

Long-lasting reduction of *Brugia timori* microfilariae following a single dose of diethylcarbamazine combined with albendazole

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Abstract

The long-term effect of a single oral dose of 6 mg/kg bodyweight of diethylcarbamazine (DEC) combined with 400 mg albendazole (ALB) on the microfilariae (mf) of the lymphatic filarial parasite *Brugia timori* was studied on Alor island, Indonesia from April 2001 to April 2002. Before treatment the geometric mean of the mf density in 96 infected study subjects was 150 mf/mL night blood (range 1–5696 mf/mL). One year after treatment 69 subjects (72%) were mf-negative and the overall geometric mean mf density reduced to 3 mf/mL (0–2456 mf/mL). The reduction of mf was more pronounced 1 year after treatment compared with 6 months after treatment. It can be concluded that a single dose of DEC + ALB leads to a long-term and progressive suppression of *B. timori* mf for at least 1 year. Therefore, DEC + ALB can be recommended as an effective strategy to control *B. timori* infection in the framework of the Global Programme to Eliminate Lymphatic Filariasis.

Keywords: lymphatic filariasis, *Brugia timori*, diethylcarbamazine, albendazole, GPELF, Indonesia

Introduction

Lymphatic filariasis has been targeted by WHO for elimination as a public health problem by the year 2020 (Bebehani, 1998; Ottesen, 2000a, 2000b). A key strategy of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) is the community-based annual treatment of the population at risk using a single dose of diethylcarbamazine (DEC) or ivermectin alone or in combination with albendazole (ALB) (Ottesen *et al.*, 1997, 1999). In Asia, DEC is the drug of choice for the treatment of *Wuchereria bancrofti* or *Brugia malayi*. Enhanced microfilaricidal efficacy of DEC has been shown when combined with ALB (Shenoy *et al.*, 1999; Ismail *et al.*, 2001) and for *W. bancrofti* some macrofilaricidal action of DEC has been reported (Noroes *et al.*, 1997). Furthermore, the use of DEC in combination with ALB may help to prevent the development of drug resistance and, due to the addition of ALB, treated persons receive an additional beneficial effect because of its helminthocidal action on intestinal helminths. In addition, there is no pharmacological reason why these drugs should not be used in combination (Horton *et al.*, 2000; Shenoy *et al.*, 2002) especially considering that ALB is provided cost-free for filariasis control by the manufacturer GlaxoSmithKline (Ottesen, 2000a, 2000b). However, such a 2-drug treatment strategy has not been evaluated for the treatment of *B. timori*.

Brugia timori is distributed on a number of islands of the lesser Sunda archipelago in eastern Indonesia, where it replaces *B. malayi*. This filarial parasite is locally of great public health importance, causing acute filarial attacks and lymphoedema, sometimes leading to severe elephantiasis (Supali *et al.*, 2002a). The absence of an important animal reservoir, the inefficient transmission by *Anopheles* mosquitoes, the low global prevalence in comparison to *W. bancrofti* and *B. malayi* together with its rather limited and isolated distribution makes the elimination of *B. timori* infection a realistic goal in the early stage of GPELF. Although there is some experience in the control of *B. timori* using DEC (50 mg on a weekly basis or 5 mg/kg for 10 consecutive days) (Partono *et al.*, 1984, 1989), a single-dose treatment regimen has never been evaluated. Recently, we were able to show that a single dose of DEC/ALB

efficiently reduces the microfilariae (mf) density in *B. timori*-infected individuals within a few days (Supali *et al.*, 2002b). This fast reduction of mf was accompanied with a number of side effects, but the treatment was judged to be safe enough to be applied for community-based treatment. In the present paper we report the long-term effect of a single dose DEC + ALB treatment and we show that even after 6 months following treatment the reduction of mf is ongoing and most treated persons became amicrofilaraemic 1 year after treatment.

Patients and Methods

Study subjects and assessment of microfilariae

The study was performed in Mainang village on Alor Island, Indonesia (Supali *et al.*, 2002a). During initial surveys in April 2001, 586 people were examined for lymphatic filariasis and 157 individuals positive for *B. timori* mf were found. No other human filarial parasite was found in the study area and none of the individuals had received antifilarial therapy since 1990. All individuals included in the study were interviewed for personal data and any history of lymphatic filariasis as well as examined for clinical signs of the disease. Weight, gender, age, and height were determined to calculate the adequate doses of DEC + ALB and for later facilitation of the dosing procedure for the community-based treatment. The mf density was determined as previously described (Supali *et al.*, 2002a). Briefly, venous blood was collected between 19:00 and 00:00 and the mf density was determined by filtration of 1 mL blood using 5 µm polycarbonate filters (Millipore, Eschborn, Germany). Filters were stained using Giemsa's solution and microscopically examined for mf. The geometric mean mf density was calculated using the log + 1 transformation. Statistical analysis of the data was performed using Epi-Info, version 6.01 (CDC, Atlanta, GA, USA).

Diethylcarbamazine and albendazole treatment and re-examination

In April 2001, 15 mf-positive individuals were treated in the hospital and 111 mf-positive individuals treated in the village using 6 mg/kg bodyweight DEC and 400 mg ALB. To study the adverse reactions and the short-term effects on mf, individuals were monitored for up to 1 week following treatment (Supali *et al.*, 2002b). In October 2001 and in April 2002, all individuals were asked to return for re-examination. With the exception of 4 subjects with mild lymphoedema (grade 1) of 1 (3 subjects) or both legs (1 subject),

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all re-examined individuals were clinically asymptomatic before treatment. Of the 126 treated individuals 66 (52%: 39 men, median age 30 years, range 7–52 years; 27 women, median age 20 years, range 7–50 years) were re-examined after 6 months. One year after treatment 96 subjects (76%: 54 men, median age 30 years, range 7–54 years; 42 women, median age 27 years, range 7–67 years) were re-examined. In all, 98 (78%) subjects could be re-examined at 6 months or 1 year after treatment. Individuals who could not attend re-examination had either moved to other villages on the island or had to stay in their fields overnight. In April 2002, the individuals were treated again using DEC + ALB during the community-based treatment that was initiated at that time.

Ethical clearance

Informed consent was obtained from each participant. The Guidelines of the Ethical Committee of the Faculty of Medicine, University of Indonesia, for the conduct of clinical research were followed in accordance to those for clinical research and human experimentation of the declaration of Helsinki, respectively Tokyo, Venice and Hong Kong.

Results

To investigate the long-term effect of a single dose of 6 mg/kg DEC in combination with 400 mg ALB on the mf density of *B. timori* 66 and 96 individuals were re-examined 6 and 12 months, respectively, after treatment. Before treatment, 41% of the individuals in both groups had an mf density of 1–100 mf/mL and 59% had an mf density of >100 mf/mL (range 1–5696 mf/mL). Six months after treatment 39% of the individuals became amicrofilaraemic, 41% had 1–100 mf/mL blood and 20% had >100 mf/mL blood (range 0–5270 mf/mL). The geometric mean mf density of this group dropped to 7% of the pre-treatment value (Table). This strong reduction in mf density was similar to the reduction observed 1 week following DEC + ALB treatment (Supali *et al.*, 2002b). Assessment of mf density 1 year after treatment showed that the process of reduction of mf density was not complete after 6 months. One year following treatment, 72% of the treated individuals were amicrofilaraemic, 21% had 1–100 mf/mL blood and 7% >100 mf/mL (range 0–2456 mf/mL). The geometric mean mf density was 2% of the pre-treatment density. One year after treatment mf were absent or strongly reduced in all treated subjects with the exception of 2 individuals (2%). One 10-year-old boy had a low mf density of 1 mf/mL blood before treatment and 1 year after treatment the mf density of 39 mf/mL remained low. Another child, a 12-year-old girl, had a high mf density of 1873 mf/mL before treatment, 6 months after it was 348 mf/mL and 1 year after treatment an even a higher mf density of 2456 mf/mL was detected.

The reduction of microfilaraemics 1 year after treatment in the study population was not correlated to the

mf density before treatment. In all, 48% of the 33 individuals with an mf density of > 500 mf/mL before treatment became amicrofilaraemic 1 year after treatment. No correlation between efficacy and gender or age was observed. During re-examination late side effects that might have occurred a week or later following treatment were not claimed by the treated individuals. All treated individuals showed no clinical signs of lymphatic filariasis during re-examination, including 4 individuals who had grade 1 lymphoedema before treatment with DEC + ALB.

Discussion

The results of the present study demonstrate that in *B. timori* infection, a single dose of DEC + ALB strongly reduces the mf density and suppresses microfilaraemia for 1 year or perhaps even longer. In addition the data suggest that the decrease of mf density is not complete at 6 months following treatment because a stronger effect of treatment was observed 1 year following treatment. Treatment of *W. bancrofti* using 6 mg/kg DEC and 600 mg ALB reduced mf levels to 17% and 7% of the pre-treatment levels at the 6 month and 1 year post-treatment examinations, respectively (Ismail *et al.*, 1998, 2001). A similar observation was made for the DEC + ALB treatment of *B. malayi* with 1 year post-treatment mf levels as low as 1.5% of the pre-treatment levels detected (Shenoy *et al.*, 1999). Since the *B. timori* geometric mean mf density dropped to 2% of the pre-treatment level 1 year after treatment, it can be said that DEC + ALB treatment of *B. malayi* and *B. timori* infections might be more effective than for *W. bancrofti*.

Although adverse reactions such as fever, headache, myalgia, itching, and adenolymphangitis are common 1–3 d following treatment (Supali *et al.*, 2002b), none of the treated individuals complained of late side effects. In *W. bancrofti* infection, scrotal post-treatment nodules can be observed in some men (Noroes *et al.*, 1997). However, such post-treatment nodules were not recorded in *B. timori*-infected individuals, even though several men, in a community-based treatment of *W. bancrofti* in coastal areas of Alor, complained of nodule formation. This could be explained by the absence of hydrocele and genital elephantiasis in *B. timori* infection (Supali *et al.*, 2002a) and by the absence of worm nests in the scrotal area as have been demonstrated for *W. bancrofti* by ultrasound (Noroes *et al.*, 1997; Shenoy *et al.*, 2000).

The long-lasting and long-term progressive decrease of mf can have different explanations. The direct effect of DEC + ALB on mf may lead to a more rapid clearance of mf. Although some female worms may resume production of mf after some time, the drugs may cause irreversible damage to the adult worms leading to a permanent cessation of mf production or even death of the adult worms. It is known that high parasite loads may lead to immunosuppression. In *B. malayi* infection, long-term DEC treatment leads to

Table. *Brugia timori* microfilariae density in Indonesian study subjects before, and 6 and 12 months after treatment with a single dose of 6 mg/kg diethylcarbamazine and 400 mg albendazole (April 2001–April 2002)

	No. of subjects examined	Microfilariae before treatment			Microfilariae after treatment		
		Positive (%)	Arithmetic mean ± SEM	Geometric mean	Positive (%)	Arithmetic mean ± SEM	Geometric mean
Six months	66	100	703 ± 138	157	61	179 ± 83	11 ^a
One year	96	100	690 ± 115	150	28	42 ± 26	3 ^a

^aDifferences to the pre-treatment microfilariae (mf) density were statistically significant (Mann–Whitney *U* test, $P < 0.000001$) as well as between the 6 months and 1 year mf density (Mann–Whitney *U* test, $P = 0.00054$).

elevated cellular immune responses and interferon- γ release (Sartono *et al.*, 1995). It is possible that the initial reduction of mf due to DEC together with the helminthocidal effect of ALB on intestinal helminths helps to improve the immune status of treated individuals. The immune system of these persons may then be more capable of killing the remaining mf. For *B. timori* it has been shown that partial immunity can develop and that immigrants from non-endemic areas acquire an infection and also develop clinical signs faster than permanent residents of an endemic area (Partono *et al.*, 1978). In our study area a high vector infection rate with *B. timori* was observed (Fischer *et al.*, 2002) and a rapid reinfection cannot be excluded. However, a cured previous infection together with an improved immune system due to DEC + ALB treatment may lead to a level of protection against reinfection.

The DEC + ALB treatment was not successful in 2 individuals. One boy had a low mf density before treatment and was unchanged after treatment. A girl with a high mf density before treatment showed a significant drop of mf 6 months after treatment, but had a high mf density exceeding the pre-treatment value 1 year after treatment. Because of the high vector infection rate it can be assumed that the young girl was reinfected with *B. timori* some time after 6 months following treatment. However, it cannot be excluded that the worm population in these 2 individuals was not susceptible to treatment. Evidence of non-susceptibility to DEC has been reported for *W. bancrofti* (Eberhard *et al.*, 1991), but is not known for *B. timori*.

Taken together, DEC in combination with ALB is an effective strategy for the treatment of *B. timori* and 1 single oral dose suppresses mf in the blood for 1 year or longer. Therefore, community-based DEC + ALB treatment can be used for the control of *B. timori* infection in the framework of the GPELF.

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