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Letter

Impairment of fertilization efficiency in mice following nano-sized titanium exposure

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ABSTRACT — Titanium dioxide nanoparticles (TiNP) are widely used commercially and exist in a broad range of applications and consumer products such as exterior wall paints, antibacterial agents, white pigments, and sunscreens. We previously reported that the testis is a fragile organ against titanium toxicity as compared to the liver; TiNP has been shown to decrease both the sperm motility and the sperm numbers, that is, TiNP quantitatively and qualitatively change the sperm functions. There are, however, few reports regarding to the influence of TiNP on fertility ability. In this paper, we evaluated the influence of TiNP on fertilization rate using *in vitro* fertility (IVF) assay. Male C57BL/6J mice were administered orally with TiNPs (10 mg/kg or 100 mg/kg). Mice were sacrificed 24 hr after the administration. As a result, TiNP (10 mg/kg group) significantly decreased the fertilization rate. In the higher dose group (100 mg/kg), the degree was weaker than in the lower dose group. Our results indicate that TiNP reduces not only the sperm motility but also the fertility, and it will be useful information in considering the influence of TiNP on next generation.

Key words: Titanium dioxide nanoparticles, Testicular function, *In vitro* fertilization, IVF, CASA, Computer-assisted sperm analysis

INTRODUCTION

Titanium dioxide nanoparticle (TiNP) is one of the most common materials used in various products such as exterior wall paints, antibacterial agents, white pigments, sunscreens (Dastjerdi and Montazer, 2010; Song *et al.*, 2016; Tsuji *et al.*, 2006). Compared to traditionally used titanium fine particles, TiNP have a larger ratio of surface area to volume; hence, TiNP may pose a potential health risk to humans. With the rapid progression of nanotechnology, the attention concerning the hazardous effects of TiNP on health is increasing in scientific society and the general public (Bouwmeester *et al.*, 2009). To date, many studies have reported the TiNP-induced toxicity to the liver (Hong and Zhang, 2016), the lung (Wang and Fan, 2014), and the intestine (Nogueira *et al.*, 2012), in addi-

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tion to the central nervous system (Czajka *et al.*, 2015).

We previously reported that the testis is a fragile organ against titanium toxicity as compared to the liver, and thought that the male reproductive system is one of the health risk target by TiNP (Miura *et al.*, 2017). TiNP has been shown to decrease both the sperm motility and the sperm numbers (Miura *et al.*, 2014; Miura *et al.*, 2017), that is, TiNP quantitatively and qualitatively change the sperm characteristics. How about sperm function, especially fertilization ability? As far as we conducted a literature survey, there are few reports regarding to the influence of TiNP on fertility. There is a report that investigated the influence of TiNP on reproductive function of female mice; orally administered TiNP to female mice reduced in mice fertility potential with disturbance in the organization of the ovarian cells ultrastructure and hormonal imbalance (Karimipour *et al.*, 2018). Thus, we evaluated the influence of TiNP on fertilization ability using *in vitro* fertility (IVF) assay.

MATERIALS AND METHODS

Preparation of TiNP suspension

TiNP suspension was prepared as previously described (Miura *et al.*, 2017). Briefly, the titanium dioxide (Aeroxide-P25) purchased from Sigma-Aldrich (St. Louis, MO, USA) was sterilized, suspended in 2 mg/mL disodium phosphate (DSP) to make the concentration of 10 mg/mL, and sonicated. The Z-average of the TiNP was about 150 d.m. (Miura *et al.*, 2017).

Animals and treatments

Eight weeks old male C57BL/6J mice (n = 3) purchased from Clea Japan (Tokyo, Japan) were used. In order to evaluate IVF rate, mice were injected orally with 20 mg/kg or 100 mg/kg TiNP (0.1 mL/20 g body weight). Control mice were administered with DSP (0.1 mL/20 g body weight). Twenty-four hr after the administration, these mice were sacrificed under carbon dioxide anesthesia followed by immediate separating the right cauda epididymides. Epididymides spermatozoa were released into 100 μL FERTIUP medium (Kyudo company, Saga, Japan) and capacitated by incubation at 37°C in 5% CO_2 incubator for at least 1 hr. Mineral oil was used to cover droplets. To measure the sperm motility abilities, a part of spermatozoa after 5 min of release into FERTIUP medium were collected and analyzed using the system of computer-assisted sperm analysis (CASA) using HTM-IVOS (Miura *et al.*, 2017).

The animal experiment was carried out in strict accordance to the recommendations in the guidelines for the care and use of laboratory animals set forth by the Institutional Animal Care and Use Committee at the National Institute of Occupational Safety and Health, Japan (JNIOSH).

IVF assays

To collect mature oocytes from oviducts, virgin females (3-4 weeks old) were super-ovulated by intraperitoneal (i.p.) injection with 0.2 mL HyperOva (Kyudo company, Saga, Japan), and 48 hr later, injected (i.p.) by 7.5 IU (0.2 mL) human chorionic gonadotropin (hCG). Approximately 14 hr after the hCG injection, female mice were anesthetized with carbon dioxide and euthanized by cervical dislocation. The cumulus-oocyte complexes were collected from both ampulla portions of the oviducts under a stereoscopic microscope and transferred to a petri dish with a drop of 200 μL CARD medium (Kyudo company, Saga, Japan). Mineral oil was used to cover droplets. Then 5 μL of sperm suspension (6×10^6 spermatozoa/mL) were added to the CARD medium containing oocytes (final 30,000 spermatozoa/CARD medium). After co-incubation of both gametes for 3 hr in CARD medium, the cumulus-free oocytes were rinsed three times in human tubular fluid (HTF) medium-based CARD mHTF (80 μL; Kyudo company, Saga, Japan). Twenty-four hr after the insemination, 2-cell stage embryos were observed and counted under inverted microscope.

RESULTS AND DISCUSSION

We have found that TiNP possesses acute testicular damage which occurs only 24 hr after the administration of TiNP (in submitted). Therefore, we examined the effect of TiNP administration on fertilization rate 24 hr after the injection. We observed no noteworthy influence of TiNP injection on body weights and organ weights of the testes, the epididymis, and the cauda epididymis (data not shown). The sperm motility was clearly decreased by TiNP administration (the value of control was 77%, whereas in the groups treated with 10 mg/kg or 100 mg/kg, these values were 44% and 48%, respectively). These results were, however, obtained from only one mouse per group, because it was technically difficult to measure the sperm motility while conducting the IVF assay. Although these values had not been statistically analyzed, based on past experimental results, we have confirmed the reproducibility of decreased sperm motility 24 hr after TiNP administration.

As a result, TiNP (10 mg/kg injected group) significantly decreased the fertilization rate (Fig. 1). We also observed a decrease in the rate in the higher dose group (100 mg/kg), but the degree was weaker than in the lower

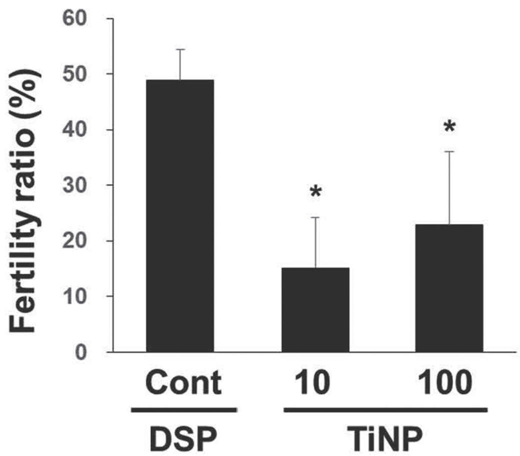

Fig. 1. Inhibition of fertility ability by TiNP administration. Mice were received an orally administration of TiNP (10 mg/kg or 100 mg/kg) followed by sacrificed at 24 hr after the administration. Spermatozoa obtained from the right cauda epididymidis were co-incubated with oocytes in CARD medium. Twenty-four hr after the insemination, 2-cell stage embryos were observed and counted under inverted microscope.

dose group. Our data clearly shows that TiNP possesses the inhibitory effect on the fertility, in addition to reducing the sperm motility.

At higher TiNP dose, the capillary vessels (and/or blood-epididymis barrier (Hoffer and Hinton, 1984; Smith *et al.*, 2015)) may become blocked owing to extensive aggregation, conversely, in the lower dose (10 mg/kg) group, vessels and/or barrier might be less likely to become blocked. For this reason, we think the possibility that the lower dose group exerts a strong suppression effect.

There is only one report that investigated the influence of TiNP on the mouse female reproductive function. In this paper, TiNP administered orally to female mice showed clear decline in IVF rates with adverse effects on the histological alterations of ovary, estrogen hormone levels, malondyaldehyde concentration and pregnancy (Karimipour *et al.*, 2018). To the best of our knowledge, there is no report investigating the influence of TiNP on the reproductive function of male mice. Therefore, our results newly present the adverse effects of TiNP on murine male reproductive system.

In previous our work, there were no change in plasma levels of sex hormones (testosterone, luteinizing hormone, follicle stimulating hormone, and gonadotropin releasing hormone) related to spermatogenesis after administration of TiNP once per week for 4 consecutive weeks (Miura *et al.*, 2017). We did not measure the levels of these hormones 24 hr after the TiNP injection, it is necessary to measure these hormone levels in the future.

Our results indicate that TiNP reduces not only the sperm motility but also the fertility, and it will be useful information in considering the influence of TiNP on next generation.

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

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