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Safety and Efficacy of Levamisole in Loiasis: A Randomized, Placebo-controlled, Double-blind Clinical Trial

Jérémy T. Campillo,[1] Paul Bikita,[2] Marlhand Hemilembolo,[2] Frédéric Louya,[2] François Missamou,[2] Sébastien D. S. Pion,[1] Michel Boussinesq,[1,a] Cédric B. Chesnais[1,a]

[1]UMI 233 TransVIHMI, Université de Montpellier, Institut de Recherche pour le Développement (IRD), INSERM Unité 1175, Montpellier, France; and [2]Programme National de Lutte contre l'Onchocercose, Direction de l'Épidémiologie et de la Lutte contre la Maladie, Ministère de la Santé et de la Population, Brazzaville, Republic of the Congo

Background. Individuals with high microfilarial densities (MFDs) of *Loa loa* are at risk of developing serious adverse events (SAEs) after ivermectin treatment. Pretreatment with drugs progressively reducing *Loa* MFDs below the risk threshold might help prevent these SAEs. We assessed the safety and efficacy of levamisole for this purpose.

Methods. A double-blind, randomized, placebo-controlled, MFD-ascending trial was conducted in the Republic of the Congo. Participants were treated in 3 cohorts defined by pretreatment MFD and levamisole dose (cohort 1: 1.0 kg and 1.5 mg/kg; cohorts 2 and 3: 2.5 mg/kg). Safety outcomes were occurrence of SAE and adverse event frequency during the first week. The efficacy outcomes were MFD reduction from baseline and proportions of individuals with at least 40% and 80% MFD reduction at day 2 (D2), D7, and D30.

Results. The 2 lowest doses (1.0 mg/kg and 1.5 mg/kg) caused no SAEs but were ineffective. Compared with placebo, 2.5 mg/kg levamisole caused more mild adverse events (10/85 vs. 3/85, $P = .018$), a higher median reduction from baseline to D2 (-12.9% vs. +15.5%, $P < .001$), D7 (-4.9% vs. +18.7%, $P < .001$), and D30 (-0.5% vs. +13.5%, $P = .036$) and a higher percentage of participants with >40% MFD reduction at D2 (17.5% vs. 1.2%, $P < .001$), D7 (11.8% vs. 6.3%, $P = .269$), and D30 (18.5% vs. 9.6%, $P = .107$).

Conclusions. A single 2.5 mg/kg levamisole dose induces a promising transient reduction in *Loa loa* MFDs and should encourage testing different regimens.

Clinical Trials Registration. NCT04049630.

Keywords. loiasis; clinical trial; levamisole; filariasis; Africa.

Loiasis is a parasitic infection caused by the filarial nematode *Loa loa*. About 140 million people live in central African regions, where this disease is endemic [1]. Currently considered as benign by the World Health Organization (WHO), loiasis is a major obstacle to the elimination of onchocerciasis, another filarial disease. Since the 1990s, onchocerciasis control is based on mass treatment with ivermectin (IVM) of all meso- and hyperendemic communities. This has led to the elimination of onchocerciasis in Latin American countries [2, 3] and a dramatic decrease in transmission in some African foci [4–6], but not in central Africa. The reason for this is that individuals with high densities of *Loa* microfilariae (mfs) in the blood can develop a potentially fatal encephalopathy after IVM treatment [7]. These serious adverse events (SAEs) probably result from the IVM-induced rapid paralysis of large numbers of *Loa* mfs, which leads to their passive drainage in the circulation and their embolization in brain capillaries. The current WHO goals are to "eliminate the transmission of onchocerciasis in 10 countries; to cease mass drug administration (MDA) with IVM in at least 1 focus in 34 countries; and to obviate the need for MDA in at least 25%, 50%, 75% and 100% of the population in at least 16, 14, 12, and 10 countries, respectively" by 2030 [8]. However, this objective is jeopardized by the fact that some areas are coendemic for loiasis and onchocerciasis.

Alternative treatment strategies have been developed to safely combat onchocerciasis in areas where loiasis is coendemic [9]. One solution to prevent post-IVM *Loa*-related SAEs would be to first treat the population with a drug to progressively reduce the *Loa* microfilarial densities (MFDs) below the threshold (30 000 mfs/mL), above which there is a risk of neurological SAEs. Various drugs and regimens have already been tested for their suitability in this application such as albendazole [10–13], antimalarials [14], or low doses of IVM [15, 16], but none of these trials was successful: the effect was either too strong or too weak or showed unsuitably large interindividual variation.

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[a]M. B. and C. B. C. contributed equally to this manuscript.

Correspondence: J. T. Campillo, Institut de Recherche pour le Développement (IRD), INSERM Unité 1175 911 avenue, Agropolis 34000 Montpellier, France (jeremy.campillo@ird.fr).

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Furthermore, individual treatment of subjects with high *Loa* MFDs remains a challenge because the therapeutic options (apheresis or 3-week daily treatment with albendazole, followed by IVM and/or diethylcarbamazine) are limited, complicated to apply, and their efficacy is moderate. Levamisole (LEV) is a long-established drug included in the WHO's List of Essential Medicines [17] and widely used in some countries, at a dose of 150 mg or 2.5 mg/kg, for its activity against soil-transmitted helminths (*Ascaris*, hookworms) [18]. LEV had been tested in the early 1980s against *Onchocerca volvulus*, *Wuchereria bancrofti,* and *Brugia malayi*. LEV showed moderate short-lasting activity in most trials [19–27] but has never been tested against *Loa*. A synthesis of previous trials on the other filarial species is provided in Supplemental Material 1.

We report results of the first trial conducted to evaluate the safety and efficacy of single-dose LEV in subjects infected with *Loa* carried out in the Republic of the Congo.

METHODS

Study Design

This adaptive double-blind, randomized, placebo-controlled trial included 3 independent cohorts with ascending *Loa* MFDs. Recruitment to the next cohort started if no SAEs occurred in the previous cohort.

To assess the safety of LEV, cohort 1 was composed of participants with low MFDs (1–1999 mfs/mL) in whom low doses of LEV were tested. Participants were allocated to 1 of 3 arms: LEV 1 mg/kg (LEV-1.0), LEV 1.5 mg/kg (LEV-1.5), or placebo. After confirmation that these doses of LEV were well tolerated in patients with low MFDs, an independent Data Safety Monitoring Board reviewed the safety and efficacy results to determine whether the dose could be increased for the next cohorts. Following the Data Safety Monitoring Board recommendations, 2 cohorts, each comprising 2 parallel arms (single dose of LEV at 2.5 mg/kg [LEV-2.5] or matched placebo) were launched: cohort 2 included subjects with MFDs between 1 and 14 999 mfs/mL, and cohort 3 included all microfilaremic subjects without upper limit of MFDs.

To assess efficacy, *Loa* MFDs were measured 5 days before treatment (D-5), and at day 2 (D2), day 7 (D7), and day 30 (D30) posttreatment. At D-5, all participants underwent a medical examination and a questionnaire to check for inclusion and exclusion criteria (see the following section). At D2 and D7, each participant underwent a medical examination and screening for any adverse events (AEs). A medical team visited the villages of all participants every day from D0 to D7 to manage AEs. All subjects received a participant card with emergency contact information.

Study Area and Selection of Participants

Participants were recruited in 21 villages located within 40 km of Sibiti (3°41′00″S, 13°21′48″E), the capital town of the Congolese administrative department of Lékoumou, a forested area where loiasis is endemic.

Participants were identified in 2 steps. In November 2019, residents were invited to participate in a survey to screen the population for loiasis. Because of the coronavirus disease 2019 pandemic, the launch of the trial testing had to be postponed to mid-January 2021. At that date, those subjects who were found microfilaremic in 2019, were aged 18–65 years, and weighed 50–85 kg for women or 45–85 kg for men, were invited to be reexamined to assess their eligibility to participate in the trial.

Volunteers underwent a medical evaluation and those with past or current history of neurological or neuropsychiatric disorders, or physical symptoms suggesting systemic disorders, were excluded from recruitment. People treated with clozapine, phenothiazines, sulfasalazine, carbamazepine, antithyroid drugs, ticlopidine, cimetidine, warfarin, or gold salts were also excluded because of a possible drug interaction with LEV. Women who reported being pregnant for less than 3 months, people with acute infection requiring treatment within the 10 days preceding the trial, and people who had received IVM, albendazole, or LEV during the previous 6 months were also excluded.

The clinical trial was conducted from January to April 2021.

Randomization, Blinding, and Drug Preparation

For the first cohort, a 1:1:1 randomization of 3 arms with blocks size of 6 was performed. For the second and third cohorts, a 1:1 randomization of 2 arms with blocks size of 4 was used. All randomizations were done by an independent statistician and stratified by sex and median age.

Sealed envelopes were prepared, containing either the number of LEV 10 mg, LEV 50 mg and matching placebo tablets required by the participant's weight and targeted dose, or 5 placebo tablets. Supplemental Material 2 provides tablet composition. Tablets were swallowed under the supervision of a single physician. All tablets were purchased from ACE Pharmaceuticals BV (Zeewolde, The Netherlands).

Laboratory Procedures

The *Loa* MFDs were assessed by examining 2 50-µL calibrated blood smears (CBS1 and CBS2) at D-5, D2, D7, and D30. All CBSs were prepared with blood taken between 10:00 AM and 3:00 PM to account for the diurnal periodicity of *Loa* mfs in peripheral blood [28]. In addition, CBSs for a given participant were prepared at the same time of the day on D-5, D2, D7, and D30. Because it is known that temperature can influence *Loa* MFDs [29], the ambient and subjects' body temperatures at the time of sampling were recorded using electronic thermometers. Blood was collected by finger-prick and spread on 2 labelled slides. The slides were dried at ambient temperature, dehemoglobinized and stained with Giemsa within 4 hours. Each slide was read independently by 2 experienced biologists who were blinded to treatment. All *Loa* mfs were counted using a microscope at 100×

magnification. Slides with an MFD difference exceeding 10% between the 2 readings were reread blind to the first result. The arithmetic means of the MFDs measured at the 4 readings (CBS1 by readers 1 and 2, CBS2 by readers 1 and 2) were used for the analyses, the results being expressed in mfs/mL.

Objectives and Outcome Measures

The primary objective of the trial was to evaluate the safety of single-dose LEV in individuals with *Loa* microfilaremia. The primary outcome measures were (1) the occurrence of an SAE and (2) the frequency of AEs during the first week posttreatment. Eighty participants provide a probability of 0.99, 0.98, 0.55, and 0.08 to detect at least 1 AE with a true frequency of 10%, 5%, 1%, and 0,1%, respectively. Classification of AEs are described in Supplemental Material 3.

The secondary objective was to assess the effect of LEV on *Loa* MFDs measured by: (1) the MFD reduction rates at D2, D7, and D30; and (2) the proportions of subjects with MFD reduction rates ≥40% and/or ≥80% at D2, D7, and D30. Reduction rates were calculated as follows: ((MFD at D-5) − (MFD at DX))/(MFD at D-5) with X = 2, 7, or 30.

Sample Size Calculation

Because no case of SAE has ever been reported after LEV treatment in central Africa, sample size calculations were performed using theoretical efficacy levels based on results of its effect on other filariasis. We made the hypothesis that <10% participants treated with placebo but ≥40% participants treated by LEV would have an MFD reduction rate exceeding 40% at D7. A sample size of 36 individuals per arm warrants an 80% power to detect a between-treatment difference at a 5% significance level. Assuming that 10% of enrolled subjects would be lost to follow-up at D7, a minimum of 40 participants had to be included in each arm.

Statistical Analysis

For the safety analyses, the numbers and proportions of participants with AEs were tabulated by AE severity score and arms. For the efficacy analyses, the arithmetic means and medians of individual MFD reduction rates were calculated and compared between arms at D2, D7, and D30 using Kruskal-Wallis test (KW test) and analysis of variance. The proportions of participants with MFD reduction exceeding 40% and 80% were compared between arms with Fisher exact tests at D2, D7, and D30.

Cohorts 2 and 3 were pooled to increase statistical power because no significant baseline differences between arms were found in each of these cohorts.

All statistical analyses were performed using Stata 15 (StatCorps LP, College Station, Texas, USA).

Trial Registration and Ethic Statement

This study was approved by the Committee on Ethics in Health Sciences Research (no. 226/MRSIT/IRSSA/CERRSSA) and an Administrative Authorization (no. 469/MSP/CAB/UCPP-19)

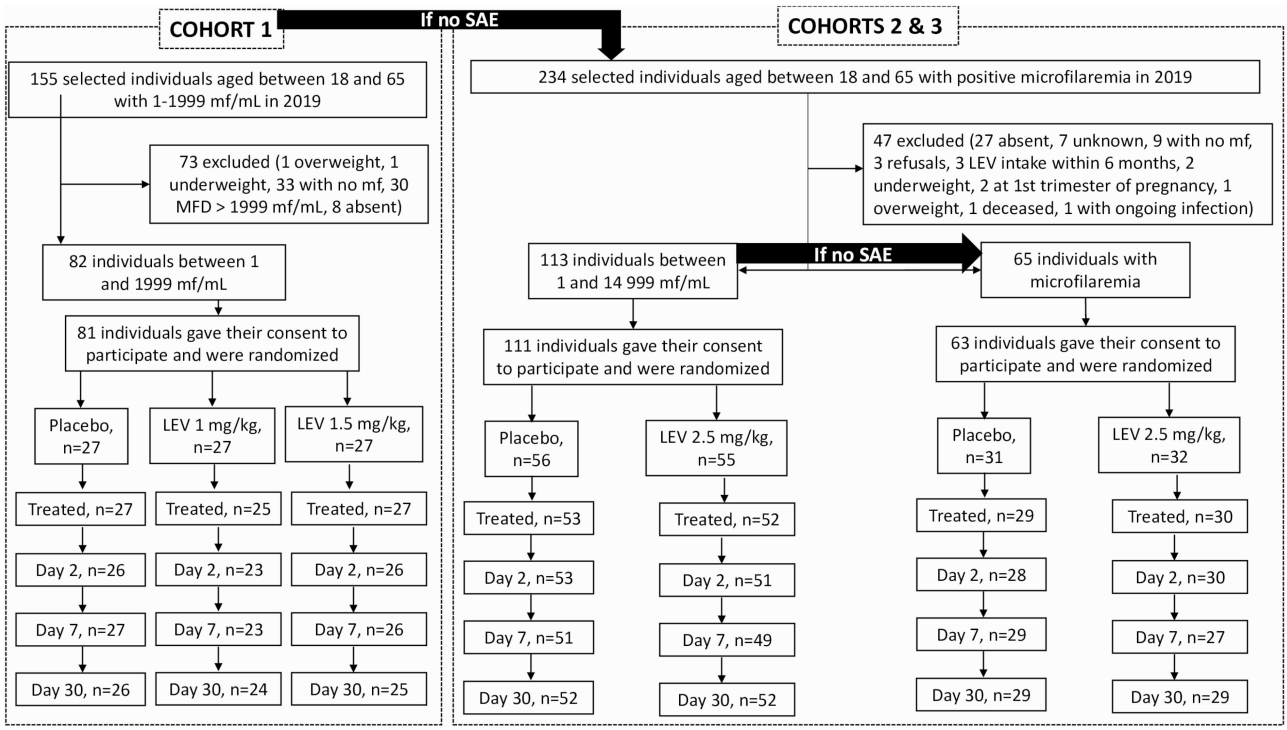

Figure 1. Flowchart of the clinical trial. Abbreviations: LEV, levamisole; MFD, microfilarial density.

Table 1. Baseline (Pretreatment) Characteristics of Trial Participants

	Cohort 1			Cohort 2		Cohort 3		Cohorts 2 and 3	
	Placebo	Levamisole 1 mg/kg	Levamisole 1.5 mg/kg	Placebo	Levamisole 2.5 mg/kg	Placebo	Levamisole 2.5 mg/kg	Placebo	Levamisole 2.5 mg/kg
Sex									
Female	9	10	10	15	16	7	7	22	23
Male	18	17	17	39	37	24	25	63	62
Age, mean ± SD	47.6 ± 15.0	47.5 ± 14.9	47.7 ± 13.6	47.3 ± 11.8	48.0 ± 12.9	45.9 ± 13.2	46.5 ± 12.4	46.8 ± 12.3	47.4 ± 12.7
Microfilaremia, mf/mL									
Arithmetic mean ± SD	636 ± 575	641 ± 536	641 ± 549	5082 ± 3826	5104 ± 3843	23 356 ± 19 714	18 146 ± 15 843	11 468 ± 14 799	9817 ± 11 742
Minimum; maximum	15; 1995	15; 1995	60; 1975	10; 14 015	5; 13 850	255; 69 085	940; 60 920	5; 69 085	10; 60 920
Geometric mean (95% CI)	350 (204–600)	412 (265–642)	359 (210–614)	2916 (1938–4388)	2832 (1816–4416)	13 320 (7936–22 355)	10 927 (7074–16 879)	4957 (3483–7055)	4614 (3255–6540)
Median [IQR]	470 [175–885]	510 [180–920]	475 [145–915]	4362 [2090–7500]	4075 [2035–7150]	16 370 [9035–34 120]	11 450 [6160–30 000]	6070 [2415–13 185]	6160 [2750–112 45]
Mansonella perstans[a] prevalence, N; %	2; 7.4	3; 11.1	3; 11.1	7; 13.0	9; 17.0	5; 16.1	4; 12.5	12; 14.4	13; 15.3
Heart rate, mean (bpm) ± SD	72.2 ± 12.6	75.8 ± 13.0	69.8 ± 10.2	78.6 ± 12.3	76.5 ± 13.7	72.3 ± 13.0	77.0 ± 10.2	76.4 ± 12.8	76.6 ± 12.5
Mean blood pressure, mean (mmHg) ± SD	101 ± 18	104 ± 15	99 ± 13	105 ± 17	105 ± 14	105 ± 18	107 ± 16	105 ± 17	105 ± 15
Systolic blood pressure, mean (mmHg) ± SD	128 ± 21	131 ± 20	127 ± 21	132 ± 22	130 ± 20	132 ± 23	133 ± 21	132 ± 23	131 ± 20
Diastolic blood pressure, mean (mmHg) ± SD	74 ± 12	76 ± 12	71 ± 9	78 ± 14	79 ± 11	77 ± 17	81 ± 13	78 ± 15	80 ± 11
Body temperature, mean (°C) ± SD	36.2 ± 0.7	36.4 ± 0.6	36.4 ± 0.6	36.6 ± 0.3	36.7 ± 0.3	36.5 ± 0.2	36.5 ± 0.3	36.6 ± 0.3	36.6 ± 0.3

Abbreviations: CI, confidence interval; bpm, beats per minute; IQR, interquartile range; SD, standard deviation.

[a]Data on *Mansonella perstans* are available in Supplemental Material 4.

was released by the Ministry of Health and Population of the Republic of the Congo. This study was conducted in accordance with the rules of Good Clinical Practices.

All participants signed an informed consent form before initiation of any study-related procedure. This trial is registered as number NCT04049630 in https://clinicaltrials.gov/.

Table 2. Mean Microfilaremia, Mean, and Median Relative Difference in Microfilaremia Between DX (X = 2, 7 or 30) and D-5, by Arm (Cohort 1)

	LEV 1.5 mg/kg			LEV 1 mg/kg			Placebo				
	MFD Arithmetic Mean	Mean Relative Difference	Median Relative Difference	MFD Arithmetic Mean	Mean Relative Difference	Median Relative Difference	MFD arithmetic mean	Mean Relative Difference	Median Relative Difference	P^a	P^b
Day 2	587.5 mf/mL	+0.7% ± 40.7%	−13.4% [−27.8%; +33.9%]	792.4 mf/mL	+17.1% ± 72.7 %	+3.1% [−35.4%; +40.9%]	600.3 mf/mL	+8.2% ± 96.6%	−2.5% [−35.9%; +34.3%]	.952	.738
Day 7	679.8 mf/mL	+33.5% ± 116.5%	+13.4% [−35.2%; +52.7%]	869.8 mf/mL	+27.9% ± 60.0%	+19.7% [−20.9%; +61.9%]	524.4 mf/mL	+4.6% ± 117.0%	−20.0% [−53.8%; +22.3%]	.036	.559
Day 30	648.6 mf/mL	+15.8% ± 58.4%	+3.8% [−18.6%; +20.0%]	704.4 mf/mL	+23.0% ± 80.3%	+2.2% [−35.8%; +75.5%]	536.0 mf/mL	−5.3% ± 93.7%	−23.3% [−49.5%; +13.8%]	.107	.563

Abbreviations: D, day; LEV, levamisole; MFD, microfilarial density.

[a]Kruskal-Wallis test.

[b]Analysis of variance.

Table 3. Proportion of Participants With a 40% and 80% Reduction in their Microfilaremia per arm in Cohort 1

	40% Decrease in Microfilaremia							80% Decrease in Microfilaremia						
	LEV 1.5 mg/kg		LEV 1 mg/kg		Placebo			LEV 1.5 mg/kg		LEV 1 mg/kg		Placebo		
	Yes	No	Yes	No	Yes	No	P^a	Yes	No	Yes	No	Yes	No	P^a
Day 2, N	4 (15.4%)	22 (84.6%)	4 (17.4%)	19 (82.6%)	5(19.2%)	21 (80.8%)	1.000	0 (0%)	26 (100%)	0	23 (100%)	3 (11.5%)	23 (88.5%)	.103
Day 7, N	5 (19.2%)	21 (80.8%)	2 (8.7%)	21 (91.3%)	9 (33.3%)	18 (66.7%)	0.108	0 (0%)	26 (100%)	1 (4.4%)	25 (95.6%)	3 (11.1%)	24 (88.9%)	.204
Day 30, N	4 (15.4%)	22 (84.6%)	8(33.3%)	16 (66.7%)	12 (48.0%)	13 (52.0%)	0.047	0 (0%)	26 (100%)	1 (4.2%)	23 (95.8%)	4 (16.0%)	21 (84.0%)	.058

Abbreviation: LEV, levamisole.

[a]Fisher exact test.

RESULTS

Screening of Eligible Participants

A total of 2052 individuals screened in 2019 met the age and weight eligibility criteria; 389 of them (18.9%, 264 males and 125 females) had *Loa* mfs in their blood. Among these 389 subjects, 344 were still microfilaremic in 2021 (88.4%).

Baseline Characteristics

After checking for inclusion and exclusion criteria, 81 participants were randomly assigned to cohort 1 (1–1999 mfs/mL), 111 to cohort 2 (1–14 999 mfs/mL), and 63 to cohort 3 (positive MFDs with no upper limit) (Figure 1). Considering all cohorts together, 112 subjects had a *Loa* MFD of 1–1999 mfs/mL, 106 an MFD of 200–14 999 mfs/mL, and 33 an MFD ≥15 000 mfs/mL.

The baseline characteristics of participants are shown in Table 1. Within each cohort, there was no difference between arms regarding age distribution, sex ratio, or mean and median *Loa* MFDs.

Results of Low Doses of LEV (1 and 1.5 mg/kg) in Subjects With Low *Loa* MFDs (Cohort 1)

The first cohort included participants with MFDs < 2000 mfs/mL. No SAEs related to treatment occurred. Thirteen patients reported 15 AEs (4 in the LEV-1.0 arm, 4 in the LEV-1.5 arm, and 5 in the placebo arm). Among the AEs reported in the LEV-1.0 arm, 2 were mild (1 epigastralgia and 1 edema) and 2 were moderate (2 cases of generalized pruritus) and required symptomatic treatment (antihistamines and corticosteroids). Among the AEs reported in the LEV-1.5 arm, 2 were not related to treatment (1 murder and 1 malaria attack) and 2 were mild (2 cases of localized pruritus). Among the AEs reported in the placebo arm, 1 was related to malaria attack, 2 were mild (2 cases of dizziness), and 2 were moderate (1 generalized pruritus and 1 epigastralgia) and required symptomatic treatment (antihistamines and proton pump inhibitor, respectively). The proportions of AEs did not differ between the 3 arms (Fisher exact test, $P = 1.000$).

Neither the mean and median MFDs (Table 2) nor the proportion of participants with a 40% or 80% MFD reduction (Table 3) were significantly different between the 3 arms at D2, D7, and D30 (Table 2).

Safety of Treatment With of LEV at 2.5 mg/kg (Cohorts 2 and 3)

In cohorts 2 and 3, no SAEs occurred. A total of 17 AEs were reported. Among them, 4 were not related to the trial (2 malaria attacks, 1 posttraumatic edema, and 1 scalp furuncle). Of the 13 AEs reported, 3 occurred in the placebo arm and 10 in the LEV arm (Fisher exact test, $P = .018$). All AEs reported were mild and transient. Table 4 summarizes the AEs possibly related to LEV in the 2

Table 4. Reported Adverse Events in Cohorts 2 and 3

Treatment	Adverse Event	Gradation	Baseline MFD (mf/mL)	Days Posttreatment	Absolute and Relative Difference in MFD From Baseline to Day 2
Placebo	Dizziness	Mild	2305	1	–1595 mf/mL (–69.2%)
Placebo	Pruritus	Mild	5990	2	–210 mf/mL (–3.5%)
Placebo	Conjunctivitis	Mild	36 990	0	+1738 mf/mL (+4.7%)
LEV 2.5 mg/kg	Conjunctivitis	Mild	4920	2	–763 mf/mL (–15.5%)
LEV 2.5 mg/kg	Blepharitis	Mild	4920	3	–763 mf/mL (–15.5%)
LEV 2.5 mg/kg	Pruritus	Mild	5985	0	–180 mf/mL (–3.0%)
LEV 2.5 mg/kg	Dizziness	Mild	6160	0	–1337 mf/mL (–21.7%)
LEV 2.5 mg/kg	Vomiting	Mild	7330	0	–3665 mf/mL (–50.0%)
LEV 2.5 mg/kg	Dizziness	Mild	24 420	0	+11 843 mf/mL (+48.5%)
LEV 2.5 mg/kg	Dizziness	Mild	30 000	0	–4170 mf/mL (–13.9%)
LEV 2.5 mg/kg	Dizziness	Mild	32 090	0	–8664 mf/mL (+27.0%)
LEV 2.5 mg/kg	Epigastralgia	Mild	30 950	0	–9130 mf/mL (–29.5%)
LEV 2.5 mg/kg	Vomiting	Mild	60 920	0	–15 473 mf/mL (–25.4%)

Abbreviations: LEV, levamisole; MFD, microfilarial density.

Table 5. Mean Microfilaremia, Mean and Median Relative Difference in Microfilaremia Between DX (X = 2, 7, or 30) and D-5, and Percentage of Individuals with ± 10% Variation in MFD, by arm (Cohort 2 and 3) and Initial MFD Stratum

		LEV 2.5 mg/kg				Placebo					
		Mean MFD	Mean Relative Difference	Median Relative Difference	Individuals With ± 10% Variation (n, %)	Mean MFD	Mean Relative Difference	Median Relative Difference	Individuals With ± 10% Variation (n, %)	P^a	P^b
Day 2	All participants	8509 mf/mL	+6.1% ± 108.6%	−12.9% [−32.4%; +19.7%]	65 (81.2%)	12 443 mf/mL	+33.0% ± 82.3%	+15.5% [−9.4%; +43.4%]	67 (82.7%)	.078	.001
	1–2499 mf/mL		+40.7% ± 212.8%	−6.1% [−34.6%; +26.0%]	16 (84.2%)		+76.4% ± 147.3%	+17.8% [−4.5%; +88.9%]	18 (90.0%)	.545	.087
	2500–6999 mf/mL		+6.7% ± 46.8%	−4.8% [−27.4%; +46.7%]	15 (68.2%)		+33.2% ± 47.0%	+23.4% [−0.4%; +63.3%]	18 (90.0%)	.075	.047
	7000–11 999 mf/mL		−3.7% ± 30.5%	−3.6% [−23.0%; +17.7%]	16 (76.2%)		+23.0% ± 25.3%	+19.3% [+3.6%; +39.4%]	14 (73.7%)	.005	.007
	≥12 000 mf/mL		−19.5% ± 30.3%	−25.2% [−40.5%; −13.9%]	18 (100%)		+2.02% ± 27.1%	−8.2% [−19.0%; +19.0%]	17 (77.3%)	.023	.008
Day 7	All participants	8847 mf/mL	+13.9% ± 122.3%	−4.9% [−31.1%; +22.5%]	57 (75.0%)	12 879 mf/mL	+29.2% ± 76.6%	+18.7% [−2.0%; +47.8%]	65 (81.2%)	.347	.001
	1–2499 mf/mL		+47.1% ± 234.3%	+4.3% [−31.1%; +27.8%]	16 (84.2%)		+62.9% ± 136.6%	+34.1% [−7.0%; +78.4%]	18 (90.0%)	.797	.084
	2500–6999 mf/mL		+12.3% ± 56.8%	−0.5% [−24.6%; +63.3%]	17 (77.3%)		+36.8% ± 45.8%	+30.8% [+7.6%; +62.4%]	15 (79.0%)	.141	.053
	7000–11 999 mf/mL		+7.3% ± 36.0%	+4.1% [−19.7%; +38.7%]	13 (72.2%)		+13.8% ± 29.0%	+19.9% [+1.5%; +26.6%]	16 (84.2%)	.548	.412
	≥12 000 mf/mL		−14.1% ± 23.1%	−10.9% [−36.0%; −4.6%]	11 (64.7%)		+5.5% ± 26.9%	+3.1% [−13.7%; +19.8%]	16 (72.7%)	.022	.020
Day 30	All participants	10 241 mf/mL	+55.6% ± 395.2 %	−0.5% [−26.6%; +24.6%]	62 (76.5%)	13 364 mf/mL	+29.9% ± 86.6%	+13.5% [−7.2%; +35.2%]	60 (74.1%)	.568	.036
	1–2499 mf/mL		+231.0% ± 805.1%	+9.1% [−33.9%; +81.5%]	14 (73.7%)		+67.7% ± 157.1%	+9.0% [−26.6%; +120.7%]	19 (90.5%)	.367	.989
	2500–6999 mf/mL		−4.0% ± 35.5%	−7.2% [−29.5%; +15.9%]	17 (77.3%)		+20.8% ± 43.4%	+17.4% [−9.0%; +31.3%]	16 (80.0%)	.048	.030
	7000–11 999 mf/mL		+11.4% ± 25.7%	+10.1% [−5.9%; +24.6%]	16 (76.2%)		+15.2% ± 29.8%	+13.5% [+1.8%; +34.5%]	12 (67.7%)	.674	.673
	≥12 000 mf/mL		−1.8% ± 31.0%	−9.0% [−26.9%; +16.0%]	15 (78.9%)		+14.2% ± 26.4%	+16.4% [−6.5%; +25.1%]	13 (59.1%)	.081	.036

Initial MFD is stratified according to interquartile range.

Abbreviations: D, day; MFD, microfilarial density.

[a]Analysis of variance.

[b]Kruskal-Wallis test.

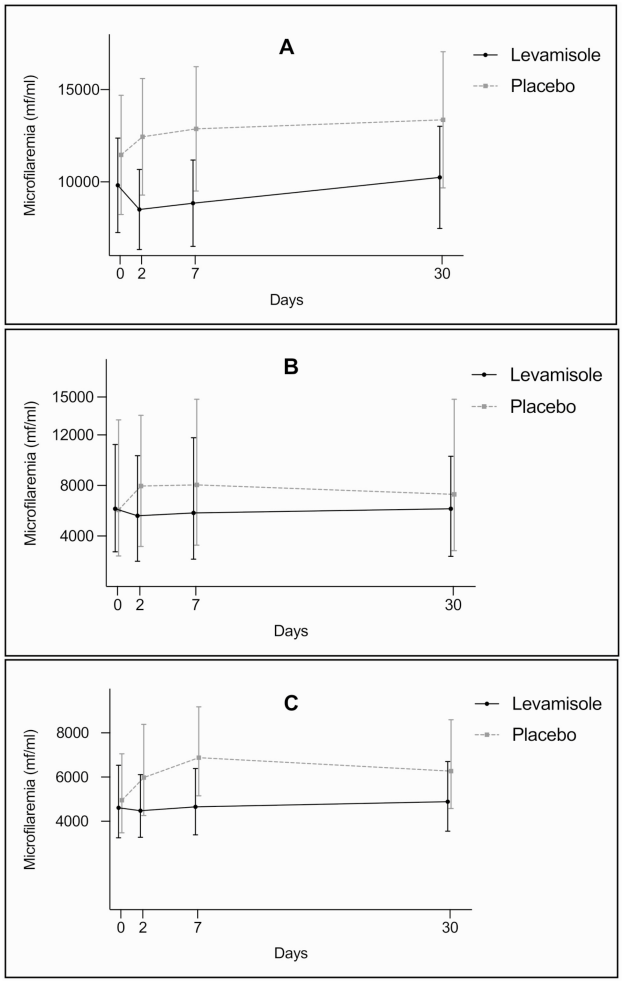

Figure 2. Evolution of mean and median microfilarial densities. *A,* arithmetic mean with 95% confidence interval; (*B*) median with interquartile range; (*C*) geometric mean with 95% confidence interval.

cohorts. In the LEV-2.5 arm, the mean initial MFDs were 19 645 (range, 4920–60 920) mfs/mL and 9877 (range, 10–49 605) mfs/mL in participants who reported an AE and in those who did not, respectively. This difference was significant (KW test, $P = .020$).

Effect of LEV 2.5 mg/kg on *Loa* MFDs (Cohorts 2 and 3)

Median MFDs were significantly lower in the LEV arm than in the placebo arm at D2, D7, and D30 (KW test, $P = .001$, .001 and .036, respectively). This effect was particularly clear in individuals with high baseline MFDs (Table 5). As shown in Figure 2, the arithmetic mean, the geometric mean, and the median of the MFDs' increase over time in the placebo arm, whereas they decrease at D2 and then increase again at D7 and D30 in the LEV arm. The high interindividual variability in response to treatment, which can be seen in Figure 3, is confirmed by the high standard errors in Table 5, notably among those with low initial MFDs. The proportion of participants with an 80% reduction in MFDs did not differ significantly between arms at D2, D7, and D30. The proportion of participants with a 40% reduction was significantly higher in the LEV arm compared with the placebo arm at D2 ($P < .001$), but not at D7 ($P = .269$) nor D30 ($P = .107$) (Table 6).

A logistic regression analysis of the proportion of patients who decreased their MFDs by at least 40% between D-5 and DX and a linear regression analysis of the absolute difference in MFD between D-5 and D2, both adjusted on temperatures and sampling time differences are available in Supplemental Materials 5 and 6, respectively. Relative differences between D-5 and D2 according to initial MFDs are provided in Supplemental Materials 7.

DISCUSSION

This trial is the first to assess the safety and efficacy of LEV in *Loa*-infected subjects. No SAEs occurred after a single dose

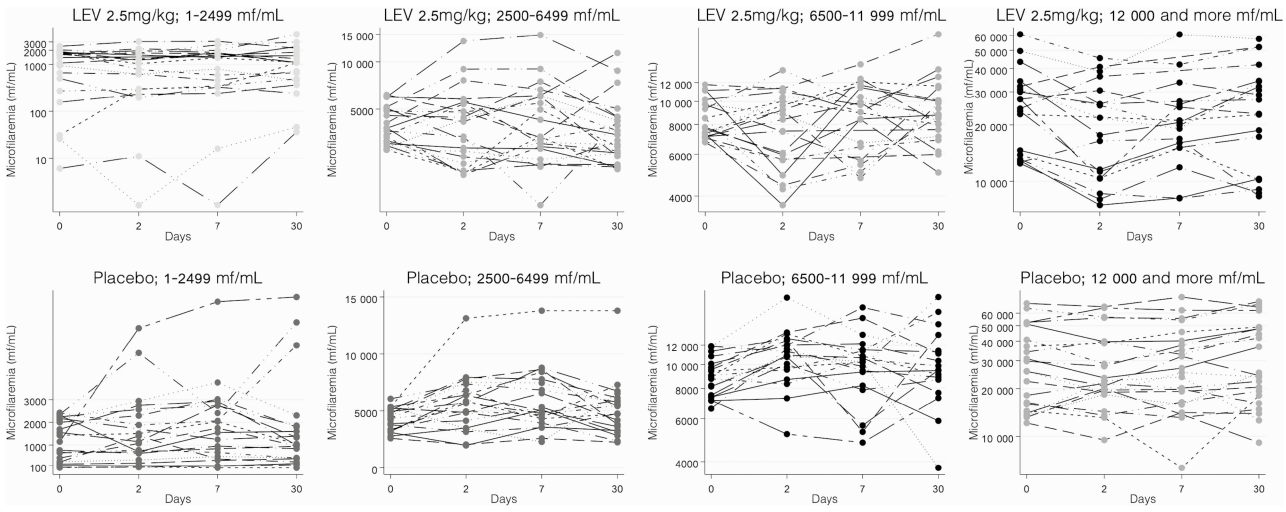

Figure 3. Evolution of individual microfilarial densities. Abbreviation: LEV, levamisole.

Table 6. Proportion of Participants With a 40% and 80% Reduction in Their Microfilaremia per arm in Cohorts 2 and 3

		40% Decrease in Microfilaremia					80% Decrease in Microfilaremia				
		LEV 2.5 mg/kg		Placebo			LEV 2.5 mg/kg		Placebo		
		Yes	No	Yes	No	P^a	Yes	No	Yes	No	P^a
Day 2, N	All participants	14 (17.5%)	66 (82.5%)	1 (1.2%)	80 (98.8%)	<.001	1 (1.3%)	79 (98.7%)	0 (0%)	81 (100%)	.497
	1–2499 mf/mL	4 (21.1%)	15 (79.0%)	1 (5.0%)	19 (95.0%)		1 (5.3%)	18 (94.7%)	0(0%)	20 (100%)	
	2500–6999 mf/mL	2 (9.1%)	20 (90.9%)	0 (0%)	20 (100%)		0(0%)	22 (100%)	0(0%)	20 (100%)	
	7000–11 999 mf/mL	3 (14.3%)	18 (85.7%)	0 (0%)	19 (100%)		0(0%)	21 (100%)	0(0%)	19 (100%)	
	≥12 000 mf/mL	5 (27.8%)	5 (27.8%)	0 (0%)	22 (100%)		0(0%)	18 (100%)	0(0%)	22 (100%)	
Day 7, N	All participants	9(11.8%)	67(88.2%)	5(6.3%)	75(93.7%)	.269	1(1.3%)	75(98.7%)	1(1.3%)	79(98.7%)	1.000
	1–2499 mf/mL	3 (15.8%)	16 (84.2%)	2 (10%)	18 (90%)		1 (5.3%)	18 (94.7%)	1 (5.0%)	19 (95.0%)	
	2500–6999 mf/mL	4 (18.2%)	18 (81.8%)	1 (5.3%)	18 (94.7%)		0(0%)	22 (100%)	0(0%)	19 (100%)	
	7000–11 999 mf/mL	2 (11.1%)	16 (88.9%)	1 (5.3%)	18 (94.7%)		0(0%)	18 (100%)	0(0%)	19 (100%)	
	≥12 000 mf/mL	0 (0%)	17 (100%)	1 (4.5%)	21 (95.5%)		0(0%)	17 (100%)	0(0%)	22 (100%)	
Day 30, N	All participants	15(18.5%)	66(81.5%)	7(8.6%)	74(91.4%)	.107	0(0%)	81(100%)	1(1.3%)	80(98.7%)	1.000
	1–2499 mf/mL	5 (26.3%)	14 (73.7%)	5 (23.8%)	16 (76.2%)		0(0%)	19 (100%)	1 (4.8%)	20 (95.2%)	
	2500–6999 mf/mL	5 (22.7%)	17 (77.3%)	0 (0%)	2 (100%)		0(0%)	22 (100%)	0(0%)	20 (100%)	
	7000–11 999 mf/mL	1 (4.8%)	20 (95.2%)	1 (5.6%)	17 (94.4%)		0(0%)	21 (100%)	0(0%)	18 (100%)	
	≥12 000 mf/mL	4 (21.1%)	15 (79.0%)	1 (4.6%)	21 (95.4%)		0(0%)	19 (100%)	0(0%)	22 (100%)	

Abbreviation: LEV, levamisole.

[a]Fisher exact test.

of LEV-1.0, -1.5, or -2.5, even in individuals with high initial MFDs. The severity and proportion of AEs observed in the LEV-treated arms were in accordance with those described in the prescription drug information [30].

The dose recommended to treat soil-transmitted helminthiases (2.5 mg/kg) induced a significant mean decrease in *Loa* MFD 2 days posttreatment, with 17.5%, 11.8%, and 18.5% of the population in the LEV-2.5 arm showing a ≥40% decrease in their MFDs at D2, D7, and D30, respectively. In the LEV-2.5 arm, only 1 participant with low baseline MFDs (25 mfs/mL) showed an 80% decrease in MFDs at D2 and D7, which may be reassuring because a large and rapid effect would raise the question of the occurrence of SAEs similar to those induced after IVM administration in individuals with high *Loa* MFDs.

Maximum mean reduction in *Loa* MFDs seems to occur about 2 days after LEV intake. It is followed by a slight mean increase in MFDs between D2 and D7 and a more marked mean increase between D7 and D30. This suggests that the mfs are not definitely eliminated and similar results have been reported from trials evaluating LEV on *W bancrofti* [27]. LEV might have a "microfilarifugal" action rather than a microfilaricidal one (ie, it may stimulate migration of microfilariae to deep organs where they could be sequestered and/or eliminated by the immune system) [31]. A second mechanism would be that mfs circulating at the time of treatment are eliminated but are replaced very rapidly by those newly released by adult worms. Mfs might also regain their muscular activity after a phase of temporary paralysis because of several mechanisms [32]. That AEs are more frequent in high baseline MFDs is consistent with the hypothesis of massive destruction of mfs. However, levamisole modes of action are multiple and complex [33] and its effects on mfs need to be clarified.

Changes in MFDs over the follow-up period varied significantly between individuals even in the placebo arm. This variability has already been described in other trials [10, 13]. This variation may be due to (1) detection error from variations in the reading of the microscopists in low MFD cases or (2) heterogeneity in physiological factors driving MFD variations between subjects. However, we collected 2 slides for each participant and each slide was read by 2 different technicians, thus reducing the risk of reading errors. More than 90% of the posttreatment CBSs were prepared within 30 minutes of time of the initial sample, ensuring that the results were not significantly impacted by the periodicity of *Loa*. Finally, small differences in temperatures on the sampling days did not significantly impact the results, as shown by the results of the regressions. Therefore, we assume that the variability found cannot be attributed to measurement accuracy but to variations in MFD at the level of the individuals. This requires further investigation.

The results of this trial are promising because they suggest a possible effect of LEV on *Loa* MFD at a dose that is well-tolerated. However, other trials using higher doses or repeated doses for several days are needed to identify the most effective administration scheme both as an alternative individual treatment for subjects with high *Loa* MFD and as a pretreatment before ivermectin administration. In view of the MFD variability found over just 30 days of follow-up, it will be necessary to define specific efficacy criteria for future trials, considering the natural MFD variability and focusing only on individuals with high *Loa* MFDs, the final objective being to achieve a significant reduction in MFDs for all the patients. Should an efficient

regimen be identified, the results would enable to determine the optimal time interval between pretreatment with LEV and safe IVM administration, considering both the pharmacokinetic-pharmacodynamic relationships and logistical constraints (the interval should not exceed a couple of weeks).

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. C. B. C., M. B., and S. D. S. participated in the conception and design of the study; C. B. C., F. M., and F. L. conducted the screening survey in 2019; C. B. C., F. M., F. L., M. H., and J. T. C. carried out the clinical trial in 2021; F. L. supervised the field laboratory and read the slides; C. B. C. and J. T. C. participated in the acquisition of data; J. T. C. performed the statistical analyses and wrote the first version of the manuscript; C. B. C., M. B., S. D. S., and J. T. C. participated in the interpretation of data and reviewed the article. All authors approved the final version for publication.

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