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ORIGINAL ARTICLE

Combination of topical liposomal amphotericin B and Glucantime in comparison with glucantime alone for the treatment of anthroponotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica*: study protocol for a randomized, controlled trial

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ABSTRACT

Background and Objectives: Cutaneous leishmaniasis (CL) treatment is a challenging issue, although numerous modalities have been introduced as candidate treatment for CL yet only antimonial agents are commonly used to treat CL, a different form of amphotericin B is used to treat visceral form of leishmaniasis but the efficacy against CL is not high. There are a few reliable clinical trials on CL, the main reason is the nature of the disease which required a well design protocol to evaluate the efficacy of any candidate treatment against CL. In this study, a protocol was developed and used to evaluate a topical formulation of a nano-liposomal form of amphotericin B in addition to glucantime to treat CL caused by *L. tropica.*

Materials and Methods: This study is a phase 3, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of topical nano-liposomal amphotericin B (SinaAmpholeish 0.4%) in combination with intralesional injections of meglumine antimoniate in the treatment of ACL caused by *L. tropica.* Overall, 130 patients, aged 12-60 years, with a diagnosis of ACL caused by *L. tropica* are recruited and treated according to the protocol.

Results: A total of 130 patients with CL lesion will be recruited and double- blind randomly treated with received intralesional injections of Glucantime weekly or Glucantime plus SinaAmpholeish for 4 weeks.

Conclusion: The results of this study showed that the protocol works well and the treatment was tolerated by both groups of patients.

Keywords: Treatment; Leishmaniasis; Cutaneous; Anthroponotic; Clinical trial; Protocol

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INTRODUCTION

Leishmaniasis is a neglected disease caused by protozoa, *Leishmania*, and transmitted through the bite of female sand flies. The four clinical patterns of this disease in humans are cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). Leishmaniasis is endemic in many parts of the world including the Middle East, 14 of the 22 countries of the EMRO region are endemic to leishmaniasis. Both forms of CL; zoonotic CL [ZCL] caused by *L. major* and anthroponotic CL [ACL] caused by *L. tropica* is endemic with high incidence rates in different parts of Iran, CL is a major health problem that leads to considerable morbidity and causes severe disfigurement (1-4).

Various modalities such as chemotherapy, curettage, cryotherapy, etc. are used to treat CL, unfortunately, with not enough efficacy, chemotherapy is usually done by using antimonials (20 mg/kg for 15-20 d, either intravenously (IV) or intramuscularly (IM) for sodium stibogluconate, and IM only for meglumine antimoniate. Antimonials are also commonly used as intralesional injections. Among the various techniques for intralesional injection, infiltration of 0.1 mL of drug per each square centimeter at the periphery of the lesion using small-gauge needles (ie, Nos. 27-30) to up to complete blanching of each lesion, is generally used, the cure rate reported with antimonial therapy is 40%-70% (5-9). There is no significant difference between intralesional and intramuscular administration. This therapeutic approach is associated with several side effects, systemic administration accompanies by myalgia, blood and liver abnormality tests, pancreatitis and cardiotoxicity (6, 9). The most common side effect of the intralesional injection is the pain at the site of injection and allergy to antimonials (4-9).

Amphotericin B is a polyene macrolide and the most effective systemic antifungal drug, which is highly effective against different species of *Leishmania*. Amphotericin B is the second -line drug for the treatment of leishmaniasis but the main drawback of this drug is the acute toxicity especially cardio-nephrotoxicity, the effect of polyene drugs is through higher affinity to ergosterol and episterol which lead to leakage of intracellular potassium and magnesium. Three lipid formulations of liposomal amphotericin B (AmBisome®), the amphotericin B colloidal dispersion (Amphocil™), and the amphotericin B lipid complex (Abelcet®) have been developed to reduce toxicity and improve the efficacy of the drug. AmBisome™ showed significantly lower toxicity compared to the other formulations and has been approved by the US Food and Drug Administration. Due to the lower toxicity of AmBisome, a higher dose might be used which is more effective. Although AmBisome is highly effective against VL, it is not very effective to treat experimental CL (10-12).

A topical form of amphotericin B has lower side effects and a small amount of the drug reaches sensitive internal organs such as the kidneys. *Leishmania* parasites live and multiply within the phagolysosomes of infected macrophages, the stratum corneum (SC) of the skin prevents penetration of topical agents, but lipid formulation penetrates into the skin probably due to the similarity to skin lipid composition (13). In order to reach an effective drug penetration through the skin, and target the *Leishmania* parasites within the phagolysosome, an effective drug-carrier system such as liposome might be useful.

Liposomes are spherical vesicles, consisting of phospholipids and cholesterol, which form bilayers and entrap water-soluble molecules in the internal water compartment and water-insoluble ones into the lipid bilayers. Liposomal formulations introduced several advantages over other delivery systems (10, 11). Liposomes are able to pass SC, and reach the viable epidermis and deep dermis, liposomes are actively engulfed by dermis macrophages, the entrapped drugs in the liposomes are released in phagolysosomes where *Leishmania* parasites live and multiply (10-17).

Liposomal amphotericin B containing 0.4% of amphotericin B was formulated using phosphatidylcholine and cholesterol (18) and was produced (under the commercial name of SinaAmphoLeish 0.4%) under GMP conditions in Minoo Pharmaceutical Co., Tehran, Iran. This formulation has been tested *in vitro* and *in vivo* against *L. major* and *L. tropica*, the results showed that the formulation is very effective. The safety of the preparation was checked *in vivo* in an animal model (Draize skin irritation test and Draize eye irritation test). Then the preparation was tested in healthy human volunteers for safety evaluation (19, 20).

In the current study, the SinaAmpholeish 0.4% preparation will be tested in human volunteer pa-

tients with cutaneous leishmaniasis caused by *L. tropica*. The efficacy and safety of this product as a combination with the national treatment protocol of intralesional injections of meglumine antimoniate will be assessed and compared with placebo according to GCP guidelines (21).

MATERIALS AND METHODS

General objectives. To evaluate the efficacy and safety of the combination of topical liposomal amphotericin B and meglumine antimoniate (Glucantime) in comparison with Glucantime alone for the treatment of anthroponotic cutaneous Glucantime (ACL) caused by *Leishmania tropica*.

Specifics objectives. To compare the rate of clinical cure in the group of patient volunteers who received the combination of topical nano-liposomal amphotericin B (SinaAmpholeish 0.4%) plus meglumine antimoniate (Glucantime) and the group who received Glucantime alone at 4 and 8 weeks and at 3 and 6 months after the treatment initiation.

Study design. This study is a phase 3, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of topical nano-liposomal amphotericin B (SinaAmpholeish 0.4%) in combination with intralesional injections of meglumine antimoniate in the treatment of ACL caused by *L. tropica*.

Sample size. The sample size was considered 49 patients in each group according to the desired response rate of 70% to treatment with the combination of a topical nano-liposomal form of amphotericin B plus national treatment and expected a response rate of 40% to National Guideline treatment and placebo. We determined a significance level of 5%, the power of 80%, and used the EpiInfo-6 statistical package. To compensate for the loss to follow up or withdrawal, 20% was added to the sample size.

Population. Overall, 120 patients, aged 12-60 years, with a diagnosis of ACL caused by *L. tropica*, will be enrolled from the endemic areas of Mashhad, 2016-2018 in the northeast part of Iran. ACL diagnosis will be done based on positive direct smear and/or culture, identification of *Leishmania* species causative agent of the lesion will be confirmed by PCR. If the PCR results indicate *L. major*, as the causative agent of any of the patients, that specific patient will be withdrawn from the trial and replaced with a new volunteer patient. The potential candidates will be fully informed about the nature of the study and only those knowingly and willingly giving written consent will be recruited.

The study was designed by the Center of Research and Training in Skin Disease and Leprosy, Tehran University of Medical Sciences, and was confirmed by EMRO/WHO.

Selection of the patients. Males and females 12-60 years of age.

Inclusion criteria.

1- Parasitologically proven CL caused by *L. tropica* (identification is done by using PCR).

2- Otherwise healthy subjects on the basis of medical history, physical examination, and results of blood tests if requested by the physician.

3- Ability to understand the principles of the trial.

4- Signed informed consent voluntarily and knowingly.

Exclusion criteria.

1- Pregnant or lactating women (woman required pregnancy test will be tested for pregnancy)

2- Concurrent or chronic illness based on previous history (patients presenting with concomitant illness will be referred for appropriate clinical care)

1- Duration of lesion more than 6 months.

2- The number of lesions is more than 5.

3- Ulcer size greater than 3 cm in their largest diameter.

4- History of the full course of standard treatment (antimonials).

5- History of allergy to Glucantime or amphotericin B.

6- Involvement in any other drug or vaccine trial during the study period.

Volunteers diagnosed with ACL but are ineligible for inclusion in the trial will be treated with the standard treatment free of charge.

Volunteer patients who were in the placebo arm and who did not respond will be given the choice of a further standard treatment plus the topical nano-liposomal form of Amphotericin B (SinaAmpholeish 0.4%).

Study development: logistic phase. The duration

of this phase is 6 months and include the following activities:

- Screening as routine clinical practice management and distribution of information of the study.
- Informed consent signing before recruitment.
- Baseline assessments, recruitment, and blind randomization into two groups.

Recruitment phase. Male and female patient volunteers aged 12-60 years, with ACL caused by *L. tropica* will be recruited from the endemic area of Mashhad, Iran. The participants are required to be healthy (except for CL lesion) based on medical history and physical examination, patient volunteers should be able to follow the principles of the trial and sign the informed consent voluntarily and knowingly. Patients with any of the following conditions will be excluded; Pregnancy, breastfeeding, duration of the lesion more than 6 months, number of lesions more than 5, ulcer size greater than 3 cm in the largest diameter. If the patient has already been treated with a full course of standard treatment (antimonials) or has a history of allergy to Glucantime or Amphotericin B or she/he is involved in any other drug or vaccine trial during the study period will be excluded.

Screening visit. During this visit, a complete clinical history and physical examination will be elaborated. The data about leishmaniasis such as the number of lesions, the location (on a diagram), size of induration, ulceration and scar (greatest diameter X perpendicular diameter in millimeters), presence of lymphangitis, and adenopathy will be recorded. The inclusion and exclusion criteria will be applied and the study will be explained to the potential candidates. The candidate patients will be requested to sign an informed consent form.

Treatment visits. The patients will be visited in the Leishmaniasis Clinic and Treatment Center in the city of Mashhad. Two groups will be treated according to the Iranian National Guideline which is either systemic treatment (20 mg/kg/day antimonate, 3 ampoules at most or weekly intralesional injections of Glucantime plus biweekly cryotherapy. Double-blind randomly one group will be treated with topical nano-Liposomal Amphotericin B (SinaAmpholeish 0.4%) twice a day for 28 d and the other group will be treated with a placebo twice daily for 28 d. In each visit, response to treatment and the characteristics of lesions will be assessed and the data will be collected. If any adverse event is detected, the patient will be referred immediately for necessary evaluation and will be reported to the adverse event committee to make ay decision.

Follow-up visits. Each patient will be visited on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 90 and 180. The number, locations, appearance, and size of the lesion(s) will be recorded and lesions will be photographed from a standard distance with a standard lighting system.

Procedure. To evaluate the response to treatment, the lesion(s) characterizations for each patient such as the number, sites, appearance, and size of the lesions will be recorded at the first visit, lesions will be photographed from a standard distance with a standard lighting system at day first, and repeated on days 56, 90 and 180. At each visit, the evolution of lesions will be assessed using a standard Evaluation of Lesion Scheme (ELS). According to ELS, cured (CC) means complete re-epithelialization with loss of induration, partially cured (PC) means More than 50% reduction in size or induration compared to baseline. If there is an equal or less than 50% reduction in size or induration compared to baseline, then is named Improved (I). No Change (NC) means no observable change in the size of the active lesion and Worsened (W) is any measurable increase in the size of the lesion(s).

Evaluation and management of adverse events. During the study, the patients will be assessed for any adverse events. The side effects such as toxicity related to infusion and nephrotoxicity are lower due to topical administration of drugs, a small amount of the drug reaches sensitive internal organs such as the kidneys. All of the adverse events will be reported to the adverse events committee for making the appropriate decisions.

Data analysis phase. The categorical variables will be described using frequency and percentage, and the quantitative findings will be described using mean and standard deviation. To analyze the efficacy of the main outcome variable of the study will be compared by the chi-squared test between the two groups. The main approach of the study will be intention-to-treat. The final results will be analyzed per protocol. Lost

data will be replaced through multiple imputation techniques.

Endpoints. The endpoints of this study are the complete re-epithelization of all lesions within 90 days, no recurrence at day on day 180, and partially cured (PC) equally or more than 50% reduction in size or induration compared to the baseline in two groups.

Final report. The results of this study will be collected and discussed. The final report will be presented to World Health Organization (WHO/EMRO) and the Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Science that is the sponsor for this study. After analysis, the results will be published in national and international journals and presented to the scientific committee.

Ethical aspects. The proposal and related documents have been submitted to the Tehran University of Medical Sciences Ethical Committee and were approved by the letter of 93/S/130/1676 dated 10/14/2014 (22/07/1393, Persian calendar), approval No 26900.

The study will be explained to the potential candidates and sufficient time will be given to the candidates to decide whether or not to participate in the study. Volunteers will be given the opportunity to enquire about the details of the study and any questions regarding the study will be answered. The PI will ensure that the consent form is signed and dated by the volunteer, physician, the legal guardian for subjects under 18 years old, and an independent witness (if applicable). The PI will ensure that the written information and the consent form are revised.

Ethical considerations. Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

RESULTS

Baseline data. At the baseline, 207 potential volunteer patients were screened and 77 were excluded from the study; 28 were excluded due to not meeting the eligibility criteria, 3 patients not consenting to participate, and 17 patients were excluded due to other reasons. After obtaining informed consent, 130 patients were allocated to SinaAmpholeish or placebo group according to a pre-defined random sequence.

The mean age of the study participants is 35.4 ± 14.2 and 34.1 ± 16.3 years, in SinaAmpholeish and placebo, respectively. 49% (24/49) of SinaAmpholeish and 60% (33/55) of the placebo group were women. In both comparison groups, the mean duration time of onset was about 12 weeks.

The number of the lesion was one in 27 (55.1%) patients in the SinaAmpholeish group and 32 (58.2%) patients in the placebo group. The highest number of the lesion was five (two patients in each arm).

DISCUSSION

Treatment of cutaneous leishmaniasis (CL) still rely on using pentavalent derivatives (meglumine antimonite, glucantime and sodium stibogluconate, Pentostam) which are used either as intralesional or systemic, the treatment of CL needs multiple injections for a long period of time which is painful and not tolerated by every patient. No topical treatment is available to facilitate the CL cure process, although, there have been numerous candidate modalities in the experimental phase (4-7), one of the reasons is the nature of the CL lesion. Clinical trials to evaluate the therapeutic effects of a candidate treatment must be performed according to a well-designed protocol (21, 22). The current protocol was designed according to the national and international guidelines and should be able to precisely demonstrate the effect of SinaAmpholeish on the treatment of ACL. The outcome of the ongoing study so far showed that the protocol is well designed and easy to follow and accepted by the regulatory authorities.

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