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Effect of mercury and zinc on some metabolically important enzymes of *Oreochromis mossambicus*

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Present study consists the effect of sub lethal levels of heavy metals - zinc and mercury on the activities of enzymes like HMG Co A reductase, lactate dehydrogenase and serine hydrolyase in *Oreochromis mossambicus*. Activities of the enzymes varied depending on the duration of exposure to the toxicants. Feasibility of measuring the enzyme activities in monitoring sub lethal metal poisoning is also discussed.

Introduction

Studies have demonstrated that structural and other properties of enzymes as well as specific activities are affected by exposure of animals to pollutants, which may possibly lead to loss of metabolic flexibility[1-6]. HMG Co A reductase (EC 1.1.1.34) catalyses the conversion of β-hydroxyl β methyl glutaryl Co A to mevalonate and is found attached to the endoplasmic reticulum. HMG Co A and mevalonate are the important intermediates in the biosynthesis of both cholesterol and ketone bodies. Enhanced synthesis of cholesterol, mediated by this enzyme may hence be important in encountering the increased influx of heavy metals into the body of the fish.

Lactate dehydrogenase (EC 1.1.1.27) is a zinc containing enzyme and is generally associated with cellular metabolic activities. A fish under stress preferentially meets its energy requirements through anaerobic oxidation. Lactate dehydrogenase can thus be used as an indicator in monitoring metal-induced toxicity in fish[9]. Serine hydrolyase (EC 4.2.1.1.3) or serine dehydratase plays a very significant role in gluconeogenesis and metabolism of amino acids. Like HMG Co A reductase, serine hydrolyase also plays a significant role in lipogenesis and hence they form an important defense mechanism against heavy metal toxicity[8]. In the present study attempts had been made to examine the effect of toxicity due to zinc and mercury on the activities of the enzymes, HMG Co A reductase, lactate dehydrogenase and serine hydrolyase in *Oreochromis mossambicus.*

Materials and Methods

Male specimens of *O. mossambicus* of an average length 10 ± 1 cm were collected from Rice Research Institute, Kochi and acclimated in the aquarium tanks maintained at *p*H 7.0, temperature 29 ± 1ºC and salinity 0 ppt. The effect of Hg^{2+} and Zn^{2+} on the enzymes, HMG Co A reductase, lactate dehydrogenase and serine hydrolyase in *Oreochromis mossambicus,* was studied by exposing the fish to water containing Hg^{2+} and Zn^{2+} at concentrations of 1/10 of 96 h LC_{50} (Hg^{2+} 0.1 ppm and Zn^{2+} 0.42 ppm). Sixteen fishes each were used for control and the two tests. Stocking density was one fish for 5 L of the medium. Medium in the tank was changed daily, maintaining the toxicant concentration constant after each replacement. Feeding was suspended 24 h prior to the sampling of the fish for assay of enzyme activity. Four fishes each were sampled from each tank after 2, 4, 6 and 8 days post-exposure. Liver was excised causing minimum disturbance to the specimen. Tissue was blotted to remove the adhering fluids and was used as the sample tissue.

HMG Co A reductase activity was estimated following the method described by Rao and Ramakrishnan[10]. Lactate dehydrogenase activity was measured following the method of Bergmeyer and Bernt[11]. The activity of serine hydrolyase was assayed following the method of Suda and Nakagawa[12]. Protein content of the extract for all the enzymes were

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estimated by the procedure of Lowry *et al* [13] with Bovine serum albumin as standard. The data obtained were analyzed using ANOVA (Two-way) technique.

Results

Studies on the effect of exposure of *O. mossambicus* to sub lethal concentrations of mercury and zinc revealed that the activity of the enzymes monitored varied depending on the dose and duration of exposure to the toxicant. Changes in the activity of HMG Co A reductase produced by exposure to zinc and mercury are summarized in Table 1. Difference between the effects of both the xenobiotics is not significant at each specific period of exposure at 5% level. But in both zinc exposed and mercury exposed animals, significant difference is noticed between the effect of different period of exposure (p<0.001). The effect of different periods of exposure show a sigmoid pattern with maximum activity attained on the 4th day of exposure to zinc and mercury. At the 2nd day of exposure both have an inhibitory effect, whereas at all other periods the enzyme is showing higher levels of activity.

The effect of exposure to zinc and mercury on activity of lactate dehydrogenase is represented in Table 2. Difference of activity of lactate dehydrogenase produced due to exposure to zinc and mercury is not significant at 5% level. But significant difference is noticed between the activity of enzyme due to different period of exposure (p<0.05). Highest activity of the enzyme as a result of zinc exposure is seen at 2nd day, after which the activity goes on decreasing. Enzyme activity in presence of mercury over different periods of exposure shows a sigmoidal curve. Effect of 2 days of exposure is inhibitory, whereas an accelerating effect is noticed at all other periods of exposure.

Serine hydrolyase activity in presence of zinc and mercury is given in Table 3. Variation between the effects of zinc and mercury on the enzyme is

Table 1—Effect of zinc and mercury on the activity of HMG Co A reductase of *Oreochromis mossambicus* expressed as the ratio of mevalonate to HMG Co A/min./g protein.

Days of exposure	Control		Mercury		Zinc	
	Activity	%	Activity	%	Activity	%
2	23.08 ± 0.05	100	18.82 ± 0.05	81.55	18.97± 0.00	82.20
4	15.58 ± 0.00	100	22.09 ± 0.05	141.78	23.94 ± 0.00	153.66
6	30.83± 0.07	100	43.56 ± 0.09	141.29	31.49 ± 0.08	102.14
8	41.44± 0.00	100	47.32± 0.66	114.19	44.76 ± 0.56	108.01

Difference between groups –not significant
Difference between exposure period –P< 0.001

Table 2—Effect of zinc and mercury on the activity of lactate dehydrogenase of *Oreochromis mossambicus* expressed as micromoles of NADH oxidized/ hour/ gm protein.

Days of exposure	Control		Mercury		Zinc	
	Activity	%	Activity	%	Activity	%
2	45.72± 6.09	100	30.78 ± 5.20	67.32	103.69 ±10.6	226.79
4	61.15 ± 8.15	100	83.81 ±12.1	137.06	73.34 ± 2.04	119.93
6	70.40 ± 11.1	100	104.58 ±20.9	148.55	69.17 ± 3.87	98.25
8	154.04 ± 3.65	100	174.78 ± 6.43	113.46	149.48 ± 51.2	97.04

Difference between groups –not significant
Difference between exposure period –P< 0.05

Table 3—Effect of zinc and mercury on the activity of serine hydrolyase of *Oreochromis mossambicus* expressed as millimoles of pyruvate formed/ hour/ gm protein.

Days of exposure	Control		Mercury		Zinc	
	Activity	%	Activity	%	Activity	%
2	22.94 ±0.82	100	27.13± 0.00	118.27	41.30±1.47	180.04
4	42.94 ±1.90	100	43.18 ±5.35	100.55	59.43 ±1.43	138.39
6	70.28 ±1.55	100	78.19 ±6.40	111.25	73.06 ±0.00	103.95
8	51.76 ±1.16	100	55.02 ±2.45	106.29	54.08 ±7.21	104.47

Difference between groups –not significant
Difference between exposure period – P< 0.001

not significant at 5% level. But the effects due to different periods of exposure to each metal varies significantly (p<0.001). Both zinc and mercury exert a stimulatory effect at all periods of exposure. Maximum activity is observed at the 2nd day of exposure and thereafter the stimulatory effect goes on decreasing in both cases.

Discussion

In presence of both the metals, HMG Co A reductase shows an initial inhibition. This may be due to the damage of the endoplasmic reticulum to which the enzyme is bound[7]. From 4th to 6th day of exposure a progressive stimulation of the enzyme is seen. This enhanced activity which can lead to increased production of cholesterol and ketone bodies works as a defense mechanism against metal toxicity[8]. Primary regulation of cholesterol biosynthesis is centered on the HMG Co A reductase reaction[7]. Hepatic lipogenesis has been reported as a defense mechanism in fish[8]. The stimulation of the enzyme may be achieved by regulation at the level of gene expression, by controlling the degradation of the enzyme or by activating the enzyme by phosphorylation[7]. A further decrease after the 6th day indicates the inefficiency of lipogenesis by the enzyme to trap the metal, the influx of which again exerts a deleterious effect on the enzyme. An increased HMG Co A reductase protein and mRNA levels in the small intestine has been reported in hyperglycemic condition[14].

In general, an enhanced activity of lactate dehydrogenase is noticed in presence of sub lethal concentrations of zinc and mercury. LDH plays a key role in energy metabolism and acts as a pivotal enzyme between the glycolytic pathway and Kreb's cycle. They catalyze the conversion of pyruvate into lactate under anaerobic conditions. This suggests that the stressed fish is meeting the lion-share of its energy requirements through anaerobic oxidation. The stimulated lactate dehydrogenase activity points to the fact that the pyruvate is preferentially transformed to lactate thus favoring anaerobic metabolism. It has been explained that the structural damage – the separation of epithelia from the underlying pillar cells of gill lamella – produced in the gill membranes increases the effective distance that oxygen must diffuse to reach the blood. This reduced efficiency of oxygen uptake leads to hypoxia and pyruvate is preferentially transformed to lactic acid[15]. Enhanced lactate dehydrogenase activity thus means a stepped up glycolytic rate. An enhanced lactate dehydrogenase activity in *Tilapia mossambica* in response to toxicant stress has been reported by Koundinya and Ramamurthi[16] and Balavenkatasubbaiah *et.al.*[9] An elevation in serum lactate dehydrogenase has been reported by Hilmy *et. al*.[17] and Christensen *et.al*[18,19].

Prolonged exposure to toxicants, however, registers a gradual decrease in lactate dehydrogenase activity which eventually approaches control. This drop in lactate dehydrogenase activity may represent a decline in the efficiency of anaerobic metabolic pathways. This may be related to the low rate of metabolism in the liver following extended exposure to sub lethal concentrations of these toxicants[20,21]. A temporary metabolic adaptation with increased dependence on glysolysis followed by a decline in their metabolism as evidenced by our study has also been reported by John[22]. A low lactate dehydrogenase activity due to impaired oxidative activities has been demonstrated by many authors[8,23-26]. The initial lactate dehydrogenase activity in the mercury dosed animals is very low and is significantly less than the control. This may refer to a situation where the regulatory mechanisms are set in fast and mercury is maintained at a level which is not enough to impair the aerobic oxidative pathways. The organism is preferentially resorting to aerobic metabolic pathways and maintains a decreased lactate dehydrogenase activity.

Maximum serine hydrolyase activity at the end of 2nd day of exposure followed by a gradual decrease on continued exposure to both the metals is observed. The glycogenic action of serine is explained by the production of pyruvic acid. The α and β carbon atoms of the amino acid can also be considered as a source of C-2 units and thus to fatty acid and cholesterol[7]. An augmented production of pyruvic acid and hence gluconeogenesis and also an elevated synthesis of fatty acids and cholesterol whose carbon residues are derived from the amino acids may also result. This lipogenesis serves as a defense mechanism against heavy metal toxicity[8]. However, extended exposure to the metal makes this defense mechanism inefficient and the metals exert direct inhibitory effect on the enzyme[27]. This may explain the gradual decrease in the enzyme activity noticed after 2nd day of exposure.

Conclusion

Considerable changes were observed in the activities of the various enzymes studied. These changes represent disturbances in the metabolic

processes of the organism. Measurement of enzyme activity could thus indicate the effects of stress at an earlier stage than when the effects become evident at the level of organ or organism and represents a valuable tool in biological monitoring.

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