Doxycycline Treatment of Brugia malayi–Infected Persons Reduces Microfilaria and Adverse Reactions after Diethylcarbamazine and Albendazole Treatment

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Background. The efficacy of doxycycline for treating the causal agent of human lymphatic filariasis Brugia malayi is unknown. Standard treatment with diethylcarbamazine-albendazole is associated with adverse reactions. We assessed whether doxycycline alone or in combination with diethylcarbamazine-albendazole would lead to sustained amicrofilariaemia and reduced incidence of adverse reactions.

Methods. A double-blind, randomized, placebo-controlled 6-week field trial of doxycycline treatment (100 mg/day) of 161 persons infected with B. malayi was conducted. Four months after receiving doxycycline (n = 119) or placebo (n = 42), participants received diethylcarbamazine (6 mg/kg) plus albendazole (400 mg) or a matching placebo. Adverse reactions were assessed 48 and 60 h after administration of diethylcarbamazine-albendazole. Treatment efficacy was evaluated at 2, 4, and 12 months after the initial doxycycline treatment.

Results. Four months after beginning doxycycline treatment, Wolbachia loads were reduced by 98%. Doxycycline treatment reduced the prevalence of microfilaria at 2, 4, and 12 months of follow-up (P < .001 for all time points). At the 1-year follow-up, prevalence was reduced by 77% and 87.5% in patients receiving doxycycline alone or doxycycline plus diethylcarbamazine-albendazole, respectively. In contrast, the reduction of microfilaria in the group receiving placebo doxycycline plus diethylcarbamazine-albendazole was merely 26.7%. Adverse reactions were lowest in the group receiving doxycycline plus placebo diethylcarbamazine-albendazole and highest in the group receiving placebo doxycycline plus diethylcarbamazine-albendazole. The proportion of persons with high fever and severe adverse reactions was significantly reduced in the group treated with doxycycline plus diethylcarbamazine-albendazole.

Conclusions. A 6-week course of doxycycline, either alone or in combination with diethylcarbamazine-albendazole, leads to a decrease in microfilaria and reduces adverse reactions to antifilaria treatment in B. malayi–infected persons.

Brugia and Wucheria species cause lymphatic filariasis which affects 120 million people worldwide [1]. Filarial disease is associated with episodes of acute and chronic inflammation that can lead to conditions such as elephantiasis and hydrocele. The global lymphatic filariasis elimination program has 2 aims: (1) to reduce microfilaria, by using filaricida drugs, to levels that are too low to sustain transmission of filaria parasites in humans and (2) to reduce morbidity associated with chronic filaria disease [1]. Current strategies involve the community-based single oral treatment with combination of diethylcarbamazine or ivermectin and albendazole, which needs to be repeated annually or semiannually over many years to reduce transmission [2, 3]. Additional control strategies are needed, because the currently used drugs show only partial activity against adult parasites [4].

The discovery of Wolbachia in filaria species has provided a new target for chemotherapy of human filariasi.
Wolbachia are susceptible to the tetracycline group of antibiotics [6, 7]. Studies in animal models have shown that treatment of infected animals with tetracycline decreases microfilarial load, inhibits development of larval worms, and renders adult female worms infertile [8, 9]. In human onchocerciasis, treatment with doxycycline (100 mg/day) for 6 weeks depleted the bacteria and led to long-term sterility of adult female Onchocerca volvulus [10]. Similarly, in bancroftian filariasis a study revealed that treatment of microfilaricemic patients with doxycycline (200 mg/day) led to a 96% reduction in Wolbachia DNA levels. Twelve months after the commencement of doxycycline therapy, there was an almost complete absence of microfilaria -emia [11, 12]. Recently, it was established that administration of doxycycline (200 mg/day) for 8 weeks [13] or 6 weeks [14] resulted in a strong macrofilaricidal activity.

Given the success of doxycycline treatment in depleting microfilaria in bancroftian filariasis as well as onchocerciasis, it is essential to determine whether doxycycline in combination with diethylcarbamazine-albendazole leads to sustained microfilaria emia in brugian filariasis because almost one-half of persons affected by filariasis in Southeast Asia are infected with Brugia malayi or Brugia timori [15]. Moreover, diethylcarbamazine treatment is poorly accepted in affected communities because of adverse reactions of microfilaria-positiv subjects to this drug [16]. There is evidence that adverse reactions to diethylcarbamazine coincide with the release of Wolbachia into the bloodstream [17]. It is therefore important to assess the effect of doxycycline on adverse reactions associated with diethylcarbamazine-albendazole treatment. In the current study, we aimed to assess the effect of targeting Wolbachia in brugian filariasis as proof of principle that targeting the endosymbiont of Brugia species is a valid strategy for discovering new therapeutic candidates, with a high rate of community acceptance, to be applied to an important, neglected disease.

**MATERIALS AND METHODS**

This European Union–funded collaborative study of European and Indonesian institutions was approved by the Commission of Medical Ethics of the University of Indonesia (Jakarta, Indonesia) and for European centers by the Research Ethics Committee of the Liverpool School of Tropical Medicine (Liverpool, United Kingdom). The trial registration number is ISRTN 37962059.

**Objectives.** The primary objective was to assess whether a 6-week course of doxycycline treatment was effective in reducing Wolbachia loads in Brugia-infected persons and, when given in combination with diethylcarbamazine plus albendazole, whether treatment would result in sustained microfilaria emia in subjects with brugian filariasis. The secondary objective was to reduce adverse reactions to standard antifilarial drugs.

**Study site and enrollment.** The study was performed from March 2002 through August 2003 in Parigi-Moutong, Central Sulawesi, and in Bonebolango, Northern Sulawesi, areas of Indonesia where B. malayi is endemic. Before the trial, the prevalences of microfilaria in the villages in Parigi-Moutong and Bonebolango were 24% and 38%, respectively. The inhabitants were informed of the purpose of the study. Informed consent was obtained from all persons (or parents) before the clinical and parasitological study and blood withdrawal in accordance with the guidelines of Indonesian Department of Health and Human Services. Eligibility requirements for participation were as follows: age range, 12–76 years; body weight, ≥40 kg; microfilaria load, >5 microfilaria/mL, and good health without any clinical condition requiring long-term use of medications.

The study included 161 participants with microfilaria emia. The median age for men was 29 years (range, 12–76 years) and for women was 28.5 years (range, 12–70 years). Exclusion criteria for all participants were abnormal hepatic and renal profile (alanine aminotransferase level, >30 U/L; γ-glutamyl transpeptidase level, >28 U/L; creatinine level, >1.2 mg/dL) measured by dipstick chemistry (Reflot on; Roche Diagnostics), and additional exclusion criteria for women included pregnancy or breast-feeding.

**Interventions and randomization.** Doxycycline (100 mg) and matched placebo capsules were provided by Pfizer. Diethylcarbamazine, albendazole, and placebo tablets were purchased from Indo Farma Pharmaceutical Company.

To prevent mix-up of the drugs and matching placebos within members of the same family, the participants were assigned to groups by cluster randomization. The drugs and placebo capsules were coded with random numbers, which were concealed from investigators and patients. Groups I and II received 1 capsule of doxycycline (100 mg) per day for 6 weeks; group III received 1 capsule of matching placebo per day for 6 weeks (fig 1). Drugs were taken under supervision of the medical team, and adverse reactions were monitored and recorded in a questionnaire completed by medical staff. During these 6-week courses of doxycycline treatment, the patients were visited by primary health care medical staff who were unaware of treatment allocation daily.

Four months after the start of treatment, groups II and III received a single dose of diethylcarbamazine (6 mg/kg) plus albendazole (400 mg), whereas group I received placebo instead of diethylcarbamazine and albendazole. For diethylcarbamazine-albendazole and placebo diethylcarbamazine-albendazole treatment, all patients were admitted to a primary health center for 3 days, which allowed adverse reactions to be monitored closely and frequently (twice daily) and for appropriate care to be provided to the patients. Tablets were swallowed under supervision after a meal. Heart rate, blood pressure, and body
Figure 1. Design of a study of doxycycline treatment plus treatment with diethylcarbamazine-albendazole among persons infected with Brugia malayi.
The calculation of sample size was based on the mean microfilarial density in a pilot study, reduction in microfilarial density by 50% after 4 months (power, 80%; α = 0.05), and a 15% dropout rate throughout the trial period. For assessment of the potential reduction in adverse reactions to treatment with diethylcarbamazine-albendazole, a group size of 13 persons per treatment allocation was derived from power calculations based on a pilot study, assuming that 50% of persons in the control group have proinflammatory cytokine levels, compared with none of the doxycycline-treated persons (power, 80%; α = 0.05).

**Outcomes.** The primary outcome measurements were the quantity of *Wolbachia* single-copy genes per microfilaria (as a surrogate for numbers of whole bacteria per microfilaria) 4 months after the start of treatment and the quantity of circulating microfilaria at 2, 4, and 12 months after the start of treatment. The numbers of *Wolbachia* per microfilaria and the microfilarial level were determined by collecting 5 mL of night blood (obtained between 8:00 and 10:00 p.m.) in an EDTA-containing tube. One mL of blood was filtered through a 5-μm-pore filter (Millipore) and stained with Giemsa to quantify microfilariae. The filtration and counting of the microfilaria was performed by the same person on all occasions. The plasma from the rest of the blood was collected by centrifugation and stored at −20°C for serological assays. The remaining blood pellet was diluted with 5 mL of distilled water and filtered to retain microfilaria for quantification of *Wolbachia* DNA.

*Wolbachia* content was quantified before doxycycline treatment and at 2 and 4 months after doxycycline treatment. Genomic DNA was extracted from microfilaria collected from blood specimens on filter with the QIAamp DNA kit (Qiagen). The single-copy *ftsZ* gene of *Wolbachia* was quantified by real-time PCR, using 1× HotStar Taq polymerase buffer (Qiagen), 3.75 mmol/L MgCl₂, 200 μmol/L dNTPs, 300 nmol/L each of forward (5′-CGATGAGATTATGGGACATATAA-3′) and reverse (5′-TTGCAATTACTGGTGCTGC-3′) primers, 50 nmol/L hybridization probe (5′-[Fam]-CAGGGATGGGTGGTCGTA-3′), 2.5 U of HotStar Taq (Qiagen), and 2 μL of DNA in a 20-μL total reaction volume. Amplification took place in a Rotorgene 3000 (Corbett Research) under the following conditions: 1 cycle of 15 min at 95°C, followed by 40 cycles of 94°C for 15 s, 58°C for 30 s, and 72°C for 30 s, with fluorescence monitored on the FAM channel. Copy numbers of *ftsZ* were calculated from a standard curve of a serially diluted plasmid containing *ftsZ* and then divided by microfilariae per microliter.

Blood samples were collected at the following time points: before doxycycline treatment; 2 months after the start of doxycycline treatment; 4 months after the start of doxycycline treatment or just before diethylcarbamazine-albendazole treatment; 3 days and 7 days after diethylcarbamazine-albendazole treatment; and 1 year after the start of doxycycline treatment.

The secondary outcome measurement was the clinical assessment of adverse reactions following antifilaria treatment. Symptoms were monitored during the 3 days after administration of diethylcarbamazine-albendazole. The scoring of symptoms was as reported elsewhere [18]. Briefly, 0 points were awarded for the absence of symptoms and a body temperature ≤37.4°C, 1 point each for the presence of arthralgia, dizziness, lymph node enlargement, or myalgia; and 2 points for a headache. Body temperatures measuring 37.5°–38.5°C were awarded 5 points, and temperatures >38.5°C were awarded 10 points.

**Table 1. Microfilaraemia before and after treatment with doxycycline (DOX) and/or diethylcarbamazine-albendazole (DEC).**

<table>
<thead>
<tr>
<th>Group, parameter</th>
<th>Before DOX</th>
<th>2 months after DOX</th>
<th>4 months after DOX</th>
<th>3 days after DEC</th>
<th>7 days after DEC</th>
<th>1 year after DEC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DOX plus placebo DEC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients (no. female/no. male)</td>
<td>54 (27/27)</td>
<td>53 (26/27)</td>
<td>54 (27/27)</td>
<td>54 (27/27)</td>
<td>54 (27/27)</td>
<td>48 (26/22)</td>
</tr>
<tr>
<td>Microfilaria, geometric mean count (95% CI)</td>
<td>328 (225–477)</td>
<td>271 (159–459)</td>
<td>37 (20–459)</td>
<td>27 (15–49)</td>
<td>24 (13–43)</td>
<td>2 (1–2)</td>
</tr>
<tr>
<td>Proportion of patients with microfilaria (%)</td>
<td>54/64 (100)</td>
<td>51/63 (96.2)</td>
<td>43/54 (79.6)</td>
<td>41/54 (75.9)</td>
<td>42/54 (77.8)</td>
<td>11/48 (22.9)</td>
</tr>
<tr>
<td><strong>DOX plus DEC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients (no. female/no. male)</td>
<td>57 (21/36)</td>
<td>56 (21/35)</td>
<td>57 (21/36)</td>
<td>57 (21/36)</td>
<td>56 (21/35)</td>
<td>54 (21/33)</td>
</tr>
<tr>
<td>Microfilaria, geometric mean count (95% CI)</td>
<td>506 (373–867)</td>
<td>455 (256–808)</td>
<td>89 (51–158)</td>
<td>12 (8–19)</td>
<td>13 (8–19)</td>
<td>1 (1–1)</td>
</tr>
<tr>
<td>Proportion of patients with microfilaria (%)</td>
<td>42/42 (100)</td>
<td>37/41 (90.2)</td>
<td>39/42 (92.9)</td>
<td>36/42 (85.7)</td>
<td>35/42 (83.3)</td>
<td>22/30 (73.3)</td>
</tr>
<tr>
<td><strong>Placebo DOX plus DEC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients (no. female/no. male)</td>
<td>42 (21/21)</td>
<td>37 (20/17)</td>
<td>42 (21/21)</td>
<td>42 (21/21)</td>
<td>39 (19/20)</td>
<td>30 (13/17)</td>
</tr>
<tr>
<td>Microfilaria, geometric mean count (95% CI)</td>
<td>373 (247–562)</td>
<td>403 (246–660)</td>
<td>227 (140–369)</td>
<td>20 (11–30)</td>
<td>39 (23–65)</td>
<td>13 (6–30)</td>
</tr>
<tr>
<td>Proportion of patients with microfilaria (%)</td>
<td>42/42 (100)</td>
<td>37/37 (100)</td>
<td>42/42 (100)</td>
<td>38/42 (90.5)</td>
<td>39/39 (100)</td>
<td>22/30 (73.3)</td>
</tr>
</tbody>
</table>

* P < 0.01 for comparison of microfilaria count between treatment groups using Kruskal-Wallis nonparametric analyses of variance.

a P < 0.01, compared with predoxycycline value (Wilcoxon signed rank test).

b P < 0.05, compared with predoxycycline value (Wilcoxon signed rank test).

c P < 0.05, compared with predoxycycline value (Wilcoxon signed rank test).

d P < 0.01, compared with predoxycycline value (Wilcoxon signed rank test).
Adverse reactions were classified into 3 categories exactly as described elsewhere [18].

**Statistical analysis.** Statistical analysis was performed in SPSS for Windows, version 11 (SPSS). Differences between variables for 3 groups were assessed by use of the Kruskal-Wallis test, whereas the Mann-Whitney U test was used for comparing differences between 2 groups. A nonparametric, repeated-measure trend test was performed to assess the effect of treatment over time. Specifically, measurements were ranked within individuals, followed by a repeated-measurement trend test. Differences in the variables before and after the treatment of the same individual were compared using the Wilcoxon matched-pairs rank test. Comparison of proportions was performed using the $\chi^2$ test.

**RESULTS**

**Adverse reactions to doxycycline and diethylcarbamazine-albendazole.** The 6-week course of 100 mg of doxycycline per day was generally well tolerated. The adverse reactions reported after doxycycline administration were mild and tolerable. None of the participants had to stop taking doxycycline because of adverse reactions. The adverse effects in the doxycycline group ($n = 119$) were myalgia ($n = 6$), nausea ($n = 4$), dizziness ($n = 5$), headache ($n = 3$), insomnia ($n = 2$), abdominal pain ($n = 2$), itching ($n = 2$), diarrhea ($n = 1$), rash ($n = 1$), malaise ($n = 1$), and drowsiness ($n = 1$); in the placebo group ($n = 42$), adverse effects were myalgia ($n = 2$), nausea ($n = 1$), dizziness ($n = 2$), insomnia ($n = 1$), and itching ($n = 1$). No report of photosensitivity reactions was recorded during or after the treatment.

The most common adverse reactions to diethylcarbamazine-albendazole were fever, headache, arthralgia, dizziness, lymph node enlargement, or myalgia. Altogether, 51 of 99 participants receiving diethylcarbamazine-albendazole and 8 of 62 receiving placebo diethylcarbamazine-albendazole experienced moderate or severe adverse reactions.

**Reduction of microfilaria count and prevalence of microfiliemia after doxycycline and diethylcarbamazine-albendazole treatment.** The microfilaria count before doxycycline treatment was the same in all 3 treatment groups. Four months after the start of doxycycline treatment, the microfilaria count in each group was reduced significantly compared with that before doxycycline treatment ($P < .001$) for all groups. The reduction in microfilaria count was highest in the groups treated with doxycycline and lowest in the group treated with placebo doxycycline (89%, 83%, and 39% for the doxycycline plus placebo diethylcarbamazine-albendazole group, the doxycycline plus diethylcarbamazine-albendazole group, and the placebo doxycycline plus diethylcarbamazine-albendazole group, respectively). During diethylcarbamazine-albendazole treatment, there was a strong reduction in microfilaria counts in both groups.
placebo doxycycline and doxycycline groups, and at 7 days after the administration of diethylcarbamazine-albendazole, the levels of microfilaria in those 2 groups were comparable. However, after 1 year, the group receiving doxycycline plus diethylcarbamazine-albendazole had significantly reduced levels of microfilaria compared with placebo doxycycline plus diethylcarbamazine-albendazole recipients.

Interestingly, at 1 year after doxycycline treatment the doxycycline plus placebo diethylcarbamazine-albendazole group had a significantly lower microfilaria count than did the group receiving placebo doxycycline plus diethylcarbamazine-albendazole (table 1 and figure 2). The reduction in prevalence of microfilaria 1 year after doxycycline treatment was 77% and 87.5% in the doxycycline plus placebo diethylcarbamazine-albendazole and the doxycycline plus diethylcarbamazine-albendazole groups, respectively; in contrast, the reduction in the placebo doxycycline plus diethylcarbamazine-albendazole group was only 26.7% (table 1). One year after initiation of doxycycline or placebo doxycycline treatment, both doxycycline plus placebo diethylcarbamazine-albendazole and doxycycline plus diethylcarbamazine-albendazole groups had lower numbers of microfilaria-positive participants and lower microfilaria density than did the placebo doxycycline plus diethylcarbamazine-albendazole group. The prevalence of microfilaria emia as well as the microfilaria count in the doxycycline plus diethylcarbamazine-albendazole group was lower than in the doxycycline plus placebo diethylcarbamazine-albendazole group, but this did not reach statistical significance (P = .09 for both prevalence of microfilaria emia and microfilaria count).

Reduction of Wolbachia levels after doxycycline treatment.

DNA samples from microfilaria trapped on filter were analyzed to determine Wolbachia ftsZ gene copy numbers per microfilaria. There were no differences in the gene copy numbers between the 3 treatment groups at the start of the trial.

The gene copy numbers of microfilaria-positive ftsZ-positive participants were reduced by 94% in both doxycycline groups at 2 months (P < .001 for both groups), compared with before doxycycline treatment. At this time point, proportions of patients with no microfilaria and no ftsZ copies were 5% and 7% in the doxycycline plus placebo diethylcarbamazine-albendazole and doxycycline plus diethylcarbamazine-albendazole groups, respectively.

At 4 months after the onset of treatment, ftsZ copy numbers were reduced by 98% in both doxycycline groups compared with levels before doxycycline treatment. At this time point, we also found a slight reduction in ftsZ copy numbers in the placebo doxycycline group (P = .28), compared with before doxycycline treatment (table 2).

Body temperature and adverse reactions after diethylcarbamazine-albendazole treatment.

Body temperatures and adverse reaction scores were lowest in the group that received doxycycline and placebo diethylcarbamazine-albendazole. In the doxycycline plus placebo diethylcarbamazine-albendazole group, no one had severe adverse reactions or a body temperature >38°C. The effect of doxycycline on adverse reactions to diethylcarbamazine-albendazole was clear from the significantly lower body temperatures and adverse reactions in doxycycline plus diethylcarbamazine-albendazole recipients, compared with those in placebo doxycycline plus diethylcarbamazine-albendazole recipients (figure 3). The percentage of persons with body temperature >38°C or severe adverse reactions was significantly higher in the placebo doxycycline plus diethylcarbamazine-albendazole group (26.3% and 42.9%, respectively), compared with the doxycycline plus diethylcarbamazine-alben-

### Table 2. Effect of doxycycline treatment on Wolbachia ftsZ gene copies per microfilaria.

<table>
<thead>
<tr>
<th>Treatment group, time (months)</th>
<th>Proportion of patients (%)</th>
<th>ftsZ gene, median copies per microfilaria (10th–90th percentile)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline plus placebo diethylcarbamazine-albendazole&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60/60 (100)</td>
<td>204 (14–1278)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>2</td>
<td>58/61 (95)</td>
<td>12 (3–73)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4</td>
<td>40/61 (66)</td>
<td>5 (1–60)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Doxycycline plus diethylcarbamazine-albendazole&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55/55 (100)</td>
<td>226 (64–1874)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>2</td>
<td>52/56 (93)</td>
<td>13 (3–102)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4</td>
<td>42/57 (74)</td>
<td>5 (1–437)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Placebo doxycycline plus diethylcarbamazine-albendazole&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39/39 (100)</td>
<td>470 (41–1723)</td>
<td>.4</td>
</tr>
<tr>
<td>2</td>
<td>35/35 (100)</td>
<td>599 (79–1420)</td>
<td>.02</td>
</tr>
<tr>
<td>4</td>
<td>41/41 (100)</td>
<td>312 (21–823)</td>
<td>.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> Changes from baseline in Wolbachia ftsZ gene copy numbers per microfilaria.
<sup>b</sup> P < .001, assessed by nonparametric repeated measure trend test. **P** = .28, assessed by nonparametric repeated measure trend test.
Body temperature (A) and adverse reactions (B) following diethylcarbamazine-albendazole treatment in groups treated with doxycycline plus placebo diethylcarbamazine-albendazole (DOX & pl DEC), doxycycline plus diethylcarbamazine-albendazole (DOX & DEC), and placebo doxycycline plus diethylcarbamazine-albendazole (pl DOX & DEC). Each mark represents 1 person; horizontal lines represent medians. *Differences between body temperature and adverse reactions in treatment groups were assessed using Kruskal-Wallis nonparametric analysis of variance.

dazole group (10.5% and 15.8%, respectively; P<.05) (table 3).

DISCUSSION

Doxycycline treatment depleted Wolbachia from B. malayi in infected patients; this is in agreement with studies of bancroftian filariasis [11–13]. Levels of Wolbachia measured before doxycycline treatment, as determined by copy numbers of the ftsZ gene per microfilaria (median, 300 copies/microfilaria) agree well with copy numbers determined for the wsp gene in a laboratory strain of B. malayi [19]. After doxycycline treatment, the Wolbachia load per microfilaria was reduced by >1 log, a 94% reduction compared with pre-doxycycline levels. Four months after treatment, the Wolbachia load was reduced by 2 logs, representing a 98% reduction compared with pre-doxycycline levels.

Our results have established that a 6-week course of 100 mg doxycycline per day leads to a strong reduction in microfilariaemia in B. malayi–infected subjects. Moreover, diethylcarbamazine-albendazole without doxycycline induced a significant reduction in microfilariaemia; however, only 27% of subjects were microfilariaemic 1 year after treatment, compared with much higher rates of microfilariaemia in the doxycycline plus diethylcarbamazine-albendazole (87.5%) or the doxycycline plus placebo diethylcarbamazine-albendazole (77%) groups. Here, we have also shown that microfilaria count as well as prevalence of microfilariaemia at 1 year after treatment was similar in participants receiving doxycycline plus diethylcarbamazine-albendazole and in those receiving doxycycline plus placebo diethylcarbamazine-albendazole, indicating that doxycycline by itself was highly effective in reducing B. malayi levels. Together, these results imply that a 6-week course of 100-mg/day doxycycline alone is sufficient to deplete Wolbachia from filaria worms and to lead to almost complete microfilariaemia at 1 year after treatment. Although the measurement of adult worm burden is not possible for brugian filariasis (unlike bancroftian filariasis [20, 21], the fact that doxycycline leads to a strong long-term (12-month) reduction in microfiliariaemia (in contrast to 1 dose of diethylcarbamazine-albendazole) suggests that doxycycline either leads to long-term sterilization of adult female Brugia or has substantial macrofilaricidal effects. A strong macrofilaricidal effect has been shown in humans infected with W. bancrofti [13, 14] as well as in gerbils infected with Brugia pahangi [8, 9].

We also observed a small but significant reduction in microfilaria counts at 4 months in the placebo doxycycline group, but the microfilariaemia prevalence remained at 100%. The levels of microfilariaemia have been shown to fluctuate seasonally [22]. Pretreatment samples had been collected at the end of the rainy season, whereas the samples before diethylcarbamazine-albendazole treatment were collected in the dry season; thus, seasonality may account for the slight reduction in the microfilaria density at 4 months after treatment in the placebo doxycycline group. The seasonal fluctuation may also account for the significant reduction in the Wolbachia load seen in the placebo doxycycline group 4 months after the start of treatment.

Diethylcarbamazine treatment is known to induce local and systemic adverse reactions [18]. The adverse reactions are thought to be caused by the rapid release of microfilarial material and Wolbachia into the bloodstream [3, 17]. Because body temperature is a continuous variable and a major adverse reaction to diethylcarbamazine, we also analyzed it separately. In contrast to the placebo diethylcarbamazine-albendazole group,
we observed higher body temperatures or adverse reactions in groups treated with diethylcarbamazine-albendazole. When the 2 groups treated with diethylcarbamazine-albendazole were compared, we found ~1.5 fold higher percentage of persons with body temperatures <37.5°C or mild adverse reactions in the group treated with doxycycline before diethylcarbamazine-albendazole than in the group who did not receive doxycycline. Moreover, we found that the percentages of persons experiencing body temperatures >38.5°C or severe adverse reactions were 2 to 3 times lower when doxycycline was received prior to diethylcarbamazine-albendazole treatment, suggesting that anti-Wolbachia treatment may prevent adverse effects of antifilaria treatment.

In conclusion, finding reported here support the notion that doxycycline, either alone or in combination with antifilaria therapy, strongly reduced Wolbachia levels in B. malayi–infected persons and, either alone or in combination with antifilaria therapy, led to a decrease in microfilaraemia that was sustained for at least 1 year after treatment. Second, doxycycline treatment reduced adverse reactions following diethylcarbamazine-albendazole treatment; this may be particularly important for patients with very high microfilaraemic loads, because they are known to most frequently experience adverse reactions to diethylcarbamazine.

The finding is important at 2 levels. At the individual level, doxycycline might be a suitable alternative for individual treatment and for those wishing to avoid adverse reactions to standard treatments. At the community level, although doxycycline is not appropriate for mass drug administration, our finding provide proof of principle that the endosymbiont of Brugia species is a valid target for new drug discovery and development [23, 24].

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### Potential conflict of interest

All authors: no conflicts

### References


