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Phase III Clinical Trial to Evaluate Ivermectin in the Reduction of *Mansonella ozzardi* infection in the Brazilian Amazon

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Abstract. The treatment of mansonelliasis is still a challenge because there are few clinical trials for the treatment of the disease. This double-blind, randomized, placebo-controlled study (phase III clinical trial) was conducted to evaluate the effectiveness of a single oral dose of ivermectin (0.15 mg/kg) in the reduction of the *Mansonella ozzardi* microfilaraemia and the occurrence of adverse effects in infected people compared with the control group treated with placebo. A total of 49 microfilaraemic patients were randomly selected from the municipality of Lábrea, State of Amazonas, in the Brazilian Amazon. Among them, 40 patients have concluded the study, 19 treated with ivermectin and 21 treated with placebo. In the first and third days after the treatment, all the patients were clinically evaluated, and the diagnostic and quantification of blood microfilariae through blood filtration in polycarbonate membranes was performed. A significant reduction of the microfilaraemia (99.9%) was observed in the patients who received ivermectin. Slight changes in laboratory test results, without clinical importance, were seen in treated and control groups. Our results suggest that ivermectin is effective and safe for the treatment of infections caused by *M. ozzardi*.

INTRODUCTION

Mansonella ozzardi is one of the filaria responsible for mansonelliasis in humans, with a geographic distribution limited to the Central and South Americas, with high prevalence in the Amazon region since the decade of 1950.[1] This parasite is transmitted by Diptera, families Ceratopogonidae and Simuliidae,[2] and was recorded in Brazil for the first time in 1949, in the municipality of Manaus, in the State of Amazonas.[3] The symptoms of mansonelliasis have been extensively studied and the infected individuals might present moderate fever, cold legs, arthralgia, adenitis, dizziness, and headaches.[4–6] Until now, few studies have evaluated the pharmacological treatment of *M. ozzardi* infection[7–10] and even less has used a placebo group.[8,9] Ivermectin has already proved to be a safe and effective drug for the treatment of other helminths.[11–13] The present study can be considered a continuation of the trial performed by Basano and cols published in 2014.[10]

MATERIALS AND METHODS

This is a study of a phase III double-blind, randomized, placebo-controlled clinical trial, with a group of patients receiving a single dose of ivermectin (0.15 mg/kg) and another group receiving a placebo (silica and talc, with presentation identical to the ivermectin). The effectiveness and tolerability of ivermectin in the treatment of *M. ozzardi* was evaluated, quantifying the microfilaraemia and recording the hematological and biochemical changes, as well as identifying the symptoms reported by the patients with *M. ozzardi* and the eventual adverse effects (AE) reported after third days of the use of the drug and placebo.

Study area. The study was conducted in the Lábrea municipality, western Amazon, State of Amazonas, Brazil: 07°15′34″ S and 64°47′59″ W (Figure 1), on the riverside population of the Purus River, where approximately 260 people live, in nine communities. The municipality had an estimated population of 44,071 inhabitants in 2016.[14]

Sample. The sample included men, women (negative pregnancy test and not breastfeeding) and children more than 4 years old and adults less than 61 years with infection by *M. ozzardi* that has not reported baseline pathologies (cardiac, renal, hepatic, and neurological diseases, malnutrition, and meningitis), or altered biochemical examinations for hepatic or renal function or that have used any anti-helminthic, neuroleptic, and anti-histamine drugs in the last 60 days and had known allergic reaction against ivermectin (Mazzoti's reaction). The individuals that met all the criteria voluntarily accepted to participate in this study.

The number of examined individuals in the general population was estimated considering a prevalence of mansonelliasis of 40%.[10] The clinical trial sample size was calculated using specific parameters related to the MacNemar Z test used to compare paired proportions, $\alpha = 0.05$ (type I error) and $\beta = 0.20$ (type II error), with 85% probability of cure among the patients who received ivermectin.[15] Following these parameters, a group of a minimum of seven individuals for each group was defined. However, anticipating the evasion of patients, all the subjects who attended the criteria were included, totalizing 49 individuals, with 40 of them remaining until the end of the study (19 in the ivermectin group and 21 in the placebo group).

The therapeutic regimen adopted was a single oral dose of ivermectin presented as 3-mg capsules, purchased from a pharmaceutical company. A single oral dose (0.15 mg/kg) was administered, according to the weight chart, following the standards used for the treatment of onchocerciasis. The

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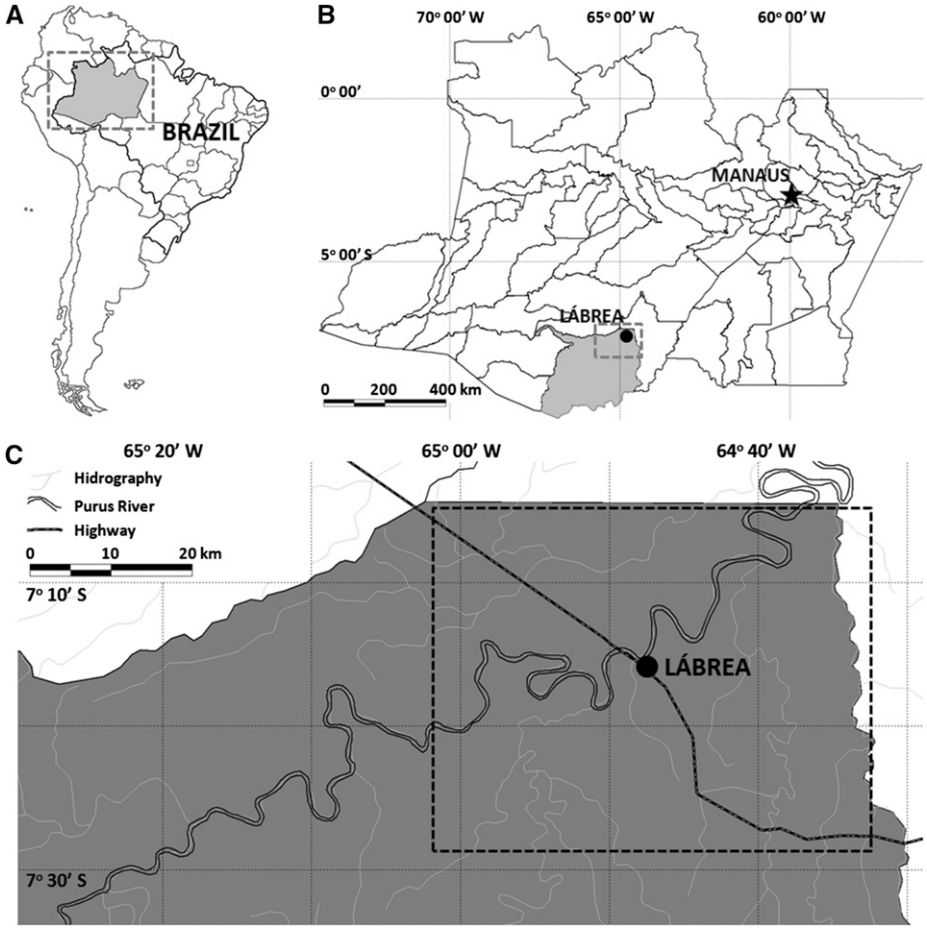

FIGURE 1. Study area location. (**A**) State of Amazonas in Brazil. (**B**) Magnified view of Lábrea municipality location. (**C**) Study area in Lábrea municipality, highlighted in dashed square.

appearance of the placebo was like the ivermectin tablet and was composed of silica and talc. The ivermectin group received tablets with the drug, and 3 days after the administration of the medication, received tablets of placebo. The control group, by its turn, received initially placebo and on the third day was administered with ivermectin, after the blood sampling and application of the clinical–epidemiological questionnaire. Therefore, all the microfilaremic individuals were treated and all the symptoms were reported in the initial day (D0) and three days after the beginning of the treatment (D3) in both groups, as well as the AE during the treatment. It should also be considered that a phase II clinical trial with results favorable to ivermectin has already been performed in 2014[10] and was essential for the decision of treating both groups at the end of the study and following up the patients for just 3 days.

The drug was administered by medical professionals and the use of any other medication was recorded in the clinical–epidemiological file. The patients submitted to the treatment were followed up by a team of investigators, well-equipped to deal with eventual adverse reactions during the 3 days after the use of the drug.

Complementary laboratorial tests (complete blood count, creatinine, hemoglobine, and transaminases) were performed in D0 (pretreatment) and D3 (posttreatment), the microfilariae (mf) density was calculated on days D0 and D3, and signs, symptoms, and eventual AE were recorded.

Diagnostic and quantification of blood mf. One milliliter of venous blood was diluted in 10 mL of saline solution 0.9% and filtered in polycarbonate membranes (Nucleopore Corporation, Pleasanton, CA) with 3 μ diameter pores,[16] in days D0 and D3. Subsequently, the membranes were placed over microscope slides, methanol-fixed, and stained with eosine-Giemsa. The counting of the mf number in the membranes was made under an optic microscope, ×400, by two independent expert professionals and the result expressed as the number of mf/mL of blood.

Evaluation of efficacy and safety. The parasitological cure was considered the primary "endpoint," and the occurrence of AE and changes in the laboratorial tests were regarded as secondary "endpoints."

AE. Clinical–epidemiological questionnaires were applied to the investigation of signs/symptoms before the medication (D0) and in D3. Signs and symptoms not present before the treatment, and those that appeared and/or worsened after the administration of the drug until D3 were considered AE. Considering that the half-life of the drug is 27 hours, the patients were monitored, for safety reasons, for 3 days after the treatment of the evaluation for the possible development of AE.

Ethical issues. The study was approved by the human research ethical committee from the Centro de Pesquisas em Medicina Tropical, registered under the number 1727294 (CAAE 51130015.7.0000.0011).

Statistical analysis. Descriptive statistical analyses were performed through the calculation of averages, variances, standard deviations, medians, quartiles, minimums, and maximums, to analyze the quantitative variables. The graphical representation of the variables was done using box-plot graphics. The comparison between the quantitative variables was made using the Mann–Whitney nonparametric test. The corrected Chi-Squared test of Yates or the Fischer test were used to compare the qualitative variables. A significance level of 0.05 was adopted in the hypothesis test of difference between the groups. The analyses were performed using software Statistica 8.0® (Palo Alto, CA), R (Vienna, Austria) and Rstudio® (Boston, MA).

RESULTS

From a total population of 260 inhabitants in the nine riverside communities in the Purus River, 208 (80%) were selected for convenience and examined by blood filtration in polycarbonate membranes, with 49 (23.5%) individuals being identified with microfilariae of *M. ozzardi*. The frequency rates of parasitized individuals in the different communities ranged from 2.0% to 48.9%.

From the positive cases, nine participants evaded the study, five before D0 and four between D0 and D3, which resulted at the end of the study in two groups of randomly distributed individuals, one with 19 people (ivermectin group) and the other with 21 people (placebo group).

In the ivermectin group, there was a higher frequency of male individuals (68.4%) with an average age of 33.9 ± 19.6 years and average weight of 55.4 ± 16.3 kg. The female subjects were most of the participants (52%) in the placebo group, with an average age of 38.1 ± 17.5 years and average weight of 65.9 ± 13.7 kg. There were no statistically significant differences in the distribution per weight and per age ($P > 0.05$ Mann–Whitney U), as well as per gender ($P = 0.214$, Fischer exact test), with both groups being considered homogeneous.

In the treated group, at D0 the average microfilaraemia was 120.5 mf/mL and at D3 was 0.1 mf/mL, with just one case remaining positive and 94.7% becoming amicrofilaremic. There was a sharp reduction of 99.9% in the microfilaraemia between D0 and D3 ($P < 0.0001$, Mann–Whitney U test). Despite the fact that the microfilaraemia has also decreased in the placebo group between D0 and D3 (average 88.48 and 25.7 mf/mL, respectively), this was not statistically significant, with $P = 0.27$ (Figure 2).

Regarding the biochemical tests in the group treated with ivermectin, between D0 and D3, only glutamic-pyruvic transaminase (GPT) presented a reduction in the blood concentration ($P = 0.008$), although without any clinical consequence.

With respect to the hematology in both groups, most of the individuals presented the total leukocyte count and hemoglobin concentration within the normal limits, with 85.7% of the leukocyte counting and 83.7% of the hemoglobin concentration being normal.

When the symptoms of the treated group were compared between D0 and D3 there was a statically significant reduction of them: visual changes ($P = 0.0008$) and tinnitus ($P = 0.01$).

Regarding the AE and the treatment, a total of seven symptoms were reported in the ivermectin group until D3: chills, headaches, chest pain, ocular pain, sickness, fever, and dizziness, amounting to 24 complaints, the most frequent being headache 34.8%, fever 30.4%, and chills 13.0%. A total of 12 (63.1%) patients reported AE, none of them classified as severe. In this group, every symptomatic patient presented an average of two symptoms, with a maximum of four reported symptoms per person.

DISCUSSION

Mansonella ozzardi is one of the eight filariae of medical importance to human beings, being the only one autochthonous to the Americas,[17] and one of the three filariae that parasitize exclusively humans present in Brazil.[3,18] It presents high prevalence in the Amazon region, with rates that vary up to 70%, based on the region, locality, and diagnostic method.[3,4,10,19–25] This work showed the presence of this parasite with a mean prevalence of 23.5% among the riverside communities in the Purus River that until then have not yet been evaluated about the presence of *M. ozzardi*, confirming the high prevalence of this filaria in the riverside populations of the state of Amazonas.

The choice for ivermectin in this study is because of its use as an anti-helminthic in humans since the decade of 1980 and, particularly, in the case of the filariasis with the highest

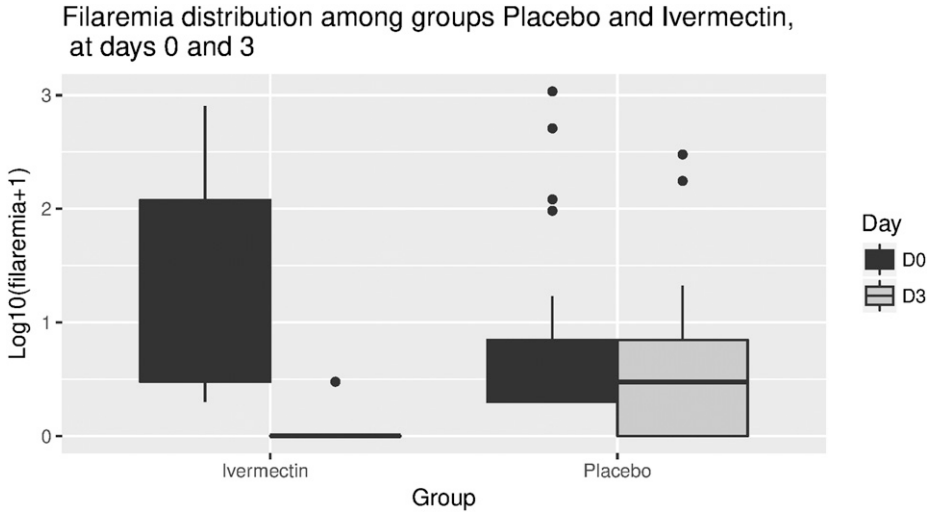

FIGURE 2. Filaremia distribution among groups placebo and ivermectin, at days 0 and 3.

medical interest, such as the so-called neglected filarial diseases (onchocerciasis and lymphatic filariasis) and other mansonelliasis present in Africa.[26–33]

For *M. ozzardi*, there are few studies about the specific treatment with favorable results conducted with this drug.[7–10,34] Therefore, the present work confirms the effectiveness of ivermectin in the treatment of *M. ozzardi*, in accordance with Chadee et al.[8] and González et al.[9] and Basano et al.[10]

Regarding the biochemical tests, despite some changes in the transaminases, such as the increase in GPT, when the treated group was compared with the placebo group between D0 and D3, and the decrease in GPT, comparing the treated with the placebo group between D0 and D3, they did not present clinical implications, which is in accordance with previous studies,[7–10] which might make ivermectin a safe treatment of mansonelliasis.

The comparison of the concentration of hemoglobin, number of leukocytes, monocytes, lymphocytes, and eosinophils in the two groups separately between D0 and D3, and between the same groups in the days D0 and D3, showed no statistically significant difference between the groups before and after the treatment.

Regarding the hematological changes in the white series in the pretreatment of all patients, in contrast to most of the previous studies, the leucogram was within the normal ranges in 85.7% of the individuals with mansonelliasis, just as recorded by Basano et al.[10]

A similar finding between this study and the previous ones was the presence of eosinophilia (47.5%) among the patients, in accordance with Batista et al.,[4] Tavares,[6] and Basano et al.[10] However, eosinophilia did not present a statistically significant variation between the pre- and posttreatment, which is demonstrated in the study of Basano et al.[10]

Another explored issue was the effectiveness of the treatment and the occurrence of AE with the use of ivermectin. The AE were neither limiting nor severe, although present in a significant amount. From the total amount, AE were found in 63.15% of the patients from the treated group, resolving in 100% of the cases until D3, as was found in other studies.[7,10,26] From the 19 individuals treated, 12 (63.1%) presented reactions without the need of any medical intervention. Such effects, in most cases, appeared in up to 24 hours after the use of medication and resolved in a similar time, in accordance with Basano et al.[10]

However, Krolewieck et al.,[34] in a study performed in Argentina, showed a higher intensity of these reactions in two patients treated with ivermectin (severe reactions), in one group of 10 patients, smaller than this study, and with no placebo group.

There is a controversy regarding the pathogenicity of mansonelliasis that has been discussed for many decades. Few authors have focused on clinical trials, especially until the half of the twentieth century, when this parasite was even declared as purely of academic interest by Biagi.[35] Batista et al.,[4] in a study about the pathogenicity of mansonelliasis, mentioned several authors who considered this parasitosis as non-pathogenic.[36–40] However, other authors in their studies have also correlated the signs and symptoms of this parasitosis.[4,6,41–44]

Analyzing all the clinical symptoms reported by the patients with *M. ozzardi* from the two groups that were evaluated in the clinical trial, before the treatment there was no clinical determinant characteristics for a specific symptom. This fact was not observed in previous studies that have identified some more frequent associations: fever, headache, arthralgia, myalgia, and cold legs.[4,6,41–44]

The present work presents ivermectin as a feasible option for the treatment of *M. ozzardi*, representing an advance compared with the study of Basano et al.,[10] as it used a placebo group, presenting similar results about the occurrence of AE in the treated group, although with significant reduction of microfilaraemia.

As there were no AE until D3, the present study confirms the findings of previous works[7,10,34] and claims that ivermectin might be considered safe for the treatment of mansonelliasis.

Based on the effectiveness and safety of this individual treatment, the health authorities should be warned about the need of structuring an adequate model of control for this Amazon-endemic disease, currently neglected by the public health system. This model, in a certain way, would also include the collective treatment of mansonelliasis and other concurrent helminthic diseases in this region, treatable with ivermectin, a multipurpose drug.

In conclusion, the groups were considered homogeneous for the analyzed variables and there was a significant statistical decrease of microfilaraemia and of occurrence of mild AE, not being a deterrent for the treatment.

Future controlled clinical trials based on these findings should also evaluate the use of ivermectin with other drugs in the same design. Another possibility would be to test the use of drugs that might be related to the interference between the symbiosis between helminths and bacteria, as the antibiotics that eliminate the bacteria *Wolbachia* sp.

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