Effects of Chronic Exposure to Lead on the Chloride Cells in the Gills of Heteropneustes Fossilis

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Abstract—*This study is designed to investigate the ill effects of lead acetate on the chloride cells in the gills of Heteropneustes fossilis. Chloride cells perform an integral role in acid-base regulation. Sublethal concentration of lead acetate i.e. 3.0 mg/litre for six weeks produced significant changes.*

Keywords*: Lead, chloride cells, sublethal*

1. INTRODUCTION

Environmental levels of lead have increased more than 1,000 folds over the past three centuries as a result of human activities. Lead is a normal constituent of the earth's crust, with trace amounts found naturally in soil, plants and water. It left undisturbed, lead is practically immobile. Most of the lead released into the environment come from vehicle exhaust. Lead is released into the air during burning coal, oil or waste. Once lead gets into the atmosphere, it may travel long distances if the lead particles are very small. Lead may remain stuck to soil particles or sediment in water for many years.

Fish readily absorbs dissolved heavy metals in the water and this serves as a reliable indicator of the of heavy metals by a consortium led by imperial College, London, Showed 72% of Delhi's food samples (spinach etc) exceeded permissible levels for lead (Times of India, April 3rd, 2003). According to Indian council of Agricultural Research, a daily intake of even small quantities of the pollutants may lead to cancer, a reduced life span and fertility and many metabolic and genetic disorders.

The gills of Heteropneustes fossilis are located near the head region and are composed of paired gill arches on both lateral sides of the pharynx. Adayemo (2008) observed the histological alterations in the gills of clarias gariepinus exposed to environmental lead.

Fresh water teleosts take Ca^{+2} in the body from the surrounding water. Ca^{2+} can enter the fish through its chloride cells passively, as lone as the Ca^{+2} channals are open and the electrochemical gradient is favourable.

Oronsaye, Brafield (1984) studied the effect of dissolved cadmium on the chloride cells in the gills of stickle.

2. MATERIALS AND METHODS

Living specimens (approx. 14 to 16 cm in length and approx. wt. 70-80 gm) of fresh water teleost fishes, *Heteropneustes fossilis* (Bloch) were collected from the unpolluted fresh water, resources of the Hastinapur (U.P.) and were acclimatize to the laboratory condition for 4 to 5 days. Prior to experimentation, fishes were treated in 1% potassium permanganate solution for 15 minutes to disinfect the fishes. Fishes were maintained in laboratory glass aquaria in dechlorinated tap water (pH=7.4, hardness 160 ppm (as $CaCo_3$), alkalinity 87ppm). Fishes were fed twice daily with commercial fish pellets and small aquatic animals. Water temperature maintained between 18^0C to 24^0c for six weeks.

The fishes were exposed to sublethal concentration *i.e.* 3mg/L lead acetate according to the ‘standard Methods (1971) of the American Public Health Association. In each experimental group, 20 fishes were treated with the calculated dose of the lead acetate and a second group of 20 fishes in dechlorinated tap water served as control. After completion of tenure both the groups of fishes were processed simultaneously.

3. TOXICANT USED

Lead acetate of analytical grade was selected as the toxicant, obtained from BDH, England.

4. METHODS OF HISTOPATHOLOGY

(A) Light microscopic studies

Gills from both the groups were taken out, washed in 1% saline solution to eliminate mucus and blood deposits and after cutting in pieces of required thickness fixed immediately in 10% buffered neutral formalin and alcoholic Bouin’s fluid for 12 hrs. Standard methods of dehydration, clearing and embedding were used. Serial sections of 5-6 µ thickness were cut and stained with Delafield haematoxylin and alcoholic eosin.

(B) Electron microscopic studies

The ultrastructural studies were carried out in Regional Electron Microscope Facility at all India Institute of Medical Sciences, New Delhi.

For ultra structural studies fishes from both the groups were dissected ventrally and heart was exposed to perfuse intracardially with normal saline followed by 3% gluteraldehyde (GA) solution in 0.1 M phosphate buffer for about 10 minutes to give proper preservation. Gills were washed in 0.1 M phosphate buffer, and placed it over night at 4^0C. Final trimming of the Gills to appropriate size was done in the buffer. The trimmed tissues of about 1mm thickness were post fixed in 1% O_sO_4.

Further processing of tissues was done in the All India institute of Medical Science, New Delhi.

5. OBSERVATION

5.1 Control

Histologically, gill epithelium that covers the gill filaments and lamella provides a distinct boundary between a fish's external environment and extracellular fluids and plays a physiological function of the fish gill. As seen under electron microscope the gill epithelium of Heteropneustes fossilis is composed of several cell types. These are pavement cells (PVCs) and chloride cells (mitochondrion rich cells), which comprise >90% and <10% of the epithelial surface area respectively. In contrast to PVCs, chloride cells occupy a much smaller fraction of the branchial epithelial surface area, but they are considered to be the primary sites of active physiological processes in the gills. PVCs are found in all regions of gill filaments, chloride cells are usually more common on the afferent edge of filaments, as well as the regions that run between individual lamellae, termed the interlamellar region. Chloride cells are usually not found on the epithelium covering the lamellae.

Some ultra structural characteristics of these cells are presence of the extensive basolateral membrane infoldings that form a tubular system associated with numerous mitochondria and the subapical tubulovesicular system.

6. LEAD ACETATE TREATMENT

Chronic exposure to sublethal concentration of lead produced a number of histopathological changes in the gills. The gills were swollen compared to the control. The lesions observed in the gills of animals exposed to lead consisted primarily of epithelial lifting, which is characterized by a lifting of the outer layer of the lamellar epithelium and hyperplasia Large number of necrotic and apoptotic cells in gill epithelium were frequently seen.

Chloride cells were large and prominent and significant proliferation in the chloride cells were also observed.

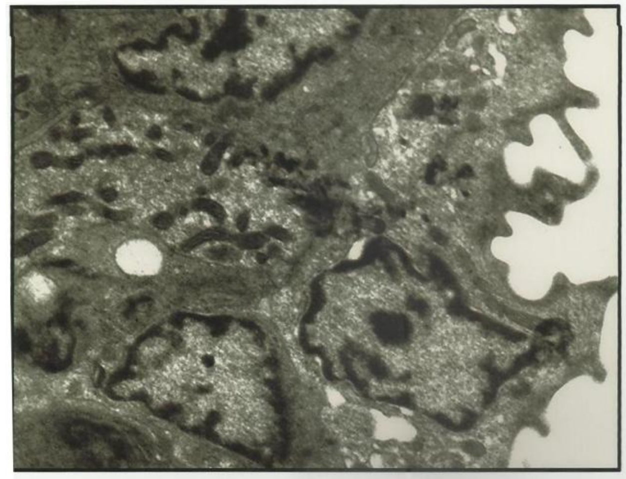

Fig.3. Electron micrograph of Mitochondria rich cell and pavement cells of Heteropneustes fossilis from control group showing normal structure of large number of Mitochondria and microridges. X-7600

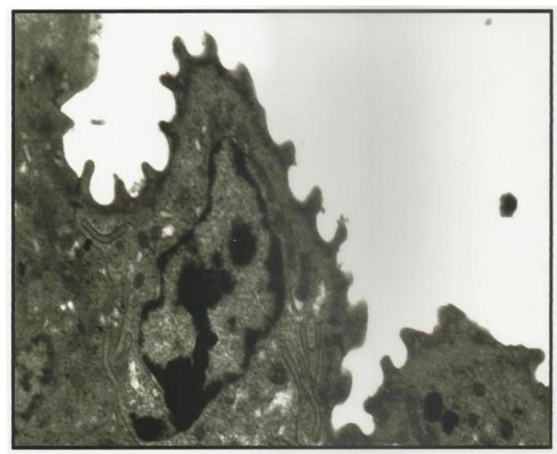

Fig.4. Enlarged view of the above showing well developed microridges in pavement cell and tubular elements. X-11600

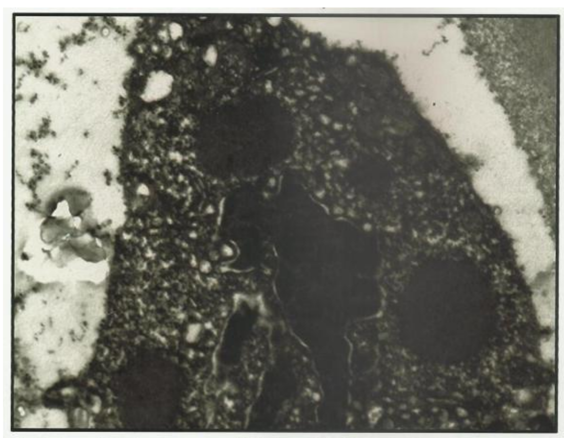

Fig.6. Apoptotic changes are visible in a chloride cell of fish of above group. Note complete degeneration of mitochondria and numerous enlarged phagosomes. X-13500

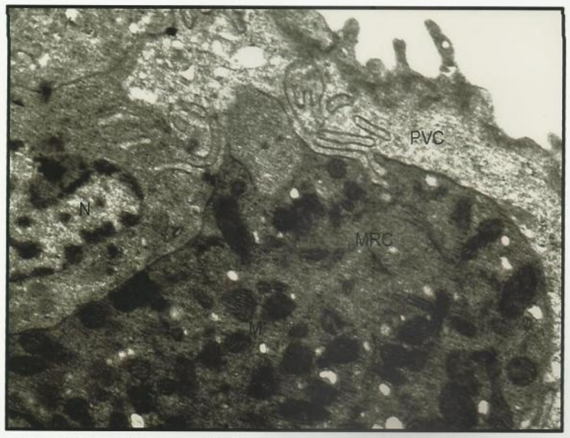

Fig.5. Mitochondria rich cell (chloride cell) and pavement cell are seen in micrograph of ultrathin section of gill of fish treated with lead acetate for six weeks. Note the degeneration of mitochondria and fragmentation of tubular element in MRC. X-13500

7. DISCUSSION

Lead can enter water column through geologic weathering and volcanic action and by various anthropogenic practices including smelting, coal burning and use in gasoline, batteries and paints (World Health Organization, 1995). The toxic effects of lead on fishes are multidirectional (Dimitrova et al, 1994).

Chronic exposure for six weeks produce increased number of chloride cells and their apical surface area. The main compensatory responses are hypertrophy and hyperplasia of the respiratory epithelial and chloride cells. Dutta et al, (1996) observed branchial defence response achieved by mucus hypersecretion, chloride cells proliferation, epithelial lifting. The number of chloride cells in the epithelial linings of both PL and SL of H. fossilis increased significantly. Rajbanshi and Gupta (1988) also observed an increased number of chloride cells in H. Fossilis following exposure to water-borne copper. An increased number of chloride cells in the SL following exposure to heavy metals salts has also been observed by Shepard and Simkiss (1978b), Lauren and McDonald (1987a, 1987b), Karlsson-Norrgren et al, (1985).

The mitochondria-rich chloride cell is believed to be the principal site of trans-epithelial Ca^{+2} and Cl^{-} influxes. These cells perform an integral role in acid-base regulation. Under certain conditions to challenge ion regulation, chloride cells proliferate on the lamellae.

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