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Intralesional Pentamidine: A Novel Therapy for Single Lesions of Bolivian Cutaneous Leishmaniasis

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Abstract. A novel therapy, intralesional (IL) pentamidine, was compared to intralesional therapy with antimony (ILSb), a World Health Organization–recommended therapy, for single Bolivian *Leishmania braziliensis* lesions. In Study 1, 90 patients were randomized equally between three injections of ILSb over 5 days, five injections of ILSb over 11 days, and three injections of IL pentamidine (120 μg/mm^2 lesion area [ILPenta-120-3]) over 5 days. Cure rates at 6 months were 57% for ILSb-3 injections, 73% for ILSb-5 injections, and 72% for ILPenta-120-3 injections. Adverse effects were local irritation and injection-site pain—ILSb (60 patients): mild (25), moderate (4); IL pentamidine (30 patients): mild (4), moderate (3). In Study 2, 60 patients were randomized equally between five injections of ILSb and three injections of a double dose of IL pentamidine (240 μg/mm^2 [ILPenta-240-3]). In Study 2, cure rates were 67% for ILSb-5 injections and 73% for ILPenta-240-3. For three IL injections of pentamidine, efficacy was optimized at a dose of 120 μg/mm^2 lesion area. The cure rate of that regimen was similar to that for ILSb-5 injections and nonstatistically larger than that of ILSb-3 injections. IL pentamidine is an attractive alternative to ILSb on the basis of efficacy for Bolivian *L. braziliensis*, the threat of Sb-resistant parasites, tolerance, and patient convenience of three visits over 5 days.

INTRODUCTION

Cutaneous leishmaniasis (CL) in the New World generally presents as a papule that enlarges and ulcerates over 1–3 months then self-cures, but *Leishmania* (*Viannia*) *braziliensis* and other members of the *Leishmania* (*Viannia*) subgenus uncommonly disseminate to the mucous membranes of the nose and mouth, causing mucosal disease that does not self-cure. The classic agent pentavalent antimony (Sb) can be administered systemically or intralesionally. Whether systemic therapy or local therapy is best to use to treat cutaneous lesions that could disseminate is presently undecided.[1]

In a previous paper,[1] we reported that intralesional Sb (ILSb) administered on days 1, 3, and 5, cured 70% of 30 patients with single CL lesions due to Bolivian *L. braziliensis*, compared with a placebo rate of 18%. We viewed ILSb for three (ILSb-3) injections as attractive based on cure rate, lack of systemic side effects, and relative convenience of three clinical visits over 1 week. However, a 70% cure rate is far from the ideal of 100% cure. One potential way to improve upon the cure rate of ILSb-3 injections would be to administer ILSb for five (ILSb-5) injections. Another approach would be to intralesionally inject an antileishmanial agent with more inherent efficacy than Sb. Systemic Sb is used in preference to the other classic antileishmanial agents, systemic amphotericin B and systemic pentamidine, because the clinical therapeutic index of systemic Sb is superior, but the inherent efficacy of Sb—antileishmanial efficacy in vitro where in vivo factors are not operative—is far inferior to that of the other two classic agents. In an early publication of the in vitro efficacy of these drugs against a CL species in human monocyte-derived macrophages, the obligatory host cell for *Leishmania*, approximately 90% of parasites were killed by 10 μg Sb/mL, 0.5 μg pentamidine/mL, and 0.5 μg amphotericin B/mL.[2] This comparison suggests that if the same amount of pentamidine or amphotericin B as Sb is delivered via IL administration to CL lesions, administration of pentamidine or amphotericin B would be more effective than administration of Sb. Because pentamidine, like Sb, is a cation and a topical formulation of amphotericin B is already the subject of a formal development program (Anfoleish[3]), we chose to compare IL pentamidine to ILSb.

Our first study compared ILSb-3 injections with ILSb-5 injections and to IL pentamidine for three injections. The IL pentamidine regimen was 120 μg/mm^2 lesion area per injection. When both the ILSb-5 injection group and the IL pentamidine-3 injection group had cure rates that were superior to ILSb-3 injections, we performed a second study in an attempt to increase the cure rate of IL pentamidine. The second study compared ILSb-5 injections to a double dose of IL pentamidine: 240 μg/mm^2 lesion area for each of three injections (ILPenta-240-3).

METHODS AND PATIENTS

Study design. Study 1 was a three-armed, open-labeled comparison of ILSb-3 injections, ILSb-5, and IL pentamidine (120 μg/mm^2 of lesion area) for three injections (ILPenta-120-3 injections), for the treatment of single lesions due to *L. braziliensis* in Bolivia. Through a randomized list generated by a computer program, 90 patients were assigned to the three groups in equal allocation (30 patients per group).

Study 2 was a two-armed, open-labeled comparison of ILSb-5 injections and ILPenta-240-3 injections for 3 days, for the treatment of single lesions due to *L. braziliensis* in Bolivia. In this study, 60 patients were randomized in equal allocation to the two groups (30 patients per group).

Formal sample size calculations were not used for this hypothesis-generating phase 2 study.

Because these trials were a continuation of the investigations of the prior publication,[1] study procedures closely followed those previously reported.[1]

Patients. Patients were from locales similar to that in the prior report[1]: Chapare, Los Yungas, and Santa Cruz, Bolivia. After signing informed consent and meeting entrance criteria,

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the patients were treated at the Hospital Dermatológico de Jorochito, Bolivia. Patients had to have one ulcerative lesion ≤ 30 mm in largest diameter and ≤ 500 mm^2 in total area. The limitation of total area ensured that a maximum of 60 mg pentamidine was injected in the initial pentamidine group (ILPenta-120-3 injections). Other entrance characteristics were identical to that for the previous study[1]: either gender, ≥ 12 years, parasitologically diagnosed, no antileishmanial therapy in the last 3 months, no mucosal lesions, and no history of concomitant diseases including immunosuppression. Patients were entered between March 2013 and November 2014.

Parasites were speciated by polymerase chain reaction.[1]

Interventions. ILSb (*N*-methylglucamine [Glucantime®; Rhodia Laboratories, Rhône-Poulenc, France]: 81 mg Sb/mL) was administered on each of days 1, 3, and 5 (ILSb-3 injections) or on each of days 1, 3, 5, 8, and 11 (ILSb-5 injections) at a dose of 650 μg Sb (8 μL)/mm^2 of lesion area per day.[1]

IL pentamidine (30 mg/mL; Pentacarinat® Sanofi-Aventis, Bogota, Colombia] was administered at a dose of 120 μg (4 μL)/mm^2 of lesion area (ILpenta-120-3 injections) or 240 μg (8 μL)/mm^2 of lesion area (ILpenta-240-3 injections) on each of days 1, 3, and 5.

On each day of drug treatment, a small button of Xylocaine® (Terbol Laboratories, Santa Cruz, Bolivia) was applied by means of a 23-gauge needle at the four cardinal points of the lesion. Then, one-fourth of the calculated total volume of drug was administered via a 23-gauge needle at each of these cardinal points, with the needle being moved in all directions to infiltrate the whole lesion.

Treatments were administered by the study staff, and the targeted number of administrations was achieved for all patients.

Apparent superinfection upon study entrance was treated with soap and water plus fusidic acid cream twice a day for 4–7 days, if necessary augmented by dicloxacillin (1.5 g orally for 7 days), before antileishmanial treatment.[1]

Outcome parameters and analysis. Efficacy was evaluated exactly as before.[1] The endpoint parameter was reduction in lesion size. Lesion size was defined as the area of the lesion ulcer computed as "maximum ulcer width" × "maximum ulcer length." Lesion size was measured at study entrance, then at 1 month, 3 months, and 6 months after the end of therapy. The change in lesion size was calculated by expressing lesion sizes after therapy as a percentage of the lesion size before therapy.

The criteria for failure were the same as before[1]: substantial enlargement (doubling) of lesion size by 1 month after therapy, nonsubstantial (< 50%) diminution in lesion size at 3 months after therapy, relapse (enlargement after previous diminution), not being completely re-epithelialized ("re-epithelialized" = lesion size of "0" mm^2) at 6 months after therapy. Any lesion that did not fail was considered "cured." Thus for a patient to be cured, the lesion could not have doubled soon after therapy (1 month), failed to make substantial progress toward healing (at least 50% resolution by 3 months), or relapsed and must have completely re-epithelialized at 6 months.

Local adverse effects were assessed on treatment days when treatments were applied by study personnel. Patients were evaluated for local pain and irritation (defined as erythema and/or edema and/or itching and/or burning sensation and/or scaling), which could be caused by any of the agents; sterile abscess, which could be due to pentamidine; myalgia and nausea, which could be due to systemic absorption of either Sb or pentamidine; and low blood pressure and hypoglycemia, which could be due to systemic absorption of pentamidine. Each adverse effect other than pain was graded on a 0–3 scale defined as follows: 0 = absent, 1 = mild (present but treatment not required), 2 = moderate (present and needed specific treatment), and 3 = severe (present with such intensity that antileishmanial therapy had to be stopped). The 0–3 scale for pain was as follows: 0 = absent; 1 = mild (present but expected with injections, treatment not required); 2 = moderate (present and more than expected with injections, treatment not required); and 3 = severe (present with such intensity that analgesics were required).

Categorical variables (number of patients cured, number of patients with lesions at specified body sites) were compared by the χ^2 test or Fischer's exact test. Continuous variables (age and lesion size at entrance) were compared by Student's *t* test.

Ethical review. The study was approved by the Comité de Bioética de la Facultad de Medicina, Universidad Mayor de San Simón, Cochabamba, Bolivia. ClinicalTrials.gov identifier no. NCT01300975.

RESULTS

Study 1. *Entrance characteristics.* Patients had a mean age of 29 years and a mean lesion size of 310 mm^2 (Table 1). Of the speciated parasites, 82% were *L. braziliensis*.

Efficacy. The cure rates per experimental group were 57% (37–73%) for ILSb-3 injections, 73% (56–86%) for ILSb-5 injections, and 72% (54–85%) for ILPenta-120-3 injections ($P = 0.18$ by χ^2 test for ILSb-3 injections versus ILSb-5 injections) (Table 1). For all groups, the time that failure criteria were met was approximately equally distributed among the 1-month, 3-month, and 6-month follow-up periods. Of the seven patients in the ILPenta-120-3 injections group in whom infection with *L. braziliensis* was documented, six cured and one was lost.

Adverse effects. Most adverse effects were local irritation and local pain (Table 2). Local irritation in the sense of mild erythema was seen in seven to eight patients in the Sb groups totaling 60 patients versus three patients in the pentamidine group of 30 patients, but was judged moderate in one pentamidine patient who experienced erythema, itching, and edema. Local pain was seen in 14 patients in the Sb groups versus three patients in the ILPenta-120-3 injections group, with four Sb patients and two pentamidine patients reporting moderate symptoms. A few instances of myalgia were reported in the Sb groups. No pentamidine patients experienced sterile abscess, hypotension, or hypoglycemia. No patient experienced grade 3 (severe) side effects such that therapy had to be stopped even transiently.

Study 2. *Entrance characteristics.* Presenting characteristics were similar to that of Study 1 (Table 3). Patients had a mean age of 27 years, a mean lesion size of 260 mm^2, and 87% of speciated parasites were *L. braziliensis*.

Efficacy. The cure rates per experimental group were 67% (49–81%) for ILSb-5 injections and 73% (56–86%) for ILPenta-240-3 injections ($P = 0.57$ by χ^2 test) (Table 3). For both groups, the time that failure criteria were met was generally 3 months after therapy. Of the eight patients in the ILPenta-240-3 injections group in whom infection with *L. braziliensis* was documented, six cured and two failed.

TABLE 1
Efficacy data Study 1

	ILSb-3 injections	ILSb-5 injections	ILPenta-120-3 injections	All patients
Number of patients	30	30	30	90
Entrance parameters				
Age (years): mean [SD]	28 [10]	30 [13]	30 [11]	29 [11]
Weight (kg): mean [SD]	59 [12]	61 [11]	65 [9]	62 [11]
Lesion size (mm^2): mean [SD]	336 [119]	280 [122]	315 [116]	310 [120]
Species*: No.	*L. braz*: 6 *L. amaz*: 1	*L. braz*: 4 *L. lain*: 2	*L. braz*: 7 *L. guy*: 1	*L. braz*: 17 *Non-L. braz*: 4
Efficacy parameters (number of patients)				
Cure	17	22	21†	60
1 month‡	4	11	12	27
3 months‡	17	21§	20‖	57
Fail	12	8	8	28
1 month	3	2	3	8
3 months	5	3	1	9
6 months	2	1	4	7
Relapse or new lesion	2	2	0	4
Lost	1	0	1	2
Intent-to-treat cure rate (95% CI)	57% (37–73%)	73% (56–86%)	72% (54–85%)	67% (56–76%)

CI = confidence interval; ILPenta = intralesional pentamidine; ILSb = intralesional therapy with antimony; SD = standard deviation.
* *L. braz/amaz/lain/guy = Leishmania braziliensis/amazonensis/lainsoni/guyanensis.*
† Includes one patient who was 96% re-epithelialized at 1 month, did not return to clinic at 3 months, but was contacted by telephone and reported complete epithelialization at that time.
‡ "Cure" at 1 or 3 months signifies lesions that 100% re-epithelialized at that time and were later shown not to relapse at 6 months.
§ One patient was 76% re-epithelialized at 3 months, 100% re-epithelialized at 6 months.
‖ One patient was 90% re-epithelialized at 3 months, 100% re-epithelialized at 6 months.

Adverse effects. As for Study 1, all adverse events were mylagia (in the Sb group), local irritation, and local pain (Table 4). There were three cases (one Sb, two pentamidine) of moderate local irritation, three cases (two Sb, one pentamidine) of moderate local pain, and two cases (one Sb, one pentamidine) of severe local pain requiring analgesics. There were no instances of adverse effects such that antileishmanial therapy had to be stopped prematurely.

Although the pentamidine dose was doubled, no pentamidine patients experienced sterile abscess, hypotension, or hypoglycemia.

DISCUSSION

We describe an attractive therapeutic index for IL pentamidine, a therapy not previously reported in the literature,[4] for single *L. braziliensis* lesions in Bolivia.

ILSb is a World Health Organization–recommended therapy for cutaneous leishmaniasis caused by essentially all Old and New World species,[5] although there is controversy[6–9] about the appropriateness of this local treatment versus systemic therapy for potentially disseminating species such as *L. braziliensis.* Factors favoring local therapy are adverse effects of systemic therapy and the presence of only one lesion, which increases the feasibility of local measures. The major factor favoring systemic therapy is the higher likelihood that subclinical disseminated parasites will be killed and mucosal leishmaniasis prevented.

Our previous study described a 70% cure rate for *L. braziliensis* at our site with three injections of ILSb.[1] In an attempt to improve upon this cure rate by increasing the number of ILSb injections, the present report first compared ILSb-3 injections versus ILSb-5 injections. In the Old World, another approach to improving the cure rate of ILSb is to combine ILSb with cryotherapy,[10–13] but with *L. braziliensis* in our hands, cryotherapy was both ineffective and poorly tolerated.[1] In Study 1 of the present report, the cure rate for ILSb-3 injections was 57% versus a cure rate for ILSb-5 injections of 73%. Although not statistically significant with 30 patients per group, these cure rates suggest that extending treatment from three injections over 5 days to five injections over 11 days may improve efficacy. We note in passing that this range of cure rates is comparable to that reported for ILSb in the literature: 56–75% for Iranian CL,[12,13] 80–83% for *L. braziliensis* in Brazil.[14,15] Adverse effects were not greater in the ILSb-5 injections group compared with the ILSb-3 injections group. On the other hand, extending the number and period of clinic visits to five visits over 11 days is an important practical disadvantage of the five-injection regimen.

TABLE 2
Adverse events in Study 1

	ILSb-3 injections			ILSb-5 injections			ILPenta-120-3 injections		
Adverse event	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Myalgias	2	0	0	1	0	0	0	0	0
Nausea	0	0	0	0	0	0	0	0	0
Sterile abscess	0	0	0	0	0	0	0	0	0
Low blood pressure	0	0	0	0	0	0	0	0	0
Low glucose	nd	nd	nd	nd	nd	nd	0	0	0
Local irritation	8	0	0	7	0	0	3	1	0
Local pain	6	2	0	4	2	0	1	2	0

ILPenta = intralesional pentamidine; ILSb = intralesional therapy with antimony; nd = not done. For definitions of mild/moderate/severe, see Materials and Methods.

TABLE 3
Efficacy data in Study 2

	ILSb-5 injections	ILPenta-240-3 injections	All patients
Number of patients	30	30	60
Entrance parameters			
Age (years): mean [SD]	25 [7]	28 [7]	27 [7]
Weight (kg): mean [SD]	61 [12]	59 [11]	60 [11]
Lesion size (mm^2): mean [SD]	243 [134]	277 [135]	260 [135]
Species*: No.	*L. braz*: 5 *L.* species: 1	*L. braz*: 8 *L.* species: 1	*L. braz*: 13 *L.* species: 2
Efficacy parameters (number of patients)			
Cure	20	22	42
1 month†	3	11	14
3 months†	16‡	22	38
Fail	10	7	17
1 month	1	0	1
3 months	8	7	15
6 months	1	0	1
Lost	0	1	1
Intent-to-treat cure rate (95% CI)	67% (49–81%)	73% (56–86%)	70% (57–80%)

CI = confidence interval; ILPenta = intralesional pentamidine; ILSb = intralesional therapy with antimony; SD = standard deviation.
L. braz/species = *Leishmania braziliensis*/species unidentified.
†"Cure" at 1 or 3 months signifies lesions that 100% re-epithelialized at that time and were later shown not to relapse at 6 months.
‡Four patients had lesions that 96%, 96%, 91%, and 91%, respectively, re-epithelialized at 3 months, then completely re-epithelialized at 6 months.

The first study also evaluated ILPenta-120-3 injections. This dose was chosen on the basis of potential therapeutic index. Pentamidine is arguably 20 times more antileishmanial on a μg/μg basis in vitro,[2] and since we administer Sb at a dose of 650 $\mu g/mm^2$ of lesion area, each pentamidine injection of 120 $\mu g/mm^2$ had the potential to be 3.5 times as effective as each Sb injection. The largest lesion in this study was set at 500 mm^2 so that a maximum of 60 mg of pentamidine would be injected each day, for a total dose over three injections of approximately 3 mg/kg. Intramuscular pentamidine at 2 mg/kg/injection × four injections—total dose of 8 mg/kg—to 42 patients, resulted in myalgias in 16 patients and nausea in 3 patients, and no instances of sterile abscess, low blood pressure, or low glucose,[16] and we anticipated that a total IL dose of 3 mg/kg would not produce systemic adverse effects.

In Study 1, ILPenta-120-3 injections had a cure rate (72%) that was nonstatistically higher than ILSb-3 injections (57%) and essentially identical to that of ILSb-5 injections (73%). Although ILPenta-120-3 injections did not meet our intent of being dramatically more effective than ILSb-3 injections, it also did not produce marked adverse effects, so we then evaluated IL pentamidine at twice the dose (ILPenta-240-3 injections) in Study 2. The cure rate for ILPenta-240 in the second study (73%) was essentially identical to the cure rate of ILPenta-120 in the first study (72%), which suggests that efficacy of three injections of IL pentamidine is optimized at a dose of 120 $\mu g/mm^2$ lesion area. The 72% cure rate of ILPenta-120 compares favorably to the 18% placebo cure rate in our immediate previous study[1] in the same patient population.

TABLE 4
Adverse events in Study 2

	ILSb-5 injections			ILPenta-240-3 injections		
Adverse event	Mild	Moderate	Severe	Mild	Moderate	Severe
Myalgias	0	2	0	0	0	0
Nausea	0	0	0	0	0	0
Sterile abscess	0	0	0	0	0	0
Low blood pressure	0	0	0	0	0	0
Low glucose	nd	nd	nd	0	0	0
Local irritation	4	1	0	4	2	0
Local pain	4	2	1	3	1	1

ILPenta = intralesional pentamidine; ILSb = intralesional therapy with antimony; nd = not done. For definitions of mild/moderate/severe, see Materials and Methods.

Our study suggests that IL pentamidine may be a more attractive agent than ILSb, at least for Bolivian *L. braziliensis*. Both three injections of IL pentamidine and five injections of ILSb may be more effective than three injections of ILSb, but the pentamidine regimen has the important advantage that the total course is completed in three administrations over 5 days. IL pentamidine for three injections may have produced less local adverse events than the Sb regimens. The lack of systemic adverse effects in patients who was administered the "double-dose" of 240 $\mu g/mm^2$ suggests that lesions as large as 1,000 mm^2 could be safely treated with ILPenta-120 in the future.

A clear advantage of pentamidine over Sb is to treat Sb-resistant parasites. Sb resistance of cutaneous *Leishmania* can be generated in vitro.[17] Sb-unresponsive CL is being seen clinically in the sense that recent cure rates of New World *L.* (*Viannia*) CL treated with systemic Sb are surprisingly low for a standard therapy, approximately 50–75%.[18–22] An undecided issue is whether there is a correlation between in vitro and clinical data, whether parasite resistance is the cause of clinical unresponsiveness. For *Leishmania tropica* from Iran, the mean effective dose (ED50) for parasites from 165 lesions that responded to systemic or ILSb was 4.6 μg/mL, whereas the mean ED50 for parasites from 16 lesions that did not respond was 19 μg/mL.[23] The authors concluded that primary Glucantime-resistant *L. tropica* field isolates were now frequent in Iran. On the other hand, for *L. braziliensis* from Peru, in vitro resistance was found for eight patients who failed systemic Sb but also for 11 patients who cured.[24] These authors concluded that there was no correlation between in vitro susceptibility to antimonials and the clinical outcome of therapy.[24]

The purpose of this report is not to decide the frequency with which Sb resistance plays a role in Sb clinical unresponsiveness, but to add this consideration to those involved in choosing an IL drug. IL pentamidine therapy for 3 days is more expensive than ILSb for 5 days: $135 for IL

pentamidine versus $25 for ILSb. However, on the basis of several other considerations—efficacy at least for Bolivian *L. braziliensis*, the threat of Sb-resistant parasites, tolerance, and especially medical and patient convenience—ILPenta-120 on each of days 1, 3, and 5, is an attractive alternative to ILSb.

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