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Metallothionein deficiency aggravates depleted uranium-induced nephrotoxicity

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ABSTRACT

Depleted uranium (DU) has been widely used in both civilian and military activities, and the kidney is the main target organ of DU during acute high-dose exposures. In this study, the nephrotoxicity caused by DU in metallothionein-1/2-null mice (MT −/−) and corresponding wild-type (MT +/+) mice was investigated to determine any associations with MT. Each MT −/− or MT +/+ mouse was pretreated with a single dose of DU (10 mg/kg, intraperitoneal injection) or an equivalent volume of saline. After 4 days of DU administration, kidney changes were assessed. After DU exposure, serum creatinine and serum urea nitrogen in MT −/− mice significantly increased than in MT +/+ mice, with more severe kidney pathological damage. Moreover, catalase and superoxide dismutase (SOD) decreased, and generation of reactive oxygen species and malondialdehyde increased in MT −/− mice. The apoptosis rate in MT −/− mice significantly increased, with a significant increase in both Bax and caspase 3 and a decrease in Bcl-2. Furthermore, sodium-glucose cotransporter (SGLT) and sodium-phosphate cotransporter (NaPi-II) were significantly reduced after DU exposure, and the change of SGLT was more evident in MT −/− mice. Finally, exogenous MT was used to evaluate the correlation between kidney changes induced by DU and MT doses in MT −/− mice. The results showed that, the pathological damage and cell apoptosis decreased, and SOD and SGLT levels increased with increasing dose of MT. In conclusion, MT deficiency aggravated DU-induced nephrotoxicity, and the molecular mechanisms appeared to be related to the increased oxidative stress and apoptosis, and decreased SGLT expression.

Introduction

Depleted uranium (DU), which has a relatively low radiation capacity and chemical properties similar to natural uranium, is a by-product formed by refining natural uranium to enriched uranium (^{235}U) (Bleise et al., 2003). Exposure to DU is primarily from nuclear waste and the manufacture and use of DU weapons. In the process of DU weapon production and use, uranium may enter the body through the respiratory tract, gastrointestinal tract or skin (ATSDR, 2013), after which DU is mainly distributed into the kidneys, bone and liver, posing possible serious threats to human health (Brugge and Buchner, 2011). The kidney is the main target organ of the acute chemical toxicity caused by DU in high-dose exposures, and DU exposure (single intramuscular doses of 0.1, 0.3 or 1.0 mg/kg uranium) has been shown to elicit dose-dependent increases in kidney concentrations of the metal (Jortner, 2008). DU toxicity can result in severe renal tubular necrosis, which can lead to renal failure and death (Vicente-Vicente et al., 2010; Cheng et al., 2010). A common, initial event in the action of all toxic metals seems to be the generation of oxidative stress that is characterized by the following effects: (a) depletion of intracellular antioxidants and free-radical scavengers, (b) inhibition of the activities of various enzymes that contribute to the metabolism and detoxification of reactive oxygen species (ROS), such as glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD), and (c) increased production of ROS (Thiebault et al., 2007).

To date, chelation therapy that uses ligands with strong metal-binding capabilities remains the only way to accelerate the excretion of a poisonous metal. Many chelating agents have been examined in animal studies over the years to determine their efficacies for removing uranium (Fukuda et al., 2008; Zhang et al., 2011; Bao et al., 2013). Catechol-3,6-bis

Abbreviations: Bax, Bcl-2 associated X protein; Bcl-2, B-cell lymphoma/leukemia-2; BUN, blood urea nitrogen; CAT, catalase; CBMIDA, catechol-3,6-bis (methyleiminodiacetic acid); Cr, creatinine; DU, depleted uranium; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; GSH-Px, glutathione peroxidase; H & E, hematoxylin and eosin; NaPi-II, sodium-phosphate cotransporter; PBS, phosphate buffer solution; PI, propidium iodide; MDA, malondialdehyde; MT, metallothionein; ROS, reactive oxygen species; SD, standard deviation; SOD, superoxide dismutase; SGLT, sodium-glucose cotransporter.

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(methyleiminodiacetic acid) (CBMIDA), which may be administered orally, has been shown to reduce the uranium burden without causing renal damage (Fukuda et al., 2009). However, CBMIDA has low gastrointestinal absorption and showed no uranium removal effect when the uranium was dissolved in a solution of pH 7 (Fukuda et al., 2007). In our previous study (Hao et al., 2012), pretreatment with zinc significantly increased the survival rates at 30 days post-DU administration and alleviated acute toxicity of DU in rats, and the induction of metallothionein (MT) by zinc might play an important role in DU detoxification.

MT is a family of low molecular weight, sulphur-containing proteins that is broadly distributed throughout the body. The family consists of four isoforms, MT-1, MT-2, MT-3 and MT-4, and MT-1 and MT-2 are the major contributors in the prevention of heavy metal toxicity (Kang, 2006; Maria et al., 2014; Gu et al., 2014; He et al., 2014). Jiang et al. (2009) has shown that MT exerts significant protective effects in nematodes exposed to DU, demonstrating a new avenue for the prevention and treatment of DU-induced injuries. We also found exogenous MT effectively inhibited DU-induced human kidney cells (HK-2) apoptosis (Hao et al., 2014). Nevertheless, the role and detoxification mechanisms of MT-1 and MT-2 in mice are not well understood.

In the present study, MT-1 and MT-2 double-knockout mice (MT−/−) and wild-type mice (MT+/+) were used to evaluate the relationship between MT and DU toxicity. Exogenous MT was also used to evaluate the correlation between kidney injuries and MT doses. The pathological changes and antioxidant activity were assessed. We also determine whether DU-induced cytotoxicity is mediated partly by the activation of apoptotic pathways, as our previous in vitro studies have suggested (Hao et al., 2014). In addition, studies (Muller et al., 2008; Vicente-Vicente et al., 2010) have reported that alterations in solute transport related to the mechanism involved in uranium nephrotoxicity. Therefore, we hypothesized that DU lead to dysfunction of transporters, and that the presence of MT protects the transporters and thus prevents DU nephrotoxicity. The relationship between MT and transporters was evaluated to explore the protective mechanisms of MT in countering DU toxicity, which would lay the foundation for future studies about the treatment of DU exposure.

Materials and methods

Animals. MT-null mice, which are deficient in MT-1 and MT-2 genes, and homozygous wild-type mice were originally obtained from the Murdoch Institute of the Royal Children's Hospital (Parkville, Australia) and bred as previously reported (Shuai et al., 2007). Male mice aged 6–8 weeks were acclimated to the laboratory for 5 days prior to the experiment, then mice with an approximately equal mean body weight were selected for experiment. Food intake, water intake, body weight, and health status were recorded daily. The study was conducted in accordance with Chinese legislation and "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) regarding the care of animals used for experimental purposes.

Treatments. Both MT+/+ and MT−/− mice were randomly divided into two groups, each consisting of 8 animals, as detailed below. In the DU group, the mice were exposed to uranyl nitrate [the source and constituent of DU was the same as in a previous study (Hao et al., 2009), composed of 99.25% DU and 0.75% titanium by weight, with $^{238}U = 99.75\%$, $^{235}U = 0.20\%$, trace ^{234}U] at a single dose (10 mg/kg body weight, intraperitoneal injection). Tris-maleate buffer pH 7.0 (Sigma-Aldrich, Santa Clara, CA, USA) was used to dissolve uranyl nitrate (weight/volume = 0.1%). The final concentration of uranyl nitrate solution was 1.0 mg/ml, and the volume of injection was 0.25 ml for 25 g mice. The second group, which was the saline group, received an equal volume of saline as the DU group. The animals were killed 4 days after DU or saline injection. Blood and kidney samples were collected and analyzed as described below. In order to further prove the correlation of DU-induced nephrotoxicity and MT, another experiment was conducted. MT−/− mice were administrated with or without exogenous MT (Sigma-Aldrich, Santa Clara, CA, USA) once at three different doses (10, 20, 30 μmol/kg body weight, intraperitoneal injection) 1 h before DU exposure, respectively. The working concentration of MT solution was 1.0 μmol/ml. The high dose of MT (30 μmol/kg) was based on relevant references (Kukner et al., 2007; Helal and Helal, 2009). The control group received an equal volume of saline as the DU group (10 mg/kg) as described above. Therefore, the mice were randomly divided into five groups [control, DU, MT(10) + DU, MT(20) + DU, MT(30) + DU], each consisting of 8 animals. After 4 days of DU exposure, the kidney pathological changes, serum concentrations of creatinine (Cr) and urea nitrogen (BUN), cell apoptosis, the levels of SOD and SGLT were analyzed as described below.

Uranium analyses in kidney tissue. The uranium content in the kidney tissue was determined using an inductively coupled plasma mass spectrometer (ICP-MS, Thermo Finnigan MAT, Bremen, Germany), as described previously (Hao et al., 2013). Values are expressed as ng/g tissue.

MT measurements in kidney tissue. Total kidney tissue MT concentrations were determined by cadmium–hemoglobin affinity assay (Eaton and Cherian, 1991), as described previously (Shuai et al., 2007). In brief, kidney tissues were homogenized in Tris–HCl buffer (50 mM, pH = 8.0), and the supernatant was collected by centrifugation at 18,000 ×*g* for 15 min at 4 °C. The supernatant was determined for the total protein and MT concentrations. The total protein concentrations were measured with a Bradford Protein Assay kit (Beyotime, Haimen, Jiangsu, China). The MT concentrations in the kidney were expressed as mg/g protein.

Biochemical assays. Femoral artery blood samples were obtained while the mice were sedated with xylazine hydrochloride. Serum concentrations of Cr and BUN were measured via an automated Konelab 20 (Thermo Electron Corporation, Cergy-Pontoise, France), as previously reported (Hao et al., 2013).

Histopathology and light microscopy. At the 4th day after the administration of DU, kidneys were dissected and fixed with 4% formaldehyde for 48 h, dehydrated and embedded in paraffin, then sliced into 5 μm sections. Hematoxylin and eosin (H & E, Beyotime) staining were used to observe pathomorphological changes. During the evaluation of tissue damage, vacuolization, necrosis, cast formation in renal proximal and distal tubules and mononuclear cell infiltration in interstitial spaces were scored by an assessment in randomly selected areas. The damage was scored as follows: 0 = none, 1 = 0–10%, 2 = 11–25%, 3 = 26–50%, and 4 = 51–100% (Inal et al., 2014). The scoring of the histological data was blinded and measured by an independent laboratory.

Analysis of malondialdehyde (MDA) and antioxidant enzymes. Kidney tissues were placed in ice-cold PBS and homogenized at 16,000 rpm for 3 min. The homogenates were centrifuged at 1000 *g* for 10 min at 4 °C. The supernatants were stored at −80 °C until analysis. Tissue MDA content was measured using the thibabituric acid method (Yoshioka et al., 1979). The activities of SOD and CAT were measured using the methods of Wang et al. (2008). All of these measurements were performed using commercial kits (Jian Cheng Institute of Bioengineering, Nanjing, China), and absorbance readings were obtained using a microplate Reader (Bio-rad 550, BioRad Laboratories, California, USA).

ROS determination. Kidneys were removed aseptically from euthanized mice of each group ($n = 8$) and single cell suspensions prepared as our previously described (Hao et al., 2012). Intracellular ROS generation was measured by the reactive oxygen species assay kit (Beyotime) according to the manufacturer's instructions. The ROS