Ecotoxicology and Environmental Safety 165 (2018) 662–670

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Response of the freshwater mussel, *Dreissena polymorpha* to sub-lethal concentrations of samarium and yttrium after chronic exposure

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ARTICLE INFO

Keywords:
Rare earth elements
Bioaccumulation
Inflammation
Genotoxicity
Gene expression
Mechanism of action

ABSTRACT

Samarium (Sm) and yttrium (Y) are commonly used rare earth elements (REEs) but there is a scarcity of information concerning their biological effects in non-target aquatic organisms. The purpose of this study was to determine the bioavailability of those REEs and their toxicity on *Dreissena polymorpha* after exposure to increasing concentration of Sm and Y for 28 days at 15 °C. At the end of the exposure period, the gene expression of superoxide dismutase (*SOD*), catalase (*CAT*), metallothionein (*MT*), glutathione-S-transferase (*GST*), cytochrome c oxidase 1 (*CO1*) and cyclin D (*Cyc D*) were analysed. In addition, we examined lipid peroxidation (LPO), DNA strand breaks (DSB), GST and prostaglandin cyclooxygenase (COX) activities. Results showed a concentration dependent increase in the level of the REEs accumulated in the soft tissue of mussels. Both REEs decreased *CAT* but did not significantly modulated *SOD* and *MT* expressions. Furthermore, Sm^{3+} up-regulated *GST*, *CO1* and *Cyc* D, while Y^{3+} increased and decreased *GST* and *CO1* transcripts levels, respectively. Biomarker activities showed no oxidative damage as evidenced by LPO, while COX activity was decreased and DNA strand breaks levels were changed suggesting that Sm and Y exhibit anti-inflammatory and genotoxic effects. Factorial analysis revealed that the major impacted biomarkers by Sm were LPO, *CAT*, *CO1* and COX, while *GST* gene expression, COX, *Cyc D* and *CAT* as the major biomarkers affected by Y. We conclude that these REEs display different mode of action but further investigations are required in order to define the exact mechanism involved in their toxicity.

1. Introduction

Rare earth elements (REEs) consists of 17 elements and are classified according to their ionic radii into light and heavy REEs (Fu et al., 2014). Contrary to their name, these elements are commonly found in the Earth's crust (Liang et al., 2014) and their concentrations are more abundant than those of mercury or silver (Environment Canada, 2012). This appellation refers to the lack of their concentration in pure ore deposits characteristic of other elements such as gold (Abhilash et al., 2015; Thomas et al., 2014). In the recent decades, REEs have undergone a steady use in several industrial, medical and agriculture applications (González et al., 2015; Pagano et al., 2015). Samarium (Sm) and Yttrium (Y) are among the most commonly used REEs (Chua, 1998). Y was the first REE discovered in 1794 (Hayashi et al., 2006) and is a promising element for high technology materials (Lobinger et al., 2005) such as electronic devices including television, computer, miscellaneous visual color displays (Rim et al., 2013), electrodes, electrolytes, electronic filters and superconductors (Takahashi et al., 2012). However, Sm is the fifth most abundant of the REEs and is extensively used for agricultural fertilizer, magnets material synthesis and analgesic treatment for tumor patients (Zhang et al., 2014). As a consequence of industrial and agricultural practices large amounts of dissolved REEs have been discharged into the aquatic environment (Fu et al., 2014; Protano and Riccobono, 2002). It has been reported that nanomolar concentrations of the metals are usually measured in natural waters and micromolar concentrations can now be found near mining sites (Miekeley et al., 1992; Noack et al., 2014). Despite their widespread applications, there is a lack of key ecotoxicological data and only few studies have examined the long-term effects of REEs in aquatic organisms. Moreover, current literature on REE toxicity is mostly confined to reports on a few REE mainly Ce, La and Gd (Pagano et al., 2016). Therefore, there is a scarcity of information about the molecular interaction between most REEs and biological molecules in aquatic organisms. Moreover, the results of toxicological studies performed with REE are controversial and the mechanisms of action and toxicity of those elements are still unknown. For this reason, monitoring of their toxicity will become crucial as REE mining activities become developed in Canada and others countries (Abhilash et al., 2015).

In vivo studies on rats showed that Y exhibited cytotoxicity and promoted neuronal cell death by the induction of apoptotic pathways

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https://doi.org/10.1016/j.ecoenv.2018.09.047
Received 22 March 2018; Received in revised form 6 September 2018; Accepted 9 September 2018
Available online 21 September 2018

(Ding et al., 2017). Toxicological evaluation with in vitro assay reveals that Y ion can inhibit the production of active oxygen free radicals at low concentration, but it turned out contrary at high concentration (Liu et al., 2000). It was also reported that Y^{3+} can modulate the expression of several hundred genes in the brain tissue of rats (Yang et al., 2006). At high concentrations, most of genes were related to cell receptor, cell signal and transmission and ionic passage, but at lower concentrations there are mainly genes related to cell skeleton and movement, immunity, and DNA binding proteins (Yang et al., 2006). Toxicological studies indicated that subchronic exposure to Sm causes testis injury in male mice with apoptosis and mitochondrial damage (Zhang et al., 2014). It has also been reported that exposure of cultured human breast cancer cells to lowest concentrations of $SmCl_3$ for 16 months increased mitotic activity and DNA synthesis (Nie, 1992). In rat liver, results showed that $SmCl_3$ could decrease the activity of peroxidase and increase the activity of SOD but no obvious morphological changes in the liver were observed (Cui et al., 1994).

To our knowledge, there is no research report regarding the biochemical and the molecular mechanisms induced by Y and Sm on the freshwater mussel *Dreissena polymorpha*. This bivalve is considered as an organism of choice for the monitoring of metallic and organic contaminants in freshwater ecosystems. Recently, gene expression profiles of proteins involved in stress (*SOD* and *CAT*), in detoxification (*MT* and *GST*), in mitochondrial alterations (*CO1*) and in cell cycle progression (*Cyc D*) were analysed in zebra mussels exposed to gadolinium salt (Hanana et al., 2017). Overall, results showed that those genes are modulated with this lanthanide and could be involved in its mechanism of action. Mechanistic understanding at biochemical levels could increase the knowledge of the survival strategies of the organisms under stressed conditions and therefore may provide efficient tools to aid in the monitoring of REE to protect ecosystem health. The aim of this study was therefore, to assess the bioaccumulation of Sm and Y in the soft tissue of zebra mussel and to investigate their effects after chronic exposure at sublethal concentrations in order to elucidate their mechanisms of action. For this purpose, we measured gene expression of several proteins such as *SOD*, *CAT*, *MT*, *GST*, *CO1* and *Cyc D*. Moreover, to determine the biochemical alterations, we monitored the activities of GST (phase II enzyme of detoxification) and COX (inflammation), we also evaluated the oxidative damage and the genotoxicity by measuring LPO and DNA strand breaks level, respectively.

2. Material and methods

2.1. Mussel sampling and in vivo exposure

Adult zebra mussels, *D. polymorpha*, were collected in july 2015 at a reference site in the Saint-Lawrence River near the City of Montréal, Québec, Canada (45°19′50″N, 73°58′12″W). Mussels were gently cut off from the rocks, quickly transferred to the laboratory in bags filled with river water. Before the experiments, mussels were acclimated in the laboratory for 2 weeks in 50 L glass-holding aquaria filled with dechlorinated and UV-treated tap water at 15 °C, 16 h light/8 h dark cycle under aeration and were fed three times per week with concentrates of phytoplankton (Phytoplex, Kent Marine, WI) and *Pseudokirchneriella subcapitata* algal preparations.

2.2. Exposure Design

A total of 300 mussels having similar shell length (20 ± 3 mm) were used in this study (Fig. 1). Mussels grouped together (byssal treads) were delicately separated and placed in 4 L containers lined with polyethylene bags. They were exposed to increasing concentrations of $SmCl_3$ and YCl_3 (10, 50, 250 and 1250 µg/L) for 28 days at 15 °C. The control group consisted of mussels exposed to aquarium water (dechlorinated and UV-treated tap water of the City of Montreal). The lanthanides exposures were conducted in triplicate (three tanks for control and three tanks for each concentration), placing in each tank (4 L) 15 mussels. Before the treatment, the baseline level of biomarkers in controls mussels between tanks was assessed and no difference was detected. Considering the high number of animals required to carry out biomarker measurements, chemical and genomic analyses, the three different exposures were conducted in the same conditions. During the exposure, the physico-chemical parameters of water were checked (Table 1S) and mussels were fed 2 h before the water renewal at every 2 days. At the end of the exposure time of 28 days, mussels exposed to the 1250 µg/L of YCl_3 were removed from this study due to the high mortality (96%) observed in this group. For each remaining concentration and for each REE, a total of 30 mussels were collected to perform chemical study (N = 10), gene expression analysis (N = 10) and biomarker measurements (N = 10). The remaining mussels were kept at −80 °C for subsequent analysis by other biomarkers. Bioaccumulation and biomarkers analyses were performed in the whole soft tissue of mussel kept at −80 °C until analysis, whereas genomic study was carried out using digestive glands of mussels. The digestive glands were excised, weighed and immediately transferred to RNA Later solution (Thermo Fisher Scientific, Ontario, Canada). Samples were then stored at −20 °C until RNA extraction and analysis.

2.3. REE bioaccumulation in mussels

All Y and Sm concentrations in water were analysed at the beginning of the exposure period (t = 1 h). As preparation of REE dilutions was done in the same way throughout the exposition, only one non-composite sample (50 mL) was taken from one tank used for each concentration tested and analysis was performed in triplicate. In addition, water was also monitored before container renewal, after 2 days of exposure, considering processes such as uptake, adsorption and precipitation that can influence concentrations in static exposure experiment. Briefly, REE concentrations were determined after acidification with nitric acid (1%) (seastar grade) and analysed by ion-coupled plasma mass spectrometry (ICP-MS, XSERIES 2 ICP-MS, Thermo Scientific, USA) with a detection limit of 0.1 ng/L.

Regarding the bioaccumulation of REE in mussel tissue, we determined their concentrations at the end of the exposure period (t = 28 days). First mussels were allowed to depurate for 24 h at 15 °C to remove unbound elements. For each replicate, pools of 2 mussels per concentration (N = 10 mussels per treatment) were sampled, weighted and frozen at −80 °C until analysis. Tissues (0.22 g wet/weight) were thawed and transferred to acid-clean (HNO_3 10%) Teflon® digestion vessels. They were acid-digested with 8 mL of high purity concentrated HNO_3, 1 mL of high purity concentrated HCl, and 2 mL of high purity 31% H_2O_2 (Baseline®, Seastar Chemicals Inc.) and then digested during 2 h at 180 °C, using a microwave digestion system (Ethos EZ, Milestone Scientific®). Digestion volumes were then adjusted to 12 mL with deionized water. Total Sm and Yt concentration was afterwards measured by ICP-MS (XSERIES 2 ICP-MS, Thermo Scientific, USA) with a detection limit of 0.2 mg/kg (wet weight^{-1}).

2.4. Biomarkers analyses

Soft tissues were weighted and homogenized in 1:5 (W/V) ratio of buffer solution containing 25 mM Hepes-NaOH buffer, 100 mM NaCl, 0.1 mM dithiothreitol and 1 µg/L of aprotinin at pH 7.4. The level of lipid peroxidation (LPO) and DNA damage (DSB) were measured in the homogenate. However, cyclooxygenase (COX) and glutathione-S-transferase (GST) activities were determined in the S15 fraction which was obtained by centrifuging the homogenate at 15,000 × *g* for 20 min at 2 °C. Protein content in the supernatant and the homogenate was estimated using a standard solution of albumin for calibration (Bradford, 1976).

LPO was assessed by the detection of thiobarbituric acid-reactive substances (TBARS) by fluorescence at 540 nm and 590 nm for