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Avaliation the Therapeutic Efficacy of Fluconazol

ORIGINAL

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Abstract

Leishmaniasis is a group of diseases with a large spectrum, cosmopolitan, but with broad and significant impact in the tropical zone. Leishmaniasis presents itself in two forms, one visceral involving hemolinfopoietic system structures, other with skin and/or mucosa involvement, often without visceralizing. The latter, known as American Cutaneous Leishmaniasis (ACL) is the aim of this study. One of the major problems in the ACL is treatment procedure using injectable formulations, with risk for complications from the injection and the risk of damage to the liver and kidney function and cardiac complications. Several tests have demonstrated satisfactory results using Fluconazole®. In order to consolidate the results described in the literature, this study sought to demonstrate the therapeutic efficacy of Fluconazole® with high-dose treatment of ACL in patients from an endemic area in the Southern State of Ceará, Brazil, City of Barbalha. It's conducted a prospective randomized study with two groups of patients. Sixty of the Group I: they were treated with 300mg or 450mg of Fluconazole® for six weeks. Sixty Group II: they were treated with Glucantime 20mg/kg/day for 20 consecutive days. The diagnosis of ACL was performed with Imprint, culture, biopsy and histopathological stain with Giemsa and immunohistochemistry. Montenegro's Intradermoreaction was also performed. All patients were evaluated clinically and followed up for ninety days. Continuous variables were evaluated by Student's t test, and for the correlation of variables the Pearson correlation coefficient (r) was used. The time healing of each group had its evaluation by Kaplan-Meier method. In all tests the hypothesis α was considered significant when less than 5% ($p < 0.05$). The therapeutic efficacy (TE) was calculated based on the reduction of relative risk. The study population was predominantly made up of individuals between 30 and 40 years, brown skin color, peasants, rural people, with a slight pre-

valence of females. The results showed therapeutic efficacy of 38.7%. The cure was related to the size of the lesion, relevant factor in the statistical p <0.0001. The lesions smaller than 30 mm (alone or sum of multiple lesions) responded promptly to Fluconazole®.

Keywords
Cutaneous Leishmaniasis.
Fluconazol. Meglumine.

Introduction

The American tegumentary leishmaniasis (ATL) is an infectious disease of the skin and mucosa, whose etiological agent is a protozoan of the gender *Leishmania sp*. The treatment is a challenge, because the drugs available on the market show, in general, high toxicity. Thus, therapeutic flaw, relapses and the resistance to the treatment motivates the search for a drug of easier use. (Lima *et al*., 2007).

The diagnosis of ATL presents a spectrum which permeates epidemiologic, clinic and laboratory elements. In general, there is the necessity of utilizing more than one among the criteria in order to achieve the diagnosis of certainty. (Gontijo; Carvalho, 2003; Manson-Bahr, 1987).

Definitive diagnosis involves the demonstration of the parasite, obtained by various parasitological techniques of direct and indirect research. The simplest technique is the direct detection of parasites in material obtained by scarification, aspiration or biopsy of the edge of the lesion, using Romanowsky colorings (Furtado, 1994). The polymerase chain reaction (PCR) is a method endowed with high sensitivity, capable of detecting trace quantities as small as a fentogram from the DNA of a leishmania, equivalent to 1/10 of the parasite (Brujin; Barker, 1992), Brazil has a sensitivity of 98.41% and specificity of 95.59% (Gontijo, 1997). The Montenegro intradermoreaction (MIDR), as professed by Montenegro in 1926, prepared based on promastigotes of Leishmania cultivated, is still widely used and useful (José *et al*., 2001). The MIDR detects the presence of delayed-type hypersensitivity, since, from the immunological point of view, LT is characterized by a cellular response during and after the cure of the infection, either spontaneously or after treatment (Carvalho *et al*., 1995). The MIDR presents positivity of 84% and 100%, estimated, in the cutaneous and mucocutaneous forms, respectively, and negative in the diffuse cutaneous form (SHAW; Lainson, 1975). Indirect immunofluorescence assay (IIFA) is the most widely used serological method (Marzochi *et al*., 1980). Demonstrates sensitivity greater than 60% in cutaneous forms, 100% in the LCM and negative in the LCD (KAR, 1995; Mendonca *et al*., 1988).

Emerged in France in the midst of World War II, the antimoniate of N-methyl glucamine, marketed as Glucantime® (Rhône-Poulenc-Rohrer) is marketed in the countries of French and Spanish languages, as well as in Brazil, being the drug of first choice (RATH *et al*., 2003). The most common side effects are arthralgia, myalgia, loss of appetite, headache, fever, vomiting, dizziness and swelling at the injection surroudings (Carvalho; Dolci, 2006). Are described in the literature complications such as cardiotoxicity, nephrotoxicity and hepatotoxicity of antimony, composing important limitation to the safe use (Carvalho, 2007). Because they are abortifacient, antimony should not be administered to pregnant women (Gontijo; Carvalho, 2003), and one should use amphotericin B. Notwithstanding the success of antimony, the fear of the emergence of resistance is a fact, considering the irregular use in some regions (Haldar; Sen; Roy, 2011). Several therapeutic alternatives are recommended, especially the pentamidines.

In the 1960s, 1970s and 1980s, drugs such as metronidazole, antituberculosis drugs (rifampicin and isoniazid), cycloguanil pamoate, nitrofurans (nifurtimox), trimethoprim-sulfamethoxazole and dapsone were tested in the treatment of leishmaniasis, with varying efficiencies, not being possible to recommend them for routine therapy on the basis of unreliable data (Berman, 2005). Alternative treatments are used with itraconazole, ketoconazole, and more recently, azithromycin. Despite the promising results of some of these drugs, studies used small numbers of patients; were published as case reports or unsuitable study design (Blum *et al*., 2004; Ouellette *et al*., 2004; Silva-Vergara *et al*., 2004; Zerpa *et al*., 2006).

Leishmania, like some fungi, use common metabolic pathways, such as the synthesis of sterols. Thus, the growth of these organisms becomes susceptible to the sterol synthesis inhibitor (Roberts *et al*., 2003). The Fluconazol® a fungistatic, inhibiting the synthesis of ergosterol (Nobre *et al*, 2002), shown promising drug in the treatment of some species of leishmania, the etiological agents of skin disease (Alrajhi *et al*, 2002; Hart *et al*., 1989). In vitro studies demonstrate Fluconazol® a significant inhibitory effect on promastigotes of Leishmania donovani, in some subspecies of *Leishmania braziliensis* and a slight or no effect on *Leishmania aethopica, Leishmania major, Leishmania tropica* and *Leishmania mexicana* (Beach; Goad; Holz, 1988). Testings in the New-World are observed in the literature (Monte Neto, 2006; SOUSA *et al*, 2011.)

The healing criterion is clinical. It is recommended monthly monitoring for three consecutive months and, after clinical cure, follow-up 12 months after treatment completion.

This paper aims to evaluate the therapeutic efficacy of Fluconazol® in patients with American Tegumentary Leishmaniasis, study its therapeutic activity, to compare the efficacy and the healing time of the treatment with Fluconazol® over the standard drug (Glucantime®) and evaluate Fluconazol® associated side effects.

Materials and methods

This is a prospective, randomized study to evaluate the therapeutic efficacy of Fluconazol®, orally, in high doses, in patients with cutaneous leishmaniasis determined by *Leishmania (V.) braziliensis*. The study presented technical rigor, followed all the rules of biosafety related to work in humans, and the disease on screen. The work considered all ethical guidelines for research *in anima nobili* following a recommendation from National Health Council (CNS) Resolution 466/12, including protecting names and personal data of individuals in the research. Patients were informed about the study and received explanations about the main objective of the work, the procedures they underwent and the guarantee of confidentiality of your information and exams. Those who agreed to participate voluntarily signed the Informed Consent Form (ICF), developed specifically for this research (Brazil, 1996).

The original project was submitted and approved (Protocol 4/2008) to the Ethics Committee of Hospital São José of Infectious Diseases - HSJ/Health Secretary of the State of Ceará and appreciated by the Ethics Committee of the Medical Program of Research in Barbalha, the Federal University of Ceará (UFC). It had its start in July 2009 and ending in December 2011.

Characterization of research subjects

Patients proceeded from Tropical Pathology Clinic of the Medical School of the Federal University of Ceará, in Barbalha. The Clinic is part of the health structure of the Department of Health of Barbalha, South Municipality of Ceará, located in the metropolitan region of Cariri, on the banks of Araripe, at 414 meters above sea level and 610 km far from Fortaleza, the capital the state. The administrative and technical guardianships are borne by the UFC

Medical School in Barbalha, home of the Clinic. The patients followed the criteria described in the following: (a) Inclusion criteria - men or women aged above 18 years, presenting lesion with parasitological confirmation for leishmaniasis and without prior treatment to agree to participate in the study by signing the Instrument of Consent; (b) Exclusion criteria - men or women under 18, pregnant or breastfeeding, patients with heart disease, liver disease (or only increases in enzymes, SGOT, SGPT), kidney disease, HIV carriers; and patients who had already developed a mucosal form of LTA and did not agree to participate.

Parasitological diagnosis

After aseptic skin with iodine alcohol (commercial, "Deshydrater" brand) and local anesthesia with 1% lidocaine (commercial, Xylestesin® brand, Cristália laboratory) were performed biopsies (three fragments) with punch of 02 mm at the edge of the lesion, in place without apparent secondary infection. A fragment of 02 mm was grown in NNN culture medium, prepared according to standard technique that has been modified (Pessoa; Martins, 1978); the other was used for imprints and the third set at 10% buffered formalin, for histopathological examination, according to standard technique (Michalany, 1998; Tolosa *et al*, 2003) and for immunohistochemical study.

The obtained smears were stained with Giemsa according to standard techniques (Michalany, 1998) and examined for Leishmania amastigotes. For the cultures, the collected material was inoculated into NNN medium containing Schneider, supplemented with 20% fetal bovine serum, 2% sterile human urine, and 100 µg gentamicin/ml. The cultures were incubated at 26°C for four weeks in BOD (brand Quimis - Q315M) and examined weekly, investigating the presence of Leishmania promastigotes.

The material, fixed in buffered formalin, was processed, three histologic sections being made, one stained with haematoxylin and eosin, according to standard techniques (Michaelany, 1998; Tolosa *et al*., 2003); the second section was stained with Giemsa according to standard techniques (Tolosa *et al*., 2003) and the third section subjected to immunohistochemistry.

The evaluation for immunohistochemical identification of Leishmania in tissues was performed on four-micrometer sections mounted on silanized slides (Dako - silanized slides number S3003), deparaffinized in xylene and ethanol, followed by hydration. Endogenous peroxidase activity was blocked with pig serum, followed by antigen retrieval in a water bath at 96°C with citrate buffer (Dako, antigen retrieval solution); following, there was inhibition of specific binding with skim milk (30 ml TRIS + NaCl + 0.3 g bovine albumin 0.3 g of Merck + skimmed milk powder). This was followed by incubation with primary anti-leishmanial antibodies (produced in Evander Chagas Institute on the basis of *Leishmania chagasi* - MHOM/BR/1974/PP75), polyclonal antibodies at a dilution of 1/4000 with a reducing non-specific binding of (Dako Unspecific Bond Reducer) in a humid chamber for 30 minutes. In the following, we used biotinylated secondary antibody (DAKO LSAB kit). For the revelation of the primary antibody was used as chromogen diaminobenzidine, and contrasted with Harris hematoxylin (Duarte; Rochael, 2006).

Definition of groups and treatments

The study was randomized, containing two treatment groups, consisting of 60 patients each group. The calculation of the sample (n) was based on the Central Limit Theorem, which advocates samples from more than 30 observations for continuous variables; the distribution of mean is Gaussian - normal (Jekel; Katz; Elmore, 2005).

Was performed exchanged randomization in blocks. In a box, were deposited 20 records; each half of the records had different color. The blue color was defined as Group I and the red as Group II. Each patient who met the inclusion criteria with-

drew, randomly, a record to define in which group it would be included.

Group I - patients were treated with Fluconazol® (PRATI-Donaduzzi Laboratory - national drug industry, in the state of Paraná); boxes with 100 hard gelatin capsules provided by the Health Department of the Municipality of Barbalha at a dose of 300 or 450 mg/day. 300 mg were administered to patients weighing less than or equal to 50 kg and 450 mg for patients weighing more than 50 kg. Patients received capsules of 126 or 168 Fluconazol® at a concentration of 150 mg/capsule, respectively, havng to eat two or three capsules at once in the morning. The number of capsules provided was sufficient quantity for a six-week treatment (42 consecutive days).

Group II - patients were treated with Glucantime® (provided by the Department of Health of Barbalha) at a dose of 20mg/kg/day. Treatment was carried out for 20 consecutive days intravenously over 30 minutes of application. The drug was diluted in normal saline serum, 250 ml, in an outpatient setting. Patients received ampoules corresponding to 20 days of treatment.

Monitoring and laboratory tests

Returns were performed after 20, 40, 60 and 90 days. Before treatment the patients underwent a complete physical examination. Then, blood was collected by venipuncture into BD Vacutainer ™ tubes of type (purple cap) for CBC; BD Vacutainer® type tubes (red and gray cover) for liver function tests (AST and ALT), renal function (urea and creatinine) and dosage of glucose. Followed the establishment of the Montenegro test, reagent obtained from PRCI (Production and Research Center of Immunobiology - Secretary of the State of Paraná Health); one ml bottles with Leishmania (L) amazonensis at a concentration of 40mg/ml of protein nitrogen. 0.1 ml was applied, with intradermal sterile hypodermic syringe on the anterior surface of the right forearm after sterilization with 70% alcohol. Once proceeded the review after 72 hours, reading was performed with metal calipers Azehheb brand. Finally, an ECG was performed by registering all the derivations.

During treatment, were carried out in two stages (in the middle and end of treatment), ALT dosages, AST, urea and creatinine (all with enzymatic kits LABTEST - Brazil) and an ECG for every return to the user of Glucantime® throughout treatment with antimony and urine analysis with standard analysis. The observed side effects were recorded at each visit. The blood counts were performed by an automated cytometer ABX Pentra 60, with internal and external quality control. The tests in biochemistry were performed by automated spectrophotometer LabMax - 240 LABTEST with control external and internal quality.

Patients were evaluated by the principal researcher for the clinical follow-up, using the protocol itself and by a blinded observer (clinical doctor's clinic), which recorded the clinical outcome and side effects in the conventional patient record. The number and appearance of the lesions were described by both. The size (measured with calipers in millimeters) and location (Head, Upper Limps, Lower Limbs, Body Joint or Miscellaneous) were recorded. A photographic record of the evolution of the lesions, with a digital camera (12 megapixels), was made. Lesions with secondary infections were treated with topical antibiotics or, when necessary, a systemic antibiotic (azithromycin). The patients were examined until the full lesion(s) healing(s), with no evidence of inflammatory activity, which is the cure criteria (Brazil, 2007).

Statistic analyzes

To mount the database, was used a licensed Excel Microsoft Corporation 2010 © spreadsheet. Statistical analyzes were carried out using SPSS© software, version 17.0, licensed. The program GraphPad Prism ©, version 3.0, licensed, was used for making graphs and calculations. The estimate of treatment effi-

cacy was obtained through the relative risk reduction of calculation. The Student Test t was used to compare, in both groups, the means of continuous variables. The level of correlation parametric data was evaluated by Pearson's correlation coefficient (r). The healing time of each group had its evaluation by the Kaplan-Meier method. In all hypothesis tests, the α was considered significant when less than 5% ($p < 0.05$).

Results

We included 120 patients with cutaneous Leishmaniasis, selected from the eligibility criteria, a total of 280 patients examined at the Tropical Diseases Clinic of UFCA and whose etiologic agent was *Leishmania (V.) braziliensis* **(Figure 1)**.

The diagnosis was confirmed by identification of the parasite in at least one of three parasitological methods used and the Montenegro intradermalreaction. Sixty patients were primary use of Fluconazol® and 60 primary use of Glucantime®, according to inclusion criteria previously described **(Table 1)**.

The patients studied were born in the city of Barbalha, resident since birth, with no travel record; predominantly in the countryside, in both groups **(Table 2)**. Most of the women were in the group Fluconazol® n = 34 (56.7%), and in equal numbers in the study group who used the Glucantime n = 30

Figure 1: Leishmanias observed by Immunohistochemistry (A), Histopathology - Giemsa (B) and Imprint (C) in material from patients with LT. Source: research data.

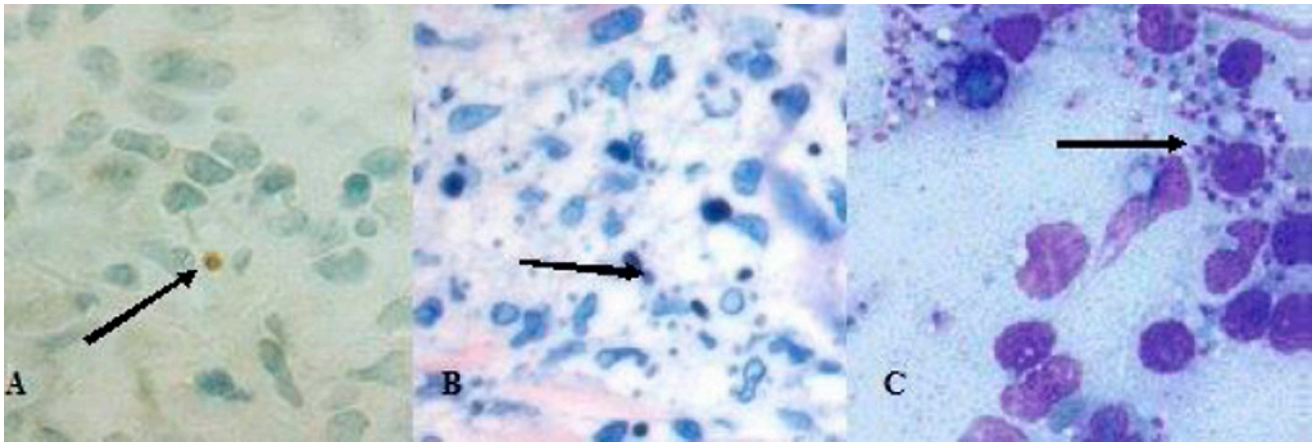

Table 1. Diagnostic methods of American Tegumentary Leishmaniasis, and its expression, positive or negative.

Method	Fluconazol		Glucantime	
	Positive (n) (%)	Negative (n) (%)	Positive (n) (%)	Negative (n) (%)
Histopathological	38 (63.7%)	22 (37.5%)	31 (51.6%)	29 (48.4%)
Giemsa	28 (46.6%)	10 (16.6%)	25 (51.6%)	06 (10.0%)
Serious threat	10 (16.6%)	28 (46.6%)	06 (10.0%)	25 (51.6%)
Imprint	58 (96.7%)	02 (03,3%)	59 (98.3%)	01 (01,7%)
Culture	38 (63.7%)	22 (37.5%)	41 (68.3%)	19 (31.7%)
Montenegro	1.61 (1.09–2.37)	0.016	2.99 (1.50–5.96)	0.002

Source: research data.

Table 2. Epidemiological variables and their distributions in patients with American Tegumentary Leishmaniasis by treatment.

Variable		Drugs	
		Fluconazol n (60)	Glucantime n (60)
Sexo			
Woman		34 (56.7%)	30 (50.0%)
Man		26 (43.3%)	30 (50.0%)
Moradia			
Rural		57 (95.0%)	60 (100.0%)
Urban		03 (05.0%)	-
Ocupação			
Farmer	man	20 (33.5%)	22 (36.6%)
	woman	08 (13.3%)	02 (03.3%)
Student	woman	08 (13.3%)	07 (11.6%)
	man	03 (05.0%)	05 (08.3%)
Other	woman	19 (31.6%)	21 (35.0%)
	man	02 (03.3%)	03 (05.0%)
Location			
Lower limb		21 (35.0%)	19 (31.7%)
Top member		21 (35.0%)	22 (36.6%)
Head		05 (08.3%)	06 (10.0%)
Joint		09 (15.0%)	06 (10.0%)
Trunk		04 (06.7%)	07 (11.7%)
Mixed		40 (66.7%)	45 (75.0%)
Race			
White		15 (20.0%)	12 (20.0%)
Black		05 (08.3%)	03 (05.0%)
Age			
Average		41 (68.3%)	33.7 (56.1%)
Minor		18	18
Of age		88	84
Evolution (in weeks)			
Average		4.9	4.6
Less time		1.0	2.0
Greatest time		12.0	12.0
Number of lesions			
Media		1.4	1.7
Minimum		1.0	1.0
Maximum		4.0	20.0
Bulbao			
Present		33 (55.0%)	34 (56.7%)
Absent		27 (45.0%)	26 (43.3%)
Weight			
<50 kg		12 (20.0%)	05 (08,3%)
> 50 kg		48 (80.0%)	55 (91.7%)
Cure			
Primary		40 (66.7%)	59 (98.3%)
Secondary		20 (33.7%)	01 (01,7%)
Size of injury (mean)		25.2 ± 18.2	27.8 ± 30.0

Source: research data.

(50.0%). This is an essentially rural population, with an average age between 30 and 40 years (group I, mean of 41 years; Group II, mean 33.7 years). The age difference between the two groups was evaluated by the Student Test t, the result of $p < 0.05$ was 0.032, a result that characterizes the case of two distinct populations with respect to age. The studied groups consisted of the resident farmers on the slopes of Araripe, tropical region of hot semi-arid type, with ombrophilous forest, home to rich flebotominic fauna (Silva 2004). Students, housewives and merchants represented the other occupation identified as others. Overall, sought health services, health care, on average, after four weeks of evolution of the lesion (Group I n = 4.9 weeks; Group II n = 4.6 weeks). It was observed in both patient groups with up to three months of their skin lesion evolution. There were no patients who sought medical care with less than a week in the populations studied. The minimum time was one week in Group I and two weeks in Group 02. All the patients of the search had only classical ulcerated lesions of varying sizes and numbers, located generally in the limbs.

Altough the size in patients with single ulcer size was unitary, in patients with multiple ulcers the final size was configured by the size of the sum of these. Holders of leishmaniasis presented with complaint of ulcerated lesion, with or without lymphadenopathy satellite. Sometimes this lymphadenopathy was accompanied by episodes of fever with chills, preceding days in ulceration **(Table 2)**. The number

of ulcers in both populations, ranged from one to 20. In Group I, the average damage was around 1.4 in Group II around 1.7. The size of the lesions as measured in mm ranged from a minimum of 8.2 mm and a maximum of 113.5 mm in Group I; in Group II, the minimum size was 8.0 mm and the maximum of 190,6mm.

After a physical examination and laboratory tests, there was a weak association with comorbidities, especially hypertension, observed in 12 patients, 07 treated with Fluconazol® **(Table 3)**. There were no significant laboratory abnormalities of clinical interest.

Table 3. Clinical data of patients with American Tegumentary Leishmaniasis treated with Fluconazol® and Glucantime®.

Clinical aspects	Patients treated		Total
	with Fluconazol	with Glucantime	
Hypertension	07	05	12
Diabates mellitus	01	00	01
Mental retardation	01	00	01
Aortic insuf.	01	00	01
Hearing deficence	00	01	01
No clinic changes	50	54	104
Total	60	60	120

Source: research data.

In **Table 4**, we observe the therapeutic effects of drugs used in the study, which allow measuring the effect of these intragroup and intergroup. Intergroup effect's measure refers to the relative risk's reduction, also known as therapeutic efficiency (TE).

This measurement is calculated considering the risk of either drug alone, subsequently applying the formula of Coutinho; Cunha (2005). It has thus the following result: F.T.S. = 40/60 = 0.6; G.T.S. = 59/60 = 0.98. The chance of healing intragroup injury, using the Fluconazol®, is 60%. The Glucantime® is 98%; however therapeutic efficacy compared to

Table 4. Therapy Success intra-group of patients with MCL treated with Fluconazol® and Glucantime®.

Treatment	Patients treated		Total
	Success n (%)	Dud n (%)	
Fluconazol	40 (66.6%)	20	60
Glucantime	59 (98.3%)	01	60
Total	99	21	120

Source: research data.

Fluconazol® Glucantime® would be 38.7%. Despite the low therapeutic effectiveness has not been observed any side effects during treatment with Fluconazol®.

$$FTE = (1 - (F.T.S./G.T.S.))x100$$

FTE = Fluconazol® Therapeutic Eficacy.
F.T.S. = Fluconazol® Therapeutic Sucess, (Intragroup eficacy).
G.T.S. = Glucantime® Therapeutic Sucess, (Intragroup eficacy)

The obtained data show that all successful results observed with Group I (treated with Fluconazol®), were found in lesions ≤ 30 mm **(Table 5)**. The relation between lesion size and the chance of cure by Fluconazol® exists and has proportional inverse correlation, as is shown by the Pearson Correlation Coefficient, equal to -0.7842 (negative and moderate correlation, p <0.0001).

Table 5. List of lesion size and the effect of treatment with Fluconazol® in patients with.

Lesion size	Effect of fluconazol		Total
	Sucess	Failure	
>30 mm	00	20	20
≤30 mm	40	00	40
Total	40	20	60

Source: research data.

The healing of the lesions occurred in all cases and in both groups. The cure criterion was essentially clinical, defined by re-epithelialization of ulcerated lesions, total regression of the infiltration and erythema, up to three months after completion of the treatment regimen; criterion assessed by the investigator and, independently, by a blinded observer as described in the methodology. The average healing time of Group I (Fluconazol®) was 45.7 days, with a minimum time of 32 days, occurred in two cases of young women, with 09 mm lesion each; maximum time of 62 days observed in lesions greater than 20 to 30 mm size, resistance to Fluconazol® and responsives to the 20 days treatment with Glucantime®. The healing of the lesions in Group II (Glucantime®) occurred in the first 20 days of clinical follow-up in 59 patients, responsive to Glucantime®. One case of tegumentary leishmaniasis in a young man of 20 lesions did not respond to conventional treatment, responding promptly to amphotericin B in 20 days, with a final cure after 40 days of clinical follow-up without recurrence.

Discussion

This randomized study, performed in a population of 120 patients, to evaluate the therapeutic efficacy of Fluconazol® in high doses in the treatment of tegumentary leishmaniasis was justified and is justified in light of the risks related to the use of first-line drugs, in general, in elderly patients, especially in cardiac, hepatopathic and nephropathic patients, to the exclusive use of these injectable, to complications at the application site, among other discomforts and contraindications. Follows the line of several studies conducted in endemic areas, including the Baturité region in order to find an alternative drug for the treatment of American tegumentary leishmaniasis by *Leishmania (V.) braziliensis* (Laffitte; Gentonb; Panizzon, 2005; Alrajhi *et al*., 2002.) The target population of this study was represented by patients in general the countryside, in the Municipality of Barbalha, randomly divided into two groups - Group I, with 60 patients using Fluconazol®, 300 or 450mg/day. 300 mg have been administered to patients weighing less than or equal to 50 kg, and 450 mg for patients weighing more than 50 kg. A Group II represented by patients using Glucantime® 20mg/kg/day treated for 20 days intravenously over 30 minutes of application.

The study subjects were represented, mostly by rural workers people in the city of Barbalha, not floating fixed residents, study site, residents on the slopes of Araripe, forest zone to the south of Ceará. This fact shows that the American tegumentary leishmaniasis in Barbalha is still zoonosis, as described in the literature (Brazil, 2015; Gontijo; Carvalho, 2003). The subjects, mostly, are mestizos (mixed skin color), slightly higher prevalence in females in the group Fluconazol® 34F (56.6%), 26H (43.7%). The GROUP II population was equivalent in gender, 50% of men and 50% women. Apparently, in Barbalha, women, like men, attend the woods. The literature shows that leishmaniasis is predominant in males (Travi *et al*., 2002). The studied population of young adults, with a mean age of 41 years (68.3%) in Group I and age of 33.7 years (56.1%) in Group II. The national literature states that ATL occurs in both sexes and all age groups, however, in the country's average, predominantly greater than ten years, in 90% of cases in males, with 74% of the sample (Brazil, 2015). The average disease duration in Group I was 4.9 weeks (± 2.2 standard deviation), a little over a month; Group II was 4.6 weeks (± 2.2 standard deviation), very close to Group I. The studies show that for LCM means dertermined by *L. (V.) panamensis*, in Colombia, the evolution period is situated around two to three months (Osorio; Castillo; Ochoa, 1998). This fact seems to show that inhabitants of Barbalha area who care about the health or immunobiology of local leishmaniasis

must have a different profile. A data of interest found was that slightly more than 50% of MCL cases studied are presented in the form bubonic (Group I: 33(55.0%) and Group II 34(56.7%)). This form of leishmaniasis, determined by *L (V.) braziliensis*, is described in the literature (Sousa *et al*., 1995). Manifested with satellite lymphadenopathy at important times, with or without fever and chills. The status preceeds days or weeks to the onset of ulceration and lymph node puncture shows plenty of amastigotes (Bomfim, *et al*., 2007, Sousa *et al*., 1995).

Regarding comorbidities, we found a low association with the LTA other synchronous disease. 12 patients had hypertension, controlled in treatment, one diabetic, one oligophrenic accompanied by the local CAPS, one with hearing loss and one with aortic insufficiency. Comorbidities did not influence the course of the disease, nor interfere with the suggested treatment. There are descriptions, increasingly growing in the ATL association with HIV-positive patients, not shown in this study (Rabello; Orsini; Disch; 2003).

In the diagnosis of ACL, the methodologies showed variable sensitivity. The Imprint as parasitological examination revealed important sensitivity in both studied groups (Group I: 58 (96.7%), 59 (98.3%)). Studies show the sensitivity of the method, easy, low cost and high sensitivity when performed with good technique and by expertise (Light *et al*. 2009; Bahamdan, 1996). The findings corroborate the literature (Gontijo; Carvalho, 2003). Montenegro, like Imprint, showed high sensitivity in both groups (Group I: 56(93.3%), Group II: 55(91.6%)). Montenegro should not be used in isolation when the investigation of patients in endemic areas, considering the high level of awareness of individuals in these regions (Person; Lopes, 1963; Marzochi, 1992). The biopsy with histopathological is not a sensitive method for diagnosing ATL, especially in mucocutaneous form (Ridley; Magalhães; Marsden, 1989) and should be used in the differential diagnosis of skin deep mycoses (chromomycosis, blastomycosis) and skin cancer, basal cell ulcerated and epidermoid (Magalhães; Chiarini; Raick, 1982). The findings of this trial confirm the literature data (Group I: 38 (63.7%), Group II: 31 (51.6%)). The culture of Leishmania from skin punch is important parasitological method with high sensitivity, but this study showed equivalence with histopathology, according to various contamination during the procedure (Group I: 38 (63.7%), Group II : 41 (68.3%)). It is an effective method, but with long-term and need for refinement in the collection, as well as during incubation time and growth monitoring of parasites (Rodrigues, 2000).

Perhaps one of the major problems, in the medical context, of tegumentary leishmaniasis is in the treatment (Reithinge *et al*., 2007). It is the penury to the sick with its wound(s) open, often deforming for leaving scar(s). Even that took place several decades of his advent by Gaspar de Oliveira Viana, pentavalent antimony, trivalent originally known as tartar emetic (TE), did not find suitable replacements (Costa, 1992; Mitropoulos; Konidas, Durkin-Konidas, 2010). Leishmaniasis is as old as human history, as civilization (Piscopo; Mallia, 2006). Since ancient times, there are reports of tegumentary leishmaniasis, existing descriptions of its clinical manifestations in the library of King Assurbanipal in the 7th century BC (Manson-Bahr, 1996). Initially, patients were treated with TL with local ointments and plant extracts. Only in the nineteenth century, in Tashkent, capital of Uzbekistan, the main city in Central Asia, in the foothills of the mountains with rich Golestan flebotômica fauna, we started to use pure lactic acid as abrasive injuries. Several other abrasives were used during this period with relative success or uncertain (Berman, 1988).

It fell to Oliveira Viana Gaspar use, for the first time, the trivalent antimony, known at the time as tartar emetic (Demicheli; Frézard, 2005). The tri-

valent antimony was already used as an emetic in the treatment of syphilis and even arthritis (Weldon *et al.*, 1983). Side effects, such as depression, coughing and chest pain, were identified as negatives, investing in the alternative treatment demand (BERMAN, 1988). These undesirable effects of TE instigated the research, culminating in the development of pentavalent antimony (SbV), as estibamina urea, estibosan and neoestibosan in 1920 (Almeida, Santos *et al.* 2011).

Treatment of tegumentary leishmaniasis, in simple ulcerated form, is recommended to accelerate the healing process and prevent the continuation or dissemination of the parasite, and minimize scarring deformations (Blum *et al.*, 2004, Weina *et al.*, 2004). Unlikely in the LCM and in the kalazar, treatment should always be performed, because these forms of leishmaniasis can complicate and culminate in death (Brazil, 2006; Davidson, 1998). In regions where leishmaniasis is endemic, there is the provision, by the official health agencies, for free treatment of all patients, although there are failures, sometimes important, in consequence to this demand, because of the quantitative limitation of the therapeutic arsenal and the lack of qualified professionals for diagnosis and monitoring treatment (Reithinger *et al.*, 2007).

The control of tegumentary leishmaniasis nowadays is only based on pharmacotherapy, since the vaccines did not show the desired effect, but there are several ongoing trials to develop a vaccine with high prophylactic efficacy (Palatnik-De-Sousa, 2008). The primary treatment remains pentavalent antimony in sodium stibogluconate formula (Pentostan®, Glaxo Wellcome, London, UK), often used in English-speaking countries or as N-methylglucamine antimoniate (Glucantime®, Rhone-Poulenc- Rohrer, Paris, France), used mainly in Latin America and French or Spanish-speaking nations (Berman, 1988; Rath *et al.* 2003). Comparative study between the two formulations was conducted in Kenya, showing better economic viability of Glucantime® (Moore *et al.*, 2001). However, the World Health Organization (WHO) states that the therapeutic efficacy of Glucantime® depends on several factors, such as geographic location and the types protocols of treatments used (Gontijo; Carvalho, 2003). Most endemic regions, with the exception of Venezuela, French Guiana and Suriname, which have their own health policies, follow the WHO guidelines for the treatment of American tegumentary leishmaniasis, the use of 20 mg/kg Glucantime® by daily for 20 consecutive days (Reithinger *et al.*, 2007). The original guidance was 10bmgSbV/kg (Glucantime®), however, with the emergence of non-responsive cases to the treatment, particularly in Indian Territory in the 1970s, was nominated this new dosage (Thakur *et al.*, 1999) Evidences describe that TL response to treatment depends on the species and strain of *Leishmania sp*. (Almeida, Santos *et al.* 2011; Arevalo *et al.* 2007; Yardley *et al.* 2006). In addition, the use of pentavalent antimony is threatened by natural development of resistance of parasites to this drug (Croft; Sundar; Fairlamb, 2006). These events triggered, in recent years, the search options for chemotherapy of leishmaniasis, based on drugs used in similar diseases, biotechnological potential isolated substances of plants and micro-organism, as well as substances used in popular medicine (Lima *et al.*, 2007).

The literature describes approximately 25 compounds with antileishmanial properties, although only a few are used in the treatment of leishmaniasis. In general, they present one or more disadvantages such as high production cost, difficulty of administration, toxicity, determine the development of resistance (Mishra; Saxena; Singh, 2007). Currently, for non-responsive to the pentavalent cases, it is used the amphotericin B, including its liposomal formulation; the pentamidinas and paromomycin (Brazil 2007). On the other hand, major side effects and the high production cost limit their use (Mishra; Saxena; Singh, 2007). Moreover, some

cases have been described resistant to treatment with pentamidine (Basselin *et al.*, 2002). Studies show four new potential drugs in the treatment of LV, including liposomal amphotericin B (Berman, 1998), oral miltefosine and sitamaquine (Sundar, Rai, 2002; Moore and Lockwood, 2010), these two in test in India; and a parenteral formulation Aminosidine, the Paromomycin (Thakur *et al.*, 2000). With regard to tegumentary leishmaniasis, topical formulations of paromomycin were introduced (Soto *et al.*, 2001; Santos *et al.*, 2008); the immunomodulator imiquimod (Garnier; Croft, 2002) and very promising use of Fluconazol® orally (Gonzalez, 2002).

Several studies demonstrate the potential therapeutic efficacy of Fluconazol® orally, for an average of six weeks from several different species of Leishmania (Alrhaji *et al.*, 2002; Sousa *et al.*, 2011; Laffitte; Gentonb; Panizzon, 2005; Minodier *et al.*, 2005). This study aimed to evaluate the therapeutic efficacy of Fluconazol® in high doses orally, compared to standard treatment recommended by the Ministry of Health of Brazil. Sixty patients with a minimum age of 18 years underwent treatment for six weeks. At the end of the study, 40 have been successful in treatment, equivalent to 66.6% (intra-effectiveness) of the study population, while individuals with the standard treatment, traditional treatment, obatained 59 satisfactory results (98.3%). The calculation of the final ET was 38.7%. No Fluconazol® group patient had adverse reactions, significant changes in hepatic, renal or cardiac function. Assessing patients treated with Fluconazol® all failures occurred in those with lesions greater than 30 mm. By withdrawing patients from Group I, with greater than 30 mm lesions, we would have a therapeutic effectiveness superior to treatment performed with Glucantime, skirting the 100%. Reviewing the literature, only one paper considers the size of the lesions (Alrhaji *et al.*, 2002). In this study of 80 patients using Fluconazol®, the lesions had an average size of 17 ± 11 mm. In this test, the average size of the lesions in Fluconazol® the group was around 25.2 mm, with a standard deviation of ± 28.2; Glucantime in the group with 27.8 mm and standard deviation of ± 30. This fact changes the look regarding the treatment of tegumentary leishmaniasis with Fluconazol® in lesions with sizes larger than 30 mm. The data obtained in the study are not the case. The relationship between lesion size and the chance of cure by Fluconazol® exists and has inverse proportional correlation, as shown by the Pearson correlation coefficient, equal to -0.7842 (negative and moderate correlation). This correlation is significant at $p < 0.05$ (0.0001). The cause of this association is not clear in the study, nor this was the object of this work.

The survival curve Kaplan-Meir and the Logrank test show there is cure, but at different times present statistical significance with Glucantime® earlier and the Fluconazol® a little later. This difference loses significance, considering that the average time difference is 20 days and the benefits from the oral treatment overlap with the time difference, reducing the risks of complication. In the present study was not observed side effect with Fluconazol®. There was, however, a downside - the cost of treatment. A treatment of tegumentary leishmaniasis with Glucantime® considering a young adult, using an average dose (two ampoules daily for 20 days), cost R$ 296.00 (two hundred ninety-six reais); treatment of cutaneous leishmaniasis with Fluconazol® considering a young adult, using an average dose of three tablets of 150 mg daily for 42 days (six weeks), was R$ 1,127.30 (one thousand one hundred and twenty seven reais and thirty cents).

In conclusion it may be said that, at least in patients with ATL derived from Barbalha, lesions larger than 30 mm, and in young adults without comorbidities the conventional treatment with Glucantime should be preferred. In other cases, assessed the patient's condition, the Fluconazole would

be the drug of choice. This would avoid the daily commute of the patient, resident in farm, from the foot of the plateau of Araripe, to the Health Centre, aiming to take the injectable formulation of Glucantime. This would also spare the patient the discomfort of the injection application and its complications, and adverse effects on liver, kidney and heart functions. There would not be, in principle, the need for control tests during treatment, only the initial routine to assess the real situation of the patient. Moreover, an Imprint, associated with the Montenegro, would be the preferred method to make the diagnosis.

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