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Acute selenium selenite exposure effects on oxidative stress biomarkers and essential metals and trace-elements in the model organism zebrafish (*Danio rerio*)

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ABSTRACT

Selenium (Se) is an essential trace-element that becomes toxic when present at high concentrations. Little is known regarding Se effects on parameters such as oxidative stress biomarkers. The aim of the present study was to investigate the effects of acute selenium exposure on oxidative stress biomarkers in a model organism, zebrafish (*Danio rerio*). Fish were exposed to selenium selenite at 1 mg L^{-1}. Reduced glutathione (GSH), and metallothionein (MT) concentrations were determined in liver, kidney and brain, with MT also being determined in bile. Essential metals and trace-elements were also determined by inductively coupled mass spectrometry (ICP-MS) in order to verify possible metal homeostasis alterations. GSH concentrations in liver, kidney and brain increased significantly (1.05 ± 0.03 μmol g^{-1} ww, 1.42 ± 0.03 μmol g^{-1} ww and 1.64 ± 0.03 μmol g^{-1} ww, respectively) in the Se-exposed group when compared to the controls (0.88 ± 0.05 μmol g^{-1} ww, 0.80 ± 0.04 μmol g^{-1} ww and 0.89 ± 0.03 μmol g^{-1} ww for liver, kidney and brain, respectively). MT levels in Se-exposed liver (0.52 ± 0.03 μmol g^{-1} ww) decreased significantly in comparison to the control group (0.64 ± 0.02 μmol g^{-1} ww), while levels in bile increased, albeit non-significantly. This is in accordance with previous studies that indicate efficient biliary MT action, leading to a rapid metabolism and elimination of contaminants from the body. Levels in the brain increased significantly after Se-exposure (0.57 ± 0.01 μmol g^{-1} ww) when compared to the control group (0.35 ± 0.03 μmol g^{-1} ww) since this organ does not present a detoxification route as quick as the liver-gallbladder route. Several metal and trace-elements were altered with Se-exposure, indicating that excess of selenium results in metal dyshomeostasis. This is the first report on metal dyshomeostasis due to Se-exposure, which may be the first step in the mechanism of action of selenium toxicity, as is postulated to occur in certain major human pathophysiologies.

1. Introduction

Selenium (Se) is an essential element for all living organisms. The effects of selenium supplementation in fish are well-known, since cultured fish show improved nutritional profiles and increased capability to withstand stress when supplemented with this element [1]. Moderate concentrations of selenium can be stored in the organism and are used to maintain homeostatic functions [2]. This element, however, can be an important toxicological hazard, due to the very narrow range between its essentiality and toxicity [1].

Selenium toxicity mechanisms are not well-understood. Toxicity has been ascribed in the past to its sulfur-like chemical characteristics, removing sulfhydryl groups essential to cellular

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oxidative processes, and its tendency to substitute in amino acids during protein assembly [3]. Recently, however, selenium toxicity has been suggested as a result of oxidative damage, with reports of increased reduced glutathione and superoxide dismutase activities in Se-exposed fish [4,5]. Studies regarding Se toxicity in model organisms are still scarce, however, with most reports dealing with its protective effects against exposure to toxic elements such as mercury, lead, chromium and cadmium [6–9]. Also, most Se toxicity studies in fish have focused only on reproductive effects, such as embryonic development and egg production [2,10], although, recently, the effects of Se-exposure on certain oxidative stress biomarkers have been described [5,6]. To the best of our knowledge, no reports are available on the effects of Se-exposure on other metals and trace-element concentrations in fish, which might be an important aspect of the mechanism of action of selenium toxicity.

In addition, some Se-exposure studies report the determination of different biochemical parameters, such as enzymes linked to oxidative stress and hematological variables, including glutathione peroxidase, GSH, lipid peroxidation, cortisol, glucose, T3 and T4 levels [5,11]. Metal exposure biomarkers in Se-exposed fish, however, such as metallothionein (MT), have not been investigated in a selenium exposure context, only in studies regarding the protective role of selenium after exposure to toxic metals, such as cadmium [12], or in studies regarding free-ranging fish environmentally exposed to selenium [13]. This metalloprotein is of interest in a Se-exposure context, since it known to bind to this element and also exhibits free radical scavenging activity [14,15].

The model organism Zebrafish (*Danio rerio*) is frequently used in different fields of research, such as genetics, regenerative medicine, toxicology and the study of cancer and neurobiology, among others [16–18]. As a model system, this species possesses several advantages, such as the fact its genome has been completely sequenced and it is extremely similar to mammal models, including humans [19].

In this context, the aim of the present study was to investigate the effects of selenium exposure on GSH, an oxidative stress biomarker, and MT, a metal-exposure biomarker that also exhibits free radical scavenging activity, and essential metals and trace-elements on a model organism, zebrafish (*D. rerio*).

2. Methodology

2.1. Samples

Zebrafish were obtained from a local pet store. All animals were the same age. Two groups, a control group ($n = 10$) and a group exposed to selenium ($n = 10$) were maintained in a static system for 96 h in 15 L aquaria containing dechlorinated tap water, pH 7.0 at $25 \pm 2\,^{\circ}C$. Selenium selenite (Synth®, Rio de Janeiro) was dissolved in 50 mL of the same water and added slowly to the exposed-group aquarium in order to obtain a final Se concentration of 1 mg L^{-1}. After the exposure period, animals were then sacrificed and dissected with the aid of a 100× stereomicroscope. Whole brains, livers, kidneys and gallbladders were removed. 10 brains, 10 livers, 10 kidneys and 4 gallbladders (bile) were used from the control group, and 10 brains, 7 livers, 10 kidneys and 8 gallbladders (bile) were used from the selenium-exposed group. As the mass for each organ was very small, samples were pooled and analyzed in triplicate.

2.2. MT extraction and determination

MT extraction followed the protocol proposed by Erk et al. [20], using heat treatment. Briefly, samples were thawed and immediately homogenized at a 3:1 ratio in sterile eppendorfs in a solution containing Tris–HCl 20 mmol L^{-1} pH 8.6, phenyl methyl sulphonyl fluoride 0.5 mmol L^{-1} as an antiproteolytic agent and β-mercaptoethanol 0.01% as a reducing agent. They were then centrifuged at 20,000 × *g* for 1 h at 4 °C. The supernatants were then carefully separated from the pellet and transferred to new sterile eppendorfs and heated at 70 °C for 10 min. Another centrifugation step was conducted at 20,000 × *g* for 30 min at 4 °C and the final supernatants containing the MTs were separated and frozen at −80 °C until analysis. The MT supernatant was treated with HCl 1 mol L^{-1} containing EDTA 4 mmol L^{-1} and NaCl 2 mol L^{-1} containing 0.43 mmol L^{-1} 5,5-dithiobis-2-nitrobenzoic acid buffered with 0.2 mol L^{-1} Na-phosphate, pH 8 [21], and incubated for 30 min. The samples were then centrifuged at 3000 × *g* for 5 min and the supernatant absorbance was evaluated at 412 nm on a spectrophotometer (Lambda 35, PerkinElmer, USA). MT concentrations were estimated utilizing reduced glutathione (GSH) as an analytical standard curve, by assuming the relationship of 1 mol MT = 20 mols GSH, as described by Kagi for fish [22]. Determinations were conducted in triplicate.

Table 1
Conditions of the ICP-MS used in the present study.

ICP-MS conditions	
Forward power	1100 W
Plasma gas flow rate	17.0 L min^{-1}
Auxiliary gas flow rate	1.2 L min^{-1}
Carrier gas flow rate	0.98 L min^{-1}
Sampling and skimmer cones	Pt
Dwell time	30 ms per isotope
Monitored isotopes	^{27}Al, ^{75}As, ^{59}Co, ^{65}Cu, ^{57}Fe, ^{55}Mn, ^{60}Ni, ^{82}Se, ^{51}V, ^{66}Zn

2.3. GSH extraction and determination

GSH extraction was conducted by sample homogenization in 0.1 mol L^{-1} sodium phosphate buffer, pH 7.0 containing 0.25 mol L^{-1} sucrose, followed by centrifugation at 17.300 × *g*. The supernatants were then separated and total glutathione content was determined using GSH as analytical standard curve [21] at 412 nm on a spectrophotometer (Lambda 35, PerkinElmer, USA) and expressed as μmol g^{-1} wet weight. Determinations were conducted in triplicate.

2.4. Metal and trace-element determinations

Samples were left overnight in sub-boiled bidistilled nitric acid (Vetec, Rio de Janeiro) and subsequently heated on a digestion block, at 80–100 °C for 4 h, in closed polypropylene tubes to avoid losses [23]. After cooling, the volume was appropriately diluted with ultrapure water. Blanks and certified material DORM-3 (Dogfish muscle, NRC, Canada) were prepared in the same way as the samples. Metal determinations were conducted using an ELAN DRC II ICP-MS (PerkinElmer Sciex, Norwalk, CT, USA) in the standard mode, without the use of a reaction cell, in the conditions described in Table 1. The sample introduction system was a Meinhard-type nebulizer with a twister cyclonic chamber. During the analysis, ^{103}Rh was used as internal standard at a concentration of 20 mg L^{-1}. Quality control was performed by strict blank control, the analysis of replicates and the certified reference material. Essential metal and trace-element determinations were conducted in triplicate. The metal results of the certified reference samples analysis demonstrated the high precision and accuracy of the analytical methods, where all elements quantified in the reference materials were in agreement with the certified values.