MALATHION INDUCED HISTOLOGICAL MODIFICATIONS IN GILLS AND KIDNEY OF *CARASSIUS AURATUS GIBELIO*

MODIFICĂRI HISTOLOGICE INDUSE DE MALATHION ÎN BRANHIILE ŞI RINICHII DE *CARASSIUS AURATUS GIBELIO*

STAICU ANDREA CRISTINA, MUNTEANU MARIA CRISTINA, DINISCHIOTU ANCA
University of Bucharest, Faculty of Biology, Bucharest

Malathion is an organophosphorous insecticide, used in agriculture and a possible source of environmental poisoning. During malathion metabolization, mitochondria generates reactive oxygen species, responsible for significant structural changes. In this study, gills and kidney histological changes in Carassius auratus gibelio exposed to 0.05 mg/l malathion were investigated. In kidney, the effects were dramatic. The 24 -72 hours exposure to malathion induced cytoplasm vacuolization and changes in cell and nuclear volumes. In addition, necrotic renal tubules appeared, nuclear malformations of epithelial cells, anisokary, nuclei pycnosis and nuclei hypertrophy, were noticed. Epithelial ruptures, secondary lamellae fusion and hyperplasia of branchial epithelium, vascular congestion were the main changes noticed soon after pollutant exposure. We suggest that structural changes in gill and kidney could be used as good response to aquatic pollution with organophosphorous insecticides.
Key words: *Carassius auratus gibelio*, malathion, histological modifications

Introduction

Malathion (*O*-dimethyl-S1-2-di(ethoxycarbonyl)-ethylphosphorodithioate) is an organophosphorous insecticide widely used in agriculture and houses for the control of diseases vectors. It is a major source of environment poisoning in the developing countries (WHO, 2003). During malathion metabolization, reactive oxygen species can be generated and subsequently oxidative stress appears as Abbdollahi-Mostafalou
(2004), Akhgari et al.(2003), Banerjee et al., 1999 reported further acute and chronic exposure of rats. The main sub-cellular target of the organophosphorous insecticides and the source generating free radicals, are the mitochondria (Carlson-Ehrich, 1999; Tos-Luty et al., 2003; Beal, 1998; Delgado, et al., 2006). Reactive oxygen species damage cellular targets and structural changes appear. Toxicological tests have shown that malathion affected the central nervous system, immune system, adrenal glands, liver and blood.

The analysis of gills and kidney histological changes following malathion intoxication was the aim of this study, taking into account that these organs are involved in detoxification processes.

Materials and Methods

Experimental lots of two years old *Carassius auratus gibelio* were exposed to a sublethal dose of 0.05 mg /L Malathion. The fish standard length and weight ranged between 18.6-19 cm and 224-247.5 g respectively. Aquamerck Compact Laboratory (Merck) kits were used for water quality testing. The pH in the experimental water was 7.3, the hardness was 1.75 nmol/l, NO_3^- concentration was 0 mg/l, NO_2^- concentration was 0.05 ng/l, and the amount of dissolved oxygen was 6 mg/l. Control fish with the same characteristics were kept in dechlorinated tap water only. During the experiment the fish were not fed. Fish kidney and gill fragments, measuring 3/3mm were removed after 24 , 48, 72 hours and respectively 6 days of Malathion exposure, then fixed in buffered formaldehyde 4 hours long and further prepared for light microscopy. Haemtoxylin-Eosine was used for the staining of 8μm thick slices. The tissues samples were investigated by Olympus CX 40 light microscope.

Results and Discussions

The posterior kidney of freshwater fishes is adaptated to produce diluted urine and it has little participation in ion or acid-base balance. It receives the vast majority of postbranchial blood and because of that we expect renal lessions when the fish are exposed to pollutants. Therefore a study of these possible kidney changes could be a good response of environmental pollution. The body fluids of freshwater fish have a higher ionic concentration compared to surrounding water, a condition referred as hyperosmotic. To maintain the concentration gradient, the removal and conservation of ions prior to the excretion of "purified" water is required. This aim is accomplished in the kidney by filtration of water through glomerular nephrons comprising of a renal corpuscle and renal tubule.

When the magnitude of the pollutant-induced stress is enough to cause cellular lesions but not the death of the organism, changes may be noticed in light microscopy.

24 hours exposure to malathion induced significant histological changes. These include cytoplasm vacuolization (Figure 1A), changes in cell and nuclear volumes. Cubic epithelial cells lining renal tubuli were detached from the basal membrane. These damages persist following 48 and 72 hours. In addition necrotic renal tubuli appeared, nuclei pycnosys, nuclei hypertrophy, anisokary and nuclear malformations of epithelial cells were noticed. 72 hours of exposure revealed frequently the contraction of glomeruli and the enlargement of Bowman’s space (Figure 1B). Lymphocytes penetrate the renal epithelium, renal tubuli lumen narrowed or was obstructed by epithelial cells haotic proliferation (Figure 1A).

Also cytoplasm acidophily increased in epithelial cells and melanomacrophages aggregates appeared (Figure 1C) frequently following 48 hours' exposure.

Monocrotofos exposure induced the same renal damage (Vermurugan et al., 2007). Because the glomerule size changed, we assumed that the total filtering surface was decreased. The changes in the size of cells and the narrow lumen could be the consequence of changes in kidney function. The fact that the physiology of the tubule cells is affected may be noticed from the nuclear changes too.

48 hours exposure to malathion induced histological changes in the gills structure. Epithelial ruptures, secondary lamellae fusion and hyperplasia of branchial epithelium were noticed. After 72 hours, vascular congestion was added. Lamellar fusion (Figure 2A) and secondary lamellae hyperplasia (Figure 2B), the most frequent changes could be protective, decreasing the gills vulnerability. Some secondary lamellae were damaged, especially at their endings due to the vascular congestion which affected the epithelium (Figure 2C).

Respiratory troubles are the early symptoms of pesticide poisoning (Murty, 1986). We assume that lamellar fusion and secondary lamellae hyperplasia would induce fish suffocation. These structural changes increase the diffusion distance between the respiratory blood and xenobiotics. As a result, they also increase the oxygen distance for gaseous exchange due to the decreased surface of the secondary lamellae (Hemalatha-Banerjee, 1997 a, b). Secondary lamellae fusion occurred when chemical composition and thickness of the mucous coat changed and a disturbance in the normal ability to recognize different cell types appeared (Daoust et al., 1984).

The rupture of the epithelium explains the ion loss from the plasma, as Peuranen et al. (1994) noticed. This effect of epithelium rupture could lead to a negative ion balance and changes in the haematocrit and hemoglobin values, but also would cause severe disturbance in gill respiration.

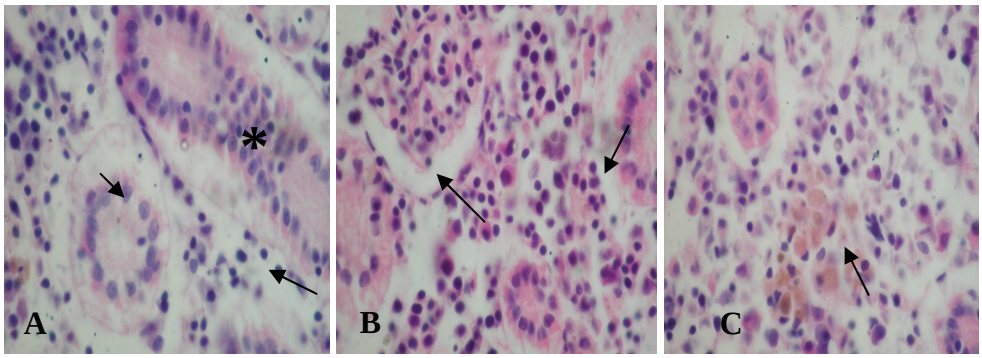

Figure 1 *Carassius auratus gibelio* kidney- A-24 hours exposure to malathion; cytoplasm vacuolization in epithelial cells lining renal tubuli (arrow), renal tubule obstruction by haotic proliferation of epithelial cells (star) (x100); B) 72 hours of exposure; Bowman's space enlargement (arrow) (x 100); C melanomacrophages aggregates (arrow) (x 100);

The rupture of the branchial epithelium is considered a direct, dose-dependent deleterious effect of the pollutant while hyperplasia, lamellar fusion and mucous hypersecretion could be signs of the branchial defence responses (Duttaet a., 1996).

Epithelial hyperplasia, aneurism, curling and fusion of secondary lamellae were noticed in *Cirrhinus mrigala* after exposure to monocrotofos (Velmurugan et al., 2007), in *Gambusia affinis*, after 30 days of exposure to deltametrin (Cengiz-Unlu, 2006).

In *Lepomis macrochirus*, malathion intoxication was responsible for supplementary changes: necrosis and oedema, (Richmonds-Dutta, 1989). Due to the serious changes in gill morphology and physiology, the fish would die through asphyxia.

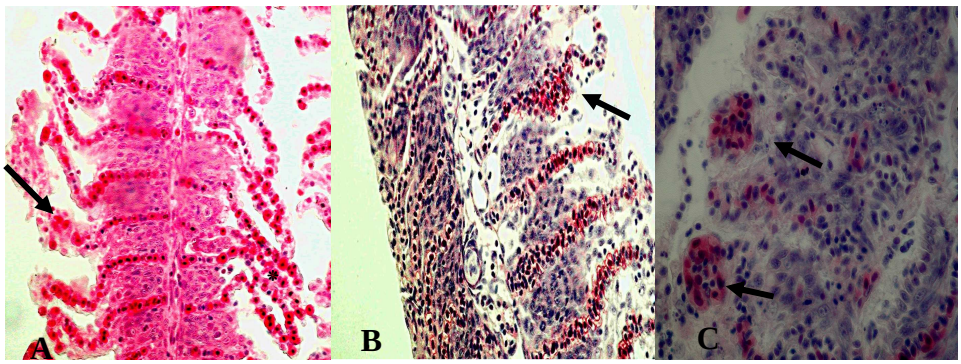

Figure 2 *Carassius auratus gibelio* gill –A) 48 hours exposure to 0.05 mg/L malathion; secondary lamellae fusion (star) and branchial epithelium disorganization appeared (arrow) (x 100), B) 72 hours exposure showing secondary lamellae hyperplasia (arrow) (x100); C) vascular congestion (arrow) (x 100); (H&E).

Conclusions

A low concentration of malathion 0.05 μg/l, close to that used for insects populations control, could be dangerous for fish populations. *Carassius auratus gibelio*, a widely spread species in freshwater basins, quickly showed structural changes as a consequence to pollutant exposure. Gills and kidney, organs which are deeply involved in organism defence against the pollutant are seriously damaged soon (48, 72 h) after fish exposure to the pollutant. Due to the serious changes in gill morphology and physiology, we assume that the fish would die through asphyxia. Because the changes in the glomerul size we assumed that the total filtering surface decreased. The changes in the size and structure of the epithelial cells and the narrow lumen of the renal tubuli could be the consequence of changes in kidney function. The frequent nuclear changes indicate that the physiology of the tubule cells is affected too.

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