

Bancroftian filariasis: effect of repeated treatment with diethylcarbamazine and albendazole on microfilaraemia, antigenaemia and antifilarial antibodies

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Mass drug
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Circulating antigen

Summary Diethylcarbamazine/albendazole (DEC/ALB) therapy is widely used in mass drug administration (MDA) programmes aimed at eliminating lymphatic filariasis. We studied the effects of repeated annual treatments with DEC/ALB on *Wuchereria bancrofti* microfilaraemia, filarial antigenaemia and IgG4 antibodies to Bm14 antigen. Fifty-seven subjects with asymptomatic microfilaraemia were treated with one or seven daily doses of DEC/ALB at time zero. All subjects were re-treated with single-dose DEC/ALB 12, 24 and 36 months later. The two treatment groups had comparable pre-treatment microfilaria counts. Multidose treatment cleared microfilaraemia more effectively than single-dose treatment. Filarial antigen levels decreased equally in both treatment groups. Total antigen clearance was observed in 29.6%, 52.0%, 63.6% and 79.5% of subjects at 12, 24, 36 and 48 months. These clearance rates are much higher than those observed in prior treatment trials with DEC or ivermectin. Antibody levels increased 4 weeks after treatment and then slowly decreased in most subjects. Antibody tests turned negative in 20%, 35%, 39.4% and 52.5% of treated subjects at 12, 24, 36 and 48 months post treatment. These results show that the studied parameters decline at different rates and to differing degrees following DEC/ALB treatment. These findings have important implications regarding strategies for monitoring the effects of MDA in populations.

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1. Introduction

Lymphatic filariasis is a mosquito-transmitted parasitic disease that affects some 120 million people in 80 countries in the tropics and subtropics (Michael and Bundy, 1997). The disease has been targeted for global elimination as a public health problem by the year 2020 (Ottesen, 2000). The main intervention tool employed by the Global Programme for Elimination of Lymphatic Filariasis (GPELF) is annual mass drug administration (MDA) to endemic populations. The regimen recommended for all areas outside of sub-Saharan Africa is a single dose of 6 mg/kg diethylcarbamazine (DEC) with 400 mg of albendazole (ALB). DEC/ALB is effective for reducing blood microfilaria (mf) counts and it also has a partial macrofilaricidal effect (killing adult *Wuchereria bancrofti*) (Hussein et al., 2004; Ottesen et al., 1997). Since *W. bancrofti* may only mature to adult worms in humans, MDA has the potential to reduce greatly the source of mf available to vector mosquitoes and to interrupt transmission of new infections.

The GPELF calls for MDA to be administered annually for 4–6 years to all people residing in lymphatic filariasis-endemic areas (estimated global target population, 1100 million; Ottesen et al., 1997). Although many millions of people have been treated with DEC/ALB in MDA programmes, relatively little information is available on the effects of repeated rounds of this therapy on parameters used to assess filariasis endemicity in populations. These include microfilaraemia (rates and levels), filarial antigenaemia (a marker for adult *W. bancrofti*), and antifilarial antibodies, which indicate infection or heavy exposure to the parasite.

We have previously reported early results from a clinical trial in asymptomatic subjects with microfilaraemia that compared effects of single-dose and multidose DEC/ALB on mf counts, filarial antigenaemia by the immunochromatographic (ICT) card test (El Setouhy et al., 2004) and viability of adult worms by ultrasound (Hussein et al., 2004). Here we report data on the effects of four annual rounds of treatment of these subjects on microfilaraemia, filarial antigenaemia by ICT card test and ELISA, and antifilarial antibody levels.

2. Materials and methods

2.1. Selection of studied subjects

Asymptomatic and clinically normal microfilaraemic men and women (≥ 18 years of age) with night blood counts

>80 mf/ml were invited to participate in the study. The 57 previously untreated subjects lived in Badrasheen district, Giza Governorate, about 45 km south of Cairo, Egypt. This area is included in the national programme for elimination of lymphatic filariasis where annual MDA based on single-dose DEC/ALB began in 2000.

2.2. Treatment groups

Prior to the first MDA, study subjects were assigned to treatment groups with block stratification for gender and blood mf count. The two groups were equivalent with respect to gender, age and infection intensity (Table 1). One group was treated with a single oral dose of 6 mg/kg diethylcarbamazine citrate (Pharmamed, Zejtun, Malta) plus 400 mg ALB (GlaxoSmithKline, Uxbridge, UK); the other group was treated with the same medications daily for 7 successive days. All subjects in the two groups were re-treated with a single dose of DEC/ALB 12, 24 and 36 months after the first treatment.

2.3. Blood samples

Venous blood samples were collected between 21:00 and 01:00 hours at time zero (just before treatment) and 4 weeks and 3, 6, 9, 12, 24, 36 and 48 months after the first treatment for parasitology and serology studies.

2.4. Detection of microfilaraemia

Microfilariae were detected by membrane filtration (5 µM, Nuclepore Corp., Pleasanton, CA, USA) of 1 ml venous blood and microscopic examination of stained filters.

2.5. Serological markers of filarial infection

Filarial antigenaemia (a marker for adult worm viability) was detected by two methods based on the same monoclonal antibody (AD12). The ICT filariasis card test (AMRAD ICT; French's Forest, NSW, Australia) was performed directly in the field from fingerprick blood samples according to the manufacturer's instructions. Note that the Binax Filariasis NOW® card test (Portland, ME, USA) was used to detect antigenaemia at 36 and 48 months (after discontinuation of the AMRAD ICT product). We also used a monoclonal antibody-based ELISA to measure filarial antigen levels in plasma relative to pre-treatment level, as previously described in

Table 1 Pre-treatment comparison of treatment groups

Parameter	Single-dose group	Multidose group	P-value
Age, mean (SE) (years)	36.7 (16.1)	30.4 (10.5)	0.14 ^a
Gender	18 M, 9 F	18 M, 12 F	0.95 ^b
Microfilariae/ml, geometric mean (range)	375.7 (90–3720)	399.4 (100–4531)	0.72 ^a
Filarial antigenaemia (card test)	26/27 (96.3%)	29/30 (96.7%)	1.00 ^b
Filarial antigenaemia (ELISA)	27/27 (100%)	30/30 (100%)	1.00 ^b
IgG4 antibodies to Bm14	25/27 (92.6%)	29/30 (96.7%)	0.9 ^b

^a Mann-Whitney *U*-test.

^b χ^2 (Fisher's exact test for filarial antigenaemia).

detail (Ramzy et al., 1991; Weil et al., 1987). The cut-off for a positive antigen test was the mean plus 3 SD (0.15) of values obtained with a panel of plasma samples from non-endemic Egyptian individuals.

Antifilarial antibodies were detected with an IgG4 antibody ELISA based on recombinant filarial antigen glutathione S-transferase fusion protein of clone Bm14 (Bm14-GST) as previously described (Chandrashekhar et al., 1994; Ramzy et al., 1995). Prior studies have shown that this assay is a sensitive marker for infection or heavy exposure to filarial parasites (Lammie et al., 2004; Weil et al., 1999). Sera were tested in parallel for antibody reactivity to Bm14-GST and to GST. The optical density (OD) value obtained with GST was subtracted from that obtained with Bm14-GST to determine the net OD value. The difference in net OD obtained with positive and negative reference plasma pools was defined to correspond to 100 antibody units and used as a reference to calculate antibody units for test sera relative to their OD values. Test sera with net OD values greater than the mean plus 3 SD of those obtained with a panel of samples from non-endemic individuals (which corresponded to a cut-off of 15 units) were considered positive in this assay.

2.6. Ethical clearance

The study was reviewed and approved by the institutional boards committees at Ain Shams University, Egypt, and at Washington University School of Medicine, St Louis, MO, USA. Written informed consent was obtained from all subjects prior to their participation in the study.

2.7. Data analysis

Data management and statistical analysis were done with a statistical software package (SPSS Inc., Chicago, IL, USA). Relative levels (RL) for mf, antigen and antibody were calculated for each subject by dividing post-treatment levels by pre-treatment values and multiplying by 100. Since it is difficult to measure low levels of antigenaemia accurately, subjects with pre-treatment antigen levels less than 50 ng/ml were excluded from the RL calculations ($n=15$). In the same manner, subjects with pre-treatment antibody levels below our cut-off value of 15 antibody units were excluded from the antibody RL calculation ($n=2$). Proportions were compared by χ^2 or Fisher's exact test (two-tailed). The Mann-Whitney U-test was used to assess the significance of group differences for continuous variables.

3. Results

3.1. Effects of therapy on microfilaremia

The mf counts fell dramatically in both treatment groups by 1 month after the first treatment, and the decreases were sustained through 48 months (Table 2). Multidose treatment was significantly more effective than single-dose treatment for clearing microfilaremia at all time points over the first year after treatment, and this difference persisted after subsequent rounds of treatment when all subjects were treated with single-dose DEC/ALB. The mf clearance rates

Table 2 Effect of diethylcarbamazine/albendazole treatment on blood microfilaremia by group

Months after treatment	Single dose			Multiple dose			RL (SE) ^b	
	No. tested	mf positive ^a		No. tested	mf positive ^a			
		%	Mean (SE)		%	Mean (SE)		
Pre-treatment	27	100	375.7 (140.5)	100	30	100	399.4 (160.4)	
1	27	96.3	64.5 (42.3)	40.2 (11.1)	30	46.7	2.7 (1.6)	
3	26	80.8	70.3 (78.7)	21.7 (4.5)	29	27.6	3.8 (2.3)	
6	26	88.5	57.0 (38.1)	22.7 (4.4)	29	34.5	6.0 (1.8)	
9	27	77.8	42.4 (49.2)	16.4 (3.7)	29	24.1	3.6 (2.5)	
12	26	76.9	30.6 (61.7)	14.3 (5.0)	28	25.0	4.2 (12.8)	
18	23	56.6	21.5 (11.2)	3.8 (1.2)	28	10.7	11.3 (39.4)	
24	23	39.1	20.4 (18.4)	1.8 (0.8)	27	3.7	70.0 (0.0)	
36	15	26.7	9.1 (6.7)	1.2 (0.8)	18	0.0	0.0	
48	18	11.1	22.7 (22.7)	2.7 (2.7)	23	0.0	0.0	

^a Microfilaria (mf) residual rates at all time points after treatment were significantly lower in the multiple dose group than the single dose group except at 48 months ($P < 0.01$).

^b RL, relative level, obtained by dividing post-treatment levels by pre-treatment values and multiplying by 100.

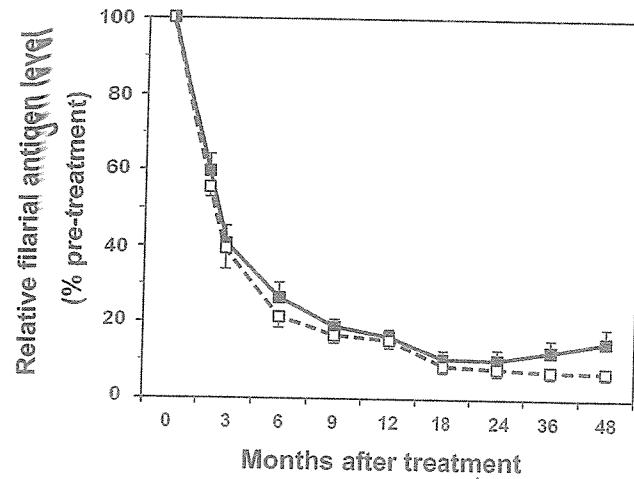


Figure 1 Effect of diethylcarbamazine/albendazole treatment on filarial antigenaemia by ELISA in the treatment groups. (■), single treatment group; (□), multiple treatment group.

at 12, 24, 36 and 48 months after the first treatment were 23.1%, 60.9%, 73.3% and 88.9% compared with 75%, 96.3%, 100% and 100% for those who were treated with a single dose or multiple doses of DEC/ALB in the first year, respectively. There was no significant gender difference in reduction or clearance of mf after treatment.

3.2. Effects of therapy on antigenaemia as determined by ELISA and by the card test

3.2.1. ELISA results

All subjects were antigen-positive by ELISA before treatment, and antigen levels were not different between treatment groups (mean \pm SE, 256 \pm 71.2 and 307.7 \pm 77.4 for single-dose and multidose groups, respectively). Antigen reductions after treatment were equivalent in the two treatment groups (Figure 1). Mean reductions for the combined groups at 12, 24, 36 and 48 months after treatment were 84.3 \pm 9.5%, 91.1 \pm 7.2%, 90.0 \pm 8.2% and 89.8 \pm 11.7%, respectively. Antigen levels fell below our ELISA threshold (0.14 OD units) in 29.6%, 52.0%, 63.6% and 79.5% of subjects at 12, 24, 36 and 48 months. There also was no significant difference between the two treatment groups with respect to antigen clearance rates.

3.2.2. Card test results

Two of 57 (3.5%) subjects had negative ICT card tests before treatment. Only one other subject cleared antigenaemia by card test at 12 months after treatment. Antigen clearance rates by card test at 24, 36 and 48 months post-MDA were 41.4%, 53.1% and 75%. As with the ELISA, there was no significant difference in clearance rates by treatment group. Table 3 summarises antigen clearance data as assessed by ELISA and the card test. This shows that while the card test remained positive in 10.0% of subjects whose antigen levels dropped below our cut-off level by ELISA, 5.1% of card-negative subjects had positive antigen tests by ELISA. Many subjects who cleared mf after treatment had persistent antigenaemia, especially after the first two rounds of treatment (Table 4). Persistent microfilaraemia

Table 3 Impact of diethylcarbamazine/albendazole treatment on filarial antigenaemia by the immunochromatographic (ICT) card test and by ELISA up to 48 months after treatment^a

ELISA results	Card test		12 months post treatment		24 months post treatment		36 months post treatment		48 months post treatment	
	Positive	Negative	Positive		Negative		Positive		Negative	
			21	3	7	17	10	2	5	16
Positive	38	0								
Negative	13	1								
Total	51	1								
			28	20			15	18		

^a Data shown are restricted to samples tested by ICT card test and ELISA.

Table 4 Persistence of filarial antigenaemia (ELISA) by microfilaraemia status up to 48 months after treatment with diethylcarbamazine/albendazole, by treatment group

Microfilaraemia status	ELISA results		12 months post treatment		24 months post treatment		36 months post treatment		48 months post treatment	
			Positive		Negative		Positive		Negative	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Single dose										
Positive	17	3	6	3	3	1	2	0	2	0
Negative	1	5	6	8	3	8	2	2	14	14
Total	18	8	12	11	6	9	4	4	17	14
Multiple dose										
Positive	6	1	1	0	0	0	0	0	0	0
Negative	14	7	12	14	6	12	4	4	17	17
Total	20	8	13	14	6	12	4	4	17	17

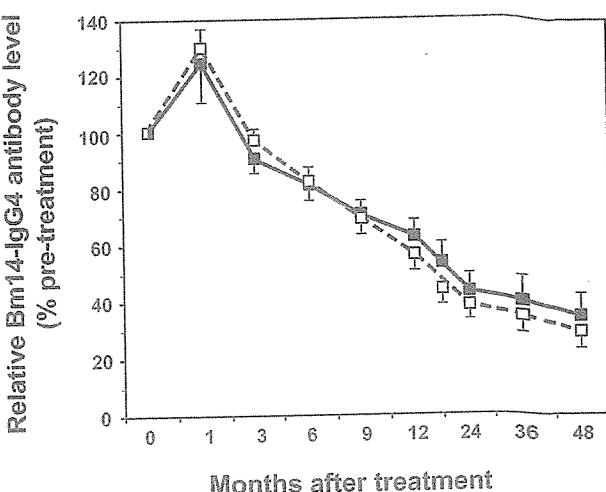


Figure 2 Effect of diethylcarbamazine/albendazole treatment on IgG4 antibodies to the filarial antigen Bm14 by treatment group. (■), single treatment group; (□), multiple treatment group.

was observed in several subjects who cleared antigenaemia by ELISA (four at 12 months, three at 24 months and one at 36 months). However, no microfilaraemia was observed in antigen-negative subjects at 48 months.

3.2.3. Effects of therapy on IgG4 antibodies

Prior to treatment, 95% (54/57) of subjects had positive antibody tests (>15 antibody units), and mean antibody levels in the two treatment groups were comparable (80.3 ± 22.4 and 80.0 ± 18.7 units for the single dose and multidose, respectively). Antibody levels increased 4 weeks after treatment and then slowly decreased thereafter in both treatment groups (Figure 2).

Antibody tests turned negative in 20%, 35%, 39.4% and 52.5% of treated subjects after 12, 24, 36 and 48 months of treatment, respectively. There was no significant difference in antibody levels between the two treatment groups at any time point. Table 5 shows the relationship between persistence of microfilaraemia, filarial antigenaemia and antibodies to Bm14. There was no significant association between clearance of antigenaemia or microfilaraemia with clearance of antibodies to Bm14 following treatment.

4. Discussion

This study aimed to compare the effects of single-dose DEC/ALB therapy with seven daily doses of the same regimen on microfilaraemia and on serological markers of infection (filarial antigenaemia and IgG4 antibodies to the recombinant filarial antigen Bm14). We also assessed the effects of re-treatment with DEC/ALB at 12, 24 and 36 months after the first treatment.

Multidose DEC/ALB was more effective in the first treatment round than single-dose treatment for reducing and clearing microfilaraemia. The difference in favour of multidose treatment persisted after several rounds of re-treatment with single doses of DEC/ALB. Multidose treatment (especially in the first round of MDA) might decrease the number of cycles needed for elimination of filariasis.

Table 5 Persistence of IgG4 antibodies to filarial antigen Bm14 by microfilaraemia and filarial antigen (ELISA) status up to 48 months after treatment with diethylcarbamazine/albendazole

Filarial infection by:	IgG4 antibodies to Bm14									
	12 months post treatment		24 months post treatment		36 months post treatment		48 months post treatment		Positive	Negative
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative		
Microfilaraemia										
Positive	20	5	8	1	4	0	1	1		
Negative	21	4	23	16	16	12	18	18		
Total	41	9	31	17	20	12	19	19		
Antigenaemia										
Positive	29	6	14	8	7	4	4	3		
Negative	12	3	17	9	13	8	15	16		
Total	41	9	31	17	20	12	19	19		

Since seven successive daily doses may be impractical for field application, additional studies are needed to determine whether 2- or 3-day multidose regimens are superior to single-dose treatment.

As expected, DEC/ALB treatment had a more dramatic effect on microfilaraemia than antigenaemia. This is because DEC/ALB is more effective against mf than against adult filarial worms. Interestingly, several subjects who cleared filarial antigenaemia at 12, 24 or 36 months had low-level persistence of microfilaraemia (Table 4). Other studies have reported persistence of mf in people who have cleared antigenaemia post treatment (McCarthy et al., 1995; Weil et al., 1991). However, this seems to be a temporary situation, since all subjects in our study who had negative antigen tests at 48 months were also mf-negative at that time.

Reductions in antigenaemia following single-dose DEC/ALB in this study were similar to those reported in earlier studies performed in Sri Lanka (Ismail et al., 2001) and greater than those observed after single-dose DEC (Ramzy et al., 2002). The same quantitative ELISA method was used in all of these studies. The filarial antigen results suggest that the first round of either single-dose or multidose treatment killed the majority of adult filarial worms. These results are consistent with ultrasound studies in the same subjects that directly observed effects of treatment on adult worms (Hussein et al., 2004). That study found that most adult filarial worms visible by ultrasound were killed after DEC/ALB treatment (90% and 97% of worm nests inactivated at 12 and 24 months, respectively), with no difference between single-dose and multidose therapy (Hussein et al., 2004).

Low-level persistence of filarial antigenaemia (indicating survival of some adult worms) in most subjects 12 months after the first round of treatment underscores the need for repeated cycles of MDA with high coverage rates if the goal of filariasis elimination is to be achieved. Our results suggest that filarial antigen prevalence rates will tend to decrease more slowly than mf prevalence rates following MDA. Our study also showed that a majority of these heavily infected subjects cleared antigenaemia after four rounds of therapy. We anticipate that the rest of these subjects will clear antigenaemia over the next year or two as their last remaining adult worms die of old age. We agree with the idea that absence (or near absence) of filarial antigenaemia in a population can be used as one criterion for filariasis elimination (WHO, 2000).

This paper reports the first data on the effects of DEC/ALB on antifilarial antibody levels in infected subjects. The early increase in antibody levels probably reflects antigen boosting from worms killed by treatment. After an initial increase, antibody levels decreased slowly over time, but over one-half of the treated subjects remained antibody-positive for years after repeated treatment rounds. We do not believe this was due to re-infection, because other studies have shown that transmission in the study area during this period was very low following MDA. To some extent, persistence of antibodies may reflect persistence of infection. However, antibodies disappeared in some subjects with persistent antigenaemia and/or microfilaraemia, and antibodies persisted in some subjects who cleared microfilaraemia and antigenaemia. It is fair to state that clearance of IgG4 antibodies to Bm14 after treatment is highly variable and

that antibodies persist for years after treatment in many subjects.

Slow clearance of antifilarial antibodies in adult subjects despite effective treatment has important implications for those interested in monitoring MDA programmes. Antibody prevalence rates in adults will tend to underestimate changes in infection prevalence rates and transmission in populations. Therefore, we favour using repeated assessments of antibody prevalence rates in young children as a means of monitoring changes in filariasis transmission in endemic areas.

In conclusion, this study has provided significant new information on serological changes following multiple rounds of DEC/ALB treatment of bancroftian filariasis. These data may be useful for those who treat and follow individual infected patients. However, our results also have important messages for filariasis elimination programmes based on MDA. Our antigen results suggest that single-dose and multidose treatments are both highly effective against adult filarial worms. However, multiple rounds of therapy were required to obtain total clearance of filarial antigenaemia in these subjects, and some subjects had persistent antigenaemia even after four rounds of treatment. Antigen clearance rates after treatment were high in subjects with low baseline levels (lighter infection). For this reason, antigen clearance rates should be faster in populations following MDA than in the heavily infected subjects included in our study. Changes in antibody levels after treatment were highly variable; many subjects had positive antibody tests 4 years after effective treatment. We do not recommend antibody testing of adults as a means of assessing the success of MDA programmes. However, repeated cross-sectional studies of antibody prevalence rates in children may be very useful for this purpose.

Conflicts of interest statement

The filariasis antigen card tests used in this study employ reagents licensed from Barnes-Jewish Hospital, Washington University School of Medicine, Campus Box 8051, 660 South Euclid Avenue, St Louis, MO 63110, USA (affiliation for Dr G.J. Weil).

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