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Chronic exposure to Cerium Oxide Nanoparticles Induce Oxidative Stress in *Balb/c* Mice

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Abstract

Significant amounts of ceria nanoparticles (CeO_2 NPs) are released into the environment, whereas their effects on living organisms are not clearly established. We investigated the long-term toxicity of CeO_2 NPs to *Balb/c* Mice. Animals were exposed to 0, 100, 200, 400 and 800 mg/kg BW by intragastric administration (IGA) for 9 weeks. Weight was weekly recorded, whereas superoxide dismutase (SOD) activity and the levels of reduced glutathione (GSH) and malondialdehyde (MDA) were measured in livers and kidneys after exposure period. Although the weight of mice was not significantly affected, we observed a depletion of SOD and GSH contents at 400 and 800 mg/kg BW in the liver and kidney. Conversely, we noticed a substantial increase in MDA level in the liver at 400 and 800 mg/kg BW and in the kidney at 800 mg/kg BW. The results suggested that long-term exposure to CeO_2 NPs induces oxidative stress in mice, and hence highlighted the toxicity of these nanoparticles to mammalians.

1. Introduction

Ceria nanoparticles (CeO_2 NPs) are used for various purposes, including catalyst, oxygen sensors, oxygen permeation membrane systems, fuel cells, and glass-polishing materials, and electrochromic thin-films [1]. Expected release of an important amount of CeO_2 NPs has raised health concerns [2] that the Organization for Economic Cooperation and Development (OECD) urged a rapid assessment of their toxicity [3]. This advice raised much attention of researchers who initiated a vast *ad hoc* campaign.

Results revealed a double face of CeO_2 NPs with regard to toxicity. On one hand, CeO_2 NPs could potentially prevent chronic inflammation and pathologies connected to oxidative stress [4-6]. This finding was confirmed in A549 and HepG2 cell lines shortly exposed to CeO_2 NPs although a long-term exposure of the same cell lines resulted in toxic effects [7]. Antioxidant effects were also noticed in mice shortly exposed to a lower and single dose of CeO_2 NPs [8]. On the other hand, cerium oxide (CeO_2) was reported to cause pulmonary fibrosis in humans [9]. Recent studies highlighted the adverse effects caused by CeO_2 NPs to humans through the generation of reactive oxygen species (ROS) [5,10-14].

Using a single dose, Nemmar *et al*. [15] have recently observed oxidative stress in mice shortly exposed to CeO_2 NPs.

With regard to previous findings, the observed effects of CeO_2 NPs on either cell lines or mice are contradictory, and therefore deserve much attention. It is imperative to mention that most of these results were collected from *in vitro* experiments involving mammalian cells exposed to low and single doses of CeO_2 NPs. In addition, the dose-dependent chronic toxicity of these nanomaterials to mammalians remains unknown. This study aimed at investigating the long-term effects of CeO_2 NPs to mice through the generation of oxidative stress.

2. Methodology

2.1 Chemical preparation

Ceria nanoparticles (<25 nm particle size and 30-50 m^2/g Surface Area) were purchased from Sigma Aldrich (St. Louis, MO, USA). From a stock solution, five solutions (0, 10, 20, 40, 80 mg/mL) were prepared in normal sterilized saline, vortexed, and sonicated for 30 min to prevent particles from adhesion.

2.2 Animals treatment

Balb/c mice of 16-18 g body weight were bought from the Experimental Animal Center of Hubei, and grown according to the company's protocol. Mice were housed in stainless steel cages under controlled environmental conditions (temperature 21 ± 0.8 °C, humidity 50 ± 3% and a 12 h light/dark cycle). Commercial normal diet and water were freely available to animals. After acclimation for 1 week, 30 males were randomly separated into 5 groups of 6 mice each. Animals were then exposed to CeO_2 NPs through intragastric administration (IGA) to set final concentrations at 0, 100, 200, 400, and 800 mg/kg BW. Control group was exposed to normal saline.

2.3 Sample preparation

After 9-week exposure, mice were humanly killed. Livers and kidneys were rapidly collected, weighed, and homogenized in a physiological saline solution to get a 10% homogenate. Homogenates were centrifuged at 3000 rpm for 10 min at 4 ºC (Eppendorf centrifuge 5415 R) and the supernatant was taken for analysis. The analyses were repeated three times and the data averaged.

2.4 Body weight and biochemical analysis

The body weight of mice was weekly recorded. Superoxide dismutase (SOD) activity was determined using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Protein concentration was assayed according to the Bradford method [16]. Absorbance was measured using a microplate reader (BIO-TEC). SOD activity was expressed as units per mg of protein. Reduced glutathione (GSH) content was determined using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Lipid peroxidation was ascertained as the content of malondialdehyde (MDA) generated by the thiobarbituric acid (TBA) reaction. 0.3 mL of sample or phosphate-buffered saline for the blank, 0.5 mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.6% TBA prepared in 10% TCA were homogenized, heated in boiling water for 15 min and rapidly cooled on ice bath for 5 min. The mixture was centrifuged at 8400 rpm for 10 min and absorbance was measured at 450, 532 and 600 nm. MDA content was calculated using the **Eqn.1.**

$$MDA\ (\mu mol/L) = 6.45\ (OD_{532} - OD_{600}) - 0.56\ OD_{450} \qquad \textbf{Eqn. 1}$$

2.5 Numerical and data analysis

Microsoft Office Excel 2016 and Origin Pro 8 software were used for calculation and data analysis, respectively. One-Way Analysis of Variance (ANOVA) was undertaken to statistically analyze the significance between exposed and control groups. P-values less than 0.05 were considered to be significant.

3. Results and discussion

Hazardous health effects of nanoparticles are emerging issues. CeO_2 NPs are widely used [1] and, therefore, concerns about their potential toxicity to humans and environmental impact have increased quickly [3]. Nanoparticles can enter within cells, leading to oxidative stress that can attack almost any component of the cell [17]. Fortunately, cells possess antioxidant mechanisms that limit the harmful effects of oxidative stress. This defense system includes antioxidant molecules, including superoxide dismutase (SOD) and glutathione (GSH) commonly used as biomarkers of oxidative. In addition, animal weight and MDA content are also assessed for the same purpose.

3.1 Body weight

To follow their growth, we weekly recorded the body weight of mice for 9 weeks. As shown in **Figure 1**, there was no significant changes in body weight of experimental groups compared with the control group. This observation is presumably due to the toxicity that may occur at molecular lever prior to organismal one [18].

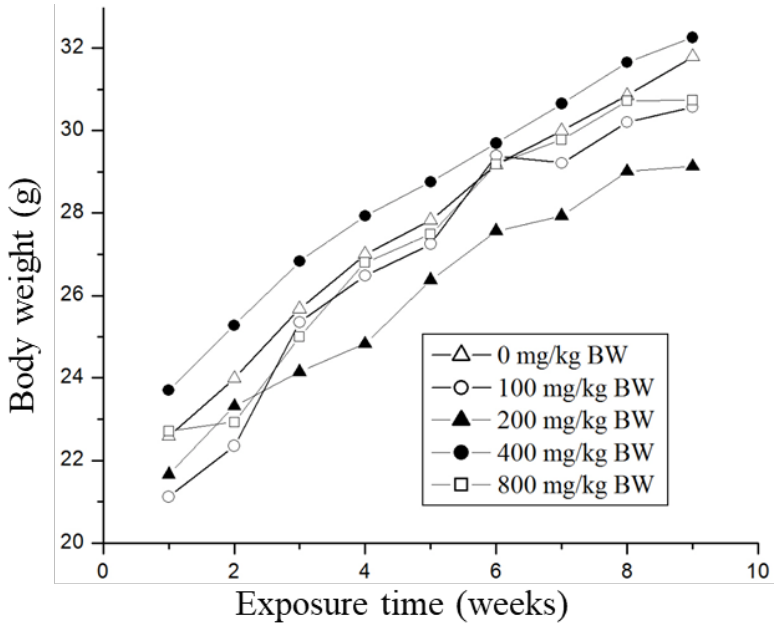

Figure 1. Body weight of *balb/c* mice exposed to CeO_2 NPs

3.2 Superoxide dismutase activity

SOD is a critical enzyme involved in antioxidant defense by breaking down harmful oxygen molecules in cells [18]. After 9 week-exposure, the activity of SOD was assayed to examine the enzymatic defense against oxidative stress in mice exposed to various concentrations of CeO_2 NPs. As shown in **Figure 2**, a light but not significant increase in SOD was observed in both liver and kidney at 100 mg/kg BW. Increased SOD activity indicates the production of ROS [19,20] following the exposure of mice to CeO_2 NPs.

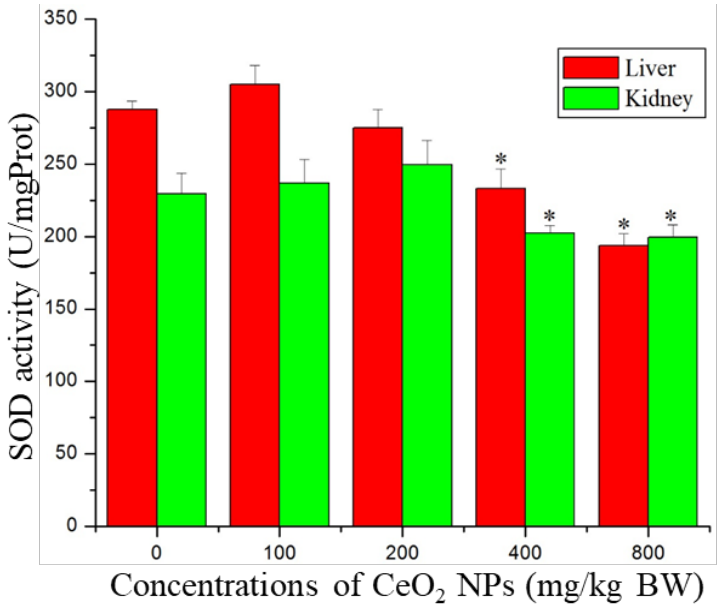

Figure 2. SOD activity in mice exposed to CeO_2 NPs for 9 weeks

The observed increase in SOD activity is most probably due to hormesis that consists in activation of antioxidant systems, including SOD to overcome the ROS excess generated by the pollutant [20]. SOD activity continued to augment in kidney up to 200 mg/kg BW, suggesting that CeO_2 NPs toxicity was more important in liver than in kidney. On the other hand, SOD activity was significantly decreased in liver at 400 mg/kg BW ($p<0.01$) and 800 mg/kg BW ($p<0.01$) in concentration-dependent manner. Similar trend was also observed in kidney at the same concentrations, namely 400 mg/kg BW ($p<0.01$) and 800 mg/kg BW ($p<0.01$). Reduction in SOD activity has been found to be linked to oxidative stress [21]. These observations suggested not only the occurrence of oxidative stress but also the failure of the antioxidant system to fight against increased ROS. Although, this failure did not affect the animal's growth (**Figure 1**), animals could be affected after exposure to CeO_2 NPs for more long period.

3.3 GSH content

Found in all mammalian tissues, GSH is a tripeptide that prevents tissue damage by proving electron in peroxide detoxification. This small molecule protects mitochondria-rich tissues against oxidative stress [22]. It has been clearly established that GSH content decreases when oxidative stress increases. **Figure 3** shows a significant depletion of GSH levels in liver at 400 mg/kg BW ($p<0.01$) and 800 mg/kg BW($p<0.01$). This trend was noticed in kidney at the concentrations of 400 ($p<0.05$) and 800 mg/kg BW ($p<0.01$). We observed a higher amount of GSH in liver where it is mostly produced [23]. The augmentation of GSH suggested an adaptive reaction that prevents the damaging effects of ROS [18]. In accordance with SOD activity, continuous diminution of GSH level confirmed the failure of the antioxidant system to protect the animals. This trend was also observed in kidneys of mice exposed to silver nanoparticles through oral administration [24].

3.4 MDA content

MDA quantification is a common and facile method for the qualification of oxidative stress in biological materials. Increase in MDA level denotes the peroxidation of polyunsaturated fatty acids due to ROS, and that can lead to cell injury [18]. Thus, MDA level was ascertained to test the existence of oxidative stress in mice.

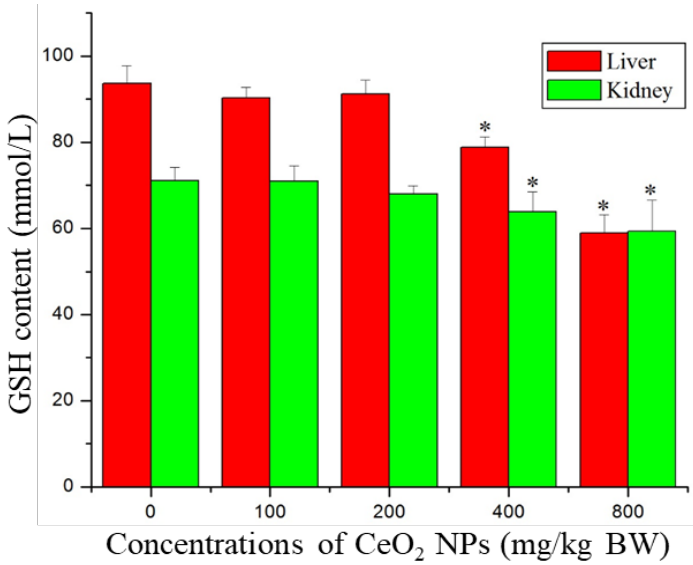

Figure 3. GSH content in mice exposed to CeO_2 NPs for 9 weeks

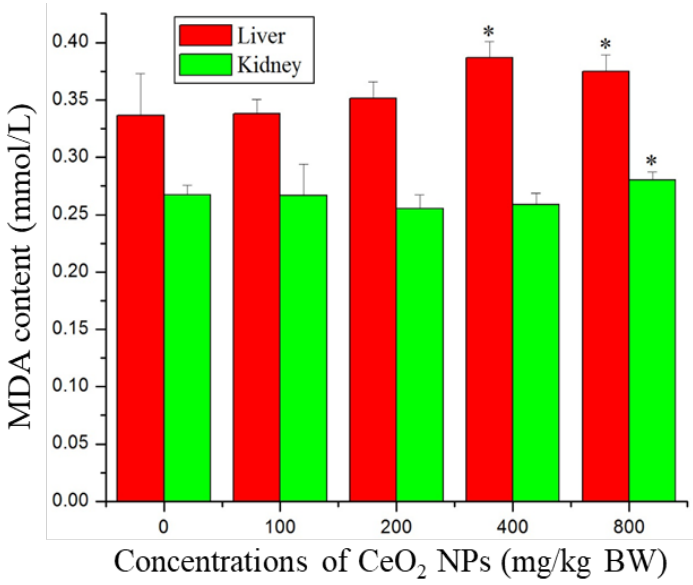

Figure 4. MDA content in mice exposed to CeO_2 NPs for 9 weeks

As depicted in **Figure 4**, MDA levels were significantly increased at 400 mg/kg BW and 800 mg/kg BW, and 800 mg/kg BW ($p<0.01$) in both liver and kidney. Increased levels of MDA have been reported to be related to oxidative stress in plants, humans and model systems [11]. In accordance with SOD activity and GSH content, these results clearly confirmed the occurrence of oxidative stress following the exposure of mice to CeO_2 NPs. Overall, the results suggested the occurrence of oxidative stress in mice exposed to CeO_2 NPs. With regard to the concentrations, the extent to which mice were affected was lower compared with the results reported by Nemmar *et al.* [15]. This discrepancy could be presumably associated with the particle size and the aggregation of CeO_2 NPs after administration.

Conclusion

Mammalians, including humans, are exposed to CeO_2 NPs whose toxic effects are not clearly established. To investigate their toxicity, *Balb/c* mice were exposed to CeO_2 NPs, and changes in biomarkers of oxidative stress were recorded. We observed a significant depletion of SOD and GSH contents in the liver and kidney, and a substantial increase in MDA level in these organs. The results of

this study highlighted the *in vivo* toxicity of CeO_2 NPs to *balb/c* mice, and hence to humans, through the generation of oxidative stress following a long-term exposure. Therefore, people must be cautious during the handling or use of materials containing CeO_2 NPs. In the light of the growing use of CeO_2 NPs, additional investigations involving a very long period of exposure and offspring are highly required.

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