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Note

Histological analysis of 70-nm silica particles-induced chronic toxicity in mice

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ABSTRACT

Nano-sized silica is a promising material for disease diagnosis, cosmetics and drugs. For the successful application of nano-sized material in bioscience, evaluation of nano-sized material toxicity is important. We previously found that nano-sized silica particles with a diameter of 70 nm showed acute liver failure in mice. Here, we performed histological analysis of major organs such as the liver, spleen, lung, kidney, brain and heart in mice, chronically injected with 70-nm silica particles for 4 weeks. Histological analysis revealed hepatic microgranulation and splenic megakaryocyte accumulation in these 70-nm silica particles treated mice, while the kidney, lung, brain and heart remained unaffected. Thus, liver and spleen appear to be the major target organs for toxicity by the chronic administration of the 70-nm silica particles.

1. Introduction

Recent progress in nanotechnology, the act of reducing size from the microscale to the nanoscale, has provided us with dramatic changes in industrial manufacturing and medicine. It also offers many benefits to revolutionize biotechnology, such as synthesis of new drugs with targeted delivery and regenerative medicine [1]. Reducing particle size increases surface area and makes modification of unique physicochemical properties, such as high conductivity, strength, durability, and chemical reactivity possible [2]. Thus, the nanotechnology has led to novel materials and innovations in the industry, bioscience and medicine.

Nanomaterials are already being used in bioscience and medicine, such as electronics, sunscreens, cosmetics and medicine for the purposes of diagnosis, imaging and drug delivery. For example, nano-sized silica particles are intended for the systemic and local delivery of drugs [3]. However, the toxicity of the manufactured nano-sized particles has not been fully evaluated.

We previously found that nano-sized particles with a diameter of 70 nm caused acute liver failure, while micro-sized particles with a diameter of 300 or 1000 nm did not [4]. In this study, we performed histological analysis of chronic toxicity induced by intravenous administration of 70-nm silica particles (SP70) for 4 weeks into the major organs, such as liver, lung, spleen, kidney, brain and heart of mice.

2. Materials and methods

2.1. Materials

Nano-sized silica particles with a diameter of 70 nm were obtained from Micromod Partikeltechnologie GmnH (Rostock, Germany). The surface was not modified. The mean diameters of these particles analyzed by Zetasizer (Sysmex Co., Kobe, Japan) were determined to be 55.7 nm. The particles were spherical and nonporous, and were stored at 25 mg/ml in aqueous suspension. The suspensions were thoroughly dispersed with sonication before use and diluted in water. The dispersion of the particles was confirmed by electron microscopy (data not shown). Reagents used were of research grade.

2.2. Animals

The 8-week-old BLAB/c male mice were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan), and housed in an environmentally controlled room at 23 ± 1.5 °C with a 12-h light/dark cycle. Mice had access to water and chow (Type MF, Oriental Yeast, Tokyo, Japan) *ad libitum*. Mice were intravenously

Abbreviations: SP70, 70-nm silica particles; HE, hematoxylin–eosin; ALT, alanine aminotransferase; HYP, hydroxyproline.

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injected with vehicle or the particles twice a week for 4 weeks. On day 3 after the last injection, the mice were sacrificed, and the serum and organs were recovered. The experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences, Osaka University.

2.3. Histological analysis

The liver, spleen, lung, kidney, brain and heart were removed and fixed with 4% paraformaldehyde. After sectioning, thin sections of tissues were stained with hematoxylin and eosin for histological observation.

2.4. Biochemical analysis

Serum alanine aminotransferase (ALT) was measured using a commercially available kit according to the manufacturer's protocol (Wako Pure Chemical, Osaka, Japan).

2.5. Hydroxyproline (HYP) assay

Hepatic HYP content was measured by Kivirikko's method with some modification [5]. Briefly, liver tissue was hydrolyzed in 6 M HCl at 110 °C for 24 h. The resultant supernatant was neutralized with 8 N KOH, and then 2 g of KCl and 1 ml of 0.5 M borate buffer were added, followed by a 15-min incubation at room temperature and further incubation for 15 min at 0 °C. Chloramines-T solution was then prepared and added. After additional incubation for 1 h at 0 °C, 2 ml of 3.6 M sodium thiosulfate was added, followed by incubation at 120 °C for 30 min. Next, 3 ml of toluene was added with incubation for a further 20 min at room temperature. After centrifugation, 2 ml of the resultant supernatant was added to Ehrlich's reagent, followed by incubation for 30 min at room temperature. Subsequently, absorbance was measured at 560 nm.

2.6. Statistical analysis

Statistical analysis was performed by Student's *t*-test. The level of significance was set at $p < 0.05$.

3. Results and discussion

We previously found that intravenous administration of SP70 induced liver injury through a single administration [4]. To investigate the chronic toxicity of SP70, 10 or 30 mg/kg of SP70 was intravenously injected into mice twice a week for 4 weeks at which point the livers were not injured or injured by the single injection, respectively [4]. During chronic administration, no significant differences were observed in the body weight between the vehicle and the SP70-treated group (Fig. 1) and no abnormal behaviors were detected (data not shown). Therefore, SP70 treatment did not show apparent toxicity in mice at the low dose.

Next, we performed histological analysis of tissues that are enriched with reticuloendothelial system (RES) such as the liver, spleen, and lungs and non-RES organs such as the heart, kidney and brain. As shown in Fig. 2A and B, treatment with SP70 induces hepatic microgranulation and increases splenic megakaryocyte accumulation. In contrast, the remaining RES organ, the lung, and all the non-RES organs did not show tissue injury with SP70 treatment (Fig. 2B–F). Thus, we examined a serum biochemical marker of liver injury, ALT, to confirm liver injury. SP70 treatment significantly elevated serum ALT levels (Fig. 3A), but a renal injury marker, blood urea nitrogen, was not elevated by these treatments

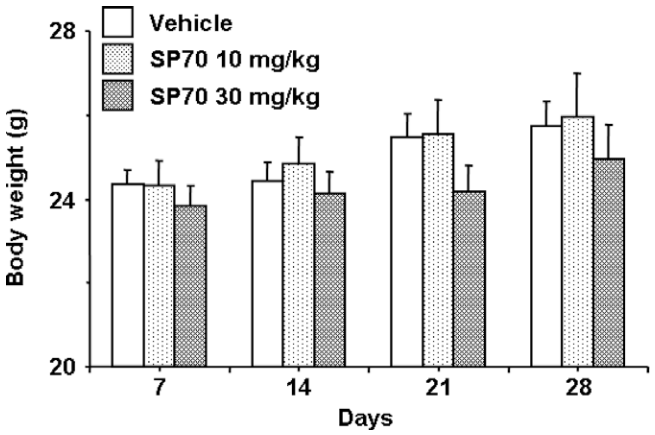

Fig. 1. Body weight changes in mice treated with SP70 for 4 weeks. Mice were intravenously administered SP70 at 0, 10 or 30 mg/kg twice a week for 4 weeks. The body weights of the treated mice were monitored on days 7, 14, 21 and 28. Each point represents mean ± SEM (n = 5–7).

(data not shown). Chronic hepatic injury causes liver fibrosis, finally leading to hepatic carcinoma. The chronic treatment with SP70 also elevated a marker of fibrosis, HYP, in the liver (Fig. 3B). Taken together, chronic SP70 treatment appears to injure the liver and spleen.

As innovative materials cover wide fields from industry to life science, nanomaterials have potential to improve the quality and performance of many consumer products as well as medical therapies. Thus, it is very critical in the field of nanotechnology to also assess the risk of nano-sized materials. As the use of nano-sized silica particles in cosmetics and the application in pharmaceutical research, e.g., drug delivery and molecular imaging [3,6] are increasing, we evaluated the toxicity of nano-sized silica particles. We have already found that SP70 causes acute liver injury in mice [4]. In the present study, we evaluate the influence of chronic administration of SP70 for 4 weeks on major organs by histological analysis. As the nano-sized particles are taken into RES organs such as the liver, lung and spleen, we expected that all the RES organs would be injured by chronic SP70 exposure. However, histological abnormalities in the lung were not observed. Kim et al. found that 50-nm silica particles were distributed into all the RES organs, but the amount of the distributed particles into the lung was smaller than that into the liver and spleen [7]. Therefore, the lack of histological abnormalities in the lung may be due to a lower distribution of SP70.

The underlying mechanism for histological injury in the liver and spleen remains to be elucidated. We previously found that the serum levels of inflammatory cytokines (interleukin-6 and tumor necrosis factor-α) were elevated by SP70 [4]. Uptake of SP70 by macrophages in the liver and spleen may cause the release of the cytokines from the macrophage, leading to histological abnormalities. Macrophage receptor with collagenous structure (MARCO), CD204 and CD36 are all macrophage silica particle receptors [8–10]. CD36 is expressed in macrophages of BALB/c mice [10].

In the present study, there is no observation of histological injury in lung, kidney, brain and heart. Regulation of liver and spleen injuries may be critical for the safe application of these nano-sized silica particles. Future analysis is necessary to determine tissue distribution of SP70. Extensive studies are also required to provide the basis for a new class of nanomaterials for drugs, proteins, and gene delivery applications. We are developing materials and methods to control the bio-distribution of these nano-sized silica particles.

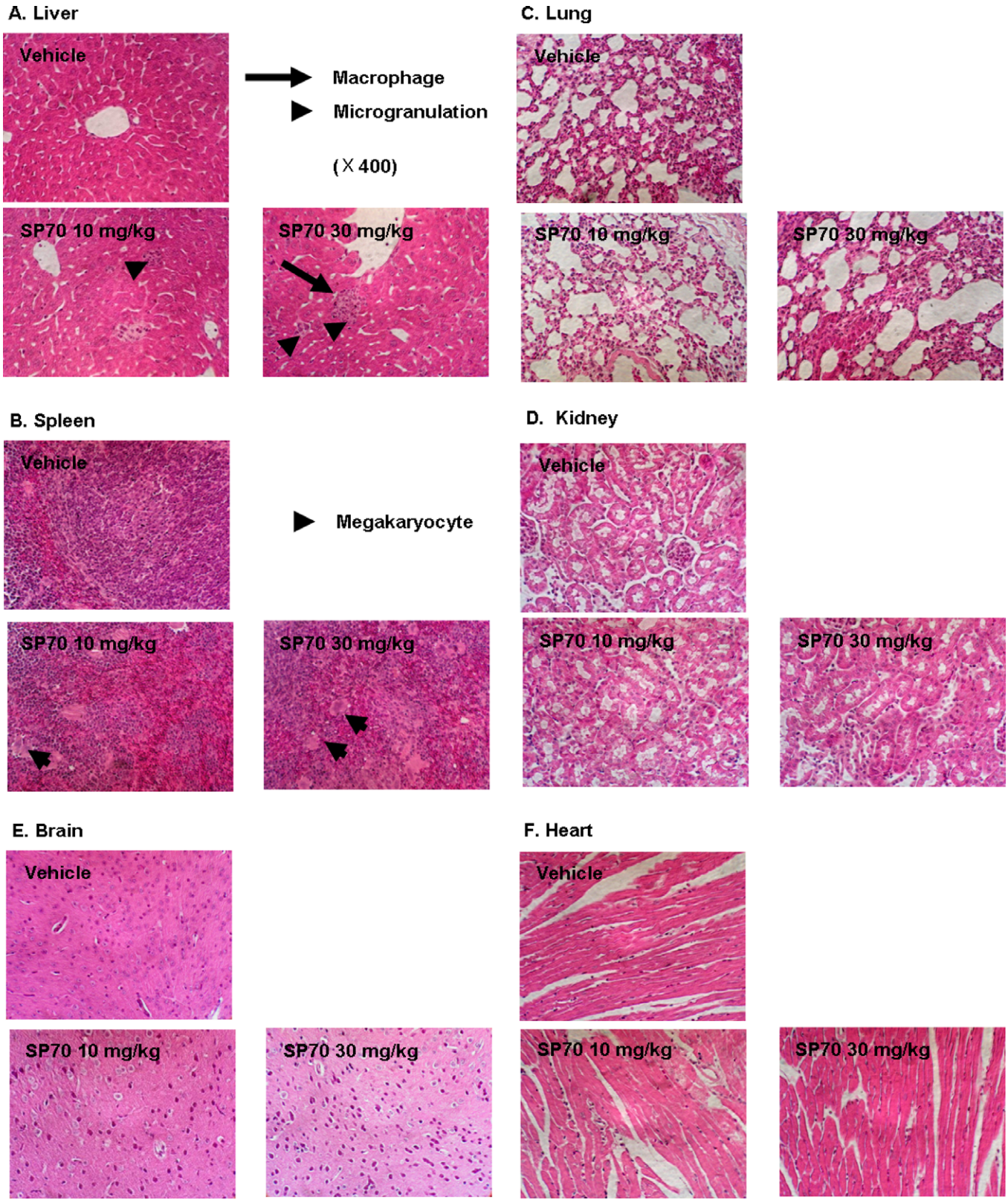

Fig. 2. Histopathological evaluation of the organs from SP70-treated mice. After chronic treatment with SP70 for 4 weeks, liver (A), spleen (B), lung (C), kidney (D), brain (E) and heart (F) were recovered and fixed with paraformaldehyde, followed by staining with hematoxylin and eosin. The arrowheads in (A) and (B) indicate microgranulation in the liver and accumulation of megakaryocyte in the spleen, respectively. The tissue sections were observed under a microscope at 400×. The pictures are representative of at least four independent sections.

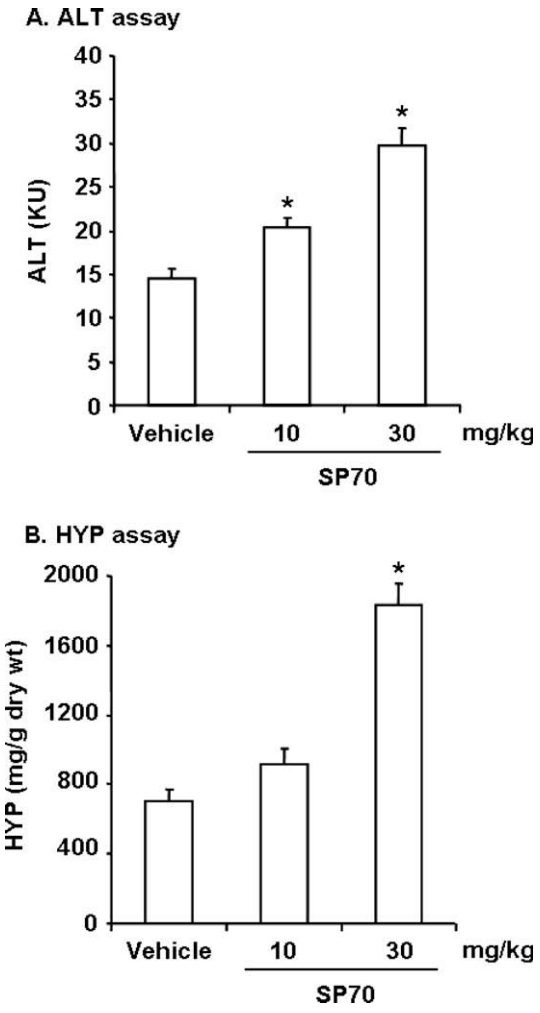

Fig. 3. Biochemical analysis of liver injury. After chronic SP70 treatment for 4 weeks, the serum and liver were collected. Then, the serum alanine aminotransferase (A) and hepatic hydroxyproline (HYP) levels (B) were measured as described in Section 2. Data are shown as mean ± SD ($n = 4$). The results are representative of at least three independent experiments. *Significant difference from the vehicle-treated group ($p < 0.05$).

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