Chemosphere 144 (2016) 168–175

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Cadmium exposure to murine macrophages decreases their inflammatory responses and increases their oxidative stress

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HIGHLIGHTS

- Oxidative stress and inflammatory responses induced by Cd were evaluated in RAW264.7 cell.
- Effects of Cd on mRNA levels of cytokines depended on the exposure time and dose.
- Cd decreased the mRNA levels and the release of cytokines in response to lipopolysaccharide.
- Cd altered the glutathione contents and activities of some anti-oxidant enzymes and their mRNA levels.

ARTICLE INFO

Article history:
Received 14 June 2015
Received in revised form
20 August 2015
Accepted 29 August 2015
Available online xxx

Keywords:
Cadmium
Oxidative stress
Inflammatory response
Macrophage

ABSTRACT

Cadmium (Cd) is an environmental contaminant that poses serious risks to human and wildlife health. The oxidative stress and inflammatory responses induced by Cd were evaluated in RAW264.7 cells. A significant decrease in the cell viability was observed in the group treated with 3 μM Cd for 24 h. The mRNA levels of *tumor necrosis factor-α (TNFα)*, *interleukin-6 (IL6)*, *interleukin-1α (IL1α)* and *Interleukin-1β (IL1β)* were generally increased or decreased by Cd exposure for 6 and 24 h, respectively. Moreover, pretreatment of the RAW264.7 cells with Cd for 24 h inhibited the transcriptional status of *TNFα*, *IL6*, *IL1α* and *IL1β* and the release of these cytokines in response to a 6-h lipopolysaccharide (LPS) treatment in a dose-dependent manner. Furthermore, the Cd exposure elicited oxidative stress not only by disturbing the transcriptional status of genes including *superoxide dismutase (Sod)*, catalase *(Cat)*, glutathione peroxidase(*Gpx), glutathione S-transferase 1 a (Gst1a), NAD(P)H: quinone oxidoreductase 1(Nqo1), heme oxygenase 1(Ho-1)* but also the enzyme activities of SOD, CAT and glutathione S-transferase (GST). The effects of Cd on the mRNA levels and activities of anti-oxidative enzymes were dependent on the exposure period and dose. These results suggested that Cd exposure generated oxidative stress and decreased the inflammatory responses in a murine macrophage cell line. Furthermore, oxidative stress may be a possible mechanism to explain the dysregulation of the immune function caused by heavy metals in this *in vitro* system.

1. Introduction

The immune system is one of the main adaptive mechanisms through which the body defends itself against harmful agents and pathogens. A disturbance of the immune system by environmental contaminants can negatively influence its ability to protect against infections and can lead to a broad range of disorders such as inflammatory and infectious diseases, oxidative stress, endocrine disruption and even tumorigenesis (Pillet et al., 2006; Ji et al., 2010; Matés et al., 2010). Today, immunotoxicology has become an important subject in mammalian toxicology, and environmental chemicals, including heavy metals, may be directly involved in this process. Supporting this idea, some previously published data has suggested that exposure to different heavy metals could affect the immune systems of humans and other test models (Bernier et al., 1995; Shen et al., 2001; Zasshi, 2009; Mishra, 2009; Sun et al., 2012).

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http://dx.doi.org/10.1016/j.chemosphere.2015.08.084
0045-6535/

Cadmium (Cd), an important heavy metal, is frequently used in many industrial applications including the production of batteries, metal plating, pigments, and plastics (Alghasham et al., 2013). These processes are known to cause Cd release into the environment through various pathways. Currently, Cd is also widely known as an environmental contaminant that poses a serious risk to human and wildlife health (Jarup and Akesson, 2009). The environmental concentrations of Cd can be greater than 1 mg L^{-1} in wastewater (Ma et al., 2008). More recently, Zhang et al. (2014) reported that the levels of Cd reached 42.3 mg kg^{-1} soil in an abandoned e-waste site in China. Importantly, Cd exposure has been associated with a wide range of toxic effects including nephrotoxicity, hepatotoxicity, oxidative stress, DNA damage, and alterations in bone formation, as well as effects on reproductive physiology (Dally and Hartwig, 1997; Yamano et al., 1998; Pillet et al., 2006; Samuel et al., 2011). Moreover, it has been reported that Cd exposure can induce immunotoxicity in various experimental systems. For example, Tsangaris and Tzortzatou-Stathopoulou(1998) reported that Cd exposure could induce apoptosis in the 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. Låg et al. (2010) observed that Cd^{2+} induced an increased release of IL6 and macrophage inflammatory protein-2/chemokine (C-X-C motif) ligand 2 (MIP-2/CXCL2) from epithelial cells and MIP-2, IL1β and TNFα from alveolar macrophages. However, to date, the mechanisms of the Cd-induced immunotoxic effects still remained unclear.

Recently, oxidative stress has become an important subject in environmental toxicity (López et al., 2007; Jin et al., 2010b), and heavy metals may be directly involved in this process. In animals, the amount of glutathione (GSH) could reflect the antioxidant potential of an organelle. The antioxidant enzyme glutathione peroxidase (GPX) catalyzes the reduction of hydrogen peroxide, but at the expense of GSH. And the antioxidant enzymes of superoxide dismutase (SOD), catalase (CAT) convert superoxide anions (O_2^-) into H_2O_2 and then into H_2O and O_2. A number of previous reports have indicated that oxidative stress induced in immune organs depresses immune function (Pathak and Khandelwal, 2006; Gao et al., 2008; Jin et al., 2010a; Sadowska-Woda et al., 2010). Thus, it is possible that heavy metal-induced oxidative stress may be involved in the decrease of inflammatory responses in macrophages; however, the mechanism still remains unclear. In this study, to investigate the inflammatory responses in macrophages following exposure to Cd in greater detail, the murine macrophage line RAW264.7 was exposed to a range of Cd doses under various conditions. The cell viability and the transcriptional levels of several genes related to immunological responses or oxidative stress were analyzed after the Cd exposure. In addition, the levels of several cytokines in the culture medium and the activities of SOD, CAT and content of GSH in the cells were examined to evaluate the immunotoxicity and the oxidative stress induced by Cd. The information acquired in the present study will be helpful in understanding the immunotoxic risk induced by environmentally relevant heavy metals in ecosystems.

2. Materials and methods

2.1. Chemicals and cell culture

Cells of murine macrophage-like cell line RAW 264.7 were obtained from the American Type Culture Collection (ATCC) and were cultured in DMEM (high glucose) supplemented with 100 U mL^{-1} of penicillin, 100 μg mL^{-1} streptomycin and 10% heat-inactivated fetal bovine serum (Gibco, USA) at 37 °C with 5% CO_2 in an incubator.

Cadmium chloride ($CdCl_2$ (Cd), purity > 99.0%, Jinshanting Xin Chemicals, Shanghai, China) was dissolved in water at 10 mM and stored at 4 °C (stock solution) and was diluted to appropriate concentrations in the final medium immediately before use. LPS (*Escherichia coli* O55:B5) was purchased from Sigma–Aldrich.

2.2. Exposure of cell cultures

To determine cell viability, the RAW264.7 cells were cultured in 96-well plates and exposed to 0.1, 0.3, 1 or 3 μM of Cd for 24 h. Six replicates were performed for each group.

To determine the mRNA levels of genes related to the immunological responses or oxidative stress, the cells were cultured in 6-well plates and exposed to 0.1, 0.3, 1 or 3 μM of Cd for 6 and 24 h and then treated with or without 1 μg mL^{-1} LPS for 6 h. The cells were harvested for measurements of the transcriptional status of related genes in the cells. Four replicates were performed for each group.

To determine the release of cytokines, the cells were cultured in 24-well plates and exposed to 0.3, 1 or 3 μM of Cd for 24 h and then treated with 1 μg mL^{-1} LPS for 6 h. After the exposure, the cell medium was sampled and centrifuged to remove cells. Four replicates were performed for each group. The supernatants were stored at −70 °C until use.

To determine the GSH contents and the activities of CAT, GST and SOD, the cells were cultured in 6-well plates and exposed to 0.1, 0.3, 1 or 3 μM of Cd for 6 and 24 h. Then, the cells were harvested for measurement of the GSH content and the activities of CAT, GST and SOD. Four replicates were performed for each group.

2.3. Cell viability determined by the MTT assay

The effect of Cd on the viability of cells were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with the MTT Cell Proliferation and Cytotoxicity Assay Kit (Beyotime, Beijing, China) according to manufacturer's instructions. The optical density values were measured at 570 nm using a microplate reader (Bio-TEK, USA).

2.4. Quantification of mRNA

Total RNA was isolated from RAW264.7 cells using TRIzol reagent (Takara Biochemicals, Dalian, China), and the cDNA was synthesized using a reverse transcriptase kit (Toyobo, Tokyo, Japan) according to the manufacturer's protocol. Quantitative real-time PCR (RT-qPCR) was performed, with *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* as an internal control, using the Eppendorf MasterCycler® ep RealPlex4 (Wesseling-Berzdorf, Germany). The expression of selected test genes was detected using the SYBR Green system (Toyobo, Tokyo, Japan). The detailed information of the primers is indicated in previous reports (Jin et al., 2011, 2014; Wang et al., 2013). The following PCR protocol was used: denaturation for 1 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The transcription of each gene in each sample was normalized against *GAPDH* and expressed as fold change compared to the control (Livak and Schmittgen, 2001).

2.5. Cytokine assays

The release of Tumor necrosis factor-α (TNFα), Interleukin-6 (IL6), Interleukin-1β (IL1β) into the culture medium was quantified using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's instructions (RapidBio, USA).