ORAL MILETOSINE plus INTRALESIONAL PENTAMIDINE FOR BOLIVIAN CUTANEOUS LEISHMANIASIS

1. Introduction

1.1 Background

Cutaneous leishmaniasis (CL) is endemic in the New World from approximately the US-Mexican border through Central America and the Northern part of South America down to the level of Rio de Janeiro.

The cure rate for New World CL is only approximately 70% for all recognized therapies. For systemic therapy with the classic agent pentavalent antimony, recent cure rates of New World CL are 50% to 75% [1-5]. For the new oral agent miltefosine in 3 of these studies, the cure rate was 57-75% [2-4]. CL can also be treated locally. For intralesional (IL) antimony, cure rates are reported as 56-75% for Iranian CL [6-7], 80-83% for *L braziliensis* in Brazil [8-9], and most recently 70% for *L braziliensis* in Bolivia when the concomitant placebo cure rate was 18% [10].

A novel approach was to evaluate intralesional pentamidine for Bolivian CL. The rationale for IL pentamidine is that little drug is administered via intralesional injection, thus the toxicity of systemic pentamidine would be unlikely to be seen. For Bolivian CL, 3-injections of IL Pentamidine over 5 days cured 72% of patients whereas 3-injections of IL antimony over 5 days cured 57% [manuscript submitted]. Adverse effects were local irritation and injection site pain: IL pentamidine (30 patients)—mild (4 patients), moderate (3); IL antimony (60 patients)—mild (25 patients), moderate (4). No pentamidine patient experienced the side effects of systemic pentamidine administration: sterile abscess, hypotension, or hypoglycemia. We concluded that IL pentamidine is an attractive alternative to standard intralesional antimony for Bolivian *Leishmania braziliensis* on the basis of efficacy, the threat of antimony-resistant parasites, tolerance, and patient convenience of 3 visits over 5 days.

1.2 Rationale

Although patients can be treated with one of these agent with the anticipation of an approximately 70% cure rate, and failures of the first treatment could then be treated with another agent, an initial cure rate > 70% would be desirable.

We propose to evaluate oral miltefosine plus intralesional pentamidine for Bolivian CL. Efficacy should be additive, since the postulated mechanisms of the drugs is different. The structure of miltefosine is similar to that of phosphotidylcholine, and may interfere with phosphatidylcholine biosynthesis or function[11]. In contrast, it is thought that pentamidine interferes with polyamine biosynthesis [12]. The advantage of the combination of oral miltefosine and IL pentamidine vs other possible drug combinations is that the systemic partner is oral rather than parenteral, and that the intralesional partner is the most active of the IL agents that we have found in Bolivia.The combination should be well tolerated, since the local side effects of pentamidine would not interact with the systemic gastrointestinal side effects of miltefosine. Finally, this combination would be

convenient, since intralesional injections of pentamidine would occur on days 1, 3, and 5 of the standard 28-day miltefosine treatment course when patients are already under medical care.

2.0 Study Aims and design

2.1 Aim:

The primary purpose of this study is to evaluate the cure rate of a standard course of oral miltefosine in combination with intralesional pentamidine for single lesions predominately due to L braziliensis in Bolivia.

A secondary purpose is to determine the tolerance of this regimen.

2.2 Design:

Open label evaluation of miltefosine (28 days) plus intralesional pentamidine (days 1, 3, 5).

After treatment, all patients will be followed for 1, 3, and 6 months.

The initial number of patients will be 30.

3.0 Experimental Plan

3.1 Site

Catchment area: **Nor Yungas, Departamento de La Paz** Bolivia
Treatment site: **Hospital Local Palos Blancos** Bolivia. The patients will be in residence at the treatment site during treatment.

3.2 Study Population (inclusion and exclusion criteria)

Gender: Male or female
Age: $\geq$12 yrs of age
Presentation: 1 ulcerative lesion, $\leq$ 30 mm in largest diameter, and with a total lesion area $\leq$**900** mm2.
Parasitology: Parasitological confirmation of the lesion will be made by visualization or culture of leishmania from the biopsy or aspirate of the lesion.
Previous treatment for leishmaniasis:
No specific or putatively specific therapy (Sb, pentamidine, amphotericin B, miltefosine, imidazoles, allopurinol) in the last 3 months
Other diseases: No concomitant diseases by history that would be likely in the PI's opinion to interact, either positively or negatively, with treatment.

3.3 Study treatments

3.3.1 Miltefosine

Miltefosine will be administered daily for 28 days as per the US Product Label:

"Administer with food to ameliorate gastrointestinal adverse reactions.
· 30 to 44 kg: one 50 mg capsule twice daily for 28 consecutive days.
· 45 kg or greater: one 50 mg capsule three times daily for 28 consecutive days."

3.3.2 IL pentamidine

IL pentamidine [Pentacarinat® Sanofi-Aventis: 30 mg/ml] will administered at a dose of 120 ug (4 ul) per mm2 of lesion area 3 times (on days 1, 3, and 5) as per our previous experience.

A small button of Xylocaine® will be applied by means of a thin needle at the four cardinal points of the lesion and then a small gauge (23g) needle will introduce the drug in each cardinal point. The needle will be moved in all directions to infiltrate of whole lesion and surrounding infiltrated area.

3.3.3 Pretreatment

If bacterial superinfection appears to be present, the lesions will receive topical fusidic acid and/or oral dicloxacillin during 7 days previous to the first day of leishmania treatment. Initial size will be measured after the antibacterial treatment.

3.4 Efficacy evaluation

For each lesion, the size of its ulcer will be measured at 1 month, 3 months, and 6 months after the end of therapy.

3.5 Adverse events recording

Re Miltefosine: Adverse events especially gastrointestinal will be recorded.

Re IL pentamidine: Patients will be evaluated for local side effects (pain, itching, irritation (erythema/edema), vesicles/bullae, sterile abscesses) and the possibility of bacterial superinfection both local and disseminated to the draining lymph nodes on each day that therapy is applied. Any other subjective complaint spontaneously referred by the patient will be registered and evaluated.

In addition, pentamidine patients will be evaluated for hypoglycemia.

3.6 Procedural Timetable

Action	Day 0	Day 1	Day28	1 M	3 M	6M
Consent	X					
Hx /PE	X		X	X	X	X
Parasitology (aspirate:smear/cult)	X					
Evaluate lesion	X	X	X	X	X	X
Treatment						
Oral Miltefosine		day 1 to day 28				
IL pentamidine		days 1, 3, 5				
Adverse events (esp. GI)	X	day 1	to day 28			
Blood Glucose	X	weeks 2	and 4			
AST	X	weeks 2 and 4				
Creatinine	X	weeks 2 and 4				

"Day 0" includes the screening period.

3.7 Efficacy response criteria (cure and failure)

A lesion will be defined as a treatment failure if:

- enlarges by 50% by 1 month after therapy
-does not diminish by 50% at 3 months after therapy
-does not completely reepithelialize by 6 months after the end of therapy.
-relapses (enlarges substantially---approximately 25%) after previously diminishing in size
-new lesion from which Leishmania can be demonstrated

Cure will be defined as complete healing of all lesions by 6 months after the end of therapy.

Thus for a patient to be cured: no lesion could enlarge by 50%, relapse, or heal incompletely; and no new Leishmania-positive lesion can have appeared.

3.8 Early withdrawal from study

- If a lesion fails.
- If the physician or patient otherwise thinks it advisable.

3.8.1 Treatment of withdrawn patients

Withdrawn patients will be treated off-protocol with best available measures as determined by the patient's personal physician.

3.9 Additional patients

After 30 patients have been evaluated, the cure rate and tolerance of the regimen will be analyzed. If the cure rate is close to 90%, further patients may be enrolled to better ascertain the cure rate of this regimen.

4.0 Data analysis

4.1 Primary outcome variable (cure)

Cure vs failure will be computed using the chi-square test or Fischer's exact test.

4.2 Tolerance

Side effects will be described by descriptive statistics and analyzed by T-test.

5.0 Investigators and Study controls

5.1 PI

Dr Jaime Soto. Dr Soto is overall in charge of the investigation.

5.2 Co-PIs

Dr. ANA VARGAS (MD, responsible of clinical control and follow up and pentamidine administration)

DR. ROLO PARRA (DO, responsible of clinical control and follow up)

LIC. RUTH ESCOVAR (RN, responsible of daily administration of miltefosine and follow up during treatment period and during f-u visits. She will also be responsible to maintain contact with patients to avoid losses).

5.3 Associate Investigators

Dr Jonathan Berman will provide consultative advice on cutaneous leishmaniasis

5.4 Data handling

Dr Soto will devise CRFs, and check CRF data vs source data. Given the relatively low numbers of patients and relatively small numbers of data points, each entry in the CRF can be checked against the source data (2-person method).

5.5 Ethical review

The protocol will be approved by the responsible ethical committee as an extension of the IL pentostam/ IL pentamidine protocol.

The protocol will be listed on www.ClinicalTrials. Gov.

6.0 References

1. Miranda-Verastegui C, Tulliano G, Gyorkos TW, Calderon W, Rahme E, Ward B, Cruz M, Llanos-Cuentas A, Matlashewski G. 2009. First-line therapy for human cutaneous leishmaniasis in Peru using the TLR7 agonist imiquimod in combination with pentavalent antimony. *PLoS Negl Trop Dis* 3:e491.

2. Vélez I, López L, Sánchez X, Mestra L, Rojas C, Rodríguez E. 2010. Efficacy of miltefosine for the treatment of American cutaneous leishmaniasis. *Am J Trop Med Hyg* 83:351-6.

3. Machado PR, Ampuero J, Guimarães LH, Villasboas L, Rocha AT, Schriefer A, Sousa RS, Talhari A, Penna G, Carvalho EM. 2010. Miltefosine in the treatment of cutaneous leishmaniasis caused by Leishmania braziliensis in Brazil: a randomized and controlled trial. *PLoS Negl Trop Dis* 4:e912.

4. Chrusciak-Talhari A, Dietze R, Chrusciak Talhari C, da Silva RM, Gadelha Yamashita EP, de Oliveira Penna G, Lima Machado PR, Talhari S. 2011. Randomized controlled clinical trial to access efficacy and safety of miltefosine in the treatment of cutaneous leishmaniasis Caused by Leishmania (Viannia) guyanensis in Manaus, Brazil. *Am J Trop Med Hyg* 84:255-60.

5. Newlove T, Guimarães LH, Morgan DJ, Alcântara L, Glesby MJ, Carvalho EM, Machado PR. 2011. Antihelminthic therapy and antimony in cutaneous leishmaniasis: a randomized, double-blind, placebo-controlled trial in patients co-infected with helminths and Leishmania braziliensis. *Am J Trop Med Hyg* 84:551-5.

6. Asilian A, Sadeghinia A, Faghihi G, Momeni A. 2004. Comparative study of the efficacy of combined cryotherapy and intralesional meglumine antimoniate (Glucantime) vs. cryotherapy and intralesional meglumine antimoniate (Glucantime) alone for the treatment of cutaneous leishmaniasis. *Int J Dermatol* 43: 281-3.

7. Salmanpour R, Razmavar MR, Abtahi N. 2006. Comparison of intralesional meglumine antimoniate, cryotherapy and their combination in the treatment of cutaneous leishmaniasis. *Int J Dermatol* 45: 1115-6.

8.Oliveira-Neto MP, Schubach A, Mattos M, da Costa SC, Pirmez C. 1997. Intralesional therapy of American cutaneous leishmaniasis with pentavalent antimony in Rio de Janeiro, Brazil--an area of Leishmania (V.) braziliensis transmission. *Int J Dermatol* 36: 463-8.

9. Vasconcellos Ede C, Pimentel MI, Schubach Ade O, de Oliveira Rde V, Azeredo-Coutinho RB, Silva Fda C, Salgueiro Mde M, Moreira JS, Madeira Mde F, Baptista C, Valete-Rosalino CM. 2012. Intralesional meglumine antimoniate for treatment of cutaneous leishmaniasis patients with contraindication to systemic therapy from Rio de Janeiro (2000 to2006). *Amer J Trop Med Hyg* 87: 257-60.

10. Soto J, Rojas E, Guzman M, Verduguez A, Nena W, Maldonado M, Cruz M, Gracia L, Villarroel D, Alavi I, Toledo J, Berman J. Intralesional antimony for single lesions of bolivian cutaneous leishmaniasis. *Clin Infect Dis*. 2013; 56:1255-60.

11. Dorlo TP, Balasegaram M, Beijnen JH, de Vries PJ. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. J Antimicrob Chemother. 2012;67:2576-97.

12. Kaur G, Rajput B. Comparative analysis of the omics technologies used to study antimonial, amphotericin B, and pentamidine resistance in leishmania. *J Parasitol Res.* 2014;726328.