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Effects of cadmium chloride on the functional state of human intestinal cells

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ABSTRACT

Toxic effects of cadmium chloride in concentration range from 1 to 300 μM on differentiated human intestinal epithelial Caco-2 cells after three hours of exposure were investigated. Processes of disorganization of the actin cytoskeleton associated with the toxic effects of cadmium were characterized by fluorescent microscopy. The cadmium-induced activation of cellular stress response processes (changes in the mRNA expression of caspase-3, heat-shock and oxidative stress genes) has been demonstrated. The study revealed dose-dependent changes in mRNA expression levels of proteins involved in the formation of adherens (E-Cadherin and p120 catenin) and tight intercellular junction contacts (Claudin 4 and ZO1). The time- and concentration-dependent trend of cell monolayer transepithelial resistance lowering, characterizing the loss of intercellular contacts density with prolongation of cell exposure cadmium chloride was estimated. Results indicates that proteins associated with tight and adhesion junctions are primary targets of cadmium. Amongst genes involved in cell junction formation, the genes encoding E-Cadherin and p120-catenin proved to be the most sensitive to cadmium influence.

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1. Introduction

Cadmium salts represent the group of highly toxic heavy metal (HM) compounds and may enter human body with food and fluids, affecting structure and function of cells and tissues through the digestive tract, including the intestinal epithelium. The toxicity of some substances is invoked by a combination of several processes, which may evolve simultaneously or independently (Boveri et al., 2004; Klaassen et al., 2009). Cadmium transport across the cell membrane occurs via divalent metal transporter 1 (DMT1) transporter (for ions) or transcytosis (for cadmium complexes with metallothionein or phytochelatin) (Langelueddecke et al., 2014; Tallkvist et al., 2001). The toxicity of cadmium is related to its ability to modulate the activity of cellular enzymes, initiate the development of oxidative stress, suppress mitochondrial functions and disrupt calcium homeostasis (Prozialeck, 2000). Cadmium was reported to diminish intracellular glutathione concentration and lessen thiol-group content of proteins, leading to the rise in reactive oxygen species (ROS), such as superoxide-anion, hydrogen peroxide and hydroxyl radicals, which results in lipid and protein peroxidation and DNA damage (Bae et al., 2001; Bertin and Averbeck, 2006; Boveri et al., 2004; Liu et al., 2009; Prozialeck, 2000; Simmons et al., 2011). *In vitro* studies have shown cadmium to initiate inflammatory cytokines production by intestinal epithelial cells due to the activation of NF-κB signaling pathway under oxidative stress (Hyun et al., 2007).

Epithelial intercellular junctions form the structural and functional barriers preventing the penetration of potentially toxic compounds from the intestinal lumen to the bloodstream. The durability of these junctions influences the efficiency of their barrier function, whereas the changes in their permeability may serve as a marker for tissue disruption in the presence of different compounds, particularly salts of the HM, including cadmium, iron, copper, lead and others. Most of these effects occur in association with enterocyte actin skeleton perturbations (Ferruzza et al., 2002; Natoli et al., 2009).

When differentiated the immortalized intestinal Caco-2 cells constitute a monolayer of viable polarized cells, exhibiting physiological and morphological features of enterocytes, in particular, forming strong intercellular junctions. These cells are of wide use

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in the studies of drug transport through the intestinal epithelial barrier, as well as in the identification of transporter proteins, their inductors and inhibitors, and *in vitro* evaluation of toxic effects exerted upon intestinal epithelium by xenobiotics (Artursson et al., 2012; Sun et al., 2008).

The increase in epithelial permeability in the presence of cadmium salts is usually explained by calcium ions substitution on the sites of E-cadherin binding, which results in protein conformational modification and the disruption of adherens junctions and, later, tight junctions. At high calcium concentration cadmium effects on paracellular permeability are diminished due to the competitive binding of these metal ions to the same site. At the same time, the dissociation constant for cadmium bound to E-cadherin is several times lower than that for calcium (Prozialeck, 2000).

Cd^{2+} exposure (at concentration of 1–100 μM) on various epithelial cells leads to the disruption of intercellular contacts within 1–4 h, while cells do not detach from the surface and no obvious signs of cell death is registered (Prozialeck, 2000). In addition changes in the level of gene expression under toxic exposure often occurs within the few hours (Murray et al., 2004; Rusanov et al., 2014). Thus, the assessment of the functional status of cells at the early stages allows operatively predict the impact of toxic effects, as well as to investigate the molecular mechanisms of its development.

However, the mechanisms of cadmium toxicity are not fully understood yet. Our study investigates a number of parameters describing functional activity of Caco-2 cells, including gene expression, actin cytoskeleton structure and transepithelial electrical resistance of cell monolayer.

2. Materials and methods

2.1. Cell maintenance and differentiation

Caco-2 cells were kindly provided by prof. U. Marx (Technical University, Berlin, Germany) and cultivated in MEM supplemented with 10% fetal bovine serum, 0.1 M non-essential aminoacids, 1 mM pyruvate, 0.1% penicillin–streptomycin at 37 °C in 5% CO_2 atmosphere. All reagents for medium preparation were purchased from Gibco (USA). Cells were initially seeded with density of 2.8×10^5 cells/cm^2 and differentiated by cultivation for 21 days (Hubatsch et al., 2007) in 24-well plates (Corning, USA), coverslips or transwell inserts (polycarbonate membrane, pore diameter 0.4 μm, surface area 0.143 cm^2) (Corning, USA). The medium was changed every other day. Cell counts were performed on automated cell counter Countess (Invitrogen, USA).

2.2. Transepithelial electrical resistance (TEER)

TEER was measured by EVOM voltohmmeter (World Precision Instruments, Inc., USA). Transwells with TEER of 200 Ω cm^2 or more were used for the experiments.

2.3. $CdCl_2$ treatment

For Cd^{2+} ion toxicity study cadmium chloride (Sigma, USA) dissolved in culture medium was used. Different concentrations of cadmium chloride (1, 50, 100 and 300 μM) in final volume of 500 μl of medium were applied to the apical side of the Caco-2 cell monolayers for 3 h, then the medium was discarded and the cells were washed three times with PBS.

2.4. Cell imaging

For fluorescent microscopy the cells were fixed in 4% formaldehyde solution in PBS, permeabilized with 0.1% Triton X-100, blocked with 0.5% BSA solution in PBS. For cytoskeleton staining Alexa Fluor 488® phalloidin (Molecular Probes®, USA) diluted 1:100 (v/v) in PBS with 0.5% BSA was used. Visualization was performed on inverted fluorescent microscope AxioObserver Z1 (Carl Zeiss, Germany).

2.5. RNA isolation and real-time PCR

Changes in mRNA expression profiles were studied by RT-PCR. The cells were lysed in 700 μl QIAsol lysis reagent (Qiagen, Germany). 10 μl of the sample were used for intracellular cadmium content estimation, the rest was used for total RNA isolation with Rneasy MiniKit (Qiagen, Germany) according to the previously described protocol (Krainova et al., 2013). cDNA synthesis was conducted by Sensiscript RT Kit (Qiagen, Germany) according to the manufacturer's recommendations, for each reverse transcription reaction 500 ng of total RNA were used.

The primer sequence design procedure and PCR protocols were described previously (Krainova et al., 2013). The primers used in this study are listed in Table 1.

RT-PCR was performed on automated amplificatory DT-Prime (DNA-Technology LLC, Russia) in 384-well plates. The product accumulation was detected in concordance with the intensity of the fluorescent signal from intercalating dye SYBR Green I (Invitrogen, USA). Target genes expression was normalized to the level of reference genes *SDHA* and *GAPDH* (Vandesompele et al., 2002; Trushkin et al., 2013).

2.6. Cellular uptake of Cd

Cadmium uptake by cells was measured by inductively-coupled plasma mass-spectrometer (ICP-MS) Agilent 7500 (Agilent, USA). 10 μl of cell lysate in QIAsol lysis reagent were dissolved in 500 μl of 1% HNO_3. Isotope ^{112}Cd was quantified in the resulting

Table 1
Sequences for primers used in the study.

Gene	Primer sequence*
GAPDH	F: 5′-GAAGGTGAAGGTCGGAGTC-3′ R: 5′-GAAGATGGTGATGGGATTTC-3′
SDHA	F: 5′-TGGTGCTGGTTGTCTCATTA-3′ R: 5′-ACCTTTCGCCTTGACTGTT-3′
GSR	F: 5′-ATGAAAATGGTCTGTGCTAA-3′ R: 5′-CTGAAGAGGTAGGGTGAATG-3′
NQO1	F: 5′-AGGTATCATTCAACTCTCCA-3′ R: 5′-GATTCAGTCTTTTCCTTCTC-3′
XDH	F: 5′-GCTGTGGAGGAGATGGGAATAA-3′ R: 5′-GGTTTGAATAAAGATGGCGAGAGG-3′
ACTB	F: 5′-CCACGAAACTACCTTCAACTCC-3′ R: 5′-CTCGTCATACTCCTGCTTGCT-3′
CDH1	F: 5′-CGGAGAAGAGGACCAGGACTTT-3′ R: 5′-GAAGATACCGGGGGACACTCA-3′
CTNND1	F: 5′-GCCATGTCTGTAGTCTCTGTGGA-3′ R: 5′-TCACAGTCTTCACTACTTTCTTGACC-3′
TJP1	F: 5′-CCCTCCGCTGATACCTTCATCT-3′ R: 5′-TGTGGCTTCATTGCTGGATCTT-3′
CLDN4	F: 5′-TCCGCCAAGTATTCTGCTG-3′ R: 5′-CGTGGCACCTTACACGTAGTT-3′
CASP3	F: 5′-TATCACTAAAGAAATGGTTGG-3′ R: 5′-TCAAAATGAGAGGGAAATAC-3′
HSP90AA1	F: 5′-GGACAGCAAACATGGAGAGAA-3′ R: 5′-GCGCAGTTTCATAAAGCAAGA-3′
HSPA1A	F: 5′-TCCAAAACAAAAACAGCAATCTTGG-3′ R: 5′-GAGAAGGACGAGTTTGAGTTTGGCAC-3′

* Sequences are listed from 5′ to 3′; F – forward primer; R – reverse primer.

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