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Anatase and rutile titanium oxide nanoparticles induce acute kidney injury by coadministration with paraquat, cisplatin or 5-aminosalicylic acid.

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Nanoparticles are used in a variety of fields; for example, titanium oxide nanoparticles are used in paints, food additives, cosmetics, and sunscreen materials. Although the use of titanium oxide nanoparticles is regulated, their safety has not been established. Furthermore, the interaction between titanium oxide nanoparticles and various chemical substances and pharmaceuticals is unknown. We co-administered rutile-type titanium oxide nanoparticles (nTR) or anatase-type titanium oxide nanoparticles (nTA) to mice together with paraquat (PQ), cisplatin (CDDP), or anti-5-aminosalicylic acid (5-ASA), and investigated the extent, if any, of liver and kidney injury. As a result, when nTA and nTR were administered alone, no increases were observed in aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are indicators of liver damage, or urea nitrogen (BUN), which is an indicator of kidney damage. Next, nTA and nTR were co-administered with PQ, CDDP or 5-ASA. Although no increase in ALT or AST was observed, BUN levels increased significantly and acute kidney injury was induced. The findings suggested that titanium oxide nanoparticles induce acute kidney injury through their interaction with chemicals and drugs.

1. Introduction

Nanomaterials are substances smaller than 100 nm in diameter. Recently, the development of nanotechnology has dramatically increased the amount of nanomaterials used in fields such as medicine, cosmetics, and chemical fiber industries (Hubbs et al. 2011). Nano-sized substances have unique physical properties. In addition to having a larger surface area than micro-sized materials, nanomaterials also have unique properties related to their chemical composition, surface structure, and solubility (Hoshyar et al. 2016). Although the increased surface area of nanomaterials can be advantageous for some applications, large surface areas can lead to increased interactions with living tissues, cells, proteins, and nucleic acids, leading to toxic effects in humans (Fischer and Chan 2007; Nohynek et al. 2008). Human exposure to nanomaterials commonly accompanies exposure to other potentially toxic substances such as foods, food additives, cosmetics and pharmaceuticals.

Titanium oxide is used as a pigment in paints, pharmaceutical additives, cosmetics, chemical fibers, and food additives (McIlwee and Alster 2018; Winkler et al. 2018; Barreau et al. 2021). Also, titanium oxide nanoparticles confer excellent UV protection and high transparency (Nohynek et al. 2007). There are three crystal forms of titanium oxide: rutile, anatase, and brookite, and only the rutile and anatase forms are used industrially. Rutile-type titanium oxide has a denser atomic arrangement and more stable physical properties than the anatase-type, so it is used in a wide variety of applications (Racovita 2022).

Abbreviations:
nTR, rutile-type titanium oxide nanoparticles; nTA, anatase-type titanium oxide nanoparticles; PQ, paraquat; CDDP, cisplatin; 5-ASA, 5-aminosalicylic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen

The effects of nanoparticles on living organisms are largely unknown. However, an increasing number of nanotoxicology studies have been undertaken to elucidate the safety, pharmacology and pharmacokinetics of nanoparticles (Vega-Villa et al. 2008; Cai et al. 2020). Silica nanoparticles have been reported to be cytotoxic, hepatotoxic, and placental toxic (Nishimori et al. 2009; Yamashita et al. 2011). Titanium oxide nanoparticles are widely used as food additives. As a result, research on silica particles has progressed and studies have shown that they accumulate in the digestive tract, liver, and kidneys, and they cause homeostatic abnormalities, such as decreased blood glucose levels (Baranowska-Wójcik et al. 2020). Although the use of titanium oxide nanoparticles in industry is regulated, safety guidelines for the application have not yet been established. Furthermore, the pharmacological effects of the interactions between titanium oxide nanoparticles and chemical substances or pharmaceuticals are unknown. Therefore, we examined the effects of coadministration of rutile-type titanium oxide nanoparticles (nTR) or anatase-type oxide nanoparticles (nTA) and the pesticide paraquat (PQ), the anticancer agent cisplatin (CDDP), or the anti-inflammatory agent salicylic acid (5-ASA) in mice, and clarified the extent of liver and renal injury attributable to the interactions among these agents.

2. Investigations, results, and discussion

Single administration of nTA and nTR to mice did not induce acute liver injury or kidney injury (Fig. 1A-C). In our previous studies, we reported that silica or gold nanoparticles interacted with PQ, CDDP, and 5-ASA to induce acute liver and kidney injury (Isoda et al. 2013, 2020). Therefore, we investigated whether acute liver and kidney injury was caused by the interaction of nTA and nTR with PQ or CDDP or 5-ASA.

To prevent interaction between the drug and titanium oxide nanoparticles before administration and absorption, the drug was injected intraperitoneally and the titanium oxide nanoparticles were injected intravenously. PQ is widely used around the world

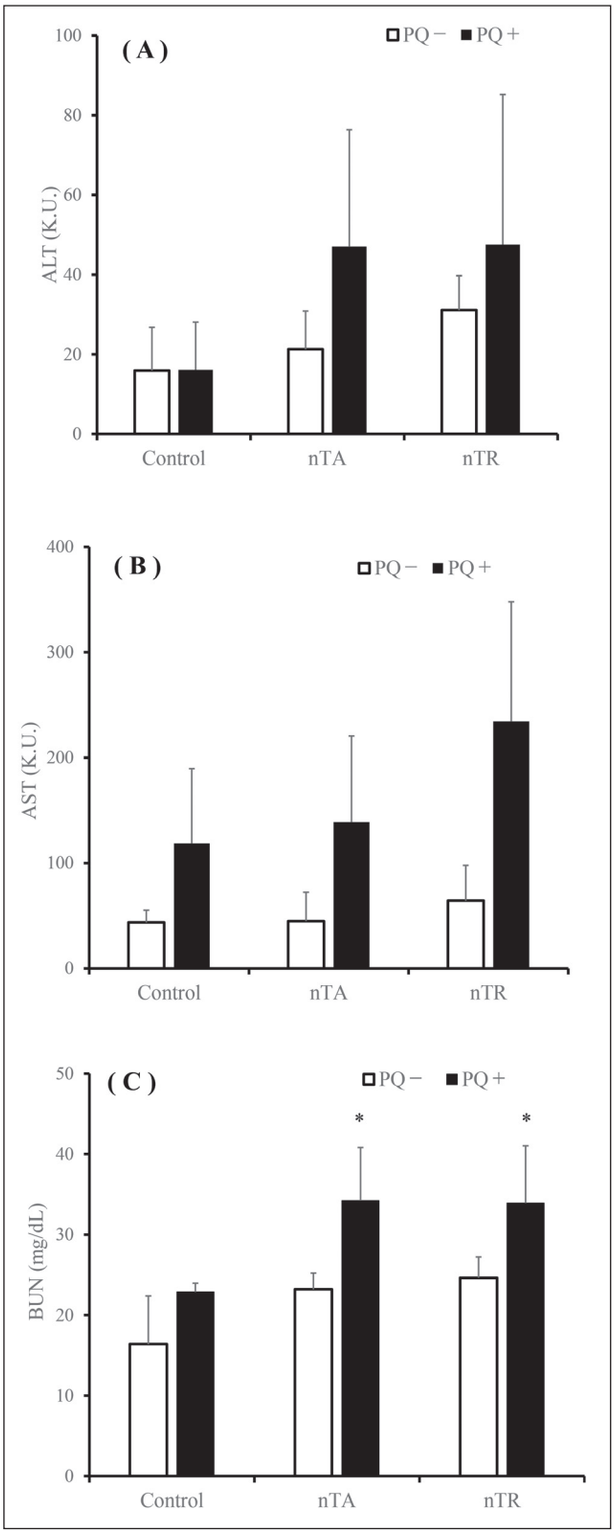


Fig. 1: Effect of nTA or nTR on paraquat-induced toxicity. Mice were injected intraperitoneally with 0 mg/kg (open column) or 50 mg/kg (solid column) paraquat together with titanium oxide nanoparticles (nTA; anatase-type titanium oxide nanoparticles, nTR; rutile-type titanium oxide nanoparticles), which were injected intravenously at a dose of 4 mg/kg. At 24 h post-injection, serum was recovered and ALT (A), AST (B) and BUN (C) levels were assayed as described in the experimental. Data are representative of three independent experiments. Data reflect the mean±SEM (n = 4). *Significant difference between vehicle and paraquat-treated group (*$p < 0.05$).

as a herbicide (Ma et al. 2018). Mice were administered PQ (50 mg/kg) at a dose that did not induce kidney injury (Fig. 1). No significant increase in ALT or AST was observed in the co-administration group of nTA or nTR and PQ, and no liver injury was induced (Fig. 1A, B). However, co-administration of nTA or nTR with PQ increased blood urea nitrogen (BUN) levels and induced acute kidney injury (Fig. 1C).

Next, we investigated the interaction between cisplatin and titanium oxide nanoparticles. Administration of cisplatin has been shown to cause side effects such as kidney and hepatic failure in mammals (Lu and Cederbaum 2006). Co-administration of a non-inducing dose of CDDP (80 μmol/kg) with nTA or nTR did not increase serum ALT and AST levels (Fig. 2A, B). However, serum BUN levels were synergistically elevated in the same mice, increasing from 31.7 to 59.6 mg/dL after co-administration with nTA and from 25.4 to 67.8 mg/dL after co-administration with nTR (Fig. 2C). Thus, nTA and nTR induced acute kidney injury by interacting with CDDP.

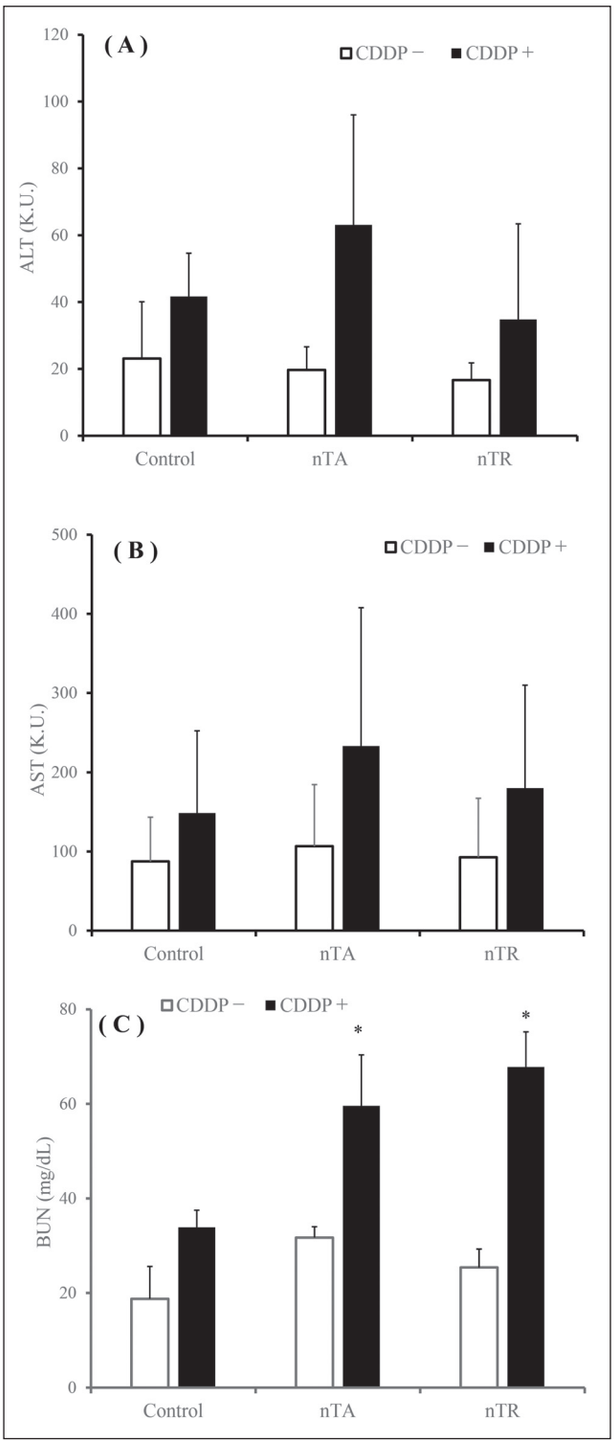
We also investigated the interaction between 5-ASA and titanium oxide nanoparticles. Administration of 5-ASA can cause side effects, such as nephritis and liver failure (Deltenre et al. 1999). Co-administration of 5-ASA (500 mg/kg) with nTA or nTR did not increase serum ALT and AST levels (Fig. 3A, B). However, the same mice showed synergistic elevations in BUN levels from 19.4 to 43.7 mg/dL and from 25.9 to 44.1 mg/dL after administration of nTA and nTR, respectively (Fig. 3C). nTA and nTR were therefore considered to induce acute kidney injury by interacting with 5-ASA.

The findings showed that co-administration of nTA and nTR with PQ or CDDP or 5-ASA induced acute kidney injury, but not liver injury.

Regarding the single administration of titanium oxide nanoparticles, no acute toxicity was observed after either intravenous administration or oral administration (data not shown). Titanium oxide nanoparticles have been reported to accumulate in the liver, heart, spleen, and kidney of mice after tail vein administration (Xu et al. 2013). We performed the same experiment more than once, but we could not observe a significant increase in ALT and AST upon co-administration of titanium oxide nanoparticles with PQ, CDDP, or 5-ASA. In addition, we performed a dose-response test in advance and co-administered titanium oxide nanoparticles at 4 mg/kg, which does not cause liver damage. No liver injury was observed in this study despite exposure of the liver to titanium oxide nanoparticles. Furthermore, no liver injury due to interactions with PQ, CDDP, and 5-ASA were observed in this study. These findings show that titanium oxide nanoparticles do not appear to cause liver damage.

It has been reported that the main exposure to titanium oxide nanoparticles may occur through inhalation and skin contact (Shi et al. 2013). In addition, it has been reported that intravenous administration is the most likely method for medical treatment (Shi et al. 2013). Therefore, in this study, we investigated the hepatotoxicity and nephrotoxicity of titanium oxide nanoparticles administered alone through the tail vein of mice (data not shown). As a result, the dose for interaction co-administration was set at 4 mg/kg, which did not induce hepatotoxicity or nephrotoxicity when administered through the tail vein of mice.

Drug interactions caused acute kidney injury when PQ or CDDP or 5-ASA was co-administered with titanium oxide nanoparticles. CDDP and PQ are mainly excreted in the urine by the kidney (Kawase et al. 1984; Safirstein et al. 1986). On the other hand, 5-ASA is metabolized into a stable acetyl form (N-acetylmesalazine) *in vivo* by N-acetyltransferase in the liver and intestinal mucosa. After metabolism, 5-ASA is partially absorbed and excreted in the urine, but mostly excreted in the feces (Matthis et al. 2016). Although PQ, CDDP and 5-ASA have different excretion routes, titanium oxide nanoparticles and acute kidney injury were observed in this study. It has also been reported that titanium oxide nanoparticles accumulate in the kidney (Valentini et al. 2019). It is therefore considered that the cause of acute kidney injury by titanium oxide nanoparticles is due to excretion failure



Fig, 2: Effect of nTA or nTR on cisplatin-induced toxicity. Mice were injected intraperitoneally with 0 (open column) or 80 µmol/kg (solid column) of cisplatin (CDDP) together with titanium oxide nanoparticles (nTA; anatase-type titanium oxide nanoparticles, nTR; rutile-type titanium oxide nanoparticles) injected intravenously at a dose of 4 mg/kg. At 24 h post-injection, serum was recovered and ALT (A), AST (B) and (BUN) (C) levels were assayed as described in the experimental. Data are means±SEM (n = 4). *Significant difference between vehicle and cisplatin-treated group (*p < 0.05).

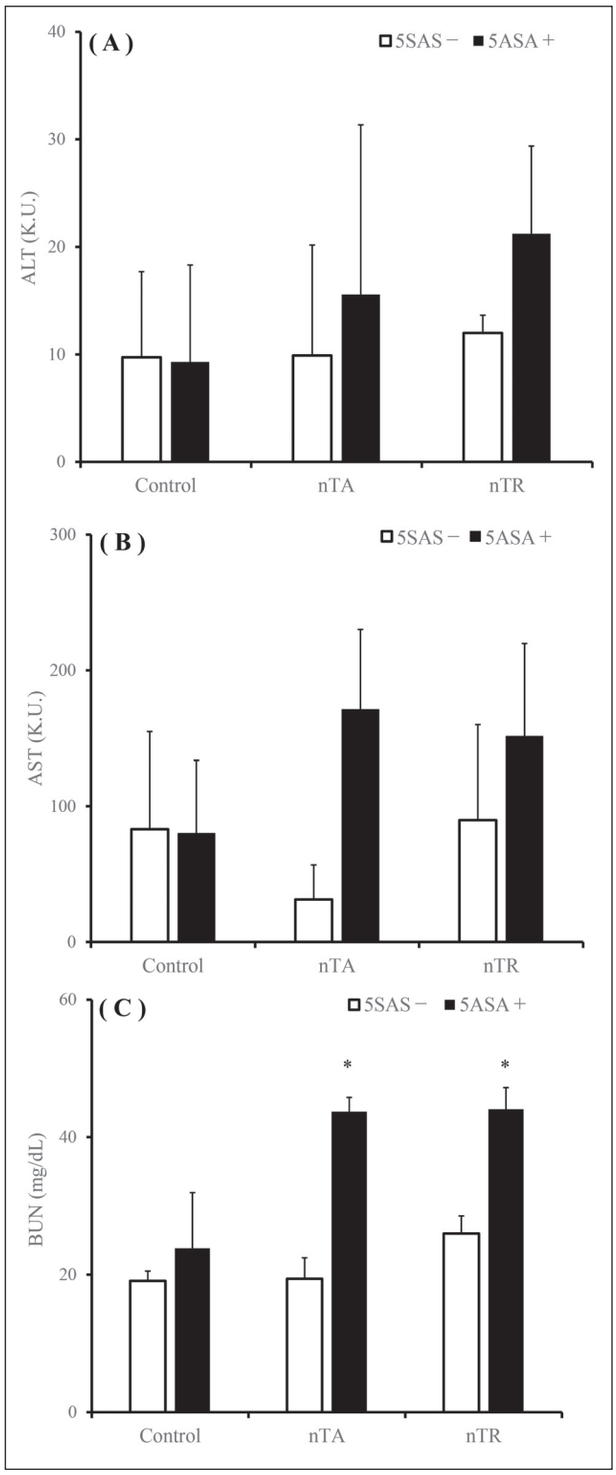


Fig. 3: Effect of nTA or nTR on 5-ASA-induced toxicity. Mice were injected with 0 mg/kg (open column) or 500 mg/kg (solid column) 5-ASA and with titanium oxide nanoparticles (nTA; anatase-type titanium oxide nanoparticles, nTR; rutile-type titanium oxide nanoparticles) at doses of 4 mg/kg, intraperitoneally and intravenously, respectively. At 24 h post-injection, serum was recovered and ALT (A), AST (B) and BUN (C) levels were assayed as described in the experimental. Data are mean±SEM (n = 4). *Significant difference between vehicle and cisplatin-treated group (*p < 0.05).

caused by accumulation in the kidney. PQ, CDDP and 5-ASA have been reported to induce acute kidney injury when administered in excess (Kawase et al. 1984; Safirstein et al. 1986; Moss et al. 2022). Titanium oxide nanoparticles have not been reported to induce acute kidney injury. This suggests that the acute kidney injury side effects of PQ, CDDP, and 5-ASA were influenced by titanium oxide nanoparticles.

In this study, we were unable to clarify the underlying causes of acute kidney injury. Consequently, future studies will examine the accumulation of titanium oxide nanoparticles in the kidney.

3. Experimental

3.1. Materials

nTA with a diameter of 25 nm and nTR with a diameter of 100 nm were obtained from Sigma-Aldrich Co. (St. Louis, USA). The size distribution of the nTA was 31.1±18.7 nm, and the size distribution of the nTR was 95.3±32.7 nm. The particles were platy and nonporous, and stored as 10 mg/mL aqueous suspensions. The suspensions were thoroughly dispersed by sonication before use and were diluted with water. An equal volume of suspension was injected in each treatment. PQ, CDDP and 5-ASA were dissolved in saline and stored at -20 °C before use. All reagents 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 one week to acclimatize before commencing the experiments. The experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences, Teikyo Heisei University, Japan.

3.3. Biochemical analysis

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were measured using commercially available kits (Wako Pure Chemical Industries, Japan) according to the manufacturer's instructions. Briefly, collected serum (0.1 mL) was combined with 1 mL of color A reagent (including urease) and incubated at 37 °C for 15 min. Following the addition of 1 mL of color B reagent, the sample was incubated at 37 °C for 10 min. Absorbance was measured at a wavelength of 570 nm. BUN was measured using a commercially available kit (Arbor Assays, Inc., USA,) according to the manufacturer's instructions. Absorbance was measured at a wavelength of 450 nm.

3.4. Statistical analysis

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

Conflicts of interest: None declared.

Availability of data and materials: All data are fully available without restriction.

Authors' contributions: I.K., K.H., N.M. and S.H. designed the study and wrote the Discussion. K.I. and K.H. performed the experiments. K.I. and M.O. wrote the manuscript. All authors read and approved the final manuscript.

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