Journal of Environmental Radioactivity 154 (2016) 25–33

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

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

Depleted uranium induces sex- and tissue-specific methylation patterns in adult zebrafish

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ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form
15 December 2015
Accepted 9 January 2016
Available online 29 January 2016

Keywords:
Zebrafish
Depleted uranium
DNA methylation
Sex dependency

ABSTRACT

We examined the effects of chronic exposure to different concentrations (2 and 20 μg L^{-1}) of environmentally relevant waterborne depleted uranium (DU) on the DNA methylation patterns both at HpaII restriction sites (5′-CCGG-3′) and across the whole genome in the zebrafish brain, gonads, and eyes. We first identified sex-dependent differences in the methylation level of HpaII sites after exposure. In males, these effects were present as early as 7 days after exposure to 20 μg L^{-1} DU, and were even more pronounced in the brain, gonads, and eyes after 24 days. However, in females, hypomethylation was only observed in the gonads after exposure to 20 μg L^{-1} DU for 24 days. Sex-specific effects of DU were also apparent at the whole-genome level, because in males, exposure to 20 μg L^{-1} DU for 24 days resulted in cytosine hypermethylation in the brain and eyes and hypomethylation in the gonads. In contrast, in females, hypermethylation was observed in the brain after exposure to both concentrations of DU for 7 days. Based on our current knowledge of uranium toxicity, several hypotheses are proposed to explain these findings, including the involvement of oxidative stress, alteration of demethylation enzymes and the calcium signaling pathway. This study reports, for the first time, the sex- and tissue-specific epigenetic changes that occur in a nonhuman organism after exposure to environmentally relevant concentrations of uranium, which could induce transgenerational epigenetic effects.

1. Introduction

Anthropogenic activities increase the levels of environmental pollutants, raising concerns about the risks associated with the exposure of human and nonhuman populations to these pollutants. The importance of genetic factors in the biological disorders and pathologies observed after chronic exposure to pollutants is well established. However, recent work has highlighted the role of the chemical modification of DNA and its associated proteins, collectively called “epigenetic” (i.e., “around genes”) mechanisms, in biological disorders. Epigenetics is defined as “the study of the changes in gene expression that occur in organisms with differentiated cells, and the mitotic inheritance of given patterns of gene expression … which [are] not based on changes in DNA sequence” (Holliday, 1994). It includes the study of three types of modification: DNA methylation (occurring primarily on cytosine when followed by guanine, the CpG configuration), histone tail modifications (acetylation, ubiquitination, phosphorylation, or methylation), and noncoding RNA molecules.

DNA methylation is one of the most frequently studied epigenetic mechanisms, playing a key role in the regulation of gene expression (Bird, 1992), and it is highly disturbed in cancer cells. The loss of DNA methylation in the promoters of oncogenes was one of the first epigenetic changes identified in human cancer (Feinberg and Vogelstein, 1983). Epigenetic changes have also been linked to neurodegenerative diseases (Garza-Manero et al., 2014). Importantly for ecotoxicology, the modification of DNA methylation patterns may be propagated across generations, with

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http://dx.doi.org/10.1016/j.jenvrad.2016.01.004
0265-931X/

transgenerational effects (Angers et al., 2010). Moreover, unlike modifications in the DNA sequence, DNA methylation may occur rapidly in response to environmental changes and may therefore be a way that an organism copes with environmental stress on a very short time scale (Rando and Verstrepen, 2007).

Within this general framework, the present study focuses on uranium (U), an actinide naturally present in the environment, whose concentrations in the geosphere are expected to increase with the growing world nuclear energy capacity (IAEA, 2012). Uranium is an isotopic mixture of three isotopes: ^{238}U (99.27%), ^{235}U (0.72%) and ^{234}U (0.0055%), and can be found naturally in the environment, in surface and ground waters at concentrations ranging from 0.01 to 12 400 μg L^{-1} (Salonen, 1994). It is widely used in many industries (nuclear power plants, weapons, airplanes, etc.) and is also present in agricultural products, such as phosphate fertilizers, thus increasing its presence and distribution in all environmental compartments. Particularly, due to it fissile properties, ^{235}U-enriched uranium is used as a combustible in the nuclear industry, following an enrichment process also producing residual material called depleted uranium (DU). DU radioactivity is half of natural uranium (NU) radioactivity (1.47 × 104 Bq g^{-1} versus 2.52 × 104 Bq g^{-1} in the natural isotopic composition) due to the depletion in ^{235}U and ^{234}U. Depleted uranium is used in many applications, such as armor penetrators, counterweights in airplanes or even in irradiation protection shields in medical facilities (Darolles et al., 2010), which represent supplemental sources of uranium contamination in the environment. Both NU and DU have a chemical toxicity (similar in both isotopic mixtures) and a low radiological toxicity (Mathews et al., 2009). However, in the radioecological context, which involves low concentrations of exposure (tens of μg L^{-1}), radioactive levels are really low. Indeed, if we consider a concentration of 20 μg L^{-1}, it represents a radiological activity of 0.294 and 0.504 Bq L^{-1} for DU and NU respectively, levels which are of the same order of magnitude than the average natural radioactivity of surface waters in France which is 0.370 ± 0.240 Bq L^{-1} (Mathews et al., 2009).

The effects of depleted uranium (DU) have predominantly been studied in freshwater organisms chronically exposed to environmentally relevant concentrations of it. Several studies of the zebrafish have been conducted, highlighting the various biological disorders caused by DU, such as reproductive and developmental toxicity (Bourrachot et al., 2014), histological changes (Barillet et al., 2010), neurotoxicity (Barillet et al., 2011; Faucher et al., 2012), genotoxicity (Barillet et al., 2011), and the disruption of transcriptional processes (Lerebours et al., 2010a, 2009). However, there are few data on the possible roles of uranium on epigenetic changes. To our knowledge, the only published data describing the epigenetic changes induced by uranium exposure deal with mice surgically implanted with DU pellets (Miller et al., 2009), and an epidemiological study of a cohort of uranium mine workers (Su et al., 2006).

The aim of this study was to establish whether chronic exposure to environmentally relevant concentrations of DU modifies DNA methylation in the zebrafish, and the possible influence of sex on this phenomenon. Two concentrations of DU were tested, 2 and 20 μg L−1, corresponding to environmentally relevant U concentrations. Indeed, for U, the 2 μg.L^{-1} concentration corresponds to the European natural geochemical background of U in surface water, which ranges from 0.02 to 6 μg L−1, and the highest concentration, 20 μg.$L^{-1,}$ can be found in the vicinity of uranium mining areas (Gagnaire et al., 2015).

DNA methylation was examined in the brain, gonads, and eyes of the fish. The two somatic organs were chosen because they are involved in important biological functions and are linked with effects manifesting at the level of the individual organism, whereas changes in gonadal tissues might exert transgenerational effects, affecting the progeny of the exposed fish. DNA methylation was determined at the genome-wide level using high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) (Ravanat et al., 2002) and at specific cleavage sites with methylation-sensitive amplified fragment length polymorphism (MS-AFLP) (Xu et al., 2000).

2. Materials and methods

2.1. Experimental procedures

Adult zebrafish (*Danio rerio*, AB strain) were purchased from a French supplier (Gis Amagen, Gif-sur-Yvette, France). Fifty males (0.24 ± 0.07 g wet weight [w.w.)], 3.00 ± 0.21 cm) and females (0.33 ± 0.07 g w.w., 3.15 ± 0.29 cm) were placed separately in 30 L tanks to avoid any interference by reproductive processes, with synthetic soft water ($CaCl_2[2H_2O]$ 42.49 mg L^{-1}, $MgCl_2[6H_2O]$ 19.30 mg L^{-1}, $MgSO_4[7H_2O]$ 24.65 mg L^{-1}, Na_2CO_3 0.78 mg L^{-1}, KCl 11.33 mg L^{-1}, and $NaNO_3$ 26.35 mg L^{-1}), oxygenated by bubbling with air. This type of water was chosen to optimize the bioavailability of DU (Denison, 2004). The fish were acclimated to the synthetic water for 3 weeks, and then exposed to DU for 24 days. A water renewal system involving peristaltic pumps was used to change 50% of the total water volume in each tank automatically daily. The fish were fed daily with standard fish pellets (5% of body mass per fish per day; Tetramin™, Melle, Germany). The temperature was set at 28 ± 1 °C, and the pH at 6.5 ± 0.1 with HCl using peristaltic pumps controlled by automated pH regulators (Consort R301, Illkirch, Belgium). The pH and water composition were chosen to ensure a good balance between the maximal bioavailability of DU and the optimal physiological conditions of the zebrafish. During acclimation and exposure, the stocking density was 1.7 fish L^{-1} and the light cycle consisted of 12/12 h (day/night).

The fish were divided into three different groups: a control group (no added DU), and two groups exposed to either 2 or 20 μg L^{-1} DU ($UO_2(NO_3)_2$-$6H_2O$; Sigma, Lezennes, France). The dissolved DU concentrations were monitored using filtered and acidified water samples, which were analyzed several times a day. To ensure that the concentration in each tank was as close as possible to the nominal concentration, the peristaltic pump system distributed DU from a stock solution at the concentration required in the contaminated tanks. The values measured throughout the exposure phase are given in the supplementary data (Table S1).

At each sampling time, five fish were taken from each tank and dissected. The brains, gonads, and eyes were cut into two parts to allow MS-AFLP and HPLC–MS/MS analyses comparison on the same fish. Given the small size of the zebrafish organs, DNA methylation and uranium accumulation could not be measured on the same individuals.

During the experiment, no differences in fish length or weight were observed between DU exposure conditions or sampling times. All procedures used in this experiment were approved by the Animal User and Ethical Committee at the Institut de Radioprotection et de Sûreté Nucléaire (Comité d'éthique IRSN n°81).

2.2. Uranium quantification

After exposure for 7 or 24 days, 3–5 fish were taken from each tank to measure the bioaccumulation of DU in the target organs. The tissues were dried at 50 °C for 48 h and the dry weight [d.w.] was measured with a microbalance (Sartorius SE2, Göttingen, Germany). The tissues were chemically digested for 3 h at 100 °C with 70% HNO_3, followed by 30% H_2O_2 until complete solubilization. The solutions of the digested organs were then evaporated to dryness. The samples were acidified with 2% (v/v) HNO_3 69% (trace-