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A pilot and open trial to evaluate topical Bacterial Cellulose bio-curatives in the treatment of cutaneous leishmaniasis caused by L. braziliensis

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ABSTRACT

The treatment of cutaneous leishmaniasis (CL) in Brazil using pentavalent antimony (Sb^v) is associated with a high failure rate and long time to heal. Moreover, standard Sb^v treatment cures only 50–60% of the cases. In this pilot clinical trial, we evaluated the topical use of bacterial cellulose (BC) bio-curatives + Sb^v in the treatment of CL caused by *L. braziliensis*, in Bahia, Brazil. A total of 20 patients were randomized in two groups assigned to receive either parenteral Sb^v alone or parenteral Sb^v plus topically applied BC bio-curatives. CL patients treated with Sb^v + topical BC bio-curatives had a significantly higher cure rate at 60 days post initiation of treatment compared to CL patients treated with Sb^v alone (P=0.01). At day 90 post initiation of treatment, cure rate was similar in the two groups as was overall healing time. Adverse effects or local reactions to topical BC application were not observed. This pilot trial shows that the potential use of a combined therapy consisting of topical BC bio-curatives and parenteral Sb^v in favoring healing of CL lesions caused by *L. braziliensis*, at an early time point.

1. Introduction

Cutaneous leishmaniasis (CL) is a neglected infectious disease associated with a single lesion or a few lesions that may heal spontaneously or evolve to metastatic, more severe forms of disease (Ramírez and Guevara, 1997). Pentavalent antimonials (Sb^v) meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) remain the first-line treatment for CL, but have important limitations such as parenteral route of administration and adverse effects that are dose-dependent (Croft and Coombs, 2003). The rate of therapeutic failure is up to 50% in CL endemic regions where *Leishmania braziliensis* is prevalent (De Prates et al., 2017; Machado et al., 2010). Amphotericin B (Annaloro et al., 2009) and Miltefosine (Machado et al., 2010) are also limited with regards to cost and/or time of treatment, reinforcing the need for new chemotherapeutic alternatives. Given that treatment options for CL are currently limited, a combination treatment of Sb^v with new formulations can increase efficacy, lower drug dosage and lead to a general decrease in side effects, favoring a positive outcome.

In the present work, we explored a combined treatment consisting of parenteral Sb^v accompanied by topical use of bacterial cellulose (BC) membranes. BC is a natural hydropolymer produced by different acetic acid bacteria strains, suitable as both a drug delivery system and as wound dressing. (Carvalho et al., 2019; Portela et al., 2019) BC is comprised by a homogenous, open-porous three-dimensional network of cellulose fibers (Laromaine et al., 2018), with high liquid absorption and retention capacity (Geyer et al., 1994). BC acts as physical barrier, inhibiting bacterial growth (Powell et al., 2016); it is biocompatible, permeable to gas and liquids, improving wound and burn healing (Barud et al., 2016). We have previously demonstrated, using a pre-clinical model of CL caused by *L. braziliensis* that BC-bio-curatives can be used as platform for drug delivery (Celes et al., 2016). Given these properties of BC, we designed a pilot and open trial to evaluate the outcome of topical application of BC bio-curatives regarding cure rate, healing time and adverse effects.

2. Methods

Ethical considerations: This trial was approved by the Ethics

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Committee of the Federal University of Bahia, Salvador, Brazil (CEP 1.331.798). It was conducted based on the principles established by the Declaration of Helsinki. Informed consent was obtained from all patients before enrollment.

2.1. Study design

Setting: This study was conducted in the health post of Corte de Pedra, an endemic area for *L. braziliensis*, in Bahia, Brazil. In this area, *L. braziliensis* causes severe forms such as mucosal and disseminated leishmaniasis and increasing therapeutic failure is observed even in localized and less severe forms. Thus, the Ministry of Health in Brazil indicates systemic treatment for CL. Healing time for CL patients in Corte de Pedra is usually 60 to 90 days after initiation of standard Meglumine antimoniate (Sb^v) (Glucantime; Sanofi-Aventis) therapy (20 mg Sb^v/kg/day for 20 consecutive days) therapy and clinical cure on day 30 occurs in ~20% of patients and on day 60 in ~ 47 to 54% patients (De Prates et al., 2017; Machado et al., 2020). A failure rate of up to 50% has been reported for Sb^v treatment of CL caused by *L. braziliensis* in Corte de Pedra (Machado et al., 2010).

Subject selection: The inclusion criteria were the presence of only 1 ulcerated lesion measuring 1–5 cm of diameter, illness duration >1 month and <3 months, in a subject 18–65 years of age. CL diagnosis and species typing was confirmed by detection of subgenus Viannia (*L. braziliensis*) DNA by polymerase chain reaction (PCR), as decribed (Weirather et al., 2011). The exclusion criteria were evidence of severe underlying disease (cardiac, renal, hepatic, or pulmonary), including serious infection other than CL; immunodeficiency or antibody to human immunodeficiency virus; pregnancy or lactation and subjects with no ability to understand or no desire to sign the informed consent.

Group assignment and treatment. Patients were randomized into two groups (Sb^v, 10 patients, or Sb^v+BC, 10 patients) (randomizer.org). One patient moved to another region after initiation of treatment and was withdrawn. Meglumine antimoniate (Sb^v) (Glucantime; Sanofi-Aventis) was administered intravenously at a dose of 20 mg Sb^v/kg/day for 20 consecutive days (maximum daily dose of 1,215 mg) to both groups. The study group (Sb^v+BC) was treated simultaneously with topical BC membranes (kindly donated by Seven Biotecnologia-Nexfill, Londrina-PR, Brazil), placed onto the ulcerated lesion, and covered with Tegaderm Film (1624 W 3 M Health Care). Seven Biotecnologia-Nexfill had no role in study design, analysis, decision to publish, or preparation of the manuscript. Nexfill Biocellulose Curatives (BC) are films composed of cellulose fibers with a nanometric structure, which is thin in thickness, non-toxic and hypoallergenic. Nexfill Biocellulose Curatives have the same selective permeability of the skin, allowing normal sweating, preventing the escape of liquids, reducing the loss of electrolytes and proteins, and preventing the entry of microorganisms. BC bio-curatives were replaced three times a week for 3 weeks. In patients treated with Sb^v+BC, lesions were cleaned with soap water before replacement of BC bio-curatives. This procedure as well as lesion dressing with Tegaderm was conducted by a health care worker. Lesions in control patients were not submitted to the soap water cleaning procedure.

Study procedures. Twenty milliliters of peripheral venous blood were collected from each patient at the time of diagnosis and 15 days after initiation of treatment in order to detect early toxicity and provide safer follow-up. Blood was used to perform a complete hemogram, tests for aminotransferases (aspartate aminotransferase, alanine aminotransferase), urea, and creatinine. All women in child-bearing age were submitted to beta human chorionic gonadotropin test to exclude pregnancy. All patients were submitted to incisional biopsy on the border of the ulcer. Patients were seen for follow up at 2 weeks (15 days), 2- (60 days) and 3- months (90 days) post initiation of treatment. Patients that did not return for follow-up were visited at home the same day or within 7 days of the missed appointment.

Clinical outcomes. The primary endpoint was final cure at 90 days after initiation of therapy. The secondary endpoints were initial cure at 60 days after the initiation of therapy, healing time in days, and clinical Adverse Effects (AEs). Cure was defined as lesions showing complete reepithelialization, without raised borders or infiltrations, at day 90 post initiation of treatment. Presence of an active ulcer or raised borders at day 90 were considered as failure. Additional courses of Sb^v were indicated when patients were diagnosed with failure at day 90 by the 2 independent physicians. At this point, the patients were treated with another Sb^v course for 30 days, according to the recommendations of the Ministry of Health of Brazil. These patients continued to be followed up until complete reepithelialization was observed. Two physicians who were unaware of the group assignment independently examined the patients on all visits. Clinical AEs were graded according to the Common Terminology Criteria for Adverse Event v3.0 of the National Cancer Institute (ctep. cancer.gov/protocolDevelopment/electronic_applications/ docs/ctcaev3.pdf)

Statistical analysis. Statistical analyses were performed using GraphPad Prism 6.00 (GraphPad Software, San Diego, CA). Values of $P < 0.05$ were considered significant. Intention-to-treat analysis was performed to establish the cure rates. For quantitative variables, difference between two groups was determined by the Mann–Whitney test. For categorical variables, the comparison between groups was calculated using the chi-squared test.

3. Results

A total of twenty patients were enrolled in the trial from January 2018 to December 2019 (Fig. 1). All were diagnosed by PCR as infected by *L. braziliensis*. One patient allocated to the Sb^v group moved to another region, consequently being excluded from follow up. Patients ranged in age from 19 to 42 years with a predominance of male sex in both groups. No significant differences between the two groups were observed regarding clinical characteristics (Table 1). Most patients presented regional lymphadenopathy associated with the ulcerated lesion and lesions were located in the lower limbs (5/9 in Sb^v alone and 9/10 in Sb^v+BC); mean duration of disease was one month (Table 1).

Sixty days (two months) after the initiation of treatment, 22% (2/9) of patients in the Sb^v group were cured compared to 80% (8/10) in the Sb^v + BC group ($P = 0.01$) (Table 2). The cure rate at 90 days (3 months post initiation of treatment) was similar for both groups: 55% (5/9) in the Sb^v group and 80% (8/10) in the Sb^v + BC group ($P = 0.25$) (Table 2). Among patients treated with Sb^v + BC, 2/10 (20%) did not cure at day 90 and needed additional treatment with of Sb^v. These two subjects were considered as failures at 6 months by ITT (Intention to Treat) analysis. Among patients treated with Sb^v alone, 4/9 (44%) did not cure at day 90 and also needed additional treatment with Sb^v. All patients who needed rescue treatment showed complete re-epithelization of lesions. The decision regarding the need for additional treatment was made by both investigators who evaluated the patients and there was a 100% of agreement between them regarding cure or failure. Overall, the healing time (in days) ranged from from 20-180 days in patients treated with Sb^v alone and 30-270 days in patients treated with Sb^v + BC, including patients who needed rescue treatment ($P = 0.29$, Table 2). Fig. 2 shows a representative CL lesion at the time of enrollment, CL lesion covered by the BC bio-curative and, 60 days later, CL lesion after complete tissue reepithelialization. In general, presentation of arthralgia and myalgia was similar in the two groups and no adverse skin reaction or any alterations in blood work parameters were observed in patients treated with Sb^v + topical BC.

4. Discussion

The parenteral route of administration, high toxicity and increasing cases of resistance have decreased therapeutic efficacy of pentavalent antimonials (Ponte-Sucre et al., 2017), nonetheless, they remain the standard CL treatment in Brazil (Machado et al., 2010; Ministério da Saúde, 2021). To this end, we evaluated a combination treatment for CL

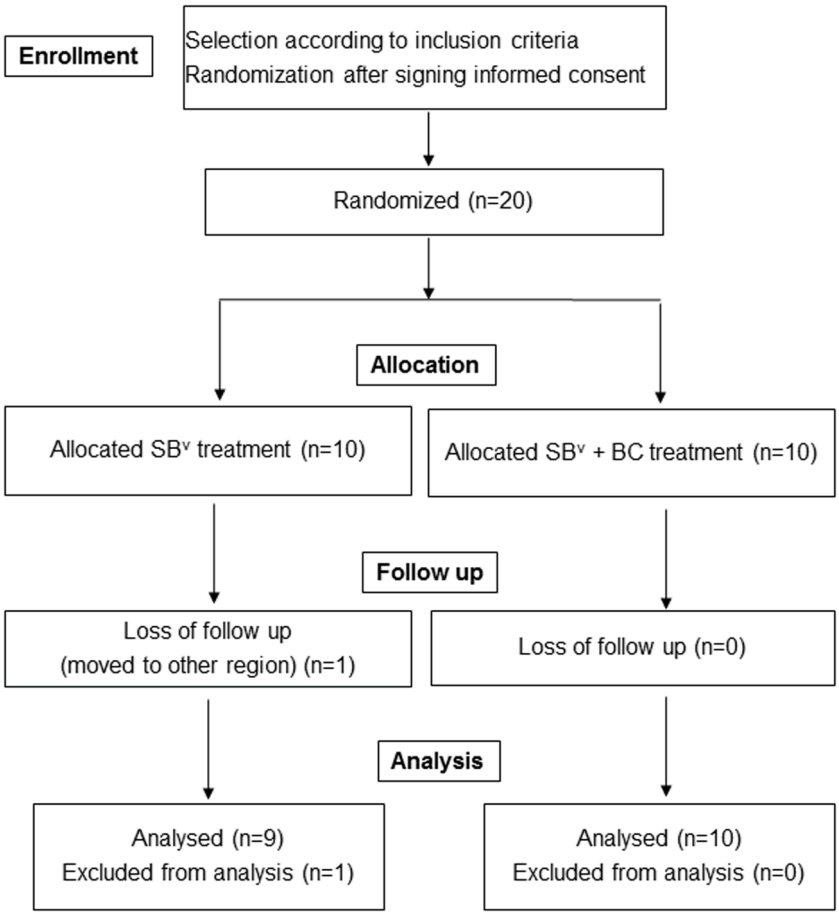

Fig. 1. Study design.

Table 1
Baseline characteristics of CL patients included in the trial and treated with Sb^v or Sb^v + BC.

Characteristic	Sb^v (n=9)	Sb^v + BC (n=10)	P value
Age, mean±SD, y	28.9 (10.7)	29.5 (9.7)	0.90¶
Male to female ratio	6/3	8/2	
Weight, mean±SD, kg	64.2 (7.4)	74.8 (16.5)	0.05¶
Lesion area (mm^2)*	220 (60-532)	209 (110-690)	0.9¶
Lymphadenopathy ratio	4/9	8/10	0.10§
Disease duration (days)*	30 (30-60)	30 (30-56)	0.49¶
Lesion site ratio (lower limbs)	5/9	9/10	0.08§

¶ Mann-Whitney test
* Median (interquartile difference)
§ Pearsonś chi-squared test

Table 2
Therapeutic outcome two and three months data in CL patients treated with Sb^v or Sb^v + BC.

Characteristic	Sb^v (n=9)	Sb^v + BC (n=10)	P value
Cure rate at 2 months (60 days)	2/9 (22%)	8/10 (80%)	0.01§
Cure rate at 3 months (90 days)	5/9 (55%)	8/10 (80%)	0.25§
Healing time (days)*	90 (20-180)	60 (30-270)	0.29¶

§ Pearsonś chi-squared test.
* Median (interquartile difference).
¶ Mann-Whitney test.

consisting of topically applied BC bio-curatives plus standard systemic Sb^v in a randomized, pilot open trial. We showed that topical use of BC bio-curatives in combination with systemic Sb^v significantly increased

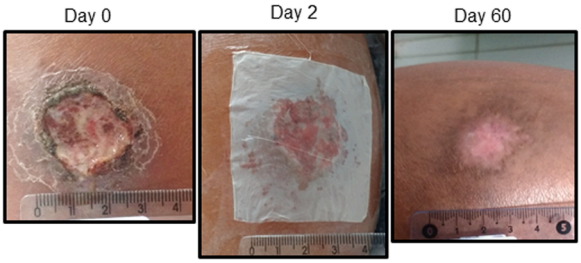

Fig. 2. Topical treatment of CL lesions with BC bio-curatives. BC bio-curatives were applied onto CL lesions and changed every two days, for 20 days. Photographs show lesion at the time of diagnosis (day 0), after placement of bio-curative (day 2) and at day 60.

cure rate at an early time point, that is 2 months (60 days) after initiation of treatment, compared to patients treated with Sb^v alone. In this study, the cure rate at 90 days post treatment was similar for both groups of patients as was the overall healing time. Our limited study size may explain these two outcomes. A failure rate of up to 50% has been reported for Sb^v treatment of CL caused by *L. braziliensis* in Corte de Pedra (Machado et al., 2010). Herein, subjects from both groups needed additional courses of Sb^v.

BC is considered an optimal dressing as it maintains high humidity at the wound site, removes excess exudates, is non-toxic and non-allergenic, allows oxygen exchange, prevents microbial invasion, is easily managed by the patient and is cost-effective (Dart et al., 2019; Felgueiras et al., 2020; Jannesari et al., 2011; Rezvani Ghomi et al., 2019). Indeed, application of BC bio-curatives decreased healing time in

patients with epidermolysis bullosa (Dwiyana et al., 2019), a condition characterized by chronic multiple wounds, and in skin graft donor patients (Alves et al., 2009; Brown and Holloway, 2018). BC when used as an implant, stimulated fibroblasts and collagen production (Helenius et al., 2006) and bacterial cellulose film supports the growth, spreading and migration of human keratinocytes (Sanchavanakit et al., 2006). We believe that such properties may have contributed towards the better CL lesion healing, at day 60 only, as reported here. To our knowledge, BC does not present antileishmanial activity but BC can be used as delivery vehicle (Barud et al., 2013; Santos et al., 2004).

Topical formulations have been developed for leishmaniasis but few have been successful in clinical trials. One example is paramomycin used alone or combination with gentamicin, which has been tested in CL caused by *L. major* (Ben Salah et al., 2013). Recently, paromomycin was also proven effective against CL caused by *L. panamensis* (Sosa et al., 2019) and *L. braziliensis* in Bolivia (Soto et al., 2019). To our knowledge, this is the first time that topical BC bio-curatives have been tested in CL wounds. We show that use of this easily managed (Vilar et al., 2016) and low cost (under U$$ 8 per bio-curative, 8cm x 10cm) addition to the current CL treatment scheme can positively impact cure rate at an early time point (60 days post initiation of treatment). Moreover, no adverse effects were observed in subjects treated with topical BC bio-curatives, evidencing their safety.

We are aware that a major limitation of our study is the limited sample size, which likely reflected in the similar cure rates at 90 days post initiation of treatment, and also in the overall time to heal observed here. Another limitation in our study may be related to differences in wound care regime as CL patients treated with BC bio-curatives had their lesions cleaned with soap water whereas control patients did not. However, in the clinical trials that evaluated the efficacy of topical paramomyicin for CL treatment (Ben Salah et al., 2013; Soto et al., 2019), lesions were cleaned with soap water, and following drug application, lesions were covered with sterile gaze and secured with tape. In both studies, authors found that the cure rate was superior only in lesions treated with drug application compared to vehicle, indicating that lesion cleansing and gauze covering do not significantly improve cure rate.

In conclusion, we believe we have evidence to pursue a larger trial, capable of addressing the limitations of the present study, to corroborate the potential use of topically applied BC bio-curatives for the treatment of CL caused by *L. braziliensis*, in addition to Sb^v.

CRediT authorship contribution statement

Fabiana S. Celes: Conceptualization, Methodology, Data curation, Writing – original draft. **Hernane S. Barud:** Conceptualization, Methodology. **Sayonara M. Viana:** Data curation, Writing – original draft. **Pedro B. Borba:** Data curation, Writing – original draft. **Paulo R.L. Machado:** Writing – review & editing. **Edgar M. Carvalho:** Conceptualization, Methodology, Writing – review & editing. **Camila I. de Oliveira:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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