Oxidative stress in the gill tissues of goldfishes (*Carassius auratus*) parasitized by *Dactylogyrus* spp

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Received: 23 December 2012 / Accepted: 2 January 2013 / Published online: 13 January 2013
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Abstract The aim of this study was to investigate the status of oxidative stress in the gill tissues of goldfishes (*Carassius auratus*) parasitized by *Dactylogyrus* spp. We therefore compared the concentrations of malondialdehyde (MDA) and total thiol groups in the gill tissues of parasitized and non-parasitized goldfishes. 15 goldfishes parasitized by *Dactylogyrus* spp. along with 15 non-parasitized goldfishes were selected for the study. A significant increase in MDA concentration ($P < 0.01$) and a significant decrease in total thiol groups in the parasitized group, were observed when compared to the non-parasitized group. This is the first study which evaluates the effect of *Dactylogyrus* spp. on the oxidative stress status of goldfish. The results of the present study revealed that parasitized goldfishes showed more severe oxidative stress and lipid peroxidation than non-parasitized fishes and enhanced lipid peroxidation may be linked to gill damage in goldfishes parasitized by *Dactylogyrus* spp.

Keywords Oxidative stress · *Dactylogyrus* · Gill · Goldfish

Introduction

All aerobic organisms must face the need to deal with reactive oxygen species (ROS). ROS are chemically reactive

molecules containing oxygen. They form as a natural by-product of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis (Vinagre et al. 2012). Mitochondrial respiration is the main endogenous source of ROS (Sevcikova et al. 2011). Mechanisms of antioxidant defenses in fish include the enzymatic and non-enzymatic antioxidants, similar to those in mammals (Di Giulio and Meyer 2008). Superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase are the main antioxidant enzymes. Low molecular weight antioxidants such as glutathione and metal-binding proteins such as ferritin and ceruloplasmin play a key role in non-enzymatic antioxidant defence (Kelly et al. 1998; Moran et al. 2001). In addition, thiol groups prevent tissue damage through reacting with free oxygen radicals and lipid peroxides and neutralization of these molecules (Erbay et al. 2003).

Excess ROS generation causes oxidative stress. Oxidative stress is an unavoidable aspect of aerobic life. Oxidative stress is defined as the imbalance between oxidants and antioxidant defenses (Almeida et al. 2009). Excess ROS cause a dangerous chain reaction that can destroy nucleic acid, proteins, lipids and other cellular compounds (Halliwell and Gutteridge 1999). Some ROS can initiate lipid peroxidation, a self-propagating process in which a peroxy radical is formed when a ROS has sufficient reactivity to abstract a hydrogen atom from an intact lipid (Halliwell and Gutteridge 1999). Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues (Magni et al. 1994). Malondialdehyde (MDA) is an end product of peroxidative decomposition of polyenic fatty acids in the lipid peroxidation process and its accumulation in tissues or biological fluids is indicative of the extent of free radical generation, lipid peroxidation and oxidative stress (Halliwell and Chirico 1993).

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In fish, oxidative stress is mostly studied in environmental contamination by pollutants, particularly heavy metals, pesticides and polycyclic aromatic hydrocarbons (Van der Oost et al. 2003; Bacanskas et al. 2004; Lushchak 2011). A possible role(s) of the ROS in the pathogenesis of parasitic infections has been an active area of research in recent years. Data reporting changes in oxidative stress and lipid peroxidation associated with parasite infection in aquatic animals are extremely scarce (Bello et al. 2000; Dautremepuits et al. 2003; Marcogliese et al. 2005; Stumbo et al. 2012).

Dactylogyrids are monogenean parasites that infect the gills of cyprinid fishes (Ogawa 1994; Simkova et al. 2007). Infection causes thickening of the gill epithelium that impairs respiratory function, negatively affects growth and can even cause death, especially in small carps (Thoney and Hargis 1991). Parasites with a direct life cycle, such as monogeneans, can reach epizootic levels very quickly when hosts and parasites are confined close together (Blazek et al. 2008; Borji et al. 2012). By tracing the available literature, there is no investigation of the oxidative stress status in fishes parasitized with *Dactylogyrus* spp. Such information will give additional insight on the pathogenesis of the disease. The aim of the present study was to determine the level of MDA as a biomarker of lipid peroxidation and total thiol groups in the gill tissues of goldfishes (*Carassius auratus*) parasitized with *Dactylogyrus* spp.

Materials and methods

Sampling and parasitological examination

15 goldfishes (*Carassius auratus*) with *Dactylogyrus* spp. infection along with 15 non-parasitized goldfishes were selected for the study. The fork length and weight of selected fishes were 4–5 cm and 10 g, respectively. For parasitological analysis, wet mounts from both the left and right gill arches were examined for each fish. Firstly, the gills were washed with their original water to remove blood, and then analyzed for the presence of parasites using dissection and light microscopes. Each parasite was collected from the gills and the general morphology, such as sclerotized and copulatory organs, was photographed using a digital camera under the light microscope. Identification of *Dactylogyrus* using light microscopy was performed based on morphological (hook, marginal hook, bars and copulatory organ shape and number) characteristics of the parasite according to existing keys reported in Yamaguti (1961). The selection of parasitized fishes was restricted to those affected with *Dactylogyrus* spp only. Also, the negative control fishes did not show any parasite in the gill tissues.

Preparation of gill homogenates

The gill tissues were dissected, washed in physiological saline solution (0.9 % NaCl) and frozen at -80°C until required for use. Prior to the analysis, tissue samples were unfrozen. Then they were homogenized by “Silent crusher M” (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) in 1:10 w/v cold 1.15 % KCl solution and then centrifuged at $9500\times g$ for 30 min at 4°C . Supernatants were used to determine the levels of MDA and total thiol groups.

MDA concentration

The concentration of MDA was estimated in the homogenized gill tissues according to the method of Placer et al. (1966). The reaction mixture consisted of 0.2 ml of homogenized gill tissue, 1.3 ml of 0.2 M Tris–0.16 M KCl buffer (pH 7.4) and 1.5 ml of thiobarbituric acid reagent. The mixture was heated in a boiling water bath for 10 min. After cooling, 3 ml of pyridine/*n*-butanol (3:1, v/v) and 1 ml of 1 N sodium hydroxide were added and mixed by vigorous shaking. A blank was run simultaneously by incorporating 0.2 ml distilled water instead of the gill tissue. The absorbance of the test sample was read at 548 nm. The nmol of MDA per ml was calculated using 1.56×10^5 as extinction coefficient.

Total thiol groups assay

Total thiol groups content is one of the most popular markers used in oxidative stress research. Total thiol groups of homogenized gill tissues were measured spectrophotometrically at 412 nm using DTNB as the reagent (Hu and Dillard 1994). After adding tris buffer to homogenized gill tissue, first absorbance was read at 412 nm (A1). Then DTNB was added and second absorbance at 412 nm was done (A2). The concentration of total thiol groups was calculated and expressed as mmol/l.

Statistical analysis

Statistical analysis was conducted using SPSS for windows (release 16, SPSS Inc, Chicago, Ill) with a *P* value of <0.05 as statistically significant. Independent-samples *T* test was used to compare measured parameters between parasitized and non-parasitized fishes. Data were expressed as mean $\pm$ SD.

Results

The results for MDA and total thiol groups in goldfishes with *Dactylogyrus* and healthy controls are shown in

Table 1 MDA and total thiol groups levels in the gill tissues of goldfishes parasitized with *Dactylogyrus* and healthy controls

Parameters	Parasitized group (<i>n</i> = 15)	Non-parasitized group (<i>n</i> = 15)
MDA (nmol/ml)	7.86 ± 1.49 ^a	5.11 ± 2.32 ^b
Total thiol groups (mmol/L)	0.035 ± 0.014 ^a	0.074 ± 0.007 ^b

Means within rows lacking a common superscript differ ($P < 0.05$)

Table 1. The level of MDA was significantly ($P < 0.05$) higher and the concentration of thiol groups was significantly ($P < 0.05$) lower in goldfishes with *Dactylogyrus* than those of healthy controls.

Discussion

Cyprinidae that are infected by *Dactylogyrus* may have symptoms that include gill hyperplasia, reduced growth and accelerated respiration. In severe infections, *Dactylogyrus* can cause hemorrhaging and metaplasia of the gills which can lead to secondary bacterial infections and death (Thoney and Hargis 1991). In the present study increased MDA and decreased total thiol levels were observed in the gill tissues of goldfishes parasitized with *Dactylogyrus* spp. This result indicates that parasite infection induces oxidative stress and a higher level of membrane damage in the fish gill due to increased ROS generation. As far as we know, this is the first attempt to demonstrate the effect of *Dactylogyrus* spp. on the oxidative stress status of goldfish.

Lipid peroxidation is essentially a toxic response to oxidative damage to cellular and tissue components (Di Giulio et al. 1989). Increased ROS generation in tissues leads to the oxidation damage to polyunsaturated lipids (lipid peroxidation), proteins and DNA (Storey 1996). Parasites have been shown to induce oxidative stress in host–parasite systems. Belló et al. (2000) reported that tert-butyl hydroperoxide initiated chemiluminescence, a measure of lipid peroxidation, was almost 2-fold higher in muscle of fishes parasitized with *Clinostomum detrunctum* than in muscle of non-parasitized ones. Marcogliese et al. (2005) demonstrated that yellow perch *Perca flavescens* parasitized with *Raphidascaris acus* and *Apophallus brevis* expressed higher levels of lipid peroxidation than those non-parasitized. They suggested that lipid peroxidation may be used as a biomarker of pathological effects caused by parasitism. Stumbo et al. (2012) evaluated the effects of experimental infections with larval trematodes *Ornithodiplostomum* spp. on induction of oxidative stress in fathead minnows, *Pimephales promelas*. Lipid peroxidation concentration in liver tissue increased 5 days after exposure. Sustained elevation in lipid peroxidation concentration for the liver trematode may be induced

by direct tissue damage caused by developing larvae in the liver, or by an immune response. These results indicated that the lipid peroxidation assay may be an effective biomonitor for parasite-induced oxidative stress in fish.

Thiol groups act as important cellular scavengers of peroxides and so help to protect cells from damage by these molecules. Decrease in thiol level not only impairs cell's response to oxidants, but also changes the functions of inflammatory cells (McKeown et al. 1984).

Shrimp *Palaemonetes argentine*s parasitized with the isopod *Probopyrus ringueleti* exhibited lower activity of the antioxidant enzyme superoxidase dismutase, which presumably lowers the capacity of the organism to deal with reactive oxygen species and thus to prevent cellular damage (Neves et al. 2000). By contrast, increased antioxidant enzymatic activities were observed in carp *Cyprinus carpio* parasitized by *Ptychobothrium* sp. when compared with healthy fish. This antioxidant response could contribute to neutralize the oxidative stress normally induced by parasitism (Dautremepuits et al. 2002, 2003).

Conclusion

Our results indicate that *Dactylogyrus* spp. infection increases ROS production in the gill tissue of goldfishes. This situation leads to oxidative stress and consequently gill damage via products from lipid peroxidation. The resulting lipid peroxidation might have a role in the pathogenesis of *Dactylogyrus* spp. infection. Further research is required to evaluate the correlation between parasite density and oxidative stress markers in fishes with *Dactylogyrus* spp. infection.

Acknowledgments The authors wish to thank technicians who kindly helped us for sample collection of this study.

Conflict of interest None of the authors have any conflict of interest to declare.

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