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Effects of surface charge and palladium on hepatic and kidney injury induced by polystyrene nanoparticles co-administered to mice with paraquat and cisplatin

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Recently, with the advancement of nanotechnology, various nanoparticles have been developed and used in fields such as electronics, cosmetics, and foods. However, the toxicity of nanoparticles has yet to be fully investigated. In particular, the interactions between nanoparticles and therapeutic drugs require further study. We previously reported that unmodified polystyrene nanoparticles with a particle size of 50 nm (NPP50) co-administered with paraquat (PQ) or cisplatin (CDDP) induce hepatic and kidney injury. Here, we determined if NPP50 modified with the amino group (NPP50-NH_2), carboxyl group (NPP50-COOH), or palladium (Pd-NPP50) caused liver or kidney injury when co-administered with PQ or CDDP. The results showed that when NPP50-NH_2, NPP50-COOH, or Pd-NPP50 was administered alone via the mouse tail vein, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (BUN) did not increase or cause injury. When NPP50, NPP50-NH_2, NPP50-COOH, or Pd-NPP50 was co-administered with PQ, serum levels of ALT and AST increased in the NPP50 group but did not increase in the NPP50-NH_2, NPP50-COOH, or Pd-NPP50 groups. When NPP50-NH_2, NPP50-COOH, or Pd-NPP50 was co-administered with CDDP, ALT, AST, and BUN values did not increase. These data suggest that injury due to the interaction of polystyrene nanoparticles with CDDP or PQ can be suppressed by changes in the surface charge of nanoparticles or by Pd modification.

1. Introduction

Recent developments in nanotechnology have led to a tremendous increase in the use of nanomaterials in industry, including pharmaceutical and technology companies. Nanomaterials are often used for information communication, microelectronics, cosmetics, and sunscreen and their potential use in drug delivery systems has also been studied (Chakraborty et al. 2011; McIntyre 2012). Although the importance of nanomaterials has become increasingly recognized with an increasing numbers of studies being conducted into their practical applications, their effects on organisms remain a concern (Kettiger et al. 2013; Oberdorster et al. 2005). Nanomaterials have unique physicochemical properties compared to micromaterials in terms of size, surface structure, reactivity, and mechanical strength. Therefore, reducing particle size from microscale to nanoscale is beneficial for many industrial and scientific applications. However, it is essential to understand the biological activity and potential toxicity of nanomaterials, as they have potential toxicities not found in micromaterials.

The physical properties of nanomaterials are changed by modification of their surface charge, which extends their possible applications. For example, charge-modified dendrimers are expected to have applications in drug-delivery systems. The physical properties and toxicity of carbon nanotubes change based on the surface charge (Simon-Deckers et al. 2009; Smith et al. 2009), as do the pharmacokinetics of liposomes (Wang et al. 2005). Thus, future research studies will undoubtedly lead to expanded applications of surface-modified nanomaterials (Bharali et al. 2009; Svenson and Tomalia 2005); however, little has been reported on their toxicity.

Polystyrene is a material that is used to make a number of consumer products such as food transport containers or food trays, as it does not have an odor or impart a taste to 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 materials for the electronic industry. The intravenous administration of polystyrene nanoparticles results in the biodistribution of nanoparticles to diverse organs such as the liver, spleen, and lungs (Sarlo et al. 2009). We previously reported that hepatotoxicity results from the synergistic effects of 50 nm polystyrene nanoparticles (NPP50) with two chemicals: cisplatin (CDDP) and paraquat (PQ) (Shimizu et al. 2012). These nanoparticles induce hepatic injury by interacting with pharmaceuticals and chemicals. Injuries from nanoparticles are reportedly due to the charge and shape of the particle surface. In a previous study, we reported that silica nanoparticle-induced hepatic injury was reduced by changing the surface charge (Isoda et al. 2011). Here, we determined if the interaction between CDDP or PQ with NPP50-COOH or NPP50-NH_2, respectively, also reduced injury. In addition, because palladium (Pd) is widely used as a medicinal product, we examined the interaction between Pd-NPP50 and CDDP or PQ.

Abbreviations: NPP50, 50 nm polystyrene particles; NPP50-NH_2, 50 nm polystyrene particles with NH_2; NPP50-COOH, 50 nm polystyrene particles with COOH; Pd-NPP50, 50 nm polystyrene particles with palladium; CDDP, cisplatin; PQ, paraquat; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen

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 when administered alone to mice,

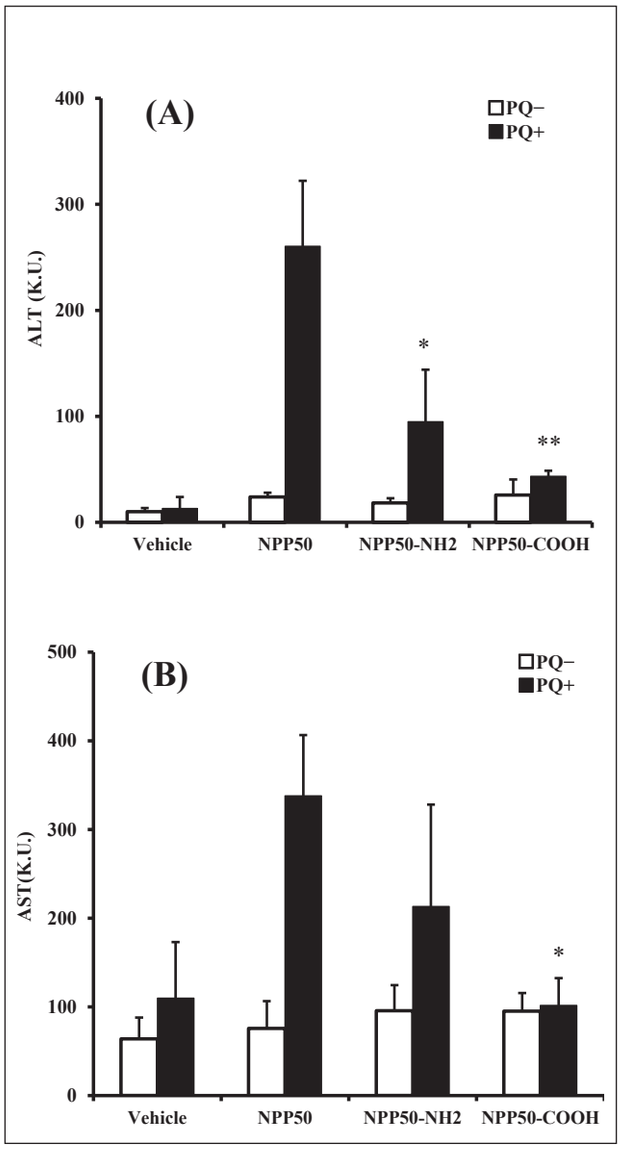

Fig. 1: Effects of NPP50-NH_2 and NPP50-COOH on PQ-induced toxicity. Mice were injected intraperitoneally with 0 (open column) or 50 mg/kg (solid column) PQ together with a polystyrene nanoparticle (NPP50, NPP50-NH_2, or NPP50-COOH) injected intravenously at a dose of 100 mg/kg. At 24 h post-injection, the 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 NPP-50 and NPP50-NH_2 or NPP50-COOH in PQ-treated groups (*$p < 0.05$, **$p < 0.01$).

they did not cause acute toxicity (Shimizu et al. 2012). However, administration of NPP50 with PQ or CDDP induced liver damage (Shimizu et al. 2012). In this study, we determined if polystyrene nanoparticles with a modified surface co-administered with PQ or CDDP also induced liver damage.

First, we injected 100 mg/kg NPP50-NH_2 or NPP50-COOH into mice via the tail vein. As shown in Fig. 1, NPP50-NH_2 and NPP50-COOH did not cause liver injury like NPP50. In addition, there was no increase in BUN values, indicating a lack of kidney injury (data not shown). However, upon co-administration of NPP50 and PQ, ALT and AST levels were significantly increased compared with vehicle, and hepatic injury was observed (Fig. 1). Co-administration of NPP50-NH_2 and PQ resulted in ALT of 102 (K.U.) and AST of 213.7 (K.U.), liver injury decreased by 50% compared with the NPP50 group. Furthermore, co-administration of NPP50-COOH and PQ resulted in ALT of 45.7 (K.U.), AST of 101.7 (K.U.), and almost no liver injury was observed.

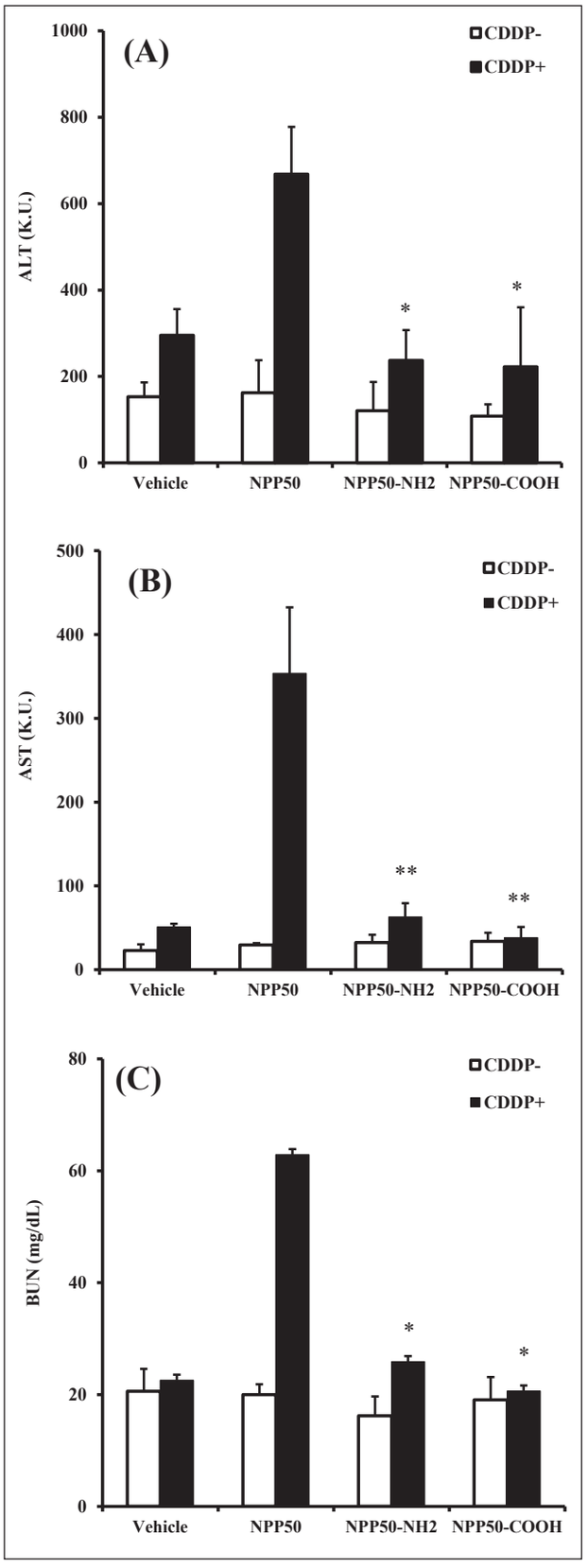

Fig 2: Effects of NPP50-NH_2 and NPP50-COOH on CDDP-induced toxicity. Mice were injected intraperitoneally with 0 (open column) or 100 mM/kg (solid column) CDDP together with a polystyrene nanoparticle (NPP50, NPP50-NH_2, or NPP50-COOH) injected intravenously at a dose of 100 mg/kg. ALT (A), AST (B) and BUN (C) levels were assayed as described in the Materials and Methods. Data are the mean ± SEM (n = 4). *Significant difference between NPP-50 and NPP50-NH_2 or NPP50-COOH in CDDP-treated groups (*$p < 0.05$, **$p < 0.01$).

Next, NPP50, NPP50-NH_2, or NPP50-COOH was co-administered with 100 mM CDDP, as shown in Fig. 2. Co-administration of NPP50 and CDDP led to significantly increased serum levels of ALT and AST compared with the vehicle group, and hepatic injury was observed. However, co-administration of NPP50-NH_2 or NPP50-COOH with CDDP did not change ALT and AST levels compared to the vehicle group, and hepatic injury was not induced. Moreover, the BUN values were also similar among groups indicating a lack of kidney injury. NPP50-NH_2 and NPP50-COOH reduced hepatic injury due to their interaction of PQ and CDDP, respectively. Thus, it appears that changing the surface charge of polystyrene nanoparticles reduces their interaction with chemicals and drugs, leading to reduced injury.

As shown in Fig. 3, administration of Pd-NPP50 alone did not lead to hepatotoxicity. Upon co-administration of NPP50 with PQ, ALT and AST levels were elevated, and hepatic injury was induced. However upon co-administration of Pd-NPP50 with PQ, ALT and

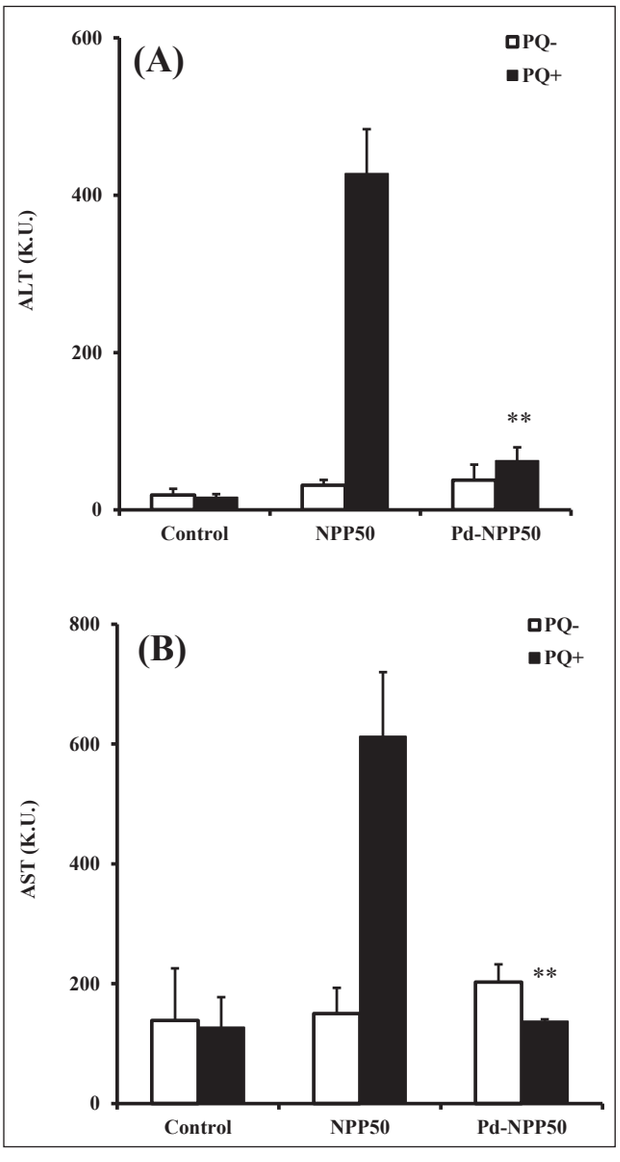

Fig. 3: Effects of Pd-NPP50 on PQ-induced toxicity. Mice were injected intraperitoneally with 0 (open column) or 50 mg/kg (solid column) PQ together with a polystyrene nanoparticle (NPP50 or Pd-NPP50) injected intravenously at a dose of 100 mg/kg. At 24 h post-injection, the serum was recovered. ALT (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 NPP-50 and Pd-NPP50 in PQ-treated groups (**$p < 0.01$).

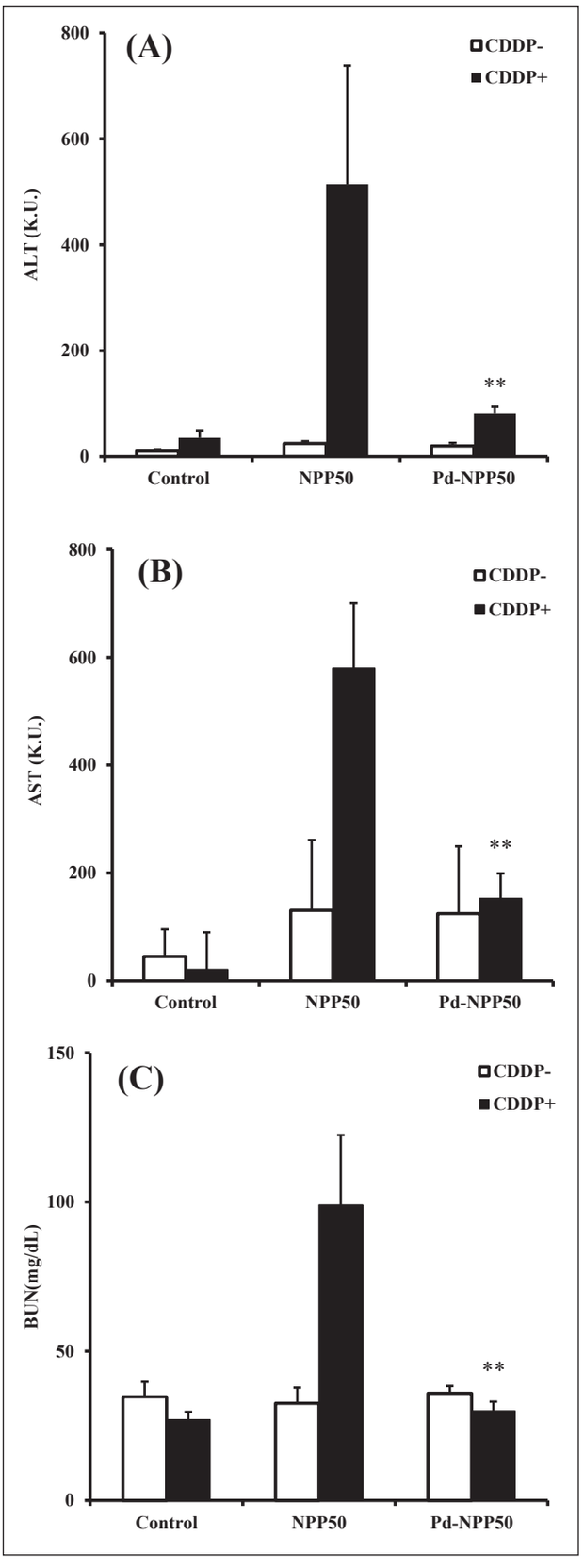

Fig. 4: Effects of Pd-NPP50 on CDDP-induced toxicity. Mice were injected intraperitoneally with 0 (open column) or 100 mM/kg (solid column) CDDP together with a polystyrene nanoparticle (NPP50 or Pd-NPP50) injected intravenously at a dose of 100 mg/kg. ALT (A), AST (B) and BUN (C) levels were assayed as described in the Materials and Methods. Data are the mean ± SEM (n = 4). **Significant difference between NPP-50 and Pd-NPP50 in CDDP-treated groups ($p < 0.01$)

AST levels were similar to those in the vehicle group, and hepatic injury was not induced. Similarly, co-administration of Pd-NPP50 with CDDP also did not lead to a change in serum levels of ALT, AST and BUN compared to the NPP50 group, and hepatic injury was not induced (Fig. 4). These data suggest that the interaction with PQ or CDDP was mostly inhibited by coating the surface of the polystyrene nanoparticles with Pd.

In this study, it was shown that NPP50-NH_2, NPP50-COOH, or Pd-NPP50 reduced liver and kidney injury caused by PQ or CDDP. A single administration to mice (100 mg/kg) of NPP50-NH_2, NPP50-COOH, or Pd-NPP50 did not cause injury (Figs. 1, 3). Injuries caused by CDDP and the toxicity of PQ are reportedly due to involvement of reactive oxygen species (ROS) and intracellular signaling factors (Gao et al. 2017; Jaiman et al. 2013; Nagano 1991). It is thought that NPP50-NH_2, NPP50-COOH, and Pd-NPP50 reduce injuries caused by PQ or CDDP by inhibiting the generation of active oxygen. Future studies should measure ROS upon co-administration of NPP50-NH_2, NPP50-COOH, or Pd-NPP50 with CDDP or PQ.

Nanomaterials are used in many products, and their use will continue to increase, as the development of nanomaterials with new functions is anticipated (Devalapally et al. 2007; Rakesh et al. 2008). Protein adsorption and cell adsorption base materials, which allow functional groups to fix to the surface of materials, have been developed (Bacakova et al. 2004; Wilson et al. 2005). The absorbance of materials is changed by surface treatment. We previously reported that liver injury by silica nanoparticles was reduced by changing the charge on their surface (Isoda et al. 2011), and protein adsorptivity was changed by changing the nanoparticle surface charge, preventing silica nanoparticles from entering the cell.

Pd inhibited the interaction of PQ and CDDP with the polystyrene nanoparticles (Figs. 3, 4). It was previously shown that modifying Pd on the surface of nanoparticles increases the dispersibility and affinity of nanoparticles (Zhong et al. 2016). In addition, Pd is a strong antioxidant, has high adsorptivity, and removes active oxygen (Shibuya et al. 2014). Together, these results indicate that Pd increases the safety of nanoparticles.

This is the first study to show that injury caused by the interaction of polystyrene nanoparticles with PQ or CDDP can be suppressed by a change in the surface charge or by Pd modification. It has been shown that the safety of polystyrene nanoparticles is improved with respect to the living body by modifying the surface. In this study, it was shown that not only the surface charge, but also Pd is important for enhancing safety.

3. Experimental

3.1. Materials

The polystyrene particles used in this study were obtained from Micromod Partikeltechnologie GmnH (Rostock, Germany). They included unmodified polystyrene particles with a diameter of 50 nm (NPP50), and those modified with the amino group (NPP50-NH_2), carboxyl group (NPP50-COOH), or Pd (Pd-NPP50). The size distribution of the modified particles was analyzed with the Zetasizer (Sysmex Co., Kobe, Japan); the mean diameters were 50.2, 61.8, and 56.3 nm for NPP50-NH_2, NPP50-COOH, and Pd-NPP50, respectively. The electric charge of the particles, also measured with the Zetasizer, was -16.7, -50.4, and -31.3mV respectively. The particles were spherical and nonporous, and stored as 10 mg/mL aqueous suspensions; they were thoroughly dispersed by sonication before use and diluted with water. An equal volume of the suspension was injected for each treatment. PQ and CDDP were dissolved in saline and stored at -20 ℃ until use. All of the 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 (Tokyo, Japan).

3.3. Biochemical analysis

Serum levels of alanine aminotransferase (ALT), 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 with Microsoft Excel and the Statcel add-in software (EMS Publication Co., Ltd., Saitama, Japan). All data are presented as the mean±standard deviation of the mean. The significance of difference between the control and experimental groups was assessed using the Dunnett's test. A P value less than 0.05 was considered statistically significant.

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Conflicts of interest: The authors declare that they have no competing interests.

References

Bacakova L, Filova E, Rypacek F, Svorcik V, Stary V (2004) Cell adhesion on artificial materials for tissue engineering. Physiol Res 53 Suppl 1: S35 - 45.

Bharali DJ, Khalil M, Gurbuz M, Simone TM, Mousa SA (2009) Nanoparticles and cancer therapy: a concise review with emphasis on dendrimers. Int J Nanomed 4: 1 - 7.

Chakraborty M, Jain S, Rani V (2011) Nanotechnology: emerging tool for diagnostics and therapeutics. Appl Biochem Biotechnol 165: 1178 - 1187.

Devalapally H, Chakilam, MM Amiji (2007) Role of nanotechnology in pharmaceutical product development. J Pharm Sci 96: 2547-2565.

Gao Y, Chu S, Shao Q, Zhang M, Xia C, Wang Y, Li Y, Lou Y, Huang H, Chen N (2017) Antioxidant activities of ginsenoside Rg1 against cisplatin-induced hepatic injury through Nrf2 signaling pathway in mice. Free Radic Res 51: 1-13.

Isoda K, Hasezaki T, Kondoh M, Tsutsumi Y, Yagi K (2011) Effect of surface charge on nano-sized silica particles-induced liver injury. Pharmazie 66: 278-281.

Jaiman S, Sharma AK, Singh K, Khanna D (2013) Signalling mechanisms involved in renal pathological changes during cisplatin-induced nephropathy. Eur J Clin Pharmacol. 69: 1863-1874.

Kettiger H, Schipanski A, Wick P, Huwyler J (2013) Engineered nanomaterial uptake and tissue distribution: from cell to organism. Int J Nanomed 8: 3255-3269.

McIntyre RA (2012) Common nano-materials and their use in real world applications. Sci Prog 95: 1-22.

Nagano T (1991) Chemical and biochemical studies on reactivities, formations and toxicities of reactive oxygen species. Yakugaku Zasshi 111: 103-119.

Oberdorster G, Oberdorster E, Oberdorster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113: 823-839.

Peralta-Videa JR, Zhao L, Lopez-Moreno ML, de la Rosa L, Hong J, Gardea-Torresdey JL (2011) Nanomaterials and the environment: a review for the biennium 2008-2010. J Hazard Mater 186: 1-15.

Rakesh L, Howell BA, Chai M, Mueller A, Kujawski M, Fan D, Ravi S, Slominski C (2008) Computer-aided applications of nanoscale smart materials for biomedical applications. Nanomedicine (London) 3: 719-739.

Sarlo K, Blackburn KL, Clark ED, Grothaus J, Chaney J, Neu S, Flood J, Abbott D, Bohne C, Casey K, Fryer C, Kuhn M (2009) Tissue distribution of 20 nm, 100 nm and 1000 nm fluorescent polystyrene latex nanospheres following acute systemic or acute and repeat airway exposure in the rat. Toxicology 263: 117-126.

Shibuya S, Ozawa Y, Watanabe K, Izuo N, Toda T, Yokote K, Shimizu T (2014) Palladium and platinum nanoparticles attenuate aging-like skin atrophy via antioxidant activity in mice. PloS one 9: e109288.

Shimizu Y, Isoda K, Tezuka E, Yufu T, Nagai Y, Ishida I, Tezuka M (2012) Influence of 50-nm polystyrene particles in inducing cytotoxicity in mice co-injected with carbon tetrachloride, cisplatin, or paraquat. Pharmazie 67: 712-714.

Simon-Deckers A, Loo S, Mayne-L'hermite M, Herlin-Boime N, Menguy N, Reynaud C, Gouget B, Carriere M (2009) Size-, composition- and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria. Environ Sci Technol 43: 8423-8429.

Smith B, Wepasnick K, Schrote KE, Cho HH, Ball WP, Fairbrother DH (2009) Influence of surface oxides on the colloidal stability of multi-walled carbon nanotubes: a structure-property relationship. Langmuir 25: 9767-9776.

Svenson S, Tomalia DA (2005) Dendrimers in biomedical applications--reflections on the field. Adv Drug Deliv Rev 57: 2106-2129.

Wang XY, Ishida T, Ichihara M, Kiwada H (2005) Influence of the physicochemical properties of liposomes on the accelerated blood clearance phenomenon in rats. J Controlled Release 104: 91-102.

Wilson CJ, Clegg RE, Leavesley DI, Pearcy MJ (2005) Mediation of biomaterial-cell interactions by adsorbed proteins: a review. Tissue Eng 11: 1-18.

Zhong H, Liu C, Zhou H, Wang Y, Wang R (2016) Prefunctionalized porous organic polymers: effective supports of surface palladium nanoparticles for the enhancement of catalytic performances in dehalogenation. Chemistry 22: 12533-12541.