**Anti-leishmanial activity of 29 daily sessions of intralesional-pentavalent antimony administration on Leishmania (Viannia)-infected BALB/c mice**

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**ABSTRACT**

**Introduction:** Intralesional-pentavalent antimonials (IL-Sb⁵) are recommended for simple cutaneous leishmaniasis (CL). Few treatment sessions (1-5) and drug volumes (1-5 ml each), relative to lesion size (LS), are recommended. There is not a validated IL-Sb⁵ protocol using doses calculated as mg/kg body weight and administered over a large number of IL-sessions, with small injection volumes.

**Objective:** The study aim was to determine the efficacy of different concentrations of IL-Sb⁵ administered in 29 daily sessions of 100 μL each, on CL infected mice.

**Methods:** Leishmania (Viannia) panamensis and L. (V.) braziliensis-infected mice (N = 6) were treated with 150, 50, and 16.6 mgSb⁵/kg/day x 29 days. Percentage of lesion area reduction, aesthetic and final (no lesions, no parasites) efficacy and effective dose (ED₅₀) were determined. In vitro-Sb⁵ activity against parasites was evaluated for both species.

**Results:** The ED₅₀ values were 72.2 and 66.3 (at the end of treatment), 54.3 and 37.7 (15-days pt.), and 145.3 and 148.6 (60-days pt.) for each species, respectively. Differences were observed between Leishmania species at 15-days pt., but not later. At 60-day pt., IL-Sb⁵-150 mg showed final cure rates of 66.6 % for L. (V.) panamensis and 33.3 % for L. (V.) braziliensis-infected mice. After 15 days pt., lesion reactivation was observed in some “aesthetically cured” mice. Glucantime was not active in in vitro assays.

**Conclusions:** The IL-Sb⁵ use with a dose calculated as mg/kg body weight and administered over a large number of IL-sessions, with small injection volumes each day could be effective against L. (V.) panamensis and L. (V.) braziliensis-CL infection. An appropriate Sb⁵-dose (higher than 150 mg/kg/day x less than 29 days) must be evaluated.

**Key words:** Glucantime; New World cutaneous leishmaniasis; intralesional drugs; Leishmania panamensis; Leishmania braziliensis.
of the genus *Lutzomyia* (Pan American Health Organization, 2018). Symptomatic NW-CL is characterized by skin lesions, such as papules, nodules, or ulcerative volcano-like lesions, with varied outcomes, in terms of lesion number and size, scar type, disability, spontaneous healing, and destructive mucosal inflammation (Bailey et al., 2017).

Pentavalent antimonials (SbV, Glucantime, and Pentostam) remain the standard treatment for all forms of leishmaniasis in some countries (Burza, et al., 2018). They are administered parentally in 10-30 doses of 20 mg/kg/day, depending on the clinical manifestations. Mild to severe side effects, including arthralgia, myalgia, cardiotoxicity, nephropathy, and pain during intramuscular administration, are observed (Burza et al., 2018; Goto & Lindoso, 2010). In some regions endemic for visceral leishmaniasis, such as Bihar, in Eastern India, therapeutic failure (TF) can be observed in up to 65 % of cases, while estimates of antimonial TF rates on NW-CL range from 15-32 % (Castro, et al., 2017; Sundar et al., 2000). In cases with simple CL (single or a few lesions, up to 900 mm²), pregnant women, and patients with kidney, heart disease, or other concomitant diseases, local/topical treatments, such as cryotherapy, localized heat, intralesional drugs, or topical application of semisolid formulations directly onto the lesion are alternative therapies (Burza et al., 2018).

Intralesional (IL)-SbV treatment is widely used in treatment of Old World-CL, and is applied alone, or in combination with cryotherapy. Further, intralesional (IL)-SbV therapy is recommended for simple NW-CL cases, where lesions do not involve the face or joints (PAHO, 2018). Intralesional-SbV injection is easily administered by trained staff, does not require hospitalization, can be given at a low dose, and results in less systemic drug absorption, side effects, and treatment costs; 1-5 sessions of intradermal IL-SbV injections, with 1-5 ml per session, at the border or base of the CL-lesion every 3-7 days are recommended (PAHO, 2018). The overall clinical efficacy of IL-SbV using an injectable Glucantime solution (the same one used for intramuscular route treatment) is reported as 75 to 77 % (de Oliveira Duque et al., 2019; Oliveira-Neto et al., 1997). In Bolivian patients with CL, three or five sessions of 2-3 ml each (650 μg SbV per mm²), every other day resulted in cure rates from 57 to 73 % at 6 months (Soto et al., 2016). Overall TF was 30 % (like parenteral SbV) and no recurrences were recorded in cured patients for ≥ 6 months (de Oliveira Duque et al., 2019; Oliveira-Neto et al., 1997). There is not a validated IL-SbV protocol using doses calculated as mg/kg body weight and administered over a large number of IL-sessions, with small injection volumes in humans. In addition, there is not an IL-SbV protocol available as a positive control for NW-CL intralesional drug discovery able to demonstrate both efficacy and some SbV-IL risks such as lesion reactivation and TF on experimental models.

The present study aim was to determine the efficacy of different concentrations of IL-SbV (Glucantime) administered in 29 daily sessions of 100 μL each, on mice infected with two common NW-CL species, *L. (V.) panamensis* and *L. (V.) braziliensis*.

**MATERIALS AND METHODS**

**Drugs and reagents:** Meglumine antimoniate, Glucantime® (Sanofi-Aventis, Brazil; lot 357345) was kindly donated by the Secretary of Health of Santander, Colombia. One bottle of Glucantime contained 1.5 g of meglumine antimoniate (405 mg of SbV). Schneider’s insect medium, RPMI medium, and heat inactivated FBS (hiFBS) were from Gibco (Grand Island, NY, USA).

**Parasites and cells:** Promastigotes of *L. (V.) panamensis* (MHOM/PA/71/LS94) and *L. (V.) braziliensis* (MHOM/BR/75/M2903) were maintained in Schneider’s medium, RPMI medium, and heat inactivated FBS (hiFBS) were from Gibco (Grand Island, NY, USA).
phorbol 12-myristate 13-acetate for 48 h, and these macrophages infected with stationary-phase promastigotes at a cell: parasite ratio of 1:5 for 24 h at 32 °C. The percentage of infection was determined microscopically by Giemsa staining. Axenic amastigotes were obtained from late-phase promastigotes after 8 days of transformation by progressive changes of temperature (from 27 to 32 °C) and pH (from pH 7.0 to 5.5). The *Leishmania* species used were susceptible to miltefosine, pentamidine and ketoconazole (Neira, et al., 2019a).

**Animals, ethics:** Female and male BALB/c mice (10-12 weeks of age) were supplied by the National Health Institute (Bogotá, Colombia). Mice were housed with a 12 h light/dark cycle, at 23 °C, 55 % ± 5 % relative humidity, with access to water and food pellets *ad libitum*. Studies were approved by the UIS-Ethics Committee (CIENCI, Code 17-2017). Animals were anesthetized by intraperitoneal injection of a ketamine/xylazine cocktail and euthanized by cervical dislocation.

**Anti-leishmanial activity in vivo:** Mice were infected by subcutaneous injection in the shaven rump with $5 \times 10^5$ stationary-phase parasites. When lesion size (LS) was in the range 40-42 mm² (8 weeks after infection), mice were randomly allocated into four groups ($N = 6$ per group); different concentrations of SbV (150, 50, and 16.6 mg SbV/kg body weight/day) and vehicle (0.9 % saline solution) (100 µL) were injected intraleisionally (IL) for 29 days. Doses of SbV were calculated for mice using mean body weight (27 g), containing 4.1, 1.4, and 0.4 mg SbV per mouse and a total of 117.5, 39.2, and 13.9 mg SbV/mouse over 29 days. Lesion size was measured weekly using a digital calliper and lesion area (mm²) calculated (Neira et al., 2019b). Follow-up period was determined in a preliminary experiment (Appendix 1). Briefly, *Leishmania* infected mice ($N = 1$ per species) were treated with IL-SbV-150 mg/kg/day × 29 days and sacrificed 15, 22, 29, and 42-days post-treatment. The chosen follow-up time was 60 days post treatment (pt.).

At the end of the experiment, animals were sacrificed, and smears (imprints) prepared from lesions fixed in methanol and stained with Giemsa for detection and calculation of parasites by microscopy. Additionally, lesion samples were collected and processed for histopathological examination. Amastigote number per microscopic field (400×) was semi-quantitatively scored [No parasites = 0, scarce = 1-5 (+), moderate = 6-10 (++) , abundant > 11 (+++) parasites] and percentage LS reduction (LSred) calculated (Neira, et al.,2019b). Aesthetic efficacy (eE) was calculated as (N mice with > 75 % of LSred /6 mice) × 100, and final efficacy (fE) as (N mice with both 100 % of LSred and no parasites/6) × 100. Reactivation was defined as the appearance of a new lesion in a previously aesthetically 100 % healed site. Mean effective dose (ED)₅₀ (dose of SbV able to reduce LS by 50 %) with 95 % confidence interval (95 % CI) was calculated by sigmoidal regression using MsxIstfTM software.

**Adverse effects:** Mice weight was measured weekly using a digital balance. Skin irritation was registered by visual inspection and signs of irritation at application sites classified from 0 = no irritation to 4 = severe irritation.

**Histopathological analysis:** Samples were fixed with 10 % neutral formalin, embedded in paraffin, and sectioned into 5 µm thick sections using a microtome. Dewaxed slices were stained with haematoxylin-eosin and examined by microscopy. Histopathological parameters and parasites were semi-quantified and scored as follows: -, absent; +, mild; ++, moderate; and ++++, severe (Neira et al., 2019b).

**In vitro assay:** Promastigotes and axenic amastigotes were incubated with serial 1:3 dilutions of Glucantime (33.3 to 900 µg/ml) for 72 h at 27 and 32 °C, respectively (Gupta et al., 2001). Control cells were incubated in culture medium. Drug activities were assessed using a resazurin colorimetric test. Absorbance values were measured using a Synergy H1 microplate reader at 570 and 600 nm. For
intracellular parasites, infected THP-1 cells were treated with Glucantime for 5 days, at 32 °C (Neira et al., 2019a). Parasite growth inhibition was determined by counting infected and non-infected cells on Giemsa-stained slides by microscopy. IC\textsubscript{50} values were calculated as described for ED\textsubscript{50}.

**Statistical analysis:** Differences were analyzed using the Student’s t-test. P values ≤ 0.05 were considered statistically significant. Area under the curve values were calculated for comparisons of Sb\textsuperscript{V} dose-response effects using GraphPad Prism software, version 6.0 for Windows and two-way ANOVA and Sidak post-hoc method.

**RESULTS**

**Efficacy of treatment with IL-Sb\textsuperscript{V}-150 mg/kg x 29:** For *L. (V.) panamensis* infection, E of 83.3 % (5/6 mice; M1–M4, M6) and LS\textsubscript{red} values of 87.0-100 % were observed both at the end of treatment and 15-days pt. At 60-days pt., LS reactivation (Re) was observed in mouse M6 (Table 1, Fig. 1). Therapeutic failure was also observed in mouse M5 failure. At the end of treatment and until 43-day pt., M5 failure showed LS\textsubscript{red} of almost 50 %; however, a subsequent increase of LS by almost 8 times was observed (Table 1). In conclusion, 29 doses of IL-Sb\textsuperscript{V}-150 mg induced a \( \bar{E} \) value of 66.6 % in *L. (V.) panamensis*-infected mice.

For *L. (V.) braziliensis* infected mice, an \( \bar{E} \) value of 83.3 % (5/6 mice, M1, M2, M4, M5, M6) and LS\textsubscript{red} from 80.9 to 100 % were observed both at the end of treatment and at 15-days pt. At 50-day pt., lesion reactivation was detected in mice M4, M5, and M6. Additionally, mouse M2, an aesthetically cured animal, tested positive for parasites in biopsy samples (Table 2, Fig. 2). Mouse M3 showed a slight decrease in LS at 15 days pt. and a complete aesthetic response, without observable parasites, at the end of the experiment. Consequently, \( \bar{E} \) was 33.3 %.

All non-cured mice showed abundant (+++) intra and extracellular amastigotes in CL-lesion samples.

None of the mice treated with 29 doses of IL-Sb\textsuperscript{V} showed body weight loss, skin irritation, or any signs of pain or loss of well-being.

**Efficacy of lower doses (IL-Sb\textsuperscript{V}-50 mg/kg x 29 days and IL-Sb\textsuperscript{V}-16.6 mg/kg x 29 days):** At 15-day pt., *L. (V.) panamensis* infected mice M4, M5, and M6 treated with IL-Sb\textsuperscript{V}-50 mg, displayed considerable

<table>
<thead>
<tr>
<th>Mice</th>
<th>Before</th>
<th>29-doses-IL-Sb\textsuperscript{V}-150 mg on <em>L. (V.) panamensis</em> infected mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm\textsuperscript{2}</td>
<td>LS\textsubscript{red} (%)</td>
</tr>
<tr>
<td>M1</td>
<td>36.6</td>
<td>3.5</td>
</tr>
<tr>
<td>M2</td>
<td>35.4</td>
<td>0</td>
</tr>
<tr>
<td>M3</td>
<td>35.2</td>
<td>0</td>
</tr>
<tr>
<td>M4</td>
<td>45.9</td>
<td>6.2</td>
</tr>
<tr>
<td>M5</td>
<td>50.3</td>
<td>35.2</td>
</tr>
<tr>
<td>M6</td>
<td>46.7</td>
<td>3.1</td>
</tr>
<tr>
<td>SS</td>
<td>13.8</td>
<td>28.7</td>
</tr>
</tbody>
</table>

The table shows details of individual lesion size (LS) before and at the end of treatment and at 15 and 60 days post treatment (pt.) (N = 6). Parasite loads were scored at the end of treatment. LS\textsubscript{red}: lesion size reduction; \( \bar{E} \): aesthetic efficacy; \%E: percentage of mice with LS\textsubscript{red} between 75-100 %; \( \bar{E} \): final efficacy: with a complete aesthetic and parasite response; SS: saline solution (a representative control mouse); Re: reactivation; ND: not determined.
Fig. 1. Response of mice infected with *L. (V.) panamensis* to IL-SbV-150 mg/kg/day x 29. Photographs of CL lesions in mice M1-M6 at the beginning, 15 days and at the end of treatment (29 days); and at 15 and 60 days pt.

improvement ($LS_{\text{red}}$ 89.5-100 %) (Appendix 2, Appendix 3) and stable effects were also observed in mice M1, M2, and M3 ($LS_{\text{red}}$ 43.7-58.4 %); however, at 60-day pt., reactivation or increase of LS was observed. In mice treated with IL-SbV-16.6 mg, $LS_{\text{red}}$ values from 0 to 51.6 % were observed both at the end of treatment and 15 days pt.; however, at the end of the experiment, $\bar{E}$ was zero and abundant (+++) parasites were observed by microscopy in imprint smears and biopsies (Appendix 2, Appendix 4).

For *L. (V.) braziliensis* infected mice, IL-SbV-50mg treatment induced $LS_{\text{red}}$ of 80-100 % in mice M2 and M4-M6 at 15 days pt.; however, reactivation occurred in two of these mice (M2 and M6) and there was an increase of LS in another (M4) with parasites present in all of
them, including M5 (Appendix 5, Appendix 6). Treatment with IL-Sb\textsuperscript{V}-16.6mg induced a LS\textsuperscript{red} of 87.2 % in one mouse (M6) at 15 days pt.; however, TF occurred at 60-day pt. Parasites were observed in lesions from all treated mice by microscopy (Appendix 5, Appendix 7).

**Cutaneous leishmaniasis lesions after relapse (Re):** Mouse M6\textsubscript{Re} treated with IL-Sb\textsuperscript{V}-150 mg and mice M4\textsubscript{Re} and M6\textsubscript{Re} treated with IL-Sb\textsuperscript{V}-50 mg after infection with *L. (V.) panamensis* had new lesions of LS of 31.6, 11.6, and 3.5 mm\textsuperscript{2}, respectively, at the end of the experiment. Mice M4\textsubscript{Re}–M6\textsubscript{Re}, treated with IL-Sb\textsuperscript{V}-50 mg and M2\textsubscript{Re} and M6\textsubscript{Re}, with IL-Sb\textsuperscript{V}-16.6 mg, following *L. (V.) braziliensis* infection, relapsed with new lesions of LS 30.6, 11.9, 8.6, 7.8, and 20.0 mm\textsuperscript{2}, respectively, at the end of the experiment. Lesions reappeared at the inoculation site and began as papules,
that gradually evolved into nodules with raised edges. In some cases, they presented as typical clean, pink ulcers, with granular tissue, and rounded with regular and raised edges.

Dose response activities of SbV:
The mean effective dose (ED)_{50} values for L. (V.) panamensis and L. (V.) braziliensis infected mice were 33.99 (CI, 18.89-61.14) mg and 36.96 (CI, 17.87-76.45) mg at the end of treatment; 28.62 (CI, 16.53-49.55) mg and 12.40 (CI, 4.41-34.91) mg at 15 days pt.; and 142.6 (CI, 57.56-353.3) mg and 157.8 (CI, 58.99-421.9) mg at 60 pt., respectively. At 43 days pt., IL-SbV was more active in mice infected with L. (V.) braziliensis than L. (V.) panamensis (P < 0.001); however, no difference was observed at the end of the experiment (60 days pt.). No significant differences were detected in the response to IL-SbV between mice infected with the two species of Leishmania based on analysis of LS or area under the curve values for lesion changes after treatment with at 150, 50, or 16.6 mg/kg/day IL-SbV × 29.

**Histopathological characteristics:**
Responders (cured) mice (M1–M4 for L. (V.) panamensis; M1 and M3 for L. (V.) braziliensis) showed none to mild changes in the epidermis and dermis (Appendix 8, Appendix 9, Appendix 10). In contrast, M5 failed or M6 for L. (V.) panamensis, M4–M6 for L. (V.) braziliensis, and negative control mice presented with severe inflammatory infiltrates, comprising lymphocytes, neutrophils, macrophages, and giant cells. Some mice presented with severe acanthosis and moderate spongiosis and necrosis (Appendix 8).

**In vitro drug activity:** Based on IC_{50} values, SbV was inactive against all live forms of parasites evaluated, since IC_{50} values were > 900 µgSbV/ml for both Leishmania strains.

**DISCUSSION**
In this work, we evaluated the responses of CL-infected mice to Glucantime, administered over a large number of IL-sessions, with small injection volumes each day (e.g., not until lesions blanched) and SbV regimens calculated by body weight (kg), instead of LS (mm²). Administration of the highest dose of IL-SbV (equivalent to 98.78 µg SbV/mm² × 29 daily, and 2 864.6 µg SbV/mm² in total) was effective in 4/6 and 2/6 mice infected with

### TABLE 2
**Efficacy of 29 doses of IL-SbV at 150 mg/kg in mice infected with L. (V.) braziliensis**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Before (mm²)</th>
<th>End of treatment</th>
<th>15-day pt.</th>
<th>60-day pt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS red (%)</td>
<td>E (%)</td>
<td>LS red (%)</td>
<td>E (%)</td>
</tr>
<tr>
<td>M1</td>
<td>55.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M2</td>
<td>34.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M3</td>
<td>14.5</td>
<td>23.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M4</td>
<td>32.8</td>
<td>6.28</td>
<td>80.9</td>
<td>83.3</td>
</tr>
<tr>
<td>M5</td>
<td>35.7</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>M6</td>
<td>43.1</td>
<td>0</td>
<td>100</td>
<td>0</td>
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<tr>
<td>SS</td>
<td>13.65</td>
<td>49.64</td>
<td>0</td>
<td>ND</td>
</tr>
</tbody>
</table>

The table shows details of individual lesion size (LS) before and at the end of treatment and at 15 and 60 days’ post treatment (pt.) (N = 6). Parasite loads were scored at the end of treatment. LS red: lesion size reduction; E: aesthetic efficacy; %E: percentage of mice with LS red between 75-100 %; E, final efficacy: with a complete aesthetic and parasite response; SS: saline solution (a representative control mouse); Re: reactivation; ND: not determined.
L. (V.) panamensis and L. (V.) braziliensis at 60 days pt.

Some positive results were obtained with IL-SbV in a hamster CL model (100% lesion reduction and no parasites in organs) (Travi, et al., 1993; Yépez, et al., 1999); however, no complete cure of CL-infected mice has been documented. Per example, treatment of mice infected with L. (L.) amazonensis, with IL-SbV 28 mg/kg/day × 5, every 4-5 days resulted in partial parasite burden reduction with no increase in footpad thickness, but not in cure (Cos et al., 2018; Fournet et al., 1996). Differences in numbers of SbV-IL sessions, dose, site of infection, parasite species, and follow-up time could explain the different results obtained in this study. In addition, type of CL lesion could be also involved; ulcerated lesions (Fig. 1, Fig. 2) without parasite dissemination or spontaneous cure are characteristic of our CL-mouse model (Neira, et al., 2019b). The final SbV doses/per mouse (at a body weight, 27 g) also differed. At 150, 50, and 16.6 mg/kg/day × 29, the final doses were 117.5, 39.2 and 13.9 mg SbV, while at 28 mg/kg × 5 the dose was 3.78 mg 224 SbV (almost three times less than the minimum non-effective dose used in our study).

IL-SbV treatment induced a dose-response effect; drug potency was higher soon after treatment than at the end of experiment, as ED_{50} values were lower at 15 than 60 days pt. Hence, the maximal SbV concentration used may have been unable to kill 100% of parasites on all treated mice, with remaining parasites able to reactivate lesions, as was observed in some mice at the end of the experiment. In a previous study we reported the histopathological characteristics of CL lesions before treatment (Neira et al., 2019b). They were characterized moderate hyperkeratosis and acanthosis, mild spongiosis in the epidermis, with a severe diffuse inflammatory infiltrate predominantly comprised of lymphocytes and plasma cells, and abundant amastigote-infected macrophages in the dermis. It is possible that a higher mgSbV/kg dose will be necessary to kill all parasites.

Next, we decided to evaluate SbV activity in vitro on promastigotes, axenic amastigotes, and intracellular amastigotes of both Leishmania strains infecting THP-1 cells, using the same batch as that used for in vivo experiments. Unfortunately, we were unable to calculate IC_{50} values (drug potency) at the maximal doses evaluated in any of the live parasite forms of either parasite species. The antileishmanial activities of SbV have been demonstrated in various in vitro models, showing some peculiarities compared with other drugs such as miltefosine or pentamidine isethionate (Fernández et al., 2014). Most notably, SbV is unable to kill promastigotes and, to a lesser extent, axenic amastigotes (Sereno et al., 1998); however, activities have been demonstrated against intracellular amastigotes infecting different types of macrophage and reference strains or isolates collected worldwide (IC_{50} 2.9-146 µgSbV/ml) (Fernández et al., 2014; Pérez-Franco et al., 2016). In general, drug susceptibility (in vitro) is supposed as a method to predict treatment outcome. However, this was not our case as SbV was active on some CL infected mice but not on free or intracellular parasites life forms. Both reference strains used are not classified as Sb-resistant strains. In contrast, SbV activity has been demonstrated against L. (V.) braziliensis /M2903 intracellular amastigotes infecting U937 differentiated cells (IC_{50} 26-50 µgSbV/ml) (Pérez-Franco et al., 2016). Before our experiments, the parasites had never been exposed to antimonials in neither in vitro or in vivo assays; however, they have been cultivated in vitro for a relatively extended period, by quarterly passage in BALB/mice. Leishmania resistance mechanisms or other factors related to drug (drug quality, intrinsic drug properties, drug physicochemical characteristics), parasite (lower intrinsic susceptibility to the drug, parasite infection by RNA viruses, parasite adaptations, manipulation skills of the parasites, higher parasite fitness) or host (immunological factors, pharmacokinetics, genetics) could be involved with SbV treatment efficacy or failure (Vanaerschot et al. 2014).
Independent of the lack of in vitro Sb\(^{V}\) response, the use of intraleisional Sb\(^{V}\) with a dose calculated as mg/kg body weight and administered over a large number of IL-sessions, with small injection volumes each day could be effective against L. (V.) panamensis and L. (V.) braziliensis-CL infection. An appropriate Sb\(^{V}\)-dose (higher than 150 mg/kg/day x 20) must be evaluated.

**Ethical statement:** The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

See Digital Appendix at: revistas.ucr.ac.cr

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**RESUMEN**

Actividad antileishmanía de 29 sesiones diarias de antimonio pentavalente intraleional en ratones BALB/c infectados con Leishmania (Viannia)

**Introducción:** Los antimoniales pentavalentes aplicados intraleionalmente (IL-Sb\(^{V}\)) se recomiendan para el tratamiento de la leishmaniasis cutánea (LC) simple. Se recomiendan pocas sesiones (1-5) y volúmenes (1-5 ml cada uno) en relación con el tamaño de la lesión (LS). No existe un protocolo de IL-Sb\(^{V}\) validado que utilice dosis calculadas según el peso corporal (en mg/kg) y administradas durante varias sesiones en pocos volúmenes de inyección.

**Objetivo:** El objetivo del estudio fue determinar la eficacia de diferentes concentraciones de IL-Sb\(^{V}\) administradas en 29 sesiones diarias de 100 µL cada una, en ratones con LC.

**Métodos:** Ratones infectados con L. (V.) panamensis y L. (V.) braziliensis (N = 6) fueron tratados intraleosomalmente con 150, 50 y 16,6 mg Sb\(^{V}\)/kg/día x 29 días. Se determinó el porcentaje de reducción del área de la lesión, la eficacia estética y final (sin lesiones, sin parásitos) y la dosis efectiva (DE)\(_{50}\). Adicionalmente de evaluó la actividad in vitro del Sb\(^{V}\).

**Resultados:** Los valores de DE\(_{50}\) fueron 72.2 y 66.3 (al final del tratamiento), 54.3 y 37.7 (15 días pt) y 145.3 y 148.6 (60 días pt) para cada especie. Se encontraron diferencias entre las especies sólo a los 15 días pt. La eficacia del tratamiento IL-Sb\(^{V}\)-150 mg, 60 días pt., fue de 66.6 y 33.3 % en ratones infectados con L. (V.) panamensis L. (V.) braziliensis respectivamente. Después de 15 días pt., se observó reactivación de la lesión en algunos ratones “estéticamente curados”. Glucantime no fue activo in vitro.

**Conclusiones:** El uso intraleosomal de Sb\(^{V}\) con una dosis calculada en mg/kg de peso corporal y administrada durante varias sesiones, con pequeños volúmenes de inyección cada día, podría ser eficaz en LC por L. (V.) panamensis y L. (V.) braziliensis. Dosis adecuadas de Sb\(^{V}\) (superiores a 150 mg/kg/día x 20) deben evaluarse.

**Palabras clave:** Glucantime; leishmaniasis cutánea del Nuevo Mundo; medicamento intraleosomal; Leishmania panamensis; Leishmania braziliensis.

**REFERENCES**


