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Short communication

Accumulation and elimination of cadmium in larval stage zebrafish following acute exposure

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Abstract

A number of recent studies have examined the impact of acute cadmium exposure on early zebrafish development at the morphological, cellular, and molecular levels. However, no information on the accumulation and elimination of cadmium during early life stages of zebrafish development has been available. Here we have quantified cadmium accumulation in larval zebrafish (*Danio rerio*) by graphite furnace atomic absorption spectroscopy following short-term acute exposure and recovery periods. Zebrafish (80 h postfertilization) were exposed to various concentrations of cadmium (0.2, 1.0, 5.0, 25, 125 μM) for 3 h. Cadmium accumulation in larvae increased with exposure concentration. After exposure at 5.0, 25, and 125 μM cadmium, the fish were allowed to recover in freshwater for 0, 12, or 24 h. Cadmium content did not show a statistically significant decrease over the recovery period when exposed to 5.0 or 25 μM cadmium, whereas significant losses over the recovery period were observed following 125 μM exposure. These results suggest that the larval zebrafish decrease total cadmium body burden only following relatively high short-term acutely toxic exposures.

Keywords: Zebrafish larvae; Cadmium; Bioaccumulation

1. Introduction

Cadmium (Cd), a heavy metal with limited biological function (Lane and Morel, 2000), is widely distributed in the environment as a result of natural and anthropogenic activities. Cd is a common pollutant in surface waters and can cause adverse effects on fish and other organisms inhabiting these bodies of water (Perceval et al., 2004; Gravel et al., 2005). Accordingly, the effect of Cd on aquatic ecosystems has been and continues to be an active area of research (e.g., Ciutat et al., 2005; Riddell et al., 2005; Stanley et al., 2005). While numerous studies have examined the effects of cadmium on juvenile and adult fish and fish cells, the mechanisms of action of cadmium in early life stages is only beginning to be addressed. Furthermore, while the long biological half-life of Cd allows it to readily bioaccumulate in exposed organisms (e.g., Ke and Wang, 2001; Savinov et al., 2003), very little about the uptake and elimination of cadmium during embryonic and larval stages of fish development is known. Elucidation of Cd accumulation is important for mechanistic interpretation of its toxic effects.

Zebrafish offer many advantages for toxicological assessment of embryonic and larval stages including small size, high reproductive potential, transparent embryos, and well-described development (reviewed in Hill et al., 2005). Larval zebrafish have been utilized to study the effects of many environmental pollutants including cadmium (Blechinger et al., 2002), insecticides (Levin et al., 2004), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Mattingly et al., 2001). The adverse effects of Cd on developing zebrafish have been investigated at the whole-body, cellular, and molecular levels. These effects include ectopic apoptosis (Chan and Cheng, 2003), morphological deformities due to altered gene expression (Cheng et al., 2000), abnormal somitogenesis (Chow and Cheng, 2003), and induction of heat shock protein 70 gene expression (*hsp70*; Blechinger et al., 2002). In the latter study, we demonstrated using a live transgenic zebrafish model that a stress-responsive

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hsp70/eGFP transgene acts as an accurate indicator of cell-specific induction of *hsp70* gene activation following acute cadmium exposure during larval development. Furthermore, this transgene responds in a dose-dependent manner at concentrations similar to those observed for morphologic indicators of early life stage toxicity and is sensitive enough to detect cadmium at doses below the median adverse effect concentration and the median lethal concentration. Here we have examined the accumulation and elimination of cadmium in whole zebrafish larvae under comparable acute exposure and recovery regimes.

2. Materials and methods

2.1. Animal care and embryo collection

Adult wild-type zebrafish were obtained from a local pet store and maintained at 28 °C in carbon-filtered tap water, with a photoperiod of 14 h. Embryos were collected and staged using standard procedures as outlined in Westerfield (1995). After collection embryos and larvae were reared in 25-mL petri dishes with system water changes daily.

2.2. Reagent and treatment solutions

Cadmium chloride hemipentahydrate ($CdCl_2 \cdot 2.5\ H_2O$; CAS No. 7790-78-5) was purchased from J.T. Baker Inc. (Phillipsburg, NJ). A 1 mM Cd stock solution was prepared in triple-distilled water. Treatment solutions are made from dilutions of the stock in carbon-filtered tap water, and exposures are conducted in sterile 25-mL petri dishes.

2.3. Cadmium treatment and sample preparation

For the acute exposures, larval zebrafish were placed in 25-mL plastic petri dishes containing 0.2, 1.0, 5.0, 25, or 125 μM Cd (0.02, 0.11, 0.56, 2.8, or 14.1 μg/mL Cd, respectively) as described previously (Blechinger et al., 2002). Untreated controls were included with all exposure groups. Exposures began when the newly hatched larvae were 80 h postfertilization (hpf) for a duration of 3 h at 28 °C. After the exposure, the larvae were rinsed several times in fresh system water to remove nonaccumulated Cd. At least three swirling rinses at 50 times dilution per rinse were performed. For recovery experiments, larvae from each Cd exposure group and control were then placed in unused petri dishes containing fresh system water to ensure no exposure to residual Cd adsorbed to original treatment dishes. Following the exposure period (i.e., no recovery) or recovery period (12 or 24 h) the larvae were transferred to preweighed microcentrifuge tubes (10 larvae per tube). Excess water was removed and the wet weight of the larvae was determined. Then 1 mL of 5% HNO_3 was added to each tube and the tubes were placed in a 65 °C water bath for 12 h to digest the tissue. The digests were allowed to settle within the tubes, and the supernatants were removed to be analyzed for Cd content. Each replicate (n) represents one set of 10 larvae; 7–15 replicates ($n = 7-15$) were analyzed and an average Cd content was calculated for each data set.

2.4. Cd quantification

Cd concentrations were measured using a graphite furnace atomic absorption spectrophotometer (Varian SpectraAA 220Z) with Zeeman background correction; 6 μL of digest sample and 6 μL 2% m/v $NH_4H_2PO_4$ (matrix modifier; Baker Analyzed A.C.S. Reagent; J.T. Baker) were hot injected at 85 °C. Operating conditions were 12 s at 95 °C and 15 s at 120 °C to dry the sample, 15 s at 500 °C, and 3 s at 2000 °C. Each sample was analyzed in duplicate. Data accuracy was monitored using quality control samples prepared from Fischer certified Cd reference standard solution and analysis of the reference material TORT-2 lobster hepatopancrease (NRC-CNRC, Ottawa, Canada). From the Cd concentration of the digested sample, the ng of Cd per g wet weight of fish tissue was determined and used for data analysis.

2.5. Data treatment

Data were normalized against average values obtained for untreated controls. One-way ANOVA with Tukey–Kramer multiple comparisons posttest was performed using GraphPad InStat version 3.05 (GraphPad Software, San Diego, CA). The limit of significance was set at $P<0.05$ throughout.

3. Results

3.1. General observations

No mortality or nonlethal effects were observed in any of the treatment or control groups over the course of the exposures and/or recovery periods. This is most likely due to the short length of the exposure periods. We have previously demonstrated that the LC_{50} and EC_{50} of Cd for zebrafish larvae determined using a 96-h acute exposure beginning at 72 hpf are 18.8 and 1.7 μM Cd, respectively (Blechinger et al., 2002), whereas the same study found no increase in mortality following a 3-h pulse exposure.

3.2. Three-hour pulse exposures and recovery

Accumulation of Cd by larval zebrafish increased with the Cd concentration of treatment solutions (Figs. 1 and 2). The Cd content in control samples averaged below 25 ng/g wet weight for exposure and recovery time points, representing any spectral interference during quantification and potential background Cd content. All samples generated following the pulse exposures had greater Cd content than control samples. Following a 3-h pulse exposure, Cd uptake was significantly different from

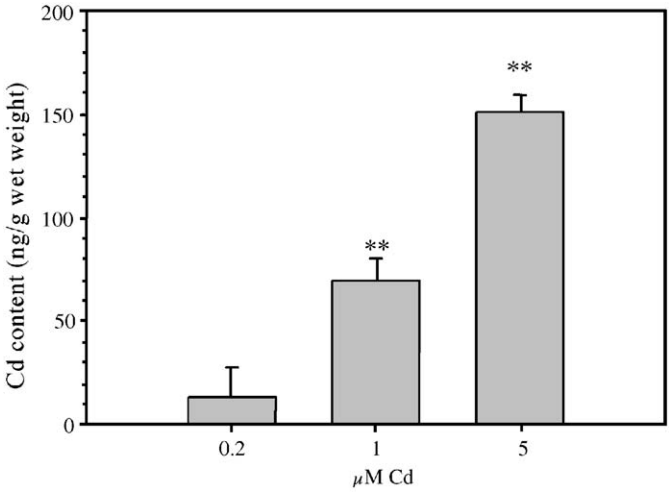

Fig. 1. Whole-body Cd content of 80-hpf larval zebrafish following 3-h exposure to 0.2, 1.0, or 5.0 μM Cd. Mean ± SE ($n = 7-15$ replicates of 10 larvae each). $^{**}P<0.001$. Data were normalized against values obtained for untreated controls.