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Strain differences in toxicity of oral cadmium intake in rats

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ABSTRACT

Influence of genetic background on toxicity of oral cadmium (Cd) administration (30 days, in drinking water; 5 ppm and 50 ppm of cadmium) was examined in Albino Oxford (AO) and Dark Agouti (DA) rats. Similar cadmium deposition was noted in gut and draining mesenteric lymph nodes (MLN) of both strains but intensity and/or the pattern of responses to cadmium in these tissues differ. Less intense intestinal damage and leukocyte infiltration was observed in gut of cadmium-exposed AO rats. While gut-associated lymph node cells of DA rats responded to cadmium with an increase of cell proliferation, oxidative activity, IFN-γ, IL-17 production and expression, no changes of these activities of MLN cells of cadmium-treated AO rats were observed. Spleen, which accumulated cadmium comparable to MLN, responded to metal by drop in cell viability and by reduced responsiveness of proliferation and cytokine production to stimulation in DA rats solely, which suggest tissue dependence of cadmium effects. More pronounced cadmium effects on MLN and spleen cells of DA rats (which accumulated similar cadmium doses as AO rats), showed greater susceptibility of this strain to cadmium. The results presented, for the first time, depict the influence of genetic background to effects of oral cadmium administration.

1. Introduction

Cadmium (Cd) is a toxic heavy metal, environmental pollutant and human carcinogen (IARC, 1993). Contaminated food and water are the most important sources of Cd exposure in general non-smoking and non-occupationally exposed human population. Approximately 7% of ingested Cd in humans is absorbed from gastrointestinal system and between 0.3 and 3.5% in rats. Upon absorption, Cd is transported by blood to different organs, where it accumulates over time. The highest deposition levels of Cd are found in liver and kidneys (Satarug et al., 2010; Joseph, 2009). Therefore, hepatotoxicity (Liu et al., 2015) and nephrotoxicity (Nordberg, 2009) of Cd are best described, although Cd affects other organs such as bones (James and Meliker, 2013), heart (Tellez-Plaza et al., 2013), testes (Marettová et al., 2015), and brain (Wang and Du, 2013). Oxidative stress and inflammation induced by leukocytes with target tissue tropism are the major underlying mechanisms of hepatotoxicity and nephrotoxicity (Horiguchi et al., 2000; Kayama et al., 1995; Ogunrinola et al., 2016; Rikans and Yamano, 2000; Rani et al., 2014; Rogalska et al., 2011; Safhi et al., 2016).

The expression of Cd toxicity is associated with host intrinsic physiological factors such as genetic background, age and sex. Host genetic variability was considered as a contributing factor concerning susceptibility to metals in general (Gochfeld, 1997; Kacew et al., 1995; Miura, 2009). Rodent strain-specific differences of

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tissue Cd accumulation were considered as responsible for susceptibility to carcinogenesis (Waalkes and Rehm, 1994; Theocharis et al., 1994; Shimada et al., 2008), hepatotoxicity (Shaikh et al., 1993; Shimada et al., 2004) and testicular toxicity (Shimada et al., 2009, 2011). However, several studies showed that host genetic background could be more important for Cd toxicity than levels of accumulated Cd. Strain specific responses to similar tissue concentrations of Cd in liver (Harstad and Klaassen, 2002; Kuester et al., 2002; Shaikh et al., 1993; Theocharis et al., 1994), testes (Dalton et al., 2005; King et al., 1998; Liu et al., 2001; Nolan and Shaikh, 1986), lungs and spleen (Demenesku et al., 2016; McKenna et al., 1997) were reported. Strain differences in susceptibility of tissues to Cd (at similar levels of accumulated metal) were ascribed to the activity of resident or infiltrating leukocytes. Liver macrophages (Kupffer cells) were considered as chief determinant of differential susceptibility of Fischer 344 and Sprague-Dawley rats to Cd-hepatotoxicity (Kuester et al., 2002). Differences in the magnitude of lung leukocyte infiltration were accounted for strain differences in the susceptibility of C57BL/6 and DBA mice to Cd-induced pulmonary injury (McKenna et al., 1997). Mechanisms of strain differences in Cd toxicity to other tissues are not known.

Cd induces changes in the intestine, the tissue which is under direct influence of the metal in settings of oral exposure. It is known from animal and human studies that oral exposure to Cd causes severe irritation of gastrointestinal epithelium (ATSDR, 2012) and interrupts the integrity of intestinal epithelial barrier (Rusanov et al., 2015). Histologically evident changes (Andersen et al., 1988; Ninkov et al., 2015) are associated with infiltration of inflammatory cells into lamina propria (Zhao et al., 2006). Cd directly impacts gut bacterial ecosystem (Breton et al., 2013) which is essential for defense against pathogenic bacteria. Cd-induced intestinal dysbiosis compromises immune-mediated intestinal homeostasis (Liu et al., 2014; Ninkov et al., 2015). In such settings, gut draining mesenteric lymph nodes are stimulated to protect vulnerable intestine (Ninkov et al., 2015).

In view of the above cited findings, the aim of the present paper was to examine the influence of genetic background on Cd-induced intestinal toxicity in rats. To this aim, intestinal response to oral Cd administration was examined in Albino Oxford (AO) and Dark Agouti (DA) rats, the strains which differ in their susceptibility to insults in different tissues including nervous system (Lukic et al., 2001), lungs (Mirkov et al., 2015), skin (Popov Aleksandrov et al., 2015), gut and joints (Kovačević-Jovanović et al., 2015). Moreover, responsiveness of DA rats to Cd administration was previously shown (Demenesku et al., 2014; Ninkov et al., 2015). Therefore, the effects of subacute (30 days) oral Cd intake on local parameters of activity in gut and associated lymph nodes (which drain antigens from gut) were examined in AO and DA rats. Furthermore, basic parameters of activity were examined in spleen, which represents the peripheral immune organ involved in the elimination of foreign substances from blood.

2. Materials and methods

2.1. Chemicals

Cadmium chloride ($CdCl_2$) was purchased from Serva (Serva, Feinbiochemica, Heidelberg, Germany). Concanavalin A (ConA), Lipopolysaccharide (LPS; type 0111: B4 from *Escherichia coli*), L-epinephrine, hexadecyltrimethylammonium bromide (HTAB), N-(1-naphtyl) ethylenediamine dihydrochloride, sulfanilamide (*p*-aminobenzenesulfonoamide), *o*-dianisidine dihydrochloride and myeloperoxidase (MPO) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Hydrogen peroxide (H_2O_2) was obtained from Zorka Farma, Sabac (Serbia). N,N,N0,N0-ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate was obtained from USB Corporation (Cleveland, OH, USA). Blotto (non-fat dry milk) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Saccharose was obtained from Lachner (Neratovice, Czech Republic). Culture medium RPMI-1640 (Biowest, Nuaillé, France) supplemented with 2 mM glutamine, 20 μg/ml gentamycine (Galenika a. d., Zemun, Serbia) and 5% (v/v) heat inactivated fetal calf serum (Biowest, Nuaillé, France) were used. For use in experiments ConA and LPS were dissolved in RPMI-1640 medium. Griess reagent was prepared by mixing equal volume of 0.1% N-(1-naphtyl) ethylenediamine dihydrochloride dissolved in water and 1% sulphanilamide in 5% phosphoric acid. [^{3}H]-thymidine (GE Healthcare, Little Chalfont, UK) was prepared in culture medium. All solutions for cell culture experiments were prepared under sterile conditions and were sterile filtered (Minisart, pore size 0.20 mm, Sartorius Stedim Biotech, Goettingen, Germany) before use. Annexin V apoptosis detection kit (allophycocyanin (APC) conjugated annexin V and propidium iodide); fluoroisothiocyanate (FITC)-labeled mouse antibodies to rat CD4, phycoerythrin (PE)-labeled mouse antibodies to rat CD8 and F(ab)2 goat anti-mouse IgG were purchased from eBioscience (eBioscience Inc., San Diego, CA, USA). Monoclonal antibody OX-42 (mouse anti-rat CD11b/CD11c) was purchased from AbD Serotec (Serotec Ltd., Oxford, UK).

2.2. Animals and Cd treatment

Animal treatment and experimental procedures were carried out in compliance with the Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes and approved by the Ethical Committee of the Institute for Biological Research "Sinisa Stankovic" (IBISS), University of Belgrade. AO and DA male rats, eight to twelve weeks of age, used in experiments, were conventionally housed at IBISS, in controlled environment (21/24 °C temperature, a 60% relative humidity and 12-h (h) ligth:dark cycle). Rats were exposed to cadmium chloride ($CdCl_2$) prepared in distilled water at concentration of 5 ppm (5 mg/l) and 50 ppm (50 mg/l) of Cd (II) ion for 30 days. Cd dose selection was based on our previous work (Ninkov et al., 2015) where these doses showed immunomodulatory effects on gut immune responses of DA rats susceptible to oral Cd administration and these doses were employed in testing strain dependent responses to Cd in the present study. These doses are relevant for human exposure (Bhattacharyya et al., 1988; Schwartz and Reis, 2000; Wang et al., 2003) and cover environmentally relevant Cd concentrations (Blanusa et al., 2002; Damek- Poprawa and Sawicka-Kapusta, 2004; Lukacinova et al., 2011; Moniuszko-Jakoniuk et al., 2009). Control rats were given distilled water solely. All rats had *ad libitum* access to standard rodent chow and water throughout the study. Cd solutions and water were replaced with freshly prepared solution or water twice a week. All functional measurements were carried out after a 30-day period of oral Cd intake, in animals anesthetized by i. p. injection of 40 mg/kg b. w. of thiopental sodium (Rotexmedica, Tritau, Germany).

2.3. Cd determination

Cd content in blood and tissue of spleen, lungs, intestine, mesenteric lymph nodes, liver and kidneys was determined by atomic absorption spectrometry graphite tube technique (AAS Varian 1275; graphite tube, GTA-95, Palo Alto, Ca, USA). Following samples homogenization and digestion of lyophilized tissue in a microwave digestion system (MBS-9, CEM Innovators, Buckingham,UK) in a mix of concentrated HCl and HNO_3 (metal-free) the samples were filtered and all dilutions were done using metal-