

The efficacy of combined topical niosomal dapsone gel and intralesional injection of meglumine antimoniate in comparison with intralesional meglumine antimoniate and cryotherapy in the treatment of cutaneous leishmaniasis

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Abstract

Objective To evaluate the efficacy of niosomal dapsone gel and intralesional meglumine antimoniate with cryotherapy and intralesional meglumine antimoniate in cutaneous leishmaniasis.

Methods This was a randomized clinical trial with 73 participants that were divided into two groups including, case group (weekly intralesional meglumine antimoniate and twice a day niosomal dapsone gel) and control group (weekly intralesional meglumine antimoniate with biweekly cryotherapy). The treatment course continued until 16 weeks or complete cure, whatever occurred earlier and participants were followed up in 4th, 8th, 12th and 16th weeks of the treatment.

Results Overall, 68 patients (33 males and 35 female) completed the study. Age, sex, size and duration of the lesions were not statistically different between two groups. At the end of the study, 82.9% of patients in case group showed complete response.

Conclusion Niosomal dapsone gel has promising results with fewer adverse effects, so, it can be used as an alternative treatment modality, especially in children and patients with contraindication of systemic drugs.

Key words

Leishmaniasis, dapsone, niosomal, topical, treatment.

Introduction

Leishmaniasis is a protozoan infectious disease caused by several species of the *Leishmania* and transmitted by infected sandfly bite.¹ The three mainstay clinical forms of the disease including cutaneous leishmaniasis (CL), mucocutaneous

leishmaniasis and visceral leishmaniasis have

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been recognized.²

Cutaneous Old World leishmaniasis is categorized into two groups, anthroponotic cutaneous leishmaniasis (ACL) and zoonotic cutaneous leishmaniasis (ZCL) which, classically are caused by *L. tropica* and *L. major*, respectively.^{2,3,4}

Annual incidence of the disease is approximately 1.5 to 2 million.^{5,6} It affects more than half of Iran's provinces and there is an increase in geographical spreading of the disease in Kerman, southeast of Iran.^{3,7,8,9}

Although CL is not a fatal disease, the treatment is necessary in order to prevent disease transmission and scar formation especially in exposed sites.^{10,11}

Currently, based on recommendation of WHO, gold standard of the disease treatment is intramuscular pentavalent antimonial with cryotherapy, but, there is limitation to the administration of standard treatment due to poor availability, high cost, serious side effects and painful injection of the drug.^{2,4,8,11,12}

In recent years, several treatment modalities such as systemic antifungal drugs, amphotericin, topical paromomycin and physical therapies were evaluated with different results.^{10,13-18} One of the systemic drugs which can be used as an alternative treatment for leishmaniasis is dapsone. This drug affects alternative complement pathway and can enhance cellular immunity through cytokines.^{19,20}

Absorption of dapsone after oral intake is slow and after absorption, it is found in internal organs such as liver, muscle, kidney and skin. Side effects of this drug are dose-dependent such as hemolysis, methemoglobinemia, peripheral neuropathy, allergic dermatitis, headache,

anemia, hepatitis and agranulocytosis.^{19,20}

Investigations show that oral intake of dapsone in the treatment of different infections leads to low efficacy and therapeutic index of the drug, due to lower concentration at the site of infection.^{19,20}

Based upon recently gathered data, some of the topical treatment regimens had superior effects compared to systemic ones or at least had equal efficacy. Topical treatment options such as niosomal drug delivery systems for the leishmaniasis have increased in recent years.²¹⁻²⁴

Considering the good efficacy and safety of 5% dapsone gel in the treatment of acne,^{20,25,26} we decided for the first time, to assess the efficacy of niosomal form of dapsone in the treatment of leishmaniasis.

Methods

This open randomized clinical trial was performed from December 2011 to October 2013 in dermatology clinic of Afzalipour hospital – Kerman (southeast of Iran). A total of 73 patients were enrolled in the study after their written consent. Patients aged ≥ 7 years and diagnosed with positive smear or demonstration of Leishman-Donovan (LD) bodies in skin biopsy were included in the study.

Exclusion criteria were pregnancy or nursing period, lesions on the face, number of the lesions more than 5, duration of the disease more than 1 year, lesion size larger than 3 centimeter, a history of the treatment of leishmaniasis during last month, a history of allergy to meglumine antimoniate or dapsone, taking immunosuppressive therapy during last six months, patients with systemic disorders such as liver, heart, kidney disease, pancreatitis, immunosuppression, hematological disease, and

sporotrichoid or lupoid forms of the leishmaniasis.

Demographic information including age, sex, size and site of the lesions were completed and induration of the lesions were assessed with ruler through Sokal method. Patients were randomly assigned to group A (intralesional meglumine antimoniate and biweekly cryotherapy) and B (niosomal dapsone gel twice a day and weekly intralesional meglumine antimoniate).

Cryotherapy was conducted by dipstick technique with liquid nitrogen. Cotton-tipped applicator was applied on the lesion until 1 mm white halo formation around the lesion. Meglumine antimoniate (Glucantime™, Rhone-Poulenc, France) was injected until blanching of the lesion.

Considering the fact that dapsone is soluble in lipid, we chose gel as drug vehicle, in order to better drug delivery into skin. In order to prepare niosomal dapsone by conventional hydrated film method, cholesterol and nonionic surfactant and dapsone were dissolved in chloroform and acetone respectively, then, the solvent was evaporated by rotary evaporator, next, the resultant lipid film was hydrated with 5ml deionized water at 70. At last, niosome was mixed with 1% carbomer934 with equal rates. Particle's size analysis, physical stability, encapsulation efficacy, percentage of drug delivery and penetration into rat's abdominal skin have been done in pharmaceutical research center.

Participants were treated for 16 weeks or until complete healing, whatever happened earlier. They were evaluated in the 4th, 8th, 12th, 16th weeks of the treatment and response rate was recorded according to ESL (evaluation of lesions scheme) as follows:

Cured (complete re-epithelialization without induration); partially cured (more than 50% re-epithelialization and decrease induration); improved (less than 50% re-epithelialization and decrease induration in the lesion); no change (without any change in the size of the lesion) or worsening (increase in the size of the lesions).

In this study we, categorized participants into three groups as: complete response (100% improvement), partial response (50%-99% improvement) and no response (improvement less than 50%). None of the patients in this study had increase in the size of the lesion.

This proposal was approved by the ethics committee of research center of medical science university of Kerman with approval code K/90/503.

Statistical analysis

Data analysis was performed by SPSS 17.0 software (SPSSINC, Chicago, USA) and Random effects mixed model, Mann-Whitney test, t test and chi-square were applied for analysis.

Results

Sixty-eight patients (33 male and 35 female) completed the study. Age of the patients ranged between 7 to 80 years). Total number of the lesion was 73, 44 (60.3%) ulcerative, 29 (39.7%) nonulcerative including papules, nodules and plaques. Sixty-three (86.3%) of the lesions were in the upper limb and 10 (13.7%) of the lesions were in the lower limb. Thirty-five of the patients with 38 lesions and 33 patients with 35 lesions were randomly assigned to groups A and B, respectively (**Table1**). The two groups were not significantly different in terms of age, sex,

Table 1 Demographic features and response rate in two treatment groups

	<i>Cryotherapy and meglumine antimoniate</i> <i>n=35</i>	<i>Dapsone and meglumine antimoniate</i> <i>n=33</i>	<i>P value</i>
Mean age (year)	31.6±20.4	29.6±15.9	0.653
Duration (month)	3.7±1.9	4.6±3.9	0.218
Sex			
Male	14 (40.0%)	17 (51.51%)	0.135
Female	21 (60.0%)	16 (48.49)	
Site of the lesion			
Upper Limb	27 (77.1%)	31 (93.9%)	0.570
Lower Limb	8 (22.9%)	2 (6.1%)	
Type of the lesion			
Non Ulcerative	21 (55.3%)	8 (22.9%)	0.005
Ulcerative	17 (44.7%)	27 (77.1%)	

Table 2 Treatment response according to percent improvement during the treatment in 2 groups, cryotherapy and meglumine antimoniate (group A) and niosomal dapsone gel and meglumine antimoniate (group B).

<i>Treatment Response</i>	<i>4 week</i>		<i>8 week</i>		<i>12 week</i>		<i>16 week</i>	
	<i>n (%)</i>		<i>n (%)</i>		<i>n (%)</i>		<i>n (%)</i>	
	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
Complete Response	-	-	11 (31.4%)	7 (18.4%)	23 (65.7%)	23 (60.5%)	29 (82.9%)	33 (86.8%)
Partial Response	26 (74.3%)	26 (68.4%)	23 (65.7%)	30 (78.9%)	11 (31.4%)	14 (36.8%)	5 (14.3%)	4 (10.5%)
Without Response	9 (25.7%)	12 (31.6%)	1 (2.9%)	1 (2.6%)	1 (2.9%)	1 (2.6%)	1 (2.9%)	1 (2.6%)

Table 3 Treatment response comparison between two groups.

<i>Response rate</i>	<i>OR (95% CI)</i>	<i>P value</i>
Total	1.03 (0.64-1.65)	0.885
Sex	1.04 (0.64-1.69)	0.866
Age	1.01 (0.62-1.64)	0.952
Type of the lesion	1.32 (0.8-2.18)	0.271
Site of the lesion	1.15 (0.68-1.93)	0.595
Size	1.14 (0.73-1.79)	0.545

duration of the disease and size of the lesions.

Table 2 shows the treatment response in two groups at week 4, 8, 12 and 16 of follow-up. 29 (82.9%) patients in group A (meglumine antimoniate and cryotherapy) showed complete response as compared to 33 (86.8%) in group B (meglumine antimoniate and niosomal dapsone gel). During the twelve months post-treatment follow-up, no recurrence was reported in the case group, but in the control group one patient developed lupoid leishmaniasis and was treated with systemic meglumine antimoniate.

No side effect with application of niosomal dapsone gel during the treatment and follow up was observed.

Discussion

Intralesional infiltration of the pentavalent antimony compounds is the treatment of choice for leishmaniasis which had variable efficacy in different studies from 81% to 97%, depending on duration of the treatment, *Leishmania* species and definition treatment response.^{1,27,28}

Combination therapy of cryotherapy and intramuscular meglumine antimoniate has more efficacy than single treatment modalities, which leads to lower therapeutic dose, shorter period of the treatment with lower cost and adverse effects.^{29,30,31}

Asilian *et al.* evaluated the efficacy of cryotherapy and intralesional meglumine antimoniate on *L. major*. Ninety percent of the patients showed complete response. This result was compatible with our results (86.8% in control group), however recent conducted studies demonstrated that *L. tropica* is more refractory to treatment than *L. major*.^{27,29}

Dapsone is an antibacterial drug that inhibits synthesis of dihydrofolic acid through competition with para-aminobenzoate. This drug also has anti-inflammatory and immunomodulatory effects by myeloperoxidase blocking.³⁸

In one study by Dogra *et al.* efficacy of oral dapsone in leishmaniasis was evaluated and oral dapsone had 82% response rate in comparison with placebo.¹³ In another study by Dogra *et al.* oral dapsone was used in dose of 2 mg/kg/day for 21 days and 80% of patients responded to treatment and there was no recurrence in the next 6 months.

Due to adverse effects of oral dapsone, 5% topical dapsone gel was used in several studies. Advantage of this form of dapsone is low systemic absorption with plasma peak which is 100 times lower than oral types. Topical dapsone gel has been evaluated in the treatment of acne with good efficacy and favorable results.^{20,25,26}

One of the newest structural drug delivery systems is niosomal form. This form consists of hydrated cholesterol and nonionic surfactants such as alkyl ether, alkyl ester and alkyl acid that induce more stability, higher absorption of drug in the skin and slow release of effective substance.^{41,42}

Niosomes have structure similar to lipids layers of biologic membrane. This structure leads to

increase of drug penetration into skin through melting of the niosome in stratum corneum and delivery of drug in deeper layer of epidermis. On the other hand, this structure enhances penetration of different substances in cells such as Langerhans cells, thus, it has been used and assessed for intracellular infection such as leishmaniasis. Other advantages of this form are lower drug toxicity and cost, as well as, easy manufacturing of the drug.^{41,42}

Our study is the first study to evaluate the efficacy of niosomal dapsone gel in the treatment of CL. Our results showed that efficacy of niosomal dapsone gel combined with intralesional meglumine antimoniate was comparable with the standard treatment including cryotherapy and intralesional meglumine antimoniate. There was no significant difference in partial and complete response rate between the two groups; also, both treatments had similar increase in efficacy during follow-up. During the twelve months of observation, no recurrence was reported in the case group whereas in the control group one patient developed lupoid leishmaniasis.

In this study we were not able to demonstrate *Leishmania* species for all of the patients, but, in the previous study in Kerman, most of the *Leishmania* species were reported as *L. tropica*.⁴³

Strengths of our study are randomization, adequate sample size and similarity between demographic features in the two groups, which increase reliability and stability of our results. We were not able to conduct a double-blind clinical trial, due to different treatment modalities.

We recommend further studies in order to evaluate, efficacy and side effects of niosomal dapsone gel compared to other topical treatment

regimens.

Conclusion

Niosomal dapsone gel can be used as an alternative and adjunctive drug with lower cost and approximately without any adverse effect in the treatment of CL, especially in children, due to painful injection of meglumine antimoniate. It can be applied as an alternative treatment, instead of cryotherapy, in sites such as ear, nose and fingers because of possible risk of scar formation secondary to vascular compromise and in individual with darker skin type due to complications such as hypo/ hyperpigmentation.

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