A SINGLE DOSE OF DOXYCYCLINE IN COMBINATION WITH DIETHYLCARBAMAZINE FOR TREATMENT OF BANCROFTIAN FILARIASIS

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Abstract. Standard treatment of lymphatic filariasis with diethylcarbamazine (DEC) is associated with systemic adverse reactions, thought to be due to the release of microfilariae material and *Wolbachia* endosymbiotic bacteria into the blood. Combination treatments with doxycycline for 3-8 weeks are more effective than standard treatment. However, long-term use of antibiotics may contribute to drug resistance and are not practical for use in remote areas. We assessed whether a single dose of doxycycline combined with the standard DEC regimen would reduce the incidence and severity of adverse reactions and increase the efficacy of standard treatment. Forty-four subjects from Tak Province were recruited into the randomized double-blind clinical trial study: 25 received DEC (300 mg) combined with a placebo, and 19 received DEC (300 mg) combined with doxycycline (200 mg). The incidences of adverse reactions to standard treatment were lower in the doxycycline group (45.5%) than in the placebo group (58.8%). Severe reactions occurred only in the placebo group (3 of 25 subjects). The severity of adverse reactions was significantly lower in the doxycycline group (mean score 0.45) than in the placebo group (mean score 1.17). The levels of IL-6 and *Wolbachia* DNA in the plasma were significantly lower in the doxycycline group. The filarial antigen levels were significantly lower in the doxycycline group at months 6 after treatment.

Key words: bancroftian filariasis, diethylcarbamazine, doxycycline, adverse reaction

INTRODUCTION

Lymphatic filariasis is caused by infection with filarial nematodes, mainly *Wuchereria bancrofti* and *Brugia malayi*. This debilitating disease is endemic in 81 tropical and sub-tropical countries, including Thailand (WHO, 2009). The International task force on disease eradication has recently identified lymphatic filariasis as one of only six potentially eradicable infectious diseases which are targeted to be eliminated by the year 2020 (WHO, 1997; Behbehani, 1998; Molyneux *et al*, 2004). To interrupt transmission, the entire population at risk must be covered by mass drug administration (MDA) using combination therapy comprised of diethylcarbamazine (DEC) and albendazole, or ivermectin and albendazole (Molyneux *et al*, 2003).

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DEC is an excellent microfilaricide, which rapidly reduces the numbers of microfilariae in the blood of infected individuals (Subrahmanyam, 1987); however, DEC has a partial macrofilaricidal effect (Weil *et al,* 1988). Systemic inflammatory-mediated adverse reactions are commonly found in patients treated with DEC (Haarbrink *et al,* 1999a), thus, compromising compliance. The systemic adverse reactions to DEC are thought to be caused by the rapid release of antigens from microfilariae and *Wolbachia,* the endosymbiotic bacteria of filariae, into the blood (Cross *et al,* 2001).

Many studies have shown antibiotics with anti-rickettsial activity (*eg* tetracycline, doxycycline) can eliminate *Wolbachia* from filarial nematodes and result in worm growth retardation, infertility, and reduced fertility of filarial nematodes (Bandi *et al,* 1999; Hoerauf *et al,* 1999; Chirgwin *et al,* 2003). Doxycycline at a dose of 200 mg daily for 3-8 weeks has been shown to decrease the development, embryogenesis, fertility, and viability of filarial worms in species that harbor *Wolbachia* (Hoerauf *et al,* 2003a,b, 2009). Moreover, doxycycline treatment results in a reduction in inflammatory cytokines, *Wolbachia* DNA levels, microfilariae levels and adverse reactions (Keiser *et al,* 2002; Hoerauf *et al,* 2003a; Taylor *et al,* 2005; Turner *et al,* 2006; Hoerauf *et al,* 2009). The long-term use of antibiotics is not practical for mass treatment and may lead to drug resistance. It has not been established whether shorter courses of antibiotic therapy in combination with antifilarial drugs may be similarly effective at reducing microfilariae levels and killing adult worms. Previous studies have reported the successful treatment of rickettsial infections using a single dose of doxycycline (Huys *et al,* 1973; Sirisanthana *et al,* 1994). In this study, we assessed whether depletion of *Wolbachia* using a single dose of doxycycline could decrease adverse reactions with DEC treatment of bancroftian filariasis. We also assessed the impact of single dose doxycycline and DEC for the treatment of bancroftian filariasis.

MATERIALS AND METHODS

Study areas and study population

The study was conducted in the 5 districts (Mae Sot, Mae Ramat, Tha Song Yang, Umphang, and Phop Phra District) of Tak Province, Thailand (Fig 1). The Vector Borne Disease Control Center (VBDC) 9.3 (Mae Sot), Department of Disease Control (DDC), Ministry of Public Health, Thailand, the heads of the villages, local health workers and local villager volunteers screened 2,594 Thai and Myanmar migrants for lymphatic filariasis by fresh blood smear or by the NOW® ICT Filariasis card test (Binax, Portland, ME) using a finger-prick blood sample. All participants were examined for microfilariae between 8:00 PM and midnight. Symptoms and signs of bancroftian filariasis were obtained from interview and physical examination.

Design and trial enrolment

This was designed as a randomized double-blind clinical trial study. Individuals who were positive for microfilaria and/or filarial antigen were recruited into the study. Individuals eligible for participation were adults aged >15 years old who lived in endemic areas, who were in good health, did not take any medications and were positive for filarial antigen. Exclusion criteria included pregnancy, lactation, doxycycline or DEC allergies, evidence of liver or renal diseases, doxycycline or DEC treatment within the previous 7 days. Endemic normals (antigen-negative and

Fig 1–Map of the study areas (◉), Tak Province, Thailand.

amicrofilaremic individuals living in endemic areas for over 10 years and having no history of lymphatic filariasis) were also recruited into the study as controls. This study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Written informed consent was obtained from each individual or child's parent or guardian in the presence of two witnesses.

Randomization and allocation

Participants were enrolled in the same order in which they were diagnosed. Randomization was performed using random allocation after screening in the field. Staff who were enrolled and provided treatment had no participation in outcome measurements.

Intervention

Drug regimens were given randomly to participants. Participants were randomized into 4 arms. Patients and controls were divided into 2 groups: those who received a 300-mg of DEC in combination with 200-mg of doxycycline and those who received DEC in combination with a placebo supplied by the same manufacturer (Fig 2).

Outcome measurements

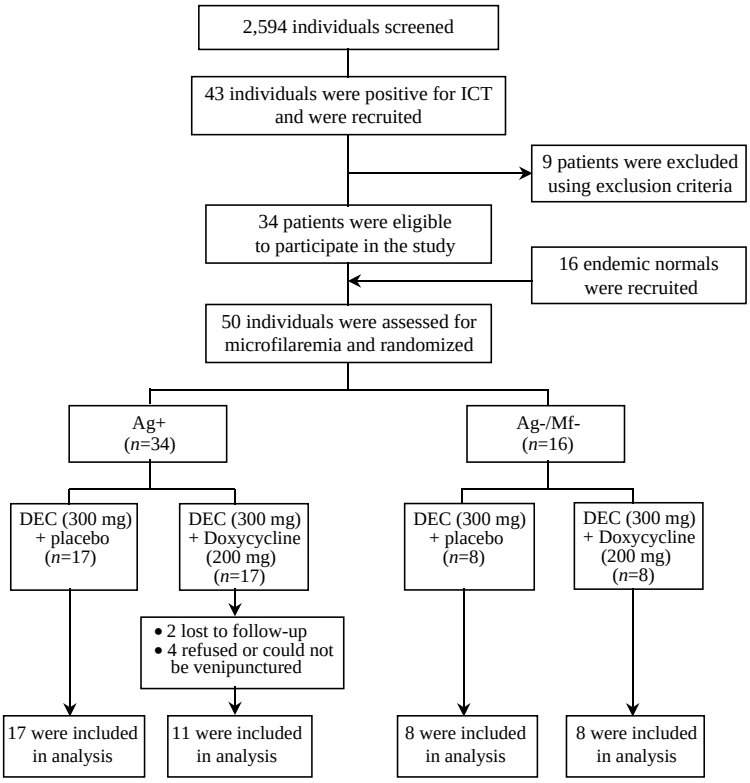
The primary outcome measurements were (1) determination of adverse reactions by clinical assessment of symptoms consistent with adverse reactions by interview and physical examination, (2) quantification of adverse reactions by determination of pro-inflammatory cytokines in plasma collected from participants, and (3) quantification of *Wolbachia* levels released in plasma using quantitative real-time PCR (qPCR) by detecting the *Wolbachia* surface protein (*wsp*) gene, a single-copy gene of *Wolbachia* (McGarry *et al*, 2004). Secondary outcome measurements were the (1) microfilaricidal and (2) macrofilaricidal effects after treatment.

Blood collections

Two to 5 milliliters venous blood was collected from subjects under sterile techniques using universal precautions. The whole blood samples were preserved in EDTA tubes. Plasma samples were separated from whole blood and stored at -70ºC until used.

Determination of microfilaremia

Thick-blood films were prepared in duplicate as described previously (Triteeraprapab and Songtrus, 1999;



ICT, Immunodiagnostic filariasis card test; Ag^+, microfiaria antigen positive; DEC, diethyl carbamazine; Ag^-, microfiaria antigen negative; Mf^-, microfilaria antigen negative

Fig 2–Flow chart showing trial profile.

Nuchprayoon *et al,* 2003). Briefly, 60 µl of blood sample was smeared onto microscope slides in duplicate. After being air-dried, the slides were stored at room temperature before examination in the laboratory. The microscope slides were stained with Giemsa stain. Identification of microfilaria species was performed under microscope by 2 technicians, independently, with consistent results. All the microfilaria-positive specimens were *W. bancrofti.*

Determination of filarial antigenemia levels

To quantitatively detect *W. bancrofti* antigen, the Og4C3 circulating antigen detection test was done using capture sandwich ELISA (More and Copeman, 1990) according to the manufacturer's instructions (TropBio, Townsville, Australia).

Adverse reaction evaluations

Adverse reactions were graded by severity from 0 to 3; Grade 0: no adverse reaction, Grade 1: mild (easily tolerated, did not interfere with daily activities), Grade 2: moderate (sufficient enough to interfere with daily activities) and Grade 3: severe (prevented normal daily activities). The participants were surveyed 48 hours after MDA, using a standard questionnaire. A translator who spoke the Thai, Karen and Myanmar languages provided translation. Signs and symptoms of adverse reactions were evaluated by interview and physical examination.

Determination of proinflammatory and anti-inflammatory cytokine levels

Plasma IL-6, TNF-α, and IL-10 levels

were measured using a specific enzyme-linked immunosorbent assay (ELISA) (eBioscience, San Diego, CA) kit according to the manufacturer's instructions. The assay sensitivities for human IL-6, TNF-α, and IL-10 on ELISA were 2 pg/ml, 4 pg/ml, and 2 pg/ml, respectively.

Determination of *Wolbachia* levels in plasma by quantitative PCR

One milliliter of plasma collected from each participant was centrifuged at 16,000*g* for 60 minutes at 4ºC to obtain a pellet of *Wolbachia.* The 800 µl of supernatant was discarded. The DNA was extracted from the pellet using a PCR template preparation kit (Roche Biochemicals, Mannheim, Germany) according to the manufacturer's instructions with modification by elution with 25 µl nuclease-free water. Quantification of *Wolbachia* DNA in plasma was performed by detecting the *Wolbachia* surface protein (*wsp*) gene, a single-copy of *Wolbachia* gene using quantitative real-time PCR (qPCR) on a LightCycler instrument (Roche Biochemicals, Mannheim, Germany).

The qPCR was performed using *wsp* conserve primers, *wsp*-F (5' TCA TGG CTG GTG GTA GTG 3') and *wsp*-R (5' TTC GCC TGG TAA GCA AAA C 3') (Invitrogen, Carlsbad, CA). Samples and the PCR master mixture were contained in 20 µl glass capillary tubes. The amplification program included an initial denaturation step for 1 cycle at 95ºC for 10 minutes and 45 cycles of denaturation at 95ºC for 10 seconds, annealing at 60ºC for 10 seconds, and extension at 72ºC for 12 seconds. Sample detection was based on SYBR Green I dye incorporation with the intended PCR products. The specificity of amplification was confirmed by melting curve analysis. All PCRs were carried out in duplicate. The number of copies of each sample transcript was then calculated from the standard curve with LightCycler software. Plasmids containing inserts of the amplified single copy of *W. bancrofti wsp* gene sequence were prepared for use as standards in the qPCR.

Data analysis

A reduction in adverse reaction severity in response to combination treatment with doxycycline and DEC was chosen as the primary study outcome. The secondary outcomes were clearance of microfilaremia, and reduction of filarial antigen in blood circulation. Data were recorded and analyzed using Microsoft Excel 6.0 and GraphPad Prism version 5 for Windows (GraphPad Software, San Diego, CA). The unpaired *t*-test was used to compare Og4C3 antigen levels, and cytokine levels between groups with $p < 0.05$ being taken as significant. The correlation was determined by the Pearson correlation test.

RESULTS

Patient participants and trial enrollment

The prevalence of filariasis was 1.6% as determined by filarial antigen detection. Of the 43 patients recruited, 34 were eligible for the study. The reasons for ineligibility were age <15 years old (7 patients), and lactating woman (2 patients). Sixteen control subjects were also recruited into the study. Therefore, a total of 50 subjects were randomized into 4 groups: 25 received 300 mg DEC and a placebo supplied by the manufacturer (placebo group), 19 received 300 mg DEC and 200 mg doxycycline (doxycycline group) (Fig 2). However, 6 patients were lost to follow-up (Fig 2), therefore, 44 individuals were included in the analysis. The baseline characteristics were not significantly different in the 4 groups (Table 1).

Adverse reaction evaluations

The incidences of adverse reactions

Table 1
Baseline characteristics of trial participants, by arm.

	Groups	*N*	Mean microfilarial count (mf/ml blood)	Sex		Mean age (range)
				Male (%)	Female (%)	
Ag+	DEC	17	670 (0-2,133)	11 (64.7%)	6 (32.3%)	33.33 ± 17.47 (15-55 years)
	DEC/Doxy	11	130 (0-450)	9 (81.8%)	2 (18.8%)	24.60 ± 8.08 (17-36 years)
Ag-	DEC	8	0	5 (62.5%)	3 (37.5%)	27.63 ± 13.19 (15-46 years)
	DEC/Doxy	8	0	4 (50%)	4 (50%)	31.88 ± 11.69 (16-47 years)
	Total	44		29 (65.9%)	15 (34.1%)	32.50 ± 14.66 (15-71 years)

Ag^+, microfilaria antigen positive; Ag^-, microfilaria antigen negative; DEC, diethylcarbamazine; Doxy, doxycycline

Table 2
Incidence and severity of the adverse reactions.

	Groups	*N*	Incidence of adverse reactions (%)	*p*-value	Mean scores for adverse reactions	*p*-value
Ag+	DEC	17	10 (58.8%)	0.0007[a]	1.17	0.03[b]
	DEC/Doxy	11	5 (45.5%)		0.45	
Ag-	DEC	8	1 (12.5%)	1.00	0.13	1.00
	DEC/Doxy	8	1 (12.5%)		0.13	
	Total	44	17 (38.6%)		0.62	

[a]Chi-square test
[b]Unpaired *t*-test
Ag^+, microfilaria antigen positive; Ag^-, microfilaria antigen negative; DEC, diethylcarbamazine; Doxy, doxycycline

were significantly lower in the doxycycline group (45.5%) than in the placebo group (58.8%) ($p = 0.0007$)(Table 2). In addition, the severity of adverse reactions was significantly lower in the doxycycline group (mean score 0.45) than in the placebo group (mean score 1.17) ($p = 0.03$). No severe adverse reactions were seen in the doxycycline group, but they occurred in the placebo group (3 of 25 subjects). One patient was hospitalized at 12 hours after DEC treatment. This severe reaction has been reported to the Ethics Committee and Serious Adverse Events (SAE) Subcommittee of the Faculty of Medicine of Chulalongkorn University, Bangkok, Thailand. The symptoms of adverse reactions reported were headache (13 of 17; 76%), dizziness (8 of 17; 47%), myalgia (8 of 17; 47%), chest pain (6 of 17; 35%), joint pain (4 of 17; 24%), diarrhea (4 of 17; 24%), nausea (3 of 17; 18%), vomiting (3 of 17; 18%),

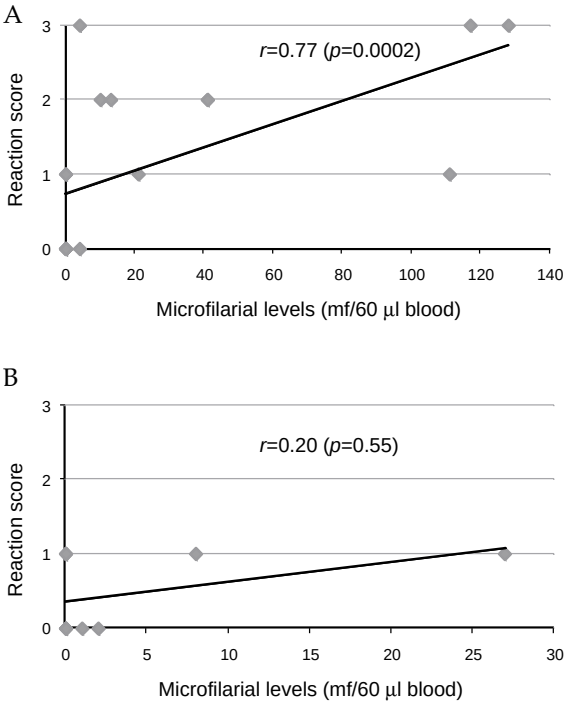


Fig 3–Correlation between baseline microfilarial levels and reaction scores in patients treated with DEC and placebo (A), and DEC and doxycycline (B). mf, microfilaria

chills (3 of 17; 18%) and abdominal pain (2 of 17; 12%). Fever (body temperature ≥38ºC) was seen in 5 of 17 (29%); most of these patients (4 of 5) were in the placebo group.

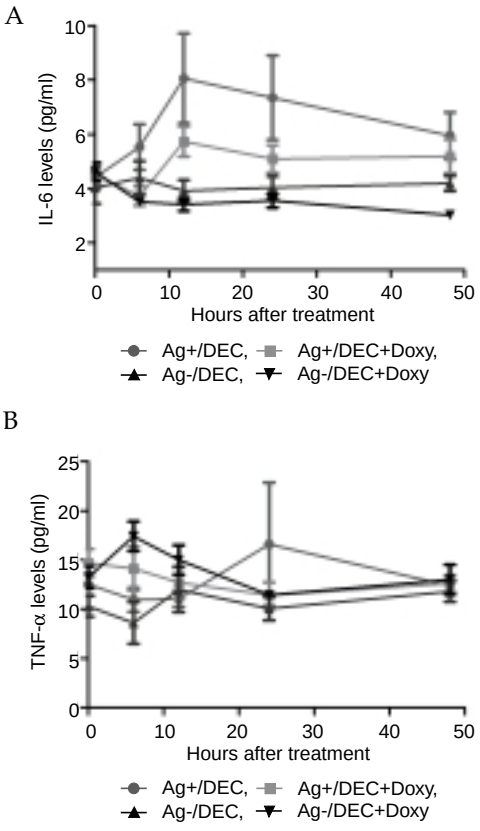
There was a strong correlation between baseline microfilarial levels and reaction scores in the placebo group (Spearman's correlation = 0.77; $p = 0.0002$) (Fig 3A). In the doxycycline group, a significant correlation between baseline microfilarial levels and reaction scores was not found (Spearman's correlation = 0.20; $p = 0.55$) (Fig 3B). These results show that after the *Wolbachia* in the microfilaria was decreased by doxycycline treatment, the severity of adverse reactions did not correlate with the antigen level in the circulation, suggesting *Wolbachia* may have contributed to the adverse reactions.

Determination of proinflammatory and anti-inflammatory cytokines

The levels of IL-6 increased significantly by 12 (p=0.044) and 24 hours (p=0.039) after treatment. While IL-6 levels correlated with severity of adverse reactions, TNF-α was elevated only in patients with severe adverse reactions (data not shown). The levels of IL-10 increased significantly in patients with moderate and severe adverse reactions by 12 hours after treatment and remained high for 24 hours (data not shown). IL-6 levels were significantly lower in the doxycycline group ($p = 0.04$)(Fig 4A), but no significant difference in TNF-α levels was seen between the doxycycline and placebo groups ($p = 0.37$) (Fig 4B).

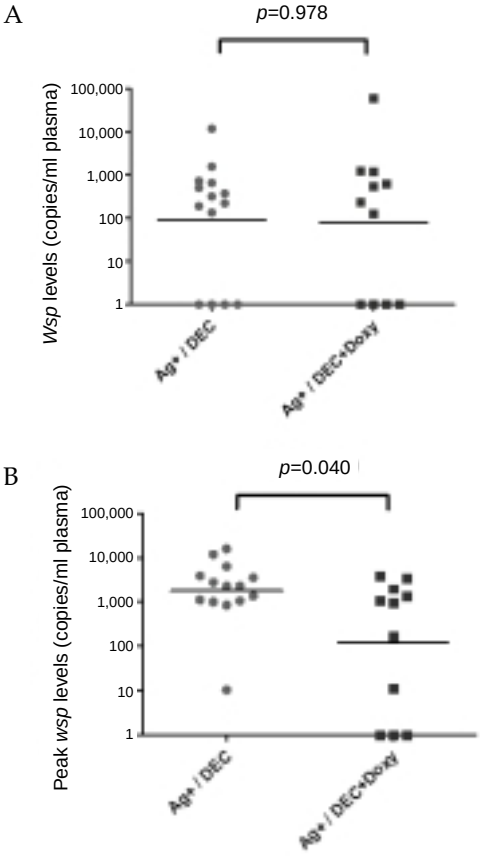
Determination of *Wolbachia* DNA levels in plasma by quantitative PCR

Wolbachia DNA was detectable in the serum at baseline in 3 patients in each group (Fig 5A). Within 48 hours of treatment, *Wolbachia* DNA was detectable in the serum of all patients treated with DEC and placebo and in 8 of 11 patients treated with DEC and doxycycline (Fig 5B). The baseline *Wolbachia* DNA levels in the patients treated with DEC and placebo and DEC and doxycycline were not significantly different from eachother ($p = 0.978$; Mann-Whitney test)(Fig 5A). However, after treatment, the levels of *Wolbachia* DNA in the plasma of patients treated with DEC and doxycycline were significantly lower than those treated with DEC and placebo ($p = 0.040$; Mann-Whitney test) (Fig 5B). The levels of *Wolbachia* DNA were significantly lower in the DEC and doxycycline treated group than in the DEC and placebo treated group at 12 and 24 hours post treatment (p=0.022 and p=0.015,



DEC, diethylcarbamazine; Ag^+, microfilaria antigen positive; Ag^-, microfilaria antigen negative; Doxy, doxycycline; TNF-α, tumor necrosis factor alpha

Fig 4–Plasma levels of interleukin-6 (A) and tumor necrosis factor-α (B) at various times after treatment with DEC and placebo or DEC combination and doxycycline.



Ag^+, microfilaria antigen positive; Ag^-, microfilaria antigen negative; Doxy, doxycycline; Wsp, *Wolbachia* surface protein

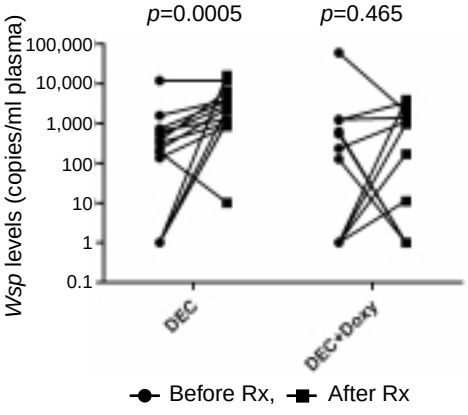
Fig 5–Baseline plasma levels of *wsp* before treatment (A), and after treatment (B) with DEC and placebo or DEC and doxycycline.

respectively), but were not significantly different at 6 and 48 hours after treatment ($p = 0.669$, and $p = 0.095$, respectively)(data not shown).

After treatment with DEC and placebo, the *Wolbachia* DNA levels increased significantly above baseline ($p=0.0005$; Wilcoxon matched pairs test), but did not increase significantly in the DEC and doxycycline treatment group ($p=0.465$; Wilcoxon matched pairs test) (Fig 6). These results suggest a single dose of 200 mg doxycycline can reduce *Wolbachia* levels in plasma.

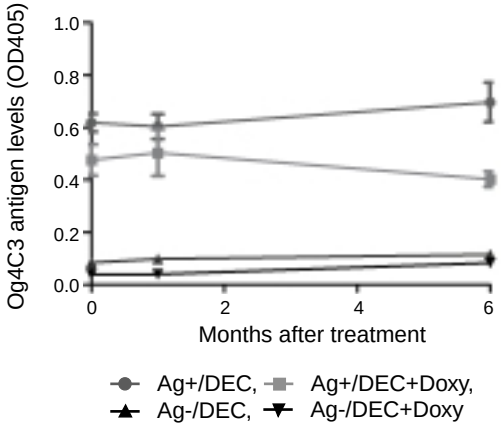
Determination of microfilaricidal effects

To assess the microfilaricidal effects of doxycycline in combination with DEC, night blood samples were collected from participants at 24 and 48 hours after treatment. Randomization was performed by



DEC, diethyl carbomizine; Doxy, doxycycline; Wsp, *Wolbachia* surface protein; Rx, treatment

Fig 6–Comparison of *wsp* plasma levels before and after treatment with DEC and placebo of DEC and doxycycline.



Ag^+, microfilaria antigen positive; Ag^-, microfilaria antigen negative; DEC, diethylcarbamazine; Doxy, doxycycline

Fig 7–Macrofilaricidal effects of doxycycline treatment in patients with bancroftian filariasis.

random allocation after screening in the field, but before the microfilarial count in the laboratory. Therefore, the microfilarial loads before treatments were blinded, resulting in the different levels of baseline microfilaria. Because DEC has an excellent microfilaricidal effect, the level of microfilaria decreased rapidly after treatment in both treatment groups (Table 3). There was a significant reduction in microfilaria in the DEC and placebo group at 24 hours (p = 0.0002; Mann-Whitney test), with a decrease in the mean microfilarial count to 1% of the baseline level and amicrofilaremia occurring in 88.2% of patients. Forty-eight hours after DEC and placebo treatment, only one patient still had microfilaria in the circulation (Table 3). The mean microfilaria level was still significantly lower than the baseline level (0.7% of the baseline level)(p=00002). In the DEC and doxycycline group, the microfilariae level was decreased to 0 within 24 hours of treatment (Table 3).

Determination of macrofilaricidal effects

To assess the macrofilaricidal effects of doxycycline in combination with DEC, adult *W. bancrofti* antigen levels were measured by Og4C3 ELISA. The filarial antigen levels were significantly lower in the DEC and doxycycline group than in the DEC and placebo group 6 months after treatment (p = 0.063) (Fig 7). Circulating filarial antigens did not significantly differ between the DEC and doxycycline and DEC and placebo groups at baseline and after one month (p=0.052, and p=0.328, respectively). However, 6 months after treatment, the filarial antigens were significantly lower in the doxycycline group (p=0.029), while filarial antigen levels had begun to increase in the placebo group (Fig 7).

DISCUSSION

The adverse reactions following DEC treatment are believed to be immune-mediated. However, the mechanism of the adverse reactions has not been clearly elucidated. Similar to other studies (Haarbrink *et al*, 1999a; Keiser *et al*, 2003),

Table 3
Microfilaricidal effects of doxycycline treatment.

	Groups	*N*	0 h		24 h		48 h	
			No. of positive (%)	Mean mf count (range)	No. of positive (%)	Mean mf count (range)	No. of positive (%)	Mean mf count (range)
Ag+	DEC	17	10(58.8%)	450 (0-2,133)	2(11.8%)	4.5 (0-33)	1(5.9%)	3.0 (0-33)
	DEC/Doxy	11	5(45.5%)	50 (0-450)	0	0	0	0
Ag-	DEC	8	0	0	0	0	0	0
	DEC/Doxy	8	0	0	0	0	0	0

h, hour(s); Ag^+, microfilaria antigen positive; Ag^-, microfilaria antigen negative; DEC, diethylcarbamazine; Doxy, doxycycline

we found the clinical symptoms after DEC treatment occurred 6 hours after treatment, reaching a peak at 12-24 hours, correlating with the onset of microfilarial killing in the circulation. The severity of adverse reactions after DEC treatment correlated with baseline microfilarial levels (Fig 3A). This finding is similar to previous studies (Moulia-Pelat *et al*, 1994; Gopinath *et al*, 2000) and supports the hypothesis adverse reactions are induced by the sudden release of antigens from the dead microfilariae.

Similar to previous studies (Haarbrink *et al*, 1999b, 2000), IL-6 levels increased after treatment and correlated with the severity of adverse reactions (data not shown). IL-10 was also found to be elevated in the patients with severe adverse reactions. A high IL-10/TNF-α ratio has been reported to be associated with poor outcomes in septic patients (Gogos *et al*, 2000) and in febrile hospitalized patients (van Dissel *et al*, 1998). In this study we found a correlation between a high IL-10/TNF-α ratio and severe adverse reactions. A high IL-6/IL-10 ratio was also found in patients with severe reactions (data not shown). These results indicate counter-regulatory mechanisms between pro-inflammatory and anti-inflammatory cytokine pathways are probably involved in the occurance of adverse drug reactions.

The discovery of *Wolbachia* bacteria in filarial nematodes raises a number of intriguing questions. It has been hypothesized that *Wolbachia* is associated with DEC-induced adverse reactions in lymphatic filariasis. Therefore, *Wolbachia* removal from filarial parasites using doxycycline should reduce adverse reactions after DEC treatment.

Treatment with doxycycline at 200 mg daily for 3-8 weeks results in reduction of inflarmmatory cytokines, *Wolbachia* DNA levels, microfilariae levels, and adverse reactions (Keiser *et al*, 2002; Turner *et al*, 2006). However, long-term use of antibiotics is not practical for mass treatment and may cause drug resistance. Single dose doxycycline has been used in the treatment of rickettsial infections (Huys *et al*, 1973; Sirisanthana *et al*, 1994). According to pharmacokinetic studies, peak serum concentrations of doxycycline are reached within 1-2 hours, while DEC is slowly absorbed orally, taking 2-3 hours to reach a peak concentration (Agwuh and MacGowan, 2006; Gschwend *et al*,

2007). The elimination half-life of doxycycline is longer than DEC (16-25 hours for doxycycline and 11-14 hours for DEC) (Bolla *et al*, 2002; Agwuh and MacGowan, 2006). We hypothesized, after administration of doxycycline in combination with DEC, doxycycline would deplete the *Wolbachia* in the parasites, resulting in lower levels of *Wolbachia* released into the plasma after the parasite is killed by DEC.

In this study, we demonstrated doxycycline treatment can reduce the incidence and severity of adverse reactions after DEC treatment. Similar to previous studies using a 3-week course of doxycycline (Turner *et al*, 2006), mild adverse reactions occurred more commonly in the DEC and doxycycline group, while moderate and severe adverse reactions were fewer in the doxycycline group. These findings suggest doxycycline can prevent or improve severe adverse reactions due to antifilarial treatment.

Few studies have investigated the quantification of *Wolbachia* in plasma samples after doxycycline treatment (Keiser *et al*, 2002). Our study demonstrated DNA from *Wolbachia* can be detected in the plasma of patients after treatment with either DEC alone or DEC in combination with doxycycline. Doxycycline treatment is associated with lower levels of detectable *Wolbachia* DNA and proinflammatory cytokines in plasma. These data imply a single dose of doxycycline is sufficient to decrease *Wolbachia* in parasites, reducing *Wolbachia* levels in plasma, leading to milder adverse reactions. This study also supported the role of *Wolbachia* bacterial products in the release of inflammatory cytokines associated with adverse reactions, and the pathology of lymphatic filariasis. The further studies of *Wolbachia* may help to explain the causes of inflammation associated with adverse reactions.

Although we observed suppression of microfilaremia and a decrease in adult filarial antigens after a single dose of doxycycline, our study showed a single dose of doxycycline is insufficient to kill adult worms. Filarial antigens were still positive in both groups after 6 months. However, doxycycline treatment significantly decreased filarial antigens 6 months after treatment, while DEC and placebo treatment induced reductions at 1 month, but these increased again by 6 month after treatment. The positive remaining filarial antigen indicates the persistence of living adult worms in the lymphatic system after treatment. This result emphasizes the need for repeat treatment of patients, and the needs for the development of macrofilaricidal drugs.

Our study showed a single dose of doxycycline is as effective as a 3-week course of doxycycline in reducing the incidence and severity of adverse reactions. Further studies with long-term follow-up are needed to confirm these micro- and macrofilaricidal effects. However, the use of a single dose of doxycycline in combination with DEC may have an advantage in a MDA program in endemic areas which are usually rural and difficult to access.

ACKNOWLEDGEMENTS

We are grateful for financial support from the National Research Council of Thailand, and the Graduate Thesis Grant from the Graduate School of Chulalongkorn University. VS was supported by the Thailand Research Fund through the Royal Golden Jubilee PhD Program (PHD/0169/2543). We would like to express our appreciation to Dr Parvapan Bhattarakosol, and Dr Kanitha Patarakul for their helpful advice and suggestions. We are thank-

ful to the health personnel from the Vector Born Disease Control Center 9.3 (Mae Sot) Tak, Department of Disease Control, Ministry of Public Health, for the field study. We also appreciate Ms Alisa Junpee, Ms Pornpun Jaratsing, and the staff at the Lymphatic Filariasis Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, for their technical assistance with the field and laboratory work.

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