Association of miltefosine with granulocyte and macrophage colony-stimulating factor (GM-CSF) in the treatment of cutaneous leishmaniasis in the Amazon region: A randomized and controlled trial

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ABSTRACT

Objectives: To compare topical granulocyte and macrophage colony-stimulating factor (GM-CSF) and miltefosine (G + M) versus placebo and miltefosine (P + M) or parenteral meglumine antimonials (MA) in the treatment of 150 patients with cutaneous leishmaniasis (CL) caused by Leishmania guyanensis in the Amazon.

Design: A randomized and double-blinded clinical trial.

Results: At 90 days after the initiation of therapy, the cure rates were 66%, 58%, and 52% for the groups P + M, G + M, and MA, respectively (p > 0.05). Cure rates at 180 days did not differ. Healing time was similar in the 3 groups, but faster in the MA group as compared to the G + M group (p = 0.04). Mild and transitory systemic adverse events were frequent in all groups (above 85%). Nausea (85%) and vomiting (39%) predominated in the miltefosine groups and arthralgia (51%) and myalgia (48%) in the MA group. One patient (group MA) stopped treatment after presenting with fever, exanthema, and severe arthralgia.

Conclusions: Miltefosine did not present a higher cure rate than MA, and the association of GM-CSF did not improve the therapeutic response. Nevertheless, because of its less toxicity, easier administration, and a similar cure rate when compared with MA, miltefosine should remain as one of the main drugs for treating CL due to L. guyanensis.

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Introduction

Different species of leishmania cause leishmaniasis in Brazil, but Leishmania braziliensis and L. guyanensis are responsible for the majority of cases of tegumentary leishmaniasis (TL), that may affect skin and mucosal, being the most common in the latter group is the nasal mucosa (Amato et al. 2007). TL is composed of distinct clinical forms such as cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML), disseminated leishmaniasis (DL), and diffuse cutaneous leishmaniasis (DCL). In the Amazon region, L. guyanensis is the main causal agent of TL, while L. braziliensis predominate in the other regions (De Guerra et al. 2015). The cutaneous ulcer is the main characteristic of CL but the disease is

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more severe in patients infected by *L. braziliensis* than *L. guyanensis* (Romero et al. 2001). CL caused by *L. braziliensis* presents positive lymphoproliferative responses and higher IFN-γ production but infections by *L. guyanensis* seem to induce a downregulation of the immune system allowing continuous parasite replication (Matta et al. 2009). While in CL due to *L. braziliensis* absence or scarce number of amastigotes forms are detected, the parasite burden in CL caused by *L. guyanensis* is high and amastigotes are easily observed in material obtained by the scarification of the lesion border (Romero et al. 2001). The initial clinical manifestation in both diseases is a papular or nodular lesion, but while a huge satellite lymph node is found in the disease caused by *L. braziliensis*, adenomegaly is small in CL caused by *L. guyanensis* (Romero et al. 2001). Moreover, the ulcer in *L. braziliensis* are well-limited and the borders are more raised than in *L. guyanensis* infection (Romero et al. 2001).

Therapy for CL range from topical to systemic; for instance, local heat therapy is associated with high efficacy in CL in the Old World (Sadeghian et al. 2007), but limitations such as the size of the ulcers, lesions in the face, and risk to the disease progress to ML in areas of *L. braziliensis* transmission indicate systemic intervention (Llanos-Cuentas et al. 1984; Blum J et al. 2012). Pentavalent antimonium was first used over 70 years ago, but it is still the main therapy for CL in South and Central America. The recommended therapy for CL in Brazil is meglumine antimonate, but the response is quite variable dependent on the species of the leishmania and the region where the disease is documented. For instance, in the Southeast and in the Northeast of Brazil, *L. braziliensis* is the main causal agent of CL but while response to therapy is observed in more than 90% of the patients in the Rio de Janeiro state (Sahel et al. 2017), the cure rate ranges from 48% to 60% in the endemic area of Corte de Pedra in Bahia (Machado et al. 2010; Prates et al. 2017).

In the Amazon region, the efficacy of meglumine antimoniate is low and pentamidine has been used as the first choice drug. While the cure rate of CL caused by *L. guyanensis* with antimony versus pentamidine in a single dose of 4 mg/Kg/weight was similar, 58.1% and 55.5%, respectively (Neves et al. 2011); pentamidine (7 mg/Kg/weight) applied in 3 doses had a cure rate of 96.2% (Gadelha et al. 2018). Both meglumine antimoniate and pentamidine have a high rate of adverse reactions that are dose-dependent (Chrusciak-Talhari et al. 2011; Gadelha et al. 2015).

Considering all the challenges involved in CL therapy, the development of new drugs and strategies is an important issue in CL regardless of the species and country.

MILtelfosine acts by blocking cytochrome C oxidase, which results in changes in mitochondrial membrane potential, reducing oxygen consumption and ATP levels in *L. donovani* (Luque-Ortega and Rivas, 2007). In addition, it also inhibits phosphatidylyethanolamine N-methyl-transferase and consequently, the biosynthesis of phosphatidylycholine (Rakotomanga et al. 2007). Miltefosine also increases phagocytosis and induces IFN-γ production, the most important cytokine that activates macrophages to kill Leishmania (Jha et al. 1999; Wadhone et al. 2009; Ponte et al. 2012). We have previously shown in CL caused by *L. guyanensis* that the cure rate with miltefosine (71.4%) was higher than with meglumine antimoniate (51.6%) (Chrusciak-Talhari et al. 2011). There are also evidences that intrasional or subcutaneous administration of granulocyte and macrophage colony-stimulating factor (GM-CSF) enhances the cure rate of CL treated with meglumine antimoniate (Santos et al. 2004; Almeida et al. 2005). GM-CSF enhances phagocytosis and leishmania killing (Shi et al. 2006). Recently, we showed that monocytes from patients using miltefosine plus GM-CSF increased the respiratory burst, the ability to kill leishmania, and increased the proliferation of CD4 and CD8 cells (Peixoto et al. 2020).

The present study is part of a multicentric project aimed to determine if the combination of miltefosine with GM-CSF enhances the cure rate of CL caused by *L. braziliensis* or *L. guyanensis*. Here, we show data from a clinical trial comparing miltefosine plus GM-CSF versus miltefosine plus placebo versus meglumine antimoniate in the treatment of CL caused by *L. guyanensis*.

**Patients and methods**

**Type of study, case definition, and study population**

A randomized placebo-controlled trial with 3 groups of CL patients comparing the efficacy of oral miltefosine combined with topical placebo (M + P), oral miltefosine combined with topical GM-CSF, and intravenous meglumine antimoniate (control group). The study was registered at clinicaltrials.gov under NCT03023111. CL was defined by the presence of at least one ulcerated lesion in any part of the body, with documentation of the presence of Leishmania amastigotes in the slit skin smears obtained from the borders. This study included 150 CL patients and was conducted from June 2018 through February 2020 at the dermatology outpatient clinics of Heitor Vieira Dourado Tropical Medicine Foundation (FMTHVD) in Manaus, Amazon State. The majority of patients (83%) came from endemic areas located in the rural and suburban areas of Manaus municipality, 14.6% from other municipalities, and 2% from other states (Rondonia and Mato Grosso).

**Study design**

The inclusion criteria were age from 18 to 65 years, one to five ulcers ranging from 10 to 50 mm of diameter, the deviation of illness between 30 to 90 days, with no previous treatment. Exclusion criteria were the evidence of chronic diseases, patients with the diagnosis of HIV or in the use of immunosuppressive drugs, pregnancy or breastfeeding mothers, women in the fertile age who did not accept to use parenteral contraceptive, and subjects with no ability to understand or no desire to sign the informed consent. Patients were randomized according to a computer list obtained in www.randomization.com and allocated at a ratio of 1:1 into 3 groups, each with 50 patients. Group 1 was treated with miltefosine (Impavido™, Paesel + Lorei, Rheinberg, Germany) at a dose of 2.5 mg/Kg of body weight (maximum daily dose of 150 mg), 3 times a day for 28 days plus placebo (polycarbophil gel twice a day per 28 days). Group 2 was treated in the same dose of miltefosine plus topical GM-CSF (polycarbophil gel 0.01%, twice a day per 28 days). Patients were instructed to clean the ulcers with water and soap before applying the gel. Both the topical GM-CSF and placebo were produced by the 4 G Company, Porto Alegre, Brazil. The group 3 was treated with meglumine antimoniate (Glucantime®, Sanofi-Aventis) in the daily dose of 20 mg/kg by intravenous route once a day for 20 days. Women in childbearing age were included only after a negative beta HCG test to exclude pregnancy and used a parenteral contraceptive during 3 months. There recombiant human granulocyte and macrophage colony-stimulating factor (rhGM-CSF) was purified according to Schwanke et al. (2009). Ointments were prepared on 1.5% (w/w) aqueous polycarbophil gel containing 10 μg/g of rhGM-CSF. Placebo was prepared in the same way, without the rhGM-CSF. This concentration was the same used in our previous trials (Santos et al. 2004; Almeida et al. 2005), and we decided to use this different application formula and schedule to facilitate adherence and avoid additional visits to the clinics.
Laboratory procedures

Identification of amastigotes in tissue

Skin fragments were collected from the border of the ulcer by scarification and the smear was visualized by optical microscopy by Giemsa stain. Skin biopsies were performed in the lesion border by a 4-mm punch. The material was divided into two parts being half used for culture in the NNN medium and half added to RNAlater solution for PCR.

PCR for determination of leishmania species

DNA extraction was performed using a commercial Quiagen kit and molecular identification was performed from the polymerase chain reaction (PCR), with the help of the primers described by Marfurt et al. (5'-TAT TGC TAT GGC AAA CTT CCG-3' and 5'-ACA GAA ACT GAT GAT TAT ATA CCG-3') (REF). The DNA solution was amplified in a final volume of 25 μL, containing 10x PCR Buffer; 50 Mm Mgcl; 50 Mm of Fme; 50 Mm of Rme; 10 mM of dNTPs; and 2U of Taq polymerase. The PCR conditions were 5 min at 94 °C followed by 40 cycles of 30 s at 94 °C, 15 s at 55 °C and 10 s at 72 °C, finally, a single 5 min cycle at 72 °C. The PCR products were separated on a 2% agarose gel. The PCR product was then sequenced in an ABI 3730 analyzer (Applied Biosystems), and the sequences were identified by Genbank’s BLAST tool.

Laboratorial exams

All patients had blood taken on day 0 and 30 days to perform hemogram, urea, creatinine, blood sugar levels, transaminases (AST and ALT), and amilase.

Evaluation of response to therapy

Cure was defined by healing of the ulcers with complete reepithelization in the absence of infiltration of the borders at 90 (primary cure) and 180 (final cure) days after the initiation of treatment. Patients who fail therapy on day 90 were treated with meglumine antimoniate in the doses of 20 mg/Kg for 30 days.

Statistical analysis

The data with normal distribution were presented by means and standard/deviation and were analyzed by Student’s t test. Categorical data without normal distribution were presented by the median and interquartile intervals [25%–75%] and were analyzed by the Kruskal-Wallis test. The proportions were analyzed by the chi-square test of Pearson. The Kaplan-Meier survival analysis was done by the log rank test for trend. The intention to treat analysis (ITT) was used to determine the cure rate. The statistical analyses were performed by STATA/MP, V.13. A value of P < 0.05 was considered statistically significant.

Results

The majority of the 150 CL subjects were male (84% of cases), with age ranging from 18 to 65 years, presented with one ulcer (80%) localized mainly in lower limbs in 44.6% of cases and also in upper limbs (41.3%). These data did not differ according to treatment groups. Demographic and clinical and laboratory data are shown in Table 1. Ulcers were characterized by round shape, flattened erythematous borders, and granulous or crusted superficial center; size ranging from 30 to 105 mm² without differences between groups. Satellite lymph nodes with less than 1 cm of diameter were detected in 42% of patients in subjacent skin near ulcerations; small satellites papules near the ulcer were found in 28%. All patients had a positive direct skin slit smear showing many amastigotes in the cutaneous tissue. A positive culture for Leishmania sp. was obtained in 83% of subjects, and PCR for L. guyanensis was positive in 94% of the cases (Table 2).

Patients were followed up monthly for 90 days and also at 180 days after starting treatment. Seven losses were documented: one due to a severe adverse event (AE) in the Sb² group; six subjects did not return to follow up, two from group 3 (meglumine antimoniate), and four from group 1 (M + P).

Cure rates at 90 days were 66%, 58%, and 52% for groups M + P, M + GM, and meglumine antimoniate, respectively (p > 0.05). The cure rates at 180 days were the same for the first two groups and dropped to 50% for the meglumine antimoniate group (Table 3). Cure rates paired analyses comparing two groups each time did not show any significant differences for 90 and 180 days.

The healing time in days was similar between the three groups (Figure 1, Kaplan-Meyer Curve), ranging from 45 ± 22.48 ± 24, and 37 ± 14 for groups M + P, M + GM, and meglumine antimoniate, respectively. Paired analysis comparing two groups each time showed a faster healing time in the control group when compared with the group treated with Miltefosine + GM-CSF (p = 0.04).

Mild and transitory systemic AEs were recorded in up to 97% of patients using Miltefosine. The most common were nausea and vomiting (85% and 39%, respectively) in the first days of therapy, and no irregular use was reported. All patients were advised to use ondanetron 4 mg when necessary. In the control group, 86% of

Table 1
Demographic, Clinical, and Laboratorical Features of 150 Cutaneous Leishmaniasis Patients from the Amazon Region.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Miltefosine + Placebo (N = 50)</th>
<th>Miltefosina + GM-CSF (N = 50)</th>
<th>Sb²(N = 50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Median (Years)</td>
<td>39 (27–50)</td>
<td>40 (29–48)</td>
<td>42 (27–50)</td>
<td>&gt;.05²</td>
</tr>
<tr>
<td>Male/Female ratio</td>
<td>44 / 6</td>
<td>44 / 6</td>
<td>38 / 12</td>
<td>&gt;.05²</td>
</tr>
<tr>
<td>Illness duration, Median (Days)</td>
<td>30 (30–45)</td>
<td>30 (30–60)</td>
<td>30 (30–45)</td>
<td>&gt;.05²</td>
</tr>
<tr>
<td>Site of the lesion [%]</td>
<td></td>
<td></td>
<td></td>
<td>&lt;.05²</td>
</tr>
<tr>
<td>Head/Neck</td>
<td>4 (8)</td>
<td>3 (6)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Trunk</td>
<td>7 (14)</td>
<td>2 (4)</td>
<td>5 (10)</td>
<td></td>
</tr>
<tr>
<td>Upper limbs</td>
<td>12 (24)</td>
<td>25 (50)</td>
<td>25 (50)</td>
<td></td>
</tr>
<tr>
<td>Lower limbs</td>
<td>27 (54)</td>
<td>20 (40)</td>
<td>20 (40)</td>
<td></td>
</tr>
<tr>
<td>Highest lesion size, Median (mm²)</td>
<td>55 (30–105)</td>
<td>55.1 (30–100)</td>
<td>50 (30–105)</td>
<td>&gt;.05²</td>
</tr>
<tr>
<td>Number of lesions</td>
<td>38 (76)</td>
<td>41 (82.9)</td>
<td>41 (82.9)</td>
<td>&gt;.05²</td>
</tr>
<tr>
<td>1 lesion [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 or more lesions [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Satellite lymph node [%]</td>
<td>19 (41)</td>
<td>22 (44)</td>
<td>22 (44)</td>
<td>&gt;.05²</td>
</tr>
<tr>
<td>Positive PCR for L. guyanensis</td>
<td>29 (58)</td>
<td>27 (54)</td>
<td>36 (72)</td>
<td>&gt;.05²</td>
</tr>
<tr>
<td>Positive PCR for L. brasilensis</td>
<td>2 (4)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>&gt;.05²</td>
</tr>
<tr>
<td>Positive PCR for L. naiff</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>0</td>
<td>&gt;.05²</td>
</tr>
</tbody>
</table>

* Data represents Medians (Interquartile ranges). Kruskal-Wallis rank test.

² Data represents proportions (Chi square test).
Table 2
Cure Rate of Cutaneous Leishmaniasis Patients from the Amazonic Region Treated with Miltefosine Plus Placebo, Miltefosine Plus GM-CSF, and Meglumine Antimoniate with Intention to Treat Analysis.

<table>
<thead>
<tr>
<th>Response to Therapy</th>
<th>Miltefosine + placebo (n = 50)</th>
<th>Miltefosine + GM-CSF (n = 50)</th>
<th>Meglumine antimoniate (n = 50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cure on Day 60 (%)</td>
<td>27 [54]</td>
<td>28 [56]</td>
<td>27 [54]</td>
<td>ns*</td>
</tr>
<tr>
<td>Cure on Day 90 (%)</td>
<td>33 [66]</td>
<td>29 [58]</td>
<td>26 [52]</td>
<td>ns*</td>
</tr>
<tr>
<td>Cure on Day 180 (%)</td>
<td>33 [66]</td>
<td>29 [58]</td>
<td>25 [50]</td>
<td>ns*</td>
</tr>
<tr>
<td>Relapses (%)</td>
<td>0</td>
<td>0</td>
<td>1 [2]</td>
<td>ns*</td>
</tr>
</tbody>
</table>

* Data represents proportions (Chi square test).

Table 3
Frequency of Adverse Reactions in Patients Treated with Miltefosine or Meglumine Antimoniate.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Miltefosine [n = 97]</th>
<th>Meglumine antimoniate [n = 49]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>20 [21*]</td>
<td>16 [32.6]</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Fever</td>
<td>9 [9]</td>
<td>11 [22.4]</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>12 [12.3]</td>
<td>25 [51]</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Mialgia</td>
<td>14 [14.4]</td>
<td>21 [42.8]</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Nausea</td>
<td>83 [85.5]</td>
<td>9 [18.3]</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Vomit</td>
<td>38 [39.1]</td>
<td>6 [12.2]</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Others</td>
<td>42 [43.2]</td>
<td>25 [51]</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>None</td>
<td>3 [3]</td>
<td>7 [14.2]</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

* Chi square test.

Figure 1. Kaplan-Meier curve showing the proportion of Cutaneous Leishmaniasis patients not cured in the three groups of treatment.

cases present AEs: arthralgia in 51% and myalgia in 48% were the most common. AEs in the three groups are showed in Table 3. One subject in the meglumine antimoniate group had to stop medication due to a severe AE, with fever, exantheme, intense arthralgia, and the elevation of amilase four times the reference value.

Discussion

CL is predominantly caused by L. guyanensis in the Amazon region (De Guerra et al. 2015). The increasing deforestation of the urban area in the periphery of Manaus, the capital of the Amazonas State, with the settlement of houses close to the fragment of forests, has contributed to an increase in the number of cases in this municipality (Chagas et al. 2018). Therefore, the identification of new drugs is necessary to improve the cure rate, decrease the high healing time, reducing the absenteeism, and stigmatization. Although in a recent open trial, pentamidine in three weekly doses of 7 mg/kg was associated with a high cure rate (Gadelha et al., 2018), its parenteral use requires further visits to the clinics, which indicate the need for more practical therapeutic strategies, including oral drugs.

In a recent study, we showed that the cure rate with miltefosine was higher than that of the treatment in CL due to L. guyanensis (Chrusciak-Talhari et al. 2011). Furthermore, in CL caused by L. braziliensis, we have shown that topical GM-CSF associated with meglumine antimoniate enhances the cure rate of CL when compared with meglumine antimoniate plus placebo (Santos et al. 2004). In an attempt to improve the cure rate of CL due to L. guyanensis, we performed this clinical trial aimed to determine if miltefosine plus GM-CSF would be more effective than monotherapy with miltefosine or meglumine antimoniate. In the present study, the cure rate in the 3 groups of the study were similar.

In a clinical trial aimed to compare the response to therapy between different groups, it is important that patients had similar characteristics, as there are host factors associated with more or less response to therapy. The main risk factors for failure therapy in CL are age, as children have a greater failure rate (Suprien et al. 2020), size of the largest lesions, and more than 1 lesion (Llanos-Cuentas et al. 2007; Blum et al. 2012). Here, there were no differences among the 3 groups with regard to these specific variables. Others have found that CL characterized by satellite papules around the ulcer are associated with treatment failure (Chrusciak-Talhari et al. 2011). However, we did not find this association in the present trial.

The ITT analysis did not show a difference in the response to therapy between the groups. The group that uses miltefosine plus placebo or GM-CSF had a higher rate of cure than that of meglumine antimoniate but this difference did not achieve significance. The end points were cure on day 90 and on day 180 for initial and final cure, respectively. The results were similar at these 2 time points indicating that the cure on day 90 may be used as the end point for responses to therapy in future studies. As it is important to reduce the healing time of CL, we also compared the cure rate on day 60 but no differences were observed among the groups. The healing time did not show differences among the groups but patients treated with miltefosine plus GM-CSF had a higher healing time than patients treated with meglumine antimoniate. In addition to increasing phagocytes and leishmania
killing, GM-CSF down modulate the immune response by enhancing IL-10 levels (Mattia et al. 2009). In L. braziliensis infection, an exaggerated inflammatory response is associated with pathology and IL-10 may benefit therapy (Mattia et al. 2009). However, as in CL due to L. guyanensis there is a low lymphocyte proliferation, high expression of IL-10, and a T cell response toward type 2 cytokines; it is possible that these differences in the immune response may explain the lack of efficacy of GM-CSF to improve the miltefosine cure rate. Another possible explanation for GM-CSF failure could be a possible low penetration and action due to the formulation and schedule used in this trial that differ from the ones used in previous studies (Santos et al. 2004; Pacheco et al., 2005). However, immunological data from subjects using topical GM-CSF in this trial showed a higher ability of monocytes to kill leishmania and an increased proliferation of CD4 and CD8 when compared with the placebo group, which indicates that GM-CSF was absorbed and had systemic effects (Peixoto et al. 2020).

Despite therapy for CL taking less than 30 days, the healing time in the majority of the patients is only observed after 60 days from the initiation of therapy. Here, we evaluate by using Kaplan–Meier the healing time of not cured patients, and despite a trend of the group, M + P have a short cure time than the others, no difference among the groups was documented.

Adverse reactions are a major problem for therapy of CL. Pentavental antimony (meglumine antimoniate) is often associated with myalgia and arthralgia, and these manifestations may be severe, imposing the interruption of the treatment as observed in one of our cases. Moreover, the need of a parenteral route for the application of the drug decreases the adhesion to the treatment due to the limitations of access to the Health Posts. Miltefosine is known to cause nausea and vomits (Machado et al. 2010; Chruskiasky-Talhari et al. 2011). We have found that the preventive use of ondansetron contributed to a better control of these symptoms, as none of the patients using miltefosine have discontinued treatment due to it. The occurrence of herpes zoster has been reported in CL patients treated with sodium stibogluconate or meglumine antimoniate (Wortmann et al. 1998; Arboleda et al. 2013). In this trial, herpes zoster was also documented in the trunk of 3 patients in the meglumine group, who presented good and quick response to systemic acyclovir.

This study showed that miltefosine did not present a higher cure rate than meglumine antimoniate, and the association of GM-CSF did not improve the therapeutic response to miltefosine. Nevertheless, due to its less toxicity and easier administration, along with a similar cure rate when compared with meglumine antimoniate, miltefosine should remain as one of the main drugs for the treatment of CL due to L. guyanensis.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

This project was developed according to international ethical guidelines for biomedical research involving human beings (Resolution 466/12 CNS, 2012) and was approved by the Ethics Committee of the Tropical Medicine Foundation Dr. Heitor Vieira Dowrado (CAAE 808624173.0000.0005).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.11.183.

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