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Free Radical Mediated Neurotoxicity and Antioxidant Status In Brain of Freshwater Indian Catfish *Hsteropneustes Fossilis* to Elevated Temperature

Ashwani Kumar Dubey*

Research & Development Unit, Godavari Academy of Science & Technology, Chhatarpur, Madhya Pradesh, India.

***Corresponding Author:** Ashwani Kumar Dubey, E-Mail: ashwani_0326@yahoo.com, Phone: +91-9425143654

ABSTRACT

Lipid peroxidation is an indicator of free radical induced membrane damage resulting from the degradation of polyunsaturated fatty acids (PUFA). Neural degeneration is a frequent consequence of excessive free radical production. Brain is rich in PUFA and therefore it is susceptible to oxidative damage. In the present investigation, the effect of different temperature for varying periods on lipid peroxidation, superoxide dismutase activity, and ascorbic acid content in brain of freshwater Indian catfish *Heteropneustes fossilis* were observed. Results demonstrated that with increasing temperature from 25°C to 37°C, the lipid peroxidation and superoxide dismutase activity increased. Ascorbic acid content increased at 32°C and 37°C while no significant change was observed at 27°C. Lipid peroxidation with the increase in superoxide dismutase activity during temperature exposure demonstrated a continuous increase in free radical production in the brain. The increase in ascorbic acid may be due to its utilization in order to prevent oxidative damage. In conclusion, temperature stress induces neuronal damage via a mechanism leading to free radical production and altering endogenous antioxidant defense system.

Key words: Lipid peroxidation, Superoxide dismutase, Ascorbic acid, Brain, Oxidative damage.

Introduction

Extreme environmental conditions are known to exert stress on aquatic animals. Very low or high temperature elicits a series of physiological responses. Standard body metabolism increase continuously with increase in temperature till the attainment of lethal temperature limit and each species display its own characteristic rate of increase in a given range of temperature. The temperature has been known to alter enzyme activities (Shaklee *et al.*, 1977; Parihar *et al.*, 1997), oxygen consumption (Duthie and Houlihan 1982), ascorbic acid (Parvatheswararao 1967; Parihar *et al.*, 1996), phospholipids (Parihar and Dubey 1995), in fish. Blood flow and phagocytes activities are enhanced by even a small increase in temperature (Cheville, 1983; Parihar and Dubey 1995). These activated phagocyte cells in rainbow trout; Salmo gairdneri has been demonstrated to produce oxyradicals (Higson and Jones 1984; Secombes et al 1988).

Antioxidant enzymes play a vital role in protecting cellular damage by the harmful effect of reactive oxygen species (Dubey *et al.*, 1997; Altan *et al.*, 2003). Antioxidant matters protecting the cell membranes from lipid peroxidation (Havsteen 2002; Hosnuter *et al.*, 2004; Tatli Seven and Seven 2008;Dubey 2012). An extensive literature appeared to oxidative stress and antioxidant activity on lipid metabolism (Ahmed and Siddqui 2007) hepatocytes (Bukowska *et al.*, 2000) red blood cells

(Duchnowicz and Koter 2003) nephrotoxicity (Sharma *et al.*, 2011) while *in vivo* oxidative activity has been shown in fish (Ozcan *et al.*, 2004).

Brain is rich in polyunsaturated fatty acids and therefore, highly susceptible to peroxidation (Sun and Sun 1974; Dubey *et al.*, 1997). In the present study the effect of different temperature for varying periods on lipid peroxidation, superoxide dismutase activity and ascorbic acid content in brain of freshwater Indian catfish *Heteropneustes fossilis* were observed.

Materials and Methods

Experimental animal and design

Adult freshwater Indian catfish *H. fossilis* (weight 50±1.8 gm, length 17±1.2 cm) were procured from the local vicinity of Ujjain (MP) and acclimatized in laboratory conditions for two weeks in dechlorinated tap water at room temperature. A photoperiod of 12-hour light: 12-hour dark cycle maintained. After acclimatization fish were randomly transferred into separate exposure glass aquaria. Four groups were maintained, of 30 each. The first group was kept as control (25°C). The second; third and fourth groups were kept for temperature exposure.

Biochemical assay

The lipid peroxidation level was measured in terms of malonaldehide equivalents (MDA) by the thiobarbituric acid test Okhawa *et al.*, (1979) as described by Parihar *et al.*, (1996). Superoxide dismutase activity was assayed by the method of Marklund and Marklund (1974). Ascorbic acid measured by the modification of the dinitrophenylhydrazine (DNPH) technique of Terada *et al.*, (1978) as described by Thomas *et al.*, (1982). Protein content was estimated by Lowry *et al.*, (1951).

Statistical analysis

All data are expressed as mean ± SEM. Control and treatment values were compared by Student's t-test. The ($p< 0.05$) level was selected as the point of minimal statistical significance in every comparison.

Results and Discussion

Changes in lipid peroxidation, superoxide dismutase activity and ascorbic acid content in the brain after temperature exposure for varying periods are shown in table 1. The lipid peroxidation level was significantly increased in brain with increase in temperature from 25°C and 37°C. Its levels were significantly increased when temperature periods extended from 60 to 240 min. Brain combines the presence of a high percentage of polyunsaturated fatty acids (PUFA) with high free radical generating system. Fish PUFA is characterized by a higher degree of interaction than that of terrestrial organisms.

Since temperature exposure induced the lipid peroxidation which is considered to be the consequence of free radical activity in those biological membranes which are rich in PUFA (Cheeseman 1982), its increase level in the brain can have serious consequence for the whole organism taking into the account of the main co-ordinary regulatory functions of the brain during temperature stress.

In the brain superoxide dismutase (SOD) activity was significantly ($P<0.05$) increased at 32°C and 37°C elevated temperature at various times 60 to 240 min in comparison to control. Whereas no significant change ($P<0.05$) was observed at 27°C. Extending the period of temperature exposure from 60 to 240 min showed various responses at the different elevated temperature. In the present study increased lipid peroxidation with the increase in superoxide dismutase activity during temperature exposure demonstrated a continuous increase in free radical production in the brain. Temperature exposures consistently revealed a relationship between the SOD activities and lipid peroxidation in brain of *H. fossilis*. The very marked increase in SOD activity with exposure to temperature is almost certainly due to generation of free radical which resulted in peroxidation of lipids in biomembranes. A similar increase in SOD activity to increase in lipid peroxidation was also reported by Radi *et al.*, (1985), in fish exposed to certain pollutants. Oxidative stress might be associated with elevated or depressed SOD activity. It might be elevated owing to induction by the stressor or depressed as a toxic response to the stressor (DiGiulio *et al.*, 1989). In the case of temperature exposure, the first process i.e. induction of SOD activity might occur. Greater increase is SOD activity during temperature exposure indicates increase dismutation of superoxide anion to hydrogen peroxide (Farber 1994).

Ascorbic acid (AsA) content was increased at 32°C and 37°C significantly at extended time period, while no significant change was observed at 27°C for varying time periods. There is growing evidence which indicates that antioxidant vitamin might act

as protective agents against free radicals. Because AsA role as an antioxidant it is important to examine its level in the brain as an indicator of the oxidative stress. In conditions of AsA deficiency symptoms of oxidative stress including cell damage, might be expected to appear as the oxidant defense system is challenged to compensate for the lack of AsA (Bendich *et al.,* 1986). In our previous study AsA deficiencies were observed in respiratory organs, gill and air sac during the temperature increase (Parihar and Dubey 1995) in fish. This finding indicated that oxidative stress reflected to AsA deficiency under temperature exposure in respiratory organs and in these reported by (Henning *et al.,* 1991) by an increase in lipid peroxidation. AsA contents were increased significantly (P<O.05) in brain at each temperature exposure for varying periods as compared with control. Studies with human subjects showed that elevated level if AsA increases phagocytic activity (Krault *et al.,* 1988, Goetzl *et al.,* 1974) and enhance humoral immune response/antibody production in channel catfish (Li and Lovell 1985). As temperature exposure resulted in a significant increase in lipid peroxidation, the increase of AsA was probably due to the reducing power of AsA in the brain during temperature dependent free radical generation.

Conclusion

In conclusion, temperature stress induces neuronal damage via a mechanism leading to free radical production and altering endogenous antioxidant defenses.

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Table 1: Changes in the concentration of MDA during temperature exposure from 25°C (control) to 37°C at various times in brain of a freshwater Indian catfish Heteropneustes *fossilis*.

Experiment	Time Period (Minute)	Lipid peroxidation (n moles MDA/mg protein)	Superoxide dismutase (unit/gm wet tissue)	Ascorbic acid (µg/wet tissue)
Control (25°C)	60	6.23±0.50	10987±316	85.99±7.71
	120	6.34±0.50	11026±338	88.97±7.69
	180	6.24±0.48	10900±318	89.88 ±7.69
	240	6.28±0.49	10890±313	89.98±7.61
27°C	60	7.38±0.52*	11038±314*	115.21±8.11
	120	7.78±0.53*	11836±311*	118.39±8.08
	180	7.21±0.58*	11948±308*	121.32±8.04
	240	7.55±0.59*	11998±302*	124.31±8.31
32°C	60	12.35±0.43*	12473±310#	123±10.84#
	120	12.85±0.41*	12800 ±312#	128±8.62#
	180	13.65±0.49*	12880±313#	136±9.23#
	240	14.63±0.50*	12890± 312#	140±7.91#
37°C	60	20.50±0.48#	12899±528#	116.66±9.58#
	120	20.68±0.49#	13.680±529#	129±9.79#
	180	21.67±0.47#	13990±531#	139.79±8.23#
	240	22.68±0.48#	14580±533#	180.08±8.21#