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Hormetic effect induced by depleted uranium in zebrafish embryos

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ABSTRACT

The present work studied the hormetic effect induced by uranium (U) in embryos of zebrafish (*Danio rerio*) using apoptosis as the biological endpoint. Hormetic effect is characterized by biphasic dose-response relationships showing a low-dose stimulation and a high-dose inhibition. Embryos were dechorionated at 4 h post fertilization (hpf), and were then exposed to 10 or 100 μg/l depleted uranium (DU) in uranyl acetate solutions from 5 to 6 hpf. For exposures to 10 μg/l DU, the amounts of apoptotic signals in the embryos were significantly increased at 20 hpf but were significantly decreased at 24 hpf, which demonstrated the presence of U-induced hormesis. For exposures to 100 μg/l DU, the amounts of apoptotic signals in the embryos were significantly increased at 20, 24 and 30 hpf. Hormetic effect was not shown but its occurrence between 30 and 48 hpf could not be ruled out. In conclusion, hormetic effect could be induced in zebrafish embryos in a concentration- and time-dependent manner.

1. Introduction

Uranium (U) is a metal contaminant widely distributed in our environment (Li et al., 2015; Shao et al., 2016). It reaches the environment from different sources including U production activities (Krishnapriya et al., 2015; Phillips and Watson, 2015), U mine tailings and ore wastes (Lee and Yang, 2010) and depleted uranium (DU) munitions in conflict areas and at test sites (Crean et al., 2013). Exposures to U have aroused public health concerns due to the internal irradiation and/or chemical toxicity (Li et al., 2015; Choy et al., 2006). As a result, immense efforts have been devoted to understanding and modeling the behavior of U in the environment, such as oxidative corrosion of carbide inclusions at the surface of U metal (Scott et al., 2011), precipitation and adsorption of U and its relationship with other contaminants within the geological materials of the surface (Phillips and Watson, 2015) and translocation of U from water to foodstuff while cooking (Krishnapriya et al., 2015), etc. Extensive effort has also been spent on characterization and remediation of U contamination in the environment including chemical extraction (Choy et al., 2006; Crean et al., 2013), enrichment and separation of U from radioactive wastewater (Shao et al., 2016), removal from aqueous solution by nanoparticles and graphene composites (Li et al., 2015), U biomineralization (Choudhary and Sar, 2011), and rhizofiltration to remediate U-contaminated groundwater (Lee and Yang, 2010). A review can also be found in Gavrilescu et al. (2009). In particular, U can be found in aquatic systems at concentrations varying from 0.01 μg/l to 2 mg/l, depending on the geological background (WHO, 2001).

The biological effects of U have also been studied in non-human species but most of these studies are related to acute exposures and bioaccumulation patterns (Bywater et al., 1991; Labrot et al., 1999; Poston, 1982). It was established that U could trigger the production of free radical species (Miller et al., 2002; Yazzie et al., 2003) and could suppress the activities of enzymes related to antioxidant defenses such as catalase and superoxide dismutase in exposures to 20 and 100 μg/l of U on zebrafish (Barillet et al., 2005, 2007). A reduction in the hatching rate and a delay in hatching were also observed on zebrafish embryos after being exposed to 20 and 250 μg/l of DU (Bourrachot et al., 2008). A range of effects from exposures to environmental relevant levels of DU were studied on adult zebrafish by Barillet et al. (2011). The authors reported that U was highly bioconcentrated in fish, according to a time- and concentration-dependent model. Information related to the effects of U exposure in freshwater fish, especially that related to sublethal concentrations of U, is essential for realistic environmental risk assessment. Such information is available in the literature (Barillet

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et al., 2005, 2007; Buet et al., 2005; Cooley et al., 2000; Kelly and Janz, 2009; Labrot et al., 1996; Lerebours et al., 2009; Lourenço et al., 2010).

One important phenomenon for a low-dose environmental stressor is hormesis, which is characterized by biphasic dose-response relationships showing a low-dose stimulation and a high-dose inhibition (Calabrese, 2008; Calabrese and Baldwin, 2002; Calabrese and Linda, 2003). A comprehensive review on the mechanisms underlying the hormetic dose-response which were mediated through receptor and/or cell signaling pathways was given by Calabrese (2013). In particular, it was remarked that the hormetic dose-response was highly generalizable and was independent of the biological model in use, the endpoint measured and the chemical class (Calabrese, 2013). In fact, hormesis has been observed for a broad range of chemicals, metals, herbicides and also physical process like radiation exposure (Azzam et al., 1996; Bond et al., 1991; Calabrese and Baldwin, 1997, 2000, 2001, 2003, 2008; Choi et al., 2012; Cohen, 1995; Damelin et al., 2000; Elmore et al., 2005; Hayes, 2007; Hooker et al., 2004; Lefcort et al., 2008; Luckey, 1982; Mitchel et al., 1999; Ng et al., 2015a; Rithidech and Scott, 2008; Shadley and Wolff, 1987; Shen et al., 2009). Recently, a reduction in the apoptotic signals induced on embryos after exposure to low concentration of U was reported by Ng et al. (2015b). Different processes, including elimination of naturally aberrant cells by early apoptosis, had been proposed to explain the radiation-induced hormetic effect (Vaiserman, 2010).

As such, for realistic risk assessment of U in the environment, it is pertinent to carry out further studies to better understand the hormetic effect induced by U in living organisms. The primary objective of the present study was to study the U-induced hormetic effect in the embryos of zebrafish (*Danio rerio*) using apoptosis as the biological endpoint. In particular, the dependence on the concentration and time of the U-induced hormetic effect was also examined. The effects of a low concentration (10 μg/l) and a high concentration (100 μg/l) DU on zebrafish embryos (with 1 h exposure time) were studied at four different time points, namely, 20, 24, 30 and 48 hpf. As PTU treatment was needed when studying the embryos at 30 and 48 hpf, separate experiments were also carried out to confirm that treating zebrafish embryos with the desired concentration of PTU (i.e., 75 μM) would not affect the amount of apoptotic signals on the embryos. Zebrafish has become a popular model for studying the toxicity and biological effects of different environmental stressors because of the considerable homology between zebrafish and human genomes which include conservation of most DNA repair-related genes (Barbazuk et al., 2000).

2. Material and methods

2.1. Zebrafish embryos

Adult zebrafish (*D. rerio*) were maintained in fish tanks at 28.5 °C with a 14/10 h light-dark cycle. Spawning was triggered when the 14 h photoperiod began. A specially designed plastic collector was used to collect embryos after the start of the photoperiod (Choi et al., 2010). All embryos were collected within 15–30 min to ensure the synchronization of developmental stages of the embryos. Immediately after collection, the embryos were transferred to an incubator maintained at 28.5 °C until they developed into 4 h post fertilization (hpf). To avoid absorption of DU in the chorions instead of the embryos themselves, the chorion of each embryo was carefully removed with a pair of forceps at 4 hpf.

2.2. Depleted uranium exposure

In the present experiments, DU contamination was achieved using uranyl acetate $UO_2(CH_3COO)_2{\cdot}2H_2O$ (Electron Microscopy Sciences). The DU stock solution as well as the working solutions were prepared as described by Ng et al. (2015b). Briefly, 0.15–0.30 g/l uranyl acetate stock solutions were prepared by dissolving uranyl acetate with MilliQ water. Since uranyl acetate is light-sensitive and would precipitate when exposed to light, all stock solutions were kept in dark and maintained at 4 °C. To minimize the effects due to potential fluctuation of the DU concentration in different sets of experiments due to precipitation, a new DU stock solution was prepared independently and separately for each set of experiments. The stock solution was prepared 1 day before preforming each set of experiments to allow enough time for all the uranyl acetate to dissolve. On the day of experiment, the stock solution was further diluted to the desired concentration in E3 medium and the pH value of the working solution was maintained at 7.

2.3. Effect of DU on zebrafish embryos

2.3.1. Exposure protocol

The number of apoptotic cells within the embryos was chosen as the biological endpoint. In the present study, the effects of DU on zebrafish embryos were studied at four different time points, namely, 20 (Case 1), 24 (Case 2), 30 (Case 3) and 48 (Case 4) hpf. For each time-point study, when the embryos developed into 5 hpf, dechorionated embryos were divided into three groups, each having 10 embryos, and were accommodated in three separate Petri dishes with a layer of biocompatible agarose lining the bottoms. The three groups were referred to as:

(A) ***Control (C) group***: in which the embryos were dechorionated without receiving any further treatment;
(B) ***Low-U-dosed (U_{10}) group***: in which the embryos were exposed to 10 μg/l of DU for 1 h (from 5 to 6 hpf);
(C) ***High-U-dosed (U_{100}) group***: in which the embryos were exposed to 100 μg/l of DU for 1 h (from 5 to 6 hpf).

Uranium can be found in aquatic systems at concentrations varying from 0.01 μg/l to 2 mg/l, depending on the geological background (WHO, 2001). In the present study, two concentrations of DU (i.e., 10 and 100 μg/l) were employed to investigate the effects of DU on zebrafish embryos since these two concentrations were within the range of environmental concentrations found close to mining sites (Antunes et al., 2007) or in drilled wells (Kurttio et al., 2006). The same concentrations of DU were also employed in previous studies (Ng et al., 2015b, 2016) which were found to have successfully induced different effects on zebrafish embryos. In fact, the accumulated levels of DU in zebrafish embryo were measured using an Inductively Coupled Plasma-Mass Spectrometry (ICP-AES, Detection Limit = 9.3 $\mu g\,l^{-1}$) after being digested in 3 M nitric acid at 105 °C for 5 h. However, the accumulated levels of DU in the zebrafish embryos were all below the detection limit of our ICP-AES. The flows of the experiments involving embryos in these three groups were schematically shown in Fig. 1.A volume of 3 ml of E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, 0.1% methylene blue), 3 ml of DU working solution with a concentration of 10 μg/l (diluted in E3) or 3 ml of DU working solution with a concentration of 100 μg/l (diluted in E3) was used in each Petri dish for the ***C***, ***U_{10}*** or ***U_{100}*** groups, respectively. The levels of U in the working solutions (10 and 100 μg/l) were measured using an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES, Optima 2100DV, Perkin-Elmer, Wellesley, MA, USA, detection limit of 9.3 $\mu g\,l^{-1}$) after acidification with 0.3 M nitric acid. Exposures of embryos in the ***U_{10}*** and ***U_{100}*** groups to DU started at 5 hpf