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Original Research Paper

Effect of Zinc and Mercury on Lysosomal Membrane Stability of the fish *Oreochromis mossambicus* (Peters)

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ABSTRACT

Lysosomal enzyme release assay technique was carried out in the fish *Oreochromis mossambicus* exposed to mercury, a non-essential heavy metal, and zinc, an essential heavy metal. Mercury has an initial stabilizing effect on the lysosomal membrane followed by a progressive labilization indicating that sequestration of mercury by lysosomes, which relieves the biochemical machinery from the toxic effects, is efficient only up to a certain limit. Thereafter, the detoxifying machinery becomes ineffective resulting in the decline of the structure linked latency of lysosomal enzymes. On exposure to zinc, an initial labilization is seen probably due to the delayed onset of operation of the detoxifying mechanisms against this essential metal. Continued exposure to zinc stabilizes the membrane either by binding to the structural components or by inhibiting metal catalysed lipid peroxidation. Lysosomal enzyme release assay (LERA) is, thus, effective as a sensitive index of heavy metal stress and can be applied as a biochemical warning of environmental alterations.

INTRODUCTION

Some structural peculiarities of the lysosomal membrane, which is not yet clear, imparts the fundamental property of structure linked latency of lysosomal enzymes. This stability is essential for the normal functioning of the cell and it protects rest of the cell from the destructive effects of the enzymes. Alteration in the membrane causes release of enzymes and is of outstanding pharmacological significance (Woessner Jr. 1971, Kocchar & Larsson 1987).

Lysosomes are one of the most fascinating targets of heavy metals. Investigations of Sternlieb & Goldfischer (1976) have demonstrated that lysosomes are the most important sites of metal compartmentation in the cell. Their capacity to store and sequester a wide range of metal ions has been acknowledged by Allison (1969). An activation of lysosomal system involving an increase in the number and size of lysosomes was reported by various researchers (Myers et al. 1987, Studnicka 1983, Daoust et al. 1984, Weis et al. 1986, Sauer & Watabe 1989). This step clearly represents an adaptive and protective response to injury.

Heavy metals, which accumulate in lysosomes, stimulate the lipid peroxidation process and at the same time, inhibit the native defence mechanisms involved in prevention of lipid peroxidation. This results in formation of lipofuscin granules within the lysosomes. Lipofuscin can complex heavy metals and is a cardinal mechanism in heavy metal homeostasis (Viarengo 1985). Lipofuscin can later get transformed into an insoluble polymer that includes part of the bound metals which then become unavailable to the cell. Thus, though the metals within the lysosomes enhance lysosomal lipid peroxidation and alter the normal physiology of these organelles, they augment the amount of lipofuscin granules that can trap toxic metals in relatively stable form.

Though lysosomes are noted for their sequestration and accumulation of metals and various other chemicals, many of these substances are capable of destabilizing the lysosomal membrane, if the storage capacity is surpassed. Activation and leakage of the previously latent degradative lysosomal enzymes, which have got a high potential for the catabolic disruption of cellular systems, ensue and is of considerable environmental consequence (Bayne et al. 1978). These lysosomal membrane changes are perhaps, the earliest detectable alterations of cellular damage and hence, sensitivity of lysosomes to environmental pollutants including heavy metals ranks lysosomal responses as early warning systems for detection of the disturbances in the surroundings.

The present study attempts to extend the lysosomal enzyme releases assay technique to *Oreochromis mossambicus* exposed to two different heavy metals. This will, in turn, helps to assess its effectiveness as a sensitive index of heavy metal stress and its applicability as a biochemical warning of environmental alterations.

MATERIALS AND METHODS

Male *Oreochromis mossambicus* of average length 10±1 cm were collected from Rice Research Institute, ICAR, Kochi.

They were brought to the laboratory and acclimated for one month. The fish were fed daily and water was changed after consumption of the supplied food.

Feeding of the fish was suspended 24 hour prior to the experiment. Fish were stocked as three groups at a density of one fish per 5 litre. One group served as the control and contained dechlorinated tap water only and the others contained 1/10th of 96 hour LC_{50} of zinc (0.42 ppm) and mercury (0.1 ppm) respectively. The medium was renewed daily with minimum disturbance for the fish. After two days of exposure to the toxicant, fish were caught, liver was excised, water blotted off and weighed accurately. The process was repeated after 4, 6 and 8 days of exposure. Samples were homogenized in ice-cold isotonic sucrose solution containing 2mM mercapto-ethanol. Stability of lysosomal membrane was determined following the procedures of Philip & Kurup (1977, 1978) and Rao & Sisodia (1986). Acid phosphatase, the marker enzyme of lysosomes, released from the organelle was assayed following the procedure of Anon (1963). To the enzyme extract, 0.01 mM EDTA (Verity & Reith 1967) was also added to chelate any heavy metal present so as to nullify the chance of direct inhibition of enzyme by the metal.

Similar procedure was followed for the *in vitro* studies of the lysosomal latency, where the lysosomal rich fraction obtained from liver of acclimated fish was suspended in isotonic sucrose solution containing mercapto-ethanol and was incubated at 2 and 20 ppm of zinc and mercury solutions.

RESULTS

The effect of zinc and mercury on the stability of lysosomal membranes is shown in Table 1. Effect of *in vitro* exposure to zinc and mercury on the cell free preparations of lysosomes is presented in Table 2. The lysosomal lability indices, obtained as a function of time and function of various xenobiotics, were analysed using ANOVA technique. Wherever, the effects were found to be significant, least significant difference (LSD) at 5% level was calculated.

DISCUSSION

Lysosomes play a crucial role in the isolation and sequestration of heavy metals and thus, relieve the biological machinery from the toxic effects of heavy metals. But any excess of the heavy metal, beyond its storage capacity, causes leakage of degradative enzymes and derangement of cell functions.

Change in the membrane stability of lysosomes is one of the first alterations due to metal exposure and bears a quantitative relationship to the magnitude of stress response (Bayne et al. 1979, 1982, Cajaraville et al. 2000, Camus et al. 2000, Marchi et al. 2004). Thus, lysosomal membrane stability is a highly sensitive measure of the functional state of the cell and provides an ideal starting point for probing into the generalized cellular deterioration.

Presence of functional groups like thiol and disulphides in the membrane plays a key role in the integrity and permeability properties of the membrane and also in the structure linked latency. Mercuric compounds irreversibly damage the limiting membrane with the formation of mercaptide bridges. Moreover, the lipid peroxidation ensuing for the production of lipofuscin granules to bind mercury for homeostasis, also leave an unfavourable alteration in the physiology of lysosomal lamina. Mercury also suppresses the natural mechanism which inhibits lipid peroxidation.

Heavy metals have an “all or none” effect on individual lysosomes (Verity & Reith 1967). The values of acid phosphatase released, represent the proportion of the lysosomes whose threshold has been surpassed. The differences in individual lysosomal responses can be attributed to morphological and biochemical parameters that may decide the relative availability of thiol groups to the metal ions in the system.

The results of the present study indicates that as with other detoxification systems, the accumulation and sequestration of mercury by lysosomes also is effective only until the storage capacity of the lysosomes is overloaded or the lysosomes are damaged directly by the accumulated contaminant as reported by Moore et al. (1985). The lysosomal membrane is showing an initial stabilization and this is followed by a progressive labilization. This indicates that sequestration of mercury by lysosomes, which relieves the biochemical machinery from their toxic effects, is efficient only up to a limit. Thereafter, this detoxifying machinery becomes ineffective resulting in the decline of the structure linked latency of lysosomal enzymes.

On exposure to zinc, an initial labilization is seen probably due to the delayed onset of operation of the detoxifying mechanisms against this essential metal. The onset of operation of detoxifying mechanisms for this essential metal is not as fast as that for the toxic nonessential mercury. Results of the *in vitro* studies with 2 and 20 ppm solutions of zinc substantiate this hypothesis. A direct effect of metals on membrane rather than on the lysosomal enzyme is possible since all the metals remaining, which can cause any difference in activity of acid phosphatase, whose rate of release is taken as the index of the lability of lysosomes, are chelated and made unavailable to the system. Continued exposure to zinc stabilizes the membrane either by binding to the structural components or by inhibiting metal catalysed lipid peroxidation. Such a direct effect on membrane, suggesting that metals, which form redox systems, catalyse the lipid

Table 1: *In vivo* effect of Zn and Hg on stability of lysosomal membrane of *O. mossambicus* as a function of period of exposure. Stability of lysosomes is assessed by following the activity of acid phosphatase (millimoles of p-nitrophenol formed/hour/g protein) released as a function of time at 37°C.

Time	Control		Mercury		Zinc	
(min.)	Activity	LLI	Activity	LLI	Activity	LLI
A. Two Days						
00	02.96 ± 0.36	16.99	04.23 ± 0.26	15.68	03.52 ± 0.32	16.36
10	03.84 ± 0.18	22.07	05.27 ± 1.95	19.53	05.32 ± 0.20	24.76
20	03.87 ± 0.26	22.24	05.50 ± 0.23	20.38	06.04 ± 0.17	28.11
30	04.22 ± 0.11	24.26	05.73 ± 0.17	21.25	06.59 ± 0.21	30.67
B. Four Days						
00	08.13 ± 0.91	34.80	11.00 ± 2.17	35.60	03.89 ± 1.11	19.13
10	08.40 ± 0.33	35.95	10.93 ± 0.88	35.12	05.39 ± 0.38	26.45
20	08.67 ± 0.37	37.12	12.80 ± 0.44	41.13	05.44 ± 0.17	26.72
30	09.00 ± 0.29	38.53	12.82 ± 0.34	41.22	06.21 ± 0.15	30.48
C. Six Days						
00	09.28 ± 1.05	22.60	15.71 ± 0.84	21.18	04.42 ± 0.26	17.87
10	09.55 ± 0.29	23.25	19.37 ± 0.85	26.12	05.58 ± 0.30	22.58
20	10.47 ± 0.45	25.50	24.51 ± 1.75	33.04	05.53 ± 0.46	22.37
30	09.84 ± 0.74	23.97	34.67 ± 1.20	46.74	05.52 ± 0.07	22.38
D. Eight Days						
00	02.53 ± 0.20	13.45	02.94 ± 0.57	09.77	02.29 ± 0.57	10.31
10	04.06 ± 0.39	21.56	09.71 ± 0.23	32.29	04.17 ± 0.31	18.83
20	04.70 ± 0.38	24.95	09.57 ± 0.29	31.85	04.81 ± 0.17	21.68
30	05.63 ± 0.49	29.92	09.61 ± 0.50	31.96	04.98 ± 0.45	22.47

Two days - (Between groups, $p<0.01$, LSD at 5% level 0.870; Between time, $p<0.01$, LSD at 5% level 0.754)
Four days - (Between groups, $p<0.01$, LSD at 5% level 1.038; Between time, $p<0.01$, LSD at 5% level 0.899)
Six days - (Between groups, $p<0.01$, LSD at 5% level 9.18; Between time, not significant 5% level)
Eight days - (Between groups, $p<0.001$, LSD at 5% level 2.62; Between time, $p<0.05$, LSD at 5% level 2.27)
Lysosomal lability index (LLI) is the activity of acid phosphatase released expressed as percentage of the total activity of lysosomal lability acid phosphatase. Values are the mean of six different experiments ± SD.

Table 2: *In vitro* effect of different concentrations of Zn and Hg on the stability of lysosomal membrane of *O. mossambicus*. Stability of lysosomes is assessed by following the activity of acid phosphatase (millimoles of p-nitrophenol formed/hour/g protein) released as a function of time at 37°C.

Time	Control		Mercury		Zinc	
(min.)	Activity	LLI	Activity	LLI	Activity	LLI
A. 2 ppm						
00	01.33 ± 0.13	12.54	01.58 ± 0.11	14.89	00.61 ± 0.06	05.80
10	01.99 ± 0.10	18.74	02.11 ±0.12	19.94	03.03 ± 0.56	28.59
20	02.34 ± 0.10	22.04	02.45 ± 0.07	23.12	03.74 ± 0.57	35.29
30	02.96 ± 0.15	27.92	02.53 ± 0.07	23.87	04.83 ± 0.25	45.62
B. 20 ppm						
00	01.64 ± 0.11	06.70	01.20 ± 0.19	04.91	00.23 ± 0.04	00.93
10	02.15 ± 0.10	08.77	01.80 ±0.13	07.34	00.61 ± 0.17	02.48
20	02.75 ± 0.14	11.23	02.63 ± 0.13	10.71	00.61 ± 0.06	02.50
30	03.30 ± 0.15	13.48	02.53 ± 0.35	10.34	00.61 ± 0.07	02.48

At 2 ppm - (Between groups, Not significant; Between time, Not significant)
At 20 ppm - (Between groups, $p<0.001$, LSD at 5% level 0.69; Between time, $p<0.05$, LSD at 5% level 0.599)
Lysosomal lability index (LLI) is the activity of acid phosphatase released expressed as percentage of the total activity of lysosomal lability acid phosphatase. Values are the mean of six different experiments ± SD.

peroxidation and cause membrane damage has also been reported by Chvapil (1973). Lysosomal enzyme release assay (LERA) thus is effective as a sensitive index of heavy metal stress and can be applied as a biochemical warning of environmental alterations. LERA has been substantiated as a sensitive indicator of numerous environmental stresses in mollusks (Sadikaji et al. 2010). Ability of zinc to stabilize lysosomal membrane has been demonstrated by Sternleib & Goldfischer (1976) also.

To sum up LERA expressed in terms of lysosomal lability index (LLI) clearly reflects breakdown of the adaptive capacity of fish to toxic injury. It is to be realized that lability

of lysosomal membrane is not indicative of any specific stress, but many factors can cause the same response and so is a general index of stress. However, LERA is a very good indicator to monitor the effects of environmental pollutants on biomembranes.

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