Antioxidant Enzymatic effects of diethylene glycol dibenzoate on Zebrafish (*Danio rerio*)

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Abstract

Endocrine disrupting chemicals (EDCs) are known to disrupt normal metabolism and can influence the incidence of obesity in animals and humans. EDCs can exert adverse effects at low concentrations, often in a non-monotonic dose-related fashion. Among EDCs, Diethylene glycol dibenzoate (DGB), an approved alternative to phthalates in the production of plastic and latex products, however, is less abundant and its effects are almost completely unknown. The present study focused on the changes elicited Diethylene glycol dibenzoate (DGB) by on the enzyme activity Catalase and Superoxide dismutase of the various organs (muscle, gill, and liver) of Zebrafish. The observation registered in this study reflects that antioxidant enzymes activities were significantly enhanced in all the tissues (muscle, gill, and liver) when compared to control (untreated DGB). This could be due to the detoxification mechanism exhibited by the Zebrafish on exposure of DGP. The finding provides a support for the hypothesis that DGB may be the environmental contaminant with stress property.

KEYWORDS:

Diethylene glycol dibenzoate, Zebrafish, Catalase, Superoxide dismutase, Antioxidant defense.

INTRODUCTION

Today a variety of endocrine disrupting chemicals (EDCs) are recognized in the group of metabolic disruptors that includes obesogens, a wide range of environmental contaminants capable of altering energy balance regulation leading to obesity (Grun and Blumberg, 2006). These chemicals act through multiple mechanisms, ranging from direct increase in number/size of adipocytes to indirect alteration of both basal

metabolic rate and hormonal control of appetite and satiety (Heindel *et al.*, 2017). Recently, obesogen list has expanded to include contaminants that deregulate lipid metabolism (Chamorro-Garcia and Blumberg, 2014). An Information on harmful effect of DGB which is an approved alternative to phthalates in the processing of plastic and latex (Kermanshahiet al., 2009) is very limited. However, a recent study demonstrated that exposure to DGB leads to the stimulation of crucial lipolyticgenes via PPARα mediated pathway.

The antioxidant defense (AD) system of organisms provides a means of dealing with oxidative stress and includes several enzymes and vitamins (Filho, 1996*;* Rudeva, 1997*;* Kelly *et al.*, 1998*;* Marcon and Filho, 1999). A primary role of the AD system is protecting cellular components from ROS damage (Kelly *et al.*, 1998). The antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, act to remove oxygen radicals produced within the cell (Filho, 1996*;* Filho *et al.*, 1993*;* Michiels*et al.*, 1994*;* Kelly *et al.*, 1998). Superoxide dismutase occurs in two forms: (1) a cytosolic form that has copper and zinc in its active site (CuZnSOD) and (2) a mitochondrial form that has manganese in its active site (MnSOD) (McCord and Fridovich, 1969). Both forms of SOD protect the cell from potential ROS damage by converting superoxide anions to $H_2O_2$and H2O (McCord and Fridovich,1969).

Antioxidant system is located within different cellular compartments. These enzymes are found virtually in all tissue of vertebrates, but show in general high activity in the liver. A major organ of xenobiotics uptake and enzymatic transformation of ROS (Lamaire*et al.*, 1992) and eventually leak to blood. Some of these enzymes, like aminotransferase and phosphatase group, can constitute good molecular bioindicators for oxidative stress and can also indicate the magnitude of response in populations chronically exposed to contaminate such as metals and other xenobiotics (Livingstone *et al.*, 1994). Keeping this in view, the aim of this study was to assay the enzyme activity of CAT,SOD of the muscle,gill and liver of Zebrafish due to exposure of Diethylene glycol dibenzoate.

MATERIALS AND METHODS

Adult female zebrafish (*Danio rerio*, wild-type strain) were maintained in 100-L aquaria with oxygenated water under controlled conditions (28.0±0.5°C under a 14/10h of light/dark period) inaccordance with protocols and procedures approved by the University of Calgary Animal Care Committee. Fish were fed four times per day, twice with commercial adult zebrafish complete diet (Zeigler Bros., Inc.) and twice with *Artemia salina.* For the DGB trial, a total of fishes were equally distributed into three aquaria (one control and two DGB experimental groups) with a total of 10 fish in each one and treated groups. At the end of the 30th day, ten Zebrafish from each of the treatments and control were collected. The Gill, liver and muscle of Zebrafish were dissected and subjected to enzyme assay, SOD and CAT. About 0.4 mg of tissue sample was homogenized in 800µl Tris-Hcl buffer (100mM, pH-7.4) using a glass homogenizer. Then the homogenate was centrifuged at 10000 rpm for 15 min at 4 ^{O}C, and the supernatant was collected for antioxidant enzymes. Catalase (EC 1.11.1.6) activity was assessed following the Aebi method (Aebi, 1984) and SOD activity was assayed based on a modified method of Marklund and Marklund (1974).

Statistical Analysis

Results of the experiment were expressed as mean and standard error of mean of different groups. The differences between the mean values were evaluated by ANOVA (16.0). The values for P< 0.001 were considered significant. Accordingly, a statistical software package (SPSS) was used.

RESULTS AND DISCUSSION

Effect of DGB exposure to Zebrafish for CAT assay

The enzyme activities of various tissues were assayed in Zebrafish exposed to DGP. The data displayed in the CAT activities were significantly decreased (p<0.05) in DGB treated *in vivo* tissues compared with control (Table 1)

Table.1 Changes in the CAT of the various tissues of Zebrafish exposed to Diethylene glycol dibenzoate

DGP Treatment	Muscle U/mg protein	Gill U/mg protein	Liver U/mg protein
Control	4.240 ± 0.065^{c}	7.123 ±0.017^{c}	9.160 ±0.028^{c}
1-ppm	3.160 ± 0.030^{b}	6.183 ±0.014^{b}	4.086 ±0.014^{b}
10-ppm	2.250 ± 0.016^{a}	3.213 ±0.010^{a}	2.195 ±0.010^{a}

***Significant at P < 0.001. In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT). Values are expressed as mean ± standard error

Effect of DGB exposure to Zebrafish for SOD assay

The enzyme activity of various tissues was assayed in Zebrafish exposed to DGP. Thedata displayed in the SOD activities were significantly increased ($p<0.05$) in DGB treated *in vivo* tissues compared with control (Table 2). SOD activity was elevated in brains of carp and the neo-tropical fish *Hoplias malabaricus* induced by heavy metals, synthetic organic pollutants, and biotoxins (Da Silva *et al.*, 2011; Xing *et al.*, 2012). Therefore, increased SOD activity following DBP exposure, may be attributed to the cells adaptive defense mechanism to eliminate surplus O-. CAT is a key enzymatic antioxidant system, which can eliminate H_2O_2 produced from ROS, catalyzed by SOD, and thus alleviate cell damage (Zhao *et al.*, 2014).

Table.2 Changes in the SOD of the various tissues of exposed to zebrafish exposed to Diethylene glycol dibenzoate

DGP Treatment	Muscle U/mg protein	Gill U/mg protein	Liver U/mg protein
Control	10.183 ±0.045^{c}	8.233 ±0.056^{c}	4.170 ±0.026^{c}
1-ppm	15.213 ±0.020^{b}	20.213 ±0.037^{b}	6.736 ±0.020^{b}
10-ppm	19.356 ±0.024^{a}	23.913 ±0.361^{a}	7.193 ±0.038^{a}

***Significant at P < 0.001. In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT). Values are expressed as mean ± standard error

SOD and CAT play important roles in protecting the cell against the potentially toxic effects of experimental pollutants (Kuthan *et al.*, 1986). SOD, catalyzes the dismutation of the superoxide ion ($o2^{-}$) to hydrogen peroxide and oxygen molecule during energy processes. The reaction diminishes the caustic oxidative processes in cells. The level of antioxidant enzymes have been extensively used as an early warming indicator of lake pollution (Lin *et al.*, 1998). The present result is in good accord with the findings of Neerajkumar *et al.* (2011) who have confirmed that activities of anti-oxidative enzymes was significantly ($P<0.01$) influenced by endosulfan in a dose dependent manner in Tilapia *Oreochromis mossambicus*. They have noticed significant ($P<0.01$) increase in the activity of CAT, SOD and GST in gill and liver of Tilapia**.** Superoxide dismutase is an antioxidant enzyme involved in the elimination of ROS (reactive oxygen species). CAT is one among the key antioxidant enzymes that catalyses the removal of H2O2 formed during the reaction catalyzed by SOD (Arun and Subramanian 2002). Biomarkers such as protein level, enzyme activity and DNA can be used to measure the interaction between biological systems and chemical, physical or biological environmental agents (Watson and Mutti, 2004*;* Hernandez *et al.*, 2010). The liver is the primary organ for detoxification of xenobiotics and excretion of toxic substances in fish (El-Naggar*et al.*, 2009). The important function of the liver is to clean of any pollutant from the blood, but these pollutants could subsequently lead to structural damage in liver (Pathan *et al.*, 2010) as seen in the present study. Liver antioxidant enzymes are used to determine if the liver is functioning normally or if it has an injury or disease. Studies showed that enzymatic techniques are inexpensive and reliable to determine the toxicities of pollutants on marine animals in the living environment (Telli Karakoc *et al.*, 1997*;* Sunmonu and Oloyede, 2006*;* Hegazi*et al.*, 2010). Superoxide dismutase is an antioxidant enzyme involved in the elimination of ROS (reactive oxygen species). The antioxidant enzymes that make up the antioxidant defense system are expected to be intrinsically linked and dependent upon the activity of one another. Therefore, one could expect to see correlative changes in the activity of SOD and CAT (Filho *et al*., 1993)

Oxidative stress occurs if the activity of the antioxidant defense systems such as SOD, CAT and GPx (glutathione peroxidase) enzymes change by environmental pollution induces the production of reactive oxygen species (Li *et al.*, 2011). SOD is a vital antioxidant defense enzyme in nearly all cells to catalyze the dismutation of superoxide into oxygen thus protecting the cell from superoxide toxicity. SOD enzyme is more sensitive to the lethal effects of superoxide generating chemicals (Gardner *et al.*, 1995). CAT is found

in highest levels in the liver as a result of breaking down toxins present in the blood and processing metabolic products for degradation (Aebi, 1984; Chelikani *et al.*, 2004). The other study indicated that CAT and SOD activities were significantly higher in liver than in brain and gill of fish exposed to pesticide (Li *et al.*, 2011). The present results agree with earlier reports of increased antioxidant enzymes in fish exposed to environmental pollutants.

Conclusion

The Diethylene glycol dibenzoate (DGB) approved by European Chemical Agency as an alternative to phthalates in the processing of plastic. The alteration in the enzyme activity observed in the present study could be due to the formation of reactive oxygen species such as hydrogen peroxide, superoxide anion ($O2^-$), hydroxyl radicals (OH^-), which has to be neutralized by antioxidant enzymes. We can conclude that DGP may not provide a safe substitute and more studies on DGP safety will be needed on safety of DGP in fish and other organisms. Our study provides a framework for better understanding of the mechanisms underlying adverse health impact of stress EDCs. DGB is characterized as a high solvating plasticizer intended for the use in the manufacturing of PVC, vinyl flooring, adhesives, latex caulks, sealants and elastomers. These publications suggest that while DGB does not last long in the organism, the intermediates of its detoxification do, and the results do not support the use of DGB as an environmentally safer alternative to phthalates.

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