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Mast Cells in Two Species of Freshwater Fishes, Grass Fish (*Ctenopharyngodon idella*) and Cat Fish (*Claris fuscus lacepede*)

Leren Xu, Xiaozhen Yang, Denghui Gao and Ping Jiang
Department of Animal Medicine, College of Animal Sciences, Guizhou University, Huaxi, Guiyang, Guizhou 550025, P R China

Abstract: Mast cells were firstly identified in the tissues of grass fish (*Ctenopharyngodon idella*) and cat fish (*Claris fuscus lacepede*) histochemically and morphologically. Mast cells were found in the mucosa and submucosa of the intestine and in the lymphoid organs including thymus, head kidney and spleen in the two species which intended to adjacent to blood vessels. It seemed that the staining properties of the mast cells in grass fish and cat fish were similar to the mucosal mast cells in mammals in some aspects. Carnoy's fluid and Alcian blue were proved to be the good fixative and the excellent dye for the mast cells, respectively, but fixation in neutral buffered formalin (NBF) and stained with toluidine blue were unsatisfactory. Some other histochemical methods previously have been used by some investigators to detect mast cells, or so called eosinophilic granule cells (EGCs) in teleostean fish, were failed to identify mast cells in the two species of fish in the present study. Under electron microscopy, the mast cells in grass fish and cat fish were similar which contained characteristic cytogranules

Key words: grass fish; cat fish,; mast cell; histochemistry and morphology

Introduction

Mast cells in fish were early demonstrated in the tissues of carp (*Cyprinus carpio*) and whitefish (*Leuciscus* Sp.) (Michels,1923) and ocean sunfish (*Orthagoriscus mole*) (Remieu, 1924). After that by employing special techniques of fixation, embedding and staining, many investigators have also been able to demonstrate the cells with metachromatically stained cytoplasmic granules characteristic of the mast cells in tissue samples from some species belonging to the teleostean families, such as cyprinids, erythrinids, esocids, salmonids, pleuronectids and molids. Besides showing metachromasia with dyes like toluidine blue and thinin, these cells can also be stained with Alcian blue, retained the blue color after sequential staining with safranin O (Ezeason and Stock, 1980; Bielek, 1981; Bergeron and woodward, 1992; Powell *et al.* 1990,1991; Sir and Vernier 1995; Reite 1996,1997,1998; Bielet *et al* 2000; Passantino *et al* 2000). Roberts *et al.* (1971) introduced the designation "eosinophilic granule cell" (EGC) for the cells in the epidermis bearing great morphological resemblance to mast cells, but unlike mast cells in mammals, with red granules after staining with haematoxylin and eosin (HE) After that the names, mast cells (MC) and EGC, or MC/EGC were used to describe the some cell type in different papers and great intentions were made to investigate the cells in different species of teleostean fish histochemically, morphologically and functionally (Ezeason and Stock, 1980; Bielek, 1981; Bergeron and woodward, 1992; Powell *et al.* 1990,1991; Sir and Vernier 1995; Reite 1996,1997,1998; Bielet *et al.* 2000; Passantino *et al.* 2000). Since our present knowledge is based on limited studies in individuals from few out of more than 400 teleostean fish families and differences of the cell type between species must be fully considered, great caution must be take before firm conclusions are reached. Numerous questions await their solution, and the methodological design used in studies on mast cells in mammals or birds may serve a guideline (Reite, 1998).

Grass fish □ *Ctenopharyngodon idella* □ is belong to *cyprinidae, cypriniformes* and cat fish (*Claris fuscus lacepede*) belong to *clariidae, siuriformes* in *Osteichthyes,* respectively. Both are the most important species in freshwater fish farming in China. To our knowledge, no mast cells or so called eosinophilic granule cells (EGC) have not been reported in the two species. The present paper mast cells in grass fish and cat fish were demonstrated and described histochemically and morphologically by using some histochemical methods we had used in the research in some domestic animals and birds (Xu *et al*, 1993 2001,2002) previously and some other methods used by some abroad investigators (Reviewed

by Reite.1998) Light and electron microscopic examination of the mast cells were also been described.

Materials and Methods

Animals and tissue samples: Tissue samples were obtained from eight grass fish and cat fish, respectively. Samples of thymus, head kidney, spleen and intestine were removed from all fish. One samples of each tissue was fixed immediately by immersion in carnoy's fluid (60% absolute alcohol, 30% chloroform and 10% glacial acetic acid), Bouin's fluid, 4% neutral buffered formalin (NBF), MFAA (methanol: formalin: acetic acid = 85:10:5), 80% ethanol. Fixed tissues were dehydrated in a graded series of alcohol and xylene, embedded in paraffin wax; sections were cut into 6 μm in thickness, and then de-waxed in xylene, re-hydrated and stained.

Electron microscopy examination: samples of thymus, head kidney and spleen were removed from three of grass fish and cat fish, respectively, and processed for ultrastructural examination. Pieces of tissue (1 mm^3) were fixed overnight in 4 % (w/v) glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.3), rinsed in 0.1 mol/L phosphate buffer and post-fixed in 1 % osmium tetroxide in 0.1 mol/L phosphate buffer for 1.5 h at room temperature. Tissues were washed in distilled water, stained with 1% aqueous uranyl acetate for 1h at 4^0C, dehydrated in graded methanol and propylene oxide and embedded in araldite resin. Ultrathin sections (50 nm) were cut and stained with lead citrate for 10 min and examined under JEOL-2000EX electron microscope.

Histochemical staining: For toluidine blue staining (TB-SO), Sections to be stained with toluidine blue were rinsed with 0.5 mol/L HCl (pH 0.5) for 5 min, stained with 0.5% (w/v) toluidine blue (Gurr, Poole, UK) in 0.5 mol/L HCl for 30 min, then washed with 0.5 mol/L HCl for 30 sec and counterstained with 0.25% (w/v) Safranin O (Gurr, Poole, UK) in 0.125 mol/L HCl for 30 sec. Finally sections were washed with distilled water, blotted dry, dehydrated rapidly through the alcohol series into xylene and mounted. For Alcian blue staining (AB-SO), Sections were rinsed with 0.7 mol/L HCl (pH 0.2) for 5 min, stained with 0.5% w/v Alcian blue (Gurr, Poole, UK) in 0.7 mol/L HCl for 10 min, washed with 0.7 mol/L HCl for 30 sec and counterstained with 0.25% Safranin O. For thionin stainings, alcolol-fixed tissues were stained as the method described by Reite (1997) and haematoxylin and eosin (HE) staining were ordinary.

Results

Histochemistry and light microscopic examination: Mast cells were identified in the mucosa and submucosa of the intestine (Fig. 1 and 2)and in the lymphoid organs including thymus, head kidney (Fig. 3) and spleen in grass fish and cat fish when the tissues fixed in Carnoy's fluid and stained with AB-SO. The mast cells were stained in blue which ovoid or round-shaped and uniform size with a nuclei located centrally or eccentrically (Fig. 1-3). In most examined tissues mast cells were intended to adjacent to blood vessels (Fig. 2). It seemed that the staining properties of the mast cells in grass fish and cat fish were similar to the mucosal mast cells in mammals in some aspects.

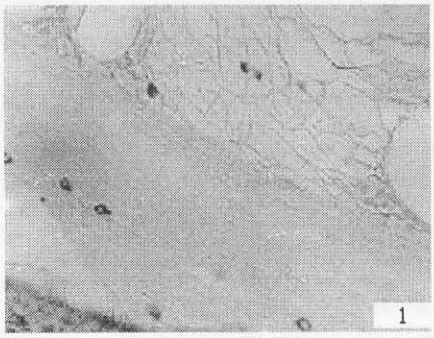

Fig. 1: Mast cells in the gut of cat fish. Paraffin section of carnoy-fixed tissue, stained with AB-SO. ×400.

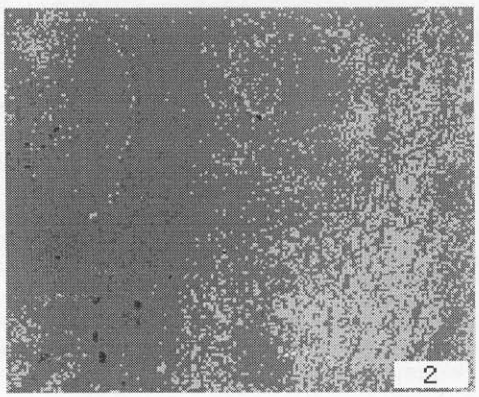

Fig. 2: Numerous mast cells adjacent to a blood vessel in serosa of the gut of cat fish. Paraffin section of carnoy-fixed tissue, stained with AB-SO. ×400.

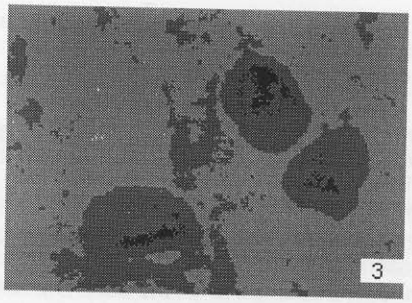

Fig. 3: Mast cells in the parenchyma of the head kidney of grass fish. Paraffin section of carnoy-fixed tissue, stained with AB-SO. ×1,000.

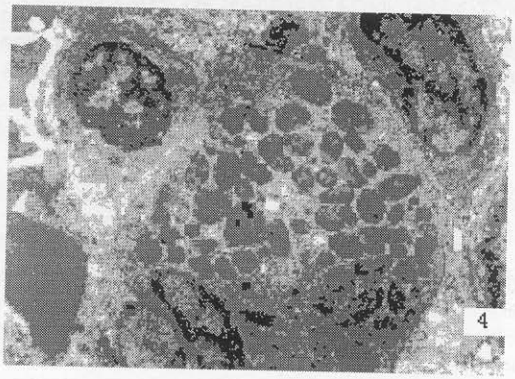

Fig. 4: Transmission electron micrograph of a mast cell in the parenchyma of thymus in grass fish. ×20,500.

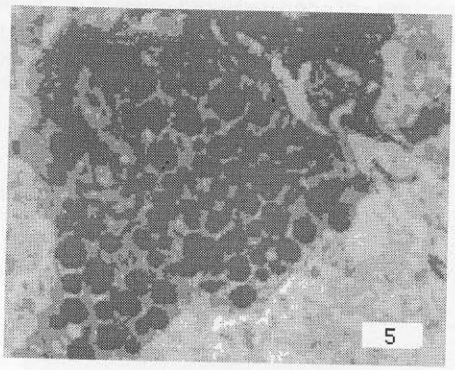

Fig. 5: Transmission electron micrograph of a mast cell in the parenchyma of head kidney in cat fish. ×20,500

Carnoy's fluid and Alcian blue were proved to be the good fixative and the excellent dye for the mast cells, respectively, but fixation in neutral buffered formalin (NBF)and stained with toluidine

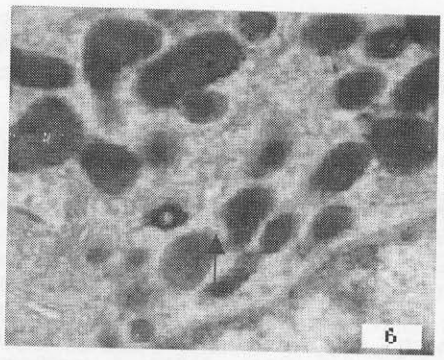

Fig. 6: Transmission electron micrograph of a hole-like cytogranules of a mast cell in the parenchyma of head kidney in cat fish. ×90,000.

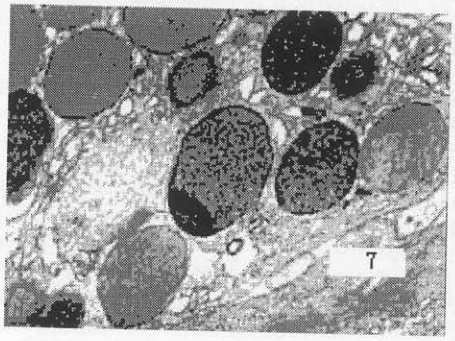

Fig. 7: Transmission electron micrograph of three cytogranules of a mast cell in the parenchyma of thymus.in grass fish ×105,000.

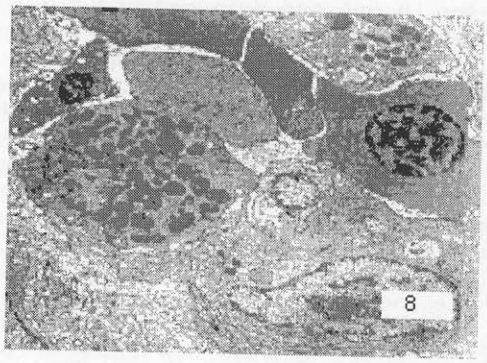

Fig. 8: Transmission electron micrograph of a mast cell with erythrocytes in the splenic sinus of spleen in grass fish. Notice numerous characteristic cytoplasmic granules in the mast cell. ×13,000.

blue were unsatisfactory. Some other histochemical methods we used repeatedly in present study such as fixed in MAFF or Bouin's fluid and stained with AB-SO or TB-SO, fixed in 80% alcohol and stained with thionin in 80% alcohol and HE staining after different fixations previously have been used by some researchers to detect mast cells, or so called eosinophilic granule cell (EGC) in teleostean fish (reviewed by Reite, 1998), were failed to identify mast cells in grass fish and cat fish in the present study.

Electron microscopic examination: Under electron microscopy, mast cells were found in the parenchyma of thymus (Fig. 4) and head kidney (Fig. 5 and 6) in the two species. Nuclei were non-segmented and located centrally or eccentrically and all mast cells contained characteristic granules (Fig. 4 and 5), The granules were round and oval in shape with a circular or outline and bounded by a unit membrane (Fig. 7). Short microvillus processes were seen on the surface of all mast cells. The granules contained amorphous granular materials but the electron density of this matrix material varied from high to low even within one granule (Fig. 7), a hole-like substructure was seen occasionally in a few granules (Fig. 6). An interesting finding is that a few mast cells could be identified within the splenic sinus (Fig. 8) which morphologically indistiguishable from those in the parenchyma of thymus and head kidney.

Discussion

In present study, mast cells were firstly identified in the intestine and some lymphoid organs such as thymus and head kidney in the freshwater fishes, grass fish and cat fish. It seemed that the staining properties of the mast cells in grass fish and cat fish were similar to the mucosal mast cells in mammals in some aspects. Carnoy's fluid and Alcian blue were proved to be the good fixative and the excellent dye for the mast cells, respectively, but fixation in neutral buffered formalin (NBF) and stained with Toluidine blue were unsatisfactory. Although mast cells or eosinophilic granule cells (EGCs) have been previously identified in many species of teleostean fish by some researchers using different histochemical methods such as fixed in MAFF or Bouin's fluid and stained with AB-SO or TB-SO, fixed in 80% alcohol and stained with thionin in 80% alcohol, stained with HE after some fixations and so on (reviewed by Reite,1998). But with those methods above in the present study we failed to identify mast cells or so called EGCs in grass fish and cat fish. Mast cells were only detected by using AB-SO staining after being fixed in Carnoy's fluid. It is possible that mast cells or EGC in different species of teleostean fish may have different histochemical properties or the staining properties of mast cells or EGC in fish may be very unstable something like mammalian mucosal mast cells.

Like mast cells in rodents, human (Irani and Schwarts, 1989) and chicken (Xu *et al.*, 2001,2003), mast cells in grass fish and cat fish were intended to adjacent to blood vessels. An interesting finding in the fish unlike in mammles is that under electron microscopy a few mast cells could be identified within the splenic sinus of grass fish which morphologically indistiguishable from those in the parenchyma of thymus, head kidney and other anatomic sites. We have reported that mast cells in chicken could be found not only outside but also inside blood vessels (Xu *et al.*, 2001,2003) The finding in present study is consistent with the report of Powell *et al.* (1990). They found that EGCs in the gills of rainbow trout (*Oncorhynchs mykiss*) showed a close association with blood capillary and some within the lumen of blood capillary. Up to now the relationship of EGCs to higher vertebrates is still not entirely clear. EGCs of fish show cytochemical and functional similarities to mammalian mast cells; for example, EGCs apparently produce histamine, and become degranulated after systemic administration of the neurotoxin, capsaicin, and the putative neurotransmitter, substance P (Ezeasor and Stokoe, 1980, Vallejo and Ellis 1990; Powell *et al.*, 1991; Naya and Lamas 1997)). In contrast, it has been reported that EGCs from the peripheral blood of some fishes are more similar to the eosinophils of higher vertebrates; for example, the granules are eosinophilic, contain eosinophil-like peroxidase, and myeloperoxidase-like protein (Bielek, 1981; Ishizeki *et al.*, 1984). So further study is needed.

Ultrastructurally, mast cells in grass fish or cat fish were similar to mast cells or EGCs in teleostean fish described previously. The characteristic cytogranules appear as membrane-bounded, homogeneous, electron dense, subspherical bodies (Ezeasor and Stokoe, 1980; Vallejo and Ellis 1990; Bielet *et al* 2000). No special substructures were found in the cytogranules of the mast cells.

Acknowlegements

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