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Effect of surface charge on nano-sized silica particles-induced liver injury

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Nanomaterials are used frequently in microelectronics, cosmetics and sunscreen, and research for the development of nanomaterial-based drug delivery systems is promising. We previously reported that the intravenous administration of unmodified silica particles with a diameter of 70 nm (SP70) caused hepatic injury. Here, we examined the acute hepatic toxicity of SP70 modified with amino group (SP70-N) or carboxyl group (SP70-C). When administered intravenously into mice, SP70-N and SP70-C dose-dependently increased the serum level of alanine aminotransferase (ALT). However, the toxicity levels of surface charge-modified silica particles were much less weaker than the level of unmodified particles. When SP70 was repeatedly administered at 40 mg/kg twice a week for 4 weeks into mice, the hydroxyproline content of the liver significantly increased. Azan staining of the liver section indicated the extensive fibrosis. To the contrary, the repeated administration of SP70-N or SP70-C at 60 mg/kg twice a week for 4 weeks into mice did not cause the hepatic fibrosis. These findings suggest that the surface charge of nanomaterials could change their toxicity.

1. Introduction

Recently, the scientific, medical, and technical applications of nanomaterials have greatly increased. Nanomaterials are frequently used in microelectronics, cosmetics and sunscreen, and their potential use in drug-delivery systems is being investigated (Dobson 2006). Nanomaterials have unique physicochemical qualities as compared to micromaterials in regard to size, surface structure, solubility, and aggregation. Thus, the reduction in particle size from the micro- to nanoscale is beneficial for many industrial and scientific applications. However, nanomaterials have potential toxicity that is not found in micromaterials, and it is, therefore, essential to understand the biological activity and potential toxicity of nanomaterials (Warheit et al. 2008).

The physical properties of nanomaterials are changed by the 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 the toxicity of carbon nanotubes change based on the surface charge (Smith et al. 2009), as do the pharmacokinetics of liposomes. Future research will undoubtedly lead to expanded applications of surface-modified nanomaterials, however, little has been reported on their toxicity.

Silica nanoparticles have been applied to diagnostic measures and drug delivery methods. Intraperitoneal administration of silica nanoparticles results in the biodistribution of the nanoparticles to diverse organs, such as the liver, kidney, spleen and lung (Kim et al., 2006). We previously found that nano-size silica particles with a diameter of 70 nm caused liver injury, while micro-size particles with a diameter of 300 or 1000 nm did not (Nishimori et al. 2009a, b). In the present study, we examined the hepatic toxicity of surface charge-modified silica nanoparticles.

2. Investigations, results and discussion

The surface modification technology has been developed in the field of nanotechnology (Schiestel et al. 2004), and many nanomaterials with new functions will be produced for cosmetics and medicinal use. Thus, it should be important to investigate the effect of surface charge of nanomaterials on living body.

We initially examined the acute toxicity of 70-nm diameter silica nanoparticles (SP70) modified with amino group (SP70-N) or carboxyl group (SP70-C) at the maximal dose of 100 mg/kg. Intravenous injection of 50 mg/kg of unmodified SP70 was lethal in mice (Fig. 1A). The acute liver toxicity of SP70-N and SP70-C increased in a dose-dependent manner (Fig. 1B, C). Intravenous injection of SP70-C was lethal in all mice at 100 mg/kg and was often lethal at 80 and 60 mg/kg. SP70-C was more toxic than SP70-N. We examined the hepatic injury caused by 40 mg/kg of unmodified SP70 and 60 mg/kg of modified SP70 (SP70-C and SP70-N). The hematoxylin-eosin staining of liver tissue from mice injected with the silica nanoparticles is shown in Fig. 2A–D. The liver injury caused by SP70 was more extensive than that caused by SP70-C and SP70-N. Significant increase in the levels of BUN, a biochemical marker of kidney injury, was not observed in mice that received the nanoparticles (Fig. 3). The less amount of unmodified SP70 induced significant liver damage than the surface-modified silica particles. Thus, the

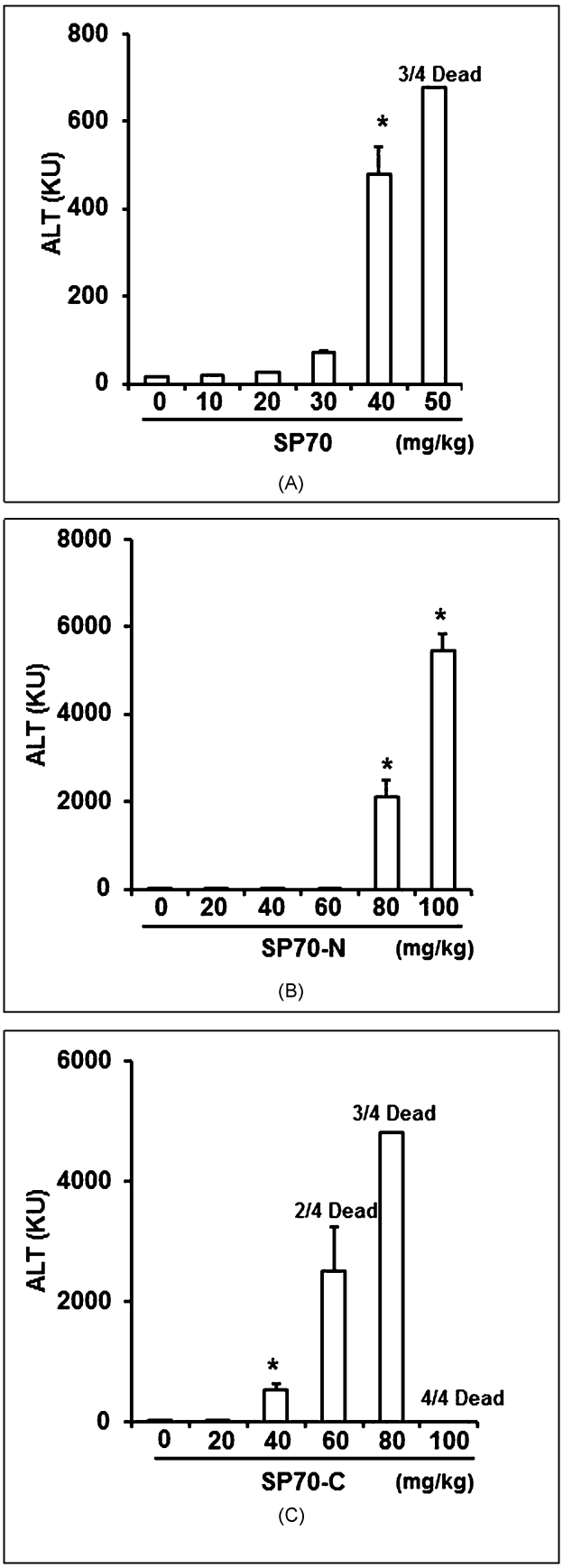

Fig. 1: Acute liver toxicity of SP70-N and SP70-C. SP70 (A), SP70-N (B) and SP70-C (C) were intravenously administered at the indicated doses. At 24 h after administration, blood was collected, and the resultant serum was used for the ALT assay. Data are means ± SEM (n = 4). *$p < 0.05$ as compared to the vehicle-treated group.

modification of the surface charge decreased the amount of acute hepatic injury caused by silica nanoparticles.

We then examined the chronic liver injury caused by 60 mg/kg of SP70-C or SP70-N as compared to 30 mg/kg of SP70. Nanoparticles were intravenously injected into mice twice a week for 4 weeks. We assessed the presence of liver fibrosis, because it is a symptom of chronic liver injury. We determined the hepatic hydroxyproline contents in the silica nanoparticle–treated mice (Fig. 4A). SP70, but not SP70-N or SP70-C, significantly increased the hepatic hydroxyproline content by 3.5-fold over the control value. Moreover, collagen, which accumulates in the fibrotic liver, was stained with Azan reagent, and blue-stained regions were observed in SP70-treated, but not SP70-C- and SP70-N-treated, liver sections (Fig. 4B-E). Thus, the chronic administration of SP70-C and SP70-N did not cause hepatic fibrosis in mice.

In this study we found that the surface modification of nanosilica particles with amino group and carboxyl group attenuated liver toxicity. We suspect that this decreased toxicity is due to a decrease in the amount of silica nanoparticles that accumulate in the liver. Oku et al. (1996) reported that the accumulation of liposomes in the liver changed depending on the surface charge of liposomes. Although we confirmed the presence of SP70-N, SP70-C and SP70 in the electron micrograph (data not shown), we were unable to compare the accumulative amounts in the liver. Therefore, an analysis of the accumulative amount of the silica nanoparticles in the liver is necessary in future studies.

The surface charge of nanoparticles might change the pharmacokinetics *in vivo*; for instance, the silica nanoparticles with a positive surface charge have increased paracellular permeability (Lin et al. 2007). Moreover, the phagocytosis of liposomes by hepatic Kupffer cells was promoted by a positive surface charge (Schiestel et al. 2004). We previously reported that the inhibition of phagocytosis by Kupffer cells increased the toxicity of nanosilica particles (Nishimori et al. 2009a). Therefore, it is thought that the nanoparticles with a positive surface charge have decreased hepatic toxicity due to increased phagocytosis by liver Kupffer cells.

This report is the first to indicate that altering the surface charge of nanomaterials changes their toxicity. Further studies based on these data will provide useful information regarding the safety of the nanomaterials.

3. Experimental

3.1. Materials

Silica particles with a diameter of 70 nm were obtained from Micromod Partikeltechnologie GmbH (Rostock, Germany). Silica particles with a diameter of 70 nm that were modified with the amino group or the carboxyl group were obtained from Micromod Partikeltechnologie GmbH (Rostock, Germany). The size distribution of the particles was analyzed using a Zetasizer (Sysmex Co., Kobe, Japan), and the mean diameters were 61.5 and 70.5 nm, respectively. The electric charge of the particles, also measured using the Zetasizer, was found to be –19.7 and –52.4 mV, respectively. The particles were spherical and nonporous and were stored at 25 mg/mL in an aqueous suspension. The suspensions were thoroughly dispersed by sonication before use and then diluted in ultrapure water. All reagents used were of research grade.

3.2. Animals

Eight-week-old BALB/c male mice were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan) and were maintained in a controlled environment (23 ± 1.5 °C; 12-h light/dark cycle) with access to standard rodent chow and water *ad libitum*. The mice were left to adapt to the new environment for 1 week before commencing with the experiment. Mice that received a single treatment of silica nanoparticles were anesthetized for sacrificing 24 h after intravenous injection. Mice in the frequent treatment group received intravenous administration of silica nanoparticles twice a week for 4 weeks. The experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences, Osaka University.

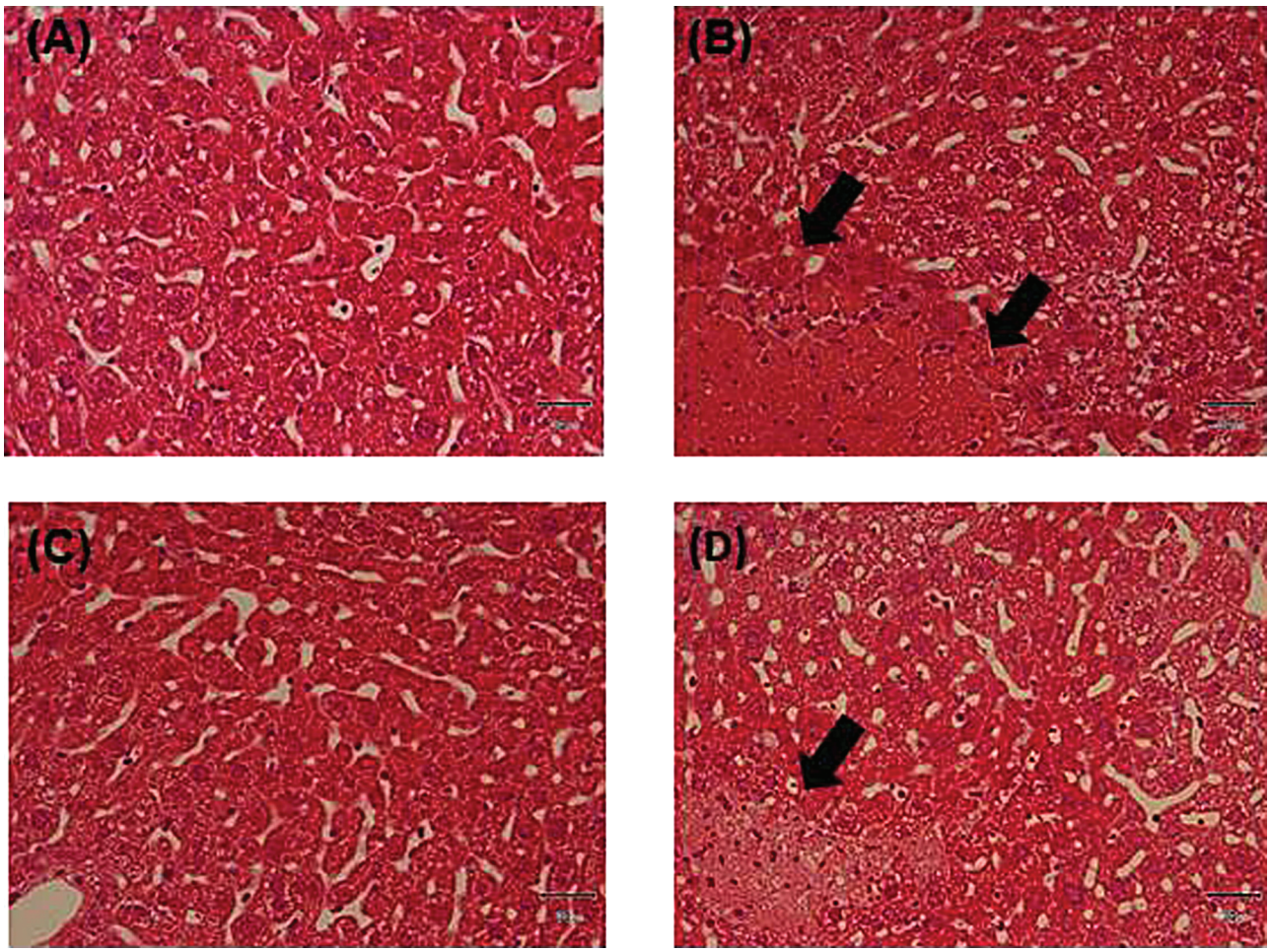

Fig. 2: Hematoxylin and eosin staining of the liver sections. Twenty-four h after administration, the liver was excised from the mice treated with vehicle (A), SP70 (B), SP70-N (C) or SP70-C (D) and fixed with 4% paraformaldehyde. Tissue sections were stained with hematoxylin and eosin and observed under a microscope. The arrows indicate areas of hepatic injury.

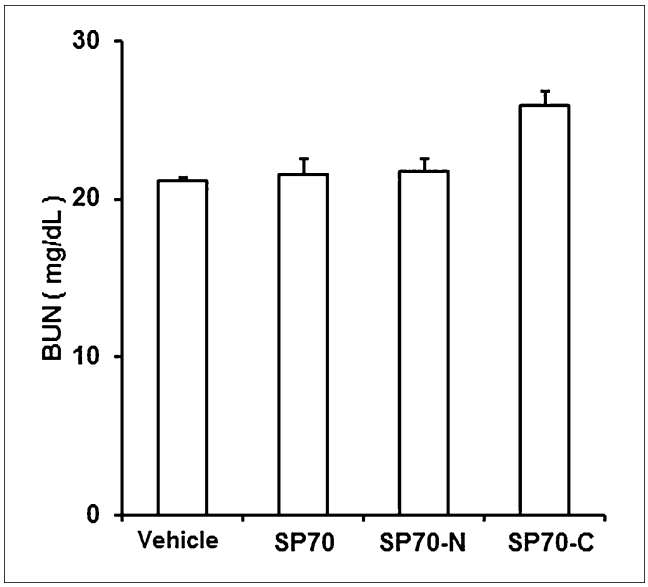

Fig. 3: Effect of SP70-N and SP70-C on kidney. SP70, SP70-N and SP70-C were intravenously administered at 40 mg/kg, 60 mg/kg, and 60 mg/kg, respectively. At 24 h after administration, blood was collected, and the resultant serum was used for the BUN assay with a commercially available kit. Data are means ± SEM (n = 4).

3.3. Biochemical analysis

Serum alanine aminotransferase (ALT) and blood urea nitrogen (BUN) were measured with commercially available kits according to the manufacturer's protocols (Wako Pure Chemical Industries, Osaka, Japan).

3.4. Histological analysis

The liver was excised and fixed with 4% paraformaldehyde. After sectioning, thin tissue sections of tissues were stained with hematoxylin and eosin for histological observation. Liver sections were stained with Azan-Mallory for observation of liver fibrosis.

3.5. Measurement of hydroxyproline content

Hepatic hydroxyproline (HYP) content was measured using Kivirikko's method (Kivirikko et al. 1967), with some modifications. Briefly, liver tissue (50 mg) was hydrolyzed in 6 mol/L HCl at 110 °C for 24 h in a glass test tube. After centrifugation at 3000 rpm for 10 min, 2 mL of the supernatant was neutralized with 8 N KOH. Two grams of KCl and 1 mL of 0.5 mol/L borate buffer were then added to the resultant solution, followed by incubation for 15 min at room temperature and a further incubation for 15 min at 0 °C. Freshly prepared chloramine-T solution was then added and the solution was incubated at 0 °C for 1 h, followed by the addition of 2 mL of 3.6 mol/L sodium thiosulfate. The samples were incubated at 120 °C for 30 min, and then 3 mL toluene was added with incubation for a further 20 min at room temperature. After centrifugation at 2000 rpm for 5 min, 2 mL of the supernatant was added to 0.8 mL of buffer containing Ehrlich's reagent and incubated for 30 min at room temperature. The samples were then transferred to a plastic tube and the absorbance was measured at 560 nm. Hydroxyproline content was expressed as micrograms of hydroxyproline per gram of liver.

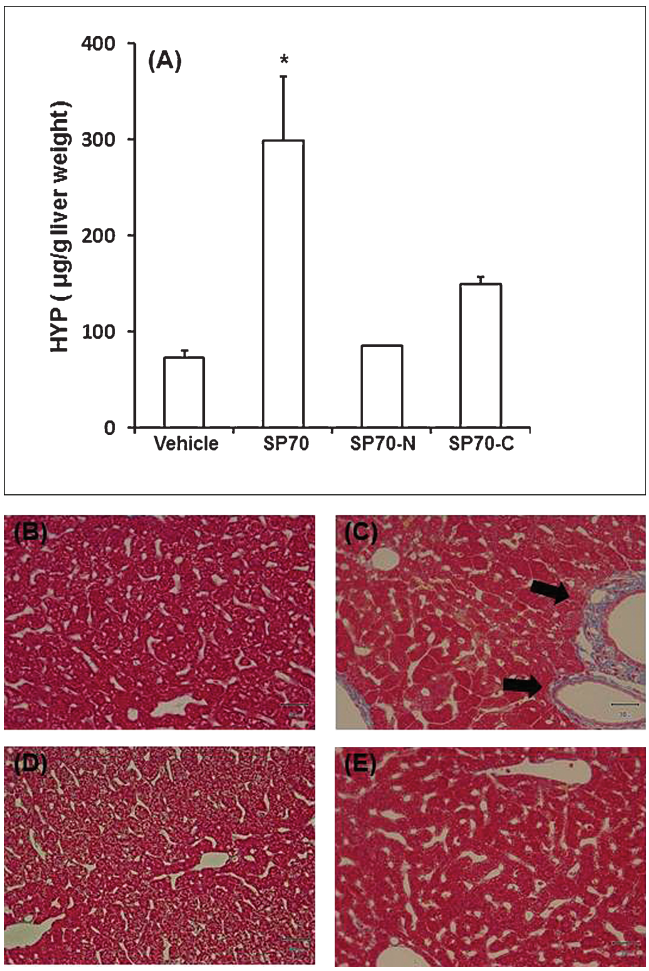

Fig. 4: Effect of SP70-N and SP70-C on chronic liver injury. SP70 was injected into mice every 3 days for 4 weeks at 30 mg/kg. SP70-C and SP70-N was injected into mice every 3 days for 4 weeks at 60 mg/kg. Three days after the last injection, the mice were sacrificed. Hydroxyproline levels (A) in the liver were measured. The liver was excised from mice treated with vehicle (B), SP70 (C), SP70-N (D) or SP70-C (E) and fixed with 4% paraformaldehyde. Tissue sections were stained with Azan and observed under a microscope. The arrows indicate areas of hepatic fibrosis. Data are means ± SEM (n = 4). *$p < 0.05$ as compared to the vehicle-treated group.

3.6. Statistical analysis

The data were analyzed for statistical significance using Dunnett's test. P values less than 0.05 were considered statistically significant.

References

Dobson J (2006) Magnetic micro- and nano-particle-based targeting for drug and gene delivery. Nanomed 1: 31–37.

Kim JS, Yoon TJ, Yu KN, Kim BG., Park SJ, Kim HW, Lee KH, Park SB, Lee JK, Cho MH (2006) Toxicity and tissue distribution of magnetic nanoparticles in mice. Toxicol Sci 89: 338–347.

Kivirikko KI, Laitinen O, Prockop DJ (1967) Modifications of a specific assay for hydroxyproline in urine. Anal Biochem 19: 249–255.

Lin YH, Mi FL, Chen CT, Chang WC, Peng SF, Liang HF, Sung HW (2007) Preparation and characterization of nanoparticles shelled with chitosan for oral insulin delivery. Biomacromolecules 8: 146–152.

Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K (2009a) Silica nanoparticles as hepatotoxicants. Eur J Pharm Biopharm 72: 496–501.

Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K (2009b) Histological analysis of 70-nm silica particles-induced chronic toxicity in mice. Eur J Pharm Biopharm 72: 626–629.

Oku N, Tokudome Y, Namba Y, Saito N, Endo M, Hasegawa Y, Kawai M, Tsukada H, Okada, S (1996) Effect of serum protein binding on real-time trafficking of liposomes with different charges analyzed by positron emission tomography. Biochim Biophys Acta 1280: 149–154.

Schiestel T, Brunner H, Tovar G.E (2004) Controlled surface functionalization of silica nanospheres by covalent conjugation reactions and preparation of high density streptavidin nanoparticles. J Nanosci Nanotechnol 4: 504–511.

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.

Warheit DB, Sayes CM, Reed KL, Swain KA (2008) Health effects related to nanoparticle exposures: environmental, health and safety considerations for assessing hazards and risks. Pharmacol Ther 120: 35–42.