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Effect of hemin, baicalein and heme oxygenase-1 (HO-1) enzyme activity inhibitors on Cd-induced accumulation of HO-1, HSPs and aggresome-like structures in *Xenopus* kidney epithelial cells

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ABSTRACT

Cadmium is a highly toxic environmental pollutant that can cause many adverse effects including cancer, neurological disease and kidney damage. Aquatic amphibians are particularly susceptible to this toxicant as it was shown to cause developmental abnormalities and genotoxic effects. In mammalian cells, the accumulation of heme oxygenase-1 (HO-1), which catalyzes the breakdown of heme into CO, free iron and biliverdin, was reported to protect cells against potentially lethal concentrations of $CdCl_2$. In the present study, $CdCl_2$ treatment of A6 kidney epithelial cells, derived from the frog, *Xenopus laevis*, induced the accumulation of HO-1, heat shock protein 70 (HSP70) and HSP30 as well as an increase in the production of aggregated protein and aggresome-like structures. Treatment of cells with inhibitors of HO-1 enzyme activity, tin protoporphyrin (SnPP) and zinc protoporphyrin (ZnPP), enhanced $CdCl_2$-induced actin cytoskeletal disorganization and the accumulation of HO-1, HSP70, aggregated protein and aggresome-like structures. Treatment of cells with hemin and baicalein, which were previously shown to provide cytoprotection against various stresses, induced HO-1 accumulation in a concentration-dependent manner. Also, treatment of cells with hemin and baicalein suppressed $CdCl_2$-induced actin dysregulation and the accumulation of aggregated protein and aggresome-like structures. This cytoprotective effect was inhibited by SnPP. These results suggest that HO-1-mediated protection against $CdCl_2$ toxicity includes the maintenance of actin cytoskeletal and microtubular structure and the suppression of aggregated protein and aggresome-like structures.

1. Introduction

Cadmium is a carcinogenic and toxic metal that occurs naturally in the environment or as a result of industrial pollution (Waisberg et al., 2003; Joseph, 2009; Templeton and Liu, 2010). Continuous exposure to cadmium has been associated with a number of disease states including Alzheimer's disease and lung cancer (Mendez-Armenta and Rios, 2007; Nordberg, 2009; Johri et al., 2010; Templeton and Liu, 2010). While many organs and tissues are adversely affected by cadmium, the kidney is particularly sensitive to its toxic effects (Waisberg et al., 2003; Nordberg, 2009; Johri et al., 2010). Cadmium exposure can result in a number of harmful intracellular effects including the production of reactive oxygen species (ROS), damage to DNA and its repair system and the formation of denatured and abnormal proteins, which can become aggregated and form aggresome-like structures (Waisberg et al., 2003; Bertin and Averbeck, 2006; Mouchet et al., 2006; Mendez-Armenta and Rios, 2007; Khan et al., 2015). Also cadmium exposure can induce the expression of a number of genes which are thought to be involved in counteracting its injurious effects including those encoding heme oxygenase-1 (HO-1) and heat shock proteins (HSPs; Alam et al., 2000; Ovelgönne et al., 1995; Somji et al., 2000; Music et al., 2014).

HO-1, which is the stress-inducible isozyme of heme oxygenase, catalyzes the breakdown of heme into CO, free iron and biliverdin (Platt and Nath, 1998; Hwang et al., 2009). While HO-1 is an ER protein, it lacks a signal peptide and a KDEL retention sequence (Gottlieb et al., 2012). However, HO-1 is posttranslationally inserted into the ER membrane *via* its C-terminal end (Yoshida and Sato, 1989; Gottlieb et al., 2012). Cadmium-induced HO-1 accumulation has been reported in a number of organisms including mouse, zebrafish, carp, and chicken (Sardana et al., 1982; Alam et al., 2000; Blechinger et al., 2007; Jancsó and Hermesz, 2015). Other agents were found to induce HO-1 accumulation in mammalian cultured cells including hemin and the plant flavonoid, baicalein (Alam et al., 2000; Brouard et al., 2000; Li-Weber, 2009). Cadmium-, hemin- and baicalein-induced *ho-1* gene expression involves the Maf recognition elements (MARE)/nuclear factor-erythroid 2 family member (Nrf2) pathway (Alam et al., 2000; Alam and Cook,

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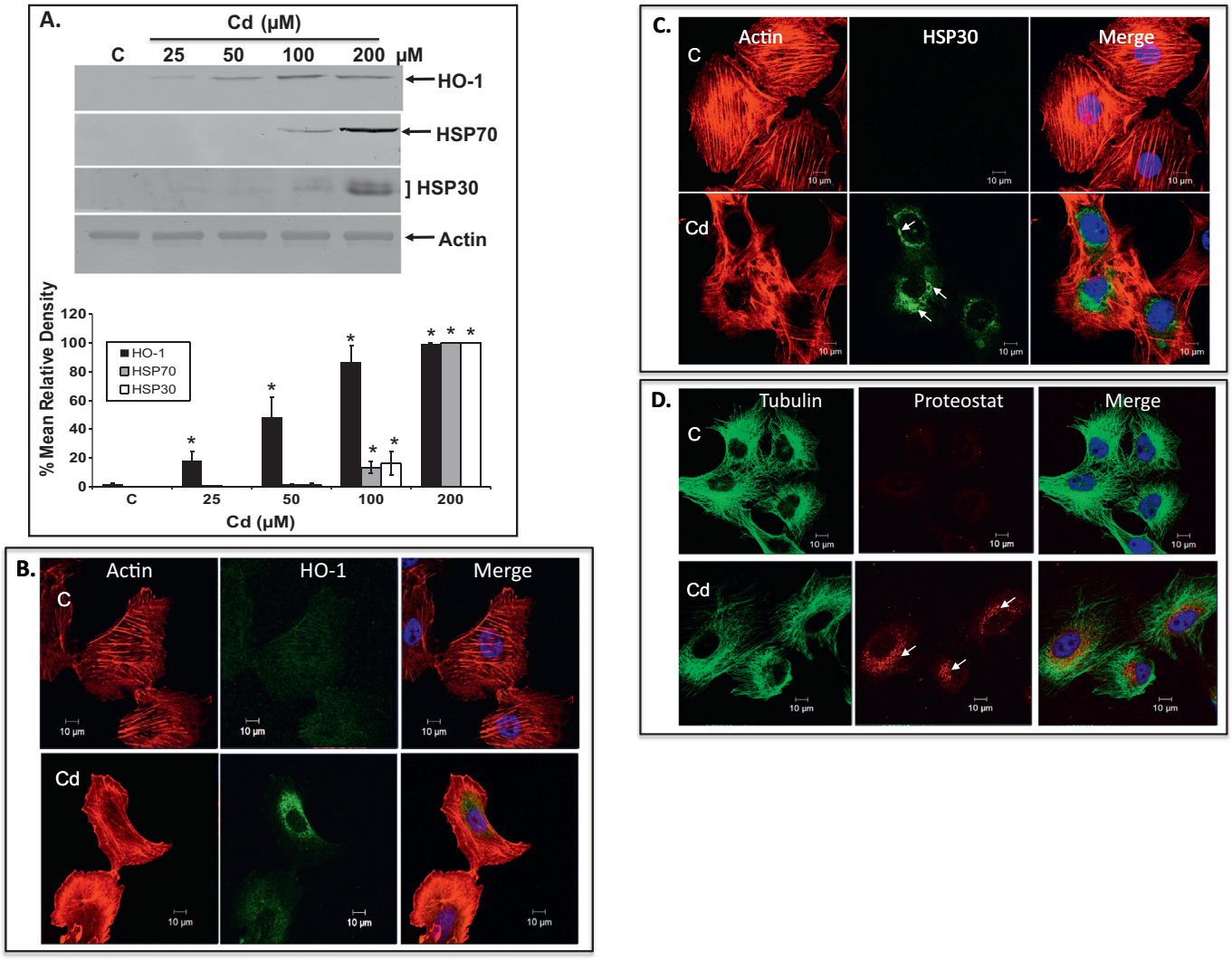

Fig. 1. Effect of $CdCl_2$ on HO-1, HSP, protein aggregation and aggresome-like structure accumulation in A6 cells. A) Cells were maintained at 22 °C (C) or treated with 25, 50, 100 or 200 μM of $CdCl_2$ (Cd) for 16 h at 22 °C. Proteins were isolated and used to generate immunoblots using anti-HO-1, anti-HSP70, anti-HSP30 and anti-actin antibodies as described in Materials and methods (representative immunoblots are shown). In densitometric analysis, the results were expressed as % mean relative density in comparison to the maximum band density obtained for each protein, which was 200 μM $CdCl_2$ for HO-1, HSP70 and HSP30. Standard error was indicated by the vertical bars. A one-way ANOVA and a Tukey's post-hoc test was used to determine significance ($p < 0.05$), as represented by an asterisk, between control cells and treated cells. These data are representative of 3 separate experiments. Immunocytochemical analysis was employed to examine the effect of $CdCl_2$ on the localization HO-1, HSP30, aggregated protein and aggresome-like structures. Cells, which were cultured on glass coverslips, were incubated in media (C) or media supplemented with 200 μM $CdCl_2$ (Cd) for 16 h at 22 °C. Nuclei and actin filaments were stained directly using DAPI (blue) and rhodamine phalloidin (red), respectively (panels B and C). HO-1 was detected with an anti-HO-1 antibody and the secondary antibody conjugate, Alexa-488 fluorophore (green; panel B). Rabbit anti-HSP30 IgG antibody and an anti-rabbit IgG antibody conjugated to an Alexa-488 fluorophore were used to detect HSP30 (green; panel C). In panel D, α-tubulin was detected using a mouse anti-α-tubulin IgG antibody and an anti-mouse IgG antibody conjugated to an Alexa-488 fluorophore (green). Aggregated protein and aggresome-like structures were detected using Proteostat dye (red). White arrows indicate aggresome-like structures. White 10 μm scale bars are indicated in the lower right of each panel. These data are representative of 3 separate trials. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2007; Macleod et al., 2009). It has been proposed that cadmium-induced *ho-1* gene expression may include the cadmium response element (CdRE) and the transcription factors HSF1 and pescadillo (Sikorski et al., 2006; Koizumi et al., 2007).

A number of studies in mammalian systems examined the cytoprotective nature of HO-1 accumulation (Brouard et al., 2000; Lang et al., 2004). For example, pretreatment of rats with hemin, which upregulated HO-1 levels, protected their kidneys and testes against damage by mercury and cadmium, respectively (Yoneya et al., 2000; Fouad et al., 2009). Baicalein-induced HO-1 accumulation was associated with protection against vascular injuries and enhanced survival of human cardiomyocytes in response to oxidative challenges (Chen et al., 2006; Cui et al., 2015). HO-1-mediated cytoprotection against oxidative stress involves its catalytic breakdown products, CO and bilirubin (Gozzelino et al., 2010; Correa-Costa et al., 2012). For example, HO-1-mediated production of CO inhibited apoptosis through its interaction with p38 mitogen-activated kinase (MAPK; Brouard et al., 2000). Also, bilirubin, a potent antioxidant, protected HeLa cells and cultured rat neurons against H_2O_2-mediated necrotic cell death (Stocker et al., 1987; Morita et al., 1997; Doré et al., 1999; Baranano et al., 2002). HO-1-induced cytoprotection against a variety of stressors was reported to be suppressed in a competitive fashion by the HO-1 enzyme activity inhibitors and heme analogs, tin protoporphyrin (SnPP) and zinc protoporphyrin (ZnPP; Srisook et al., 2005; Varga et al., 2007; Miyake et al., 2010; Wong et al., 2011). The addition of SnPP was found to sensitize mouse T cells to hypoxia by inhibiting HO-1 enzyme activity while ZnPP