a dose of 100 mg/kg, intraperitoneally and intravenously, respectively. At 24 h post-injection, serum was recovered. ALT (A), AST (B) and BUN (C) levels were assayed as described in the Materials and Methods. Data are mean ± SEM (n = 4). **Significant difference between the vehicle and 5-ASA-treated groups were observed ($p < 0.01$).

we found that administration of NPP50 to mice together with carbon tetrachloride or paraquat induced liver damage. In this study, we investigated whether there are interactions between four pharmaceutical products and the polystyrene particles.

To prevent interactions between the pharmaceutical products and the polystyrene particles prior to administration and absorption, the pharmaceutical products were injected intraperitoneally and the polystyrene particles were injected intravenously. APAP is widely used globally as a painkiller. We administered APAP (200 mg/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 APAP and NPP50 resulted in raised ALT and AST levels (Fig. 1A, B).

We next investigated the interaction between 5-ASA and the polystyrene particles. Administration of 5-ASA causes adverse effects such as hepatic failure (Deltenre et al. 1999). Co-administration of 5-ASA (500 mg/kg) with NPP200 or 1000 did not result in elevated serum ALT and AST levels. However, NPP50 synergistically elevated serum ALT levels from 34.9 to 270.1 KU, and serum AST levels from 112.2 to 325.4 KU (Fig. 2A, B).

We also investigated the interaction between TC and the polystyrene particles. Co-administration of TC (100 mg/kg) and NPP200 or 1000 did not elevate serum ALT and AST, whereas NPP50 synergistically elevated serum ALT levels, from 26.0 to 1104 KU (Fig. 3). Finally, we investigated the interaction between VPA and the polystyrene particles. VPA is widely used globally as an antiepileptic and antimanic drug, but produces side effects such as liver damage (Gram and Bentsen 1985). Co-administration of VPA (200 mg/kg) with NPP50, NPP200 or NPP1000 did not elevate serum ALT or AST (Fig. 4A, B).

In this study, we investigated the toxicity induced by four pharmaceutical products combined with nano-sized particles, and found that APAP, 5-ASA and TC produce synergistic toxic effects, whereas VPA did not produce synergistic toxic effects, when combined with 50-nm polystyrene particles.

The liver damage caused by the side effects of APAP is induced when APAP is taken in large quantities and is caused by glutathione depletion in the cells (Saito et al. 2010; McClain et al. 1999). Clift et al. (2010) reported that polystyrene nanoparticles reduced glutathione levels in J774.A1 cells *in vitro*. This finding suggests that polystyrene nanoparticles invade hepatic cells and reduce glutathione levels. In the current study, since liver damage due to APAP was observed, we believed that the ALT/AST

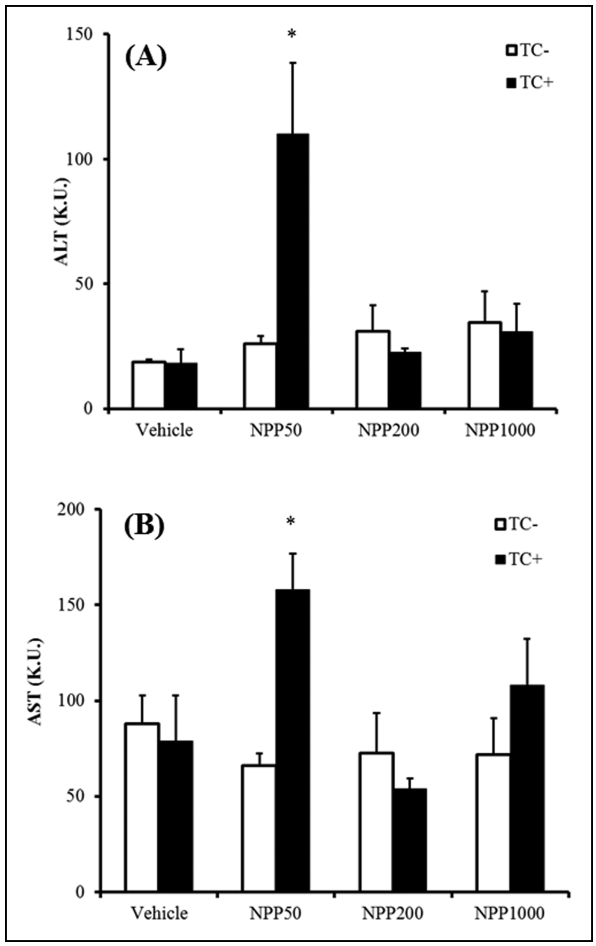

Fig. 3: Effect of NPP50 on TC-induced toxicity. Mice were injected with TC at 0 (open column) or 100 mg/kg (solid column) and with polystyrene particles (NPP50, NPP200 or NPP1000) at a dose of 100 mg/kg, intraperitoneally and intravenously, respectively. At 24 h post-injection, serum was recovered. ALT (A) and AST (B) levels were assayed as described in the Materials and Methods. Data are representative of three independent experiments. Data are the mean ± SEM (n = 4). *Significant difference between the vehicle and tetracycline-treated groups were observed (*$p < 0.05$, ** $p < 0.01$).

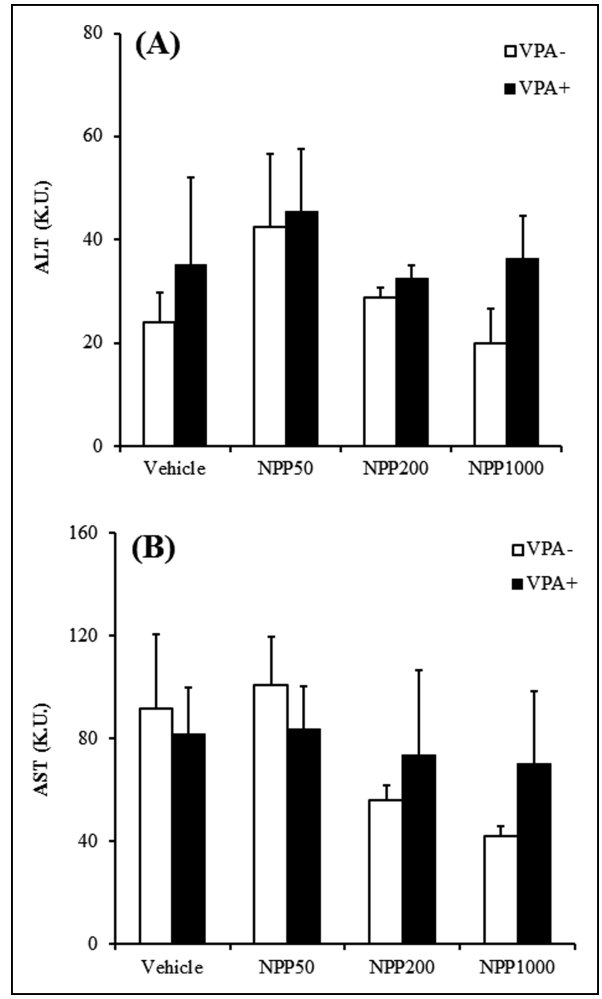

Fig. 4: Effect of NPP50 on VPA-induced toxicity. Mice were injected with VPA at 0 (open column) or 100 mg/kg (solid column) and with polystyrene particles (NPP50, NPP200 or NPP1000) at a dose of 100 mg/kg, intraperitoneally and intravenously, respectively. At 24 h post-injection, serum was recovered. ALT (A) and AST (B) levels were assayed as described in the Materials and Methods. Data are representative of three independent experiments. Data are the mean ± SEM (n = 4).

level rose (Fig. 1A, B). However, there was no evidence that polystyrene nanoparticles invaded hepatic cells. Therefore, in future we need to examine the kinetics of polystyrene nanoparticle entry into the liver.

TC induces liver damage by inhibiting the autoimmune system or by cholestasis (Hunt and Washington 1994; Czaja 2011; Thiim and Friedman 2003). Liver damage is a side effect of 5-ASA, although the mechanism of damage is unknown (Deltenre et al. 1999). Polystyrene nanoparticles induced liver damage by interacting with TC or 5-ASA (Fig. 2, 3). VPA is also known to induce severe liver damage (Cotariu and Zaidman 1988), although the mechanism is unknown. In the current study, polystyrene nanoparticles did not induce liver damage when combined with VPA (Fig. 4), whereas polystyrene nanoparticles did induce liver damage when co-administered with APAP, TC or 5-ASA. In future, it will be necessary to elucidate the mechanism of liver damage by these drugs interacting with polystyrene nanoparticles.

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

3. Experimental

3.1. Materials

Polystyrene particles with a diameter 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, respectively. The particles were spherical and nonporous, and stored as 10 mg/ml (50 nm), 25 mg/ml (300 nm) and 50 mg/ml (1000 nm) aqueous suspensions. The suspensions were thoroughly dispersed using sonication before use and were diluted with water. An equal volume of suspension was injected for each treatment. APAP, 5-ASA, TC and VPA were dissolved in saline and stored at −20 °C before use. All reagents used were of 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 analysis was performed by two-way ANOVA, followed by Student's t-test. $P < 0.05$ was considered statistically significant.

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