Effects of heavy metal ions in the ovary of sexually mature Channa punctatus (Bloch)

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Abstract

Biochemical perturbations in the amounts of lysosomal hydrolases (AcPase and Alk Pase) lactate dehydrogenase (LDH) in the ovary of sexually mature breeding females of Channapunctatus (weighing 60.0±5.0 gm) challenged by sublethal concentration of $Pb(NO_3)_2$ (10 mg/L) and $ZnSO_4.7H_2O$ (20 mg/L) after 7, 21 and 35 days caused varying degrees of perturbation. These alterations appear to be time dependent. The ovarian AcPase manifested perceptible biochemical shifts in response to Pb and Zn over a period of time, nearly 50% increase was observed in both heavy metals after 7 days of exposure. In case of Pb decremental trend started from day 21 and continued till day 35, but a rebound recovery was seen in day 35 in case of Zn. Increase in AcPase may be due to leaching out of enzyme from cell organelles due to lesions. Alk Pase profile was differentially altered in response to Pb and Zn. A reduction in response to Pb on day 7 and 21 of treatment noted but recovered on day 35. These values showed 30-40% increase in case of Zn after 21 days of exposure. But the values dropped on day 35. This may be due to slow and steady bioaccumulation of Zn and crosses its threshold after this duration Pb and Zn caused decrease in amount of LDH after 7 days of exposure. Restoration of these in the ovary occurred after day 21 and 35. Reduction in LDH values seems to indicate that the preferred intermediate substrates are other than lactates.

Key Words

Channapunctatus, ovary, $Pb(NO_3)_2$, $ZnSO_4.7H_2O$ AcPase, AlkPase ovary, Sublethal, Biochemical, LDH.

Introduction

The cytogenous and endocrine functions in the ovary of teleost fishes are distinctly compartmentalised as they are in other vertebrates. The 'milieu interior' of the ovary contains a vast array of substrates eg. carbohydrates, protein and lipids, enzymes such as lysosomal hydrolases, oxido-reductases and dehydrogenase etc. Enzyme-substrate interactions are responsible for maintenance of pools of such vital precursors as amino acids, sugars (C_5 and C_6), nucleotides and cholesterol. Growing, dividing, vitellogenic and maturing cells of the ovary have the inherent albeit variable potential to utilize these via enzymatic intervention[1-3].

Biochemical estimates of enzymes and substrates provide significant complimentary evidence to delineate the importance of metabolite and enzyme that are suspected to play a vital role in ovarian functions. Many of these display cyclicity which can be linked with such diverse ovarian states 'spent state', recrudescence, initiation and maintenance oogenesis, folliculogenesis, maturation and eventual spawning. Structural integrity, viability and hatchability of eggs of teleosts depend on successful completion of these events.Heavy metal ions are known to induce a variety of histo-pathologies in gonadal tissues. In addition, they also cause severe aberrations in metabolic pathways that culminate in cell death[4,5]. Mutagenic, Carcinogenic, Co-carcinogenic and teratogenic effects of Lead (Pb) salts are well documented. Severe disturbances are reported to occur during gametogenesis in fishes exposed to differential doses of metal ions for an extended period of time[6,7]. Contrary to Pb, salts of Zinc (Zn) express their effects only when they are either deficient or in excess. Fecundity and cellular metabolism are significantly altered due to Zinc deficiency. Higher doses of Zinc induce histo and cytopathologies. Zn competes with deleterious metals e.g. Lead (Pb) by dislodging them from sub-cellular binding sites and thus imparts protection[8-12].

Deleterious effects of heavy metal ions on ovarian metabolism have been fragmentarily studied using some species of oviparous and viviparous teleost[13,14]. The present report deals with the biochemical perturbations in the amounts of lysosomal hydrolases (AcPase & Alk Pase), lactate dehydrogenase (LDH) in the ovary of sexually mature breeding females of Channa punctatus challenged by sublethal concentration of $Pb(NO_3)$ and $ZnSO_4.H_2O$ for varying durations.

Material and methods

Sexually mature breeding females of C.punctatus (60.0+5 gm) were collected from the non polluted aquatic vicinity of Udaipur during their breeding season (May-September). They were acclimated to laboratory conditions as described before [15].Preliminary toxicity tests indicated that 10 mg/L $Pb(NO_3)_2$ and 20 mg/L $ZnSO_4.7H_2O$ were sublethal concentration to this fish for 35 days. Control and experimental groups of fishes were set up, sacrificed and homogenate was prepared as per method described earlier[15]. The supernatant was used for the biochemical estimation of Ac Pase, Alk Pase and LDH[15].

Result and discussion

The ovary of sexually mature C. punctatus exposed to $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$ for varying duration exhibited characteristic variation in the biochemical milieu of AcPase, Alk Pase and LDH (Table 1). The ovarian AcPase of Channa punctatus manifested perceptible biochemical shifts in response to Pb and Zn over a period of time. The latter was more effective. Thus, nearly 50% increase in AcPase level was discerned in response to Pb and Zn after 7 days of exposure. These values dropped on day 21 in case of Pb (but remained above control) while in case of Zn this reduction was more than 50% on day 21 with a rebound recovery that was almost similar to control on day 35. However, in case of Pb the decremental trend continued till day 35. Increase in AcPase amounts can be related to a 'surge' in lysosomal activity or simply due to leaching out of enzyme from the organelles due to lesions caused by metal ions. Such a release would cause cell necrosis and eventual phagocytosis.

Lysosomal hydrolases have the ability to catabolize intracellular and extracellular proteins, lipids, carbohydrates and nucleic acids to generate a characteristic biochemical milieu in growing dividing and maturing cells[16-18].Increase in AcPase levels as observed in the present studies may be deleterious to synthesis of nucleic acids and proteins. Further, intracellular diffusion of this enzyme from lysosome due to submicroscopic lesions induced by Pb and Zn may be responsible for cell necrosis and eventual autolysis.

Total ovarian AlkPase of C punctatus manifests differential values that appear to relate well with the cation tested and the duration of exposure. Thus AlkPase values showed a dramatic reduction in response to Pb on day 7 and 21 of treatment but recovered to a value almost similar

to control on day 35. However, in response to Zn, these values showed a 30-40% increase on day 7 and 21. However, on day 35, these values dropped to more than 50%. These differential responses indicate that Pb impairs AlkPase functions more intensely than Zn. Further Zn appears to induce decremental aberrations only after 35 days. This may be due to slow and steady bioaccumulation of Zn in the ovary which crosses its toxic threshold after this duration including a sudden and drastic reduction in AlkPase values.

A comparison of these findings with other reports reveals interesting similarities as well as differences. Thus, Banerji (1992)[3], observed an increase in the ovarian AlkPase amounts of C.punctatus in response to Pb after 30 days of exposure. However this increase subsequently declined significantly by day 60. He interpreted the initial increase as an indication of injury and necrosis of cells or by substantial leaching of this enzyme from the fragile cells, affected by Pb. Further the subsequent decrease was attributed due to saturation of binding sites on the cell membrane. These results are at variance with the present findings. The differential result obtained with Zn-vis-a vis Pb indicate that the former is known to have a protective role at low concentration. Sastri and Agarwal[19] (1979) reported a Pb induced decrease in ovarian AlkPase of Heteropneustes fossilis. This is compatible with the present study though the extent of change vary. Passow at al[20] (1961)suggested that heavy metal ions may induce inhibition of enzyme activities by binding directly to sites available on the enzymatic protein.

Total ovarian LDH pattern of Channa punctatus on day 7, 21 and 35 of treatment by Pb and Zn manifested considerable variability. Thus Pb and Zn caused decrease in LDH values by day 7 of treatment vis-a-vis control. Restoration of LDH amounts in the ovary occurred after day 21 and 35 of exposure by Pb but the values steadily rose in surpassed control after 21 and 35 days in response to Zn. Reduction in LDH values by day 7 seems to indicate that the preferred intermediate substrates are other than lactates. Alternatively, the cells may metabolize intracellular glucose, via enzymes of the Krebs cycle. However, as the cations accumulate in the ovarian tissues after day 21 and 35 of exposure, the induced stress to the cell or oxygen debt may act as a trigger for switching ‘on’ of glycolytic pathways for meeting the energy demand of cell for survival etc.Limited comparisons are feasible due to lack of information on the Pb and Zn induced alteration in the ovarian LDH of teleost. Thus Banerji (1992)[3] reported increase in ovarian LDH concentration which was highly significant on day 30th of exposure to Pb. Increase

in LDH was explained due to hypoxia. Results of present studies provide evidence for this. It is plausible that lactates are preferentially utilized in releasing minimal 'Pulses' of energy that are pre-requisite for meeting the demands of ovarian cell types in order to maintain their cytoarchitecture and basal metabolic rate.

Table 1

Effects of $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$ on the ovarian biochemical milieu of sexually mature breeding females of C.punctatus.

S.No.	Duration	AcPase mg/dl%)	AlkPase mg/dl%	LDH mg/dl%
1.	$Pb(NO_3)_2$			
(a)	7 day Control Experimental	40.33±0.08 ***72.15±2.1 (+78.89)	5.40±0.56 ***0.51±1.42 (-90.55)	496.01±1.02 ***74.08±1.02 (-85.06)
(b)	21 day Control Experimental	42.08±1.54 **55.21±1.54 (+31.20)	4.95±0.98 ***1.05±1.61 (-78.78)	493.05±1.08 477.02±2.00 (-3.25)
(c)	35 day Control Experimental	40.05±1.21 **52.97±1.38 (+32.25)	5.15±0.82 5.01±1.26 (-2.72)	497.06±1.25 440.04±1.81 (-11.47)
2.	$ZnSO_4.7H_2O$			
(a)	7 day Control Experimental	40.05±1.52 ***79.40±1.08 (+98.25)	5.80±0.55 **8.05±1.84 (+38.79)	497.02±1.02 ***50.06±2.01 (-89.92)
(b)	21 day Control Experimental	41.08±1.28 ***8.20±2.12 (-55.69)	5.25±0.82 ***9.31±1.05 (+77.33)	496.25±0.92 **681.31±1.93 (+37.29)
(c)	35 day Control Experimental	39.19±1.82 42.29±2.42 (+7.91)	4.85±0.95 ***2.1±2.15 (-56.70)	494.09±1.63 **621.05±1.36 (+25.70)

*****P<0.01, ***P<0.001, ± S.E., ( ) values in parenthesis are decrease or increase vis-a-vis controls.***

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