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ORIGINAL ARTICLE

Evaluation of the efficacy of intralesional Glucantime plus niosomal zinc sulphate in comparison with intralesional Glucantime plus cryotherapy in the treatment of acute cutaneous leishmaniasis, a randomized clinical trial

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Abstract Current treatment modalities in cutaneous leishmaniasis have low efficacy and high toxicity as well as high rate of resistance to treatment. In this study, for the first time we decided to evaluate efficacy of intralesional Glucantime plus niosomal zinc sulphate in comparison with intralesional Glucantime plus cryotherapy in the treatment of acute cutaneous leishmaniasis. This is a case–control study on 64 patients with cutaneous leishmaniasis in Kerman-Iran. Patients were categorized in 2 groups A and B whom were treated with weekly intralesional meglumine antimonite plus twice daily niosomal topical zinc sulphate versus weekly intralesional Glucantime plus every other week cryotherapy, respectively. We assessed the efficacy of treatment modalities (as partial and complete response) and their adverse effects by measuring size of the lesions every 2 weeks up to maximum of 12 weeks and 3 months after the end of the treatment. Partial response rate was 16.6% and 12.9% in group A and B, respectively ($P = 0.784$). Complete response rate was 73.3% and 80.6% in group A and B, respectively ($P = 0.784$). Complete response rate was achieved in 4.73 ± 0.29 weeks and 4.69 ± 0.28 weeks in group A and B, respectively ($P = 0.925$). Partial response rate was achieved in 2.92 ± 0.23 weeks and 2.65 ± 0.18 weeks, respectively ($P = 0.365$). Combination of niosomal zinc sulphate with intralesional Glucantime has equal efficacy versus combination of cryotherapy plus intralesional Glucantime in the treatment of acute cutaneous leishmaniasis. So, it can be used in cases that have resistance to first-line treatments.

Keywords Noisomes · Leishmaniasis · Zinc sulphate · Glucantime

Introduction

Leishmaniasis is an endemic protozoan parasitic infection transmitted by *phelebotumous* sand fly in more than 80 countries. It affects annually 1.5–2 million people's worldwide and nearly 20,000 cases in Iran (Lerner and Grervelink 1996; Lopez and Hay 2004; Norouzinezhad et al. 2016). Based on site of involvement it is classified to cutaneous, mucosal and visceral types. Literally based on geographical location, leishmaniasis is categorized to old world and new world types. Old world cutaneous leishmaniasis is grouped into 2 types of anthroponotic (urban, dry type) or zoonotic (rural, wet type) (Al Jaser et al. 1995; Desjeux 2001; Markle and Makhoul 2004). The most common cause of leishmaniasis in Kerman is *Leishmania tropica* (Sharifi et al. 1998).

Treatment response rate depends on immune status of patients and *Leishmania* parasite type. The first-line treatment for leishmaniasis is meglumine antimonite (Glucantime) that depending on the number and size of the lesions and type of the involvement (disseminated, diffuse, sporotrichoid) can be used as intralesional or parenteral (Lerner and Grervelink 1996). Side effects including toxic effects on heart or liver, renal failure, pancreatitis, painful injection, variable efficacy as well as high cost of treatment

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and treatment resistance have been reported with Glucantime (Oslen and Berdanier 1994). Other treatment modalities that have been used with variable efficacy are amphotericin, miltefosine, pentamidine, paromomycin, allopurinol, dapson, fluconazole, cryotherapy, CO_2 laser and heat therapy. So, search for new treatment modalities (with lower cost and toxicity as well as more efficacy and safety) is always proposed (Dogra et al. 1990; Al-Majali et al. 1997; Esfandiarpour and Alavi 2002; Prata et al. 2003).

Previous studies have showed that zinc has immunomodulatory effects. Zinc deficiency facilitates the entrance of *Leishmania* parasite to macrophages that leads to less efficacy of T lymphocytes and lower treatment response rate, higher resistance to treatment and disease chronicity (Griffiths and Sodeify 1976; Sorkhroodi et al. 2010). Zinc deficiency leads to reduction in cytokines related to Th1 such as interferon-α, tumor necrosis factor-α and interleukin 2. In contrast zinc deficiency leads to increase in cytokines related to Th2 (Mishra et al. 2010).

Oral and intralesional zinc sulphate has been used in the treatment of cutaneous leishmaniasis. Oral formulation of zinc sulphate is associated with variable absorption and efficacy as well as gastrointestinal adverse effects (Sharquie et al. 2001; Yazdanpanah et al. 2011). Intralesional form of zinc sulphate has relatively good efficacy, but painful injection and tissue necrosis especially in acral areas are among the important side effects of treatment (Firooz et al. 2005; Najim et al. 2006).

In this study, for the first time we evaluate efficacy of intralesional Glucantime plus niosomal zinc sulphate in comparison with intralesional Glucantime plus cryotherapy in the treatment of acute cutaneous leishmaniasis.

Materials and methods

This is a case–control study on 64 patients with cutaneous leishmaniasis in Kerman. Diagnosis of leishmaniasis confirmed via positive smear from the lesions and in cases with negative smear skin biopsy was performed. Inclusion criteria were patients with duration of disease less than 6 months and with washout period of 3 month. Exclusion criteria were pregnancy, lactation, patients younger than 2 years old, those with more than 5 lesions, lesions greater than 5 cm, lesions on the face and joints and history of sensitization to zinc sulphate or Glucantime.

First we obtained informed consent from patients and their parents of those who were less than 16 years old, and then we recorded demographic features including age, sex, site, size and duration of the lesions. Based on simple randomization the patients were categorized in 2 groups (A and B). Patients in group A were treated with weekly intralesional meglumine antimonite (Glucantime) plus twice daily niosomal topical zinc sulphate. Patients in group B received weekly intralesional Glucantime plus cryotherapy (by cotton-tipped swap every other week by liquid nitrogen) in 2 freeze–thaw cycle, until formation of 2 mm white halo in the periphery of the lesion. The treatment course was 12 weeks or until complete cure whatever occurs earlier. We evaluated efficacy and adverse effects of treatment modalities every 2 weeks until maximum 12 weeks and then 3 months after the end of the treatment protocol. Also we evaluated efficacy of treatment by measuring size of the lesions (largest diameter) by transparent paper. Complete cure was based on negative leishmaniasis smear.

Response to treatment categorized into 3 groups: complete response (100% clinical improvement with negative smear result), partial response (reduction in size of the lesion more than or equal to 60%), no response (reduction in size of the lesion less than 60% or increased lesion size) (Bahamdan et al. 1997). We evaluated side effects during treatment visits.

This study was approved by ethics committee of Kerman University of Medical Sciences with code IR.KMU.AH.REC.1395.6

Preparation of niosomal zinc sulphate

The dried lipid film results from evaporation of 300 μmol Sorbitan monostearate (SpanTM 60) and cholesterol (70/30 molar percent) in a rotary evaporator (Heidolf, Germany) and then we put it in a vacuumed oven overnight for elimination of organic solvent. Zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$) was dissolved in deionized water and then kept in a rotatory oven with dried lipid forming at 65 °C for 30 min. Non-ionic surfactant vesicles (niosomal suspension) was prepared by lipid film hydration method and maintained at room temperature for 24 h and then kept at refrigerator for study. All chemicals and solvents (Sorbitan monostearate (SpanTM 60), zinc sulfate heptahydrate (ZnSO4.7H2O) and cholesterol) were obtained from Merck, Germany. Evaluation of physical properties of noisomes including analysis of size, physical stability were as in previous studies (Pardakhty et al. 2011, 2012).

Results

This is a single-blind randomized clinical trial that 64 patients with leishmaniasis (32 patients in each group) enrolled the study. Most of the patients were male (53.2% male) and mean age of the patients was 39.56 ± 18.12

Table 1 Demographic characteristics and clinical features of patients at baseline between 2 treatment groups

Variables	Case group	Control group	*P* value
Mean age of patients (years)	35.87 ± 2.84	43.25 ± 3.44	0.104
Mean duration of the lesions	3.59 ± 0.24	3.32 ± 0.21	0.411
Sex			
Male	16 (50%)	18 (56.2%)	0.616
Female	16 (50%)	14 (43.8%)	
Site of the lesions			
Upper limb	27 (84.4%)	25 (78.1%)	0.608
Lower limb	5 (15.6%)	7 (21.8%)	

(range 8–80) years. Table 1 demonstrates demographic features of the patients and clinical features of the lesions.

Mean size of the lesions at base-line visit was 1.32 ± 0.15 cm and 1.32 ± 0.16 cm in group A and B, respectively with no significant difference ($P = 0.99$). Although reduction in size of the lesions during the treatment and follow up sessions was not statistically significant between 2 groups, this parameter was statistically significant in each group during the same period of time ($P = 0.001$). Table 2 shows mean size of the lesions during the treatment visits and after 3 months follow up. Side effects of the treatment in 2 groups are showed in Table 3 with no significant difference.

Partial response rate was 16.6% and 12.9% in group A and B, respectively ($P = 0.784$). Complete response rate was 73.3% and 80.6% in group A and B, respectively ($P = 0.784$). Response rate less than 75% was observed in 10% and 6.5% of the patients in group A and B, respectively ($P = 0.784$). Complete response rate was achieved in 4.73 ± 0.29 weeks and 4.69 ± 0.28 weeks in group A and B, respectively (0.925). Partial response rate was achieved in 2.92 ± 0.23 weeks and 2.65 ± 0.18 weeks, respectively ($P = 0.365$).

Table 2 Size of the lesions (mean ± SD) during the sessions of the treatment in case and control groups

Visits	Case group (cm)	Control group (cm)	*P* value
Base line	1.32 ± 0.15	1.32 ± 0.16	0.990
2 weeks	1.29 ± 0.16	1.13 ± 0.16	0.501
4 weeks	0.95 ± 0.17	0.87 ± 0.16	0.740
6 weeks	0.84 ± 0.17	0.74 ± 0.16	0.678
8 weeks	0.69 ± 0.18	0.58 ± 0.15	0.645
10 weeks	0.50 ± 0.17	0.36 ± 0.13	0.524
12 weeks	0.45 ± 0.17	0.31 ± 0.12	0.505
3 months after end of treatment	0.43 ± 0.17	0.26 ± 0.12	0.444
P value	0.001	0.001	

Table 3 Comparison between side effects between case and control groups

Variables	Case group	Control group	*P* value
Scar	21 (70%)	25 (80.6%)	0.334
Post inflammatory pigmentation	10 (33.3%)	18 (58.1%)	0.053
Blister formation	6 (20%)	11 (35.5%)	0.178
Pain	4 (13.3%)	9 (29%)	0.134

Discussion

Current treatment modalities in the treatment of leishmaniasis are associated with low efficacy due to increased resistance of *Leishmania* parasite (especially *L. tropica*). The main contributing factor for resistance to treatment is reduced concentration of drug in parasitic cells through inhibition of drug activation or stimulation of drug metabolism and drug inactivation (Yasinzai et al. 2013). Newer drug-delivery systems (such as noisomes, liposomes and nanoparticles) provide better penetration of drug to stratum corneum, higher uptake, release of higher concentration and attachment of drug in parasite and macrophage cells (Aflatoonian et al. 2017). In this article, we evaluated efficacy of topical 2% niosomal zinc sulphate plus intralesional Glucantime in comparison with intralesional Glucantime and cryotherapy. Complete and partial response rate were nearly equal in case (90%) and control (93.5%) groups ($P = 0.784$).

In one study by Farajzadeh and colleagues, complete response rate of intralesional 2% zinc sulphate solution plus cryotherapy was 47.5%. In the current study, complete response rate in topical 2% niosomal zinc sulphate plus intralesional cryotherapy was 73.3% that was higher than Farajzadeh study (2016). This difference can be explained by increased level of niosomal zinc sulphate in *Leishmania* parasite and Langerhans cells due to enhanced absorption of niosomal formulation and selective delivery of drug in target organ (Pardakhty et al. 2012).

In another study, Iraji et al. (2004) evaluated efficacy of intralesional Glucantime in comparison with intralesional zinc sulphate that showed complete response rate of 60% vs. 83.8%, respectively. Complete response rate in our study was 73.3% and 80.6% for intralesional Glucantime plus niosomal zinc sulphate versus intralesional Glucantime and cryotherapy, respectively. Iraji study was done on *Leishmania major* and our study was done on *L. tropica* which is more resistance to treatment (Yasinzai et al. 2013). So, this can explain higher response rate in Iraji study than present study. Also, in our study there was no side effect as tissue necrosis, but in Asilian study, tissue necrosis was reported in 10 patients (2004).

Sharquie in a study in 2017, evaluated efficacy of topical zinc sulphate solution 25% and complete cure was observed in 24.4% of the cases. Sharquie accomplished his in Baghdad where most of *Leishmania* cases are due to *L. major* (60%), while our study was done in Kerman with majority of cases are *L. tropica* (Sharifi et al. 1998). Higher efficacy of niosomal 2% zinc sulphate in our study in comparison with 25% zinc sulphate solution in Sharquie et al. (2017) study demonstrates enhanced uptake and bioavailability of niosomal zinc sulphate than conventional form.

Currently, there are only 2 studies evaluating niosomal formulation of anti-leishmaniasis drugs. In one study in 2012, Asadi et al. (2012) evaluated niosomal paromomycin formulation in vitro that showed effectiveness of drug on *Leishmania* parasite. In another study in 2017, Aflatoonian et al. (2017) evaluated efficacy of niosomal dapsone gel plus intralesional Glucantime in Kerman that showed lower complete response rate at 12 weeks of treatment (60.5%) than our study (73.3%). Dapson has anti-inflammatory effects and stimulates cellular immune system by secretion of cytokines, improving alternative complement pathway and inhibition of myeloperoxidase (Zhu and Stiller 2001; Raimer et al. 2008). In contrast, mechanism of action in zinc sulphate is through induction of inflammatory reaction and infiltration of inflammatory cells and tissue damage. Furthermore, zinc inhibits enzyme activity in *Leishmania* parasite and shifts immune system response rate from Th2 to Th1 (Griffiths and Sodeify 1976; Sorkhroodi et al. 2010).

Conclusion

Combination of niosomal zinc sulphate and intralesional Glucantime has equal efficacy versus standard protocol (cryotherapy plus intralesional Glucantime) in the treatment of leishmaniasis. So, it can be used as second-line or alternative treatment in patients with leishmaniasis who don't respond to standard treatment or have no access to cryotherapy. Furthermore, using topical niosomal zinc sulphate is painless and without risk of skin necrosis especially in acral areas.

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Author contributions MA wrote the manuscript and proposal, and acted as Corresponding author. SF involved in the conception, data screening and manuscript writing. MKH helped in data interpretation, literature search and manuscript writing. SM supervised development of work, helped to evaluate and edit the manuscript. AP provided niosomal drug and contributed in manuscript writing. RA helped in data acquisition, manuscript writing. All authors contributed in initial design, data analysis, reviewed, revised, and confirmed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests

Ethical approval In this study, ethical permission (No. IR.K-MU.AH.REC.1395.6) was granted through the Science and Ethics Committee of Kerman University of Medical Silences. All performed procedures were in accordance with the ethical standards of the Iranian institutional and/or national research committee and with the standards of 1964 Helsinki declaration.

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