PATHOLOGICAL TISSUE LESIONS INDUCED BY CHRONIC MERCURY INTOXICATION IN SILVER CRUCIAN CARP CARASSIUS AURATUS GIBELIO

LEZIUNI PATOLOGICE TISULARE INDUSE DE INTOXIICAȚIA CRONICĂ CU MERCUR LA CARASUL ARGINTIU CARASSIUS AURATUS GIBELIO

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The purpose of this work was to describe the histopathological effects of chronic inorganic mercury intoxication on liver, gills, kidneys, small intestine and skin in silver crucian carp Carassius auratus gibelio. 20 immature 1+-year-old crucian carp were obtained from a private fishfarm and acclimatized to laboratory conditions. After a 3 weeks exposure to a sublethal mercury concentration (0.25 ppm from a HgCl₂ stock solution), liver, gills, kidney, small intestine and skin were sampled and processed for histological examination. The main effects observed: numerous interstitial leukocytic infiltrates, followed by glomerulonephritis and tubulonephritis there are at the renal level; fibrosisation of peri- and interlobular conjunctive tissue, including ectasiated blood vessel and numerous limphocytic infiltrates enlarged both in perilobular and intralobular conjunctive tissue at the liver level; an disorganization process of gill lamellae by superficial layer alteration, at the gill level; cells of skin epiderma exhibit hiperplasic hypertrophy, epithelial desquamation, intraepithelial edema and citoplasmatic vacuolization; light epithelial distrophic processes and an abundant leukocytic infiltrate both in vilositaire chorion and basal chorion at the small intestine level.

Key words: fish, chronic mercury intoxication, histopathological change

Introduction

Mercury is the only metal that is liquid at room temperature. Its elemental symbol is Hg, which is derived from the Greek word hydrargyrias, meaning "water silver." Mercury is found in organic and inorganic forms. The inorganic form can be further divided into elemental mercury and mercuric salts. Organic mercury can be found in long and short alkyl and aryl compounds.

Mercury in any form is toxic. Neurologic, gastrointestinal, and renal systems are the most commonly affected organ systems in mercury exposure.
Inorganic mercury (usually mercuric, \( \text{Hg}^{2+} \), salts) most often target the kidney. Mercuric chloride \([\text{HgCl}_2]\) is toxic to renal tubular lining cells.

The purpose of this work is to describe the histopathological effects of inorganic mercury intoxication in silver crucian carp \textit{Carassius auratus gibelio}.

**Materials and Methods**

The experiment was performed on a group of 20 silver crucian carps, \textit{Carassius auratus gibelio} with a body weight of 36.40 g ±1.2 g, that were obtained from Cornesti’ fishfarm and aclimatized 2 weeks in laboratory conditions; the fish were housed in a 60 l capacity glass aquaria containing mercury contaminated water and the water replaced twice a week with stored dechlorinated water. They were fed twice a day with commercial dry pellets.

The physico-chemical parameters of the laboratory water (during experimental period) were as follows: dissolved oxygen 8.28±0.10 mg/l, water temperature 19.7±0.3°C, NO\textsubscript{2} 0.2±0.05 mg/l, NO\textsubscript{3} 3.5±0.5 mg/l, pH 8±0.5, hardness of water 6 dH\textsuperscript{o} (soft water) and was provided a 12h light: 12h dark photoperiodicity. All these parameters were daily measured: (water temperature and dissolved oxygen - movable oxygen-meter with water resisting microprocessor Hanna Hi 9145; pH, NO\textsubscript{2}, NO\textsubscript{3}, hardness of water – Germany TERMATEST kits).

Fish were exposed at a chronic mercury intoxication in concentration of 0.25 ppm from a \( \text{HgCl}_2 \) stock solution. The sublethal treatment (25% of Lc\textsubscript{50}) was calculated from percentage mortalities of fish as described by Veena et al. (1997). Mercury circulation in the aquaria was ensured by 2 air pumps AC 9904.

After lethal anesthesia, gill, kidney, intestine, skin and liver specimens sampled were collected, fixed in an ethanol solution (80\%), dehydrated, clarified, impregnated and embedded in histological paraffine for examination of morphophysiological changes induced by sublethal mercury concentrations on histological structure of these tissue. Five-micrometer-thick sections were obtained and stained for optic differentiation of tisular and cellular structure. We used two staining methods: HE and Mallory’ trichromic method.

Microscopical analyzing was performed using an Olympus light microscop with 10x lens and 40x respectively and an 10x glass field.

**Results and Discussions**

Kidney microscopical sections relieve numerous interstitial leukocyte infiltrations and vascular ectasias.

Endothelial wall thickening occur (Figure 1a), at the glomerular capillaries level from renal corpusculs structure, the process being due to interstitial conjunctive tissue fibrosis.
Infiltrative cells of macrophage–mesangial type are frequently concentrated at the vascular pole level and entire glomerul as well, (Figure 1b), implicating glomerulonephritis installation. An often encountered aspect in renal parenchym is capsular metaplasia and glomerular shrinkage, capsular space being enlarged in this case. Morphologic lesions enlarge at proximal and distal contort tub level. These consist in nephrocite hyperplasic processes, nucleus piknosis, the observed changes leading to epithelial atrophy, and nephrocite detachment from basal subepithelial membrane.

Similar observations concerning cell disintegration and cell disorganization were obtained by other authors as well. Thus, Khan M.S. et al. (2004), found out mild to sever tubular epithelial degeneration, karyolysis, dilation and shrinkage of Bowman’s capsule and glomerulus in the grass carp *Ctenopharyngodon idella* exposed chronically to sublethal concentrations of mercuric chloride. Head kidney showed large necrosis areas, increased number of melano-macrophages centers, phagocytic areas, intercellular space among parenquimal cells and atypical cells in the neotropical fish *Hoplias malabaricus* fed with methylmercury contaminated prey (Mela M. et al., 2007).

At the liver level, microscopic imagines relieve peri- and interlobular conjunctive tissue fibrosis integrating ectasiated blood vases and numerous limphocyt infiltrations both in the perilobular and intralobular conjunctive tissue (Figure 2a and Figure 2b).

Hepatic lobules consist in hepatocyte cords, that converge unto centrilobular vein.
Hepatocytes have an polygonal shape with round, light eccentrically situated and slight chromatic nucleus. Cytoplasmes are perinuclearly light granular, but generally they have a vacuolar aspect. Numerous hypertrophic cells with piknotic nucleus and clear cytoplasm occure in the lobular parenchime structure; according with the above findings, these cells suffer an atrophic process. Intralobular conjunctive stroma is slight fibrosated and slight capilare ectasia and high enlarged lipocyte infiltrations between hepatocytes cords there are.

A powerful mercury hepatotoxic effect was incriminated by Mela M. et al., (2007) and Khan M. et al., (2004) as well. The liver of methylmercury exposed individuals presented leukocyte infiltrations, increased number of melano-macrophage centers, necrotic areas and lesions in Disse’s space.

On gill sections can be seen gill arcs containing numerous gill primary lamellae; gill secondary lamellae come off from primary lamellae.

The bilayer epithelium covering gill lamellae contains an inner cubic cell layer disposed on basal membrane and an outer cell layer, principally containing pavimentous cells (Figure 3a), between last type cells appear sparsely scattered mucous cells.
Microscopic analyzing performed on permanent histological preparats show a disorganization process of gill lamellae by superficial layer alteration. Thus, superficial cells stand out from inner cell layer on large areas and suffer distrophic processes leading to theirs disappearance (Figure 3b). Lesions enlarge on low areas in the epithelial inner cell layer as well.

Khan et al., (2004) reported histopathologic changes, showed hyperplasia of epithelial cells of gill filaments, fusion of secondary lamellae giving a club shaped appearance of filaments and contraction and sloughing of respiratory epithelium in mercury exposed group.

Serious injuries to secondary lamellae were observed after only 12 h of exposure to water-borne inorganic mercury in the arctic char (Salvelinus alpinus): exfoliative epithelia with detachment, vacuolation of epithelial cells, and edema (C.A. de Oliveira Ribeiro et al., 2002)

The respiratory surface of an air-breathing fresh water fish, *Channa punctatus* exposed to chronically sublethal concentrations of mercuric chloride was found to exhibit lesions, lifting of lamellar epithelium and increased number of mucus gland openings (Gupta N. et al., 2002).

These microscopical aspect suggests that mercury sublethal concentrations affect respiratory barrier decreasing gas change area between organism and environment.

At the skin level there is a powerful derm, containing dense conjunctive tissue with numerous collagen fibres.

Epiderm is superficially situated, in which structure, cells exhibit hiperplazic hypertrophy, epithelial desquamation, intraepithelial edema and cytoplasmatic vacuolization (Figure 4), suggesting once again a decrease of change area with environment.

Khan et al., (2004) sustain our statement, they remarked that histology of epidermis was disturbed with increased number of immature cells. Overall, skin layers were atrophied and withered in mercury intoxication group.
Microscopical sections through small intestine show that mucous chorion consist in lax conjunctive tissue, including an abundant leukocytic infiltrate (Figure 5) existing both in vilositaire chorion and basal chorion.

Light distrophic processes and numerous in transit leukocytes there are at the vilositaire epithelium.

Although no histopathological changes were observed in the intestinal epithelium of S. alpinus, some alterations were described in Channa punctatus after chronic and nonlethal doses of mercuric chloride (Banerjee and Bhattacharya, 1997).

Conclusions

Mercury sublethal concentration used in our study, induces changes in all examined organs as follow:

1. Numerous interstitial leukocytic infiltrates, followed by glomerulonephritis and tubulonephritis there are at the renal level.
2. Fibrosation of peri- and interlobular conjunctive tissue, including ectasiated blood vessel and numerous limphocytic infiltrates enlarged both in perilobular and intralobular conjunctive tissue at the liver level.
3. Hepatic lobules consist in hepatocytic cords with round light eccentrically rested and slight chromatic nucleus. Cytoplasms are perinuclearly light granular, but generally they have a vacuolar aspect. Numerous hypertrophic cells with piknotic nucleus and clear citoplasme occure in the lobular parenchime structure; according with above findings, these cells suffer an atrophic process. Intralobular conjunctive stroma is slight fibosated and slight capilare ectasia and high enlarged limphocyte infiltrations between hepatocytes cords there are.
4. A disorganization process of gill lamellae by superficial layer alteration occur at the gill level.
5. Cells of skin epiderma exhibit hiperplasic hypertrophy, epithelial desquamation, intraepithelial edema and citoplasmatic vacuolization.
6. Light epithelial distrophic processes and an abundant leukocytic infiltrate exist both in vilositaire chorion and basal chorion at the small intestine level.

Bibliography


