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Xenobiotic Induced Functional Alterations in Hepatopancreas of the Penaeid Shrimp *Metapenaeus dobsoni* (Miers)

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Abstract

Xenobiotic-induced imbalance between production and utilisation of cytosolic lipid provides biochemical evidence of structural and functional alterations in animal tissues. The present study investigates metal induced accumulation of unsaturated neutral lipid in the hepatopancreas of the penaeil shrimp *Metapenaeus dobsoni* (Miers) exposed to culture medium containing 0.05 and 0.15 ppm copper and 0.005 and 0.015 ppm mercury. Accumulation of neutral lipid in animals exposed to 0.05 ppm copper seems to be higher than that in animals exposed to 0.15 ppm, suggesting the possibility of a biphasic effect of themetal. Shrimp exposed to mercury showed reasonably high concentration of neutral lipid in the hepatopancreas, the mobilization being more at higher metal concentration. A significant observation made during the present study was on the formation of greatly enlarged heterogenous lysosomes as a result of exposure to heavy metals.

Introduction

Cellular accumulation of neutral lipid as a result of exposure of organisms to xenobiotics and the use of this phenomenon as a sensitive biochemical index to assess the cellular reactions to stress from pollution have been reported by Moore (1985), Capuzzo and Leavitt (1988), Lowe (1988), and others. Among the studies on the toxic effects of copper in crustaceans are those of Chen (1985) on *Penaeus monodon*, Liao and Hsieh (1988, 1990) on *P. japonicus* and *Macrobrachium rosenbergii*, Koivisto *et al.*, (1992) on cladocerans, and Weis and Weis (1992) on *Balanus eburneus*. Mercury, which has no established biological functionk also is one of the highly toxic heavy metals, affecting biological systems even at very low concentrations. Harmful effects of this heavy metal have been reported in *Penaeus japonicus* (Chuang *et al.*, 1991), *Penaeus* shrimp and crab (Palmer *et al.*, 1992), *P.aztecus* (Palmer and Presley, 1993), *Callianassa tyrrhena* (Thaker and Haritos, 1993) and *Pleoticus muelleri* and *Artemesia longinaris* (Marcovecchio, 1994). It has been noticed that mercury toxicity causes structural and functional alterations at the molecular level, interferes with biochemical functions and cellular respiration and affects growth and development in crustaceans. It is in this context that a study has been undertaken on the effect of copper and mercury on the effect of copper and mercury on accumulation of neutral lipid in a commercially important penaeid shrimp, *Metapenaeus dobsoni* distributed abundantly in tropical waters.

Material and Methods

Juvenile *Metapenaeus dobsoni* measuring 25-35 mm in total length (from the tip of rostrum to the tip of telson) were collected from an aquaculture farm at Vypeen (76° 10' Long E and 10° 0' Lat N); transported to the laboratory in oxygen filled polythene bags and acclimatised to a salinity of 20 ± 2 ppt. The test animals were fed *ad libitum* on fresh clam meat. Copper sulphate and mercuric chloride were used as sources of heavy metals. Test animals were exposed in batches of ten to sublethal doses of 0.05 and 0.15 ppm copper and 0.005 and 0.015 ppm mercury in a semi flow-through system for 15 days at room temperature (28 ± 20C). Experiments were run using 3 replicates for each treatment and control. Test medium was well-aerated. Left over feed and faecal matter were siphoned out and 2/3 rd of the test solution in each treatment was replenished every 24h. Three animals (belonging to the intermoult stage) were selected from each replicate of the different treatment on closure of the experiment for histochemical studies. Hepatopancreas was dissected out immediately after sacrificing each animal and fixed ad a whole tissue in hexane using liquid nitrogen. Sections, 10 microns in thickness, were taken using a Bright Cryostat, ;maintaining a chamber temperature of -20 ± 1°C; post-fixed in calcium formal at 4°C for 15 min; rinsed in distilled water and placed in 60% triethylphosphate (in distilled water) for 3min. Staining was done in a 1% solution of Oil Red O in 60% triethylphosphate, for 15 min at 20°C (Moore, 1988). The sections were then rinsed in 60% triethylphosphate for 30 seconds, rinsed in distilled water and mounted in an aqueous mounting medium, glycerin jelly. Unsaturated neutral lipid content of the epithelial cells as indicated by a red colouration was determined by microscopical assessment of the sections.

Results and Discussion

The intensity of colour and the area of colouration indicate the quantity of neutral lipid available in the hepatopancreatic tissue from various treatments. Hepatopancreas necessarily has a storage, though small, of neutral lipid as evidenced by the colouration in the control animals (Fig. 1.1). Difference in the intensity of neutral lipid concentration in the hepatopancras of animals exposed to 0.05

and 0.15 ppm copper gives the evidence that lipid accumulation is more when the medium contains 0.05 ppm copper (Fig. 1.2 and 1.3). Since there is no mechanism by which the bulkness of the organelles containing lipids could be assessed based on colour, it is not possible to ascertain whether the reduced number and size of pigmented areas in the hepatopancreas of animals exposed to 0.15 ppm copper does really indicate, variation in the quantities of lipid distributed in the cytoplasm and lysosomes or whether the metal shows biphasic effect on lipid accumulation in the hepatopancreas of *Metapenaeus dobsoni.* Fatty degeneration and increased cellular lipid content associated with heavy metal stress includes neutral lipid within the enlarged secondary lysosomes as well as lipid droplets distributed in cytoplasm. Evidently, the quantitative distribution of neutral lipid in the secondary lysosomes and cytoplasm of the hepatopancreas of animals exposed to 0.05 ppm copper seems to differ considerably from that seen in the animals exposed to 0.15 ppm copper.

In the case of shrimp exposed to mercury, the variation in the neutral lipid concentration was cospicuous. Animals exposed to 0.015 ppm mobilized more lipid to the hepatopancreas (Fig. 1.4). However, from the intensity of colour it looks that animals exposed to 0.005 ppm also have reasonably high concentration of lipid in the hepatopancreas (Fig. 1.5) In this case also accumulation of neutral lipid was much ;more than what was observed in the control group.

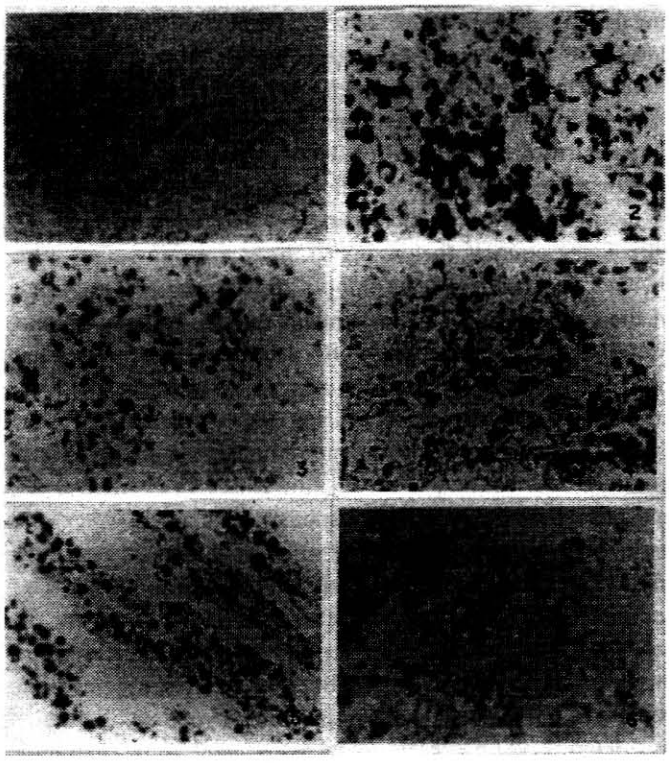

Fig.1.1. Hepatopancreas of *M. dobsoni* maintained under controlled conditons showing neutral lipid.X50

Fig.1.2. Heavily accumulated neutral lipid in the hepatopancreas of *M. dobsoni* exposed to 0.05 ppm copper. X100

Fig.1.3. Hepatopancreas of *M. dobsoni* exposed to 0.15 ppm copper with lesser accumulation of neutral lipid. X100

Fig.1.4. Hepatopancreas of *M. dobsoni* showing increased accumulation of neutral lipid when exposed to 0.015 ppm mercury. X100

Fig.1.5. Lesser accumulation of neutral lipid in the hepatopancreas of *M. dobsoni* exposed to 0.005 ppm mercury. X100

Fig.1.6. Hepatopancreas secondary lysosomes in the hepatopancreas of *M. dobsoni* exposed to 0.05 ppm copper. X200

A significant observation made during the present study was the formation of a different type of greatly enlarged secondary lysosome in metal abused animals. These lysosomes containing granular matrix, were found in very large numbers in the epithelial cells of the shrimp exposed to 0.05 ppm copper (Fig. 1.6). The heterogenous lysosomes, resulting probably from the fusion of smaller lysosomes, were surrounded by layers of discrete lipid droplets. The number of heterogenous secondary lysosomes observed in animals exposed to 0.15 ppm copper and 0.015 ppm mercury was comparatively less (Fig. 1.3, 1.4 and 1.5). The hepatopancreas of the control animals was totally devoid of the heterogenous secondary lysosomes.

Xenobiotic induced sublethal cellular pathology reflects perturbations of function and structure at the molecular level and the primary events are generally associated with particular types of subcellular organelle such as lysosomes, endoplasmic reticulum and mitochondria (Moore, 1985). According to Capuzzo and Leavitt (1988) changes in the lipid content and the lipid: protein ratio of digestive gland of *Carcinus maenas* were reflected along an ascending pollution gradient. However, not much literature is available on this phenomenon in crustaceans in general and shrimps in particular. In crustaceans, hepatopancreas plays a major role in the absorption and storage of lipid material. Mobilisation, accumulation and metabolism of lipid reserves influence growth, moulting, reproduction and adaptive responses to stress.

Moore (1988) reporting fatty degeneration and associated lysosomal accumulation of unasaturated neutral lipid in the digestive cells of *Mytilus edulis* and *Littorina littorea* from polluted waters, suggested this phenonmenon as a useful indicator of pathological alterations. Such enlarged and lipid enriched lysosomes frequenly showed reduced membrane stability, resulting in fragility and thereby augmenting transference of part of the increased cytoplasmic lipid to the lysosomes by autophagy. Storvh and Kleinfeld (1985) also provided evidence for increased autophgy associated with reduced lysosomal membrane stabiltiy. Increased quantity of neutral lipid notived in the hepatopancreatic cells of shrimp in the present study also could probably be associated with accumulation in the enlarged secondary lysosomes and distribution as droplets in the cytoplasm, as a consequence of exposure to sublethal doses of heavy metals.

An increase noticed in the neutral lipid content of the hepatopancreatic cells of *Metapenaeus dobsoni*, at a higher concentration of mercury in the test medium indicates that the enhanced lipid content is toxic dose-dependent. This is in agreement with the inference drawn by earlier workers. However, the maximum lipid accumulation observed in

individuals exposed to lower concentration of copper needs explanation. Some xenobiotics have been known to exert biphasic effects on organisms. Moore (1988) in a study on *Mytilus edulis* exposed to a diesel oil and copper mixture observed greatest lysosomal membrane stability in the low-exposure individuals. Here the xenobiotic is expected to have induced a stabilising effect on the lysosomal membrane. A similar biphasic effect of copper could probably be the reason for the larger quantity of neutral lipid found in the *M. dobsoni* exposed to lower concentration of the metal, in the present study also.

Most of the structural alterations observed in the invertebrate digestive epithlium brought about by exposure to pollutants are known to be due to increased lipid accumulations associated with enlarged secondary lysosomes. Pathologically enlarged heterogenous secondary lysosomes, developed as a consequence of pollutant exposure, were first reported by Lowe (1988) in a study on *Mytilus edulis* exposed to different levels of diesel oil and copper mixture. According to the author their numbers and sizes seem to be directly proportional to the levels of xenobiotic the animal is exposed to. Occurrence of heterogenous secondary lysosomes in large numbers in the hepatopancreatic epithelial cells of *Metapenaeus dobsoni* exposed to 0.05 ppm copper as noticed in the present study is probably an indication of cellular pathology. Their absence in the control animals supports Lowe's (1988) statement that these lysosomes are developed as a result of exposure to pollutants. However, a reduction in the number, in animals exposed to a higher level of copper (0.15 ppm) might indicate a certain level of biphasic effect exerted by the metal. Further, the lesser number of heterogenous secondary lysosomes, as noticed in animals exposed to sublethal levels of mercury would indicate a less pronounced induction of such lysosomes as a result of mercury toxicity. The fact that the heterogenous secondary lysosomes are surrounded by layers of lipid droplets could result in the distributioh of lipid in the cytoplasm as well. The present study clearly shows that the lipid content in heavy metal stressed animals is at any point of time, more than that in the control, suggesting histochemicsl estimations of neutral lipid as useful tool to assess chronic stress from sublethal doses of xenobiotics.

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