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# Hepatic oxidative stress and inflammatory responses with cadmium exposure in male mice

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ABSTRACT

Cadmium (Cd), a non-essential heavy metal, is one of the major environmental contaminants with grave toxicological consequences globally. In the present study, the effects of Cd on hepatic oxidative stress and inflammatory responses in mice were evaluated. Male adult mice were orally exposed to 3, 10 and 30 mg/L $CdCl_2$ supplied in the drinking water for 7 and 21 days. Histopathological changes and the alterations of the main parameters related to oxidative stress and inflammatory responses in the liver were observed. Hepatic malondialdehyde (MDA) contents increased significantly after treatment with 30 mg/L $CdCl_2$ for 21 days, and the contents of glutathione (GSH) increased significantly in both 10 and 30 mg/L $CdCl_2$ treated groups. The hepatic activities of glutathione peroxidase (GPX), catalase (CAT) and glutathione S-transferase (GST) increased significantly after the treatment with 30 mg/L $CdCl_2$ for 21 days. In accordance with the enzyme activities, the transcription status of hepatic *superoxide dismutase 1* (*Sod1*), *superoxide dismutase 2* (*Sod2*), *Cat*, *Gpx*, *Gstα1*, *glutathione synthetase* (*Gss*), *glutathione reductase* (*Gr*) and *heme oxygenase 1* (*Ho1*) were also increased by high dose (30 mg/L) or long period (21 days) exposure. In addition, the serum levels of *tumor necrosis factor α* (TNFα), *interleukin 6* (*IL6*) and *interleukin 1β* (*IL1β*) increased significantly in the groups treated with 30 mg/L $CdCl_2$ for 21 days. And the genes of *TNFα*, *IL6*, *interleukin 1α* (*IL1α*), inducible *nitric oxide synthase* (*iNOS*) and *interferon γ* (*IFNγ*) were also increased in the liver of mice when exposed to relative high dose of $CdCl_2$ for 7 or 21 days. Taken together, the results of this study suggested that the exposure to Cd had the potential to induce immunotoxicity accompanied with oxidative stress in the liver of mice.



## 1. Introduction

Cadmium (Cd), a non-essential heavy metal, is frequently detected in the environment around the world. High concentration of Cd was observed in the aquatic system, soil and sediment in the real environment especially in the developing countries. For example, Smolders et al. (2003) found that the concentrations of Cd even reached at 315 μg/L in unfiltered water and 107 mg/kg in sediment in the different reaches of the Tarapaya River, South America. Liang et al. (2011) reported that the concentration of Cd reached at 194.5 μg/L in the surface water of Linglong Gold Mining area, China. Zhang et al. (2014) reported that the levels of Cd even reached 42.3 mg/kg

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soil of an abandoned e-waste site in China. Nowadays, Cd and other heavy metals are widely known as environmental contaminants which constitute a serious risk for human and wildlife health (Burger, 2008; Waheed et al., 2014).

Recently, oxidative stress and immunotoxicity in animals were considered as two of the most important responses to environmental chemicals (Valko et al., 2006; Ashry et al., 2010; Jin et al., 2012). As the primary organ for detoxification and metabolism of exogenous chemicals, the liver was considered as the most important tissue for detecting oxidative stress (Cichoż-Lach and Michalak, 2014). In fact, according to previous studies, the liver was also known to be the highest depot of heavy metals including Cd in soft tissues followed by the kidney (Mudipalli, 2007; Breton et al., 2013). In addition, the liver was also considered as one of the main tissues for analyzing inflammatory response except the main immune organs (Zwacka et al., 1997; Cui et al., 2011). Recently, some studies indicated that heavy metals, including Cd, have the potential to induce oxidative stress and immunotoxicity in different tested models (Elbekai and El-Kadi, 2005; Pathak and Khandelwal, 2006). For example, Tsangaris and Tzortzatou-Stathopoulou (1998) reported that Cd exposure could induce apoptosis of immune system cell lines of Raji, CCRF-CEM and Molt-3 cells, suggesting that Cd may disturb the normal growth and development of the immune system. However, to date, studies describing the mechanisms of Cd-induced hepatic oxidative damage and inflammatory responses in mammals are limited.

In this study, to investigate the functional changes in the liver following exposure to Cd in greater detail, male mice were orally administered different doses of Cd for 7 or 21 days. Firstly, to evaluate the oxidative stress induced by Cd, the malondialdehyde (MDA) and glutathione (GSH) contents, the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione S-transferase (GST) and the transcriptional levels of the genes related to oxidative stress in the liver were examined. Then, the levels of main cytokines in the serum and the transcriptional levels of the immune system-related genes in the liver were determined after Cd exposure. The information acquired in the present study will be helpful in understanding the environmental risk induced by Cd in ecosystems.

## 2. Materials and methods

### 2.1. Chemical, animals and experimental design

Totally 56 five-week-old ICR male mice were purchased from the China National Laboratory Animal Resource Center (Shanghai, China). The mice were kept in our animal facilities (illuminated with strip lights, 200 lx at cage level with a photoperiod of 12 h light to 12 h dark; $22 \pm 1\,^{\circ}C$) for 1 week prior to the experiments. All the mice were randomly divided into 8 groups ($n = 7$). Differences in body weights were not observed among these groups at the beginning of the experiment. All the groups were fed a basic diet and treated with 3, 10 and 30 mg/L $CdCl_2$ supplied in the drinking water for 7 or 21 days, respectively. Water and food were available *ad libitum* during the experimental period.

After treatment, all the mice were sacrificed after anesthetization with ether on the last day of the treatment. Blood sera were collected, and serum was separated by centrifugation (5000 rpm for 5 min) and stored at $-40\,^{\circ}C$ before use. The livers were quickly removed and weighed. Partial samples of the livers collected from 3 randomly selected mice from each group were fixed directly in a 4% paraformaldehyde solution for histological analysis. Then the liver tissues were immediately frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ until use. Every effort was made to minimize animal suffering in each experiment. All experiments were performed in accordance with the Guiding Principles in the Use of Animals in Toxicology.

### 2.2. Histopathological analysis

Liver tissues fixed in 4% paraformaldehyde solution were processed sequentially in ethanol, xylene and paraffin. Then, the tissues were embedded in paraffin wax, sectioned (6 μm) using a Leica RM2235 (Germany) and mounted on slides. The sections were stained with hematoxylin and eosin before examination with a microscope (Olympus, Japan). And the nucleoplasmic ratio was analyzed by the software of Image-pro plus 6.0.

### 2.3. Determination of the contents of MDA, GSH and the activities of SOD, CAT, GPX and GST in the livers

The livers were defrosted and homogenized with 10 volumes of cold buffer consisting of 250 mmol/L sucrose, 5 mmol/L Tris–Hcl and 0.1 mmol/L EDTA-2Na (pH 7.5). The homogenate was centrifuged at $4000 \times g$ at $4\,^{\circ}C$ for 15 min to obtain the supernatant for the further assays. The contents of GSH, MDA and the activities of SOD, GPX, CAT and GST in the supernatant and serum were determined using kits purchased from the Nanjing Jianchen Institute of Biotechnology (Nanjing, China) according to the manufacturer's instructions. The protein concentrations were determined using bicinchoninic acid (BCA) as a detecting reagent for $Cu^{+}$ following the reduction of $Cu^{2+}$ by protein in an alkaline environment (BCA protein kit, Sangon Company, China). Measurements were obtained with a microplate reader (Power wave XS, Bio-TEK, USA) according to the manufacturer's instructions.

### 2.4. Gene expression analysis

Total RNA from livers was isolated using TRIzol reagent (Takara Biochemicals, Dalian, China). Then, the cDNA synthesized by a commercial reverse transcriptase kit (Toyobo, Tokyo, Japan). Real-time quantitative polymerase chain reaction (PCR) was performed on an Eppendorf MasterCycler® ep RealPlex$^2$ (Wesseling-Berzdorf, Germany). Oligonucleotide primers were used to detect the transcription of genes of *GAPDH*, *Sod1*, *Sod2*, *Gpx1*, *Gpx2*, *Cat*, *Gsta1*, *glutathione synthetase* (*Gss*), *glutathione reductase* (*Gr*), *heme oxygenase 1* (*Ho-1*), *NAD(P)H: quinone oxidoreductase 1* (*Nqo1*), *Tumor necrosis factor-α* (*TNFα*), *Interleukin-6* (*IL6*), *Interleukin-1α* (*IL1α*), *Interleukin-1β* (*IL1β*), *inducible nitric oxide synthase* (*iNOS*) *and interferon-γ* (*IFNγ*) using the SYBR Green system (Toyobo, Tokyo, Japan). The sequences of the primers were according to