Histopathological study of grass carp (Ctenopharyngodon idella) experimentally infected with Ichthyophthirius multifiliis

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Little information is available regarding the histopathological changes of grass carp (Ctenopharyngodon idella) after infection with Ichthyophthirius multifiliis. In the present study, the pathological changes of the gill, skin and muscle from the grass carp exposed to I. multifiliis were observed experimentally by the light microscopy (LM) and transmission electron microscopy (TEM), respectively. Results showed that I. multifiliis were observed in gill, skin and muscle sections of exposed fish and infections induced by this parasite were associated with epithelial hyperplasia, focal areas of cellular disruption and disappearance. Notably, the histological changes in ultrastructure of the mitochondria and nuclei, such as dislocation of nuclei, brown atrophy (pigmentation accumulation) destruction of striation were observed by TEM compared to control groups. In conclusion, the results obtained indicated that invasion intensified occurrence of morphological lesions in the gill, skin and muscle of grass carp exposed to I. multifiliis, which may lead to respiratory insufficiency of infected fish, bacterial or fungal secondary infection, and even cause mass mortality.

Key words: Ichthyophthirius multifiliis, grass carp (Ctenopharyngodon idella), pathological change, transmission electron microscopy (TEM), light microscopy (LM).

INTRODUCTION

The parasitic ciliate Ichthyophthirius multifiliis, the etiologic agent of “white-spot” disease, is a well-known and widely distributed parasite of freshwater fish species causing significant economic losses to the aquaculture industry (Hewlett et al., 2009; Mahmoud et al., 2009; Rakauskas and Blaževičius, 2009). Also, it has been detected in other aquatic vertebrates such as amphibians (Gleeson, 1999). It is well documented on the invasion and transmission, as well as its cell biology and life cycle of I. multifiliis, whose life cycle consists of three stages: an infective theront, a parasitic trophont and a reproductive tomont (Ewing and Kocan, 1992; Ling et al., 2010). Infections with I. multifiliis occur between spring and fall when the water temperatures are between 18 and 25°C (Davis et al., 2002). Mature parasites (trophonts) leave the host to settle upon a suitable substrate and transform to the tomocyst stage by secreting a double-layered cyst wall (Clayton and Price, 1988). Eventually, up to one thousand or so free-swimming daughter cells (theronts) are released and become infective. The theronts that feed on mucus and tissue parasitized in the epidermis of the fish, resulting in an impaired osmotic balance and impaired gaseous exchange (Hines and Spira, 1974a; Tumbol et al., 2001). In the host tissue, infections can cause localized lymphocytic infiltration, excess mucus, cellular necrosis, and varying degrees of epithelial proliferation, and even necrotic epidermis in severe cases (Hines and Spira, 1974a, b; Hines and Spira, 1974b;
Cross and Matthews, 1993).
In view of this background, the aim of this study was to examine the histological damages in grass carp (Ctenopharyngodon idella) experimentally infected by I. multifiliis, especially electronic microstructures, in order to confirm the locus of grass carp that the parasite targets and apply the findings to prevent the disease.

MATERIALS AND METHODS

Fish
Grass carp, 10 g in body weight, reared under pathogen-free conditions, were maintained in aerated tap water at 20°C in 100 L aquaria with Eheim biofilters until use.

Parasites
Laboratory cultures of I. multifiliis obtained from a fish farm (Guangzhou, China) were maintained at 20°C by serial passage to C. idella.

Experimental infection
I. multifiliis was harvested from the skin of grass carp following the procedure described previously (Sigh et al., 2004). A total of 20 grass carp were transferred to a 100 L aquarium with a concentration of 10,000 parasites per fish. Similar aquarium 20 fish were submitted to a sham infection with aerated tap water instead of I. multifiliis experimentally infected by grass carp, in order to confirm the locus of grass carp that the parasite targets.

Light microscopy (LM)
After I. multifiliis exposure, five moribund fish were gently transferred to a small plastic aquarium containing a mild anaesthetic (MS-222, 20 mg/L). In the laboratory the fish were killed quickly with an overdose of MS-222 (200 mg/L), the gill, skin and muscle were aseptically dissected and fixed by immersion in 10% neutral buffered formalin for 24 h. After dehydration in gradient ethanol and rehydrated, the sections were stained with hematoxylin and eosin (H&E). All stained slides were observed under Nikon E400 Microscope (Nikon, Tokyo, Japan). Photographs were taken by DT-2000 software. Tissue samples were likewise taken from the 5 uninfected control fish.

Transmission electron microscopy (TEM)
Fish were anesthetized as described previously. Then, the I. multifiliis infected tissues (gill, skin and muscle) from control and experimental groups of fish were fixed at 4°C with 2.5% glutaraldehyde in phosphate buffer (0.1 M), pH 7.2, post fixed in 1.0% osmium tetroxide (OsO4). After rinsing with phosphate buffer again, the specimens were dehydrated in a graded ethanol and then embedded in Epon 812 (EpiKote resin). Ultrathin sections were sliced with glass knives on a UCT ultramicrotome (Leica Ltd, Germany), stained with uranyl acetate and lead citrate, and examined under a Tecnai 12 electron microscope (FEI, Acht, The Netherlands).

RESULTS AND DISCUSSION

Clinical signs of disease in grass carp
I. multifiliis invades the surface tissues of fish, including the epidermis of the gills, the skin, and the thymus causing characteristic ‘white spot’ disease (Lu and Guo, 1990; Aydoğan et al., 2010; Pakk et al., 2011). In the present study, the infected fish showed emaciation, lethargy, dyspnea, multiple pin-point sized white spots on the body surface (Figure 1). The parasites were mostly located on or above the basal lamina, but occasionally may be found in deeper tissues when infections were extremely heavy. Moreover, twenty individuals of the affected fish had a swollen spleen and kidneys. Some diseased fish also had eye cataracts, pale gill and gill artery tumefaction. Trophonts were also detected within the peritoneal cavities of infected channel catfish (Maki et al., 2001).

Histological changes in gill, skin and muscle
In this study, a large number of I. multifiliis with a C- or horseshoe-shaped nucleus (Figure 2B), as Ni and Li, (1960) described, were predominant in gill, skin, and muscle of grass carp, which may cause histological changes in these tissues of exposed fish.

No significant changes were observed in the gill of fish from the control group. The gills comprised of rows of long, thin primary lamellar epithelium attaching to a gill (Figure 2A), and the integrity structure of capillary red cell, the epithelial cell and gill cartilage cell were revealed by ultrastructural examination (Figure 2D). However, in the infection group, a large number of I. multifiliis were observed on the gill lamellae examined with a light microscope (Figures 2B, C and G). In some cases, the gill filaments were markedly degenerated and desquamated in superficial cells forming vacuolar. The gill capillary epithelial cell and gill cartilage cell structure were destroyed and disappeared by TEM, respectively (Figures 2E and F).

Skin of control grass carp presented normal histology, including regularity of epithelial tissue and the spindle nuclei (Figures 3A and C). However, epidermal hyperplasia, disrupted cellular integrity and pyknotic nuclei were observed in infected fish (Figure 3B). In ultrastructural analysis, their nuclei with rhexis were faint or not visible (Figure 3D). I. multifiliis of variable sizes were also seen in the subepithelia, and caused marked hyperplasia of mucus cells. Empty spaces were present along with vacuolated and necrotic cells (Figure 3E).

Muscle in untreated group showed normal arrangement
Figure 1. White spots on the grass carp. Note trophonts of spherical shape under light microscopy (arrow; ×100).

Figure 2. Pathological observation in gill of the grass carp exposed by *I. multifiliis* compared with control groups. A-C: Light photomicrograph; D-G: Transmission electron micrograph; A,D: The control group; B, C, E-G: The Ichthyophthirius group; A: Reticular formation regularity, rich capillary and epithelial cell (H&E ×100); B: The desquamated filaments, *I. multifiliis* (arrow; H&E ×100); C: Vacuolation of cytoplasm of lining epithelium, congestion of blood spaces, *I. multifiliis* (arrows; H&E ×400); D: The integrity structure of capillary red cell, the epithelial cell and gill cartilage cell (×1900); E: The gill cartilage cell structure were destroyed and disappeared (arrow; ×1000); F: Gill cartilage cell structure were destroyed and disappeared (arrows; ×1900); G: *I. multifiliis* and destroyed epithelial cell (arrows; ×1900).
Figure 3. Pathological changes in skin of the grass carp infected by *I. multifiliis* compared with control groups. A-B: Light photomicrograph; C-E: Transmission electron micrograph; A: The control group; B: Epidermal hyperplasia, disrupted cellular integrity and pyknotic nuclei (H&E ×400); D: Skin structure disorder and karyorrhexis (arrows; ×1900); E: *I. multifiliis* (arrow; ×1900).

of longitudinal muscles, normal nucleus and other cardiac muscular layers and mitochondria of muscle were abundant in cytoplasm by ultra-structural examination (Figures 4A and C). Compared to the control group, the pathological changes of fish exposed to *I. multifiliis* were splitting of muscle fibers, disappearance of nuclei, brown atrophy (pigmentation accumulation), destruction of striation, splitting of longitudinal tissues (Figures 4B and E). In ultra-structural analysis, the mitochondria that proceeded to lose cristae with vacuolization were observed (Figure 4D). In some cases, *I. multifiliis* were examined in the perimysial tissues of the somatic musculature, which were associated with necrotic foci (Figure 4F).

Limited information was reported about histopathological changes of *C. idella* exposed to *I. multifiliis* that fed on fish gill epithelia by destruction of the cells or by ingesting blood from the ruptured blood vessels (Raissy and Ansari, 2011). In the present study, the pathological changes in grass carp were investigated, and like another study’s observations, ulcerated gill of fish, the partly disappeared epithelium, and the distorted gill filament were also found in our study (Zhang and Chen, 2005). Another study revealed that *I. multifiliis* invading skin, gill, and cirrus of the juvenile *Leioeassis longirostris Gunther* can result in congestion, ulceration, and the pigment layer partly disappeared (Chen et al., 2004).

Szarek et al. (2006) found that parenchymatous degeneration and vacuolar degeneration in the hepatocytes of *Cyprinus carpio* which was infected by *I. multifiliis*, besides, hypertrophy in the connective tissue on the walls of blood vessels were also observed. Here, our ultra-structural observations also revealed that both the gill capillary epithelial cell and gill cartilage cell were destructive, karyorrhexis and cristae were disappeared in the mitochondria of grass carp exposed to *I. multifiliis*.

Conclusions

This study revealed that *I. multifiliis* was the most predominant pathogen that parasitize in gill, skin, and muscle of grass carp and severe branchial destruction caused by *I. multifiliis* affected fish respiration, leading to reduced feeding, weight loss and general deterioration of health. In addition, destruction of skin and muscle by *I. multifiliis* can also lead to bacterial or fungal secondary infection and caused mass mortality.

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Figure 4. Pathological changes in muscle of the grass carp. A-B: Light photomicrograph; C-F: Transmission electron micrograph; A:C: The control group; B, D-F: The Ichthyophthirius group; A: Arrangement of longitudinal muscles, normal nucleus and other cardiac muscular layers (H&E×400); B: Splitting of muscles, dislocation of nuclei, brown atrophy, destruction of striation, splitting of longitudinal tissues and necrosis (H&E×400); C: Muscle fibers regularity and rich mitochondria (arrow; ×2900); D: The mitochondria proceeded to lose cristae with vacuolization observed (arrow; ×2900); E: Destruction of striation (×2900); F: I. multifiliis (arrow; ×1900).

