

A study on the provocative day test effect of ivermectin and albendazole on nocturnal periodic *Wuchereria bancrofti* microfilaraemia

Samuel K. Dunyo¹, Francis K. Nkrumah¹ and Paul E. Simonsen² ¹Noguchi Memorial Institute for Medical Research, University of Ghana, P.O. Box 25, Legon, Ghana; ²Danish Bilharziasis Laboratory, Jaegersborg Alle 1 D, 2920 Charlottenlund, Denmark

Abstract

We conducted a randomized double-blind placebo-controlled study, in the Ahanta West District of Ghana, on the provocative day test effect of ivermectin and albendazole alone and in combination on nocturnal periodic *Wuchereria bancrofti* microfilaraemia. Sixty-three individuals with high night-time microfilaria (mf) intensities were identified in 1997 or 1998 and randomized into 4 groups. Blood samples for mf were then collected from the same individuals in the daytime (between 09:00 and 15:00) immediately before and 30–50 min after treatment. Groups 1–4 were treated with ivermectin alone (150–200 µg/kg), albendazole alone (400 mg), the combination of ivermectin and albendazole, and placebo, respectively. Intensities of mf in daytime samples were considerably lower than in night-time samples. Neither ivermectin or albendazole alone nor their combination provoked significant liberation of *W. bancrofti* mf into the peripheral circulation after the daytime treatment.

Keywords: filariasis, *Wuchereria bancrofti*, nocturnal periodicity, provocative day test, ivermectin, albendazole, diagnosis, Ghana

Introduction

Throughout the endemic regions of Africa, microfilariae (mf) of *Wuchereria bancrofti* have been found to exhibit nocturnal periodicity with the highest number in the peripheral blood during night-time and few or none during daytime (GATIKA *et al.*, 1994; SIMONSEN *et al.*, 1997a; DZODZOMENYO *et al.*, 1999). For optimal accuracy of diagnosis, examinations for mf therefore have to be conducted during the night, preferably close to the periodic peak intensity around midnight. However, night blood sampling, especially in field surveys, is inconvenient to both the technical staff and the patients.

The mf of nocturnally periodic *W. bancrofti* can be stimulated ('provoked') to appear in the blood during daytime by the administration of a low dose of diethylcarbamazine (DEC) (KATAMINE, 1970; MANSON-BAHR & WIJERS, 1974; SIMONSEN *et al.*, 1997b). Since the search for mf in day blood specimens after a provocative dose of DEC appears to be as sensitive a method for detecting microfilaraemia as the examination of night blood specimens (MCMAHON *et al.*, 1979a), the DEC provocative day test has commonly been used for daytime diagnosis, and in some communities even for daytime field surveys, for lymphatic filariasis (WIJERS, 1977; MCMAHON *et al.*, 1981). However, DEC is not recommended for use in areas endemic for *Onchocerca volvulus* or *Loa loa* infections because of the risk of inducing Mazzotti reactions (WHO, 1995), and therefore the DEC provocative day test cannot be used in many parts of Africa.

Ivermectin has now been recommended as the drug of choice for treatment and control of lymphatic filariasis in areas of Africa that are co-endemic for onchocerciasis and/or loiasis (OTTESEN *et al.*, 1997). A role for albendazole in combination with ivermectin has furthermore been suggested for such areas (OTTESEN *et al.*, 1997). While carrying out a field trial on the efficacy of ivermectin and albendazole alone and in combination for the treatment of lymphatic filariasis in coastal Ghana (DUNYO *et al.*, 2000) we therefore decided to investigate also the provocative day test effect of the intervention drugs in a double-blind placebo-controlled study.

Materials and Methods

The study was conducted in 2 lymphatic filariasis endemic villages, Miamia (July 1997) and Asemko (August 1998), in the Ahanta West District of Ghana.

Address for correspondence: Dr Paul E. Simonsen, Danish Bilharziasis Laboratory, Jaegersborg Alle 1 D, 2920 Charlottenlund, Denmark; phone: (+45) 77 32 77 32, fax: (+45) 77 32 77 33, e-mail pes@bilharziasis.dk

The residents were first screened by finger-prick night blood sampling between 21:00 and 24:00 to identify mf-positive individuals. Sixty-three individuals (39 males, 24 females), aged 8–72 years, with high mf intensities and on normal daily activities were selected and randomly assigned to 4 treatment groups. Then, finger-prick blood was sampled from each participant during the day, between 09:00 and 15:00, immediately before and 30–50 min after drug administration. Groups 1–4 were treated blindly with ivermectin alone (150–200 µg/kg bodyweight) plus albendazole placebo, albendazole alone (400 mg) plus ivermectin placebo, the combination of ivermectin and albendazole, or placebo for both drugs, respectively.

The ivermectin and albendazole tablets and their placebos were part of supplies donated by Merck & Co., Inc., USA and SmithKline Beecham Pharmaceuticals, UK, respectively, for a field trial approved by the Ministry of Health, Ghana (DUNYO *et al.*, 2000). Oral informed consent to participate was obtained from adults and from the parents of children aged <15 years. Pregnant women and children aged <6 years were excluded. The drugs were coded, and neither the participants, the treatment team or the technicians who examined the blood specimens knew which treatment combination was given to individuals. The codes were revealed at the end of the main field trial.

Blood specimens were examined for mf by the counting-chamber technique (MCMAHON *et al.*, 1979b). In brief, at each time of sampling, 100 µL of finger-prick blood were drawn into a heparinized capillary tube and immediately thereafter transferred into a tube containing 900 µL of 3% acetic acid. The specimens were later examined in a counting chamber by microscopy. Intensities of mf were expressed as mf/mL of blood. Geometric mean intensities (GMIs) of mf were calculated as $\text{antilog}[(\sum \log(x+1))/n] - 1$, with x being the number of mf/mL of blood and n the number of individuals examined. GMIs of mf were compared statistically by t -test or 1-way analysis of variance, as appropriate, on log-transformed mf intensities using a SPSS computer software programme. P values < 0.05 were considered statistically significant.

Results

Baseline characteristics of the 4 study groups and data on their night-time and pre- and post-treatment daytime mf intensities are shown in the Table. The age and sex distribution of the participants were not significantly different between the 4 treatment groups (1-way; $P = 0.3$, χ^2 test; $P = 0.9$). Individual night sample mf intensities ranged between 450 and 14 630 mf/mL blood

Table. Characteristics of the four study groups in Ghana, and their night-time and pre- and post-treatment daytime *Wuchereria bancrofti* mf intensities

Treatment group	No. of individuals (males/females)	Mean age in years (range)	Geometric mean mf intensity in mf/mL (range)		
			Night-time	Daytime Pre-treatment	Daytime Post-treatment
Ivermectin	18 (10/8)	38.4 (8-72)	2534 (450-14 630)	39 (0-1000)	31 (0-1160)
Albendazole	16 (10/6)	34.9 (12-67)	2274 (670-14 250)	18 (0-2550)	36 (0-3430)
Combination	16 (10/6)	27.4 (12-45)	2689 (470-14 350)	26 (0-830)	40 (0-1920)
Placebo	13 (9/4)	34.3 (14-60)	2100 (460-7840)	21 (0-250)	43 (0-270)

and there was no statistically significant difference in the night-time mf GMIs between the 4 groups (1-way; $P = 0.9$).

Of the 63 pre-treatment daytime samples 51 (81.0%) were mf positive but had very low intensities. Thus, daytime pre-treatment mf GMIs were 1.5%, 0.8%, 1.0% and 1.0% of night-time intensities in the ivermectin, albendazole, combination of ivermectin and albendazole, and placebo groups, respectively. There was no statistically significant difference in daytime pre-treatment mf GMIs between the 4 groups (1-way; $P = 0.7$).

The time interval from treatment to post-treatment blood sampling ranged between 30 and 49 min (average 39 min). Of the 63 post-treatment daytime samples 55 (87.3%) were mf positive and, as in the pre-treatment daytime samples, mf intensities were very low. Thus the mf GMIs were 1.2%, 1.6%, 1.5% and 2.0% of the night-time intensities in the ivermectin, albendazole, combination of ivermectin and albendazole, and placebo groups, respectively. There was no statistically significant difference between the post-treatment mf intensities in the 4 groups (1-way; $P = 0.97$). Furthermore, when analysed pairwise, there were no significant differences between pre- and post-treatment mf GMIs in any of the treatment groups (paired t -test; $P = 0.4, 0.2, 0.06$ and 0.1 in the ivermectin, albendazole, combination of ivermectin and albendazole, and placebo groups, respectively).

Discussion

The collection of blood samples at night for diagnosis of lymphatic filariasis with nocturnal periodic microfilaraemia is inconvenient for patients and investigators, and may be a major cause for the general scarcity of investigations on this widespread infection. In lymphatic filariasis endemic areas the co-operation of communities for night blood surveys is furthermore difficult to obtain, partly because sampling time is often past the local bedtime and partly because of suspicion. Nocturnal periodic lymphatic filariasis is endemic in Ghana (DUNYO *et al.*, 1996) as well as in many other parts of Africa (MICHAEL & BUNDY, 1997) but, on account of the possibility of the occurrence of onchocerciasis and/or loiasis in the endemic communities, the use of DEC in even the provocative day test dose is contraindicated (WHO, 1995). Ivermectin alone or perhaps its combination with albendazole is now considered the treatment of choice for lymphatic filariasis in such regions (OTTESEN *et al.*, 1997), and the present study was conducted to investigate whether these drugs administered orally alone or in combination would induce a provocative day test effect on nocturnally periodic *W. bancrofti* mf.

Ivermectin, albendazole or their combination did not exhibit any significant ability to provoke daytime liberation of mf into the peripheral circulation. The drugs were administered in the dosages recommended for treatment, and the timing between treatment and post-treatment blood sampling was similar to that normally used for the DEC provocative day test (MANSON-BAHR & WIJERS, 1974; MCMAHON *et al.*, 1979a). It cannot be excluded that a provocative effect of the drugs occurred earlier or later than the 30-50 min target interval or

would have occurred with either lower or higher dosages of the drugs. A change in timing or dosage would, however, make the provocation test less convenient for practical use, especially under field conditions.

The marked contrast between the rapid provocative day action of DEC and the seeming absence of such an effect by ivermectin, although both drugs are microfilaricidal, is probably a reflection of differences in the pharmacodynamics and mode of action of these drugs. Both drugs are rapidly absorbed after oral administration. Although DEC has remained the mainstay of the treatment of lymphatic filariasis for decades, its mode of action as well as the mechanism by which it provokes daytime liberation of mf of nocturnally periodic *W. bancrofti* into peripheral circulation are still uncertain. Ivermectin generally causes muscle paralysis of nematode worms (DE SILVA *et al.*, 1997) and one could have expected an outpouring of paralysed mf into the peripheral circulation after ivermectin treatment, but this did not happen. Albendazole is poorly absorbed and mainly appears to have an embryotoxic and/or adulticidal effect on filarial worms (DE SILVA *et al.*, 1997), and therefore it is less surprising that no provocative day effect on mf was seen with this drug.

In recent years, simple and reliable immunological tests based on detection of specific circulating adult *W. bancrofti* antigens have appeared on the market (SIMONSEN & DUNYO, 1999) which can also be used on daytime blood samples for lymphatic filariasis diagnosis. A major limitation of these tests is that they are expensive and not easily available in most endemic areas. Furthermore, they cannot be used to evaluate directly the parasitological response to filariasis treatment programmes if the drug involved has no effect on adult worms. Thus, since ivermectin is not adulticidal (DE SILVA *et al.*, 1997), circulating filarial antigen tests cannot be used to evaluate the parasitological response to ivermectin treatment programmes on the short term. The search should, therefore, continue for a diagnostic test for mf that is cheap, non-invasive, acceptable and convenient to patients and investigators to replace the current night blood sampling technique, especially in areas where the DEC provocative day test is contraindicated.

Acknowledgements

We thank the chiefs, elders and study individuals of Miami and Asemko for their co-operation and participation. We also thank the staff of the Ahanta West District Health Administration, especially Dr Godfried Asamoah and Mr David Newton, for their involvement. We are grateful to Messrs Collins Ahorlu, David Zanu, Elias Dzakpasu and James Okine for technical assistance and to Mrs Cynthia Ahorlu for data entry and secretarial assistance.

References

- De Silva, N., Guyatt, H. & Bundy, D. (1997). Anthelmintics. A comparative review of their clinical pharmacology. *Drugs*, **53**, 769-788.
- Dunyo, S. K., Appawu, M., Baffoe-Wilmot, A., Nkrumah, F. K., Pedersen, E. M. & Simonsen, P. E. (1996). Lymphatic filariasis on the coast of Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **90**, 634-638.
- Dunyo, S. K., Nkrumah, F. K. & Simonsen, P. E. (2000).

- Randomized double-blind placebo-controlled field trial of ivermectin and albendazole alone and in combination for the treatment of lymphatic filariasis in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, (in press).
- Dzodzomenyo, M., Dunyo, S. K., Ahorlu, C. K., Coker, W. Z., Appawu, M. A., Pedersen, E. M. & Simonsen, P. E. (1999). Bancroftian filariasis in an irrigation project community in southern Ghana. *Tropical Medicine and International Health*, **4**, 13–18.
- Gatika, S. M., Fujimaki, Y., Njuguma, M. N., Gachihi, G. S. & Mbugua, J. M. (1994). The microfilarial periodic pattern of *Wuchereria bancrofti* in Kenya. *Journal of Tropical Medicine and Hygiene*, **97**, 60–64.
- Katamine, D. (1970). Studies on the periodicity of microfilariae. In: *Recent Advances in Researches on Filariasis and Schistosomiasis in Japan*, Sasa, M. (editor). Tokyo: University of Tokyo Press, pp. 123–144.
- Manson-Bahr, P. E. C. & Wijers, D. J. B. (1974). Banocide induced appearance of *Wuchereria bancrofti* microfilariae in the peripheral blood by day. In: *Parasitoses of Man and Animals in Africa. Proceedings of the 1972 annual scientific conference of the East African Medical Research Council*, Anderson, C. & Kilama, W. L. (editors). Dar es Salaam: East African Literature Bureau, pp. 354–357.
- McMahon, J. E., Marchall, T. F. de C., Vaughan, J. P. & Kolstrup, N. (1979a). Tanzania Filariasis Project: a provocative day test with diethylcarbamazine for the detection of microfilariae of nocturnally periodic *Wuchereria bancrofti* in the blood. *Bulletin of the World Health Organization*, **57**, 759–765.
- McMahon, J. E., Marchall, T. F. de C., Vaughan, J. P. & Abaru, D. E. (1979b). Bancroftian filariasis: a comparison of microfilariae counting techniques using counting chamber, standard slide and membrane (nuclepore) filtration. *Annals of Tropical Medicine and Parasitology*, **73**, 457–464.
- McMahon, J. E., Magayuka, S. A., Kolstrup, N., Mosha, F. W., Bushrod, F. M., Abaru, D. E. & Bryan, J. H. (1981). Studies on the transmission and prevalence of bancroftian filariasis in four coastal villages of Tanzania. *Annals of Tropical Medicine and Parasitology*, **75**, 415–431.
- Michael, E. & Bundy, D. A. P. (1997). Global mapping of lymphatic filariasis. *Parasitology Today*, **13**, 472–476.
- Ottesen, E. A., Duke, B. O. L., Karam, M. & Behbehani, K. (1997). Strategies and tools for the control/elimination of lymphatic filariasis. *Bulletin of the World Health Organization*, **75**, 491–503.
- Simonsen, P. E. & Dunyo, S. K. (1999). Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific circulating antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, 278–282.
- Simonsen, P. E., Niemann, L. & Meyrowitsch, D. W. (1997a). *Wuchereria bancrofti* in Tanzania: microfilarial periodicity and effect of blood sampling time on microfilarial intensities. *Tropical Medicine and International Health*, **2**, 153–158.
- Simonsen, P. E., Meyrowitsch, D. W. & Makunde, W. H. (1997b). Bancroftian filariasis: long-term effect of the DEC provocative day test on microfilaraemia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **91**, 290–293.
- WHO (1995). *Onchocerciasis and its control. Report of a WHO Expert Committee on Onchocerciasis Control*. Geneva: World Health Organization, Technical Report Series, no. 702.
- Wijers, D. J. B. (1977). Bancroftian filariasis in Kenya. I. Prevalence survey among adult males in the Coast Province. *Annals of Tropical Medicine and Parasitology*, **71**, 313–331.

Received 21 May 1999; accepted 3 August 1999

Announcements

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE Denis Burkitt Fellowships

The Denis Burkitt Fund was set up by his family in memory of Denis Burkitt, FRS, who died in 1993; it is administered by the Royal Society of Tropical Medicine and Hygiene.

One Fellowship (maximum value £7000) or two separate Fellowships (of £3500 each) are awarded annually for practical training, travel, or direct assistance with a specific project (preferably clinico-pathological, geographical or epidemiological studies of non-communicable diseases in Africa).

Applications must be made at least six months before the commencement of the proposed study (by 15 March or 15 September in each year). A short report on the study should be submitted, within 3 months of the recipient's return. Application forms are available from the Administrator, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W1N 4EY, UK; fax +44 (0)20 7436 1389, e-mail mail@rstmh.org

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE Robert Cochrane Fund for Leprosy

The fund, in memory of the great leprologist Robert Cochrane, is administered by the Royal Society of Tropical Medicine and Hygiene. It is used to finance up to three travel fellowships each year to a maximum value of £1000 each.

The fund will support travel for

- Leprosy workers who need to obtain practical training in field work or in research
- Experienced leprologists to provide practical clinical training in a developing country

There is no restriction on the country of origin or destination providing the above requirements are met.

Applications must be made at least six months ahead of the proposed trip, sponsored by a suitable representative of the applicant's employer or study centre and agreed by the host organization. A short report on the travel/study should be submitted, within one month of the recipient's return. Application forms are available from the Administrator, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W1N 4EY, UK; fax +44 (0)20 7436 1389, e-mail mail@rstmh.org