Lipid peroxidation and antioxidant responses in zebrafish brain induced by Aphanizomenon flos-aquae DC-1 aphantoxins

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A B S T R A C T
Aphanizomenon flos-aquae is a cyanobacterium that is frequently encountered in eutrophic waters worldwide. It is source of neurotoxins known as aphanotoxins or paralytic shelfish poisons (PSPs), which present a major threat to the environment and human health. The molecular mechanism of PSP action is known, however the in vivo effects of this neurotoxin on oxidative stress, lipid peroxidation and the antioxidant defense responses in zebrafish brain remain to be understood. Aphanotoxins purified from a natural isolate of A. flos-aquae DC-1 were analyzed using high performance liquid chromatography. The major components of the toxins were gonatoryxins 1 and 5 (GTX1 and GTX5, 34.04% and 21.28%, respectively) and neosaxitoxin (neosTX, 12.77%). Zebrafish (Danio rerio) were injected intraperitoneally with 7.73 μg/kg (low dose) and 11.13 μg/kg (high dose) of A. flos-aquae DC-1 aphanotoxins. Oxidative stress, lipid peroxidation and antioxidant defense responses in the zebrafish brain were investigated at various timepoints at 1–24 h post-exposure. Aphanotoxin exposure was associated with significantly increased (>1–2 times) reactive oxygen species (ROS) and malondialdehyde (MDA) in zebrafish brain compared with the controls at 1–12 h postexposure, suggestive of oxidative stress and lipid peroxidation. In contrast, reduced glutathione (GSH) levels in the zebrafish brain exposed to high or low doses of aphanotoxins decreased by 44.88% and 41.33%, respectively, after 1–12 h compared with the controls, suggesting that GSH participated in detoxification to ROS and MDA. Further analysis showed a significant increase in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) compared with the controls, suggesting elimination of oxidative stress by the antioxidant response in zebrafish brain. All these changes were dose and time dependent. These results suggested that aphanotoxins or PSPs increased ROS and MDA and decreased GSH in zebrafish brain, and these changes induced oxidative stress. The increased activity of SOD, CAT and GPx demonstrated that these antioxidant enzymes could play important roles in eliminating excess ROS and MDA. These results also suggest that MDA, ROS, GSH and these three antioxidant enzymes in the brain of zebrafish may act as bioindicators for investigating A. flos-aquae DC-1 aphanotoxins or PSPs and algal blooms in nature.

1. Introduction
Toxicogenic cyanobacteria-secreted paralytic shelfish poisons (PSPs) are frequently encountered in freshwater systems worldwide as a result of human activities (Ballot et al., 2010; Ledreux et al., 2010). Blooms dominated by Aphanizomenon flos-aquae DC-1 have occurred in Dianchi Lake, in Yunnan province, China, in most years in recent decades, as a result of the favorable nutrient supply and water temperature (Xing et al., 2007; Wu et al., 2010). At present, it is worrying that the prevalent algae in these blooms have been shown to secrete neurotoxic PSPs (Liu et al., 2006), which can damage the brain ultrastructure and influence locomotor function in zebrafish (Danio rerio) (Zhang et al., 2013a, b). This lake is an important source of freshwater for drinking and agricultural irrigation, and an important location for recreational activities and as a tourist destination for a population of over five million in the vicinity of Kunming city, Yunnan province (Liu et al., 2006). The blooms thus increase the potential danger to human health and environmental safety, and are having adverse effects on the local economy because of the risk of paralytic shelfish poisoning.

PSPs are potent alkaloid neurotoxins, discovered principally in the marine ecosystem where they are synthesized by dinoflagellates, but also in freshwater cyanobacteria and in bacteria (Ballot et al., 2010; Ledreux et al., 2010). PSPs can accumulate to a very high concentration in aquatic biota with no...
apparent harm to animals that filter-feed on these dinoflagellates and cyanobacteria (Zaccarioni and Scaravelli, 2008). However, subsequent human consumption of PSP-contaminated foodstuffs commonly leads to the development of paralytic shellfish poisoning, with significant morbidity and mortality (Ferrão-Filho and Kozlowsky-Suzuk, 2011). There is currently no effective antidote for PSP intoxication, and artificial respiration and fluid therapy remain the only available treatments (Wiese et al., 2010).

Concerning the effects of PSP exposure on fish, previous studies showed that PSPs could be transferred from toxic algae to a high-trophic-level fish (Kwong et al., 2006; Jiang et al., 2007). When PSPs entered fish in these ways, they delayed hatching and led to malformations and mortality in zebrafish (Oberemm et al., 1999; Lefebvre et al., 2004), and accumulated in the muscles of Geophagus brasiliensis (Clemente et al., 2010). Along with the physiological effects, PSPs altered the sensorimotor function and spontaneous swimming behavior of larval zebrafish and herring (Lefebvre et al., 2004, 2005). All these results demonstrate that PSPs exert potent toxicities in fish, not only by inducing the physiological effects, but also by causing behavioral changes in both larval and adult fish (Ferrão-Filho and Kozlowsky-Suzuk, 2011).

Lipid peroxidation is considered to represent an imbalance, with increased reactive oxygen species (ROS) and decreased activity of the antioxidant protective system (Boveris et al., 2008). The spectrum of ROS includes superoxide radicals, hydrogen peroxide, and hydroxyl radicals. The main protective system against ROS and their toxic by-products includes enzymes such as SOD, CAT, GPx and GST, as well as non-enzymatic systems such as GSH (Halliwell and Gutteridge, 2007; Melegari et al., 2012).

Several recent studies have demonstrated that extracted saxitoxin (STX) or PSPs from Cylindrospermopsis raciborskii may induce oxidative stress, lipid peroxidation in cultured neuro-2A cells and in brains of the fish Hoplias malabaricus, and antioxidative responses in the hepatopancreas of clams (da Silva et al., 2011; Melegari et al., 2012). These results also show that PSPs could induce oxidative stress, lipid peroxidation and antioxidative defense responses in both nervous cells and the nervous system, as well as in other non-nervous organs.

To the best of our knowledge, the effects of sublethal doses of A. flos-aquae DC-1 aphanotoxins or PSPs on oxidative stress, lipid peroxidation and the antioxidative defense responses in the zebrafish brain have not yet been established. Therefore, we investigated the changes in oxidative ROS and MDA, and antioxidative GSH, SOD, CAT and GPx in zebrafish brain at 1−24 h after exposure to sublethal doses of A. flos-aquae DC-1 aphanotoxins or PSPs. The results of this study will improve our understanding of biochemical changes and physiological function in the zebrafish brain, and the effects of neuromuscular stress induced by A. flos-aquae DC-1 aphanotoxins or PSPs. These results will also enable us to further understand the mechanisms responsible for brain damage and antioxidation induced by neurotoxins or PSPs.

2. Materials and methods

2.1. Chemicals

Reference standards for PSP toxins including STX group toxins (dSTX, STX, neoSTX) and gonyautoxin (GTX) group toxins (GTX1−5, dGTX2, 3) were purchased from the National Research Council in Canada, (Halifax, Nova Scotia, Canada). Chemical kits for SOD, CAT, GPx, GSH, ROS and MDA were provided by the Nanjing Bioengineering Institute, China. All other chemicals were of the highest grade available from commercial sources, unless otherwise indicated.

2.2. Algal culture, toxin extraction, purification and analysis

The algae collected from Lake Dianchi water blooms were cultured in sterile BG11 vehicle, and harvested and stored at −20 °C for further analysis.

The toxin extraction was carried out twice using 0.01 M acetic acid solution. The toxin purification was operated by precipitating, filtering and concentrating to dryness using a rotary evaporator (R-210; Buchi, Flawil, Switzerland), and finally passing through Sep-Pak C18 cartridges (Waters, Milford, MA, USA).

The analysis was operated by a LC20A HPLC system (Shimadzu, Kyoto, Japan), combined with fluorescence monitoring (LC20A, RF-10AXL, Shimadzu). Data processing was performed with Shimadzu Class-CR10 software (Shimadzu).

STXs and GTXs in extracts were identified by comparing the chromatograms with those obtained with reference standards. Toxic concentrations were determined using the factor response (peak area/toxin concentration) obtained by injection of reference standards (Diener et al., 2006). The overall toxicities of the samples were calculated as STX equivalents based on the amount of toxin and its relative toxicity compared to STX (Asp et al., 2004; Usup et al., 2004). Purified toxins were stored at −20 °C.

2.3. Determination of toxin dosage

A total of about 300 fish were used to determine toxin dosage. The preliminary experiment was based on previous toxicological studies (Bruce, 1985; Oshima, 1995; Lu and Tomchik, 2002). Two different toxin doses were finally selected: 7.73 and 11.13 μg/kg (5.3 and 7.61 μg STXeq/kg bw) as low- and high-doses, respectively. The lower dose caused evident behavioral signs of toxicity but no death, while the higher dose caused severe behavioral signs in all animals but low mortality. Control animals received vehicle alone (0.01 M acetic acid).

2.4. Preparation of brain samples

A total of 150 healthy male zebrafish (D. rerio) (each replicate) (Zebrafish Center of the State Key Laboratory of Freshwater Ecology and Biotechnology, Wuhan, China) from the same fertilization, batch and culture were used in this study.

After 10 days of acclimatization, 150 zebrafish were randomly assigned to control, low- and high-dose groups. The low- and high-dose groups received 30 μL 0.01 M acetic acid i.p. containing aphanotoxins at 7.73 and 11.13 μg/kg (5.3 and 7.61 μg STXeq/kg), respectively. Fish exposed to 30 μL 0.01 M acetic acid vehicle only served as controls.

Five fish from each group were euthanized at 1, 3, 6, 9, 12 and 24 h following toxin treatment, using cold shock by embedding into ice granules (−8 °C). The removed brains were stored at −40 °C until analysis.

The protein concentrations of enzymatic extracts were quantified using the Coomassie Brilliant Blue G-250 method (Bradford, 1976) using bovine serum albumin (Sigma) as the standard.

2.5. Examination of physiological parameters

ROS, MDA, GSH, SOD, CAT and GPx parameters were measured using six commercial detection kits (Nanjing Jiancheng Bioengineering Institute). The MDA content was expressed as nmol TBA reactive substance (TBARS)/mg protein/h. One unit (U) ROS reduced 1 μmol/L H2O2/mg protein/min. The GSH content was expressed as μg GSH/mg protein. SOD activity was defined as one unit of enzyme activity when 50% SOD activity was inhibited in 1 mL solution of the reaction system per mg of protein [U/(mg prot)]. One unit CAT activity corresponded to degradation of 1 μmol H2O2/mg protein/min.
One unit GPx activity corresponded to reduction of 1 μmol GSH/mg protein/min. All of these experiments were carried out in triplicate.

2.6. Statistical analysis

Data for MDA, ROS, SOD, CAT, GSH and GPx were analyzed statistically by one-way analysis of variance (ANOVA) using SPSS 13.0 statistical software (SPSS 13.0, Chicago, IL, USA). All data were based on three independent experiments. Significant differences between groups were assessed by ANOVA combined with least significant difference post-hoc tests. Data were expressed as mean ± SD. A value of p < 0.05 was considered significant, and p < 0.01 was considered extremely significant.

3. Results

3.1. Analysis of Aphanizomenon flos-aquae DC-1 toxins

HPLC-fluorescence derivatization (FLD) analysis revealed that the toxins extracted from cultured A. flos-aquaeDC-1 algae contained the three toxic components neoSTX, GTX1 and GTX5, which were identical to the respective control standards. The total content of PSP toxin from A. flos-aquae DC-1 was 9.52 ng/mg dry cell weight. The overall toxicity of the samples was 6.51 ng STXeq/mg dry cell, according to the relative toxicity ratios of GTX1, GTX5 and neoSTX versus STX, respectively. Moreover, HPLC-FLD revealed that the extracted toxins had a purity of 69.57%. GTX1 was the predominant toxin, accounting for 34.04% of total PSPs, whereas GTX5 and neoSTX represented 21.28% and 12.77% of total PSP, respectively.

3.2. Changes in ROS in zebrafish brain

ROS content in the whole brain was unchanged in the control group, but significantly increased in the aphantoxin-exposed groups (p < 0.05). At 3–12 h after exposure, the ROS content was 3.28 and 2.49 times higher than in the control group, respectively. However, at 12–24 h after exposure, there was no obvious difference in ROS level. The ROS content in the brain was elevated at 1 h after exposure, and there was a more obvious increase at 3–9 h after exposure. A maximum was reached at 9 h, with an average increase of 4.45 and 3.31 times at high and low doses, respectively. ROS content in exposed zebrafish brain gradually recovered at 24 h (Fig. 1).

3.3. MDA changes in zebrafish brain

The whole-brain MDA content was unchanged in the control group, but significantly increased in the aphantoxin-exposed groups (p < 0.01). The MDA content increased significantly in the high- and low-dose groups between 1 and 12 h after exposure; levels were 2.46- and 2.08-fold higher than in the control group, respectively. Brain MDA content in exposed zebrafish was increased at 1 h post-exposure, and increased further in time- and dose-dependent manners. The most evident increases in MDA contents occurred at 12 h and 9 h in the high- and low-dose groups, respectively, with 3.67- and 2.53-fold increases. The brain MDA contents of exposed zebrafish then gradually recovered, with a faster recovery in the low-dose compared with the high-dose group at 24 h (Fig. 2).

3.4. GSH level in zebrafish brain

The whole-brain GSH level was normal in the control fish, but significantly decreased in the aphantoxin-exposed groups (p < 0.01). Brain GSH level in the exposed zebrafish was significantly decreased at 3–12 h after exposure. In the high- and low-dose groups, GSH level was reduced to 44.88% and 41.33% of the control level, respectively. There was an obvious increase at 1 h and partial recovery at 12–24 h after exposure. The minimum GSH level occurred at 12 h after exposure, at 54.5% and 53.4% in the high- and low-dose groups, respectively. At 1 h after exposure, GSH content increased 1.34 and 1.09 times in the high- and low-dose groups, respectively. GSH level showed partial recovery between 12 and 24 h after exposure (Fig. 3).

3.5. SOD activity in zebrafish brain

SOD activity was normal in whole brains of control zebrafish, but significantly increased in the aphantoxin-exposed groups (p < 0.01). Compared to controls, the SOD activities increased significantly in the high- and low-dose groups from 1 to 9 h postexposure, with an average of 2.04- and 1.66-fold increases, respectively. The maximum SOD activity was at 1 h, with 2.53- and 2.2-fold increases in the high- and low-dose groups, respectively. On the contrary, SOD activity did not significantly differ among the groups with partial recovery from 12 to 24 h (Fig. 4).
3.6. CAT activity in zebrafish brain

CAT activity was normal in brains of control zebrafish, but significantly increased in aphanthoxin-exposed groups \((p < 0.01)\). Compared to controls, CAT activity increased significantly in the high- and low-dose groups at 1–12 h after exposure, with average 2.61- and 2.12-fold increases, respectively. The maximum CAT activity occurred at 1 and 6 h after exposure, with 3.43- and 2.62-fold increases in the high- and low-dose groups, respectively. On the contrary, CAT activity almost recovered at 12–24 h after exposure (Fig. 5).

3.7. GPx activity in zebrafish brain

GPx activity was normal in the control group, but significantly increased in the aphanthoxin-exposed groups \((p < 0.01)\). Compared to controls, GPx activity increased significantly in the high- and low-dose groups at 1–9 h after exposure, with average 2.04- and 1.69-fold increases, respectively. The maximum GPx activity was at 1 h after exposure, with 2.74- and 1.86-fold increases in the high- and low-dose groups, respectively. On the contrary, GPx activity almost recovered at 12–24 h after exposure (Fig. 6).

4. Discussion

We confirmed that A. flos-aquae DC-1 aphanthoxins or PSPs increased ROS and MDA levels, increased activity of antioxidant enzymes SOD, CAT and GPx, and decreased levels of antioxidant GSH in zebrafish brain, demonstrating oxidative stress, lipid peroxidation and antioxidant responses, respectively. These changes were time and dose dependent. The results suggest that the neurotoxicity of aphanthoxins or PSPs increases ROS and MDA levels and decreases GSH in zebrafish brain, and these changes might induce oxidative stress. Increased activities of SOD, CAT and GPx demonstrate that these antioxidant enzymes could play important roles in eliminating excess ROS and MDA.

4.1. ROS changes in aphanthoxin-exposed zebrafish brain

We studied changes in brain ROS content in zebrafish exposed to A. flos-aquae DC-1 aphanthoxins or PSPs. ROS level is widely utilized as a biomarker of environmental exposure and there are a variety of studies in the literature concerning the effects of environmental organic pollutants, toxins, heavy metals and other chemicals on ROS content in fish brain (Xing et al., 2012). However, data are limited about the effects of cyanobacterial neurotoxin or PSP exposure on ROS levels. Our results indicate an increase in the effect of the toxins on ROS content in zebrafish brain, suggesting that the toxins promote the production of free radicals and induce oxidative stress, which is similar to previous studies (da Silva et al., 2011).

ROS production is a metabolic response that attempts to increase hydrolysis of the toxins and eliminate damage to fish brain, which is similar to previous results (Chovanec et al., 2003). This situation is particularly obvious in fish brain compared with other organs. The major reason is that brain possesses a higher level of membrane polyunsaturated fatty acids (PUFAs) and consumes/needs more oxygen for metabolizing toxicants compared with other organs (Wang and Michaelis, 2010). The basic ROS components induced by many toxicants include superoxide anion, \(H_2O_2\) and \(OH^-\) radical. Thus, increased oxygen consumption in fish brain during metabolism of these toxins may increase production of superoxide anion and set off a series of cascade reactions to form \(H_2O_2\) and \(OH^-\), which leads to ROS formation (Olinski et al., 2002; Malhotra and Kaufman, 2007). In the present study, increased ROS, MDA, SOD, CAT and GPx levels suggested that the toxins induced direct or indirect ROS production and accumulation in zebrafish brain. In contrast, ROS accumulation may be related to the imbalance between ROS production and elimination, although there are high levels of antioxidant enzymes that may need time to eliminate ROS, suggesting the possible physiological response in fish brain (Lushchak, 2011).

4.2. Lipid peroxidation in aphanthoxin-exposed zebrafish brain

Increased ROS may attack cellular molecules that induce oxidative damage, thus we also assessed changes in lipid peroxidation in zebrafish brain by measuring MDA content. MDA content is considered to be one of the most important manifestations of lipid peroxidation induced by many toxins or toxicants (Hopps et al., 2010; Paskerová et al., 2012). In fish brain, several studies have shown that lipid peroxidation can be induced by insecticides, cyanobacterial toxins and ethanol (Vieira et al., 2009; Xing et al., 2012). However, data are limited about lipid peroxidation in fish brain induced by neurotoxins and PSPs. The increased MDA levels in zebrafish brain in our study may be attributed to the elevated ROS content, suggesting induction of lipid peroxidation, which is similar to previous studies (Kavitha and Rao, 2007; da Silva et al., 2011).
Increased MDA may result from increased ROS attack of cellular molecules, such as membrane lipids and proteins and nuclear DNA, which leads to membrane instability and lipid peroxidation (Ebadi et al., 2001; Valko et al., 2006). In the brain, lipid peroxidation may be easier than in other organs. The main reason is that brain has a large membrane surface area due to axon extensions and neuronal dendrites, which are rich in PUFAs and have high oxygen consumption, which makes them particularly vulnerable to ROS attack (Wang and Michaelis, 2010; Friedman, 2011). Previous studies have also shown that some organelles, such as mitochondria, endoplasmic reticulum and nucleus, may be vulnerable to attack by toxicants, and these are the major ROS-producing organelles (Inoue et al., 2003; Bouaïcha and Maatouk, 2004). Our previous results have also confirmed that A. flos-aquae DC-1 aphanotoxins or PSPs can influence the mitochondria, endoplasmic reticulum and nucleus and their membrane system, such as membrane blebbing, mitochondrial swelling, expansion of the endoplasmic reticulum, and nuclear chromatin condensation in zebrafish brain cells (Zhang et al., 2011, 2013a). These results are also supported by other previous studies (Nakamura and Lipton, 2010).

4.3. Antioxidant and enzyme activity responses in aphanotoxin-exposed zebrafish brain

To scavenge increased ROS and MDA and to avoid possible oxidative damage, organisms start an emergency response mechanism, such as increased activity of antioxidant enzymes SOD, CAT and GPx, and reduced endogenous antioxidant (GSH) to defend themselves against oxidative damage from free radicals (Sen et al., 2010; Xing et al., 2012).

4.3.1. SOD activity in aphanotoxin-exposed zebrafish brain

We also investigated changes in zebrafish brain SOD activity after toxin exposure caused by possible production of superoxide anion. SOD activity is generally considered as the primary line of protection against damage induced by increased ROS and lipid peroxidation (Wang et al., 2011). SOD can catalyze the dismutation reaction of superoxide anion to form H2O2, which is a precursor of the highly reactive OH– radical (Khan et al., 2012). It has been shown that many toxicants such as microcystin, insecticides, chemical dyes and heavy metals increase SOD activity in the liver, kidney, gills and gastrointestinal system of fish (Xu et al., 2009; Xing et al., 2012). However, a few studies have reported that increased SOD activity in the brain of common carp and H. malabaricus is induced by insecticides, heavy metals and cyanobacterial toxins (Xing et al., 2012; da Silva et al., 2011). In our study, the increased SOD activity in zebrafish brain suggested that oxidative stress resulted from the accumulated superoxide radicals induced by the neurotoxins, which is similar to previous studies (Pinho et al., 2005; Burmester et al., 2012).

Increased SOD activity may be an adaptive response to exposure to neurotoxins, which tries to degrade the increased superoxide anions to reduce the possible damage to zebrafish brain (Singh et al., 2010; Xing et al., 2012). Like many other organisms, fish can combat increased levels of superoxide radicals by utilizing their defense systems of ROS-scavenging enzymes such as SOD, which convert ROS into oxygen (Khan et al., 2012). Thus, the elevated SOD activity eliminates surplus superoxide anions in the brain, suggesting that the antioxidative defense response of elimination of superoxide anions still operates well under the current conditions, and increased SOD can be a physiological adaptation for the elimination of superoxide anions (Sun et al., 2006). Previous studies have also reported similar findings in other organisms. For example, da Silva et al. (2011) has reported that saxitoxin (STX) exposure produced by C. raciborskii induced a significant increase in SOD activity in the freshwater fish H. malabaricus. Therefore, the increase in SOD activity after toxin administration in our study may be attributed to an adaptive response that eliminated surplus superoxide anion (Singh et al., 2011; Xing et al., 2012). In contrast, SOD elevation may be related to an increase in its synthesis in response to toxin exposure, which is also supported by a previous study (Cabrera et al., 2010).

4.3.2. CAT and GPx activities in aphanotoxin-exposed zebrafish brain

We detected changes in CAT and GPx activities in zebrafish brain exposed to toxins, due to possible increased H2O2, which is formed by SOD dismutation to superoxide anion. CAT and GPx are two important enzymes that can work in cooperation for conversion of H2O2 into water and molecular oxygen (Wang et al., 2011). Previous studies have demonstrated that many toxins or toxicants, such as cyanobacterial toxins, heavy metals and insecticides, can cause elevation of CAT and GPx activities in the liver, kidneys and gills of fish (Xu et al., 2009; Jin et al., 2010). Recently, several studies have reported increased CAT and GPx activities in fish brain induced by atrazine, ethanol and other chemicals (Garcia and Martinez, 2012; Xing et al., 2012). We showed increased CAT and GPx activities in zebrafish brain induced by toxins, suggesting increased production of H2O2 with accumulated toxicity, which is similar to previous studies (Correia et al., 2007; Garcia and Martinez, 2012).

Increased CAT and GPx activities may be an adaptive response to toxin exposure, which tries to degrade the surplus H2O2 to reduce oxidative damage (Prieto et al., 2006; Xu et al., 2009; Xing et al., 2012). In contrast, the increased CAT and GPx activities may be attributed to increased synthesis in response to increased oxidative stress and lipid peroxidation (Gebringer et al., 2004). In addition, the elevation of CAT and GPx activities in the present study suggests that the defensive mechanism still operates well after toxin exposure, which is supported by previous studies (Moreno et al., 2005; Sun et al., 2006).

4.3.3. GSH level in aphanotoxin-exposed zebrafish brain

We also studied changes in GSH level in zebrafish brain exposed to toxins. GSH is involved in several key cellular functions, such as ROS scavenging, detoxification of electrophiles, maintenance of thiol–disulfide status, and signal transduction (Valko et al., 2007). Some toxins and xenobiotics (such as microcystin and atrazine) are reported to cause depletion of GSH levels in the liver, kidney, heart, gastrointestinal system, and muscles of silver carp, common carp and zebrafish (Atencio et al., 2008; Jin et al., 2010). Depleted GSH levels have also been reported in the brain of rats and hens, induced by atrazine and other chemicals (Kaushik and Kaur, 2003). We confirmed that GSH level was reduced in zebrafish brain by toxin exposure, suggesting increased oxidative stress and lipid peroxidation, which is similar to previous studies (Zhang et al., 2007).

GSH depletion may be attributed to its increased utilization in response to increased oxidative stress and lipid peroxidation induced by toxin exposure. For example, GSH, as an essential enzyme substrate, may react with GPx to protect against oxidative damage from endogenous ROS, which is supported by previous results (Huang et al., 2008). Thus, in the present study, the obvious increase in GPx activity after toxin exposure indicated that the rapid depletion of GSH participated in the elimination of ROS (Zegura et al., 2006).

4.4. Response time and recovery in aphanotoxin-exposed zebrafish brain

In the present study, we found that increased ROS and MDA content were apparent as early as 1 h after exposure, which was in accordance with the increased antioxidant defense response of GSH, SOD, CAT and GPx at 1 h. The partial recovery of ROS and MDA
content from 12 to 24 h after exposure was similar to that of GSH, SOD, CAT and GPx from 12 to 24 h after exposure to the toxins. The apparent increase in oxidative stress, ROS and MDA, as well as antioxidant defense SOD, CAT, GPx and GSH at 1 h after toxin exposure suggest a rapid response to the toxins in zebrafish brain, which is similar to previous studies (Lefebvre et al., 2005; Ahmed et al., 2007). The incomplete recovery in oxidative stress of ROS and MDA, as well as antioxidant defense SOD, CAT, GPx and GSH from 12 to 24 h after toxin exposure suggest the depuration of these antioxidant defense systems in zebrafish brain, which is supported by previous studies (Prieto et al., 2007).

4.5. Overall discussion in aptophoxin-exposed zebrafish brain

We discovered that increased ROS and MDA followed by decreased GSH and increased SOD, CAT and GPx activities suggested oxidative stress, lipid peroxidation and antioxidant response in zebrafish brain induced by A. flos-aquae DC-1 aptophoxins or PSPs. The increased ROS is attributed to metabolism of the neurotoxins that may occur in mitochondria and endoplasmic reticulum of the zebrafish brain cells, through increased oxygen consumption during exposure (Valko et al., 2007). The increased ROS may attack the cell membrane and lead to lipid peroxidation (Ebadi et al., 2001; Valko et al., 2006). The increase in superoxide radicals in turns increases ROS, which reduces damage but can also produce the harmful molecule H2O2 (Pinho et al., 2005; Burmester et al., 2012). Accumulation of H2O2 increases CAT and GPx activity, which can scavenge surplus H2O2 thus reducing brain damage in toxin-exposed zebrafish (Moreno et al., 2005; Sun et al., 2006). GPx eliminates H2O2 which consumes GSH as its reaction substrate, which may lead to depletion of GSH (Valko et al., 2007). The increase in CAT, SOD and GPx activities and the decrease in GSH in toxin-exposed zebrafish brain suggests that the antioxidant protection system still works well under such conditions. This may be an important physiological adaptation for the elimination of ROS and lipid peroxidation induced by neurotoxins (Xu et al., 2009).

5. Conclusions

In conclusion, this study demonstrated that sublethal doses of A. flos-aquae DC-1 toxin or PSPs caused oxidative stress, lipid peroxidation, and antioxidant defense response in zebrafish brains. Both high-and low-dose toxins increased the ROS and MDA levels, indicative of oxidative stress and lipid peroxidation. Further analysis revealed that GSH was depleted and SOD, CAT and GPx activities were increased, suggesting elimination of ROS and MDA through the antioxidant defense response in zebrafish brain. All these changes were dose and time dependent. These results provide direct evidence that the neurotoxicity of aptophoxins or PSPs is induced by increased ROS and MDA and depletion of GSH in zebrafish brain, and these changes induce oxidative stress. Increased activities of SOD, CAT and GPx demonstrate that these antioxidant enzymes play important roles in eliminating excess ROS and MDA. The results also suggest that oxidative stress, lipid peroxidation and antioxidant enzymes can act as bioindicators for investigating A. flos-aquae DC-1 aptophoxins or PSPs and environmental algal blooms in the future.

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