

## HUMAN LOIASIS IN A CAMEROONIAN VILLAGE: A DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSSOVER CLINICAL TRIAL OF A THREE-DAY ALBENDAZOLE REGIMEN

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**Abstract.** Because of the life-threatening, post-treatment reactions that have occurred in patients with loiasis treated with ivermectin, evaluation of a short-course albendazole regimen was undertaken in a *Loa*-endemic region of Cameroon. In a placebo-controlled, double-blinded, crossover study, 99 subjects with microfilaremia (100–3,3837/mL) were assigned to receive albendazole (400 mg; n = 48) or placebo (n = 51) for three days and were followed for 180 days; at day 180, the groups were crossed over and followed for an additional six months. In those initially receiving albendazole (ALB/PLAC), microfilarial levels decreased significantly by day 90 ( $P < 0.043$ ), but returned to baseline by day 180. In those receiving albendazole at day 180 (PLAC/ALB), microfilarial levels also decreased following albendazole ( $P = 0.005$ ). Blood eosinophil and antifilarial IgG levels did not change significantly for either group, although antifilarial IgG4 levels did in the ALB/PLAC group at day 180. Most subjects continued to have elevations in microfilaremia, suggesting that more intensive regimens of albendazole will be necessary to reduce *Loa* microfilaremia to levels safe enough to allow for ivermectin use.

### INTRODUCTION

Treatment of *Loa loa* infection remains problematic despite recent advances made in anthelmintic chemotherapy.<sup>1</sup> The current recommended drug of choice, diethylcarbamazine (DEC), is contraindicated in patients with elevated microfilaria (mf) levels because of its association with severe neurologic adverse effects.<sup>2,3</sup> Although the treatment of onchocerciasis was revolutionized following the discovery and use of ivermectin,<sup>4–8</sup> its administration to some patients with concomitant *Loa loa* infection has been associated with severe post-treatment effects, some resulting in fatalities.<sup>9–12</sup> Studies carried out by Gardon and others<sup>11</sup> and Boussinesq and others<sup>12</sup> have shown that these life-threatening reactions occurred in patients with extreme elevations of *L. loa* mf (>20,000/mm<sup>3</sup>). As a consequence, the use of ivermectin for onchocerciasis control has had to be re-examined with respect to areas where *L. loa* and *Onchocerca volvulus* are co-endemic.

Albendazole, a well-tolerated benzimidazole used primarily in the treatment of intestinal nematodes and some cestodes,<sup>13,14</sup> has also been shown to be partly efficacious in loiasis when used for three weeks at doses of 200 mg twice a day.<sup>15,16</sup> Indeed, this dose reduced microfilaremia significantly with few side effects and with kinetics suggestive of purely macrofilaricidal activity. Because the administration of a shorter regimen could have an enormous potential practical advantage over the 21-day course, we performed a double-blind, placebo-controlled trial to compare both the efficacy and the tolerance of a short-course albendazole regimen (400 mg for three days) with the objective of finding a way to reduce *L. loa* microfilaremia to levels at which subsequent ivermectin administration would be safe. Studies being performed concomitantly<sup>17</sup> with the present study indicated that a single 600-mg dose of albendazole when given with a fatty meal was able to reduce *L. loa* mf loads at 10 months by approximately 64%.

### MATERIALS AND METHODS

**Subjects and study design.** All subjects were residents of Ngali II, a village situated 24 km from Yaounde, the capital of Cameroon. Ninety-nine men and women who consented to participate were selected on the basis of having *L. loa* mf in peripheral blood collected at midday. Subjects were randomly assigned to one of two treatment groups. Medication was administered by and directly observed by the study team. Albendazole (donated by SKB International, Brentford, United Kingdom) was given at a dose of 400 mg once a day for three consecutive days to one group (ALB/PLAC) (n = 48). The second group (PLAC/ALB) (n = 51) received a similarly designed placebo (also provided by SKB International) once a day for three days. The demographics of both groups are shown in Table 1. At the end of the first 180 days, those who received the placebo were given albendazole, and those who had received the albendazole previously were given placebo. Follow-up was exactly the same as during the first 180 days. The crossover study design is shown in Figure 1. Ethical clearance to conduct the research was obtained from the Ethical Committee of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I.

**Clinical evaluation.** Subjects were examined for pruritus, rash, eye worm, angioedema (Calabar swellings), and subcutaneous migration of the adult worm before treatment. They were seen on a daily basis for the first week after drug or placebo administration and on days 28, 90, and 180. Similar observations were performed following the crossover (first week, days 208, 270, 360). Because the members of the study team did not reside in the village, a team of villagers was selected to inform researchers in the event of a serious side effect occurring during those times when the study teams were not in the village.

**Laboratory evaluation.** Calibrated thick smears were used to quantify the levels of mf in the blood. Capillary blood (70  $\mu$ L) was collected after a finger prick for each patient be-

TABLE 1  
Baseline characteristics of the study populations\*

Characteristics		PLAC/ALB (n = 51)	ALB/PLAC (n = 48)
Age (years)	GM	39.9	42.3
	(Range)	(15–78)	(17–77)
Sex	Female/male	26/25	24/24
	Microfilaraemia (mf/ml)	5,507.9 (Range)	2,550.3 (100–33,837)
Eosinophil count (mm <sup>3</sup> )	GM	1,049.0	787.6
	(Range)	(270–4,130)	(78–2,028)
PCV (%)	GM	39.3	38.9
	(Range)	(32–48)	(30–46)
Filaria-specific IgG (µg/mL)	GM	53.0	46.8
	(Range)	(5.2–6811.1)	(11.8–6,040.9)
Filaria-specific IgG4 (µg/mL)	GM	36.3	27.8
	(Range)	(2.3–268.4)	(1.9–369.2)

\* PLAC = placebo; ALB = albendazole; GM = geometric mean; PCV = packed cell volume.

tween 10:00 AM and 3:00 PM. Efforts were made to draw the blood at the same time of day for each patient to minimize the effect of the periodicity of *L. loa* mf. The blood was placed on two slides from which thick smears were made, then stained with Diff-Quick (Baxter International, Deerfield, IL). Parasite load was measured by counting the number of mf on both slides at 100 × magnification. The sum of the counts on both slides was multiplied by 100/7 to obtain mf density per milliliter.

Total leukocyte counts were performed using heparinized capillary blood, Lazarus' solution (Biotechnology Center, Yaoundé, Cameroon), and a Neubauer counting chamber. Thin blood smears from each patient were stained with Diff-Quick and used for the differential white blood cell count. The packed cell volume (PCV) was read after centrifuging whole blood in microcapillary tubes at 5,000 rpm for five minutes. All analyses were performed on days 1, 7, 28, 90, and 180 following the administration of albendazole/placebo (i.e., also on days 181, 188, 209, 270, and 360).

**Antigens.** Due to the difficulty of maintaining adult *L. loa* experimentally, the closely related human filarial parasite *Brugia malayi* was used as the source of soluble antigen. Adult and mf stages of *B. malayi* were harvested from infected jird peritoneal cavities and processed into adult and mf antigens as described previously.<sup>18,19</sup>

**Enzyme-linked immunosorbent assay (ELISA) for total and parasite-specific IgG.** Filaria-specific IgG and IgG4 were measured by an ELISA exactly as described previously.<sup>19</sup> Data are expressed in µg/mL for IgG and in µg/mL for IgG4.

**Statistical and data analyses.** The code (albendazole versus placebo) was broken only at the end of the 12 months needed to complete the entire crossover study. All data were double entered into a computer and analyses were performed. Differences between the two groups were assessed by the Mann Whitney U test and the Wilcoxon signed rank test. Statistical significance was inferred by having *P* values less than 0.05.

## RESULTS

**Pretreatment findings.** Ninety-nine subjects were initially enrolled in the study and randomly assigned to one of two treatment groups (48 in the albendazole group and 51 in the placebo group). As seen in Table 1, these groups were similar

with respect to sex and age distribution. Pretreatment mf densities varied from 100 to 33,837 mf/mL for those initially receiving albendazole (ALB/PLAC) and from 164 to 24,050 mf/mL for those initially receiving the placebo (PLAC/ALB). Geometric mean levels were not significantly different between the two groups. These values, together with pretreatment eosinophil counts, PCV, and antifilarial antibody levels, are shown in Table 1. As seen, there are no significant differences between the two groups for any of these parameters.

**Post-treatment participation.** Participation in the study was similar for both groups (Figure 1), with 27 of the initial 48 subjects who received albendazole initially (56.3%) and 28 of 51 subjects who initially received placebo (54.9%) followed up at all time points throughout the study. The most common reason for abandoning participation was the frequency and inconvenience of the finger pricks. Because no serious adverse events were seen among the study participants, such events were not given as reasons for failure to participate.

**Post-treatment adverse/side reactions.** There were few post treatment reactions, the most frequently cited being pruritus (30% and similar in both groups), abdominal discomfort (12%), and urticaria (2%). One individual developed urticaria 14 days after placebo administration, and another devel-

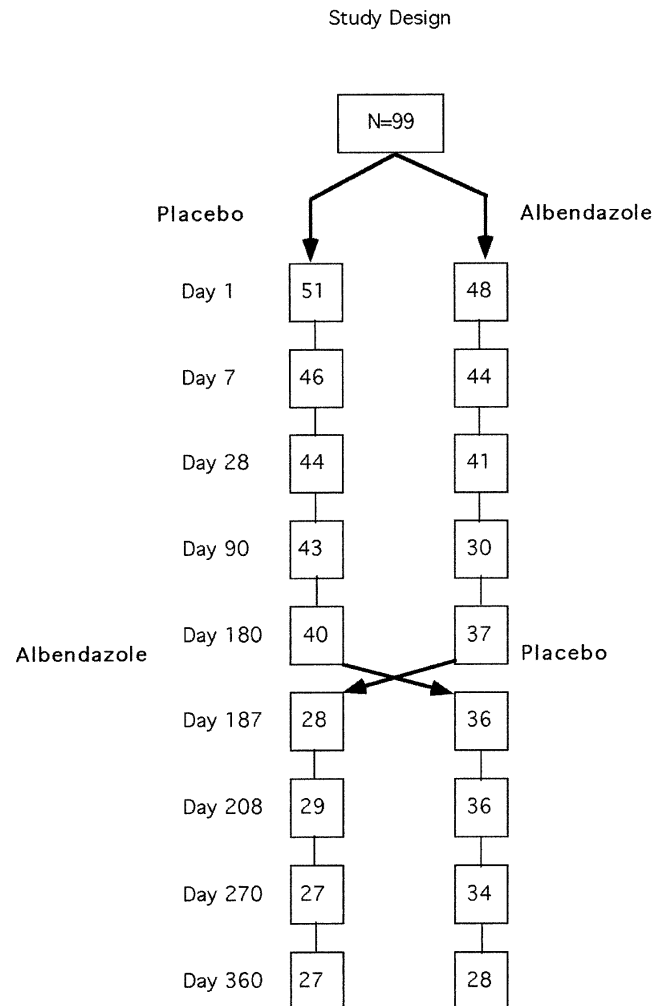


FIGURE 1. Crossover study design and number of patients participating at each assessment time point.

oped urticaria and intense pruritus one month after albendazole administration. Both responded well to administration of cetirizine. In addition, four subjects passed intestinal worms.

**Post-treatment mf.** Microfilarial counts remained stable until day 90 in the ALB/PLAC group. On day 90, we noticed a significant decrease in microfilariaemia ( $P = 0.0428$ ), as seen in Figure 2; however, the difference was no longer significant by day 180. In the PLAC/ALB group, mf counts did not vary throughout the first 180 days. Ninety days after these patients were given albendazole (day 270), there was a similarly significant decrease in mf counts ( $P = 0.0143$ ). Eleven of the 49 patients followed to the end of the study still had parasitemias  $> 20,000$  mf/mL. Only two became amicrofilaremic, both of whom had initial counts less than 500 mf/mL. The findings were similar irrespective of initial mf count (i.e., subset analysis based on initial mf counts  $< 1,000$  mf/mL or  $> 1,000$  mf/mL).

**Post-treatment eosinophilia.** Eosinophil counts remained comparable in both groups until day 360, when we noted a significant decrease in the PLAC/ALB group when compared with day 1 values (geometric mean eosinophil count on day 1 = 1,049 versus day 843 on day 360;  $P = 0.005$  and  $P = 0.017$  when compared with the ALB/PLAC group on day 360) (Figure 3).

**Post-treatment PCV.** The PCV did not vary significantly throughout the study either within each group or between the two groups.

**Post-treatment immunoglobulins.** Parasite-specific IgG levels were similar in both groups both at the beginning and at the end of the study. The difference between the two groups

was not significant at any time during the study. Parasite-specific IgG4 levels (Figure 4) were equivalent in both groups at baseline, but decreased in the ALB/PLAC group by day 180 ( $P = 0.022$ ). By day 360, this difference was no longer significant.

## DISCUSSION

Given that the clinical manifestations of loiasis are often not dependent on parasite load, any treatment to be undertaken should be safe, well tolerated, and effective, especially when mass treatment is entertained. Ivermectin has been increasingly implicated in post treatment encephalitis in *L. loa* infected patients with high microfilariaemia.<sup>20</sup> This has been shown to develop even after a single ivermectin dose. An alternative regimen, a 21-day course of albendazole, while safe, is not well suited for population-based chemotherapy. Therefore, the objective of this study was to evaluate the efficacy and tolerance of a shorter (three-day) albendazole regimen for decreasing mf to levels below which ivermectin administration would be safe. Thus, the present double-blinded, placebo-controlled crossover study was undertaken. It should be noted that a study performed after the present study was completed examined albendazole at a dose of 800 mg for three days and showed a small degree of efficacy against *L. loa* mf levels.<sup>21</sup>

As noted earlier, the two groups (ALB/PLAC and PLAC/ALB) were comparable at the start of the study. Albendazole was well tolerated, with few subjects (equivalent to those receiving placebo) requiring post-treatment intervention. The

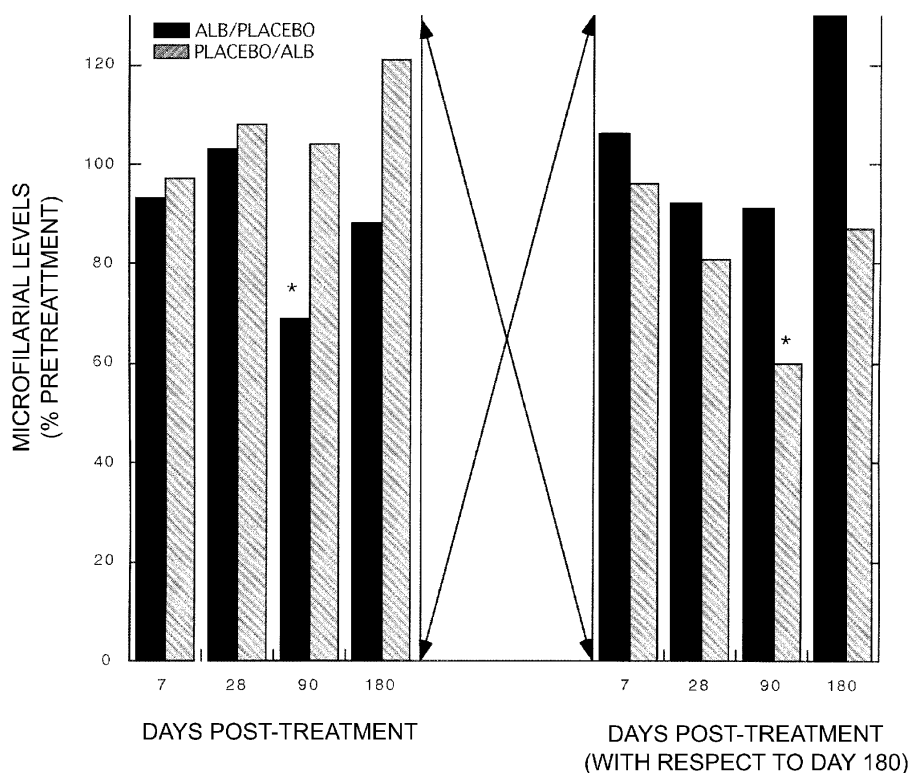


FIGURE 2. Effect of albendazole on *Loa loa* microfilaria (mf) counts. Data are expressed as the geometric mean percent pretreatment mf levels in those given albendazole on day 1 followed by placebo on day 180 (ALB/PLAC) or those given placebo on day 1 followed by albendazole on day 180 (PLAC/ALB), with the crossover depicted by the crossed arrows. Asterisks indicate statistically significant diminution of mf levels with respect to pre-albendazole levels.

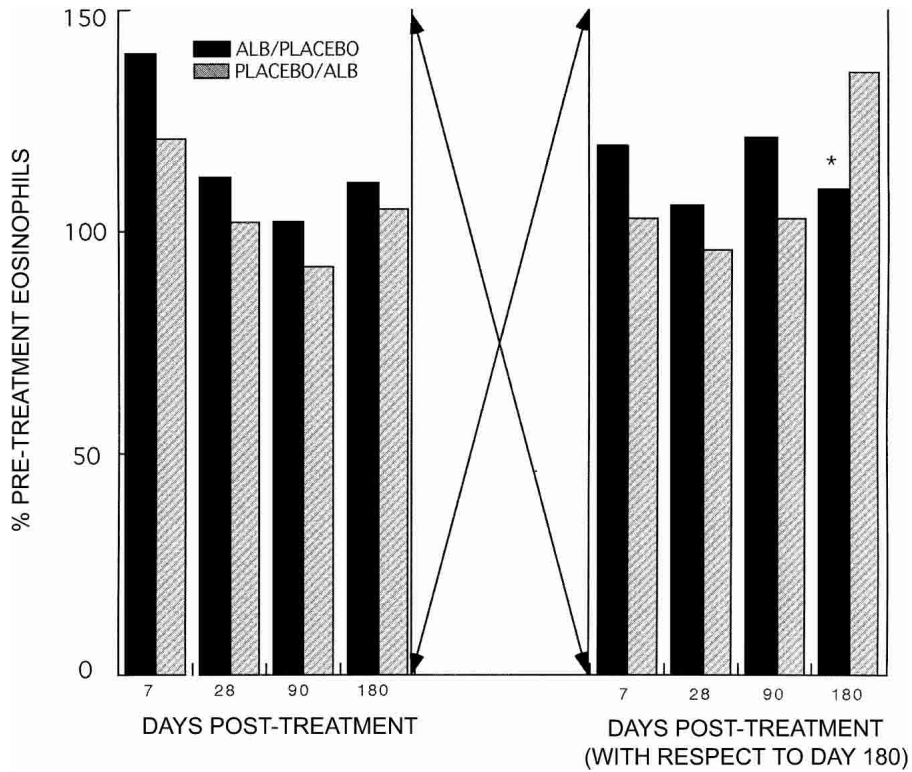


FIGURE 3. Effect of albendazole on peripheral eosinophil counts. Data are expressed as the geometric mean percent pretreatment eosinophil levels in those given albendazole on day 1 followed by placebo on day 180 (ALB/PLAC) or those given placebo on day 1 followed by albendazole on day 180 (PLAC/ALB), with the crossover depicted by the crossed arrows. The asterisk indicates a statistically significant change in eosinophil levels with respect to the two groups on day 360.

paucity of adverse reactions can likely be attributed to the pharmacokinetics and mode of action of albendazole. This drug is poorly absorbed through the gastrointestinal tract. Average maximal concentrations of albendazole sulfoxide

(the active metabolite) range from 0.13 to 0.25  $\mu\text{g/mL}$  following a single oral dose of 400 mg in healthy subjects.<sup>22</sup> Although its mode of action is not fully understood, it likely acts by blocking parasite glucose uptake and microtubule func-

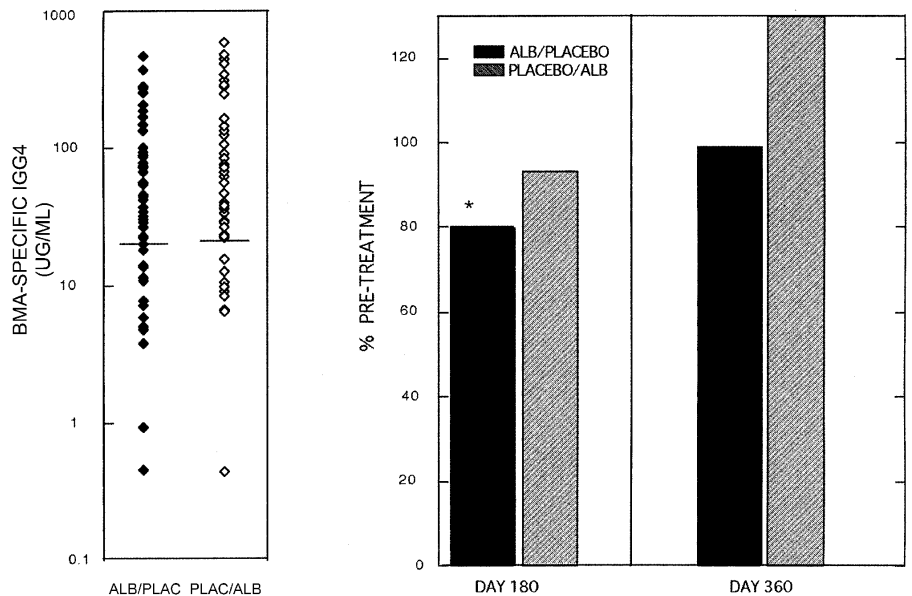


FIGURE 4. Effect of albendazole on parasite-specific IgG4. **Left panel.** Adult *Brugia malayi* (BmA)-specific IgG4 levels ( $\mu\text{g/mL}$ ) for all individuals at baseline. Each dot represents an individual patient, and the horizontal bars represent the geometric mean for the groups either receiving albendazole on day 1 followed by placebo on day 180 (ALB/PLAC) or those given placebo on day 1 followed by albendazole on day 180 (PLAC/ALB). **Center and right panels.** Geometric mean BmA-specific IgG4 levels on day 180 and day 360 as a function of the pretreatment levels in those given albendazole on day 1 followed by placebo on day 180 (ALB/PLAC) or those given placebo on day 1 followed by albendazole on day 180 (PLAC/ALB). The asterisk indicates a statistically significant change in antibody levels at day 180 compared with baseline levels.

tion.<sup>23</sup> The death of the parasite is slow, and parasite antigen is not liberated at once. This makes it well tolerated even in individuals with high parasite burdens.

Determination of drug efficacy in loiasis ideally would require quantification of adult worms as well as mf. Quantification of adults is difficult because these live in the subcutaneous tissue; however, diminution of mf levels is a good enough indicator of antiparasite activity. Albendazole was proven to decrease microfilaremia (typically at day 90, consistent with activity on the adult and on the mf indirectly). Klion and others<sup>16</sup> postulated that albendazole acted on the adult worm by blocking embryogenesis. Subjects in the PLAC/ALB group (who received albendazole on day 180) also showed a decrease in microfilaremia 90 days after they were given albendazole. Unfortunately, mf counts for both groups returned to baseline by 180 days following albendazole administration. The reason for this lack of sustained effect is not clear. It could be considered that the effect of albendazole lasted only 90 days, after which the adult worms were released from the inhibitory effect of the drug. It is also possible that the drug affected only some adult worms, with the remaining worms continuing to produce mf. An alternative explanation is that within 180 days, new infections with adult worms could have been established, since transmission of *L. loa* was ongoing in this area.

Antibodies to filarial have been shown to decrease with effective treatment of loiasis. As such, they constitute a good marker of therapeutic response. IgG4, which is believed to represent active infection,<sup>24</sup> decreased in the ALB/PLAC group by day 180 ( $P = 0.022$ ), suggesting that there was perhaps a lowering of the parasite burden.

The fact that 11 of the 49 subjects followed to the end still had parasitemias  $> 20,000$  mf/mL shows that this regimen does not entirely solve the problem of subsequent ivermectin safety. We think other short-term regimens should be considered, with particular emphasis on those in which higher doses of albendazole are used.

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