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Some morphological aspects of *Wuchereria bancrofti* uterus after treatment with diethylcarbamazine

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Abstract

Confocal and EM analyses revealed that some female *Wuchereria bancrofti*, obtained from volunteers that received recommended diethylcarbamazine dose regimens, showed few or no embryos. Furthermore, inside the gravid uterus of female *W. bancrofti* treated with DEC we observed a finely granular, electron-dense material organised as strings of pearls, ~70 nm in maximal length surrounding intra-uterine microfilariae and apparently secreted by the embryo. Over the eggshells a similar material was also observed, possibly secreted by the uterine wall. The surface of intra-uterine microfilariae presented a material with identical electron-density to the scattered structures observed inside the egg. Similarly, the sheath of blood microfilariae of *W. bancrofti* also showed electron-dense projections, with shape and size similar to that observed inside the uterus.

Keywords: *W. bancrofti*; Uterus; Diethylcarbamazine; Electron microscopy; Confocal analysis

1. Introduction

Lymphatic filariasis remains a major public health problem in tropical countries around the world, and an estimated 120 million people are infected, more than 90% with *Wuchereria bancrofti* (OMS, 2002). Studies on oogenesis, fertilization and development of the sheaths of several filarial species have been described at ultrastructural level (Harada et al., 1970; McLaren, 1972, 1973; Kagei, 1960). An amorphous surface coat covering the fertilised ova of *Dipetalonema vitae* was described by McLaren (1973), which is retained by the embryo as the microfilarial sheath. Likewise, Rogers et al. (1976) studying the development of filarial embryos of *Brugia pahangi* using light and electron microscopy confirmed previous studies showing the formation of the microfilarial sheath from the eggshell. These authors reported a well-defined eggshell closed applied to a true unit membrane (oolemma). At the 5th division state, the eggshells separate from the blastulas and come into close apposition forming narrow channels in which electron-dense material is seen. Other ultrastructural studies described identical electron-dense projections on eggshell and microfilarial sheath of *Brugia malayi*, suggesting that the sheath is derived from this structure (Zaman, 1987).

Diethylcarbamazine (DEC) has been used in control programmes as an effective microfilaricide, however, the ability of this drug to kill *W. bancrofti* adult worms, which may live up to 12 years or more, is not yet completely understood. However, some recent studies demonstrated that DEC exerts a direct effect on microfilariae of *W. bancrofti* (Peixoto et al., 2003; Florêncio and Peixoto, 2003).

In the present paper, we described for the first time some morphological aspects of the *W. bancrofti* uterus at the ultrastructural level. In addition, we also demonstrated that treatment with diethylcarbamazine possibly decreases the worm's fecundity.

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2. Material and methods

Adult parasites. Living adult *W. bancrofti* were obtained from voluntary donors. Preoperative ultrasonography had shown the presence of an intrascrotal lymphatic dilation of ~20 mm, containing living adult worms. Female worms that remained alive after 12 days of treatment with DEC (6 mg/kg/day) were obtained from one volunteer. The study protocol and procedures were reviewed and approved by the Ethics Committee of the Hospital das Clínicas, Universidade Federal de Pernambuco, Recife.

Microfilariae of W. bancrofti. Initially, screening for microfilaremia was done by microscopic examination of 60 μl of night-blood smears. After patients were detected as positive, subsequent 5–10 ml of venous blood was collected in saline-EDTA (final concentration 0.5 M) for filtration by Nucleopore (Costar, Waltman, MA) as described by Freedman et al.(1994). No volunteer had received any previous DEC treatment.

2.1. Transmission electron microscopy

The living parasites were washed twice with phosphate-buffered saline (PBS) and fixed for 1 h in a solution containing 2.5% glutaraldehyde and 4% formaldehyde (freshly prepared from paraformaldehyde) in 0.1 M cacodylate buffer, pH 7.2. After fixation, the nematodes were washed twice with PBS, dehydrated in acetone, and embedded in SPI-Pon 812 resin (Sigma Company, St Louis, MO). Polymerization was done at 60 °C for 2 days. Ultrathin sections were collected on 300-mesh copper grids, counterstained with uranyl acetate and lead citrate, and examined with a ZEISS EM 109 transmission electron microscope.

2.2. Confocal scanning laser microscopy

The nematodes were fixed at 70 °C in AFA solution: ethylic alcohol 70% (93 parts), formol 37% (5 parts), and glacial acetic acid (2 parts), as described by Amato (1985). After fixation, they were washed in distilled water, mounted in lacto-phenol, and examined with a ZEISS LSM 410.

3. Results

Fig. 1 shows the general uterine morphology of an adult female *W. bancrofti* after treatment by DEC, observed by confocal scanning laser microscopy. The two branches of the uterus are evident, with some regions containing microfilariae, and others almost empty (Figs. 1 and 2). Details of the parturition revealed the passage of only one microfilaria (Fig. 3).

Longitudinal thin sections of an adult female of *W. bancrofti* ovary showed elongated primary oocytes measuring ~8–10 μm in diameter, containing numerous small mitochondriae, sparse elements of endoplasmic reticulum, and a central nucleus (Fig. 4). Until the female gametes become mature they remain attached by pseudopod-like evaginations to an axial structure, the rachis (Fig. 4). Inside the spermatheca, each oocyte was surrounded by several amoeboids and aflagellate spermatozoa (Fig. 5). Ameboid and aflagellate spermatozoas

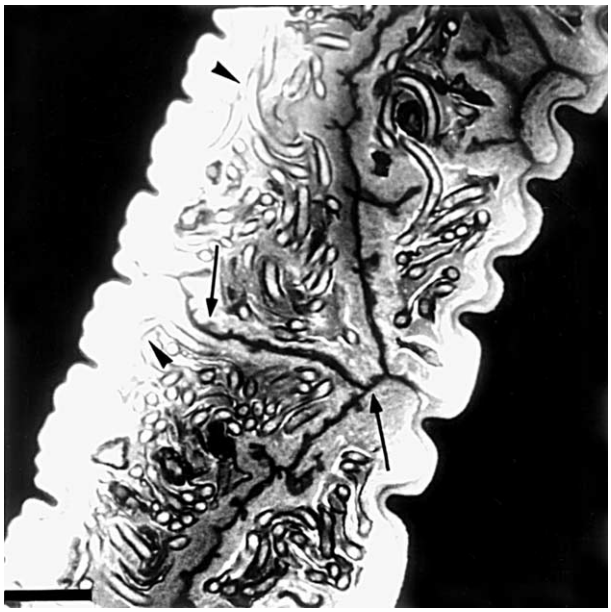

Fig. 1. Confocal scanning laser microscopy of an adult female of *W. bancrofti* after treatment with DEC. Note the uterus completely filled with microfilariae (arrows). Bar = 3 μm.

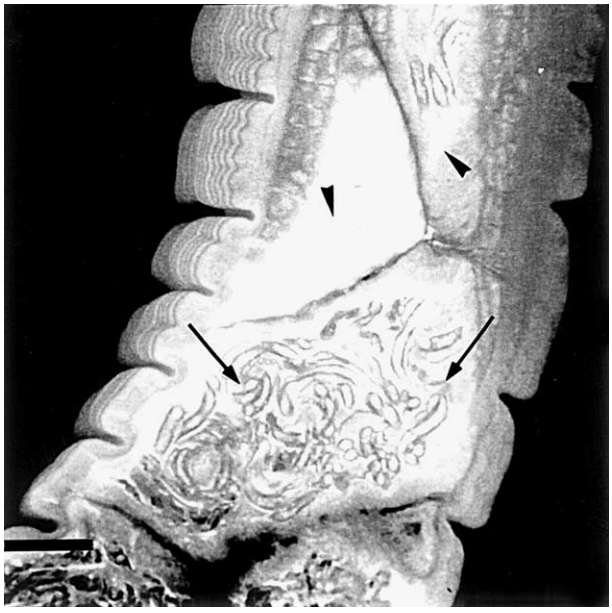

Fig. 2. Confocal scanning laser microscopy of an adult female of *W. bancrofti* after treatment with DEC. Some regions were replete with microfilariae (long arrows), and others almost devoid of embryos (short arrows). Bar = 3 μm.