Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.2.201414J1

Toxicological effects of ammonia on gills of *Cyprinus carpio* var. *communis* (Linn.)

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PEER REVIEW

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Comments

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ABSTRACT

Objective: To monitor the response of gills of *Cyprinus carpio* var. *communis* to LC_{50} levels of ammonia over a period of 96 h by bath exposure, using scanning electron microscopy (SEM).
Methods: *Cyprinus carpio* procured from a local fish farm in Vadalore, Cuddalore District, Tamil Nadu, India were acclimatized under laboratory conditions for 20 d. Fish were placed into experimental tanks with 20 L of water and 22 mg/L ammonia. A total of 10 fish were collected from each experimental tank every 24 h for histopathological studies.
Results: Histopathological studies showed these changes in the gills: secondary lamellar fusion, haemorrhage, oedema, epithelial hyperplasia, and chloride cell proliferation. Occasionally, multifocal necrosis of inter-lamellar regions of gill filaments but with no apparent haemorrhage was observed under electron microscopy.
Conclusion: The present study shows that histopathological and ultrastructural alterations in gills are useful indicators for ammonia toxicity in *Cyprinus carpio*.

KEYWORDS
Ammonia toxicity, Gill histology, scanning electron microscopy, *Cyprinus carpio*

1. Introduction

Fish gills are a multifunctional organ that is the dominant site for gas exchange, ion regulation, acid-base balance and nitrogenous waste excretion[1,2]. Gills are the primary site of toxicities due to many water-borne pollutants, because of the close association between gills and the medium in which the fish lives[3,4]. Hence, gills are good indicators of the health of aquatic organisms and its environment[5].

Ammonia is a serious and common pollutant of aquatic habitats, and enters the water bodies from several sources such as sewage effluent, industrial wastes, agricultural run-off and decomposition of biological wastes[6]. In aqueous solution, ammonia exists as the more toxic unionized ammonia (NH_3) under conditions of alkaline/neutral pH, and relatively less toxic ionized ammonium (NH_4^+) under conditions of acidic pH[6,7]. Ammonia is a serious problem in fish cultured in recirculation systems, aquaria and fish ponds due to reduced water exchanges. There are numerous reports on acute and chronic ammonia toxicities in various fish species[8,9].

Histopathological changes have been reported in gills of many fishes as a result of exposure to different

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Foundation Project: Supported by the Annamalai University Grants Commission [39-562/2010(SR)].

Article history:
Received 7 Jan 2014
Received in revised form 22 Jan, 2nd revised form 28 Jan, 3rd revised form 5 Feb 2014
Accepted 22 Feb 2014
Available online 28 Feb 2014

toxicants[10]. Chezhian *et al*.[2] reported that ammonia at different pH levels induced varying changes in fish gills, such as secondary lamellar fusion, oedema, hyperplasia, and chloride cell proliferation in *Cyprinus carpio* (*C. carpio*). Senthamilselvan *et al*.[11] observed similar changes in the gills of *Lates calcarifer* with exposure to nickel, mercury and nickel plus mercury. Studies by Tomasso *et al*.[12] and Thurstion *et al*.[13] have shown that exposure of fish to ammonia are associated with histopathological changes in gills and liver. Likewise, elevated ammonia and low pH induced gill damage in juveniles of brook trout (*Salvelinus fontinalis*) was reported by Mueller *et al*[13]. Histopathological studies with electron microscopy will aid the description and evaluation of potential lesions in aquatic animals exposed to various toxicants[14]. The aim of the present study is to study the histopathological and ultrastructural changes in the gills of *C. carpio* induced by acute ammonia toxicity under scanning electron microscopy.

2. Materials and methods

2.1. Test species

In this study, clinically healthy *C. carpio* were procured from a local fish farm in Vadalore, Cuddalore District, Tamil Nadu, India and were acclimatized under laboratory conditions for 20 d prior to start of experiments. The fish were kept in tanks with daily partial water exchanges, and fed rice bran and groundnut oil cake twice a day *ad libitum*.

2.2. Experimental design

Fish with a size range of 7-8 cm length, and weighing 8-10 g were selected for use in the experiments. Water quality measurements taken throughout the experiments according to APHA methods[15] were recorded as follows: Dissolved oxygen (6.2±0.15) mg/L; pH (7.20±0.17); water temperature (25.2±0.6) °C; salinity (0.20±0.00) ppt; total hardness (13.1±0.3) mg/L; calcium (5.0±0.1) mg/L; magnesium (8.0±0.1) mg/L and total alkalinity (20.1±0.6) mg/L. Preliminary studies were carried out to determine the median lethal concentration (LC_{50}) of ammonia by 96 h bath exposure, according to the probit analysis method by Finney[16].

The LC_{50} for 96 h ammonia bath exposure was determined to be 22 mg/L. Four replicate test tanks with ammonia at 22 mg/L, and a control tank consisting of the same water source without ammonia added were used. All experimental tanks were filled with 20 L water and ammonia added to each test tank but not control tanks, to achieve a concentration of 22 mg/L. Twenty fish were introduced into each tank. A common control (pH 7.2) was also maintained. The toxicant was renewed daily in the experimental tanks. No mortality was observed throughout the experimental period. A total of 10 fish were collected from each experimental tank on a 24-hourly basis for histopathological studies.

2.3. Scanning electron microscopy

Excised gill arches were fixed overnight in 2.5% glutaradehyde in 0.1 mol/L phosphate buffer (pH 7.4) at 4 °C for 2 h, and post-fixed in buffered 1%-2% osmium tetraoxide at 4 °C for 1-3 h. Post-osmium fixed gill tissues were subjected to serial dehydration in ethanol, critical point drying with CO_2, mounted onto aluminium stubs, coated with silver paint, and placed in a vacuum evaporator. Processed gill tissues were examined using scanning electron microscope (Leo 435 VP, LEO Electron Microscopy, Cambridge and England). The surface changes, chloride cell density and chloride cell apical surface area of the gills were analysed using the inbuilt imaging software. At least six different fields of observation were analysed, and values were calculated as mean±SD.

3. Results

Gills are the primary organs for respiration, ion and osmoregulation, acid base regulation and nitrogen excretion. Any environmental stress influencing the function of gills may therefore cause homeostatic disorders in fish[17]. In control fish kept in water without ammonia, numerous regularly arranged, slender finger-like secondary gill lamellae were observed along the length of the primary gill filaments under scanning electron microscopy (SEM). Pavement cells of the primary gill filaments from control fish had intact surfaces with distinct microridges, whereas pavement cells of the secondary lamellae had smooth surfaces (Figure 1A). In test fish subjected to 24 h of ammonia bath exposure, secondary lamellae showed multifocal swelling and multifocal loss of its distinctive finger-like structure

under SEM. Histopathologically, moderately extensive fusion of secondary lamellae, epithelial hyperplasia and proliferation of chloride cells were observed (Figure 1B). In fish subjected to 48 h of ammonia bath exposure, shortened and thickened secondary gill lamellae were observed under SEM. Histopathologically, this corresponded to hyperplasia and swelling of gill epithelial cells and hyperplasia at the tips of the primary lamella (Figure 1C). Severe and extensive fusion of secondary lamellar fusion, were observed in fish 72 h post exposure to ammonia under SEM (Figure 1D). In fish 96 h post exposure to ammonia bath, secondary lamellae fusion is so severe that there is complete loss of distinctive finger-like secondary lamellae under SEM (Figure 1E). At 96 h post-exposure, corresponding extensive gill epithelial hyperplasia, hemorrhage and necrosis were observed histopathologically.

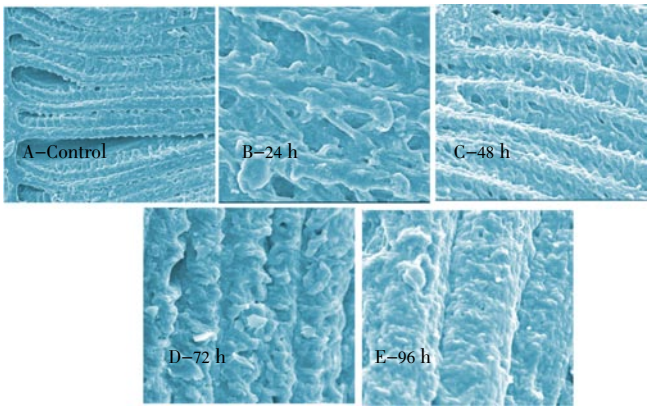

Figure 1. Photographs showing the scanning electron micrographic section of gills of fish *C. carpio* exposed to acute toxicity (96 h) of ammonia (×400).

4. Discussion

Gills are highly susceptible to toxic chemicals of environmental pollutants, because of direct contact between gills and the external environment. The absorption of toxic chemicals through gills is enhanced by increasing the permeability to water and ions through gill epithelium and by inhibition of ions exchange activity of the chloride cells[18].

The gill, main osmoregulating organ in fishes, highly sensitive to many factors, including stress, pollution and changes in the environment[19,20]. The generalized chloride cells, proliferation of chloride cells and hyperplasia were observed in the present study. Similar observations have been reported by Ghanbousi *et al.*[21] in the fish (*Aphanius dispar*) exposed to deltamethrin showed hypertrophy, hyperplasia of chloride cells, while in additional changes like vacuolization, lifting of the lamellar epithelium and fusion of secondary lamellae. Deng *et al.*[22] reported that the gill lesions (epithelium hyperplasia and lamellar fusion) observed in the Sacramento splittail (*Pogonichthys macrolepidotus*) larvae fed MeHg-added diets. The observed hyperplasia of the cell epithelium and lamellar fusion could have interfered with the efficiency of the gills resulting in reduced gas exchange. Hyperplasia of secondary lamellae has also been reported in organisms exposed to environmental pollutants often associated with the complete fusion of two neighbouring secondary lamellae[9,23]. Similar responses (hypertrophy and hyperplasia of chloride cells) have already been reported in fish exposed to cadmium[24].

Gill chloride cells are the most active cells in fish gill as they absorb and secrete many ions and electrolytes[1]. Because of these functions, increase in the apical vesicles of chloride cells of *Acipenser persicus* gills could be a result of an increase of their ion exchange activity. The above authors further stated that these lesions may reduce gill functional surface for gaseous exchange, impairing respiratory function. Smart[25] reported that the lamellar fusion can result from hyperplasia of epithelial cells, indicating advanced structural damage. The author further stated that the epithelial lifting involves epithelium separation from the basement membrane and usually indicates edema or fluid increases. In the present study also, a similar mechanism might be operating, thus reducing the gill functional surface for gaseous exchange.

Arellano *et al.*[26] suggested that the edema of the filament and lamellar epithelium has also been described in fish exposed to different pollutants. This thickening may be due to the proliferation of cells rich in mitochondria and stem cells[27], which causes partial or complete fusion of secondary lamellae. Sorour and Harbey[28] reported that major changes are hypertrophy and hyperplasia of the epithelial cells, partial fusion of some secondary lamellae, lamellar aneurism, besides epithelial lifting and edema. The author further stated that the changes may be early responses of the gills to the harmful substances. These alterations are examples of defense mechanisms because the lifting lamellar epithelium and edema increased the distance between the external environment and the blood, thus serving as a barrier to the entrance of contaminants[29]. Similar alterations in the gills have also been reported in the fish exposed to metals[30,31], organic contaminants[32] and after acute exposure to insecticides[33,34] and acute exposure of ammonia[2]. Khoshnood *et al.*[35] suggested that the lamellar fusion, gill epithelial hyperplasia and epithelial necrosis with blood emergence were some of the

effects of exposure to mercuric chloride that may be causes of respiratory and osmoregulatory disorders. The author further state that these alterations also may play a defensive role against contamination rather than have an irreversible toxic effect. However, these modifications can produce adverse effects on fish health, and may increase their susceptibility to secondary infectious diseases and even death[28]. A similar situation may prevail in fish from acute treatment in the present study supporting the observation of the above authors.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors thankful to Dean and Director of CAS in Marine Biology, Faculty of Marine Sciences, Parangipettai and authority of Annamalai University for providing necessary facilities and also thankful to University Grants Commission (39-562/2010(SR)) for providing financial support.

Comments

Background

Ammonia can have severe toxic effects on gills, which are in direct close contact with the aquatic environment. Ammonia is the main form of nitrogenous waste excreted by fish, and produced by decomposing organic matter such as uneaten feed or decaying vegetation. Any damage to gills will affect its vital functions of oxygen uptake, and carbon dioxide and ammonia excretion.

Research frontiers

This study reports on the response of gills, a vital fish organ, to a commonly occurring highly toxic environmental pollutant, ammonia. It presents observations at the light and scanning electron microscopic levels, of serial 24-hourly tissue samples taken over a period of 96 h, under controlled experimental conditions.

Related reports

Most published papers report on gill damage caused by various toxic chemicals at a point in time, as a result of natural exposure to chemical pollutants.

Innovations and breakthroughs

This study reports on the observations in gills tissues in serial 24-hourly samples taken under controlled experimental conditions, over a 96 h period of exposure to LC_{50} levels of ammonia.

Applications

Observations of the response of gills to 96 h experimental exposure to ammonia examined at 24-hourly intervals will aid future studies of gill pathology as a result of environment pollutants.

Peer review

This study is an important step forward to the increased understanding of gill response to toxic chemical insults over a period of 96 h. It adds to the knowledge available from previous publications which are mainly reports of gill damage caused by various toxic chemicals at one point in time.

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