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Letter

Silica nanoparticle-induced toxicity in mouse lung and liver imaged by electron microscopy

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ABSTRACT — Nanomaterials have been proposed as novel substrates for medical and commercial applications. However, such materials also may have novel toxicities, thus posing environmental and health concerns. We previously reported hepatic injury in mice following the intravenous administration of unmodified silica particles with diameters of 70 nm (SP70); this toxicity was not observed following administration by the same route of micro-size particles with diameters of 300 nm (SP300) or 1,000 nm (SP1000). In the present study, we used electron microscopy to investigate the dynamics of silica nanoparticles administered in mice. SP70 was observed in hepatocytes and in lung epithelial cells. Inclusion within hepatocytes was associated with accumulation of SP70 in the liver sinusoidal endothelial cells and passage through the space of Disse. In contrast, SP300 and SP1000 were not observed within the hepatocytes. To our knowledge, our report represents the first demonstration that silica nanoparticles accumulate in hepatocytes, liver sinusoidal endothelial cells, Kupffer cells, and lung tissue; accumulation of SP70 in liver sinusoidal endothelial cells correlated with the induction of liver injury.

Key words: Silica nanoparticles, Electron microscope, Hepatocytes, Liver sinusoidal endothelial cells, Kupffer cells, Lungs

INTRODUCTION

Recently, nanomaterials have been proposed for use in a widening range of industrial, pharmaceutical, and technical applications. Nanomaterials are frequently used in microelectronics, cosmetics, and sunscreens, and their potential use in drug-delivery systems is being investigated (Dobson, 2006; Caputo *et al.,* 2008; Nohynek *et al.,* 2008). Nanomaterials have unique physicochemical qualities compared to micromaterials with regard to size, surface structure, solubility, and aggregation. Thus, the reduction in particle size from the micro- to nanoscale is expected to facilitate many industrial and scientific applications. However, nanomaterials have potential toxicities that are not found in micromaterials, and it is, therefore, essential to understand the biological activity and potential toxicity of nanomaterials (Warheit *et al.,* 2008; Bystrzejewska-Piotrowska *et al.,* 2009). Silica nanoparticles are intended for use in cosmetics and for systemic and local delivery of drugs (Vallet-Regi *et al*., 2007). Previously, we found that intravenous administration of 70-nm, but not 300- and 1000-nm, silica particles caused liver injury (Nishimori *et al.,* 2009c; Nishimori *et al.,* 2009a; Nishimori *et al.,* 2009b). However, the mechanism of hepatotoxicity of the silica nanoparticles remains poorly understood.

In the present study, we investigated the dynamics of the silica nanoparticles using electron microscopy (EM). As we report here, silica nanoparticles invaded hepatocytes following parenteral administration in whole animals.

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MATERIALS AND METHODS

Materials

Silica particles with diameters of 70, 300, or 1,000 nm (SP70, SP300, or SP1000, respectively) were obtained from Micromod Partikeltechnologie GmnH (Rostock, Germany). The size distribution of the particles was analyzed using a Zetasizer (Sysmex Co., Kobe, Japan), and the mean ± S.D. diameters were 57.5 ± 20.3, 326 ± 32.1, and 966 ± 36.3 nm, respectively. The silica particles were amorphous substance with chemically unmodified surfaces. The particles were spherical and nonporous and were stored as aqueous suspensions at 25 or 50 mg/mL. The suspensions were thoroughly dispersed by sonication before use and then diluted in ultrapure water. All reagents used were of research grade.

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-hr/12-hr light/dark cycle) with *ad libitum* access to standard rodent chow and water. The mice were permitted to adapt to the new environment for 1 week before commencing with the experiment. Mice were intravenously (i.v.) injected with silica nanoparticles (10, 30, or 100 mg/kg body weight) or vehicle. Mice were then anesthetized, bled for serum, and sacrificed at 24 hr after i.v. injection. The experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences, Osaka University.

Biochemical analysis

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

Ultrastructure of liver tissue

The mice were sacrificed on 24 hr after injection of nanoparticles. The liver and lung tissues were removed, immersed in 2.5% glutaraldehyde fixative, dissected into small blocks, and then returned to the same fixative at 4°C for 2 hr. Liver and lung fragments then were washed several times in 0.1 M phosphate buffer (pH 7.4), and post-fixed in phosphate-buffered 1% osmium tetroxide for 60 min at 4°C. After dehydration by passage through a series of ethanol concentrations and QY-1, liver and lung fragments were embedded in EPON resin (TAAB). Semi-thin (1-μm) sections were stained with toluidine blue and examined by light microscopy. Ultra-thin (10-nm) sections were stained with uranyl acetate and lead citrate, and examined under a Hitachi EM (H-7650, Tokyo, Japan).

Statistical analysis

Statistical analysis was performed by two-way ANOVA, followed by *post-hoc* Student's t-test where overall significance was indicated.*P < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

We initially observed the silica nanoparticles by EM. The shapes of SP70 (Fig. 1A), SP300 (Fig. 1B), and SP1000 (Fig. 1C) were globular, and none of the silica nanoparticles exhibited flocculation. Intravenous injection of SP70 at 100 mg/kg resulted in liver damage in mice, as demonstrated by a significant rise in serum ALT levels at 24 hr post-injection (Fig. 1D). Next, to examine the mech-

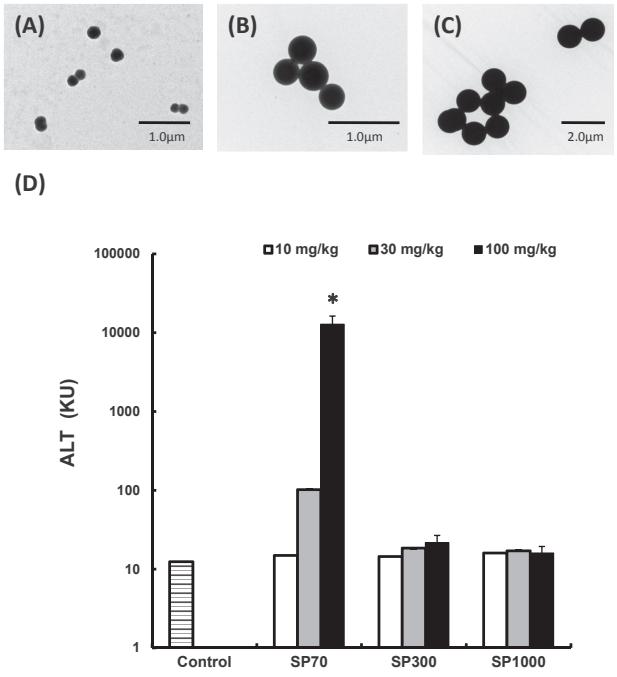

Fig. 1. Ultrastructure and acute liver toxicity of silica nanoparticles. Ultrastructure of silica nanoparticles SP70 (A), SP300 (B), or SP1000 (C) examined under the electron microscope. Silica nanoparticles of the indicated sizes were administered i.v. at the indicated doses. At 24 hr after administration, blood was collected, and the resultant serum was used for the ALT assay (D). Data are mean ± S.E.M. (n = 4). Significant differences were observed between the vehicle groups (*p < 0.05).

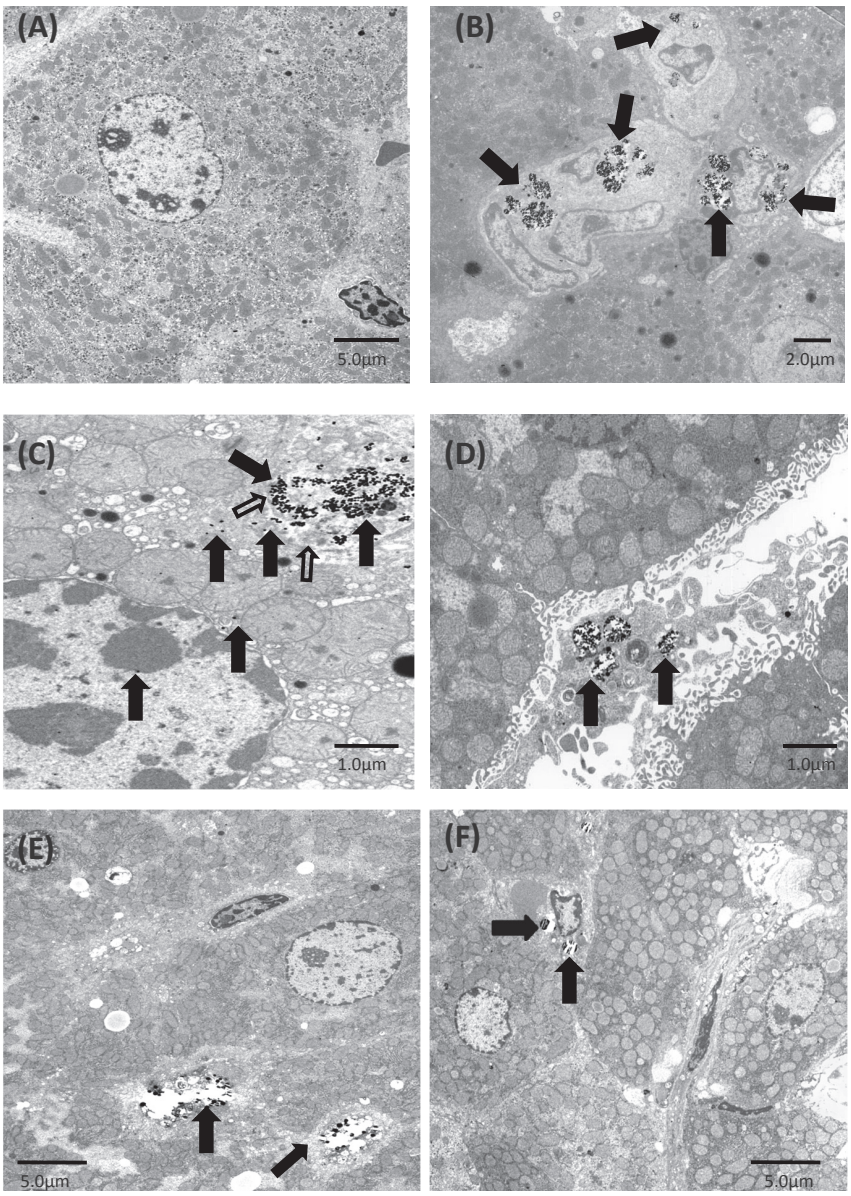

Fig. 2. Ultrastructure of liver tissue following injection of silica nanoparticles. Ultrastructure of liver tissue in silica nanoparticle-treated mice. Animals were administered i.v. with vehicle (A) or with silica nanoparticles (at 100 mg/kg) SP70 (B, C, D), SP300 (E), or SP1000 (F). At 24 hr after administration, livers were collected, fixed, and processed; ultrathin sections were stained with uranyl acetate and lead citrate and examined under the electron microscope. Black arrows indicate silica nanoparticles. White arrows indicate the space of Disse.

anism whereby silica nanoparticles induced liver toxicity, we used EM to observe liver tissue in silica nanoparticle-injected mice at 24 hr post-injection (Figs. 2, 3). SP70 was detected in the hepatocytes, with associated necrosis (Fig. 2B). In addition, SP70 accumulated in the liver sinusoidal endothelial cells; particles apparently accessed the hepatocytes by passing through the space of Disse (Fig. 2C). SP70 was in some instances observed within the nuclei of the hepatocytes. Particles were engulfed by, and accumulated within, Kupffer cells (Fig. 2D). In contrast, SP300 (Fig. 2E) and SP1000 (Fig. 2F) were not observed within the hepatocytes of 30 sections. The results of these EM observations confirmed our previous observation (Nishimori *et al.,* 2009c) that SP70 nanopar-

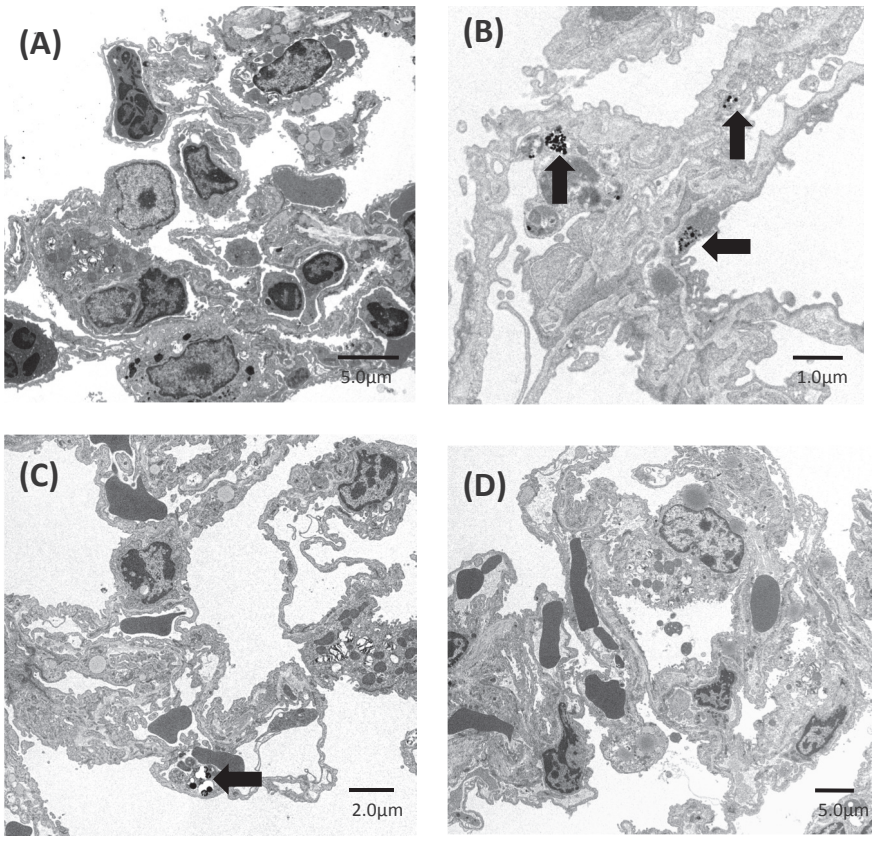

Fig. 3. Ultrastructure of lung tissue from mice treated with silica nanoparticles. Ultrastructure of lung tissue from mice injected i.v. with vehicle (A) or with silica nanoparticles (at 100 mg/kg) SP70 (B), SP300 (C), or SP1000 (D). At 24 hr after administration, lungs were collected and fixed with 2.5% glutaraldehyde; ultra-thin sections were stained with uranyl acetate and lead citrate and examined under the electron microscope. Black arrows indicate silica nanoparticles.

ticles, but not SP300 or SP1000, caused hepatocellular necrosis. The results of the present study further suggested that liver sinusoidal endothelial cells contributed to the internalization of silica nanoparticles by hepatocytes.

We extended our analysis by examining lung following i.v. administration of the silica nanoparticles (Fig. 3). We observed SP70 (Fig. 3B) and SP300 (Fig. 3C) within the lung epithelial cells. SP1000 was not observed in the lung epithelial cells of 28 sections (Fig. 3D). Similarly, silica nanoparticles were not observed in kidney tissue from any of the nanoparticle-dosed animals (SP70, SP300, or SP1000; data not shown).

Thus, we observed by EM that SP70 accumulated in the liver endothelial cells and hepatocytes. Liver sinusoidal endothelial cells were shown to participate in the accumulation of SP70 nanoparticles. In addition, we observed the phagocytosis (engulfment) of SP70 by Kupffer cells. These processes presumably contribute to liver injury. Our EM observations confirmed our previous results (Nishimori *et al.,* 2009c) indicating that liver sinusoidal endothelial cells are directly involved in silica nanoparticle-induced liver injury. The necrosis observed in the present work (e.g., Fig. 2B) was similar to the liver necrosis detected following exposure to aryl alcohol or to hepatitis B virus (Sell, 1997). Other sources have reported that silica nanoparticles induce necrosis in human epithelial cells (Napierska *et al.,* 2010).

The present work also demonstrated that SP70 and SP300 silica nanoparticles could be recovered from lung epithelial cells following i.v. injection. We are not aware of previous reports of nanoparticle accumulation in the lung following parenteral administration. The possible role of these nanoparticles in lung injury is unclear and will require further investigation.

To our knowledge, this report represents the first demonstration that silica nanoparticles are incorporated intra-

cellularly within hepatocytes, liver sinusoidal endothelial cells, Kupffer cells, and lung epithelial cells. Further studies based on these data are expected to provide useful information regarding the safety of these and other nanomaterials.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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