Ultra Structure of Excretory Organ (Kidney) in Juvenile Grouper, *Epinephelus coioides*

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Abstract

In order to study of kidney tubules, twenty specimen of 45 days old juvenile grouper (*Epinephelus coioides*) were prepared after proper biometry. Five mm thick sections were removed from the middle and posterior end of the kidney and fixed in Bouin and glutaraldehyde. The routine procedures of preparation of tissues were followed for light and transmission electron microscopic study. In macroscopic observations, it was observed that kidney was located retroperitoneal and attached to the vertebral column with an average length of 4±0.2 Cm. The microscopic results showed that nephrons consisted of glomerules, proximal, distal and collective tubules. The proximal tubules were detectable with their brush border. Distal tubules had cuboidal cells with round nucleus that were positioned in the middle part of the cell. The lumen of these tubules were PAS negative due to lack of brush border. Collecting tubules were also PAS negative as their cuboidal cells lacked brush border and their ellipsoid to round nuclei were positioned central to the cell. TEM analyses of sections showed more details of tubules structure, e.g. plasma membrane showed an extensive system of depressions in all tubules, except in collecting tubules.

Keywords: Ultra structure, Excretory organ, Grouper, Epinephelus coioides.

1. Introduction

Grouper fishes belong to Serranidae and live in waters of countries south-east of Asia. Groupers are environmentally hypo-osmotic marine fishes. These fish species lose water and uptake salt actively. Instead, fishes drink water and actively excrete monovalent ions through the gills and some bivalent ions through their kidney (Cataldi et al., 1995; Grindstaff et al., 1996; Heemstra and Randall., 1993). Kidney is very important for osmoregulation in marine fishes, and does this by changes in rate of urine secretion and balance between secretion and reabsorption (Hickman, 1968; Lin et al., 2004). Nephrone in bony fishes is composed of glomeruli, a ciliated neck segment, a
proximal tubule, an intermediate segment, a distal tubule and a collecting tubule. Kidney in teleost is without henle arcuate and some are without glomeruli (Hickman et al., 1969; Hoar et al., 1983). In fish, maintenance of blood osmolality and ion concentration is performed differently than to the external environment (Nebel et al., 2005). This is based on active transport of ions particularly Na$^+$ and Cl$^-$ by absorption and excretion depending on the salinity of environment (Lin et al., 2004). Because of the importance of these fishes in nutrition and osmoregulation, this study was performed.

2. Materials and Methods

Fish samples were collected from Imam Khomeini Marine Research Station, affiliated with the Institute of Aquaculture in South of Iran. For this purpose, 20 juvenile grouper with 5±0.1 g weight were prepared and then anesthetized by carnation.

Then, middle and posterior part of kidney were removed, fixed in the Bouins solution and transferred to the histological laboratory. After 24 h, samples were removed from Bouin solution cleaned up and washed in 70% ethanol multiple times. The routine procedures of preparation of tissues were followed and paraffin blocks were cut in 6 micron sections, stained with H&E, PAS and then, studied under light microscope (Hickman and Trump, 1969).

Some samples were prepared for TEM analysis. These samples were fixed in 2.5% glutaraldehyde primary fixative for 8 h and in secondary fixative for 12-24 h (Sodium Phosphate 1%). Finally, sections were prepared routinely for TEM analysis (Caberoy and Quinitio, 2000).

3. Results

Results of microscopic study indicated that nephrons in grouper kidneys consisted of glomerulus, neck segment, proximal, distal and collecting tubules. Glomeruli included Bowman's space, Bowman's capsule, endothelial cell, mesangial cell, visceral and parietal epithelium of the renal capsule, podocyte cells, vascular bridge and capillaries (Fig. 1).

The neck segment connecting glomeruli to first proximal tubule, composed of short cuboidal epithelial cells with round nuclei located basally (Fig. 2).


Fig. 2: Cross section of mesonephric part of kidney in (E. coioides). Bouin, H&E, ×40. NS: neck segment, PI: first proximal tubule, PII: second proximal tubule, C: collecting

Proximal segments I and II, consisted of cuboidal cells basally positioned nuclei but in PII was drawn toward to the middle part. Also, collecting tubules were PAS negative as their cuboidal cells lacked brush border and their ellipsoid to round nuclei were positioned central to the cell (Fig. 3).
The cells were longer in PII than the PI. There was brush border in both segments on apical surface of epithelia, extended into the lumen but in a reduced rate from segment I towards the segment II (Fig. 4&5).

In PAS staining, proximal tubules were positive by their microvilli (Fig.6).

Distal tubules had cuboidal cells with a round nucleus positioned centrally to the cells and tended to be larger in cross section. Lumen of tubules were PAS negative due to lack of brush border. As mentioned, collecting tubules with their cuboidal cells, lacked brush border, were PAS negative and their ellipsoid to round nuclei positioned central to the cell (Fig. 7).

The height of cells in collecting tubules were greater than distal and proximal tubules but conversely, the diameter of lumen of the former was smaller.
In Transmission Electron Microscopic study, five different segments were distinguished: (1) neck segment (2) first proximal segment, (3) second proximal segment, (4) distal tubule and (5) collecting tubule, which joined to a collecting duct.

Neck segment: The epithelial cells of this segment were short-cuboidal with round nuclei and a few number of mitochondria. Cell membrane depression in the basal or lateral portion was shallow and the apical surface of cells showed no brush border but short microvilli and cilia were visible (Fig. 8).

First proximal tubule: The cuboidal cells of this portion had round nuclei with a well-developed brush border in the apical surface. There were numerous tightly packed microvilli more than the other segment (Fig. 9).

Second proximal tubule: The epithelium of this segment like the first proximal segment consisted of cuboidal cells that were longer and with low-density brush border. Also, the nuclei of the cells were oriented toward the center. Other specifications of the cells were similar to that of the first proximal tubule (Fig. 11).

The wall of cells in proximal tubules were extensively invaginated along their basolateral surfaces. Mitochondria were distributed in cytoplasm except in the area below the brush border (Fig. 10).


Distal tubule: As seen in TEM pictures, in this region, cells were cuboidal and larger than other parts. Mitochondria were distributed into the cytoplasm. Epithelial cells were cuboidal with elliptical to round nuclei centrally in the cell. Also, this segment lacked brush border and basolateral invagination of membrane was more observable compared to other segments.

Collecting tubule: Collecting tubules recognized by high-cuboidal cells with round nuclei positioned centrally with no brush border. Also, basal membrane invaginations were observed in other tubules (Fig. 12).

Fig. 12: Electron micrograph of collecting tubules. TL: tubule lumen, N: nucleus, M: mitochondria, IV: invaginations. Magnification: ×4900.

Features Common to all Segments: All tubular segments were surrounded by smooth muscle cells. Mitochondria and basal membrane depression were observed in all epithelial cells of tubules.

4. Discussion

About 5% of teleosts are euryhaline which can tolerate salinity of their environment. Grouper is euryhaline and lives in tropical and sub tropical region (Ogawa, 1961). Adults live in rigid and coral reef area while larvae and juvenile live on surface of aquatic plants and estuarine (Caberoy and Quinitio, 2000). The morphologic structure of kidney in marine organisms was devided to five groups by Ogawa in 1961. Based on this categorization, E. coioides has type III of morphological characteristics. Like the osteichthyans and teleostean, the kidney of E. coioides is divided to three sections: the head, trunk (mesonephric portion) and caudal section. Histological studies of trunk in juvenile grouper showed that nephrons consisted of glomeruli, neck segment, proximal, distal and collecting tubules. Renal corpuscle consisted of Bowman’s space and capsule, endothelial cell, mesangial cells, paretial and visceral epithelium, podocyte cells, vascular bridge and capillaries. The glomeruli was a coil of capillary which occupied the Bowman’s spaces (Hyman, 1942; Kumar and Tembhre., 1996). The mesangial cells are barrier for free molecules and ions during the filtration process (Kumar and Tembhre., 1996). As, Hickman in 1969 described, mesangial cells are essential for rapid adaptation to different environments. The presence of mesangial cells in glomerulus in other species such as Huso huso and Acipenser persicus was reported (Hickman and Trump., 1969). Viviane and Carolina (2006) described that the epithelial cells of kidney (composed of mesonephric convoluted tubules) were mitochondria-rich with plasma membrane depression (Viviane et al., 2006). Basolateral membrane invaginations were observed in all segments but with a greater abundance in basal section. Neck segments with short- cuboidal cells, were short and connected the glomeruli to the first proximal segment (PI). Also mitochondria were less frequent and positioned in apice of the nucleus. Height of epithelial cells increased gradually towards the first proximal segment. Depression of membrane in basal and lateral portion was shallower and the apical surface of the cells showed no brush border but some short microvilli and many cilia were present. These results corroborated findings of Ottosen (1978) on marine flounder, Pleuronectes platessa. As Kumar and Tembhre (1996) reported Huso huso and Acipenser persicus proximal tubules constituted cuboidal cells with basal nuclei with a well developed brush border (Kumar and Tembhre, 1996). Due to their brush border, this tubules differentiated from distal and collecting tubules by PAS.
staining (Tang et al., 2010). The proximal tubules of euryhaline teleost have two segments, the first and second (Hoar and Randall, 1983). Proximal tubule of (*E. coioides*) as an euryhaline species, included two segments like Elasmobranches, (*Huso huso*) and (*Acipenser persicus*) (Tang et al., 2010). The number of microvilli reduced from segment I to the segment II (Charmi et al., 2010). The brush border of proximal tubules was specialized for increasing surface area for absorption. The difference between the first and second proximal segments was not very pronounced, but reduced in density of brush border and cells nuclei toward the central section of the cell.

Ottosen (1978) showed kidney of *Pleuronectes platessa* lacked distal tubules. But, the results of this study on *E. coioides* was similar to the findings of Tang et al. (2010), Lin et al. (2004) and Charmi et al. (2010). Other studies on *Chanos chanos*, *Tetraodon nigroviridis*, *Huso huso* and *Acipenser persicus* showed that distal tubules were present in nephrons (Cataldi et al., 1995). Also, Hickman and Trump (1969) reported that in the euryhaline environment, southern flounder had both distal and collecting tubules, but in this fish no intermediate segment had been reported. Based on results of this study, like Elasmobranches distal tubules in *E. coioides* had cuboidal epithelium with no brush border (Viviane et al., 2006). Round nuclei were specific of distal tubules located centrally.

Distal tubules were not specialized for absorption but functioned in pumping ions, for example sodium out of lumen into the surrounding intracellular space, via active transport pumps. In distal tubules, ion passage control is vital, and the only way that ions are allowed to excrete out of the tubule via controlled active transport. The structure of collecting tubules in *E. coioides*, because of longer cuboidal epithelium with central nuclei differ from other tubules, similar to sturgeons (Prodocimo and Freire., 2006; Olsen, 1970). In ultrastructural study on *Pleuronectes platessa* a distinct segment with strongly basophilic cells without any brush border had been described (Flik et al., 2002). This segment receives the filtrate from the distal tubule and excretes it. Collecting tubules were larger than proximal and distal tubules. Large collecting ducts were formed by the fusion of number of collecting tubules and drainage several nephrons. Cells joined at the top with an occluding junction. Occluding junctions are impermeable to ions, and their presence implies that there's a good reason to prevent free percolation of materials from one side to the other (Youson et al., 1989). In conclusion, the study clearly showed that ultra structure of excretory organ in juvenile grouper was identical to the previously description, presented for different fish species.

**References**


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