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Note

Effect of sub-lethal level of copper and mercury on digestive gland cells and lysosomal enzyme activity in the green mussel (*Perna viridis* L.)

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Abstract

The activity pattern of two lysosomal enzymes, lysozyme and acid phosphatase, and histological changes in digestive gland cells were studied in the green mussel *Perna viridis* exposed to 25μgl^{-1} of either copper or mercury by sampling on day 0, 7 and 14. In general both the metals induced higher enzyme activity than control mussels. On day 7, the lysozyme activity in mercury exposed mussels was significantly ($p<0.01$) higher than that of copper, whereas, on day 14, the difference in enzyme activity was not significant ($p<0.05$) between metals. On the contrary, acid phosphatase activity on day 14 was significantly ($p<0.01$) higher in copper exposed mussels than mercury. Histopathology of the digestive gland showed tubular dilatation, tubular cell necrosis and destruction of cilia in the digestive diverticula. On day 7, the incidence of tubular changes was significantly ($p<0.01$) higher in mercury exposed group than copper whereas, on day 14, the incidence of tubular changes was not significant ($p<0.05$) between metals.

The green mussel *Perna viridis* is one of the commercially important marine bivalves found all along the east and the west coasts of India (Rao, 1974). These bivalve molluscs typically inhabit the estuaries and coastal areas that are increasingly contaminated with anthropogenic chemicals (Oliver *et al.*, 2000). It has been well established that bivalve molluscs accumulate and concentrate potentially toxic pollutants including heavy metals within their tissues far in excess of environmental levels (Roberts, 1976). Therefore, these species have been proposed as a biomonitor for trace metals in tropical waters (Krishnakumar and Pillai, 1990). Lysosomal enzymes are used as an ideal starting point for investigations of generalized cellular injury in marine molluscs (Sumner, 1969). Several reports say that heavy metals can affect the functions of cellular constituents including lysosomes (Moore *et al.*, 1984; Cheng and Sullivan, 1984). In addition, digestive gland could be an organ of choice in mussels for studying lysosomal enzyme response to stress factors since cells of the digestive gland are the major interface for the uptake of environmental contaminants (Moore and Allen, 2002). Lysosomes can accumulate diverse range of chemical contaminants, which can cause damage leading to their destabilization (Moore and Lowe, 1985). Subsequent to destabilization, lysosomes burst and release their contents into the extra cellular environment (Lauwerya and Buchet, 1972). Quantitative measurement of lysosomal enzymes has been suggested as a reliable indicator of stress imposed by environmental pollutants (Cheng, 1983). Published information in this area is meager though physiological and cellular responses to copper and mercury in the green mussel, *Perna viridis* has been reported (Krishnakumar *et al.*, 1990). In the present study, lysosomal enzyme activity and cellular changes induced by sub-lethal concentration of copper (Cu) and mercury (Hg) in the digestive gland of the green mussel, *P. viridis* are reported.

Materials and methods

Mussels (65-75 mm shell length) were collected from a natural mussel bed off Calicut (Lat, 11°30'N, Long. 74°40'E), Kerala (salinity: 32‰, temperature: 28°C, *p*H: 7.5) and were immediately transported to the laboratory. The mussels were acclimatized in UV filtered sea water (salinity: 32 ±1 ‰, temperature: 28 ±0.5°C, *p*H: 7.5 ±0.2, DO_2: >80% saturation) for 2 days. On day 3, the mussels were divided into three groups of 30 each in triplicates (a total of 90 mussels in each group) and maintained in plastic basins with 30 l of sea water (salinity: 32 ±1‰, temperature: 8 ±0.5°C, *p*H: 7.5±0.2, DO_2 >80% saturation). The first group was exposed to copper ($CuSO_4$ 5 H_2O, E.Merck, Mumbai, 25$\mu g\ l^{-1}$), the second group to mercury ($HgCl_2$, E.Merck, Mumbai, 25$\mu g\ l^{-1}$) and the third group served as control. The sub-lethal concentra-

tion of the metals was selected after acute toxicity experiment (96 hr LD_{50} for copper, 80μg l^{-1}; mercury, 155μg l^{-1}) by Krishnakumar *et al.* (1987). The mussels were not fed during the experimental period of 14 days.

For the enzyme activity studies, digestive gland was dissected out from 30 mussels representing each group on 0, 7, and 14 days. Lysozyme activity was determined as per Shugar (1952) with slight modifications. Digestive gland was weighed and homogenised in 0.06 M phosphate buffer (*p*H, 4.6) and centrifuged at 10000 x g at 4 °C for 30 min. An aliquot (20μl) of the supernatant was dispensed into the wells of a 96 well microtitre plate containing 280μl of lyophilized powder of *Micrococcus lysodeicticus* (0.15 mg/ml of phosphate buffer) as substrate. Triplicate wells were prepared for each sample. The decrease in the absorbance was read at 450 nm in a Biotek ELx 800 Microplate reader at 60 sec interval for 5 min. Standard curve was constructed with varying dilutions of hen egg white lysozyme in phosphate buffer. Lysozyme activity was defined as decrease in the absorbance of 0.001/min/g tissue and expressed as activity equal to μg of hen egg white lysozyme. For acid phosphatase activity assay, the method of Andersch and Szcypinski (1947) was employed with slight modifications. The weighed sample of digestive gland was homogenized in 1 ml of 0.1M citrate buffer (*p*H, 3.6). The homogenate was centrifuged at 10000 x g at 4°C for 20 min. An aliquot (10μl) of the supernatant was transferred to a clean test tube containing 1 ml of 0.1M citrate buffer (*p*H 3.6) and placed in a water bath at 37°C. 0.1 ml of *p*-nitrophenyl phosphate di-sodium salt (2 mg in 0.1ml of citrate buffer) as substrate was added to start the reaction. After an incubation of one hour, the reaction was stopped by adding 2 ml of 0.25 N NaOH. The absorbance was read at 410 nm in a Jenway 6300 spectrophotometer. Standard curve was constructed using different concentrations of *p*-nitrophenol (Himedia, Mumbai) in 0.25N NaOH. The enzyme activity was expressed as milli moles (mM) of *p*-nitrophenol liberated/ g of tissue/ hour. For histopathological studies, formalin fixed tissues were processed and paraffin embedded tissue blocks were cut at 5μ thickness and stained with hematoxylene and eosin using standard protocols. The percent incidence of tubular changes was evaluated by observing under the microscope at 400X magnification.

The activity pattern of acid phosphatase and lysozyme and the percent incidence of tubular changes between treatment and control groups were statistically analyzed by paired sample *t*-test (Systat Software Inc, USA, Version: 10.2.01).

Results and discussion

The activity pattern of lysozyme and acid phosphatase in the green mussel exposed to either 25μg l^{-1} of Cu or Hg is depicted in Figures 1 & 2 respectively. The lysozyme activity values on day 7 and 14 in metal exposed mussels were significantly ($P<0.01$) higher than that in the control mussels. Also, on day 7, the lysozyme activity was significantly ($P<0.01$) higher in Hg exposed mussels than those exposed to Cu. Acid phosphatase activity in metal exposed mussels was not significantly ($P<0.01$) different from control mussels on day 7. However, acid phosphatase activity in metal exposed mussels was significantly ($P<0.01$) higher than control mussels on day 14. Also, acid phosphatase activity was significantly ($P<0.01$) higher in Cu exposed mussels than those exposed to Hg. The increased lysozyme activity in the metal exposed mussels can be attributed to release of hydrolytic enzymes from the lysosomal granules within the digestive gland subsequent to destabilization of the lysosomal membrane by the metals as reported by Moore *et al.* (1984). Alter-

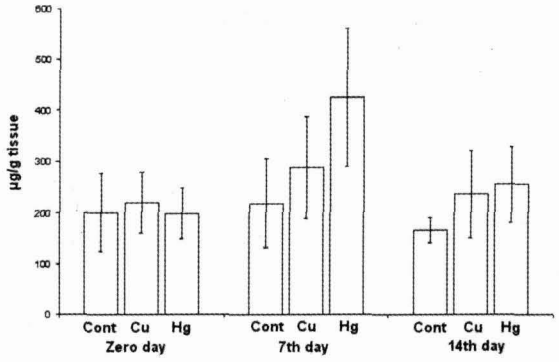

Fig. 1. Lysozyme activity pattern in the digestive gland of *P. viridis* exposed to copper and mercury. Vertical lines indicate standard deviation (n = 30).

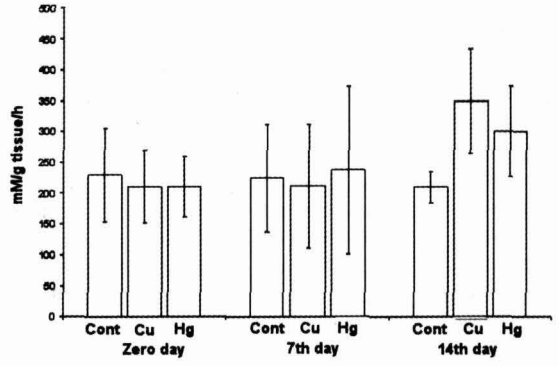

Fig. 2. Acid phosphatase activity pattern in the digestive gland of *P. viridis* exposed to copper and mercury. Vertical lines indicate standard deviation (n = 30).

natively, the increased enzyme activity in metal exposed mussels may also be due to hypersynthesis of the enzymes induced by the metals and subsequent release into the cytosol as suggested by Lauwerya and Buchet (1972). Absence of higher acid phosphatase activity in metal exposed mussels in the first week of experiment may be because even in a single cell, lysosomes are quite variable in enzymatic constitution and the enzymes of lysosomes are divergent in their functional attributes due to the heterogeneity in their size and shape (Suresh and Mohandas, 1989) or not all the cytoplasmic granules contain acid phosphatase activity and hence the vesicles represent a chemically heterogeneous population (Yoshino and Cheng, 1976). Lysozyme activity in metal exposed mussels was significantly ($P<0.01$) lower on day 14 when compared to the values on day 7. This decline implies inactivation of hypersynthesis by the metal ions. Suresh and Mohandas (1989) reported that when metal ions concentration is low, the hypersynthesis is not immediately inactivated by the metal ions and this is reflected as increased enzyme activity in the early time periods. However, as mussels continue to be exposed to copper, hypersynthesis of the enzyme is inactivated and hence, lowered lysozyme activity values on day 14 in metal exposed group when compared to the values on day 7.

Percent incidence of tubular changes is shown in Figure 3. Distinct pathological features such as cellular degeneration, necrosis, sloughing of cells, tubular dilatation leading to compression of the adjacent tubules, break down of tubules with desquamation of the cells into the lumen of the digestive gland and destruction of cilia in the digestive diverticula were observed in both the treatment groups (Figs. 4, 5, 6). The occurrence of tubular changes was significantly ($P<0.01$) higher in the metal exposed group than the control. The occurrence of tubular changes in the digestive gland indicates autolysis induced by re-

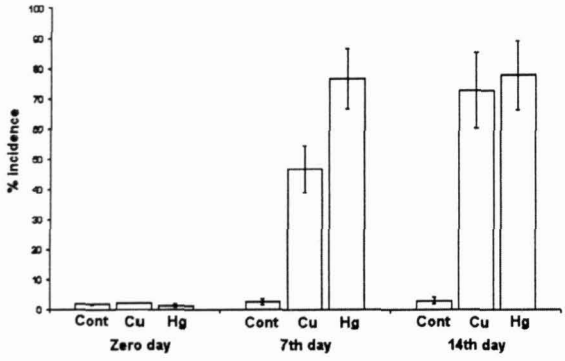

Fig.3. Percent incidence of cellular changes in the digestive gland of mussels exposed to copper and mercury. Vertical bars indicate standard deviation (n=15).

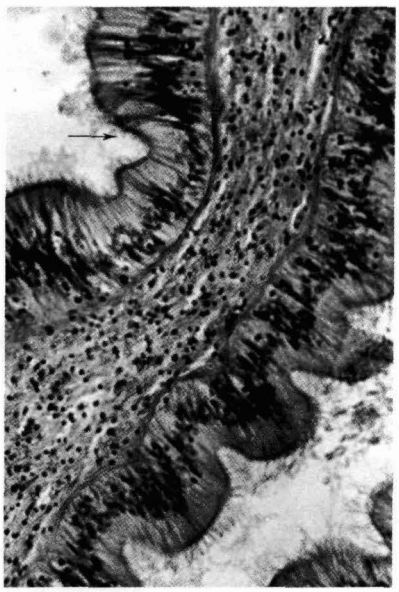

Fig.4. Extensive dilatation (→) of the digestive tubules leading to compression of adjacent tubules after two weeks of exposure to copper (H & E X 400).

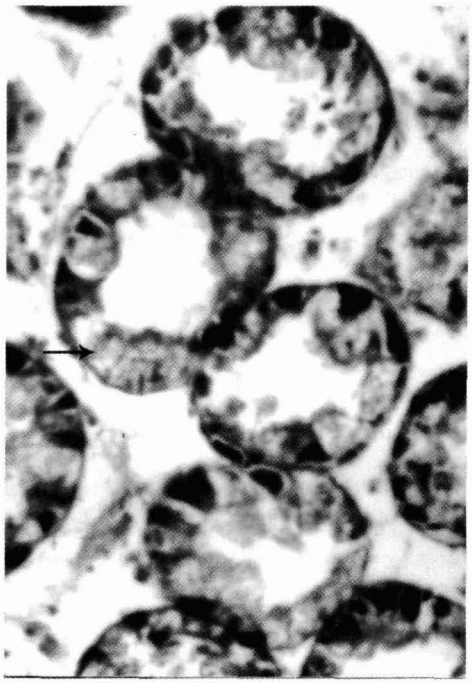

Fig.5. Vacuolation and degeneration (→) of tubular cells after one week of exposure to copper (H & E X 400)

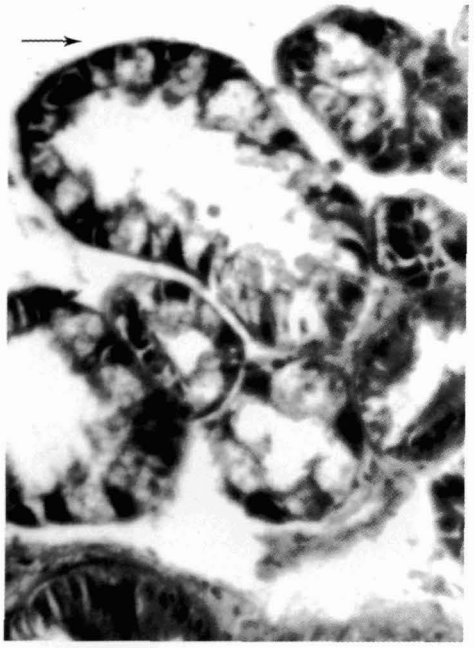

Fig.6. Digestive diverticula (→) showing loss of cilia after one week of exposure to mercury (H & E X 400)

leased enzymes. Krishnakumar *et al.* (1990) reported that leakage of lysosomal enzymes into the cytosol is responsible for tubular changes. On day 7, mussels exposed to Hg showed significantly higher incidence of tubular changes than that of Cu, whereas, on day 14 there was no significant difference in the percent incidence of tubular changes induced by both the metals. The incidence of tubular damage induced by Cu on day 14 was significantly ($P<0.01$) greater than those observed on day 7. The incidence of tubular changes in the present study was comparable to the activity pattern of the enzymes. The increased lysozyme activity and increased incidence of tubular changes in Hg exposed group on day 7 substantiate the predominant role of lysozyme in inducing the tubular lesions. The cellular pathology caused by acid phosphatase was also more conspicuous in Hg exposed group. This indicates that Hg induces comparatively higher hydrolytic enzyme activity than Cu. The tubular changes caused by both Cu and Hg on day 14 show the cumulative effect of both the hydrolytic enzymes.

Therefore, the present study concludes that both Cu and Hg could cause hypersynthesis of lysosomal enzymes. The significant hypersynthesis of lysozyme by Hg and of acid phosphatase by Cu indicates the ENZYME-METAL specificity. Both the metals could cause distinct pathology in the digestive gland cells at the given (25μg l^{-1}) concentration.

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