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Oxidative stress in the kidney injury of mice following exposure to lanthanides trichloride

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HIGHLIGHTS

- Exposure to lanthanides impaired renal function in mice.
- Exposure to lanthanides induced cell necrosis in kidney.
- Exposure to lanthanides promoted ROS accumulation in kidney.
- Exposure to lanthanides caused the reduction of antioxidant capacity in kidney.
- The order of kidney damages was Ce exposure > Nd exposure > La exposure.

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ABSTRACT

Environmental pollution from lanthanides (Ln) has been recognized as a major problem due to a grab exploitation of Ln mine in China. Exposure to Ln has been demonstrated to cause the nephrotoxicity, very little is known about the mechanism of oxidative damage to kidney in animals. In order to understand Ln-induced nephrotoxicity, various biochemical and chemical parameters were assayed in mouse kidney. Intragastric exposures of $LaCl_3$, $CeCl_3$, and $NdCl_3$ at doses of 2, 5, and 10 mg kg^{-1} BW for 90 consecutive days caused nephritis or epithelial cell necrosis and oxidative stress to kidney. An increase in coefficients of the kidney, La, Ce, and Nd accumulation and histopathological changes in the kidney could be observed, followed by increased reactive oxygen species production and peroxidation levels of lipid, protein and DNA, and decreased activities of superoxide dismutase, catalase, glutathione-S-transferase and glutathione reductase as well as antioxidants such as glutathione, ascorbic acid and thiol contents. Furthermore, La, Ce, and Nd significantly suppressed expression of genes and proteins of these antioxidative enzymes in mouse kidney. In addition, kidney functions were disrupted, including an increase of the creatinine, and reductions of uric acid, urea nitrogen, calcium and phosphonium. These findings suggest that nephritis generation or epithelial cell necrosis in mice following exposure to Ln is closely associated with oxidative stress. Of these damages, the most severe was in the Ce^{3+}-exposed kidneys, next in the Nd^{3+}-exposed kidneys, and the least in the La^{3+}-exposed kidneys, which may be attributed to the 4f electron of Ln.

1. Introduction

China has the largest deposits of lanthanides (Ln) and supplies >90% of these elements to the rest of the world. As we know, Ln belongs to the IIIB family in the periodic table of elements. Because of their diverse physical, chemical and biological effects, Ln has been used industrially in various areas, such as metallurgy, color TV, lasers, photographic cameras, semiconductors, binoculars and movie films; and also in medicine as anticancer, anti-inflammatory and antiviral agents (Kostova, 2005; Wason and Zhao, 2013). Ln-enriched fertilizers (mainly consisted of lanthanum, cerium, and neodymium nitrates) are known to be able to increase the yields of crops in China (Ni, 2002; Hu et al., 2004). The rapid development and widespread application of novel Ln technologies in the industrialized countries requires additional information on the potential health effects derived from possible exposure to Ln compounds.

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Recently, environmental pollution from Ln has been recognized as a major problem due to a grab exploitation of Ln mine in China (Wu et al., 2003; Tong et al., 2004; Zhao et al., 2008). Ln-related pneumoconiosis in Chinese mineworkers has been reported (Chen et al., 2005). However, the Ln-related health impacts are still not fully understood and there still lacks the systemic and basic information (He and Rambeck, 2000; Yu et al., 2007; He et al., 2010). Numerous studies have definitely showed that Ln exposure are able to cause injuries in various animal organ types, including lung (Li et al. 2010), liver (Cheng et al., 2011, 2012a; Fei et al., 2011a,b; Ze et al., 2011; Li et al., 2013), spleen (Liu et al., 2010; Cheng et al., 2011) and brain (He et al., 2008; Zhao et al., 2011a,b; Cheng et al., 2012b, 2013; Wang et al., 2013). The toxicity of Ln to kidneys has also been reported. Hayashi et al. (2006) and Tanida et al. (2009) had found that scandium and yttrium were accumulated in the kidney, leading to damage of renal functions in rats. We speculated that the Ln-induced renal damage might be associated with oxidative stress of kidney. Kidney is also equipped with an advanced defence system of enzymatic and non-enzymatic antioxidants, which are known as scavengers of reaction oxygen species (ROS). Renal injury following exposure to Ln may occur when the balance between oxidant production and the antioxidant system is altered. In this study we investigated the effect of $LaCl_3$, $CeCl_3$, and $NdCl_3$ on mouse kidney after intragastric administrations for 90 consecutive days; the oxidative injury in the kidney was assessed by histopathological tests, and measuring Ln accumulation and biochemical parameters of renal functions, the production of O_2^- and H_2O_2, and the level of lipid, protein and DNA peroxidation. The intracellular levels of GSH, AsA and thiol, and the activities and expressions of various antioxidant enzymes that are known to regulate cellular oxidative tone are also examined in the mouse kidney.

2. Materials and method

2.1. Reagent

$LaCl_3$, $CeCl_3$, and $NdCl_3$ were purchased from Shanghai Chem. Co. (China) and were analytical-grade.

2.2. Animal and treatment

Four-week-old male ICR mice were purchased from Soochow University Experimental Animal Center. (Suzhou, China). All mice were housed in stainless steel cages in a ventilated animal room. Room temperature of the housing facility was maintained at 24 ± 2 °C with a relative humidity of 60 ± 10% and a 12-h light/dark cycle. Distilled water and sterilized food were available for mice *ad libitum*. Prior to dosing, the mice were acclimated to this environment for 5 d. All procedures used in animal experiments conformed to the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 1996).

150 mice were randomly divided into 10 groups: one normal control group, La-treated, Ce-treated, Nd-treated groups of three gavage doses (2, 5 and 10 mg kg^{-1} body weight [BW], $n = 30$ in each group). Ln chloride solutions were administered by gavage to mice daily for 90 d. And controls were administered with equivalent amount of distilled water. Any symptoms or mortality were observed and recorded carefully everyday during various experimental stages. After 90 d, all mice were weighed firstly, and then sacrificed after being anesthetized using ether. Blood samples were collected from the eye vein by removing the eyeball quickly. Serum was collected by centrifuging blood at 2500 rpm for 10 min. Kidneys were collected and weighed.

2.3. Coefficients of kidney

After weighing the body and tissues, the coefficients of kidney to body weight were calculated as the ratio of tissues (wet weight, mg) to body weight (g).

2.4. Biochemical analysis of renal functions

Renal functions were determined by uric acid (UA), blood urea nitrogen (BUN), creatinine (Cr), calcium (Ca) and phosphonium (P). All biochemical assays were performed using a clinical automatic chemistry analyzer (Type 7170A, Hitachi, Japan).

2.5. Histopathological examination

For the pathologic studies, all histopathologic examinations were performed using standard laboratory procedures. The kidneys were embedded in paraffin blocks, then sliced (5 μm thick) and placed onto glass slides. After hematoxylin-eosin (HE) staining, the stained sections were evaluated by a histopathologist unaware of the treatments, using an optical microscope (Nikon U-III Multipoint Sensor System, Japan).

2.6. Ln content analysis of kidney

The kidneys were thawed and approximately 0.3 g of the kidney was weighed, digested and analyzed for lanthanum, cerium and nedymium contents. Inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Elemental X7, Thermo Electron Co., USA) was used to analyze the lanthanum, cerium and nedymium concentrations in the samples. An Indium concentration of 20 ng mL^{-1} was utilized as an internal standard, and the detection limit of lanthanum, cerium and nedymium was 0.089 ng mL^{-1}. The data were expressed as nanograms per gram fresh tissue.

2.7. Oxidative stress assay of kidney

ROS (O_2^- and H_2O_2) production and levels of malondialdehyde (MDA), protein carbonyl (PC), and 8-hydroxy deoxyguanosine (8-OHdG) in the kidney tissues were assayed using commercial enzyme-linked immunosorbent assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) according to the manufacturer's instructions.

2.8. Assay of antioxidant enzymes and antioxidants of kidney

The kidneys were homogenized in 1 mL of ice-cold 50 mM sodium phosphate (pH 7.0) that contained 1% polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 30,000*g* for 30 min and the supernatant was used for assays of the actives of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and glutathione reductase (GR).

The activity of SOD was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Each 3-mL reaction mixture contained 50 mM sodium phosphate (pH 7.8), 13 μM methionine, 75 μM NBT, 2 μM riboflavin, 100 μM EDTA, and 200 μL of the enzyme extract. Monitoring the increase in absorbance at 560 nm followed the production of blue formazan (Beauchamp and Fridovich, 1971).

The CAT activity was measured by the decrease in the H_2O_2 concentration for 15 s, reading the absorbance at 240 nm on a UV-3010 absorption spectrophotometer according to Claiborne (1985). The reaction volume was 1 mL and contained 500 μL of sample homogenate and 500 μL of sodium phosphate buffer 50 mM, pH 7.0 and 15 mM H_2O_2. The control was assayed without

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