Environ Sci Pollut Res (2015) 22:6912–6919
DOI 10.1007/s11356-014-3923-3

RESEARCH ARTICLE

# Impact of acute $Cd^{2+}$ exposure on the antioxidant defence systems in the skin and red blood cells of common carp (*Cyprinus carpio*)

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Received: 27 August 2014 / Accepted: 26 November 2014 / Published online: 5 December 2014


**Abstract** $Cd^{2+}$-induced oxidative stress and its effects on the expression of stress biomarkers and on macromolecule damage in the skin and blood of common carp were studied. Both tissues play important roles in the defence mechanisms against external hazards, serving as an anatomical barrier and as connecting tissue between the organs. In the skin, the production of peroxynitrite anion and hydrogen peroxide was almost doubled after exposure to 10 mg/L $Cd^{2+}$. The accumulation of these oxidant molecules suggests an intensive production of superoxide anion and nitrogen monoxide and the development of oxidative and/or nitrosative stress. Although the metallothioneins and the components of the glutathione redox system were activated in the skin, the accumulation of reactive intermediates led to the enhanced damage of lipid molecules after 24 h of metal exposure. In the blood, the basal levels of metallothionein messenger RNAs (mRNAs) were 2–2.5-fold of that measured in the skin. This high level of metallothionein expression could be the reason that the blood was less affected by an acute $Cd^{2+}$ challenge and the metallothionein and glutathione systems were not activated.

**Keywords** Antioxidant · Cadmium · Erythrocyte · Nitrosative stress · Peroxynitrite · Skin

## Introduction

Aquatic organisms are at high risk of exposure to a variety of pollutants that may enter the aquatic environment from agricultural and industrial sources. Such pollutants may not undergo biodegradation and therefore accumulate in fish, oysters, mussels, sediments and other components of the aquatic ecosystems (Abdullah et al. 2007). Fish in particular are subject to environmental stressors, because of their permanent exposure to dissolved substances through their gills and skin, and to the incorporation of harmful substances via their food (Mzimela et al. 2003).

Cadmium ($Cd^{2+}$) is a widely distributed metal that enters the aquatic environment from natural and anthropogenic sources (Satarug et al. 2003). $Cd^{2+}$ is recognized as one of the most deleterious heavy metal pollutants (Hallenbeck 1984): It has been classified as a group 1 human carcinogen (Waalkes et al. 1992) and causes a variety of adverse health effects, such as cardiotoxicity (Limaye and Shaikh 1999), hepatotoxicity (Tzirogiannis et al. 2003) or neuropathy (Antonio et al. 1999). Exposure to $Cd^{2+}$ can result in the delay of ontogenetic development, low hatchability, a high level of morphological abnormalities (Jezierska et al. 2009), reductions in survival and growth (Hansen et al. 2002), disturbances in osmotic and ion regulation, and altered activities of enzymes and cell injuries (Heath 1995) in aquatic animals. Moreover, $Cd^{2+}$ inhibits the mitochondrial electron transfer chain and leads to a mitochondrial dysfunction (Wang et al. 2004). The disturbed redox balance gives rise to the enhanced formation of reactive oxygen and nitrogen species (ROS and RNS), such as the superoxide anion ($O_2^{\cdot-}$), the hydroxyl radical (·OH), hydrogen peroxide ($H_2O_2$) and the peroxynitrite anion ($ONOO^-$) (Han et al. 2007).

The rapid elimination of excessive reactive species is essential for the organism to survive. To protect themselves against oxidative stress, aerobic organisms have evolved complex antioxidant defence systems. A number of antioxidant defence enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx), have been identified in most organisms,

Responsible editor: Thomas Braunbeck

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among them are teleosts (Basha and Rani 2003; Cunha Bastos et al. 2007). SOD catalyses the conversion of $O_2^{\cdot-}$ to $H_2O_2$ and $O_2$ (Fridovich 1989), and CAT takes part in the elimination of $H_2O_2$ (Lü et al. 2010). The classical GPx1 and the phospholipid hydroperoxide GPx4, together with reduced glutathione (GSH) and some other thiols, also take part in the reduction of $H_2O_2$ (Maiorino et al. 1998; Imai and Nakagawa 2003). The GSH redox system and the metallothioneins (MTs) are the two major lines of metal detoxification. GSH itself plays a critical role, as an antioxidant, enzyme cofactor and major redox buffer (Dringen et al. 2000). γ-Glutamylcysteine synthetase and glutathione synthase (GSS) catalyse steps of the GSH synthetic pathway (Rahman et al. 1996), while oxidized GSH, i.e. glutathione disulfide (GSSG), is recycled into GSH through the action of GR. The antioxidant role of GSH in cells relies on its concentration, rate of turnover and rate of synthesis (Potter and Tran 1993). $Cd^{2+}$ also causes alterations in the turnover of GSH (Wang and Ballatori 1998).

MTs are low molecular mass, cysteine-rich intracellular proteins that bind metal ions with high affinity. MTs are also involved in the detoxification of certain heavy metals and in the scavenging of free radicals (Karin and Herschman 1980; Kito et al. 1984). The induction of MT genes has been evidenced in response to stress situations such as oxidative stress (Andrews 2000) and hypoxia (Murphy et al. 1999).

To date, we have identified and characterized a large number of genes coding for antioxidant molecules, such as heat shock proteins (*hsps*) (Ali et al. 2003), *mt-1* and *mt-2* (Hermesz et al. 2001), *gpx4a* and *gpx4b* (Hermesz and Ferencz 2009), *cat*, *gpx1*, *gr*, and *gss* (Dugmonits et al. 2013), and *ho-1* and *ho-2* (Jancso and Hermesz 2014) in one of the most important cultured fish species, the common carp (*Cyprinus carpio*). Our previous studies focused on the liver and kidney, the organs most involved in the processing and excretion of toxic agents. Little is known, however, as concerns the antioxidant defence in tissues with no apparent immediate role in detoxification, e.g. the skin and the blood.

In this paper, we report on transcriptional changes in the expression of the *mt-1* and *mt-2*, *gpx4a* and *gpx4b*, *gpx1*, *cat*, *gr* and *gss* genes in the skin and erythrocytes of common carp after 24- and 48-h $Cd^{2+}$ exposure, from the aspects of the accumulation of $ONOO^-$ and $H_2O_2$. We also measured the activities of SOD and CAT and the levels of GSH and GSSG and followed the molecular damage in lipid molecules.

## Materials and methods

### Animals and treatments

Common carp (*Cyprinus carpio* L. 1758) weighing 800–1000 g, obtained from the Tisza Fish Farm, Szeged, were acclimatized in well-aerated 400-L water tanks at 16 °C. Water was kept oxygen-saturated by aeration and was changed twice a week. Fish were transferred into 100-L water tanks (two fish per tank) at the end of acclimatization period. For metal treatment, the carp were exposed to 10 mg/L $Cd^{2+}$ ($Cd(CH_3COO)_2 \times 2H_2O$, Fluka) for up to 48 h, under static conditions. $Cd^{2+}$ at this concentration is not lethal to common carp at least for 21 days. Three to five $Cd^{2+}$-treated and six control animals were sacrificed at each time point (0, 24, 48 h) for tissue harvesting. Tissues were frozen immediately in liquid nitrogen and stored at −80 °C. Experiments were conducted in accordance with national and institutional guidelines for animal welfare.

In the preparation of red blood cells, approximately 1.0 mL of blood sample was taken from the caudal vein of each fish. Blood coagulation was inhibited by EDTA (K3 EDTA, Greiner Bio-One International AG). The blood samples were centrifuged at 2200×*g* for 10 min at 4 °C, and the plasma and the buffy coat were removed. Red blood cells were washed three times with two volumes ice-cold sodium chloride solution (0.62 %). The samples were kept at −80 °C until processing.

### RNA extraction, reverse transcription and PCR amplification

Approximately 100 mg of frozen tissues were homogenized in RNA Bee reagent (Tel-Test, Inc.), and total RNA was prepared according to the procedure suggested by the manufacturer. Total RNA was routinely treated with 100 U RNAse-free DNAseI (Thermo Scientific) to avoid any DNA contamination.

For the quantification of *mt-1* and *mt-2*, *gpx4a* and *gpx4b*, *cat*, *gpx1*, *gr* and *gss* messenger RNAs (mRNAs), reverse transcription followed by PCR amplifications (RT-PCRs) was performed. First-strand cDNAs were synthesized by using total RNA as template, prepared either from the skin and the erythrocytes of untreated and $Cd^{2+}$-exposed animals using 5 μg total RNA, 200 pmol of each dNTP (Thermo Scientific), 200 U Maxima H Minus reverse transcriptase (Thermo Scientific) and 500 pmol random hexamer primers (Sigma) in a final volume of 20 μL, and incubated for 10 min at 37 °C, followed by 1 h at 52 °C. One-microlitre reverse transcription product was added to 25-μL DreamTaq Green PCR Master Mix 2× (Thermo Scientific). Amplification was performed in a PTC 200 Peltier Thermal Cycler (MJ Research) using 23 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s for the *β-actin* mRNA, used as internal reference, and 30 cycles for *mt-1* and *mt-2*, *gpx4a* and *gpx4b*, *cat*, *gpx1*, *gr* and *gss* mRNAs, respectively. The amplified products were detected on a 2 % agarose gel. The relative levels of mRNAs are expressed as ratios (mRNA/*β-actin*×10).

### Primers

The following primers were used: *gpx1*: F: tggcttygagcccaaattcca and R: tcaatgtcgctggtgaggaa; *cat*: F: cgtcatatgaacggatacgg and R: tcagcctgctcaaaggtcat; *gr*: F: attgctgtgcaaatggctgg and R: cctgcacgagtggtgttctgga; *gss*: F: gtccatcggcacattctgaa and R: ggcatgtatccattacggaa; *gpx4a*: F: ggaaccaggaacaaattccc and R: agatcyttctccaccacgcttg; *gpx4b*: F: gtaaaaccccagtaaactac and R: cttggatcgtccattggtcc; *mt1*: F: atggatccttgcgattgcgcca and R: cgaacaggttcacataggtga; *mt2*: F: atggatccttgcgattgcgcca and R: acaagttcacattgctgtag. For the normalization of *gpx*s, *cat*, *gr gss* and *mt* mRNAs, the level of carp *β-actin* mRNA was used as internal standard, detected with primer pairs F: caagagaggtatcctgacc and R: ccctcgtagatgggcacagt.

### Densitometry

Images of the ethidium bromide-stained agarose gels were digitized with a GDS 7500 Gel Documentation System and analysed with the GelBase/GelBlot™ Pro Gel Analysis Software (UVP).

### Sample preparation

For the measurement of GSH and $H_2O_2$ levels, lipid peroxidation and the activities of antioxidant enzymes, a 1-g skin sample from each individual fish was homogenized in 4 volumes of ice-cold physiological saline solution, using a double glass homogenizer immersed in an ice-water bath. The homogenate was centrifuged at 13,000*g* for 15 min at 4 °C to obtain supernatant. The RBCs were haemolysed by 1 volume of RBC to 9 volumes of distilled water. The quantity of protein was determined with Folin-Ciocalteu reagent, using bovine serum albumin as standard (Lowry et al. 1951). Spectrophotometric measurements were carried out with Biomate 5 Double-Beam UV–vis (Thermo Spectronic) and GENESYS 10S UV–vis (Thermo Scientific) spectrophotometers.

### GSH measurement

The GSH content was determined by the method of Sedlak and Lindsay at 412 nm by using Ellman's reagent (5,5-dithiobis-nitrobenzoic acid). Proteins were precipitated with trichloroacetic acid (TCA) 0.05 g/mL and were then centrifuged at 12,000×*g* for 10 min. The reaction mixture contained 500 μL of Tris buffer (0.4 M, pH 8.9), 300 μL of supernatant and 30 μL of DTNB. The blank sample was distilled water (Sedlak and Lindsay 1968; Tietze 1969).

### Lipid peroxidation estimation assay

In biochemical evaluations of metal toxicity, the level of thiobarbituric acid-reactive substances (TBARS) is regarded as an appropriate indicator of the extent of lipid peroxidation (LPO) (Nogueira et al. 2003). LPO was estimated by a TBARS assay at 532 nm against a blank that contained the thiobarbituric acid (TBA) reagent (0.15 g/mL TCA, $3.75\times10^{-3}$ g/mL TBA and 0.25 M HCl) as described by Serbinova et al. (1992).

### Determination of $H_2O_2$ level

For the assay of $H_2O_2$, 0.05 mg/mL horseradish peroxidase and 0.1 mg/mL *o*-dianisidine in sodium phosphate buffer (100 mM, pH 6.5) were used. The $H_2O_2$ concentration was determined spectrophotometrically at 400 nm and was calculated as nanomoles per milligram protein (Villegas and Gilliland 1998).

### Determination of $ONOO^-$ level

$ONOO^-$ was assayed by diluting samples into 1.0 M NaOH (60:1) and measuring the increase in absorbance at 302 nm. As a control, samples were added to 100 mM potassium phosphate (pH 7.4) (60:1). The decrease in absorbance was measured at neutral pH as $ONOO^-$ decomposes (Huie and Padmaja 1993; Beckman et al. 1996).

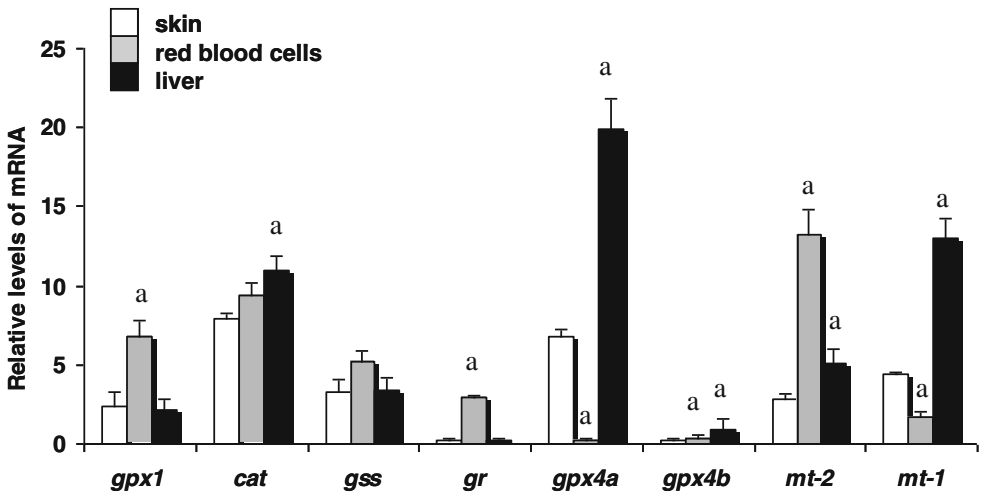


**Fig. 1** Transcriptional study of basal gene expression in the skin, red blood cells and liver. For normalization of the amount of mRNAs, the level of *β-actin* mRNA was used as internal standard in the PCR reaction. Significant differences compared to the values measured in the skin (*a*)

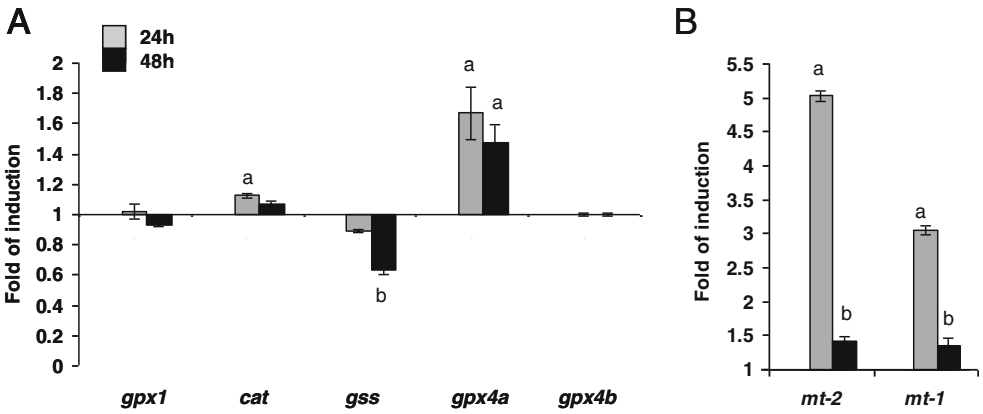


**Fig. 2** The expressions of the *gpx1*, *cat*, *gss, gpx4a* and *gpx4b* (**a**), and *mt-1* and *mt-2* (**b**) genes, in the skin following treatment with 10 mg/L $Cd^{2+}$. A significant difference between the control level (C) and that at a given time point (*a*) and a significant difference between the two examination time points (*b*). All data are means±S.D. of the results of measurements on three to six fish at each time point

### Enzyme activity measurements

CAT (EC 1.11.1.6) activity was determined spectrophotometrically at 240 nm by the method of Beers and Sizer (1953), and specific CAT activity was expressed in Bergmeyer units (BU) per milligram protein (1 BU=decomposition of 1 g $H_2O_2$/min at 25 °C).

SOD (EC 1.15.1.1) activity was determined on the basis of the inhibition of the epinephrine–adrenochrome autoxidation (Misra and Fridovich 1972). Spectrophotometric measurement was carried out at 480 nm. The result was expressed in units per milligram protein.

### Statistical analysis

For each time point of the experiments, three to six fish were used. RT-PCR reactions were performed in triplicate to increase the reliability of the measurements. Statistical differences were calculated with one-way analysis of variance (ANOVA) (MedCalc Statistical Software version 9.4.2.0, Broekstraat, Belgium) with a Student–Newman–Keuls follow-up test. Significant difference was accepted at $P<0.05$.

## Results

### Basal gene expression

The basal expression levels of the selected genes were measured in the skin and red blood cells, along with the liver, the organ with a high detoxification potential. The expressions of *gpx1*, *cat, gss*, *gr* and *mt-2* were found to be comparable in the skin and liver. The levels of *gpx1*, *gr* and *mt-1* mRNAs in the red blood cells proved to be 2–2.5-fold higher than in the skin or liver tissues. The expressions of *gpx4a* and *mt-1* predominate in the liver. The ratio of *mt* mRNAs (*mt-1*/*mt-2*) was 3:2 in the skin and about 1:8 in the erythrocytes (Fig. 1).

### $Cd^{2+}$-induced alterations in gene expression

$Cd^{2+}$ at 10 mg/L transiently upregulated the expressions of *cat*, *gr*, *gpx4a* and *mt*s in the skin. In the case of *gpx4a*, the increase was about 1.5-fold, while *mt-2* and *mt-1* were induced 5- and 3-fold, respectively (Fig. 2). The *gr* expression was around the threshold of detectability in the untreated animals but was highly induced after the 24-h exposure,

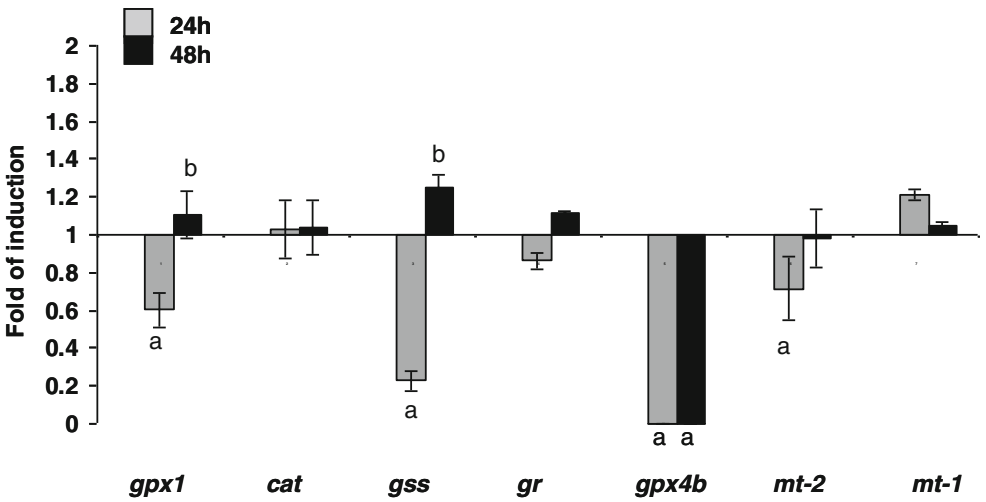


**Fig. 3** The expressions of antioxidant genes in the red blood cells following treatment with 10 mg/L $Cd^{2+}$. A significant difference between the control level (C) and that at a given time point (*a*) and a significant difference between the two examination time points (*b*). All data are means±S.D. of the results of measurements on three to six fish at each time point

whereas after 48 h of $Cd^{2+}$ exposure, the level of *gr* was around the control value (data not shown).

In the red blood cells, $Cd^{2+}$ transiently decreased the expressions of *gpx1*, *gss*, *gpx4b* and *mt-2* after 24 h of treatment and had no effects on the expressions of *cat*, *gr* and *mt-1*. However, following 48 h of $Cd^{2+}$ exposure, the levels of *gpx1*, *gss* and *mt-2* mRNAs had returned to the control values (Fig. 3).

### $Cd^{2+}$-induced alterations in the generation of $ONOO^-$ and $H_2O_2$, the activities of antioxidant enzymes, and the levels of GSH and lipid peroxidation (LPO)

In the skin, an increased level of free radical production was measured, with significant elevations in $ONOO^-$ and $H_2O_2$ content (~1.5–2-fold). SOD in cooperation with CAT catalyses the conversion of $O_2^{\cdot-}$ to $H_2O$. The activity of CAT was increased (~2.5–3-fold), while there was no substantial change in the activity of SOD.

In the erythrocytes, there was no appreciable change in $ONOO^-$ content, and the level of $H_2O_2$ was only slightly elevated. The activities of SOD and CAT increased ~1.5-fold on $Cd^{2+}$ exposure.

The antioxidant role of GSH in cells relies on its concentration. In the skin, the levels of GSH and GSSG were found to be equally increased, and as a consequence, the ratio GSH/GSSG did not change. In the red blood cells, the $Cd^{2+}$ treatment was followed by a pronounced depletion of the GSSG level (70 %), which resulted in a notable rise in the ratio GSH/GSSG (~2.5-fold).

The induced damage of the lipid molecules was followed via the LPO. The level of thiobarbituric acid reactive substances (TBARS) is regarded as an appropriate indicator of the extent of LPO (Nogueira et al. 2003). In the erythrocytes, no damage was detected during $Cd^{2+}$ exposure. In the skin, the level of TBARS underwent a gradual increase: At highest, a 4.5-fold elevation was measured (Table 1).

## Discussion

The aims of this study were to determine the activation of the antioxidant defence system and to investigate the degree of macromolecular damage, together with free radical production in the skin and red blood cells of common carp exposed to 10 mg/L $Cd^{2+}$ (Fig. 4). It is well known that heavy metals and other pollutants that accumulate in the environment can be hazardous for ecosystems, acting on specific enzymes, proteins and genes. Aquatic organisms absorb $Cd^{2+}$ directly from water (AMAP 1998), and it can then exert toxic effects (Goyer et al. 1989). The stress response induced by $Cd^{2+}$ exposure is associated with MTs, because of their high metal-binding and free radical-scavenging capacities, and with GSH and certain enzymes involved in the synthesis of GSH. The skin, an

**Table 1** Data of antioxidant markers following treatment with 10 mg/L $Cd^{2+}$ for 24 h

| | | $ONOO^-$ (nmol/mg) | $H_2O_2$ (nmol/mg) | SOD (EU/mg) | CAT (BU/mg) | GSH (nmol/mg) | GSSG (nmol/mg) | GSH/GSSG | TBARS (nmol/mg) |
|---|---|---|---|---|---|---|---|---|---|
| Skin | c | 1.715±0.191 | 0.68±0.035 | 4.736±1.3 | 0.38±0.15 | 4.348±0.73 | 1.19±0.253 | 3.68±0.193 | 3.72±2.15 |
| | $Cd^{2+}$ treatment | **2.57**±0.605 | **1.313**±0.257 | 3.55±0.33 | **1.038**±0.226 | **7.94**±0.867 | **2.27**±0.176 | 3.492±0.162 | **17.67**±4.117 |
| Red blood cells | c | 5.33±0.565 | 2.218±0.024 | 1.145±0.53 | 1.252±0.27 | 8.165±1.32 | 0.41±0.045 | 20.225±5.76 | 6.38±0.295 |
| | $Cd^{2+}$ treatment | 5.56±0.251 | **2.552**±0.012 | **2.051**±0.796 | 1.813±0.204 | 7.235±0.597 | 0.13±0.0085 | **53.67**±5.45 | 5.57±0.234 |

Bold fonts indicate significant elevation relative to the respective controls. All data are means±S.D. of the results of measurements on three fish at each time point

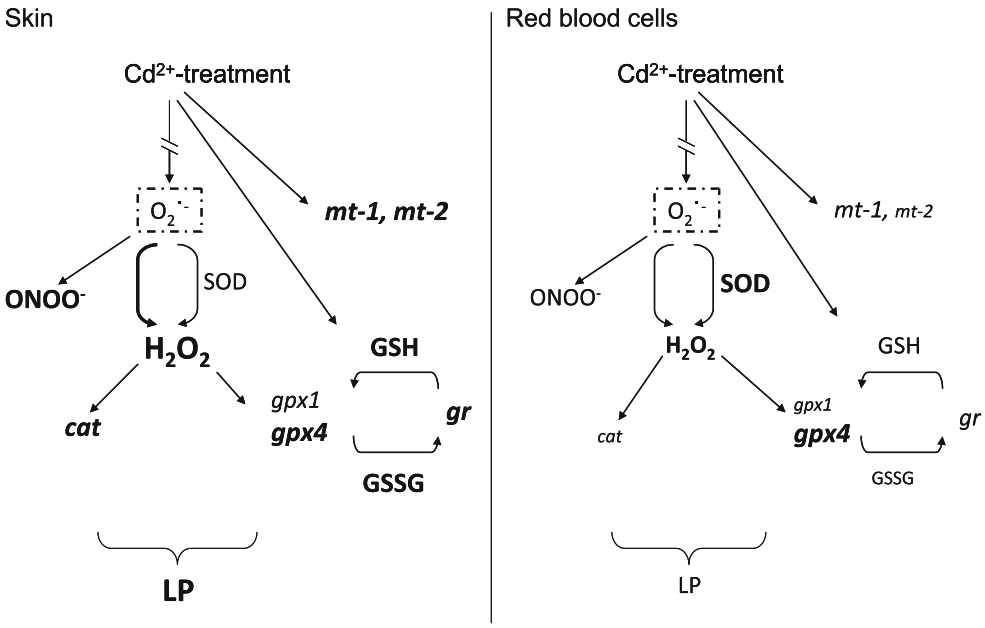


**Fig. 4** A schematic illustration of the responses of the biomarkers in the skin and the red blood cells following treatment with 10 mg/L $Cd^{2+}$ for 24 h. *Font sizes* are approximately proportional to measured levels. *Bold fonts* indicate an elevated level and *smaller fonts* a decreased level relative to the respective controls. The frame with the interrupted line indicates the nonmeasured parameter

anatomical and physiological barrier against external hazards, is a metabolically active tissue. Our molecular studies indicated that the basal expressions in the skin of selected genes coding for antioxidant molecules are comparable to those measured in the liver, the metabolically most active organ. There are only two exceptions: *gpx4s* and *mt-1*. GPx4 can react with $H_2O_2$ and a wide range of lipid hydroperoxides as substrates, including those derived from cholesterol and cholesterol esters, and it is the only GPx that is able to reduce phospholipid hydroperoxides (Maiorino et al. 1991). The expressions of *gpx1* and *gr* are roughly equal with regard to the measured mRNA levels in the liver. It is likely that the activities of Gpx1/Gpx4 and GR in the skin are sufficient to balance the GSH/GSSG redox level under physiological condition.

The present study also addressed the accumulation of free radicals and antioxidant responses induced by $Cd^{2+}$. $Cd^{2+}$ interferes with the antioxidant defence mechanisms, stimulates the production of ROS and enhances the synthesis of nitric oxide (NO) (Han et al. 2007). The simultaneous generation of NO and $O_2^{\cdot-}$ in sufficiently high concentrations in the same compartment favours the production of the toxic reaction product $ONOO^-$ (Radi et al. 2001). Although $ONOO^-$ itself is not a free radical, it is a powerful oxidant, whose toxicity is manifested among others in LPO (Radi et al. 2001). We present evidence here that continuous contact with a high concentration of $Cd^{2+}$ induces free radical production in the skin. The concentrations of $ONOO^-$ and $H_2O_2$ were almost doubled even after only 24 h of exposure. The accumulation of $ONOO^-$ suggests the intensive production of NO and the development of nitrosative stress. The increased level of $ONOO^-$ also serves as indirect evidence of elevated $O_2^{\cdot-}$ production. The increased $ONOO^-$ and $O_2^{\cdot-}$ levels in the skin were clearly reflected by a 4.5-fold increase in the level of TBARS. Thus, the induction of NO synthesis and $O_2^{\cdot-}$ production may contribute to the cytotoxicity of $Cd^{2+}$.

It is interesting to mention that the increased $H_2O_2$ production did not coincide with an elevated activity of SOD. A major source of $H_2O_2$ is the dismutation of $O_2^{\cdot-}$; besides spontaneous dismutation, mostly SOD catalyses this reaction. It is likely that another route than the SOD pathway is activated for $H_2O_2$ production in the skin.

Our data on the efficiency of the defence mechanisms in response to $Cd^{2+}$ challenges indicated that both the MT and GSH pathways are activated in the skin. The *mt-1* and *mt-2* expressions were highly elevated but only in the first 24 h of the treatment. These elevated *mt* values are comparable to those measured in the liver of the same group of animals, but the expression induced in the liver persists even at 72 h of the treatment. The GSH redox cycle was also activated: Both the de novo GSH synthesis and its oxidation and regeneration were doubled in the first 24 h of $Cd^{2+}$ treatment. The induced GSH metabolism was confirmed by data on the upregulation of the *gr* and *gpx4a* expressions. Similar results were earlier found in cultured human skin fibroblast cells: *mt-1g* and enzymes associated with GSH biosynthesis and homeostasis (e.g. glutathione S-transferases, *gss*, *gpx*, etc.) were upregulated after $Cd^{2+}$ treatment (Prins et al. 2014).

It is noteworthy that the ratio GSH/GSSG was unchanged despite the active metabolic changes. This serves as an example that the ratio GSH/GSSG cannot be used as a measure of cellular toxicity (Schafer and Buettner 2001).

The present study also addressed the molecular response in the red blood cells following acute $Cd^{2+}$ exposure. In contrast with most mammals, the red blood cells of lower vertebrates are nucleated and able to synthesize proteins (Fulladosa et al.

2006). The red blood cells are considered to be the major site for the production of ROS due to their roles in the $O_2$ transport via haemoglobin and in its $O_2$ utilization. Furthermore, since toxic chemicals are absorbed by the gills, skin and gut and are transported to other tissues through the bloodstream, they come into direct contact with the red blood cells, which in turn are among the first cells to suffer from toxic effects (Kunimoto et al. 1985).

Our measurements on red blood cells indicated no significant changes in the concentrations of $ONOO^-$ and $H_2O_2$ and in the level of LPO at 24 h of $Cd^{2+}$ exposure. This was probably not a consequence of the induced antioxidant capacity, since the expressions of selected genes coding for antioxidant molecules were at approximately the control levels. However, the high basal expressions of *gpx1* and *mt-2* genes, which were 3–4-fold those measured in the liver, suggest a sufficiently high capacity to cope with $Cd^{2+}$-induced free radical production in the first 48 h. This conception is supported by the elevated SOD and CAT activities with no significant increase in the $H_2O_2$ content.

The present study also demonstrated a significant elevation of the ratio GSH/GSSG, induced in the red blood cells by $Cd^{2+}$ exposure. However, this increased ratio was an outcome not of increased de novo GSH synthesis but of the dramatic depletion in GSSG. These results further indicate that reliance on the ratio GSH/GSSG alone in an attempt to characterize the oxidative stress status might possibly be misleading (Schafer and Buettner 2001).

## Conclusions

This comparison of the free radical production and antioxidant responses induced by $Cd^{2+}$ in the skin and in the red blood cells has indicated that tissue specificity is involved. Besides oxidative stress, the cells in the skin have to cope with an increased level of nitrosative stress, and the induced antioxidant defence system is unable to cope with the $Cd^{2+}$-generated toxic effects. In the red blood cells, the acute $Cd^{2+}$ exposure-induced free radical production can most likely be handled by the basal antioxidant capacity.

**Conflict of interest** The authors declare that there are no conflicts of interest.

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