Activity of Hydroxyurea against *Leishmania mexicana*\(^\text{\textregistered}\)

Hugo Martinez-Rojano,\(^1,2\) Javier Mancilla-Ramirez,\(^1,3\) Laura Quiñónez-Diaz,\(^1,4\) and Norma Galindo-Sevilla\(^3\)*

Departamento de Posgrado, Escuela Superior de Medicina, Instituto Politecnico Nacional, Mexico City, Mexico;\(^1\) Departamento de Urgencias Pediatricas, Hospital de Gineco-Pediatrica 3A, IMSS, Mexico City, Mexico;\(^2\) Subdireccion de Investigacion Clinica, Instituto Nacional de Perinatologia, Mexico City, Mexico;\(^3\) and Division Academica de Ciencias de la Salud, Universidad Juarez Autonoma de Tabasco, Villahermosa, Tabasco, Mexico\(^4\)

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*Leishmania mexicana* is a protozoan parasite that causes a disease in humans with frequent relapses after treatment. It is also highly resistant to the currently available drugs. For this reason, there is an urgent need for more effective antileishmanial drugs. Hydroxyurea, an anticancer drug, is toxic to replicating eukaryotic cells and has been proven to be effective in arresting the *Leishmania major* cell cycle. In this study, hydroxyurea was tested in an in vitro model of intracellular *Leishmania* infection in macrophages. The parasite density in infected macrophages was measured by microscopy after incubation for various times and treatment with hydroxyurea at different concentrations. Viable parasites that could be transformed into promastigotes by shifting the temperature to 26°C were counted every other day after the replacement of hydroxyurea with fresh medium. Meglumine antimoniate, the standard drug treatment for *Leishmania mexicana*, was used as a reference drug under the same experimental conditions. Hydroxyurea completely eliminated *Leishmania* parasites when it was used at a dosage of 10 or 100 \(\mu\)g/ml. Differences in the length of treatment needed to achieve elimination were as follows: the 10-\(\mu\)g/ml doses required 9 days, while 3 days was sufficient when 100 \(\mu\)g/ml was used. Hydroxyurea had a 50% effective dose of 0.015 \(\mu\)g/ml in vitro, which was observed on day 6 after exposure. Hydroxyurea is highly effective in killing intracellular amastigotes in vitro.

The choices of drug therapy for human leishmaniasis include pentavalent antimonials, namely, sodium stibogluconate and meglumine antimoniate, which have been widely used since the 1940s, and amphoteracin B in its liposomal formulation. Pentamidine and paromomycin (2, 3, 8, 11) have also been used. The disadvantages of these drugs include their high cost, the need for long-term treatment, the lack of an oral formulation, and serious side effects that require intense patient monitoring (2). Furthermore, the emergence of antimony-resistant parasites has been reported (2, 16, 21); this has compelled a search for new antileishmanial agents. New therapeutic approaches with orally administered drugs are being investigated. The oral formulation of the alkylphosphocholine miftefiose has shown clinical efficacy against visceral leishmaniasis. However, it is not certain that miftefiose will be as useful in the treatment of diffuse cutaneous leishmaniasis or in immunocompromised patients coinfected with human immunodeficiency virus (HIV) (7); these patients may frequently have relapses of leishmaniasis after treatment.

Leishmaniasis is caused by *Leishmania*, a eukaryotic parasite with a rapid in vitro replication rate. This characteristic makes it extremely susceptible to anticancer drugs, such as miftefiose and acridine compounds (11, 22). In light of this, hydroxyurea, an antineoplastic agent that is able to arrest the *Leishmania major* cell cycle (34), was investigated in the study described in this paper for its possible action against *Leishmania mexicana*. In the more than four decades since the ribonucleotide reductase inhibitor hydroxyurea was first evaluated clinically, a number of diverse applications of the drug in malignant and nonmalignant diseases have been identified (5, 12, 24, 26).

The primary site of action of hydroxyurea is the ribonucleotide reductase enzyme. This enzyme catalyzes the reductive conversion of ribonucleotides into deoxyribonucleotides; and when it is inactivated, the reduction of intracellular concentrations of the deoxynucleoside triphosphates, the inhibition of DNA synthesis, the inhibition of DNA repair in quiescent cells, cell cycle arrest, and apoptosis are induced (26). Hydroxyurea is a radical scavenger and inactivates the R-2 subunit of ribonucleotide reductase by reducing its tyrosyl radical to a normal tyrosine residue by the transfer of one electron. The drug is specific for the S phase of the cell cycle, in which large amounts of deoxyribonucleotides are required and which is also when the DNA of the R-2 subunit is transcribed. As a consequence, the arrest of the cell cycle at the interface of the G\(_1\) phase-S phase is seen (26).

Hydroxyurea is conventionally an orally administered drug. Oral administration has the definite advantage of offering patients convenience (24). Additionally, hydroxyurea may be helpful for patients with leishmaniasis and compromised T-cell function, such as those infected with HIV (32), in whom infections may become difficult to treat and drugs must be used at higher concentrations and for longer periods of time (10). The aim of this study was to investigate the possible effects that hydroxyurea has on the survival and cell cycle of *Leishmania mexicana*.

*Corresponding author. Mailing address: Instituto Nacional de Perinatología, Subdirección de Investigación Clínica, Torre de Investigación, 4to. Piso, Montes Urales #800, Col. Lomas de Virreyes, Mexico City 11000, Mexico. Phone: 52 (55) 5520 9900, ext. 513. Fax: 52 (55) 5294 2949. E-mail: NGalindoSevilla@hotmail.com.

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**MATERIALS AND METHODS**

**Compound formulation.** A stock solution of hydroxyurea (CH\(_2\)_N\(_2\)O\(_2\); catalogue number H 8627; Sigma-Aldrich, St. Louis, MO) was made fresh at 10 mg/ml in Dulbecco’s modified Eagle medium (DMEM; Gibco, Grand Island, NY) without fetal calf serum (FCS; Gibco) and was diluted to the appropriate...
Concentrations of meglumine antimonate (Rhone-Poulenc Rorer, Mexico) were used as the reference drug. Each ampoule of 5 ml contained 1.5 g of meglumine antimonate, which corresponded to 0.405 g of pentavalent antimony at 81 mg/ml. The concentration of pentavalent antimonial compounds was calculated by weight over volume of pentavalent antimony.

**Parasites.** Reference strains *L. mexicana* MNYC/BZ/62/M379 and MHOM/MX/00/Tab3, isolated in Tabasco, Mexico, from a patient with diffuse cutaneous leishmaniasis (15), were used. The parasites were maintained as promastigotes at 26°C in DMEM (Gibco) containing 10% heat-inactivated FCS (Gibco) or were transformed to an amastigote-like form by setting the temperature at 32°C. Overnight incubation was enough to obtain complete transformation at neutral pH. The doubling time was about 12 h and was identical for both forms: promastigotes at 26°C and amastigote-like forms at 32°C. The parasites were also able to be transformed into promastigotes from amastigote-like forms just by changing the temperature from 32 to 26°C.

**Effects of hydroxyurea on Leishmania mexicana in a cell-free system.** The susceptibilities of the promastigotes or the amastigote-like forms of *Leishmania* to hydroxyurea were tested by culturing them in cell-free medium at 26 and 32°C, respectively. The efficacy of hydroxyurea was tested at 0.01, 0.1, 1, 10, and 100 μg/ml. Parasite density was counted with a hemacytometer every other day.

**Effects of hydroxyurea on intracellular macrophage infection by Leishmania mexicana.** To test the effectiveness of hydroxyurea on intracellular parasites, a macrophage monolayer was prepared in 24-well plates with peritoneal resident macrophages harvested from BALB/c male mice weighing 20 to 25 g. Macrophages were collected after irrigation of the peritoneal cavity with 10 ml of DMEM. After pooling of the macrophages from several mice, three washes were performed with cold medium; the macrophages were counted in a hemacytometer and adjusted to 10^6 mononuclear cells/ml. One milliliter of the cell suspension was placed on each sterile 24-well culture plate. The culture plates were incubated at 32°C under an atmosphere of 5% CO₂ for 24 h. Nonadherent cells were removed by washing the plates with prewarmed, sterile phosphate-buffered saline (PBS), and adherent cells were infected with 1 ml of 10^7 mid-logarithmic phase amastigote-like forms in DMEM plus 10% FCS. The cultures were incubated for 48 h at 32°C to allow the parasites to be internalized. The cultures were then washed three times with prewarmed PBS to remove extracellular parasites, and 900 μl of fresh medium was added to each well. The ratio cells in the intracellular infection was usually 10 parasites to 1 macrophage. Various concentrations of hydroxyurea (0.01, 0.1, 1, 10, and 100 μg/ml) were added at 100 μl to each well, as was meglumine antimonate as dosages of 0.01, 0.1, 1, 10, and 100 μg of pentavalent antimony/ml as a reference. The cultures were returned to 32°C under an atmosphere of 5% CO₂ for 3, 6, 9, or 12 days. After each of these times, the drug was removed and fresh medium was added. Subsequently, the plates were transferred to 26°C to promote the transformation of the parasites to the motile form and to cause their release from the macrophages. The parasites were counted with a hemacytometer on days 2, 4, 6, and 8 after drug removal. Each point was evaluated in triplicate. The percentage of growth inhibition was calculated by using the following formula: 100 × (Tc - Tm)/Tm, where Tc is the number of parasites/ml in the control wells and Tm is the average number of parasites/ml corresponding to each dosage on days 3, 6, 9, and 12 after drug exposure. The 50% effective dose (ED₅₀) was defined in this study as the drug concentration that reduced the survival of *Leishmania* parasites by 50%.

**Viability of macrophages assayed by trypan blue exclusion.** Macrophage survival in medium containing hydroxyurea (0.01, 0.1, 1, 10, and 100 μg/ml) was tested after 6 days of incubation by staining with trypan blue at a final concentration of 0.2% in phosphate buffer. Macrophage viability was verified by microscopic quantification of the number of viable macrophages among 100 macrophages.

**Viability of Leishmania under hydroxyurea exposure.** *Leishmania* survival in hydroxyurea was evaluated with the dye propidium iodide (PI) after incubation for 48 h with 1, 10, and 100 μg/ml of hydroxyurea at room temperature. The parasites were then washed three times with PBS containing 0.02 M EDTA and were then resuspended in 0.5 ml of PBS containing PI (25 μg/ml). The stained parasites were analyzed after 20 min in a fluorescence-activated cell sorter (EPICS-ALTRA flow cytometer; Beckman-Coulter, Fullerton, CA).

**Cell cycle analysis.** To demonstrate whether hydroxyurea affected the *Leishmania* cell cycle, parasites growing in mid-logarithmic phase were incubated for 48 h with 1, 10, or 100 μg/ml of hydroxyurea at 26°C. Afterward, the parasites were washed three times with PBS containing 0.02 M EDTA to avoid clumps and were then fixed with cold methanol for 24 h. The parasites were resuspended in 0.5 ml of PBS containing RNase I (50 μg/ml) and PI (25 μg/ml) and were then incubated at 25°C for 20 min. The material was kept on ice until analysis. The stained parasites were analyzed in a fluorescence-activated cell sorter.

**Statistical analysis.** Experiments were conducted four times, and the results of each experiment were analyzed individually. For each experiment, the data were recorded in triplicate and were analyzed for statistical significance by one-way analysis of variance (ANOVA). A probability (P) value of <0.05 was considered significant. The ED₅₀s were calculated by polynomial regression analysis.

**RESULTS**

**Effect of hydroxyurea on growth of Leishmania promastigotes in vitro.** The activity of hydroxyurea against promastigotes of *Leishmania* strains M379 and Tab3 was assessed in a cell-free system. A decrease in their growth was noticed when the parasites were treated for 48 h with 10 and 100 μg/ml of hydroxyurea. Six days of treatment resulted in promastigote survival rates of 11.6% for strain M379 and 3.1% for strain Tab3 (Fig. 1). An ED₅₀ of 0.05 μg/ml was observed for hydroxyurea for promastigotes when it was measured on day 6.

**Model of intracellular infection of Leishmania.** We sought to determine whether hydroxyurea was able to reach intracellular parasites in an in vitro model of intracellular infection on macrophage monolayers. By infecting macrophages with different numbers of amastigote-like forms grown at 32°C, different rates of infection were observed. To test the efficacy of hydroxyurea, a density of 10^7 parasites/ml was used to infect the monolayer because we expected that 90% of the adherent macrophages may become infected and that each cell would harbor approximately 10 intracellular parasites after 48 h of incubation (Table 1).

**TABLE 1. Infectivity of axenic mid-log-phase amastigote-like forms of Leishmania mexicana to mouse macrophages**

<table>
<thead>
<tr>
<th>No. of axenic amastigote-like forms (10^6/ml)</th>
<th>Ratio of no. of intracellular parasites to one host cell</th>
<th>% Infected cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.5:1</td>
<td>23</td>
</tr>
<tr>
<td>1</td>
<td>1:1</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>5:1</td>
<td>73</td>
</tr>
<tr>
<td>10</td>
<td>10:1</td>
<td>90</td>
</tr>
<tr>
<td>100</td>
<td>&gt;10:1</td>
<td>92</td>
</tr>
</tbody>
</table>
Effect of hydroxyurea on intracellular infection with *Leishmania* in vitro. The effects of different concentrations of hydroxyurea on intracellular amastigotes treated for 3, 6, 9, and 12 days are shown in Fig. 2. A notable inhibitory effect of hydroxyurea on *Leishmania* growth was confirmed in the intracellular model of parasitism by reading the promastigote density at the mid-log phase of growth on day 6 after hydroxyurea removal and a shift of the temperature to 26°C. For the lowest hydroxyurea dosage of 0.01 µg/ml, 44% inhibition was observed after 3 days of incubation, with a maximum of 67% inhibition observed after 12 days. With 0.1 µg/ml of hydroxyurea, 72% inhibition was seen after 3 days of exposure and a maximum of 85% inhibition was reached after 12 days. When 1 µg/ml was tested, 86% inhibition was observed after 3 days and a maximum of 94% inhibition was reached after 12 days of hydroxyurea incubation. With 10 µg/ml, the level of inhibition began at 93% after 3 days and went up to 98% after 12 days. Intracellular amastigotes could not survive a hydroxyurea dosage of 100 µg/ml, even when the parasites were exposed for only 3 days (ANOVA, *P < 0.001*). For strain M379, annihilation of the parasites was effectively achieved at a minimal dose of 10 µg/ml of hydroxyurea after 6 days of exposure. In the absence of parasites, macrophages were viable after 6 days of incubation, even in the presence of 100 µg/ml hydroxyurea.

Antileishmanial activity of hydroxyurea compared to that of meglumine antimoniate. Macrophages with intracellular amastigote infection were incubated with 0.01, 0.1, 1, 10, and 100 µg/ml of either hydroxyurea or meglumine antimoniate. Both drugs reduced the level of multiplication of *Leishmania*; however, the level of multiplication of *Leishmania* Tab3 parasites was reduced more by hydroxyurea, since more than 85% parasite growth inhibition was achieved with hydroxyurea at a dosage of 1 µg/ml on day 6 of exposure, whereas with meglumine antimoniate the level of reduction was 50% at the same dosage on day 6 of exposure (Table 2). The data show that maximum inhibition of both *Leishmania* strains was reached at 100 µg/ml of hydroxyurea. On day 6, ED$_{50}$ for intracellular amastigotes of 0.015 µg/ml for hydroxyurea (Fig. 3) and 0.95 µg/ml for meglumine antimoniate were observed. The results were similar for both *Leishmania* strains, with minimal differences. Strain M379 was more susceptible to hydroxyurea than strain Tab3.

![Graph showing the effects of hydroxyurea on parasite density](Image)

**FIG. 2.** Effects of hydroxyurea on *Leishmania* in a model of intracellular infection. *Leishmania*-infected mouse peritoneal macrophages were treated with different concentrations of hydroxyurea (0.01, 0.1, 1, 10, and 100 µg/ml) for 3, 6, 9, or 12 days and incubated at 32°C to sustain the intracellular amastigote-like form of the parasite. Subsequently, hydroxyurea was removed and the culture temperature was changed to 26°C to support Leishmania transformation to extracellular promastigotes. The growth curves for *Leishmania* were plotted for each hydroxyurea concentration and duration of treatment. The results presented here contain only the values of parasite density on day 6 after hydroxyurea elimination. Open bars, 3 days of treatment; dotted bars, 6 days of treatment; hatched bars, 9 days of treatment; solid bars, 12 days of treatment. The results were obtained from three experiments performed in duplicate and are shown as means ± standard errors of the means. *P < 0.001*, ANOVA.

![Graph showing the effects of various concentrations of hydroxyurea on the rate of growth of *Leishmania*.](Image)

**FIG. 3.** Effects of various concentrations of hydroxyurea on the rate of growth of *Leishmania*. Intracellular amastigotes were tested with hydroxyurea or meglumine antimoniate while they were inside the adherent macrophages. The cultures were maintained for 6 days at 32°C, and then hydroxyurea or meglumine antimoniate was replaced with fresh medium, without drug, and the 24-well plates were incubated at 26°C. Six days after the removal of the hydroxyurea or meglumine antimoniate, the parasite density was counted in a hemacytometer. The data are expressed as the rates of inhibition relative to the rate for the untreated control. For promastigotes, data were obtained on day 6 of parasite culture at 26°C in the presence of hydroxyurea. For intracellular amastigotes exposed to hydroxyurea, intracellular amastigotes exposed to meglumine antimoniate; promastigotes exposed to hydroxyurea. The ED$_{50}$ was measured on day 6 after hydroxyurea elimination for intracellular amastigotes in hydroxyurea at 0.015 µg/ml and intracellular amastigotes in meglumine antimoniate at 0.95 µg/ml. For promastigotes, the ED$_{50}$ was measured in the presence of hydroxyurea at 0.05 µg/ml.

![Table showing inhibition of *Leishmania mexicana* growth by hydroxyurea and meglumine antimoniate in an intracellular in vitro infection](Image)

**TABLE 2. Inhibition of *Leishmania mexicana* growth by hydroxyurea and meglumine antimoniate in an intracellular in vitro infection**

<table>
<thead>
<tr>
<th>Compound and concn (µg/ml)</th>
<th>MHOM/MX/00/Tab3</th>
<th>MNYC/BZ/62/M379</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyurea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Meglumine antimoniate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>10</td>
<td>54</td>
<td>69</td>
</tr>
<tr>
<td>25</td>
<td>77</td>
<td>93</td>
</tr>
</tbody>
</table>

*Parasites were counted after 6 days of drug exposure and the subsequent 8 days of growth at 26°C. The results reflect the averages of five different experiments.*
Effect of hydroxyurea on viability of Leishmania promastigotes. Parasite viability after hydroxyurea treatment was evaluated by flow cytometry after 48 h of drug exposure. After staining of the parasites with PI, it was determined that viability was dose dependent. Increases in the percentages of parasites with permeable membranes in the presence of higher concentrations of hydroxyurea were observed and were 1% for the control parasites (no treatment), 12% for parasites treated with 1 μg/ml of hydroxyurea, 26% for those treated with 10 μg/ml, and 55% for those treated with 100 μg/ml (Fig. 4). In parallel with this experiment, a reduction in the parasite density was also observed as the dosages of hydroxyurea increased. Furthermore, hydroxyurea treatment of the parasites was carried out for 48 h. After this length of exposure, 100 μl of the cell culture was moved to 8 ml of fresh medium. After 8 days, the parasite densities were $62 \times 10^6$ parasites/ml for the control and $8.5 \times 10^6$ parasites/ml, $4.3 \times 10^6$ parasites/ml, and 0 parasites/ml for the parasites treated with hydroxyurea at doses of 1, 10, and 100 μg/ml, respectively. The level of parasite growth was less than expected, as judged from their viabilities.

Cell cycle arrest of Leishmania by hydroxyurea. Hydroxyurea at 10 and 100 μg/ml induced the arrest of the G2 phase-M phase of the cell cycle in Leishmania mexicana after 48 h of incubation; no effect on the cell cycle was observed with 1 μg/ml (Fig. 5). The dosages that caused disruption of the cell cycle were considered quite high compared to the concentrations used to arrest the cell cycle of the parasite at G1 phase-S phase, and 48 h was considered a long exposure time (34).

DISCUSSION

Hydroxyurea has in vitro activity against both the promastigote and intracellular amastigote forms of L. mexicana and at a dosage of 10 or 100 μg/ml is able to completely eliminate the parasite within a relatively short period of treatment. The reductions in the survival rates for both amastigotes and promastigotes were significant after day 3 of exposure for all dosages tested. The effect of hydroxyurea against amastigotes and promastigotes was dose and time dependent; however, 6 days of treatment of both forms of the parasite proved to be
sufficient, with ED₅₀ of 0.015 μg/ml (0.2 μM) for amastigotes and 0.05 μg/ml (0.65 μM) for promastigotes. This also demonstrated that hydroxyurea works with similar efficacies against both forms of the parasite. The effect of hydroxyurea against *Leishmania mexicana* was shown to be more effective than that against *Plasmodium falciparum*, which has an ED₅₀ of 792 μM (18), and to have a 50% inhibitory dose for antitumor activity in vitro of 500 μM (13).

Hydroxyurea reportedly affects the cell cycle by arresting the G₁-phase-S phase (20, 34), characterized by DNA replication, in Saccharomyces cerevisiae yeasts (20), vascular smooth muscle cells (4), and Leishmania major (34). Inhibition of DNA synthesis by inhibition of an enzyme, ribonucleotide reductase, represents the most probable explanation for the mechanism by which hydroxyurea inhibits the parasite at this level (26). It is important to note that the time of hydroxyurea exposure needed to arrest the cell cycle of the parasite and to allow it to recover after drug removal was usually 1 to 2 h. However, some organisms, such as yeast, displayed an arrest in the G₂ phase-M phase after 8.5 h of hydroxyurea exposure (1); this was the case here in the presence of high hydroxyurea concentrations of 10 and 100 μg/ml. Under these conditions, the mechanism may shift to the rescue of DNA replication by inducing the MluI cell cycle box-containing genes (19). At the same time, replication forks may be altered by hydroxyurea, leading to a size increase and the asymmetric accumulation of single-stranded DNA, which would subsequently impede replication after drug removal (27). The mechanism by which hydroxyurea inhibits *Leishmania* is different from that described for miltefosine, the other anticancer drug commonly used to treat visceral leishmaniasis. Miltefosine works by inhibiting the phospholipids and sterol biosynthesis of trypanosomatids (28).

Hydroxyurea is conventionally administered orally, and this has a definite advantage of convenience for the patient; it is also practical in areas with few resources. In humans, hydroxyurea is readily absorbed from the gastrointestinal tract. The concentrations in plasma then peak at 0.8 mM (14), 0.26 mM (17), and 0.135 mM (30) at 1 to 2 h after the administration of oral doses of 2,000, 1,200, and 500 mg, respectively. The half-life in plasma is about 2 h. Approximately 80% of the drug is recovered in the urine within 12 h following oral or intravenous administration (17, 24). These dosages are used for actual clinical practice in the treatment of HIV (17, 30), glioblastoma multiforme (12,) and myeloproliferative disorders (5).

Perhaps the most significant recent advance in the treatment of leishmaniasis has been the effective oral treatment of visceral leishmaniasis through the use of miltefosine, an alkylphosphocholine originally developed as an anticancer drug. A major limitation of miltefosine is perhaps the various therapeutic responses of *Leishmania* species from the New World to the drug that have been reported both in vitro and in vivo (28, 33). An *L. mexicana* isolate from Peru was insensitive to miltefosine in a macrophage-amastigote model (33), as well as clinical cases of cutaneous leishmaniasis in Guatemala, where *L. mexicana* and *L. braziliensis* are common; these clinical cases were, however, less responsive than clinical cases in Colombia (28). Observations from India suggest that patients with relapses showed resistance to miltefosine (29). Furthermore, miltefosine offers limited efficacy for the treatment of diffuse cutaneous leishmaniasis (7). In addition, laboratory studies have predicted that multidrug resistance may affect sensitivity to miltefosine and its analogs (9). For these reasons, other drugs, such as hydroxyurea, might be favored for testing in additional preclinical studies in order to evaluate their possible use as alternatives to miltefosine.

Meglumine antimoniate was used as a reference control during the development of this study because it was previously reported to be effective against *L. mexicana*, *L. infantum*, *L. tropica*, and *L. donovani* infections in human monocye-derived macrophage cultures (6, 23, 25). The concentration used to treat the infection in this study is based on the findings of previous studies as well as the approximate levels in the plasma of humans treated with this drug (6). When the efficacy of hydroxyurea was compared with that of meglumine antimoniate, the former was shown to be more effective in eliminating parasites.

To our knowledge, the use of mouse peritoneal macrophages for drug susceptibility tests with *Leishmania* and the transformation of *Leishmania* amastigotes to promastigotes is still relatively unexplored (6, 14, 23, 31, 33). Once these methods are established, they may provide an opportunity to test drugs in a model that more accurately resembles the conditions in the host and may provide other advantages, such as accessibility and reproducibility.

The transformation of *Leishmania* amastigote-like forms to promastigotes did not seem to be affected by hydroxyurea, once the parasites were able to transform from amastigote-like forms to promastigotes, as long as the cell culture temperature changed from 32 to 26°C.

In conclusion, hydroxyurea is a good candidate for drug therapy for leishmaniasis because it induces parasite death and cell cycle arrest in the G₂ phase-M phase when it is used at concentrations ranging from 10 to 100 μg/ml.
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