Case Report

Ultrastructural aspects of the intestinal cells surface of *Angiostrongylus cantonensis* by using of transmission electron microscopy

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Summary

The rat lungworm *Angiostrongylus cantonensis* is a zoonotic parasite and the main cause of eosinophilic meningitis in the world. Its main source of nutrients originates from the degradation of host hemoglobin in blood-feeding helminths, such as *A. cantonensis*. The purpose of this study was to analyze the ultrastructure of the intestinal cells by using of transmission electron microscopy (TEM). *A. cantonensis* worms obtained from *Rattus norvegicus* (norway rats) from endemic area were used for TEM. The ultrastructural analysis was performed using fragments cut from the middle part of the worms, and the TEM study revealed the cells with microvilli and nuclei containing areas of loose and condensed chromatin and the presence of macrovesicles and microvesicles of digestion and it was observed in this study that intestinal epithelium is look like a syncytium. The ultrastructural aspects of the intestinal cells surface of *A. Cantonensis* the indicate that the intestinal epithelium is a multi-nucleate mosaic or syncytium.

Keywords: *Angiostrongylus cantonensis*; *Rattus norvegicus*; Eosinophilic meningitis; Pernambuco; Brazil

Introduction

Angiostrongylus cantonensis or rat lungworm inhabit the pulmonary arteries of rodents, primarily synanthropic commensals species. Terrestrial snails serve as intermediate hosts until they are ingested by definitive hosts (Ramirez-Avila *et al.*, 2009). Humans can be incidentally infected by eating poorly cooked or raw intermediate snails where some of them developed the eosinophilic meningitis (Thiengo *et al.*, 2010).

A. cantonensis digests large amount of haemoglobin in order to obtain aminoacids (Huttemann *et al.*, 2007), and the degradation process occurs in a lysosome-type digestive vacuole (Halliwell & Gutteridge, 2015).

The nematode digestive system is divided into three parts include

the stomodeum, intestine, and proctodeum. The stomodeum consists of a buccal cavity and esophagus. The intestine is a simple tubular structure formed by just a one-layered epithelium and the third part incorporates in male cloaca and female the rectum. However, the internal surface of the intestinal cells may be variable depend on the species of nematodes compounded by cilia associated with basal granules (Hetherington, 1924), or have a brush border lining the gut lumen (Chitwood & Chitwood, 1938).

Although *A. cantonensis* was described for more than eighty years, the digestion process of this nematode is still not clearly understood. The purpose of this study is to observe the ultrastructure of the intestinal cells by using of transmission electron microscopy (TEM).

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Materials and Methods

A. cantonensis worms obtained from *Rattus norvegicus* from endemic area were used for TEM. The ultrastructural analysis was performed using fragments cut from the middle part of the worms, not more than 1 mm in length, which were fixed in Karnovsky solution in 0.1M sodium cacodylate buffer, pH 7.4. Some pieces were

fixed in 2 % osmium tetroxide and inclusion medium (Epoxy/Sigma resin) was performed. The material was placed in an oven at 60°C for 72 hours for polymerization of the resin. The obtained blocks were sectioned in ultramicrotome with the use of glass knife. All cross sections semithin were stained with toluidine blue II and after drying, examined under a light microscope.

After selecting the area to be studied, the blocks were made in

Fig. 1. (A) Transmission Electron Microscopy of the intestinal epithelium of *A. cantonensis*. cross section of the intestinal epithelium showing a brush border composed of microvilli (Mi) and the presence of macrovesicles (red arrow) and microvesicles (blue arrow) of digestion; The epithelium is a multinucleate mosaic (N) or syncytium pattern showed no limit between these cells and the presence of basal lamina (BL); (B): The intestinal lumen is filled with a granular material, which sometimes contains a few intermixed membranous structures, vesicles and partially digested red blood cells (BC) originating from host blood feeding; (C): Transmission Electron Microscopy of the intestinal epithelium of *A. cantonensis*. The epithelial cells with microvilli (Mi), nuclei (N) and nucleolus (Nu) containing areas of loose and condensed chromatin. The presence of macrovesicles (larger arrow) and microvesicles (smaller arrow) of digestion; (D): Macrovesicles of digestion (red arrow) and short microvilli of different sizes.

ultrathin sections with a thickness of 40 nm, using diamond razors. The obtained cuts were placed in copper grids and contrasted with alcohol solution of 5% uranyl acetate and final treatment with lead citrate. After these procedures, the screens were taken to the TEM (Tecnai G2 200 kV / FEI) and the electromicrographs were obtained.

Ethical Approval and/or Informed Consent

The study was approved by the Research Ethics Committee of Universidade Federal Rural de Pernambuco (CEUA/UFRPE, process No. 120/2015). The research related to animals has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Results and Discussion

It was observed in this study that intestinal epithelium indicate that the intestinal epithelium is a multinucleate mosaic or syncytium, because the lateral boundaries between the cells were not well defined. (Fig. 1A). This aspect was only reported by Byers and Anderson (1972) when studied the morphology and ultrastructure of the phytophagous nematode *Tylenchorhynchus dubius*.

The TEM study revealed the cells with microvilli and nuclei containing areas of loose and condensed chromatin and the presence of macrovesicles and microvesicles of digestion (Fig. 1A and C). The presence of many vesicles suggests a vesicular transport of the material as describe in *A. cantonensis* by Hüttemann *et al.*, (2007). The cytoplasm of the intestinal cells showed a moderate number of mitochondria that are concentrated in the basal portion of the cells.

The lumen, including the spaces between the microvilli, is filled with a granular material, which sometimes contains a few intermixed membranous, vesicles and partial digested red blood cells originating from host blood (Fig. 1B). These aspects were reported by Byers & Anderson (1972) and Hüttemann *et al.*, (2007) in different nematodes respectively.

Part of the epithelium showing a brush border composed of short microvilli of different sizes (Fig. 1D). The microvilli aspects was also observed by Hüttemann, Schmahl & Mehlhorn (2007) in *A. cantonensis* by light and electron microscopic studies in order to clarify the mode of nutrition of this nematode.

The ultrastructural aspects of the intestinal cells of *A. cantonensis* showed that although terminal bars delimit the apical margins between cells, the frequent lack of complete lateral boundaries and extensive length of the fasciculi indicate that the intestinal epithelium is a multinucleate mosaic or syncytium. Because we did not find the presence of gap junctions in the intestinal epithelium, it is suggested that the syncytium allows the communication between the cells during the capture of the digested hemoglobin and formation of the digestive vesicles.

Conflict of Interest

Authors state no conflict of interest.

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