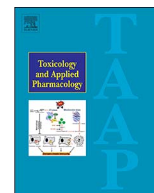
Toxicology and Applied Pharmacology 342 (2018) 108–115

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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



Cadmium disrupts signaling of the hypoxia-inducible (HIF) and transforming growth factor (TGF-β) pathways in placental JEG-3 trophoblast cells via reactive oxygen species

Oluwadamilare A. Adebambo[a], Damian Shea[a], Rebecca C. Fry[b,*]

[a] Department of Biological Sciences, North Carolina State University, USA

[b] Department of Environmental Science and Engineering, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, USA

ARTICLE INFO

Keywords:
Reactive oxygen species
Hypoxia
Placentation
Growth factor
Gene expression
Cadmium

ABSTRACT

Epidemiologic studies indicate an association between exposure to cadmium (Cd) and placental-related pregnancy disorders. While a precise mechanism is unknown, oxidative imbalance and dysregulation of the hypoxia inducible factor (HIF) and transforming growth factor beta (TGF-β) pathways have been implicated in placental disease pathogenesis. Here we investigated key oxidative and placentation pathways in JEG-3 placental trophoblast cells treated with Cd alone, environmental water samples predominated by Cd with low concentrations of other metals (e.g. inorganic arsenic (iAs)) collected from a waste-site, and a matched mixture of Cd and iAs prepared in the laboratory. The induction of cytosolic reactive oxygen species (ROS), expression of metallothionein (MT) isoforms, *HIF1α* and downstream targets, and expression of TGFβ pathway-associated genes and proteins were assessed. Additionally, the effect of pre-treatment with the antioxidant *N*-acetyl cysteine (NAC) on ROS generation and effects on HIF, MT and TGF-β signaling pathways was examined. Cd and Cd-mixture treated cells displayed higher levels of ROSs with accompanying disruption of HIF and TGFβ pathway signaling versus controls, with the Cd-mixture eliciting a greater effect. Conversely, pretreatment with NAC reduced Cd-induced ROS production and disruption of HIF, MT and TGFβ pathway signaling. The results indicate that treatment of placental trophoblast cells with Cd results in increased production of ROSs that disrupt placentation pathways involved in disease pathogenesis. Also, co-occurrence of Cd with other toxic metals, particularly arsenic, may induce detrimental health effects that are currently underestimated when analyzed as single metals.

1. Introduction

Proper placental development requires invasion of extravillous trophoblast cells into the maternal decidua (Gude et al., 2004; Charnock-Jones and Burton, 2000). In many placental disorders, there is insufficient trophoblast differentiation and invasion (Kaufmann et al., 2003; Chisolm and Handorf, 1996; Bronsens et al., 1972). This dysfunction in trophoblast differentiation/invasion can result in pregnancy complications such as preeclampsia (PE) and restricted fetal growth (Bronsens et al., 1972; Chisolm and Handorf, 1996; Walsh and Wang, 1995).

In relation to the environment, exposure to the toxic metal cadmium (Cd) has been associated with pregnancy disorders (Laine et al., 2015; Fowler, 2009; Zhang et al., 2004; Chisolm and Handorf, 1996). The mechanisms for Cd toxicity include production of oxidative stress (Liu et al., 2009; Jomova et al., 2011; Shaikh et al., 1999; Waisberg et al., 2003) and modulation of reactive oxygen species (ROS)-related gene expression (Liu et al., 2009). In addition, imbalances in the oxidant/antioxidant activity in utero–placental tissues play pivotal roles in the development of placental disorders (Myatt and Cui, 2004; Jauniaux et al., 2006; Hempstock et al., 2003; Poston and Raijmakers, 2004; Sugino et al., 2007; McCarthy and Kenny, 2016).

Placental trophoblast differentiation is oxygen-regulated and mediated by the transforming growth factor beta (TGFβ) pathway through hypoxia-inducible factor 1 (HIF-1) transcription factors (Caniggia et al., 2000). HIF-1 is a heterodimer comprising HIF-1α and HIF-β subunits that mediates adaptive responses to changes in tissue oxygenation (Caniggia et al., 2000; Semenza, 2003). In relation to pregnancy, placental HIF-1α and its targets have been shown to be altered in circulating blood of women with PE (Jauniaux et al., 2006;

* Corresponding author at: Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, 135 Dauer Drive, CB 7431, University of North Carolina at Chapel Hill, NC 27599, USA.

E-mail address: rfry@unc.edu (R.C. Fry).

https://doi.org/10.1016/j.taap.2018.01.010
Received 28 September 2017; Received in revised form 9 January 2018; Accepted 17 January 2018
0041-008X/ © 2018 Published by Elsevier Inc.

Redman and Sargent, 2009; Caniggia et al., 2000). HIF-1 also increases the transcription of genes including the TGFβ pathway and angiogenic factors such as the vascular endothelial growth factor (VEGF). TGFβs are multifunctional growth factors that regulate cell proliferation, migration, differentiation, and apoptosis (Lala and Chakrabothy, 2003; Piek et al., 1999; Goumans et al., 2003). In-vitro, TGFβs are involved in the regulation of trophoblast differentiation and invasion during placental development (Lala and Chakrabothy, 2003; Caniggia et al., 1999; Xu et al., 2016). Elevated expression of TGFβ3 has been demonstrated in the preeclamptic placentae and TGFβ3 inhibition of trophoblast differentiation and invasion (Caniggia et al., 1999; Pang and Xing, 2003).

In the present study, we investigated ROS induction and HIF-1α and VEGF gene expression in JEG-3 cells exposed to Cd alone or in the context of a mixture with inorganic arsenic (iAs). We further investigated the mRNA expression and protein secretion of TGFβ pathway members following Cd treatments. The mixture was collected as a sample from the Longjiang River, the site of the 2012 cadmium spill in Guangxi Province China (Zhang et al., 2013). To assess uniformity to the environmental mixtures, comparable laboratory samples were prepared using the same concentrations of iAs and Cd present in the environmental samples. Previous studies, including from our laboratory, have shown that concurrent exposure to mixtures of iAs and Cd may result in additive or synergistic effects that are not seen in single component exposures (Madden, 2002; Wang and Fowler, 2008; Liu et al., 2000; Adebambo et al., 2015). While it has been shown that Cd exposure can result in oxidative stress (Choi and Alam, 1996; Andrews, 2000; Jomova and Valko, 2011), few studies have investigated the mechanistic association between Cd exposure and placental disease outcomes via oxidative stress (Llanos and Ronco, 2009; Jomova and Valko, 2011). This is among the first studies to examine differences in regulation of placentation pathways relative to metal exposure and oxidative stress as it relates to disease pathogenesis.

2. Materials and methods

2.1. Water sample collection

Environmental water samples were collected from the Longjiang River in China using a diffusive gradient in thin film (DGT) passive sampler (DGT Research Ltd., Lancaster, UK) as detailed elsewhere (Adebambo et al., 2015). The waters had dissolved Cd ranging from 27 ± 2.5 μg/l (0.24 ± 0.02 μM) up to 623 ± 67 μg/l (5.54 ± 0.61 μM). Thus, these samples represent very high levels of real-world exposure to Cd. The extracts eluted from the DGT samplers were analyzed using inductively coupled mass spectroscopy (ICP-MS). For the purposes of this study, the concentrations of iAs and Cd metal-mixture from the DGT elution samples used for the in-vitro assays are shown in Table 1. Our analysis focused on the combined effects of iAs and Cd as they have known similarities in their toxicity mechanisms (He and Ma, 2009; Fowler et al., 2004). Although other metals (copper, lead and zinc) were present in these waters, their concentrations were either several orders of magnitude below those of Cd and/or they have no known interactive effects with Cd (Supplementary Data, Table 1).

Table 1
Sample composition, pre-dilution and post-dilution concentrations. Post-dilution concentrations are the treatment/exposure concentrations used in in-vitro assays.

		Pre-dilution concentration (μM)		Post-dilution concentration (μM)	
Sample ID	Sample Source	iAs	Cd	iAs	Cd
0.6 μM Cd-EM	Longjiang River	0.21	6.07	0.02	0.6
1.0 μM Cd-EM	Longjiang River	0.21	6.07	0.034	1.0
0.6 μM Cd-LM	$NaAs_2O_3$/ $CdCl_2$	–	–	0.02	0.6
1.0 μM Cd-LM	$NaAs_2O_3$/ $CdCl_2$	–	–	0.034	1.0

2.2. Study treatment design

Six total treatments were used for the in vitro assays. The first two treatments were carried out at 0.6 and 1.0 μM Cd respectively. The next two represented dilutions of the environmentally collected samples with Cd, referred to as the Cd-environmental mixture (Cd-EM). These were diluted to the same concentrations of Cd as the single cadmium exposure but also contained iAs (0.6 μM Cd; 0.06 μM iAs) and (1.0 μM Cd; 0.1μM iAs). The last two were laboratory-generated treatments referred to as Cd-laboratory mixture (Cd-LM) with the same concentrations of Cd and iAs as the environmental samples. The concentrations of Cd selected in this study are similar to those observed in the placentae of women globally (Kippler et al., 2010; Esteban-Vasallo et al., 2012).

2.3. Cell culture and treatment

The JEG-3 choriocarcinoma cell line was purchased from the ATCC (Manassas, VA). JEG-3 cells were grown in Dulbecco's modified Eagle's Minimum Essential Medium, supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin and 1 mM sodium pyruvate at 37C in 5% CO_2. JEG-3 cells were cultured in a 6-well culture plate for 24 h at 0.5×10^6 cells per well. Cells were serum starved overnight in 2 ml of serum-free DMEM supplemented with 1% penicillin/streptomycin, and 1 mM sodium pyruvate. Cells were then either immediately exposed to one of the six Cd treatments listed above (0.6 μM Cd, 1.0 μM Cd, 0.6 μM Cd-EM, 1.0 μM Cd-EM, 0.6 μM Cd-LM and 1.0 μM Cd-LM) or pre-treated with *N*-acetyl cysteine for 1 h followed by Cd treatments. NAC pre-treated and non-pretreated control cells were treated with dilute nitric acid at (pH 5.0). All the cells in each treatment group were exposed in triplicates for a total of 48 h. Cells were then harvested for RNA isolation. To assess cytotoxic effects of the treatment conditions, cells were treated with exact conditions above followed by a 3 h incubation with 1.5 mg/ml Resazurin. Cytotoxicity was assessed through the ability of viable cells to convert resazurin to the fluorescent resorufin product (Supplementary Data, Fig. 1). None of the treatment concentrations selected were found to be cytotoxic at the 48-h time-point and were well below the IC50 of Cd in JEG-3 cells of 38 μM (data not shown).

2.4. Intracellular ROS detection

The intracellular ROS concentration was measured using OxiSelect intracellular ROS assay kit with green fluorescence (Cell Biolabs, San Diego, CA, USA). JEG-3 cells were seeded in a black, clear-bottom 96-well culture plate overnight at 1.0×10^5 cells per well. Cells were then either immediately exposed to one of the six Cd treatments or pre-treated with *N*-acetyl cysteine for 1 h followed by one of the Cd treatments. After 48 h, the cells were incubated with 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA) probe (20 μM) for 1 h at 37 °C and 5% CO_2. DCFH-DA diffuses into cells and is deacetylated by cellular esterases to non-fluorescent DCFH, which is oxidized to fluorescent 2′, 7′-dichlorodihydrofluorescein (DCF) by ROS (Eruslanov and Kusmartsev, 2010). Following incubation, the fluorescence intensity of the cells was read on Promega® micro-plate reader at an excitation/emission wavelength of 560/590 nm. The fluorescence intensity is proportional to cytosolic ROS levels and was measured against a DCF standard.

Download English Version:

https://daneshyari.com/en/article/8538801

Download Persian Version:

https://daneshyari.com/article/8538801

Daneshyari.com