Chemosphere 75 (2009) 56–62

Contents lists available at ScienceDirect

Chemosphere

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

Trivalent chromium induces oxidative stress in goldfish brain

Oleh V. Lushchak [a], Olha I. Kubrak [a], Ihor M. Torous [a], Tetyana Yu. Nazarchuk [a], Kenneth B. Storey [b], Volodymyr I. Lushchak [a,*]

[a] Department of Biochemistry, Precarpathian National University named after Vassyl Stefanyk, 57 Shevchenko Str., Ivano-Frankivsk, 76025, Ukraine
[b] Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

ARTICLE INFO

Article history:
Received 26 September 2008
Received in revised form 18 November 2008
Accepted 18 November 2008
Available online 21 January 2009

Keywords:
Oxidative stress
Antioxidant enzymes
Glutathione
Lipid peroxides
Protein carbonyls

ABSTRACT

Although information on the effects of Cr^{6+} in biological systems is abundant, Cr^{3+} has received less attention. Toxic effects of chromium compounds are partially associated with activation of redox processes. Recently we found that Cr^{6+} induced oxidative stress in goldfish tissues and the glutathione system was shown to play a protective role. The present study aimed to investigate free radical processes in brain of goldfish exposed to $CrCl_3$. Trivalent chromium at a concentration of 50 mg L^{-1} was lethal and therefore we chose to examine sublethal dosages of 1.0–10.0 mg L^{-1} in aquarium water. The levels of lipid peroxides and protein carbonyls (measures of oxidative damage to lipids and proteins) in brain increased after 96 h exposure of goldfish to Cr^{3+}. However, exposure to 1.0–10.0 mg L^{-1} Cr^{3+} decreased total glutathione concentration in brain by ~50–60%. Oxidized glutathione levels also fell by ~40–60% except at the 10.0 mg L^{-1} dosage where they decreased by 85%. Therefore, 10.0 mg L^{-1} Cr^{3+} significantly reduced the ratio [GSSG]/[totalGSH] to 35% of the control value. Chromium treatment did not affect the activity of superoxide dismutase, but reduced the activities of catalase by 55–62% and glutathione-*S*-transferase by 14–21%. The activities of glucose-6-phosphate dehydrogenase and glutathione reductase were unchanged under any experimental conditions used. Therefore, it can be concluded that although Cr^{3+} exposure induced oxidative stress in goldfish brain, it failed to enhance the efficiency of the antioxidant system in the organ.

1. Introduction

Chromium is abundant in the earth's crust and is extensively used by humans in stainless steel manufacturing, leather tanning, wood treatment, etc., which leads to environmental pollution. In the workplace it occurs predominantly in two valence states – hexavalent chromium (Cr^{6+}) and trivalent chromium (Cr^{3+}), but intermediate valences – tetra- and pentavalent chromium ions also occur. Cr^{6+} compounds are extensively used in diverse industries whereas Cr^{3+} salts such as chloride, niacin, picolinate and polynicotinate appear often in dietary supplements and micronutrients. Chromium compounds do not disappear in ecosystems – they only change their state and valence.

It is widely believed that Cr^{6+} is more toxic than Cr^{3+} (Blasiak and Kowalik, 2000; Valko et al., 2005). Two main Cr^{6+} forms are dominant in the environment – as CrO_7^- and $Cr_2O_4^-$. They can readily cross cellular membranes with the help of nonspecific anion carriers (Danielsson et al., 1982; Hneihen et al., 1993), whereas Cr^{3+} is poorly transported across membranes. This difference in the ability to cross biological membranes may be one of the reasons for their different effects on biological systems. Therefore, the effects of these two chromium forms should be discussed with special attention to the valence state. This is important, because in many publications only total chromium concentrations are given, which may reduce the value of the studies (Parvez et al., 2006).

Information on the effects of Cr^{6+} in biological systems is abundant, particularly in connection with hydrobionts, including fish. In laboratory-reared guppies *Poecilia reticulata* Cr^{6+} at certain concentrations increased the maximum lifespan (Perez-Benito, 2006). It also affected health parameters in Chinook salmon *Oncorhynchus tshawytscha* (Farag et al., 2006), and the immune system in tilapia *Oreochromis mossambicus* (Prabakaran et al., 2006) and freshwater catfish *Saccobranchus fossilis* (Khangarot et al., 1999). Hexavalent chromium increased ventilation frequency and coughing rate, and in blood it enhanced hemoglobin, hematocrit, plasma glucose and lactate levels in rainbow trout *Salmo gairdneri* (Van der Putte et al., 1982). In the coastal teleost *Periophthalmus dipes* Cr^{6+} inhibited transport enzymes – ion-dependent ATPases in gills, kidney and intestine (Thaker et al., 1996). In rainbow trout *Oncorhynchus mykiss*, Hook et al. (2006) found that Cr^{6+} specifically changed the expression of 137 genes.

Toxic effects of Cr^{6+} are associated, at least partially, with the modification of redox processes. For example, in goldfish tissues

* Corresponding author. Tel./fax: +38 0342 714683.
E-mail address: lushchak@pu.if.ua (V.I. Lushchak).

0045-6535/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2008.11.052

(Lushchak et al., 2008) and isolated hepatocytes (Krumschnabel and Nawaz, 2004), treatment with $K_2Cr_2O_7$ induced oxidative stress. The mutagenic effects of Cr^{6+} and the induction of DNA-protein cross-links (Coogan et al., 1991; Kuykendall et al., 2006) are also probably associated with intensification of free radical processes. Oxidative stress development and genotoxic effects of Cr^{6+} were found in gills and kidney of European eel *Anguilla anguilla* (Ahmad et al., 2006).

Hexavalent chromium in environmental water may be reduced to Cr^{3+} by bacteria and other organisms. Fish mucus may be an important element of animal protection against this toxicant, reducing Cr^{6+} to Cr^{3+}. Skin mucus components of trout *Salmo gairdneri*, probably protein-bound sulfhydryl groups, were found to carry out the reduction of Cr^{6+} (Arillo and Melodia, 1990). It is now believed, that Cr^{6+} is reduced by glutathione and glutathione reductase (Shi and Dalal, 1989, 1990; Raghunathan et al., 2007), cytochrome b_5 (Borthiry et al., 2007), and ascorbate (Kaczmarek et al., 2007).

Although it is commonly accepted that Cr^{6+} is more toxic than Cr^{3+}, the latter one can still have toxic effects (Blasiak and Kowalik, 2000). It is also carcinogenic and can induce DNA damage (Qi et al., 2000). Trivalent chromium effects on organisms also depend on which form of Cr^{3+} organisms are exposed to, because it can exist as a free ion, but more commonly in complexes with organic acids.

Previously we investigated the effects of Cr^{6+} on free radical process in goldfish tissues and showed a Cr^{6+}-dependent development of oxidative stress and the possible involvement of the glutathione system in protection (Lushchak et al., 2008). But taking into account that in biological systems Cr^{6+} is reduced to Cr^{3+}, we logically asked if similar effects may be caused by Cr^{3+}, because it also can enter redox reactions and induce oxidative stress. Therefore, the present study analyzed Cr^{3+} effects using a similar protocol to our previous study of Cr^{6+} effects (Lushchak et al., 2008), but with a focus on the most critically important organ – brain.

2. Material and methods

2.1. Reagents

Phenylmethylsulfonyl fluoride (PMSF), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), oxidized glutathione (GSSG), glucose-6-phosphate (G6P), ethylenediamine-tetraacetic acid (EDTA), xylenol orange, cumene hydroperoxide, ferrous sulphate, 2,4-dinitrophenylhydrazine (DNPH), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), Tris(hydroxymethyl) aminomethane and potassium phosphate monobasic were purchased from Sigma–Aldrich Corporation (USA). $NADP^+$ and NADPH were obtained from Reanal (Hungary) and guanidine-HCl was from Fluka (Germany). All other reagents were of analytical grade.

2.2. Animals and chromium exposure

Goldfish (*Carassius auratus* L.) weighting 50–70 g were obtained commercially in December 2007 and held in a 1000 L tank under natural photoperiod in aerated and dechlorinated tap water at 20 ± 2 °C and pH 6.0. The experiments were carried out in 120 L glass aquaria, in a static mode, under the same conditions. Fish were not fed during preadaptation to laboratory conditions and experimentation.

We found no literature on Cr^{3+} toxicity for goldfish. A review by Eisler (1986) reported that the LD_{50}^{96} for Cr^{6+} and Cr^{3+} were very similar in rainbow trout and salmon fingerlings. On the basis of a LD_{50}^{96} for Cr^{6+} for goldfish reported by Lemly (1994) and our previous experience with Cr^{6+} in goldfish (Lushchak et al., 2008), in initial experiments we found that 50.0 mg L^{-1} $CrCl_3$ was lethal (all six tested fish died after 96 h exposure). Therefore, further we reduced the concentration to 10.0 mg L^{-1} and below. The necessary Cr^{3+} concentrations (analytical grade chromium chloride $CrCl_3$ was used) were prepared in dechlorinated tap water. For experiments, groups of six fish were placed in 120 L aquaria with different nominal chromate concentrations in the range 1.0–10.0 mg L^{-1} and were exposed to these conditions for 96 h. Fish in the control group were treated in the same manner, but chromium salt was omitted. Water was not changed during the course of the experiment in order to avoid stressing the animals. Fish were sacrificed after a 96 h exposure time and brains were quickly dissected out, rinsed in 0.9% sodium chloride solution and placed in pre-chilled homogenization buffers.

The concentration of Cr^{3+} was measured by a colorimetric method developed by Honchar and colleagues (2008) that is based on the reaction of Cr^{3+} with chromazurol *S* in the presence of surfactants.

2.3. Indices of oxidative stress

Brain samples were homogenized (1:10 w:v) using a Potter–Elvejhem glass homogenizer in pre-chilled 50 mM potassium phosphate (KPi) buffer, pH 7.5, containing 0.5 mM EDTA; a few crystals of phenylmethylsulfonyl fluoride (PMSF) were added prior to homogenization. A 250 μL aliquot of this homogenate was mixed 1:1 v:v with 40% w:v trichloroacetic acid and then centrifuged for 5 min at 7000*g*. Protein carbonyl (CP) content was measured in the resulting pellets, by the reaction with 2,4-dinitrophenylhydrazine (DNPH) as described previously (Lushchak and Bagnyukova, 2006). The content of carbonyl protein (CP) was evaluated spectrophotometrically at 370 nm using a molar extinction coefficient of 22×10^3 M^{-1} cm^{-1} (Lenz et al., 1989). Data are expressed as nanomoles CP per milligram total protein.

Lipid peroxide (LOOH) concentrations were assayed by the xylenol orange method (Hermes-Lima et al., 1995). For that, tissue samples were homogenized in five volumes of ice-cold 96% ethanol, centrifuged at 10000*g* for 10 min at 4 °C, and supernatants were used for assay as described previously (Lushchak et al., 2005). The concentration of LOOH was expressed as nanomoles of cumene hydroperoxide equivalents per gram wet weight (gww).

Total and oxidized glutathione concentrations were measured as described in Lushchak et al. (2005). Briefly, tissue samples were homogenized in 5 volumes of cold (~4 °C) 5% w:v sulfosalicylic acid and then centrifuged at 10000*g* for 5 min in a centrifuge Eppendorf 5415 R (Germany). Supernatants were removed and used immediately for measurement of total glutathione ([totGSH] = ([GSH] + 2[GSSG]). Total glutathione content was determined by following the rate of reduction at 416 nm of 0.6 mM 5,5′-dithio bis(2-nitrobenzoic acid) by GSH at 20 °C in 100 mM KPi buffer (pH 7.5) containing 0.25 mM NADPH, 1 mM EDTA, 2–5 μL of samples, and 1 U mL^{-1} yeast glutathione reductase. The concentration of oxidized glutathione was then evaluated after GSH derivatization with vinylpyridine. Reduced GSH levels were calculated by subtracting the concentration of oxidized GSSG from total glutathione content. The concentrations of glutathione in brain were expressed as micromoles per gram wet weight (gww).

2.4. Assay of antioxidant and associated enzyme activities

Tissue homogenates were prepared as described above for the carbonyl protein assay and centrifuged at 4 °C for 15 min at 15000*g* in an Eppendorf 5415 R centrifuge (Germany). Supernatants were removed and used for enzyme activity assays using a Specord M-40 (Karl Zeiss Jena, Germany). The activities of primary antioxidant enzymes, SOD and catalase, as well as associated en-