J Parasit Dis (July-Sept 2016) 40(3):935–939
DOI 10.1007/s12639-014-0609-1

ORIGINAL ARTICLE

Comparison between intralesional injection of zinc sulfate 2 % solution and intralesional meglumine antimoniate in the treatment of acute old world dry type cutaneous leishmaniasis: a randomized double-blind clinical trial

Saeedeh Farajzadeh · Maryam Hakimi Parizi · Ali Akbar Haghdoost · Azadeh Mohebbi · Saman Mohammadi · Abbas Pardakhty · Sana Eybpoosh · Amireh Heshmatkhah · Behrooz Vares · Simin Saryazdi · Ali Reza Fekri · Elham Mohebbi

Received: 30 September 2014 / Accepted: 1 November 2014 / Published online: 22 November 2014

Abstract Zinc sulfate (ZS) has been used for the treatment of acute cutaneous leishmaniasis (CL) in both forms of in vivo and in vitro recently. The aim of the present study was to compare the efficacy of intralesional injection of ZS 2 % solution with intralesional glucantime in the treatment of acute CL. In this double-blind randomized clinical trial, 80 cases with acute old world dry type CL were enrolled in the study. The treatment protocol in the first group consisted of intralesional injection of ZS 2 % vials once a week for 10 weeks or sooner in case of complete resolution of the lesions. In the second group, intralesional glucantime once a week for 10 weeks or sooner in case of complete resolution of the lesions were used. In both groups cryotherapy was performed once every other week for 10 weeks. In ZS versus second group, partial and complete clinical response was observed with fewer injections although this difference was not statistically significant. In addition, we found that the trend of treatment in second group was faster but again it was not significant [partial treatment: hazard ratio (HR) 1.4, 95 % CI 0.7–2.9; complete treatment: HR 1.3, 95 % CI 0.6–2.8]. The results of this study showed that the intralesional injection of ZS 2 % solution was as effective as glucantime on the healing of the acute old world dry type CL.

Keywords Zinc sulfate · Cutaneous leishmaniasis · Intralesional · Glucantime

S. Farajzadeh · M. Hakimi Parizi (✉)
Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran
e-mail: hakimiparizi@yahoo.co.uk

A. A. Haghdoost
Regional Knowledge Hub, and WHO Collaborating Centre for HIV Surveillance, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran

A. Mohebbi · S. Mohammadi · A. Heshmatkhah · B. Vares · S. Saryazdi · A. R. Fekri
Department of Dermatology, Kerman University of Medical Sciences, Kerman, Iran

A. Pardakhty
Pharmaceutics Research Center, Kerman University of Medical Sciences, Kerman, Iran

S. Eybpoosh
Research Center for Social Determinants of Health, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran

E. Mohebbi
Research Center for Modeling in Health, Institute of Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran

Introduction

Leishmaniasis is a parasitic disease that is endemic in some developing countries (Ashford 2000) such as Iran. The most common pathogens are *Leishmania major* and *Leishmania tropica*. Anthroponotic cutaneous leishmaniasis (ACL) caused by *L. tropica* is found mainly in areas of Bam and Kerman (Sharifi et al. 1998), southeast of Iran. The clinical features of ACL consist of dry or late ulcerative lesion (Firooz et al. 2004). The first line-therapy for ACL, according to WHO recommendation, is the pentavalent antimony compounds such as meglumine antimoniate (Glucantime) (Minodier and Parola 2007). Other treatments for cutaneous leishmaniasis (CL) are antifungal agents, azithromycin, paromomycin ointment, and physical treatment like cryotherapy (cryosurgery) (Prata et al. 2003; Dogra et al. 1990; Al

majalio and Routh 1997; Esfandiarpour and Alavi 1997). In recent researches ZS has been studied to treat the disease, although there are some controversies regarding the efficacy of ZS in the treatment of cutaneous leishmanniasis (Firooz et al. 2004; Iraji et al. 2004).

The idea behind the usage of ZS for CL is that zinc deficiency can change immune functions prematurely from predominantly cellular Th1 responses to humeral Th2 responses (Prasad 2009a, b). On the other hand, the inhibitory effects of ZS on both *L. major* and *L. tropica* amastigotes in vivo and in vitro have been confirmed. Moreover, promising results have been reported on the treatment of CL with both oral and intralesional administrations of ZS (Firooz et al. 2004). The aim of the present study was to compare the efficacy of intralesional injection of ZS 2 % solution with intralesional glucantime in the treatment of ACL which is named as acute old world dry type CL. According to literature review, this is the first study on the efficacy of intralesional ZS in Kerman Province.

Materials and methods

Study area

This study was carried out in some clinics of Kerman University of Medical Sciences, from October 2008 to December 2010. Kerman is located in the southeast of Iran, surrounded by mountains, has a moderate climate and the average annual rainfall is 135 mm. Kerman is known as an endemic focus of *L. tropica* (Sharifi et al. 2012).

Preparation of ZS 2 % solution

ZS solution (2 % w/v) was prepared by dissolving 3.56 g $ZnSO_4{\cdot}7H_2O$ = (equal to 2 g ZS base) in de-ionized water Hydrated zinc sulfate ($ZnSO_4{\cdot}7H_2O$) was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

The ZS solution was sterilized in two steps: Firstly, the ZS solution was filtered through a 0.22 micron syringe filter (Millipore, USA), then it was re-sterilized in an autoclave (Mahzad-Kala, Iran) at 121 °C for 20 min in 5 ml borosilicate screw capped vials with Neoprene rubber caps. The sterility test was carried out according to USP direct method. The injection process was: The selected lesions were first cleaned by Betadine, then 1–3 ml of glucantime or ZS was injected intradermally via an insulin syringe for the entire lesion until the surface blanched out (according to WHO suggestion for glucantime injection) (World Health Organization 1984).

Sampling

One hundred and fifty patients were screened for this double blinded randomized clinical trial. Eighty CL patients were signed an informed consent and enrolled and randomized with random number table. A sample size of 40 participants per treatment group was planned, a probability of a type I error at $\alpha = 0.05$ and $\beta = 0.1$ to determine a 20 % difference between intralesional injection of ZS 2 % solution with intralesional glucantime. The inclusion criteria were as fallow; the presence of parasitological confirmed lesion(s) of CL and the age of 5–60 years. To confirm the leishmaniasis, direct smear was taken by scraping of the active border of all lesions and stained with Giemsa to find leishman bodies. In the case of negative smear a biopsy was done. No concomitant treatment was allowed and a washout period of at least one month was required. Informed consent was taken from all of the patients and for those below 18 years a parental one was written before randomization.

Patients with the following criteria were excluded: disease duration more than 6 months, those with a history of hypersensitivity to glucantime or ZS, pregnant or nursing women, those with more than five lesions, those with lesions with size of 5 cm or more (Firooz et al. 2004), and a history of any anti-leishmanial therapy during last four weeks.

Review board in ethics committee of Kerman University of Medical Sciences approved the study, under number K-85/30 and the Australian clinical trial registration (ACTR) number was: ACTRN12609000115235.

For the first group intralesional ZS 2 % solution was injected once every week until 10 weeks or until complete healing if happened earlier, plus cryotherapy at −196 °C (liquid nitrogen) was done in the same session, as contact cryotherapy for 5–10 s to the infected site, once every other week until 10 weeks or until early complete healing. For the second group intralesional glucantime (435 mg of sb5+, active antimony) (Rhone-Poulenc, Paris, France) 15 mg/kg of active ingredient (antimony) once every week until 10 weeks or until early positive response plus cryotherapy the same as the first group.

Clinical assessment

The size of indurations' lesions was measured in two perpendicular directions using transparent paper at the baseline, weekly during treatment, and at the end of the course by observer blinded to the treatment option.

The criteria for recovery

The response to the treatment was defined as fallow; complete response (100 % clinical response plus negative smear), partial response (more than 75 % reduction of the size of the lesion in comparison to the baseline), no response (less than or equal to 75 % reduction of the size of the lesion in comparison to the baseline or an increase in the size of the lesion) (Firooz et al. 2004).

Statistical analysis

Having compared demographic and baseline data of subjects in two groups, the data file were converted in long format in a way which each record showed the data of every subjects in one session. Then, the area of each lesion was computed based on its dimensions. In our analysis, no response to treatment was defined if the area of a lesion decreased less than 75 %, partial treatment if decreased between 75 and 99 %, and complete treatment if decreased 100 % compared to its baseline area.

In order to compare the response to treatment of subjects in two groups, we used Cox regression model. Two types of models were constructed: (1) the event of interest was complete treatment, (2) the event of interest was partial or complete treatment. Since the area of lesions and the duration of lesion before treatment had significant difference in two groups, in Cox regression models, crude and adjusted hazard ratios (HR) were computed to compare the rate of response to treatment of subjects in two groups. These analyses were done using Stata version 10.

Results

Of eighty patients participated in the study, 46 patients completed the study, consisting of 22 patients (10 males and 12 females) in the ZS-treated group and 24 patients (12 males and 12 females) in the glucantime-treated group. The main reason of drop out was lost to follow up in all of the patients except in one which had severe necrosis in the ZS-treated group. In average, each participant was followed for 4.1 ± 2.6 weeks. Participants' mean age was 24.5 ± 14.2 years; most of them were female (n = 42, 52.5 %). There was no significant difference between compared groups' baseline characteristics (Table 1). The results of this study showed that, most participants had one lesion in their body (ZS: n = 32, 80 % vs. glucantime: n = 29, 72.5 %; P = 0.37), the most frequent location of the lesions was patients' hand (ZS: n = 35, 67.3 % vs. glucantime: n = 36, 65.45 %; P = 0.2). The most frequent shape of lesions was plaque in zinc group (n = 17, 32.7 %) versus nodule in glucantime group (n = 19, 34.5 %); this difference was statistically significant (P = 0.00); similarly there was a significant difference in zinc versus glucantime group regarding the mean size, and duration of lesions at baseline. Other lesion characteristics did not show significant difference between both groups (Table 1). In zinc versus glucantime group, partial and complete treatment was observed with fewer injections, although none of these differences were statistically significant. Before showing partial treatment, in average each lesion got 4.5 ± 2.6 injections (ZS: 4.7 ± 2.6 vs. glucantime group: 4.3 ± 2.7; P = 0.4); moreover, before showing complete treatment, in average each lesion got 6.8 ± 2.2 injections (ZS: 7.4 ± 1.9 vs. glucantime group 6.2 ± 0.7, P = 0.16). Having used Cox regression model, we found that the trend of treatment in second group was rapidly, but it was not statistically significant (partial treatment: HR 1.4, 95 % CI 0.7–2.9; complete treatment: HR 1.3, 95 % CI 0.6–2.8). Adjustment for the initial size of the lesion and its duration did not change the pattern (Table 2). There were no major side effects in both groups. Pain was observed in all patients of both groups. In Zinc group four patients developed necrosis of the site of the injection while this was not observed in

Table 1 Comparing main characteristics of subjects classified by their received treatments

Variable	Glucantime (n = 40)	ZS (n = 40)	*P* value
Mean (SD) of age (year)	22.12 ± 1.99	26.84 ± 2.47	0.14
Mean (SD) of duration of lesions (month)	2.66 ± 0.29	3.93 ± 0.31	0.004
Mean (SD) of number of lesions	1.37 ± 0.11	1.3 ± 0.1	0.62
Mean (SD) of the size of lesions in the first visit (mm^2)	3.61 ± 0.63	8.33 ± 2.35	0.056
Mean (SD) of follow up (week)	5.73 ± 0.48	5.03 ± 0.45	0.29
Sex			
Male	21 (52.5 %)	17 (42.5 %)	0.37
Female	19 (47.5 %)	23 (57.5 %)	
Response to treatment			
No response	19 (47.5 %)	19 (47.5 %)	0.36
Partial	2 (5 %)	1 (2.5 %)	

Table 2 Comparing of the response to treatments

	Hazard ratio (95 % confidence interval)			
	Crude	Adjusted for the		
		Lesion size in the first visit	Duration of lesion	Size and the duration of lesion
Complete treatment	1.37 (0.66–2.86)	1.26 (0.57–2.77)	1.39 (0.66–2.95)	1.28 (0.57–2.86)
Partial or complete treatment	1.32 (0.63–2.76)	1.21 (0.55–2.67)	1.39 (0.64–3.01)	1.27 (0.56–2.91)

glucantime group at all ($P = 0.11$). Assessing the site of necrosis in these patients was forearm in two, face in one, and dorsum of the foot in one of them. The necrosis was mild in three patients and was alleviated spontaneously after one week treatment, but it was severe in one of the patient which resulted in the cessation of the therapy. In this patient the location of injection was on the dorsum of the foot and the necrosis was not resolved by medical treatment, therefore, surgical intervention by full thickness graft
was done.

Discussion

The results of this study indicated that intralesional injection of ZS 2 % solution was as effective as glucantime in the matter of healing of the acute old world dry type CL. This study showed that no significant differences between the effects of ZS 2 % solution and glucantime, except the mean of duration of lesions, that ZS 2 % treatment would take longer to heal lesions.

Zinc plays a role in determination of the leishmanolysin molecule's structural features. The leishmanolysin molecule mediates attachment and internalization of the parasite through an interaction with macrophage surface molecules. Also, was showed that zinc was less effective than in the control subjects in CL patients. The authors claimed that the mentioned changes could be a part of defense strategies of organism (Firooz et al. 2004; Prasad 2009a, b).

Najim et al. studied in vitro sensitivities of promastigotes and axenic amastigotes of both *L. major* and *L. tropica* to ZS. In their study the lethal dose 50 % (LD_{50}) was calculated and compared to the pentavalent antimony compounds. The results showed that the two forms of both strains were sensitive to ZS and their respective LD_{50} were lower compared to the pentavalent antimony compound. To confirm the results, they administered oral ZS to the mice with CL and they found the positive effects of oral ZS in the treatment of CL lesions (Najim 1998).

In another clinical trial, the efficacy of intralesional administration of ZS solution 2 % was compared with sodium stibogluconate or 7 % sodium chloride solution in the treatment of ACL. Thirty-three of a total of the 38 lesions (84.8 %) that were treated by intralesional ZS 2 % solution were cured after only one injection and 94.7 % of the lesions were completely healed following administration of a maximum of two intralesional injections with a 10–15 day interval (Sharquie et al. 1997; Najim et al. 2006). The results of another trial conducted on 130 ACL patients showed that administration of oral ZS had a dramatic effect on the healing of the lesions, in which, 83.9 % of the lesions in those treated with 2.5 mg/kg/day oral ZS were cured after a 31.8 ± 1.8 days. The cure rate was 93.1 and 96.9 % in the groups that were treated with oral ZS 5 mg/kg/days for 29.9 ± 1.7 days and 10 mg/kg/days for 28.3 ± 1.4 days, respectively (Sharquie et al. 2001). In another study, in 2004, Iraji et al. evaluated 66 ACL patients of which 35 patients received meglumine antimonate (MA) and 31 received ZS. The cure rates were 60 and 83.8 % for MA and ZS respectively. After the second and fourth weeks, the efficacy of treatment with ZS was higher than that with MA ($P = 0.01$), but after 6 weeks of the treatment no significant differences were observed between the two groups ($P = 0.05$) (Iraji et al. 2004). Recent study proposed that one intralesional injection of ZC 2 % has had lower healing rate versus those received. Glucantime (33.3 vs. 80 %) (Maleki et al. 2012).

In a recent study in Iran, 72 patients with CL lesions less than 8 weeks were recruited in a double-blinded randomized clinical trial, in an area endemic for *L. major* (Firooz et al. 2004). They were treated with six injection of weekly intralesional glucantime and ZS. Thirteen patients with 19 lesions in the glucantime group and 22 patients with 31 lesions in the ZS group completed the trial. Complete re-epithelialization was observed in 2 (10.5 %) and 19 (61.3 %) lesions in the ZS and glucantime groups, respectively, 1 week after the end of treatment ($P < 0.05$). They concluded that a six-week course of weekly intralesional injections of ZS 2 % solution was less effective than glucantime in the treatment of acute old world CL (Firooz et al. 2004).

In our study accomplished in an endemic area for *L .tropica* the efficacy of ZS was better in comparison with Firooz et al. (2004) study in an area endemic for *L. major*.

The discrepancy could be due to the causative *Leishmania* species (*L. tropica* vs. *L. major*). Different susceptibility of the parasites to zinc derivatives in different geographical regions should also be a probable factor.

The side effects of ZS 2 % treatment was the necrosis at the site of injection in four cases that this effect was also seen in the study of Iraji et al. (2004). We suggest that not to be injected ZS in places with thin skin such as dorsum of the foot. The main limitation of the current study was that we did not follow up the patients after the end of the treatment. We also did not assess the degree of pain at the site of injection in these two groups.

Conclusion

In the current study interalesional injection of ZS 2 % solution has the same efficacy as glucantime. So, it seems that in case of hypersensitivity and/or resistance to pentavalent antimony compounds we may use intralesional administration of ZS, it could worthy to mention that although pentavalent antimony compounds are expensive and their administration could be associated with several side effects, they are still the first-line drugs in the antileishmanial armamentarium.

Acknowledgments The authors would like to acknowledge the reviewers of our drafts and Afzalipour hospital of University of Medical Sciences, Kerman, Iran.

Conflict of interest The authors confirmed that they have no financial or personal relationships which may have inappropriately influenced them in writing this paper.

References

Al majalio O, Routh HB (1997) A two year study of liquid nitrogen therapy in cutaneous leishmaniasis. Int J Dermatol 36(6):36–460

Ashford RW (2000) The leishmaniasis as emerging and reemerging zoonoses. Int J Parasitol 30:1269–1281

Dogra J, Aneja N, Lal BB, Mishra SN (1990) Cutaneous leishmaniasis in India: clinical experience with itraconazole. Int J Dermatol 29:661–662

Esfandiarpour I, Alavi F (1997) Evaluating the efficacy of allopurinol and meglumine antimoniate (Glucantime) in the treatment of cutaneous leishmaniasis: a pilot study. Int J Dermatol 36(1):59–60

Firooz A, Khatami A, Khamesipour A, Nassiri-Kashani M, Behnia F, Nilforoushzadeh M, Pazoki-Toroudi H, Dowlati Y (2004) Intralesional injection of 2 % zinc sulfate solution in the treatment of acute old world cutaneous leishmaniasis: a randomized, double-blinded, controlled clinical trial. J Drug Dermatol 4:73–77

Iraji F, Vali A, Asilian A, Shahtalebi MA, Momeni AZ (2004) Comparison of intralesionally injected zinc sulfate with meglumine antimoniate in the treatment of acute cutaneous leishmaniasis. Dermatology 209(1):46–49

Maleki M, Karimi Gh, Tafaghodi M, Raftari S, Nahidi Y (2012) Comparison of intralesional two percent zinc sulfate and glucantime injection in treatment of acute cutaneous leishmaniasis. Indian J Dermatol 57(2):118–122

Minodier P, Parola P (2007) Cutaneous leishmaniasis treatment. Travel Med Infect Dis J 5:150–158

Najim RA (1998) Zinc sulfate in the treatment of cutaneous leishmaniasis: an in vitro and animal study. Inst Oswaldo Cruz 93:831–833

Najim RA, Sharquie KE, Al-Zubaidy SA (2006) Possible mechanisms of action of the compounds injected intralesionally in the treatment of cutaneous leishmaniasis, in addition to their direct effects on the parasites. Ann Trop Med Parasitol 100(1):33–38

Prasad AS (2009a) Zinc: role in immunity, oxidative stress and chronic inflammation. Curr Opin Clin Nutr Metab Care 12:646–652

Prasad AS (2009b) Impact of the discovery of human zinc deficiency on health. J Am Coll Nutr 28:257–265

Prata A, Silva-Vergara ML, Costa L, Rocha A, Krolewiecki A, Silva JC, de Paula EV, Pimenta Junior FG, Giraldo LE (2003) Efficacy of azithromycin in the treatment of cutaneous leishmaniasis. Rev Soc Bras Med Trop 36(1):65–69

Sharifi I, Fekri AR, Aflatonian MR, Nadim A, Nikian Y, Kamesipour A (1998) Cutaneous leishmaniasis in primary school children in the south-eastern Iranian city of Bam, 1994–1995. Bull World Health Organ 76(3):289–293

Sharifi F, Sharifi I, Zarean M, Hakimi Parizi M, Aflatoonian M, Harandi MF, Zahmatkesh R, Mashayekhi M, Kermanizadeh A (2012) Spatial distribution and molecular identification of *Leishmania* species from endemic foci of south-eastern Iran. Iran J Parasitol 7(1):45–52

Sharquie KL, Najim RA, Farouj IB (1997) Comparative controlled trial of intralesionally administered zinc sulfate, hypertonic sodium chloride and pentavalent antimony compound against acute cutaneous leishmaniasis. Clin Exp Dermatol 22:169–173

Sharquie KE, Najim RA, Farjou IB, Al-Timimi DJ (2001) Oral zinc sulfate in the treatment of acute cutaneous leishmaniasis. Clin Exp Dermatol 26:21–26

World Health Organization (1984) The leishmaniasis. Report of a WHO expert committee. WHO Tech Rep Ser 701