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Full Length Article

Cadmium-induced IL-6 and IL-8 expression and release from astrocytes are mediated by MAPK and NF-κB pathways

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ABSTRACT

Chronic exposure to cadmium has been linked to brain cancers, learning disabilities and memory deficits. Previous studies of cadmium toxicity in the central nervous system report cadmium induces oxidative stress in neurons and astrocytes. In the peripheral system, cadmium promotes interleukin-6 (IL-6) and IL-8 production and release. Elevation of IL-6 expression is linked to the pathogenesis of neurodegenerative diseases and astrogliosis. IL-8 plays a role in angiogenesis of gliomas and neurodegenerative diseases. Herein, the effects of non-toxic concentrations of cadmium on the production of IL-6 and IL-8 and the underlying mechanisms were investigated. U-87 MG human astrocytoma cells and primary human astrocytes were exposed to cadmium chloride. At 24 h post-exposure to 1 and 10 μM, levels of intracellular cadmium in U-87 MG cells were 11.89 ± 3.59 and 53.08 ± 7.59 μg/g wet weight, respectively. These concentrations had minimal effects on cell morphology and viability. IL-6 and IL-8 mRNA levels and secretion increased in dose- and time-dependent manners post cadmium exposure. Acute exposure to cadmium increased phosphorylation of ERK1/2, p38 MAPK, and p65 NF-κB. Pretreatment with U0126–an inhibitor of MEK1 and MEK2 kinases–SB203580–a p38 MAPK inhibitor–and SC-514–an IKKβ inhibitor–suppressed cadmium-induced IL-8 expression and release. Upregulation of cadmium-induced IL-6 was inhibited by U0126 and SC-514, but not SB203580. On the other hand, SP600125–a JNK inhibitor–and celecoxib–a selective COX-2 inhibitor–had no effect on production of both cytokines. In conclusion, non-toxic concentrations of cadmium can stimulate IL-6 and IL-8 release through MAPK phosphorylation and NF-κB activation. Suppressing IL-6 and IL-8 production could be novel approaches to prevent cadmium-induced angiogenesis in gliomas and inflammation in the brain.

1. Introduction

Cadmium is used extensively in various industries and produced as a by-product of metal smelting. It is also a contaminant in cigarette smoke. Cadmium is classified as a human carcinogen by the Department of Health and Human Services (DHHS) and the International Agency for Research on Cancer (IARC) (Jarup et al., 1998; Waisberg et al., 2003). After being absorbed into the bloodstream, cadmium can destroy the structural integrity of the blood-brain barrier (BBB), allowing cadmium to enter and accumulate in the brain (Wang and Du, 2013; Provias et al., 1994). Epidemiological studies identify cadmium as a risk factor for brain cancers and Alzheimer's disease (Min and Min, 2016; Wesseling et al., 2002). Cadmium exposure is associated with the lack of attention in children, adult with high consumption of contaminated foods, and workers in cadmium-contaminated areas (Ciesielski et al., 2012, 2013; Viaene et al., 2000). The average and maximum levels of cadmium detected in brain tissue of brain tumor patients were 2.02 ± 10.99 and 72.78 μg/g wet tissue, respectively (Al-Saleh and Shinwari, 2001).

Inflammation is one of the hallmarks of neurodegenerative diseases and cancers, and upregulation of many proinflammatory cytokines and chemokines including interleukin-6 (IL-6) and IL-8

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has been associated with these diseases. Cadmium increases IL-6 and/or IL-8 production in various types of human peripheral cells, including bronchial epithelial cells (Rennolds et al., 2012; Cormet-Boyaka et al., 2012), THP-1 monocytic cells (Freitas and Fernandes, 2011), and HepG2 hepatocyte cells (Souza et al., 2004). IL-6 is an inflammatory cytokine with neurotrophic and detrimental effects depending on its concentrations and expression of its receptors. IL-6 protects neurons from excitotoxicity; however, high concentrations of IL-6 induce neuronal death. IL-6 stimulates astrocyte proliferation and secretion of inflammatory mediators and growth factors (Spooren et al., 2011). IL-8 is a chemoattractant chemokine responsible for innate immunity by recruiting neutrophils and macrophages to inflammation sites (Brat et al., 2005). Over production of IL-8 in the brain promotes tumor growth and angiogenesis in gliomas (Brat et al., 2005). In humans, increased IL-6 and/or IL-8 levels in the central nervous system (CNS) were linked to pathogenesis of neurodegenerative diseases such as Alzheimer's disease (Liu et al., 2014), viral infection (Liu and Kumar, 2015; Nookala and Kumar, 2014; Vivithanaporn et al., 2010) and epilepsy (Pernhorst et al., 2013). In these disorders, astrocytes are one of the major sites of IL-6 and IL-8 production. Furthermore, IL-6 and IL-8 production in astrocytes is upregulated by MAPK and/or
NF-κB pathways (Nookala and Kumar, 2014; Shah and Kumar, 2010; Shah et al., 2012). In the peripheral system, cadmium-induced IL-6 and IL-8 is mediated by ERK1/2 and/or NF-κB pathways (Rennolds et al., 2012; Cormet-Boyaka et al., 2012; Freitas and Fernandes, 2011; Hyun et al., 2007; Rajendran et al., 2016).Previous studies report cadmium toxicity on astrocytes is mediated by glutathione depletion and oxidative stress (Im et al., 2006). Little is known about cadmium's effect on astrocytic inflammation. Herein, we determined whether cadmium is transported into astrocytes and subsequently induced inflammation. In addition, the mechanism of cadmium-induced inflammation was investigated. Cadmium transport into human astrocytes was observed, as was cadmium's stimulation of IL-6 and IL-8 expression and release via the ERK1/2, p38, and NF-κB pathways.

2. Materials and methods

2.1. Cell cultures and reagents

U-87 MG astrocytoma cells (U-87 MG) were obtained from American Type Culture Collection (ATCC) and cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, 1% sodium pyruvate, and 1% penicillin-streptomycin (Gibco, USA). Primary human astrocytes (PHA) were provided by Prof. Christopher Power (Department of Medicine, University of Alberta, Canada). PHA were prepared according to protocol 1420 approved by the University of Alberta Human Research Ethics Board (Biomedical) as reported previously (Vivithanaporn et al., 2010). PHA were grown in MEM containing 10% fetal bovine serum, 1% L-glutamine, 1% sodium pyruvate, and 1% penicillin-streptomycin. All cultures were grown and maintained at 37 °C in a humidified chamber with 5% CO_2. Completed medium was renewed every 1-2 days. Both cell types were used from the fifth to the tenth passage.

Cadmium chloride ($CdCl_2$) was dissolved in sterile water at 1000 mM while manganese chloride ($MnCl_2$), nickel chloride ($NiCl_2$), copper(II) sulfate ($CuSO_4$) and mercury chloride ($HgCl_2$) were dissolved in sterile water at 100 mM. All stock solutions of heavy metals were stored at −20 °C until use. U0126 (Cell-signaling, USA), SB203580 (Tocris, United Kingdom), SP600125, SC-514 and celecoxib were dissolved in dimethyl sulfoxide (DMSO). All chemicals were from Sigma-Aldrich unless otherwise indicated. Cells were treated with inhibitors one hour prior to cadmium exposure. The final concentration of DMSO was 0.1%.

2.2. Measurement of intracellular cadmium

U-87 MG cells were plated in 100-mm dishes. At 70–80% confluence, cells were treated with 1 and 10 μM of $CdCl_2$ for 24 h in serum-free MEM. Level of cadmium uptake was measured as previously described (Soodvilai et al., 2011). Briefly, cells were washed twice with phosphate-buffer saline (PBS) containing 10 mM disodium EDTA and twice with PBS without EDTA. Cells were trypsinized with 0.1% trypsin and centrifuged at 3000 rpm for 10 min. Cell pellets were digested with 1 ml of 65% nitric acid and evaporated at 90 °C three times. Lysate was incubated with 2 ml of 65% nitric acid overnight and then diluted with 18 ml of distilled water. Cadmium content was measured using a flame furnace atomic absorption spectrophotometer (FFAAS) (PinAAcle 900T, Perkin Elmer). Amount of intracellular cadmium content was expressed as μg of cadmium/g protein of wet weight.

2.3. Observation of cell morphology

U-87 MG cells were plated at 5×10^5 cells in 60-mm dishes. At 70–80% confluence, cells were treated with 1 to 30 μM $CdCl_2$ for 6 and 24 h. Cell morphology was observed with an inverted light microscope (Eclipse E200, Nikon).

2.4. MTT cell viability assay

U-87 MG and PHA cells were treated with $CdCl_2$ from 0.01 to 100 μM for 6 and 24 h in serum-free MEM. At 2 h before the indicated incubation time, MTT solution was added to each well to a final concentration of 0.5 mg/ml. The formazan crystals were then dissolved in DMSO and measured spectrophotometrically at 562 nm using a microplate reader (ELx808™, Biotek). The percentage of cell viability was calculated as percentage in comparison to untreated cells.

2.5. Quantitative real-time PCR

U-87 MG cells were treated with 0.1, 1 and 10 μM $CdCl_2$ for 3 and 6 h. Total RNA was extracted and purified by Total RNA Purification kit according to manufacturer's protocol (Jena Bioscience, Germany). Complementary DNA (cDNA) was synthesized using 1 μg of total RNA mixed with Primer Random (Roche Diagnostics, USA) and superscript III reverse transcriptase (Invitrogen, USA). The primer sequences for human IL-6 were: forward, 5′-ACCCCTGACCCAACCACAAAT-3′; and reverse, 5′-AGC TGCGCAGAATGAGATGAG-3′. The primer sequences for human IL-8 were: forward, 5′-CACCGGAAGGAACCATCTCAC-3′; and reverse, 5′-TGGTCCACTCTCAATCAC

TCTCAG-3′. The primer sequences for human GFAP were: forward, 5′-ACACGTCTGACCCTCTCCAC-3′; and reverse, 5′-TGCTCGTGCCTCAGTTTTAC-3′. The primer sequences for human GAPDH were: forward, 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′; and reverse, 5′-CGGAGTCAACGGATTTGGTCG-3′. Quantitative real-time PCR analysis was performed using SensiFAST SYBR LO-ROX (Bioline, Canada) on an Applied Biosystems Real-time PCR 7500 system (ABI 7500, Appied Biosystems). Threshold cycle (C_t) value of each target gene was normalized to the expression of housekeeping gene, GAPDH. The difference between C_t value of the target gene and GAPDH of cadmium-treated cells was subtracted from the difference between C_t value of the target gene and GAPDH of untreated cells and expressed as relative fold