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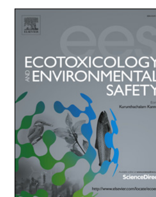

Oral cadmium exposure affects skin immune reactivity in rats

Dina Tucovic[a], Aleksandra Popov Aleksandrov[a], Ivana Mirkov[a], Marina Ninkov[a], Jelena Kulas[a], Lidija Zolotarevski[a], Vesna Vukojevic[b], Jelena Mutic[b], Nikola Tatalovic[c], Milena Kataranovski[a,d,*]

[a] Immunotoxicology Group, Department of Ecology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, 142 Bulevar despota Stefana, 11000 Belgrade, Serbia

[b] Innovation Center of the Faculty of Chemistry, University of Belgrade, 12-16 Studentski trg, 11000 Belgrade, Serbia

[c] Department of Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, 142 Bulevar despota Stefana, 11000 Belgrade, Serbia

[d] Institute of Physiology and Biochemistry, Faculty of Biology, University of Belgrade, 16 Studentski trg, 11000 Belgrade, Serbia

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ABSTRACT

Skin can acquire cadmium (Cd) by oral route, but there is paucity of data concerning cutaneous effects of this metal. Cd acquired by oral route can affect skin wound healing, but the effect of Cd on other activities involved in skin homeostasis, including skin immunity, are not explored. Using the rat model of 30–day oral administration of Cd (5 ppm and 50 ppm) in drinking water, basic aspects of immune-relevant activity of epidermal cells were examined. Dose-dependent Cd deposition in the the skin was observed (0.035 ± 0.02 μg/g and 0.127 ± 0.04 μg/g at 5 ppm and 50 ppm, respectively, compared to 0.012 ± 0.009 μg/g at 0 ppm of Cd). This resulted in skin inflammation (oxidative stress at both Cd doses and dose-dependent structural changes in the skin and the presence/activation of innate immunity cells). At low Cd dose inflammatory response (nitric oxide and IL-1β) was observed. Other inflammatory cytokines (IL-6 and TNF) response occurred at 50 ppm, which was increased further following skin sensitization with contact allergen dinitro-chlorobenzene (DNCB). Epidermal cells exposed to both Cd doses enhanced concanavalin A (ConA)-stimulated lymphocyte production of IL-17. This study showed for the first time the effect of the metal which gained access to the skin *via* gut on immune reactivity of epidermal cells. Presented data might be relevant for the link between dietary Cd and the risk of skin pathologies.

1. Introduction

Cadmium (Cd) is toxic pollutant which is widely spread in the environment through industrial production and agriculture. Being non-biodegradable, Cd is persistent in the environment and is important water and food contaminant. Because of its long half-life and low rate of excretion from the organism, Cd is of both occupational and environmental concern. Despite efforts to decline anthropogenic activities which result in environmental pollution, Cd has remained global health concern greatly because of accumulation in food. This draw attention to non-occupational exposure to Cd and possible health risks (Satarug et al., 2003; Satarug and Moore, 2004).

Epidemiological data revealed that life-time exposure to environmentally-relevant levels of Cd posed a risk of variety organ diseases including chronic kidney, heart and lung diseases, and nervous tissue disorders (Adams et al., 2012; Buck Louis et al., 2012; Garcia-Esquinas et al., 2014). Experimental studies of Cd exposure by oral route, the main way for this metal to reach the organism, showed broad spectrum of toxic effects in variety of tissues, with kidneys and liver as the most affected organs (Sugawara and Sugawara, 1974; Muller et al., 1986; Friberg et al., 1986: Jonah and Bhattacharyya, 1989; Saygi et al., 1991; Hiratsuka et al., 1999). Long-term exposure to Cd affects also male reproductive tissues (Waalkes, 2003) and exerts osteotoxicity (Ohta et al., 2000). Increased levels of Cd noted in lungs of mice exposed to oral Cd administration affected neuronal pathways involved in regulation of bronchial tone (Chandler et al., 2016).

Cd deposition was noted also in skin hair (Wibowo et al., 1986; Lauwerys et al., 1994). The metal may be taken by growing hair in the hair follicles from the blood and/or be absorbed by hair following excretion by sebaceous and sweat glands. Cd which gained access to the skin can affect physiological processes in the skin as well. In this regard, it was shown that both topical skin as well as oral exposure to Cd impaired cutaneous wound healing in mice (Lansdown et al., 2001; Mei et al., 2017). Recently explored relationship between cadmium and

* Corresponding author at: Immunotoxicology Group, Department of Ecology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, 142 Bulevar despota Stefana, 11000 Belgrade, Serbia.
E-mail address: milena@ibiss.bg.ac.rs (M. Kataranovski).

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psoriasis suggested positive association between blood cadmium and severity of psoriasis (Liaw et al., 2017). This throws light on effects of Cd on other aspects of activities which maintain skin homeostasis, including skin immunity, which are not explored yet. Skin has essential immunological functions which are required for active defense against pathogens and variety of physical and chemical insults (Nestle et al., 2009). Epidermal cells are critical immune sentinels in immunosurveillance of skin exposed to external or endogenous/systemic stimuli (Nestle and Nickoloff, 2007).

The aim of this study was to examine whether oral exposure to Cd affects cutaneous immune activity. The rat model of 30–day oral administration of Cd in drinking water was used to examine basic aspects of immune-relevant activity of the epidermal cells. The obtained data showed for the first time influence of Cd which gained access to the skin *via* gut on skin immune system.

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, N-(1-naphtyl) ethylenediamine dihydrochloride, sulfanilamide (*p*-aminobenzenesulfonoamide). 3-(4,5-dimethyl-thiazol-2-yl)-2, 5 diphenyl-tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-Chloro-2,4 dinitrochlorobenzene (DNCB) was obtained from BDH Chemicals Ltd. 2,4- dinitrobenzenesulfonic (DNTB) acid, sodium salt, was purchased from Aldrich Chemical Company (Milwaukee, WI, 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). Sucrose was obtained from Lachner (Neratovice, Czech Republic). Dispase II was obtained from Boehringer (Manheim, Germany) and trypsin solution from Difco (Lawrence, KS, USA). Sodium nitrite ($NaNO_2$) and reduced L-Glutathione (GSH) were purchased from Fluka Chemie (Buchs, Switzerland). 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 dispase II were dissolved in RPMI-1640 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.

2.2. Animals and cadmium 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, Serbia. Dark Agouti (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). Four to six animals were assigned to each treatment group per experiment, in at least two independent experiments. Rats were exposed to cadmium chloride ($CdCl_2$) prepared in distilled water at two concentrations, 5 ppm (5 mg/l) and 50 ppm (50 mg/l) of Cd (II) ion in a period of 30 days, while control rats were given distilled water solely. In some experiments, at day 30, 100 μl of 0.4% DNCB dissolved in acetone/olive oil was applied for 2 consecutive days to the upper part of dorsum (approximately 16 cm^2) of treated rats, as described previously (Belij et al., 2012). All rats had *ad libitum* access to standard rodent pellets and water throughout the study. Twice a week Cd solutions and water were replaced with freshly prepared solution/water. One day before cessation of cadmium treatment (day 29th) animals were put for 24 h into metabolic cages in order to measure food consumption, urine volume and weight of feces. All functional measurements were carried out after a 30-day period of oral Cd intake, or 24 h following second DNCB application, in animals anesthetized by i.p. injection of 40 mg/kg b.w. of thiopental sodium (Rotexmedica, Tritau, Germany).

2.3. Cadmium determination

Cd content in blood and tissue of liver, kidney, intestine, hair, skin, epidermis and dermis was determined by inductively coupled plasma mass spectrometry, ICP-MS (ICAP, Q, X Thermo Scientific series 2). The entire system is controlled with Qtegra Instrument Control Software. Samples were decomposed in the microwave digestion system (Ethos One, Milestone Italy) at 180 °C, in a mix of concentrated HNO_3 (65%, Distill acid, Hotel Berghof (Germany)) and H_2O_2 (30%) by applying the following temperature program: 10 min warm-up to 180 °C and heating for 15 min. Samples were filtered and all dilutions were done using metal-free ultrapure water. All reagents were of analytical grade supplied by Merck (Darmstadt, Germany). Single-element stock solution containing 1000 mg/L of Cd was used for the standards preparation for analysis by ICP-MS. As internal standards were used: ^{6}Li, ^{45}Sc, ^{115}In and ^{159}Tb (VHG standards, Manchester, UK). The concentrations were expressed as μg of Cd per g of wet tissue.

2.4. Clinical biochemistry and hematology

Blood was withdrawn from abdominal artery for determination of leukocyte counts and hematologic parameters and for isolation of plasma or serum. Differential leukocyte counts and hematological parameters were determined automatically by Siemens ADVIA 120 flow cytometer (Terytown, New York, USA) using commercially available reagents. Plasma fibrinogen was measured by Siemens-Dade Behring-BCT analyzer using Multifibren *U* test for quantitative determination in plasma. Haptoglobin and albumin were measured in serum by BN (Dade Behring) immunochemical system for human blood proteins measured by Siemens BNII (Dade Behring) BCT analyzer. Cross-reactivity with rat blood proteins was checked using serum obtained from turpentine-induced inflammation in rat, known inflammatory model of acute phase reaction in these animals (Giffen et al., 2003). Changes in plasma or serum proteins are expressed as the relative changes (values from controls were taken as 1). Plasma levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were measured using an autoanalyzer (Ciba Corning Express, Oberline, OH) and commercially available reagents. Biochemical parameters in freshly catched urine were measured using semi quantitative Combur[10] Test® M (Roche Diagnostics GmbH, Mannheim, Germany).

2.5. Histology

Full thickness dorsal skin (approximately 1 cm^2) tissue specimens were fixed in 4% formaldehyde (pH 6.9), and after preparation molded into paraffin wax for subsequent sectioning at 5 μm. Samples were stained with hematoxylin and eosin (H&E) and subsequently analyzed by a certified histopathologist using a Coolscope digital light microscope (Nikon Co, Tokyo, Japan).

2.6. Measurements of skin explant's viability

Full thickness dorsal skin was excised, cleared of subcutaneous tissue, weighted, cut into pieces of approximately 0.3 cm^2 and placed in duplicate in 0.2 ml of complete culture medium. MTT reduction assay was employed for skin viability determination as described (Klein et al., 1996). MTT was added immediately, in a concentration of 0.5 mg/ml of culture, and skin explants were incubated for 3 h at 37 °C in a

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