ORIGINAL PAPER

Comparative analysis of a chemotherapy effect on the cuticular surface of *Wuchereria bancrofti* adult worms in vivo

A. Oliveira-Menezes · R. Lins · J. Norões · G. Dreyer · R. M. Lanfredi

Abstract *Wuchereria bancrofti* is the main species responsible for human lymphatic filariasis and remains a major public health problem in tropical countries around the world. Diethylcarbamazine (DEC) has been used for decades in control programs as an effective microfilaricide, although its efficacy in killing adult worms is only around 50% and its direct mode of action is unclear. Recently, in an attempt to control and eliminate lymphatic filariasis, WHO has recommended albendazole (ALB), a broad-spectrum anthelminthic combined with DEC or ivermectin for mass treatment. Some studies have shown that DEC alone blocks oogenesis, fertilization in adult worms, and loss of the microfilarial sheath of several filarial species, whereas ALB is thought to target nematode tubulin. So far, the direct effect of ALB in combination with DEC has not been described in *W. bancrofti* adult worms. Therefore, the purpose of this study is to investigate by scanning electron microscopy if DEC coadministered with ALB can induce in vivo morphological alterations of the *W. bancrofti* adult worm surface obtained from a patient in whom the adult worm remained alive, checked serially by ultrasonography for 2 months after antifilarial treatment. Our analysis demonstrates that worms presented morphologic alterations in some regions suggesting cuticular surface damage. On the other hand, adult worms that were recovered from a patient treated with DEC alone after a single dose did not show such any abnormalities.

A. Oliveira-Menezes · R. M. Lanfredi (✉)
Laboratório de Biologia de Helmintos Otto Wucherer,
Instituto de Biofísica Carlos Chagas Filho,
Universidade Federal do Rio de Janeiro,
Edifício do Centro de Ciências da Saúde, Bloco G, Av. Carlos
Chagas Filho, s/n, Cidade Universitária, Ilha do Fundão,
21941-902 Rio de Janeiro, RJ, Brazil
e-mail: reylanf@biof.ufrj.br

R. Lins · J. Norões · G. Dreyer
Núcleo de Ensino, Pesquisa e Assistência em Filariose (NEPAF)—
Hospital das Clínicas, Universidade Federal de Pernambuco,
Cidade Universitária,
50670-901 Recife, PE, Brazil

J. Norões
Serviço de Urologia, Departamento de Cirurgia, Centro de
Ciências da Saúde, Universidade Federal de Pernambuco,
Cidade Universitária,
50670-901 Recife, PE, Brazil

Introduction

Wuchereria bancrofti is the main species responsible for human lymphatic filariasis and remains a major public health problem in tropical countries (WHO 2002).

In 1998, the World Health Organization announced the Global Program to Eliminate Lymphatic Filariasis (GPELF), with a goal of eliminating lymphatic filariasis as a public health problem. In filariasis-endemic areas, the strategy most commonly used to interrupt transmission of the parasite is annual single-dose mass treatment for 4–5 years with albendazole (ALB) 400 mg coadministered with diethylcarbamazine (DEC) 6 mg/kg, in areas free of *Onchocerca volvulus* and *Loa loa*, or ivermectin (IV) 150–200 µg/kg. The addition of ALB to DEC or IV in mass treatment campaigns also provides a broad public health benefit due to its action against a range of intestinal helminthes (Ottesen et al. 1999).

DEC, the drug most widely used as an effective antifilarial drug, has been used for decades in treating patients (Ottesen 1985), although its efficacy for killing adult worms is only around 40–50% when estimated by ultrasonography, even when high doses are used (Norões

et al. 1997). Its direct mode of action is still unclear (Maizels and Denham 1992), although DEC has a great number of direct biochemical effects on different metabolic enzymes. These activities, however, do not seem to be directly responsible for the microfilaricidal effect of the drug (Subrahmanyam 1987), although Peixoto (2005), studying worms obtained from a single patient, suggests that DEC alone on a 12-day schedule blocks oogenesis and decreases fecundity in female *W. bancrofti*.

ALB is a benzimidazole drug that is widely used against human intestinal helminthes and in veterinary practice because of its broad spectrum and low toxicity (Horton 1990) and has shown activity either in vitro or in vivo in cattle and sheep against larval stages of *Haemonchus placei*, as well against adult worms of *H. placei*, *Trichostrongylus* sp., and *Cooperia* sp. (Cambell 1990).

Patients infected with *Brugia malayi* treated with ALB 500 mg three times a day, for 21 days, had decreased microfilaremia in the first week and all patients showed amicrofilaremia status by the second post treatment week (Mak and Chan 1983). Unfortunately, no data is available about adulticidal effect of such high doses. The detailed mechanism of action of ALB is unclear and experimental evidence with several intestinal helminthes shows that ALB, like other benzimidazoles, acts to inhibit β-tubulin polymerase, causing disruption of cytoplasmic microtubule formation (Lacey 1990; Horton 2000).

The DEC effects on *W. bancrofti* adult worms and cuticular surfaces of other nematodes are unknown. In contrast, papers have been published about the use of ALB and other benzimidazoles producing morphological alterations in several organisms. A study in vivo against *Glugea anomala* (Microsporidia), parasite of the connective tissue of the naturally infected stickleback, shows that ALB or mebendazole 1–50 mg/ml during 2–6 h stimulate the disorganized spores and irreversible malformations in different stages of fungi (Schmahl and Benini 1998). Ultrastructural experimental studies carried out with *Giardia* spp. in vivo with 100 mg/kg and in vitro suggest that ALB induces morphological changes not seen with any other drug (Morgan et al. 1993). Similar in vitro effects were observed in *Entamoeba histolytica* and *Trichomonas vaginalis* trophozoites (Cedillo-Riviera et al. 2002).

A previous transmission electron microscopy study shows that DEC alone induces release of microfilariae *Onchocerca volvulus* cuticle components (Gibson et al. 1976), a loss of microfilarial sheath of *W. bancrofti* (Peixoto et al. 2003), and fewer embryos inside the uterus of a *W. bancrofti* female (Peixoto 2005), although the effects on the surface morphology of these parasites are unknown. Scanning electron microscopy (SEM) of the in vitro effect of ALB, IVM, and DEC used alone or in combination on infective third-stage larvae of nocturnally subperiodic *B.*

malayi showed that only IVM induced damage to the surface as well as loss of regular cuticular annulations, and the cuticle was grooved in the middle region of the nematode body (Tippawangkosol et al. 2004).

In the current study, we compare the topology of *W. bancrofti* adult worms collected from naturally infected patients treated with a single dose of DEC combined with ALB (400 mg) and with DEC (6 mg/kg) alone. As far as we know, this is the first description by SEM of the effect on the adult worms of *W. bancrofti* obtained from patients treated with the drugs currently recommended for mass chemotherapy.

Materials and methods

Parasites

After informed consent, parasites were obtained from adult male patients living in the Greater Recife-Brazil, harboring living *W. bancrofti* adult worms detected at intrascrotal lymphatic vessels by ultrasonography. Both were microfilaremic, and because of the severity (Norões et al. 1996) and progression of lymphatic damage (Dreyer et al. 2002), the patients had a medical indication to be operated on to avoid rupture of dilated segment of lymphatic vessel and release of lymph fluid into surround tissues, which could complicate the urological presentation of the disease. Apparently unchanged living adult worms continued to be visualized by ultrasound at 2 and 3 months after patients received a single dose of the combination DEC (supplied by Farmanguinhos—FIOCRUZ, Brazil) and ALB (Zentel®, GlaxoSmithKline) or DEC alone, respectively. Patient screening, ultrasonography, physical examination, antifilarial treatment, follow-up, and surgeries took place at a public tertiary referral service for bancroftian filariasis (NEPAF), located at the Hospital das Clínicas—UFPE—Brazil. The patients did not receive any other drug up to surgery procedure and adult worm extraction. Serial physical examination of intrascrotal contents, performed in parallel with ultrasonography pre and after treatment (24 h, 7 days, and weekly up to surgery day) did not show any nodule formation a characteristic of adult worm death (Norões et al. 1997). The urologist (JN) was unaware of the treatment schedule of the patients. Segments of lymphatic vessels containing only living adult filariae were surgically removed from treated patients and from a control untreated patient during a hydrocele repair. For this study, 1 male and 2 female worms were analyzed from DEC-treated patient; 3 females from a patient treated with DEC coadministered with ALB and 2 females and 1 male worm from untreated patient. The worms were alive and apparently not damaged, and microfilariae were seen by transparency under microscope in the

females' uterus. The patients were allocated in studies approved by the ethical committee of Hospital das Clínicas (UPPE—Brazil).

Scanning electron microscopy

For SEM, very active adult worms were surgically removed from a segment of tied intrascrotal lymphatic from treated and untreated patients. The worms showed a typical movement within the vessels, before and after they were opened and released in to a Petri dish with lymph from the vessel segment. The worms were gently transferred and washed in a Petri dish with NaCl 0.9% and immediately fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2; postfixed in 1% osmium tetroxide (OsO_4) and 0.8% potassium ferricyanide ($K_3 Fe(CN)_6$). The worms were dehydrated in a graded ethanol series (30–100%), critical-point dried in CO_2, mounted on stubs, gold sputter-coated, and examined using a Jeol JSM 5310 scanning electron microscope.

Results

Male and female *W. bancrofti* worms from an untreated patient were analyzed by SEM. The cuticular surface was transversally striated except at the anterior end and the caudal tip, where the surface was wrinkled. The anterior end was bulblike, bearing two pairs of cephalic papillae (Fig. 1a). The transversal striations along the body (Fig. 1b–d) presented spherical structures called bosses that were more densely distributed from the median region to the posterior region of the body (Fig. 1d) than at the anterior region (Fig. 1b). The female posterior region was slightly dorsally bent (Fig. 1c), the anal opening had one lip and was ventrally located in close proximity to the posterior end (Fig. 1c,d). At this region, the number of bosses increased (Fig. 1d) but decreased again toward the extremity (Figs. 1c, inset). Two phasmids were laterally displayed at the tail tip (Fig. 1c inset).

The nematode surface of female worms from a DEC-treated patient had a normal appearance when compared with untreated worms. The cuticular striations and distribution of the bosses (Figs. 1a and 2a,c) showed the same pattern. At the posterior end of the female, as also observed in untreated worms, the anus was surrounded by bosses (Fig. 2b,c) and the tip of tail with the cuticular wrinkled pattern where the phasmids were inserted (Fig. 2d) was seen.

Analysis of the three female worms obtained from a patient treated with DEC + ALB presented topological alterations on some regions of the cuticular surface. The classical cuticular pattern was maintained, as well the body cuticular striations and distribution of bosses, the anterior (Fig. 3a) and posterior end (Fig. 3b). Nevertheless,

Fig. 1 Scanning electron microscopy of *Wuchereria bancrofti* adult worm from untreated patients. **a** The anterior end is bulblike where two pairs of cephalic papillae are seen (*thin arrow*; *scale bar* 10 μm). **b** The cuticular surface of *W. bancrofti* showing transversal striations along the body (*asterisk*) and bosses (*large arrow*; *scale bar* 5 μm). **c** Female posterior end dorsally curved of (*empty head arrow*) showing anal opening with one lip, ventrally located (*empty arrow*; *scale bar* 50 μm). *Inset*, detail of female tail tip showing two lateral phasmids. (*Scale bar* 10 μm) **d** Female posterior end showing transversal cuticular striations (*asterisk*), densely distributed bosses (*large arrow*), and anal opening (*empty arrow*; *scale bar* 10 μm)
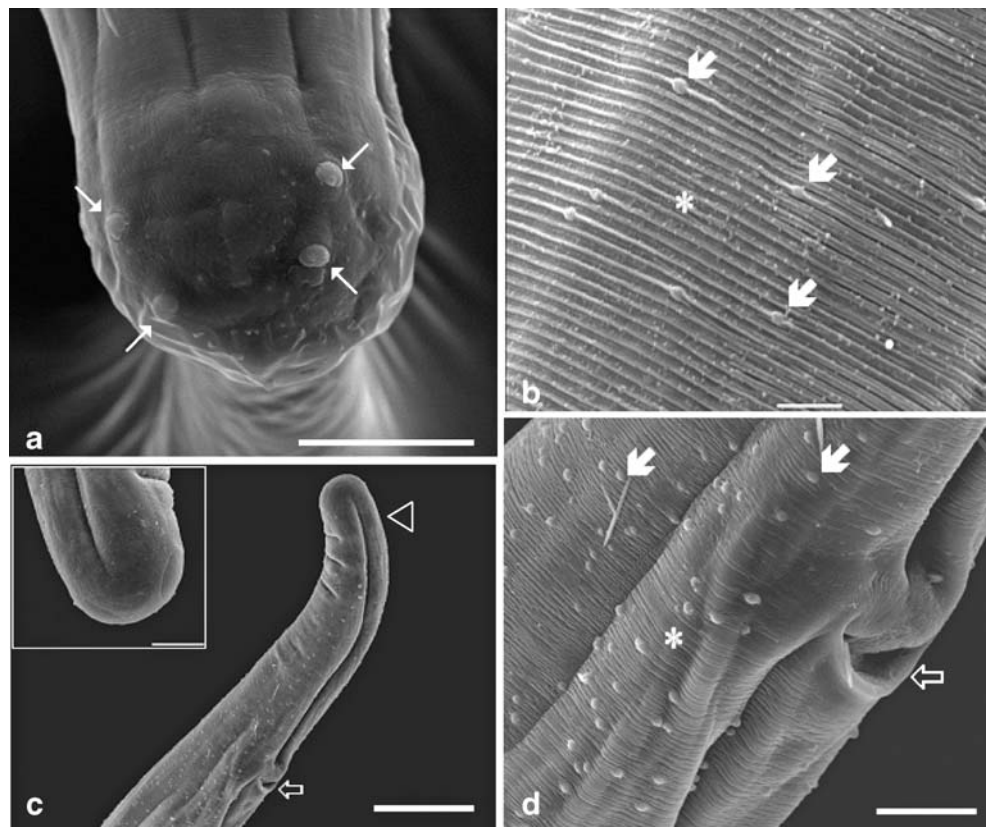
significant alterations were observed at the lateral line level (Fig. 3a,f), where the surface of the cuticle formed a swollen band whose midregion presented a row of puffed-up and deflated structures (Figs. 3a,e, and 4c). At the other side of the same body region, the cuticle presented long digit form (Figs. 3a,c,d), spherical (Figs. 3a,c), and spike-like (Fig. 3a) projections besides a row of spherical protuberances nearby (Fig. 3d). In other regions of the body, the cuticular surface presented different amorphous alterations; these were larger cuticular projections like a ruffled leaf (Fig. 4a–d), but even in these structures, cuticular striations and bosses could be seen at the base line of the normal cuticle where the projections were formed (Fig. 4b,d). Meanwhile, male worms from control and from DEC-treated patients did not present any cuticular alteration.

Discussion

W. bancrofti adult worms were described by SEM by Araújo et al. (1995), and this is the only previous SEM study of adult forms of this exclusive human filarid. The authors did not reveal if worms were collected from treated or untreated patients. In the present study, the topography of the surface of the adult worms from patients treated with DEC or DEC + ALB was compared with adult worms from an untreated patient. The cuticular surface of male worms from DEC-treated patients was similar to the male worm collected from untreated patient, agreeing with the results presented by Araújo et al. (1995).

Our results from control and DEC-treated worms are very similar to those presented by Araújo et al. (1995), so it seems that DEC does not induce cuticular or surface alterations in female adult worms. A similar outcome was observed in immature females of *Dirofilaria* sp. (Franz et al. 1982). On the other hand, DEC has an effect on the loss of *W. bancrofti* microfilarial sheath and induces a wrinkled appearance of this surface (Peixoto et al. 2003). Chandrashekar et al. (1984) analyzing microfilariae of *Litomosoides sigmodontis* and *Brugia pahangi* in vitro suggested that changes in its surface occur because the loss of the sheath may lead to exposure of antigenic determinants and trigger secondary immunological damage. Therefore, DEC seems to have different pharmacological mechanism of action for different filarial species and development stages for the same species (Ottesen 1984).

On the other hand, the association of DEC + ALB seems to produce morphological alterations to the cuticular surface of the female adult worms, not seen when DEC was administered alone, although it does not seem to damage the worm enough to induce death up 2 months after drug intake, confirmed by unchanged ultrasound image of filarial dance sign over time; because there was no nodule formation in intrascrotal contents (by physical examination) and more important, to the naked eye, there was no nodule on the lymphatic vessel and the recovered worms were very active, with a uniform pattern of transparency nor disrupted, showing the appearance expected of normal worms. On addition, an examination under light stereomicroscopy did not show any calcification in any part of the body, and finally the same method for SEM analyses was used in all

Fig. 2 Scanning electron microscopy of *Wuchereria bancrofti* adult worm from DEC treated patients. **a** Anterior region nematode body showing cuticular striations (*asterisk*) and sparsely distributed bosses (*large arrow*; scale bar 10 μm) **b** Female posterior end (*empty arrow*) showing anal opening (*empty arrow*) and bosses (*large arrow*; scale bar 50 μm). **c** Surface of posterior region of female body with cuticular striations (*asterisk*), densely distributed bosses (*large arrow*), and anal opening (*empty arrow*; scale bar 10 μm). **d** Female tip of tail with the cuticular wrinkled pattern where the phasmids are inserted (*short thin arrow*; scale bar 5 μm)
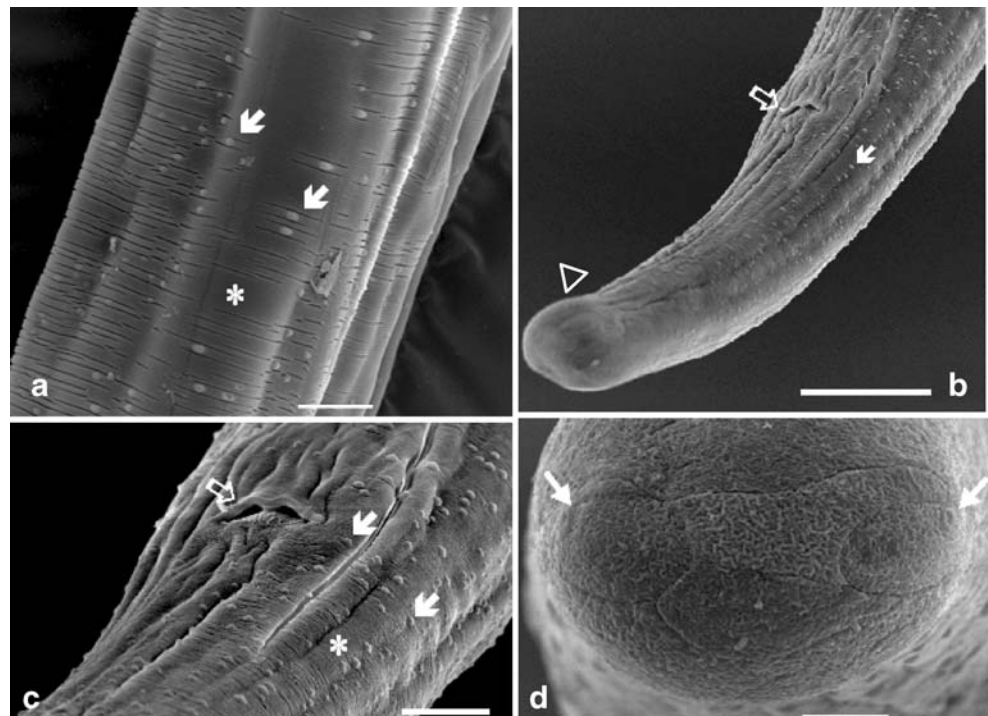
Fig. 3 Scanning electron microscopy of *Wuchereria bancrofti* adult worm from DEC + ALB treated patients. **a** Anterior region of nematode body showing a row of swollen bands (*full head arrow*), long digitform, spherical, and spike-like projections (*scale bar* 100 μm). **b** Detail of female tail tip (*scale bar* 5 μm). **c** Anterior region of *Wuchereria bancrofti* with details of cuticular alterations like long digitform, spherical, and spike such as projections (*scale bar* 50 μm). **d** Detail of spike-like projections close to a row of spherical protuberances (*scale bar* 10 μm). **e** Surface of *Wuchereria bancrofti* showing the cuticle with a row of puffed up and deflated structures (*full arrow head*) and bosses (*large arrow*; *scale bar* 10 μm). **f** Surface of *Wuchereria bancrofti* showing in detail lateral region with cuticle forming a swollen band (*full arrow head*). (*Scale bar* 10 μm)
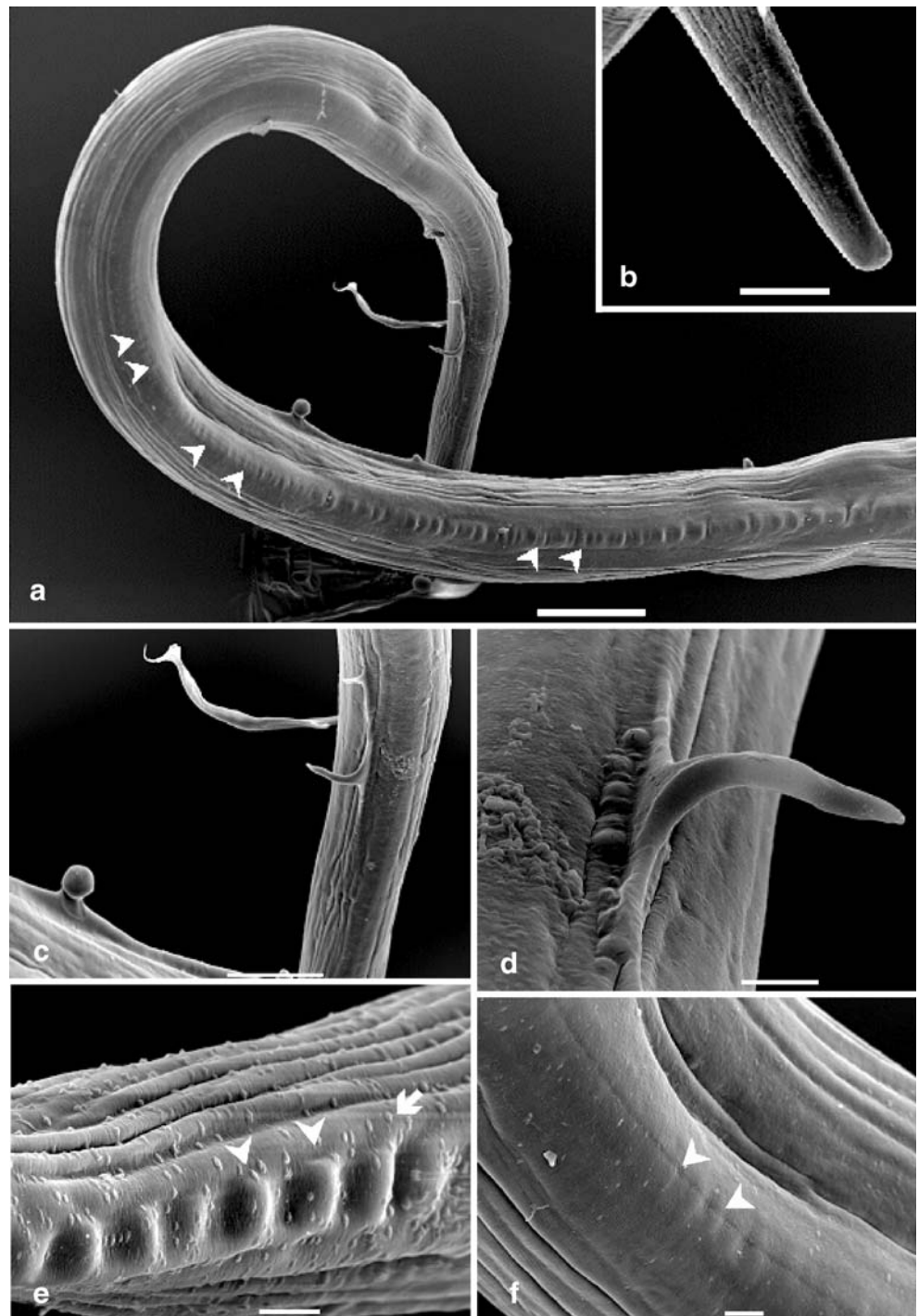
worm samples. Additionally, in all three female worms analyzed, micrographs show that the worm surface forms the cuticular protrusions and they occur in regions where no mechanical handling lesion could be seen. Another interesting fact is that the alterations arise in distinct locals of the nematode body, leaving large regions apparently without alterations.

Taken together, the facts described above, in principle, eliminate the hypothesis of the cuticular alterations due to technique artifacts and confirm that the cuticular alterations seen in worms from DEC + ALB treated patients is a drug (s) induced surface effect.

No data retrieved from the literature so far report surface morphological changes caused by ALB alone or associated

Fig. 4 Scanning electron microscopy of *Wuchereria bancrofti* adult worm from DEC + ALB treated patients. **a** Cuticular surface with larger cuticular projections like leaf ruffled (*scale bar* 100 μm). **b** Detail of larger leaf-like cuticular projections and bosses (*large arrow*; *scale bar* 50 μm). **c** Cuticular surface showing larger cuticular projections like a leaf ruffled and row of swollen bands (*full arrow head*; *scale bar* 5 μm). (*Scale bar* 10 μm)
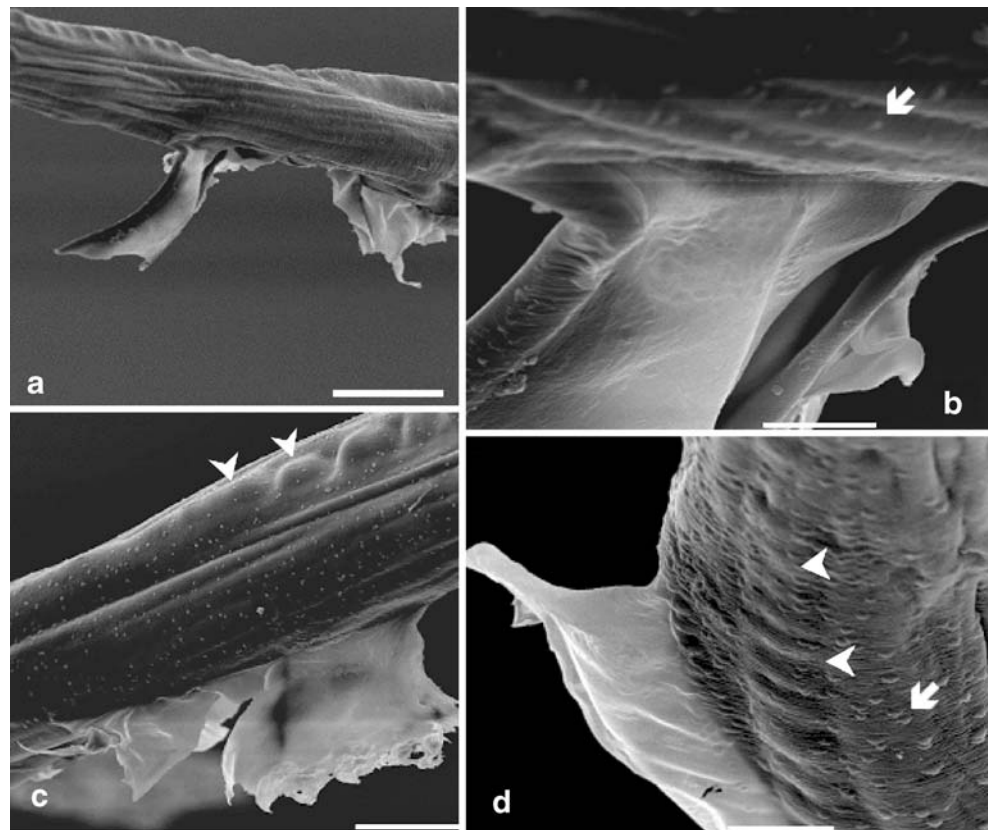
with DEC against filarial or other adult nematode parasites, and recent studies by SEM with the third stage of subperiodic *B. malayi* showed damage on cuticular surface with ivermectin treatment, but no morphological changes were observed when DEC or ALB was used alone or in combination (Tippawangkosol et al. 2004).

The main mechanism of action of ALB in parasites is based on blockage of microtubules β-tubulin polymerization (Lacey 1990; Robinson et al. 2004), producing cellular modifications (Osman et al. 1994; Horton 2000). Females of *Ascaris lumbricoides* eliminated from humans treated with a single oral dose of ALB 250 mg and dissected did not contain embryonated eggs, suggesting high effectiveness of this drug against embryogenesis (Carvalho et al. 1992). In *Ascaris suum*, its primary site of action is the intestine where, apparently, it affects secretory and absorptive functions of intestinal cells (Borges and Nollin 1975). The same was observed in *Haemonchus contortus* and *Trichuris globulosa* (Kaur and Sood 1996). This mechanism could induce inanition and consequently death of *Ascaris* sp. (Borges and Nollin 1975), *Enterobius vermicularis* (Georgiev 2001), *Strongyloides stercoralis* (Ochoa et al. 2003) and consequently parasite expulsion (Lacey 1990; Carvalho et al. 1992). An aberrant form, severe degradation or death in first-stage larvae of *Trichinella native*, *T. nelsoni*, and *T. spiralis* was observed when parasites were exposed to mebendazole, ALB, or thiabendazole (Martinez et al. 2002).

On the other hand, the results presented might emphasize the possibilities for the potential adulticidal effect of higher doses of ALB, at least for individual antifilarial treatment, as already suggested by Jayakody et al. (1993).

Based on the present study, comparing the worms obtained from untreated and treated patients with DEC alone or coadministered with ALB single dose, no major deleterious effects on *W. bancrofti* adult worms were detected. SEM showed some minor cuticular changes at ultrastructural levels when DEC + ALB were used, however, movement and activity of the parasite were apparently normal. It is not possible, however, to understand whether or not these subtle alterations have biological significance on the parasite itself or in the host-parasite interplay at longer time or if would have an accumulative damage after yearly mass treatment of DEC + ALB, recommended by World Health Organization (Ottesen et al. 1997) for transmission interruption.

References

Araújo A, Figueredo-Silva J, Souto-Padrón T, Dreyer G, Norões J, De Souza W (1995) Scanning electron microscopy of adult *Wuchereria bancrofti* (Nematoda: Filarioidea). J Parasitol 81:468–474

Borges M, Nollin S (1975) Ultrastructural changes in *Ascaris suum* intestine after mebendazole treatment in vivo. J Parasitol 61:110–122

Cambell WC (1990) Benzimidazoles: veterinary uses. Parasitol Today 6:130–136

Carvalho OS, Guerra HL, Massara CL (1992) Developmenet of *Ascaris lumbricoides* eggs from females eliminated after chemoterapy in man. Mem Inst Oswaldo Cruz 87:49–51

Cedillo-Riviera R, Chavez B, Gonçalez-Robles A, Tapia A, Yepez-Mulia L (2002) In vitro effect of nitrazoxanide against *Entamoeba histolytica*, *Giardia intestinalis* and *Trichomonas vaginalis* trophozoites. J Eukaryot Microbiol 49:201–208

Chandrashekar R, Rao UR, Subrahmanyam D (1984) Effect of diethylcarbamazine on serum-dependent cell-mediated immune reactions to microfilariae in vitro. Tropenmed Parasitol 35:177–182

Dreyer G, Addiss D, Roberts J, Norões J (2002) Progression of lymphatic vessel dilatation in the presence of living adult *Wuchereria bancrofti*. Trans R Soc Trop Med Hyg 96:157–161

Franz M, Volkmer KJ, Lenze WA (1982) A case of dirofilariasis in man (subgenus *Nochtiella*): a scanning electron microscope study. Tropenmed Parasitol 33:31–32

Georgiev V (2001) Chemotherapy of *Enterobius* (Oxiuridae). Expert Opin Phamacother 2:267–275

Gibson DW, Connor DH, Brown HL, Fuglsang H, Anderson J, Duke BO, Buck AA (1976) Onchocercal dermatitis: ultraestructural studies of microfilarie and hot tissue, before and after treatment with diethylcarbamazine (Heterazan). Am J Trop Med Hyg 25:74–87

Horton RJ (1990) Benzimidazoles in the wormy world. Parasitol Today 6:106

Horton RJ (2000) Albendazol: a review of anthelmintic efficacy and safety in humans. Parasitology 121:113–132

Jayakody RL, De Silva CSS, Weerasinghe WMT (1993) Treatment of bancroftian filariasis with albendazole: evaluation of efficacy and adverse reactions. Trop Biomed 10:19–24

Kaur M, Sood ML (1996) In vitro effects of anthelmintics on the histochemistry of *Haemonchus contortus* and *Trichuris globulosa*. Appl Parasitol 37:302–311

Lacey E (1990) Mode of action of Benzimidazoles. Parasitol Today 6:112–115

Maizels RM, Denham DA (1992) Diethylcarbamazine (DEC): immunopharmacological interactions of an anti-filarial drug. Parasitology 105:S49–S60

Mak JW, Chan WC (1983) Treatment of *Brugia malayi* infection with mebendazole and levamisole. Southeast Asian J Trop Med Public Health 14:510–514

Martinez J, Perez-Serrano J, Bernadina WE, Rodriguez-Cabeiro F (2002) Expression of Hsp90, Hsp70 and Hsp60 in *Trichinella* species exposed to oxidative shock. J Helminthol 76:217–223

Morgan UM, Reynoldson JA, Thompson RCA (1993) Activities of several benzimidazoles and tubulin inhibitors against *Giardia* spp. in vitro. Antimicrob Agents Chemother 37:328–331

Norões J, Addiss D, Santos A, Medeiros Z, Coutinho A, Dreyer G (1996) Ultrasonographic evidence of abnormal lymphatic vessels in young men with adult *Wuchereria bancrofti* infection in the scrotal area. J Urol 156:409–412

Norões J, Dreyer G, Santos A, Mendes VG, Medeiros Z, Addiss D (1997) Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti* in *vivo*. Trans R Soc Trop Med Hyg 91:78–81

Ochoa MD, Ramirez-Mendoza P, Ochoa G, Vargas MH, Alba-Cruz R, Rico-Mendez FG (2003) Bronchial nodules produced by *Strongyloides stercoralis* as the cause of bronchial obstruction. Arch Bronconeumol 39:524–526

Osman AM, Jacobs DE, Plummer JM (1994) In vivo effects of sublethal concentrations of albendazole metabolites on the structure of reproductive organs of *Dictyocaulus viviparous*. J Helminthol 68:161–166

Ottesen EA (1984) Immunological aspects of lymphatic filariasis and onchocerciasis in man. Trans R Soc Trop Med Hyg 78:9–18

Ottesen EA (1985) Efficacy of diethylcarbamazine in eradicating infection with lymphatic-dwelling filariae in humans. Rev Infect Dis 7:341–356

Ottesen EA, Duke BO, Karam M, Behbehani K (1997) Strategies and tools for the control/elimination of lymphatic filariasis. Bull World Health Organ 75:491–503

Ottesen EA, Ismail MM, Horton RJ (1999) The role of albendazole in programmes to eliminate lymphatic filariasis. Parasitol Today 15:382–386

Peixoto CA (2005) Some morphological aspects of *Wuchereria bancrofti* uterus after treatment with diethylcarbamazine. Micron 36:17–22

Peixoto CA, Alves LC, Brayner FA, Florencio MS (2003) Diethyl-carbamazine induces loss of microfilarial sheath of *Wuchereria bancrofti*. Mícron 34:381–385

Robinson MW, Mc Ferran N, Trudgent A, Hoey L, Fairweather I (2004) A possible model of benzimidazole binding to β-tubulin disclosed by invoking an inter-domain movement. J Mol Graph Model 23:275–284

Schmahl G, Benini J (1998) Treatment of fish parasites. 11. Effects of different benzimidadole derivates (albendazole, mebendazole, fenbendazole) on *Gluglea anomalia*, Moniez, 1887 (Microsporidia): ultrastructural aspects and efficacy studies. Parasitol Res 84:41–49

Subrahmanyam D (1987) Antifilarials and their mode of action. Ciba Found Symp 127:246–264

Tippawangkosol P, Choochote W, Na-Bangchang K, Jitpakdi A, Pitasawat B, Riyong D (2004) The in vitro effect of albendazole, ivermectin, diethylcarbamazine, and their combinations against infective third-stage larvae of nocturnally subperiodic *Brugia malayi* (Narathiwat strain): scanning electron microscopy. J Vector Ecol 29:101–108

WHO (2002) Lymphatic Filariasis Elimination in the Americas. Regional Program Managers Meeting, Port-Au-Prince, Haiti, 1–184, 4–6 September