SHORT COMMUNICATION

Effects of cadmium chloride on mouse inner medullary collecting duct cells

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ABSTRACT

Cadmium is a known renal toxin. The cytotoxic effect of cadmium chloride (CdCl₂) was evaluated on renal inner medullary collecting duct cells (mIMCD3). The 24 hr LC₅₀ value for CdCl₂ in mIMCD3 cells was 40 μM. The present study showed that mIMCD3 cells were sensitive to CdCl₂ exposure.

KEY WORDS: cadmium chloride; cytotoxicity; kidney; mIMCD3 cells

Introduction

Cadmium exposure is a public health concern for renal diseases, even at low levels of exposure (Ferraro et al., 2010; Kobayashi et al., 2009; Thomas et al., 2009) because the kidney is the organ most sensitive to cadmium toxicity (Järup et al., 1998). Most renal cell studies have focused less on the inner medulla although it is often exposed to high concentrations of common nephrotoxins (Burg, 2002; Rocha et al., 2001; Yancey et al., 1982). Renal inner medullary collecting duct cells (mIMCD3), which are an immortalized cell line derived from the mouse renal inner medulla, have proven a useful system to investigate effects of nephrotoxins (Cai et al., 2003; Kojima et al., 2011; Park et al., 2007; Park et al., 2008; Schenk et al., 2010). The present study investigated the effect of cadmium chloride on mIMCD3 cells.

Materials and methods

Cell culture and chemicals

This experiment was performed as previously described (Park et al., 2007; Park et al., 2008). All reagents for cell culture were purchased from Life Technologies (Carlsbad, CA, USA). Briefly, mIMCD3 cells were grown in the presence of 45% Ham’s F-12, 45% Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum (FBS), 10 milliunits/ml penicillin and 10 μg/ml streptomycin. The final osmolality of isosmotic medium was 300±5 mosmol/kg medium, which was confirmed by a microosmometer (Model 3300, Advanced Instruments, Norwood, MA, USA). Cells were grown at 37 °C and 5% CO₂. Cadmium chloride (CdCl₂) was purchased from Sigma (St. Louis, MO, USA) and dissolved in Milli-Q water (Millipore, Bedford, MA, USA) freshly.

Cytotoxicity assays

Cell viability to determine the cytotoxic effect of CdCl₂ was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Roche Applied Science, IN, USA) as described previously (Park et al., 2007; Park et al., 2008). Briefly, mIMCD3 cells were grown, trypsinized, and seeded evenly with 100 μL of medium into each well of a flat-bottomed 96-well cell culture plate (Nalge-Nunc, Rochester, NY, USA). Once confluent, the desired concentrations of CdCl₂ for testing were diluted from a stock solution, added to the wells and incubated in a humidified incubator of 5% CO₂ at 37 °C for 24 hr. Controls were the cells without CdCl₂ treatment. MTT assay was performed according to the manufacturer’s instruction. Briefly, 10 μL MTT reagent was added into each well and cells incubated for 4 hr, followed by addition of 100 μL of solubilization solution into each well. After 24 hr incubation, the ratio of absorbance at 560 nm versus 750 nm was measured with a SpectraFluor Plus microplate reader (Tecan, Durham, NC, USA).
ratio represented a measure of viable cells in each well and this ratio was normalized to controls that were run in parallel in the 96-well plate. Each condition was repeated in 8 wells and experiments were independently replicated 5 times. The concentration at which after 24 hr half of the cells for each of concentration of the toxins tested were viable (LC50) was determined. The results were expressed as percentage of cell survival compared to the control. Data were presented as mean ± S.E.M.

Results and discussion

Control (water) had no influence on the survival of mIMCD3 cells. The 24 hr LC50 value for CdCl2 in mIMCD3 cells was 40 μM in this experiment (Figure 1). The results of this study demonstrated that CdCl2 is directly toxic to mIMCD3 cells, which are well suited for this study. Previous studies reported that cadmium chloride (CdCl2) caused damage to the proximal tubular epithelium of the mammalian kidney (Järup, 2002; Prozialeck et al., 1993; Van Vleet & Schnellmann, 2003). A similar toxic effect of CdCl2 in LLC-PK1 cells (pig renal proximal tubule cell line) was found with a 24 hr LC50 value of 50 μM (Gennari et al., 2003). The cell viability at 9 hr was decreased by 38% and 45% at 25 and 50 μM CdCl2, respectively (Gena et al., 2010). CdCl2 was reported to cause DNA strand breaks, lipid peroxidation, reactive oxygen species, induction of necrosis and apoptosis, and to inhibit Na, K-ATPase (Kinne-Saffran et al., 1993; Mao et al., 2007; Mao et al., 2011; Valverde et al., 2001).

Overall, the present study revealed that cadmium chloride has a toxic effect on inner medulla areas and that mIMCD3 cells could be suited for studying the mechanisms related to CdCl2 toxicity.

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