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Original Article

Field Efficacy of Topical Nano-Liposomal Amphotericin B (Sina Ampholeish®) Alone or in Combination with Glucantime® and Cryotherapy on Human Cutaneous Leishmaniasis

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Abstract

Background: Cutaneous leishmaniasis (CL) is a parasitic disease that presents a broad spectrum of clinical features. Treatment of CL is problematic. We aimed to compare the field therapeutic efficacy of topical nanoliposomes containing 0.4% amphotericin B (Nano Lip-AmB) alone and in combination with cryotherapy and/or Glucantime® on human CL in the endemic areas of Iran.

Methods: This retrospective study was performed based on the results of using Nano Lip-AmB alone or with Glucantime® and/or cryotherapy in the treatment of zoonotic cutaneous leishmaniasis (ZCL) in patients referred to health centers of Isfahan, Golestan and Ilam Provinces of Iran as endemic foci of ZCL caused by *Leishmania major* besides Mashhad and Bam cities as endemic foci of anthroponotic cutaneous leishmaniasis (ACL) caused by with *L. tropica*.

Results: Two hundred and seventy-eight patients with CL were included in the current study. All of the patients (100%) who received Nano Lip-AmB alone or in combination with Glucantime® and/or cryotherapy based on guideline of Iranian national committee for the treatment of CL. Two patients with 7 skin lesions, who was resident in ACL endemic area and received Nano Lip-AmB plus Glucantime® and another patient was a resident of ZCL endemic area and received Nano Lip-AmB plus cryotherapy showed clinical relapses after treatment.

Conclusion: Sina Ampholeish® in combination with other standard protocols of treatment of CL is well tolerated and with acceptable clinical efficacy rate.

Introduction

Leishmaniasis is a neglected tropical and subtropical disease and characterizes a complex of diseases with an important clinical and epidemiological diversity. Leishmaniasis has a broad spectrum of clinical manifestations including ulcerative skin lesions, destructive mucosal inflammation, and disseminated visceral infection (kala-azar) (1). The most common is CL which can be caused by several *Leishmania* spp. and it is rarely fatal (1). *L. tropica*, the causative agent of ACL, and *L. major*, causes ZCL. Iran is endemic area for old world CL and CL is one of the differential diagnosis for chronic skin diseases (2).

Mashhad from Khorasan Razavi Province, Bam and Kerman in Kerman Province are the most endemic areas of ACL in Iran (2). Almost 85% of reported CL in Iran showed epidemiological and clinical pattern of ZCL (2).

The highest annual incidence rate of ZCL was reported from Ilam, Fars, Semnan, Isfahan, Golestan and Khuzestan Provinces during 2013 – 2020 (2-4).

CL treatment is a challenging subject that is an imperative step in the development of lesion management (5). Several paradigms have been introduced as a candidate treatment for CL such as chemotherapy, curettage, cryotherapy, but the efficacy against CL is not high (6-8). Amphotericin B (AmB) is the most effective drug for the treatment of fungal diseases as well as protozoan infections such as the *Leishmania* parasite but it is highly nephrotoxic (9, 10).

In CL, the *Leishmania* parasites grow inside infected macrophages in the dermis of the skin. Nano-liposomal amphotericin B (Nano Lip-AmB) with a size of about 100 nm contains the drug amphotericin B, which increases the penetration rate of the drug into the epidermis and dermis and finally the macrophages infected with the *Leishmania* parasites, and as a result, the drug is released with a high concentration in the vicinity of the parasite; Therefore, the use of liposome significantly increases the effectiveness of amphotericin B (10-12).

Considering the many years of use of the Nano Lip-AmB for the treatment of CL alone or together with Glucantime® and/or cryotherapy in the health-treatment centers in CL endemic regions in Iran, in this study, recorded data over several years was collected and evaluated the therapeutic effect of Nano-liposomal form of AmB alone or together with other specific treatments on CL.

Methods

Study design

This retrospective study was conducted at the health care centers in Isfahan, Golestan and Ilam Provinces of Iran as Endemic foci of ZCL caused by *L. major* and Mashhad and Bam cities as endemic foci of ACL caused by *L. tropica*.

Ethical approval was obtained from the Ethics Committees at Tehran University of Medical Sciences, Tehran, Iran ((IR.TUMS.SPH.REC.1400.199)

We evaluated the safety and efficacy of the following treatments for skin lesions caused by *L. major* and *L. tropica* using topical Nano Lip-AmB (Sina Ampholeish®) alone, Nano Lip-AmB combined with Glucantime®, Nano Lip-AmB plus cryotherapy and Nano Lip-AmB plus Glucantime® and/or cryotherapy.

Data collection

A questionnaire including ID code, number, type, size, and location of the skin lesion, existence and type of underlying or accompanying diseases, confirmation diagnostic method (s) of CL, treatment method (s), duration of treatment, alone or combination of Nano Lip-AmB, type and time to use of other drugs, side effects of each kind of the treatment method, recovery times for lesion, recurrence of CL after a period of improvement was completed.

Statistical analysis

Statistical significance was evaluated using SPSS version 24.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9 software.

Results

Response to treatment of CL, in total of 278 with 731 lesion (n= 157, 56.4% male, n= 121, 43.5% female) confirmed CL patients were evaluated in the current study. Of 278 patients; 106 (38.1%), 61 (21.9%), 78 (28.0%) were residents of Isfahan, Ilam and Golestan Province respectively, as endemic foci ZCL. Also, 23 (8.2%) and 10 (3.5%) patients were residents of Mashhad and Bam cities as endemic foci ACL.

Twelve of 278 patients had received Nano Lip-AmB alone twice daily for 28 days. 2 of 278 patients had received national standard treatment of weekly intralesional Glucantime®. 84 of 278 patients had received weekly intralesional Glucantime® plus biweekly cryotherapy (3 or 4 sessions) and Nano Lip-AmB alone twice daily for 28 days. 175 of 278 patients had received Lip-AmB alone twice daily for 28 days plus weekly intralesional Glucantime®. 1 of 278 patinets had received weekly intralesional Glucantime® plus biweekly cryotherapy. 4 of 278 patinets had received Nano Lip-AmB alone twice daily for 28 days plus biweekly cryotherapy.

Patients were treated by different formulation according to the treatment guidelines of the Infectious Diseases Management Center (Table 1).

Table 1: Drug formulation received in CL patients

Data	*N*	*%*
Drug formulation/ ACL endemic areas		
Ampholeish	3	9.09
Ampholeish, Glucantime® and Cryotherapy	2	6.06
Ampholeish plus Glucantime®	28	84
Drug formulation/ ZCL endemic areas		
Ampholeish	9	3.67
Glucantime® plus Ampholeish and Cryotherapy	79	32.2
Glucantime®	2	0.81
Ampholeish plus Glucantime®	151	61.6
Ampholeish and Cryotherapy	4	1.63

Out of 33 patients in Mashhad and Bam cities, 30 (90.9%) patients had Glucantime® in their treatment regimen that 19 (57.5%) in the form of intra-lesion injection, 9 (27.2%) in the form of systemic treatment, and 2 (6.06%) in the form of intra-lesion injection and systemic treatment. Out of 245 patients in Isfahan, Ilam and Golestan Province, 236 (96.3%) patients had Glucantime® in their treatment regimen that 138 (56.3%) in the form of intra-lesion injection, 67 (27.3%) in the form of systemic treatment, and 31 (12.6%) in the form of intra-lesion injection and systemic treatment. Lesion size in patients was categorized in two group including; small (≤ 5 cm) and large (> 5 cm). In the cities of Mashhad and Bam, out of 33 patients, all patients (100%) had small lesion, and in the Isfahan, Ilam and Golestan Province, 167 out of 245 patients (68.1 %) had a small lesion and 78 (31.8%)

had a large lesion. None of the patients in Mashhad and Bam cities had any underlying disease. Out of 245 patinets in Isfahan, Ilam and Golestan Province, 12 (4.8%) patients had any underlying disease.

Clinically dry and wet types of lesions were observed in 26 (78.7%) and 7 (21.2%) patients in Mashhad and Bam cities and 59 out of 245 (24.08%) and 186 (75.9%) patients in Isfahan, Ilam and Gorgan Provinces (Table 2).

Table 2: Glucantime® Route in treatment regimen

Data	*N*	*%*
Glucantime® Route in treatment regimen/ ACL endemic areas		
Inter-lesional	19	57.5
Systemic	9	27.2
Inter-lesional and Systemic	2	6.06
Glucantime® Route in treatment regimen/ ZCL endemic areas		
Inter-lesional	138	56.3
Systemic	67	27.3
Inter-lesional and Systemic	31	12.6

In 26 (78.7%), 7 (21.2%), it was diagnosed by microscopy and culture method in Mashhad and Bam cities. In 225 (91.8%), 2 (0.81%), 17 (6.9%), 1 (0.40%) it was diagnosed by microscopy, clinical, culture and microscopy& culture method in Isfahan, Ilam and Gorgan Provinces (Table 3).

Table 3: Method of diagnosis

Data	*N*	*%*
Method of diagnosis/ ACL endemic areas		
Microscopy	26	78.7
Culture	7	21.2
Method of diagnosis/ ZCL endemic areas		
Microscopy	225	91.8
Clinical	2	0.81
Culture	17	6.9
Microscopy and Culture	1	0.40

There was no treatment-related toxicity, adverse events, infection, or deterioration of any treated in patients. Of 278 patients enrolled, 278 (100%) had 100% wound epithelialization but two patients had recurrences after the initiation of treatment that one of them was a resident of Isfahan Province that treated with combined treatment methods including Nano Lip-AmB plus Glucantime® and cryotherapy and one of them was a resident of Mashhad city that treated with Nano Lip-AmB plus Glucantime® (Table 4).

These two patients were referred to a dermatologist for further examination of treatment. There was no significant relationship between the type of treatment formulation and recovery.

Table 4: Recurrence after treatment

Data		*N*	*%*
Recurrence after treatment/ ACL endemic areas			
	Yes	1	3.03
	No	32	96.9
Recurrence after treatment/ ZCL endemic areas			
	Yes	1	0.4
	No	244	99.5

Discussion

CL is an infectious, parasitic disease of skin caused by different species of *Leishmania* genus (13). CL is endemic in 98 countries of both new and old world with annual new cases varies from 0.2 to 0.4 million for VL and 0.7–1.2 million for CL (14).

In 2018, more than 85% of new CL cases has reported from countries of Afghanistan, Algeria, Bolivia, Brazil, Colombia, Islamic Republic of Iran, Iraq, Pakistan, the Syrian Arab Republic, and Tunisia (15). Referring to activities for CL control program in Middle East region, the number of CL cases reduced from 23202 in 2008 (Incidence rate 32 per 100000) to 13124 in 2019 (Incidence rate 15.8 per 100000) (15).

CL are endemic in different provinces located in north, north-eastern, central, western, south-western, southern and south-eastern Iran (14). In Iran two major types of CL exist: ZCL and ACL that ZCL is due to *L. major* and ACL is caused by *L. tropica* (16). CL is often self-limited. Localized, non-healing, often ulcerative skin lesion is typical clinical presentation of CL (17).

The treatment of CL has been of interest for a long time. Despite a wide range of treatments, poor therapeutic responses and adverse effects are common (15). Many old and new therapeutic approaches have been used to treat CL with three treatment policies, which include local treatment, physical treatment and systemic treatment (18). Local treatment includes the use of plant extracts and mineral substances, intralesional injection using Mepacrine, Emetine and acid berberine sulfate, Glucantime®, and the use of ointments such as paromomycin and Imidazole. Systemic treatment includes the use of medicinal compounds such as Glucantime®, amphotericin B, aromatic diamides, allopurinol, paromomycin, imidazole derivatives, etc. (19). Physical treatments include wound curettage, radiation therapy, thermotherapy, and cryotherapy (20). These drugs present several side effects, long period of treatment and low efficacy, this mainly due to the appearance of parasites resistant (21).

The polyene antibiotic amphotericin B is an antifungal agent has been used as a second-line treatment for mucocutaneous leishmaniasis (MCL) and CL especially when resistance to pentavalent antimonial drugs (10). The mechanism of this drug is the effect on the metabolism of sterols of *Leishmania* and fungi. This drug binds to ergosterol present in the membrane of *Leishmania*, and by causing changes in the cell membrane, it causes cell permeability and kills the parasite (22). The use of amphotericin B is restricted by its acute toxicity (23). In 25% of patients who used it, kidney disorders, malaise, lethargy, fever, chills,

changes in electrolytes and rarely cardiac arrest occurred. In 1997, the FDA approved liposomal amphotericin B for treatment of visceral leishmaniasis (10). Response rates of 90% have been reported in treatment of mucosal leishmaniasis in HIV-negative patients (24). The objective of this study was to assess the efficacy and safety of Sina Ampholeish® alone, together with Glucantime® and Cryotherapy on human CL in endemic areas of the disease in Iran.

In this process research study liposomal amphotericin B alone also together Glucantime® and Cryotherapy showed good efficacy and no side effects. L-AmB in comparison with sodium stibogluconate (SSG) for treatment of *L. braziliensis* CL in travelers treatment is effective, better tolerated, and more cost effective (25). Intralesional Amphotericin B as an alternative treatment for CL have good efficacy in patients infected CL and all of the patients had recovered completely (26). Nano-liposomal form of amphotericin B is safe in animal model as a candidate for treatment of CL and could be further checked in human trials (27). In a randomized, double-blind study, Nano-liposomal form of amphotericin B was safe on Healthy Volunteers (28). Jaafari et al. showed topical liposomal amphotericin B was able to inhibit the growth of promastigote and amastigotes forms of *L. major* in culture media and it also was able to reduce the sizes of skin lesions produced by *L. major* in BALB/c mice (29). Another study demonstrated that combination of topical liposomal amphotericin B and Glucantime® for the treatment of ACL caused by *Leishmania tropica* is effective in patinets with CL lesions (30).

The safety and efficacy of topical liposomal amphotericin B alone or in combination with national standard treatment for CL using Glucantime® on the CL patients with satisfactory results (31).

It seems that Nano Lip-AmB is a promising formulation for treatment of CL. Also 2 patients with 7 lesions, who was resident of Mashhad city and received Lip-AmB plus Glucantime® and another patient was a resident of Isfahan Province and received Lip-AmB plus cryotherapy showed recurrence after treatment. In Guery et al., study 5 of 43 patients experienced a relapse after a median duration of 6 months post treatment (32). In Solomon et al., study 10 of 34 patients had relapses and needed additional treatment (25). Indicating that liposomal amphotericin B may have a suppressive rather than a curative effect (33). In this study, no adverse events associated with the drugs used to treatment were observed. Adverse drug events are important for patient care, quality improvement, drug safety research, and post-marketing surveillance (34). In another study 53% of patients experienced at least one adverse event (32). Pentamidine had fewer adverse events than other drugs in the treatment of mucosal leishmaniasis (35).

Since this study is retrospective study, had some major limitations including limited cases in the group treated with Nano Lip-AmB and some missing necessary data for final statistical analysis.

Conclusion

Depending on the type and number of lesions and the type of CL, SinaAmpholeish® with intralesional injection of Glucantime® or cryotherapy can be effective for the treatment of CL in both endemic areas of ZCL and ACL. Further field evaluation of Sina Ampholeish® alone for the treatment of CL is recommended in larger populations and longer periods.

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Conflict of interest

The authors declare that there is no conflict of interest.

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