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Deleterious effects in reproduction and developmental immunity elicited by pulmonary iron oxide nanoparticles

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ABSTRACT

With the extensive application of iron oxide nanoparticles (FeNPs), attention about their potential risks to human health is also rapidly raising, particularly in sensitive subgroups such as pregnant women and babies. In this study, we a single instilled intratracheally FeNPs (1, 2, and 4 mg/kg) to the male and female parent mice, mated, then assessed reproductive toxicity according to the modified OECD TG 421. During the pre-mating period (14 days), two female parent mice died at 4 mg/kg dose, and the body weight gain dose-dependently decreased in male and female parent mice exposed to FeNPs. Additionally, iron accumulation and the enhanced expression of MHC class II molecules were observed in the ovary and the testis of parent mice exposed to the highest dose of FeNPs, and the total sex ratio (male/female) of the offspring mice increased in the groups exposed to FeNPs. Following, we a single instilled intratracheally to their offspring mice with the same doses and evaluated the immunotoxic response on day 28. The increased mortality and significant hematological- and biochemical- changes were observed in offspring mice exposed at 4 mg/kg dose, especially in female mice. More interestingly, balance of the immune response was shifted to a different direction in male and female offspring mice. Taken together, we conclude that the NOAEL for reproductive and developmental toxicity of FeNPs may be lower than 2 mg/kg, and that female mice may show more sensitive response to FeNPs exposure than male mice. Furthermore, we suggest that further studies are necessary to identify causes of both the alteration in sex ratio of offspring mice and different immune response in male and female offspring mice.

1. Introduction

Iron, a representative particulate material-bound heavy metal, has been suggested as an important cause that provokes the respiratory symptoms by forming reactive oxygen species (ROS) when we inhaled ambient particles (Aust et al., 2002; Jacobs et al., 2012; Wang et al., 2014). Meanwhile, iron oxide nanoparticles (FeNPs) have been widely studied with the great potential for revolutionizing applications, such as drug delivery, magnetic resonance imaging agents, soil and groundwater remediation, and as photocatalysts (Liu, 2006; Penn et al., 2003). Therefore, it is anticipated that the level in the environment and human exposure to FeNPs may notably increase over the coming decade, enhancing the potential risks to human health, particularly in sensitive subgroups such as pregnant women and babies.

Nanoparticles (NPs) can easily penetrate through biological membranes due to their small size (Chu et al., 2010; Guarnieri et al., 2014; Lee et al., 2013; Schädlich et al., 2012; Wang et al., 2013). NPs also have an increased surface area ratio to mass compared to the micro-sized particles of the same substance, thus their chemical/catalytic reactivity are markedly enhanced. For example, carboxyl-coated FeNPs (10, 20, 30, and 40 nm) is primarily distributed in the liver and the spleen, the smallest size (10 nm) penetrated more readily into the brain and the uterus than other sizes, and smaller FeNPs (10 and 20 nm) effectively altered the expression level of oxidative stress-, iron transport-, metabolism-, and apoptosis-related genes (Yang et al., 2015). In addition, growing evidences suggest that exposure to harmful environmental particles during pregnancy period can cause adverse health effects on the offspring (Liu et al., 2007; Srám et al., 1999; Yokota et al., 2013). Therefore, reproductive and developmental toxicity has been recently raised as an important issue among the concerns about the

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potential adverse health effects following exposure to NPs (Campagnolo et al, 2012; El-Sayed et al, 2015; Hougaard et al., 2015; Kadar et al., 2013). However, only very little information is still available about them, especially in mammals (Ema et al., 2010).

FeNPs induced programmed cell death by generating oxidative stress in immune cells (Couto et al., 2014; Park et al., 2015) and altered immune homeostasis in body remaining in the body for a long time (Ban et al., 2013; Park et al., 2010). Additionally, occurrence of hyperresponsiveness disease in early-life following exposure to environmental risk factors increased rapidly with industrial development and has been considered as a major health problem worldwide (Caminati et al., 2015; Huang et al., 2015; Miller and Peden, 2014; Wegienka et al., 2015). In our previous study, we found that pulmonary FeNPs (0.5, 1, and 2 mg/kg) induces Th1-type immune response, stimulating function of antigen presenting cells on day 90 after a single intratracheal instillation (Park et al., 2015). In this study, we aimed to identify reproductive toxicity and developmental immunotoxicity following consecutive pulmonary exposure of FeNPs to the parent and their offspring mice. Soluble iron of 20 mg/kg dose is toxic for humans (Velez and Delaney, 2006). Therefore, we a single instilled intratracheally FeNPs (1, 2 and 4 mg/kg) to the parent mice and performed reproductive toxicity according to the modified Organization for Economic Co-operation and Development (OECD) TG 421. Additionally, we a single instilled intratracheally the same dose of FeNPs to their offspring mice (5 weeks-old) and observed the immunotoxic response on day 28.

2. Material and methods

2.1. Preparation of FeNPs

As reported previously (Park et al., 2014, 2015), FeNPs was prepared by the well-known reduction method, which was suggested by Kang et al. (1996). The synthesis process of FeNPs is follows; $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ were dissolved in HCl solution (0.04 M), and then Fe precursor solution was added to NaOH solution by drop-wise method (1.5 M). After the first drop of Fe solution, the color of mixture was gradually changed from brown to black. The mixture was left for 5 min after addition of NaOH solution. The precipitated FeNPs was separated by centrifugation (13,000 rpm), and washed 3 times with deionized water (DW). The resulting FeNPs was re-dispersed in DW, and then HCl solution (0.01 M) was added in FeNPs solution. The prepared was heated at boiling temperature and HCl solution (1 M) was added for 3 min. The solution was cooled down to room temperature (RT), and the rod-shaped FeNPs, known as FeOOH (iron oxyhydroxyde) NPs, was finally obtained (Fig. 1). Hydrodynamic diameter of FeNPs in phosphate-buffered saline (PBS), a vehicle used for dosing, and Gamble's solution, an artificial lung fluid, were 209.4 ± 98.0 and 45.1 ± 2.6 nm, respectively, and their surface charges were 11.9 ± 2.6 and −219.1 ± 14.0, respectively.

2.2. Animal care and FeNPs treatment

ICR mice (6-weeks) were obtained from Orient Bio Inc. (Gyeonggi-do, Korea) and acclimated for 1 week in our specific-pathogen-free facility (23 ± 3 °C, relative humidity of 50 ± 10%, 12 h light/dark cycle, and ventilation of 10–20 times/h). The parent mice (10 mice/dose/sex) and their offspring mice (5-weeks, female (21–24 g) and male (27–29 g), 12 mice/dose/sex) were a single exposed intratracheally to FeNPs (1, 2 and 4 mg/kg, Fig. 2), and the control group was instilled with a vehicle which was made by performing the same steps without the iron compounds. The experiment (IACUC No. 2014-0037) was assessed by the Institutional Animal Care and Committee (IACUC) of Ajou University (Suwon, Korea) and performed in accordance with the "Guide for the Care and Use of Laboratory Animals", an Institute for Laboratory Animal Research publication. Changes in body weight of parent and offspring mice following exposure to FeNPs were checked one time per week, and development of offspring mice following exposure to the parent mice was evaluated on 6–12 h, day 14 and day 21 after birth.

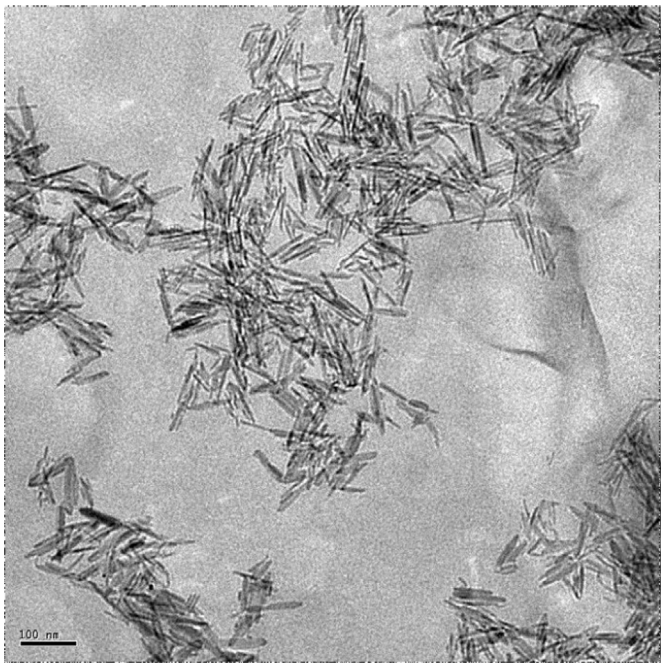

Fig. 1. TEM image of FeNPs.

2.3. Blood analysis

Blood was obtained via postcaval vein and a part of whole blood was centrifuged at 3000 rpm for 10 min to obtain serum for biochemical analysis. Hematological and biochemical analysis were performed using a blood autoanalyzer (HemaVet850, CDC Technologies, Inc., Dayton, Ohio, USA) and chemistry analyzer (BS-400, Mindray, Shenzhen, China), respectively, in Neodin Veterinary Science Institute (Seoul, Korea).

2.4. Histopathological analysis

The tissues were obtained from the parent (ovaries and testes, 5 samples/group) and offspring (lungs, 4 samples/group) mice under carbon dioxide gas anesthesia. The ovaries and lungs and the testes were fixed in 10% neutral buffered formalin and Bouin's solution, respectively. All the process for the evaluation of histopathological lesion was performed according to the standard operating procedures of the Korea Institute of Toxicology (Daejeon, Korea), a good laboratory practice institute in Korea.

2.5. Microarray and ingenuity pathway analysis

To investigate effects of the male parent mice to reproductive toxicity, the testes were obtained from the male parent mice of the control and the highest dose of group after mating, and microarray analysis (Macrogen Inc., Seoul, Korea) was performed using Illumina MouseRef-8 v2 Expression BeadChip (Illumina, Inc., San Diego, CA, USA) as previously described method (Park et al., 2014). Finally, ingenuity pathway analysis (IPA) was performed using microarray data (Macrogen Inc., Seoul, Korea).

2.6. Iron staining of tissue sections

The ovaries and the testes from the parent mice were embedded in