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Goldfish brain and heart are well protected from Ni^{2+}-induced oxidative stress

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ABSTRACT

After 96 h goldfish exposure to 10, 25 or 50 mg/L of Ni^{2+} no Ni accumulation was found in the brain, but lipid peroxide concentration was by 44% elevated in the brain, whereas carbonyl protein content was by 45–45% decreased in the heart. High molecular mass thiol concentration was enhanced by 30% in the heart, while in the brain low molecular mass thiol concentration increased by 28–88%. Superoxide dismutase activity was by 27% and 35% increased in the brain and heart, respectively. Glutathione peroxidase activity was lowered to 38% and 62% of control values in both tissues, whereas catalase activity was increased in the heart by 15–45%, accompanied by 18–29% decreased glutathione reductase activity. The disturbances of free radical processes in the brain and heart might result from Ni-induced injuries to other organs with more prominent changes in the heart, because of close contact of this organ with blood, whereas the blood–brain barrier seems to protect the brain.

1. Introduction

Human activities, including mining, smelting, refining, alloy processing, scrap metal reprocessing, fossil fuel combustion, and waste incineration, contribute to nickel contamination in aquatic ecosystems. As a consequence, the concentration of nickel in aquatic systems receiving urban and industrial wastewaters has increased over the past decades (Pyle and Couture, 2012). At present Ni concentrations in contaminated freshwater environments can reach up to 2500 μg/L whereas in unpolluted freshwater bodies they usually range from 0.1 to 10 μg/L (Eisler, 1998).

Nickel is known to be genotoxic, immunotoxic, mutagenic and carcinogenic and thus potentially hazardous to living organisms (Eisler, 1998; Denkhaus and Salnikow, 2002; Kasprzak et al., 2003). Its accumulation in different organs leads to alterations of metabolism, disturbances in metal concentrations (Misra et al., 1990; Funakoshi et al., 1996), lipid peroxidation (Athar et al., 1987; Misra et al., 1990; Rodriguez et al., 1991; Chakrabarti and Bai, 1999), oxidation of nucleic acids (Datta et al., 1991; Kasprzak et al., 1992), and oxidative stress (Athar et al., 1987; Salnikow et al., 1994; Chen et al., 2003). Ni-induced oxidative stress may be the consequence of either enhanced generation of reactive oxygen species (ROS) via a Haber–Weiss reactions involving the Ni^{2+}/Ni^{3+} redox couple (Torreilles and Guerin, 1990; Klein et al., 1991), or weakening of the antioxidant system such as glutathione depletion (Torreilles and Guerin, 1990; Klein et al., 1991) and/or inactivation of antioxidant enzymes (Misra et al., 1990; Rodriguez et al., 1990; Cartañá et al., 1992).

Toxic effects of nickel related to stimulation of free radical processes have been well studied in mammalian systems, whereas there is still very little information concerning the modes of its toxic action in aquatic organisms, particularly fish (Agrawal et al., 1979; Jha and Jha, 1994; Ptashynski et al., 2001; Pane et al., 2005) in contrast to other transition metals, for instance, copper, which ROS-mediated toxicity has been well documented (Craig et al., 2007; Eyckmans et al., 2011; Jiang et al., 2011). To our best knowledge, there are no systematic experimental studies, indicating that environmental Ni may induce oxidative stress in fish (Pyle and Couture, 2012). Few reports exist in this field showing that nickel triggers oxidative stress in rainbow trout erythrocytes *in vitro* (De Luca et al., 2007) and dietary Ni enhanced lipid peroxide levels in plasma of lake whitefish (*Coregonus clupeaformis*)

Abbreviations: CP, carbonyl protein groups; GPx, glutathione peroxidase; GR, glutathione reductase; G6PDH, glucose-6-phosphate dehydrogenase; GST, glutathione-S-transferase; H-SH, high molecular mass thiols; LOOH, lipid peroxides; L-SH, low molecular mass thiols; Ni^{2+}, nickel divalent cation; Ni, nickel collective name as a chemical element; ROS, reactive oxygen species; SOD, superoxide dismutase.

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(Ptashynski et al., 2002). Meanwhile, fish, occupying upper levels of trophic chains can integrate the effects of metal pollutants on the lower trophic levels (Kövecses et al., 2005). Fish receive metals from water by three main routs: via skin and gills and with water/food (Dallinger and Kautzky, 1985). Taking into account the increased possibility of pollution of the aquatic ecosystem with waterborne nickel due to increased global use of this metal, it is important to understand the basic Ni toxicity to fish (Pyle and Couture, 2012).

Previously we have found that up to 50 mg/L of waterborne nickel did not induce oxidative damage of proteins and lipids in goldfish liver and white muscles (Kubrak et al., 2012a) in contrast to tissues of Ni-administrated mammals (Athar et al., 1987; Misra et al., 1990; Rodriguez et al., 1991). Contrary in kidney of Ni-exposed goldfish Ni accumulation increased renal iron content and led to oxidative stress development (Kubrak et al., 2012b) as it was also observed for kidney of Ni-treated mammals (Misra et al., 1990). Thus, we postulate that goldfish exposure to Ni^{2+} disturbs free radical metabolism and the observed effects are tissue-specific, indicating involvement of diverse mechanisms in different tissues and high antioxidant potential of goldfish tissues towards Ni ions in comparison with mammalian tissues. Additionally, Ni, similarly to Co, is a known mimetic of hypoxia, because it causes HIF-1α stabilization and triggers expression of HIF-1α mediated genes (Andrew et al., 2001). Previously we have found that goldfish exposure to waterborne nickel induced hyperglycemia accompanied with glycogenolysis in goldfish liver and white muscles (Kubrak et al., 2012a). These findings are in a good agreement with data of other authors for different fish species, where a decrease in stored glycogen with simultaneous increase of plasma glucose and/or lactate was found in liver and muscles of nickel exposed fish (Chaudhry, 1984; Chaudhry and Nath, 1985; Ghazaly, 1992). The intensification of glycogenolysis in concert with enhanced hyperglycemia shows some similarities with a hypoxic stress response. In the hypoxic context, brain and heart are organs of the critical importance.

In the present study, therefore, we aim to address further issues: *(i)* if a short-term goldfish exposure (96 h) to waterborne nickel at toxic concentrations (10–50 mg/L) leads to the metal accumulation in the brain and heart, *(ii)* whether waterborne nickel induces perturbations of free-radical processes in these organs and *(iii)* how defense mechanisms are involved in coping with nickel hazard to freshwater fish.

2. Material and methods

2.1. Animals and experimental conditions

Goldfish (*Carassius auratus* L.) with a body mass of 40–65 g were obtained from local suppliers twice: in June and November 2010. In both cases, fish were acclimated to laboratory conditions for 4 weeks in a 1000 L tank under natural photoperiod in aerated and dechlorinated tap water, which was changed by 2/3 of volume every second day. Water parameters in the tanks during fish acclimation correspond to those in aquaria during experiments (shown in Table 1). Fish were fed *ad-libitum* with commercial pellets for *Cyprinid* (Koi Growner, The Netherlands), containing 44% protein, 11% fat and 150 mg/kg vitamin C. Fish were fed during the acclimation period (4 weeks), but were fasted for 4 days prior and during experimentation. All experiments were conducted in accordance with institutional and international guidelines for the protection of animal welfare (EU Directive 2010/63/EU for animal experiments, http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm).

Experiments were carried out in 120 L glass aquaria (containing 100 L of water), in a static mode. Groups of 5–6 fish were placed in aquaria with different nominal concentrations of nickel ions: 10, 25

Table 1
The Ni^{2+} concentrations (mg/L) and main water parameters (temperature (°C), pH value, oxygen level (mg/L) and hardness (Ca^{2+} concentration, mg/L)) in aquarium water after goldfish exposure to control conditions (without Ni^{2+} supplementation) or 10, 25 or 50 mg/L of Ni^{2+} for 96 h.

Fish group	Parameter	Exposure time (h)				
		0	24	48	72	96
Control	Ni content (mg/L)	ND	ND	ND	ND	ND
	Temperature (°C)	19.0 °C^a	19.0 °C^a	19.5 °C^a	19.5 °C^a	19.0 °C^a
		23.0 °C^s	23.5 °C^s	23.0 °C^s	24.0 °C^s	23.5 °C^s
	pH	7.75 ± 0.03	7.71 ± 0.02	7.69 ± 0.03	7.72 ± 0.03	7.70 ± 0.02
	Oxygen level (mg/L)	9.0^a	9.3^a	9.0^a	9.4^a	9.2^a
		7.6^s	7.3^s	7.6^s	7.0^s	7.1^s
	Hardness (Ca^{2+}, mg/L)	39.3 ± 0.02	39.0 ± 0.02	38.6 ± 0.03	38.8 ± 0.02	39.3 ± 0.04
10 mg/L	Ni content (mg/L)	9.8	9.7	9.8	9.7	9.7
	Temperature (°C)	19.5 °C^a	19.0 °C^a	19.5 °C^a	19.0 °C^a	19.0 °C^a
		23.5 °C^s	23.5 °C^s	24.0 °C^s	23.0 °C^s	23.5 °C^s
	pH	7.68 ± 0.03	7.66 ± 0.04	7.69 ± 0.02	7.71 ± 0.03	7.70 ± 0.03
	Oxygen level (mg/L)	9.3^a	9.5^a	9.4^a	9.5^a	9.2^a
		7.7^s	7.4^s	7.5^s	7.1^s	7.2^s
	Hardness (Ca^{2+}, mg/L)	38.6 ± 0.02	38.5 ± 0.02	38.9 ± 0.03	38.8 ± 0.01	39.0 ± 0.02
25 mg/L	Ni content (mg/L)	24.2	24.0	23.7	24.2	23.7
	Temperature (°C)	19.0 °C^a	19.0 °C^a	19.5 °C^a	19.5 °C^a	19.5 °C^a
		24.0 °C^s	23.0 °C^s	23.0 °C^s	23.5 °C^s	23.5 °C^s
	pH	7.69 ± 0.02	7.68 ± 0.02	7.70 ± 0.03	7.72 ± 0.03	7.69 ± 0.03
	Oxygen level (mg/L)	9.0^a	9.2^a	9.5^a	9.3^a	9.2^a
		7.3^s	7.6^s	7.0^s	7.2^s	7.0^s
	Hardness (Ca^{2+}, mg/L)	39.0 ± 0.02	39.0 ± 0.03	38.7 ± 0.01	38.6 ± 0.02	39.1 ± 0.03
50 mg/L	Ni content (mg/L)	49.6	49.4	49.0	49.8	49.3
	Temperature (°C)	19.5 °C^a	19.5 °C^a	19.0 °C^a	19.0 °C^a	19.0 °C^a
		23.5 °C^s	23.5 °C^s	23.0 °C^s	23.0 °C^s	24.0 °C^s
	pH	7.68 ± 0.02	7.71 ± 0.02	7.77 ± 0.04	7.75 ± 0.03	7.70 ± 0.02
	Oxygen level (mg/L)	9.1^a	9.3^a	9.3^a	9.5^a	9.2^a
		7.5^s	7.6^s	7.2^s	7.5^s	7.4^s
	Hardness (Ca^{2+}, mg/L)	38.8 ± 0.02	39.0 ± 0.03	38.6 ± 0.04	38.2 ± 0.02	39.1 ± 0.02

ND—measured values of nickel concentration in aquarium water were below detection limit of the method used. All assays of nickel concentration were done for two independent experiments; data of the typical experiment are presented.
"s" and "a"—water parameters, monitored for summer and autumn experiments, respectively. The water parameters were measured every 24 h twice in summer experimental runs and once—during autumn experiments. Data of representative measurements are shown for temperature of aquarium water and dissolved oxygen, whereas for pH values and hardness data are shown as mean ± S.E.M for all tree experiments.