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Report

Cerium (IV) Oxide Nanoparticles Enhance Hepatotoxic and Nephrotoxic Effects of Paraquat, Cisplatin, or Acetaminophen in Mice

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Nanomaterials are central to nanotechnology and have many useful properties. Cerium(IV) oxide nanoparticles (nCeO) have catalytic and antioxidant activity, and hold promise in industrial and medical products. However, the potential biological hazards of nCeO have not been addressed. We therefore investigated the *in vivo* effects and drug interactions of nCeO. Tail vein administration of nCeO did not induce liver and kidney injury in mice but induced liver or kidney damage when co-administered with paraquat, cisplatin or acetaminophen. Therefore, our findings indicate that nCeO are potentially hepatotoxic and nephrotoxic due to drug interactions.

Key words nanoparticle, liver, kidney, drug interactions

INTRODUCTION

Recent progress in nanotechnology in diverse fields such as the materials, quantum and life sciences has brought technological innovations to the environmental, energy, information and electronics fields.[1–3] Nanomaterials with a particle size of 100 nm or less are widely used in, for example, medical products, cosmetics, and electronic devices.[4–6] The global demand for nanomaterials is continually increasing due to the development of new materials and technological innovation. Nanomaterials exhibit novel properties not seen in micromaterials, such as responses to exogenous stimuli, including heat, light and voltage.[7,8] However, nanoparticles are artificial materials that have unknown effects on living organisms. Nano-sized materials may have unique physico-chemical properties due to their larger surface area and smaller size, chemical composition, surface structure, solubility and shape than micro-sized materials. Due to their increased surface area, nanomaterials have increased interactions with living tissues, cells, proteins, and nucleic acids, potentially leading to toxic effects in humans.[9–11] Human exposure to nanomaterials commonly accompanies exposure to other potentially toxic substances such as foods, food additives, cosmetics and pharmaceuticals.

Cerium(IV) oxide is mainly used as industrial abrasives and catalysts, and in fuel cells and sunscreen creams.[12] Cerium(IV) oxide is a white solid with high melting and boiling points and a stable structure. Lung damage due to long-term exposure to cerium(IV) has been reported, but acute toxicity in animal experiments has not been reported.[13,14] Cerium(IV) oxide is a highly safe chemical with no reports of reproductive toxicity or carcinogenicity.[14] Cerium oxide nanoparticles (nCeO) are being successfully developed as fuel cell materials and as regenerative medicine products with strong antioxidant capacity.[15] In particular, cerium oxide nanoparticles have antioxidant ability to remove reactive oxygen species (ROS) *in vivo*, and cerium oxide nanoparticles have been developed as anti-inflammatory biomaterials. However, there are no reports on the safety of these nanoparticles in living organisms.

The field of nanotoxicology has recently expanded as researchers investigate the safety, pharmacology, and pharmacokinetics of nanoparticles. To date, we have focused on the interactions between nanoparticles and chemical substances. Silica nanoparticles have been shown to be cytotoxic, hepatotoxic, and cause placental damage,[16,17] and induce liver injury through interactions with drugs.[18] Carbon nanotubes have been reported to induce pulmonary mesothelioma.[19] Pharmacological effects resulting from drug interactions with cerium oxide nanoparticles are largely unknown. In this study, we investigated the toxicity of cerium oxide nanoparticles in mice, and the interactions between these nanoparticles and those that have interacted with nanoparticles in previous studies,[18] such as paraquat (a globally used pesticide), cisplatin (a widely used antitumor agent), or acetaminophen (an anti-inflammatory drug), could synergistically exert toxic effects.[20,21]

MATERIALS AND METHODS

Materials Cerium(IV) oxide nanoparticles (nCeO) with a diameter of 25 nm were obtained from SIGMA-ALDRICH Co. (St. Louis, MO, USA). The size distribution of the particles was analyzed using a Zetasizer (Sysmex Co., Hyogo, Japan); the mean diameter was 24.3 ± 8.7 nm. The particles were spherical and nonporous, and stored as 100 mg/mL 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. Paraquat (PQ), cisplatin (CDDP) and acetaminophen (APAP) (FUJIFILM Wako Co., Osaka, Japan) were dissolved in saline and stored at −20ºC before use. All reagents used were of research grade.

Animals Eight-week-old BALB/c male mice were purchased from Funabashi Farm Co., Ltd. (Chiba, Japan). The

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mice were maintained in a controlled environment (temperature: 23 ± 1.5ºC; 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.

Drug Interactions of nCeO The nCeO administered intravenously at a dose of 0.3 mg/kg body weight; simultaneously, paraquat (500 mg/kg) or cisplatin (80 μmol/kg) or acetaminophen (400 mg/kg) was administered intraperitoneally. Blood was recovered 24 h after the co-administration. The doses of cisplatin, paraquat and acetaminophen were previously determined experimentally and did not induce toxicity.

Biochemical Analysis Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were measured using commercially available kits (FUJIFILM Wako Co.) according to the manufacturer's protocols. Briefly, collected serum (10 μL) was combined with 1 mL of color A reagent (containing urease) and incubated at 37ºC for 15 min. Following the addition of 1 mL of color B reagent, the sample was incubated at 37ºC for 10 min. Absorbance was measured at a wavelength of 570 nm. Blood urea nitrogen (BUN) was measured using commercially available kits (ARBOR ASSAYS, Inc., Ann Arbor, MI, USA) according to the manufacturer's protocols. Absorbance was measured at a wavelength of 450 nm.

Statistical Analysis Statistical analyses were performed with Statcel 3 add-in forms for Microsoft Excel (EMS Publication Co., Ltd., Saitama, Japan). All data are presented as means ± SEMs. Significant differences between control groups and experimental groups were determined using the Dunnett test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

First, we examined the acute toxicity of nCeO at a dose of 0.3 mg/kg and found that these nanoparticles alone do not cause acute toxicity (Fig. 1A). Next, we investigated whether there is an interaction between several chemicals and nCeO. To avoid interactions between the chemicals and nCeO prior to administration and absorption, the chemicals were injected into mice intraperitoneally and nCeO were injected intravenously.

PQ induces liver and renal damage after intraperitoneal administration.[20] PQ (50 mg/kg) was administered to mice at doses that did not induce hepatic and renal damage. Co-treatment with nCeO caused toxicity. Co-administration of nCeO and PQ increased ALT, AST and BUN levels (Fig. 1A, B).

Next, we investigated the interaction between CDDP and nCeO. Administration of CDDP causes side effects such as renal and hepatic failure. Co-administration of CDDP (80 μmol/kg) and nCeO synergistically increased serum ALT levels from 8.4 to 187.8 K.U. and serum AST levels from 59.1 to 617.4 K.U. (Fig. 2A). Serum BUN levels increased synergistically from 20.4 to 89.0 mg/dL (Fig. 2B). We also investigated the interaction between APAP and nCeO. Co-administration of APAP (400 mg/kg) and nCeO synergistically increased serum ALT levels from 5.8 to 331 KU (Fig. 3).

In this study, we investigated the toxicity induced by chemicals combined with nCeO and found that PQ, CDDP and APAP produced synergistic toxic effects when combined with nCeO. The reason why the AST value, which is an index of liver injury, was large in the results was due to the procedure

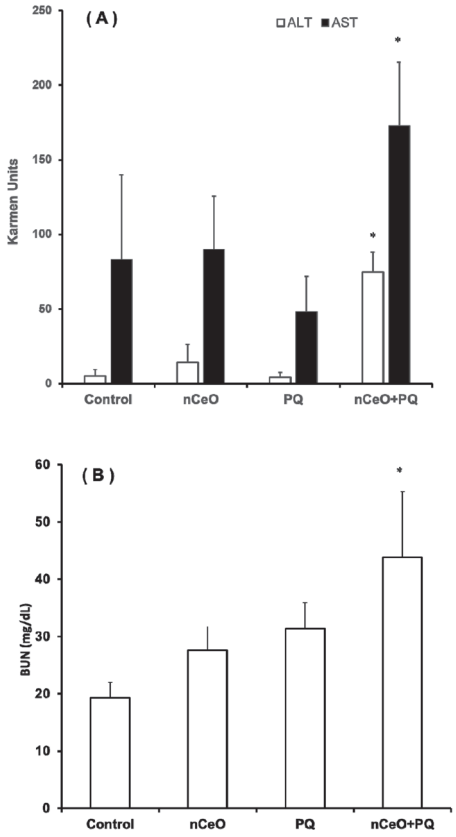

Fig. 1. Effect of nCeO on PQ-Induced Toxicity

Mice were injected intraperitoneally with PQ at 500 mg/kg together with intravenous injection of vehicle or nCeO (0.3 mg/kg). (A): At 24 h post-injection, serum levels of liver enzymes alanine transferase (ALT; open columns) and aspartate transferase (AST; solid columns) were measured. (B): Plasma levels of blood urea nitrogen (BUN). Data are representative of three independent experiments, and are presented as mean ± standard error of the mean (SEM; n = 4). ** Significant difference ($p < 0.01$) between the vehicle- and the PQ-treated group

when blood was collected from the mouse. It is thought that AST leaked into the blood from the myocardium when blood was collected from the heart to ensure the volume of blood. Liver injury induced by PQ and CDDP is caused by oxidative stress.[22,23] APAP-induced liver injury results from metabolic toxicity due to cytochrome P450.[24] The administration of nCeO to rats results in their accumulation in the liver and spleen, then they are captured by phagocytic cells in the liver.[25] Li *et al*. reported that nCeO are toxic to liver cells and increase ROS levels.[26] Our results show no hepatic toxicity resulting from nCeO accumulating in the liver, although nCeO do induce synergistic toxicity with chemicals known to cause oxidative stress. In addition, paraquat and cisplatin have been reported to be nephrotoxic, inducing acute kidney injury in a dose-dependent manner.[20,21] This suggests that the increase in BUN values in Fig. 1 (B) and 2 (B) was due to the increased nephrotoxicity of paraquat and cisplatin due to nCeO. Further biochemical and other analyses, such as proteome and genome assays, will be performed in our laboratory to determine the mechanism of these synergistic effects.

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

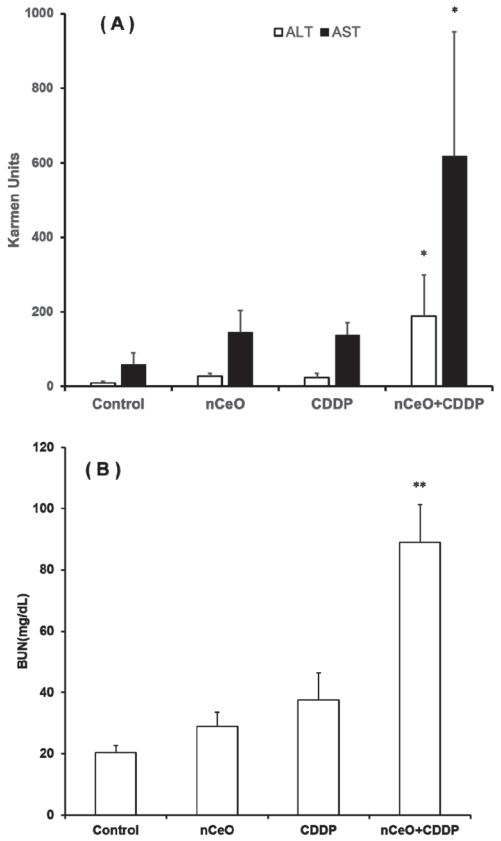

Fig. 2. Effect of nCeO on CDDP-Induced Toxicity

Mice were injected intraperitoneally with CDDP at 80 μmol/kg together with intravenous injection of vehicle or nCeO (0.3 mg/kg). (A): At 24 h post-injection, serum levels of liver enzymes alanine transferase (ALT; open columns) and aspartate transferase (AST; solid columns) were measured. (B): Plasma levels of blood urea nitrogen (BUN). Data are representative of three independent experiments, and are presented as mean ± standard error of the mean (SEM; n = 4). ** Significant difference ($p < 0.01$) between the vehicle- and CDDP-treated group.

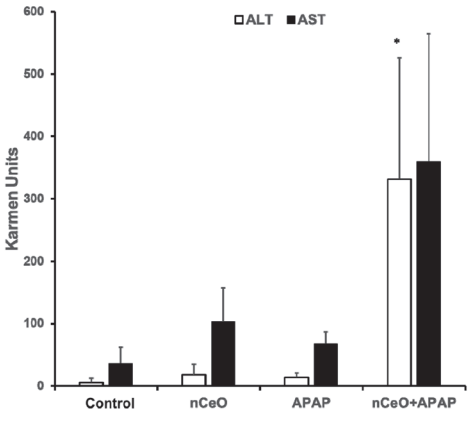

Fig. 3. Effect of nCeO on APAP-Induced Toxicity

Mice were injected intraperitoneally with APAP at 400 mg/kg together with intravenous injection of vehicle or nCeO (0.3 mg/kg). At 24 h post-injection, serum levels of liver enzymes alanine transferase (ALT; open columns) and aspartate transferase (AST; solid columns) were measured. Data are representative of three independent experiments, and are presented as mean ± standard error of the mean (SEM; n = 4). ** Significant difference ($p < 0.01$) between the vehicle- and APAP-treated group.

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Conflict of interest The authors declare no conflict of interest.

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