GLUCANTIME EFFICACY IN THE TREATMENT OF ZOONOTIC CUTANEOUS LEISHMANIASIS

B Pourmohammadi[1,3], MH Motazedian[1], F Handjani[2], GHR Hatam[4], S Habibi[5] and B Sarkari

[1]Department of Parasitology and Mycology, [2]Department of Dermatology, School of Medicine, Shiraz University of Medical Sciences, Shiraz; [3]Damghan School of Health, Semnan University of Medical Sciences, Semnan; [4]Basic Research Center in Infectious Diseases, Shiraz University of Medical Sciences, Shiraz; [5]Valfagr Health Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract. Pentavalent antimony (SbV) compounds are still considered the first line of treatment for all forms of leishmaniasis. There have been reports of drug resistance and unresponsiveness to treatment with these drugs. We investigated the clinical response to treatment of cutaneous leishmaniasis with glucantime, the drug of choice for all forms of leishmaniasis in Iran. All individuals suspected of cutaneous leishmaniasis from October 2007 to March 2008 were included in the study if met specific criteria. After laboratory diagnosis and parasite identification by PCR, 43 patients agreed to participate and complete the protocol for treatment. Meglumine antimoniate (glucantime) was given at a dose of 20 mg/kg/day for 20 days (two 10-day periods) according to a World Health Organization (WHO) recommended protocol. Response to treatment was evaluated 6 weeks after initiation of treatment. Fifteen patients (34.9%) were clinically unresponsive to glucantime treatment while the remaining 28 patients (65.1%) responded to treatment. There were no statistically significant differences by occupation, gender, chronicity of the disease before starting treatment, number of lesions, or age between the glucantime sensitive and resistant patients. Our study showed a significant level of unresponsiveness to glucantime among patients with cutaneous leishmaniasis caused by *Leishmania major* in Iran. These findings highlight the need for new treatment regimens.

Keywords: zoonotic cutaneous leishmaniasis, treatment, glucantime, unresponsiveness, Iran

INTRODUCTION

Leishmaniasis is a disease with worldwide distribution caused by different species of protozoan parasites of the genus *Leishmania*. This disease is an emerging yet neglected infection (Ouellette *et al*, 2004; Santos *et al*, 2008). Depending on the species of parasite and host immune state the infection severity can vary from spontaneous healing of the cutaneous form to usually fatal visceral disease if left untreated (Santos *et al*, 2008). Both forms of the disease, cutaneous and visceral, are endemic in Iran (Hadighi *et al*, 2006).

Correspondence: Prof Mohammad Hossein Motazedian, Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
Tel: 98 711 2305291; Fax: 98 711 2305291
E-mail: motazedm@sums.ac/Motazedian33@yahoo.com

Cutaneous leishmaniasis (CL) is a public health burden in tropical and subtropical regions (Boggild *et al*, 2007). It is one of the ten leading diseases among tourists returning from tropical countries reported in 3% with skin diseases (Blum and Hatz, 2009). The rise in leishmaniasis prevalence is due to multiple factors, including the AIDS epidemic, increased international travel, lack of effective vaccines, difficulty in controlling vectors, international conflicts, and emerging resistance to chemotherapy (Lianos-Cuentas *et al*, 2007; Sereno *et al*, 2007).

Different methods have been used to control the disease, including vector control and vaccination. Since vaccination is not available for most *Leishmania* species, disease control relies on chemotherapy (Papadopoulou *et al*, 1998; Ouellette *et al*, 2004).

Pentavalent antimony (SbV) compounds, introduced as anti-*Leishmania* drugs more than 6 decades ago, still remain the treatment of choice for all forms of leishmaniasis (Papadopoulou *et al*, 1998). Clinical response to these drugs varies depending on host factors, such as site and chronicity of the lesions, underlying disease, concomitant infection, acquired resistance, pharmacokinetic properties of the drug and *Leishmania* species variation (Lucumi *et al*, 1990).

Unfortunately, resistance to SbV appears to be occurring; an alarming study from Bihar, northern India of visceral leishmaniasis (VL) cases reported more than 60% of patients were unresponsive to SbV (Decuypere *et al*, 2005). A study of CL caused by different species of *Leishmania* in Peru reported a failure rate of 24.4% at 6 months, with 96% of failures occurring within the first 3 months after treatment (Lianos-Cuentas *et al*, 2008). In Iran, a failure rate of 11% has been reported among CL cases caused by *L. tropica* (Hadighi *et al*, 2006). Mohebali *et al* (2007) reported 5 of 31 patients with CL treated with intramuscular failed treatment. Currently, glucantime is the drug of choice for treating CL and VL in Iran. It is essential to monitor the efficacy of treating patients with this drug. We evaluated the clinical status of CL patients treated with glucantime in Shiraz, Iran.

MATERIALS AND METHODS

Subjects

All patients with skin lesions suspected of having CL referring to the Health Center in Shiraz, Iran from October 2007 to March 2008 were included in the study if they met the following criteria: aged 12 to 70 years, not pregnant or lactating, did not have underlying disease, such as diabetes, acute or chronic liver or renal disease, cardiac disease, or immunodeficiency, had normal blood testing (CBC, SGOT, SGPT, BUN, and creatinine) and the presence of at least 3 lesions. Patients with fewer than 3 lesion were generally treated with intralesional injections, so they were not included in the study. Participants should not have previously received glucantime or other anti-*Leishmania* drugs for at least one month prior to the study and the drug delivery route had to be through intramuscular injection. Study goals were explained to all patients and informed consent was obtained from each patient. The study protocol was reviewed and approved by the Ethics Research Committee of Shiraz University of Medical Sciences.

Sampling and parasite identification

Patients included in the study filled out a personal profile form and sampling was done from the lesion edge. At least two slides were prepared from each patient and two samples were cultured in NNN medium for use later with *in vitro* tests.

One of the slides was stained with Giemsa and studied under a light microscope. The other slide was used for species identification with polymerase chain reaction.

DNA extraction

Each fresh or dried smear was scraped off the slide with a sterile scalpel and the scrapings were added to 200 l of lysis buffer [50 mM Tris-HCl (pH 7.6), 1 mM EDTA, 1% (v/v) Tween 20] containing 8.5 l of a proteinase K solution (19 g/ml), in a 1.5 ml tube. The tube was incubated for 2 hours at 56ºC before 200 l of a phenol:chloroform:isoamyl alcohol mixture (25:24:1, by volume) was added. After being shaken vigorously, the tube was centrifuged at 6,000*g* for 10 minutes, then the DNA in the supernatant solution was precipitated with 400 l cold absolute ethanol, resuspended in 50 l double distilled water and then stored at -20ºC until tested.

PCR amplification

The PCR was performed on all 149 methanol fixed and/or Giemsa-stained samples. PCR used to amplify the variable area of the minicircle kDNA of Leishmania from the smears was a slight modification (Pourmohammadi *et al*, 2008) of that described elsewhere (Aransay *et al*, 2000). The forward LINR4 (5'-GGG GTT GGT GTA AAA TAG GG-3 2) and reverse LIN17 (5'-TTT GAA CGG GAT TTC TG-3') were used. Each 25 l reaction mixture contained 250 M each of deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 1 U Taq polymerase (CinnaGen Tehran), 1 M LINR4, 1 M LIN17 Primers, 100 pg DNA extract, and 2.5 l PCR buffer. Each reaction mixture was overlaid with mineral oil before being transferred to a CG1-96 thermo cycler (Corbett Research, Sydney, Australia) set to give 5 minutes at 94ºC, followed by 30 cycles, of 30 seconds at 94ºC, 30 seconds at 52ºC, 1 minute at 72ºC, and then a final extension at 72ºC for 5 minutes. A 5 l sample of each PCR product was subjected to electrophoresis in 1.5% agarose gel. The bands were then stained with ethidium bromide and visualized under ultra violet trans illumination. The parasites were identified by comparing the size of the band produced from a test sample with those produced from reference strains of *L. infantum* (MCAN/IR/96/LON49), *L. tropica* (MHOM/IR/89/ARA2) and *L. major* (MHOM/IR/54/LV39). A band of 720 bp, for example, would have indicated that *L. infantum* (or at least *L. infantum* kDNA) was present in the tested smear.

Treatment and follow-up of patients

After laboratory confirmation of *Leishmania major* infection and for the first 10 days of treatment, glucantime (Glucantime, Paris, France) at a dose of 20 mg/kg/day was given IM. During the second 10 day course of treatment, glucantime was given if there were no increase in liver enzymes, BUN, creatinine, or skin reactions at the injection site. The patient was then asked to return to the clinic 6 weeks later for physical examination and testing. The patients were considered unresponsive to treatment if amastigotes were found on light microscope examination of Giemsa-stained slides prepared from the lesion edge 6 weeks after termination of therapy. For cases with positive results, either the drug was administered for another week or other methods of treatment were chosen according to the physician's decision.

RESULTS

Forty-three patients with confirmed CL participated in the study and completed the treatment course of glucantime. Fifteen patients (34.9%) were clinically unresponsive to glucantime while the remaining 28 patients (65.1%) were responsive to treatment.

Thirty patients (69.8%) were male and mainly laborers (48.8%). The minimum age among subjects was 13 years old and the maximum was 70 years old. Seventy-five percent of cases who were cured were >20 years old. The mean ages of cured and uncured patients were 31.1±14.53 and 25.4±11.36, respectively. No correlation was observed between age and resistance to treatment (p=0.19, t-test). Eighty percent and 60% of uncured patients were males and employed, respectively. Most participants (48.8%) had more than 6 lesions. The median number of lesions in cured and uncured patients were 5 (3-25) and 9 (3-26). There were no corollation between number of lesions and responsive to treatment (p=0.1, Mann-Whitney test). The median lesion diameters were 12.5 cm (2-100 cm) and 10 cm (2-50 cm) in those who were cured and those not cured, respectively, and there was no corollation between median lesion diameter between cured and uncured patients (p=0.21; Mann-Whitney test). Seventy-three percent of unresponsiveness patients had ulcerative lesions (Table 1).

DISCUSSION

Comparison of clinical trials worldwide with those in Iran, show different results regarding the efficacy of glucantime in the treatment of CL. In our study, 43 patients completed the 20-day course of treatment, 15 (34.9%) were unresponsive to therapy. Response to treatment was considered as improvement in appearance of the lesions, hyperkeratinization, lesion shrinkage, and most importantly, absence of the parasite from skin samples prepared at least 1 month after the last injection of the drug. Four cases had positive culture results with NNN medium 6 weeks after completion of treatment, with no significant change in the appearance of the lesion compared to pre-treatment (Table 1). In patients who were unresponsive to treatment, some were given an additional course of the drug and others were given other treatment based on the physician's decision.

A clinical trial in eastern Iran without causative agent determination, showed a 93% cure rate using glucantime intramuscular standard treatment (Nilforoushzadeh *et al*, 2008). Cryotherapy was reported as a significantly better treatment in children compared with intralesional glucantime (Pouran *et al*, 2009).

Another study from northern Iran found 83.3% of patients with CL who received intramuscular meglumine antimoniate for 14 days responded to treatment (Mohebali *et al*, 2007). Eleven percent *in vivo* and *in vitro* drug resistance by *L. tropica* was reported in another study (Hadighi *et al*, 2006).

A 59% resistance rate was reported in the same area as our study in one clinical trial without species determination (Zerehsaz *et al*, 1999), but in another study in the same region, a response rate of 84% was seen (Beheshti *et al*, 2007), evaluation of treatment and determination of causative agents were not clear in that study. It appears resistant species are more prevalent in the southern than in the northern and eastern parts of Iran, indicating possible genomic variation in *L. major* (Hatam *et al*, 1999; Motazedian *et al*, 2002).

The cure rate with meglumine was lower in our study compared to higher cure rates from other clinical trials, in Bolivia (83%), Colombia (83%) and Pakistan (81%) (Soto *et al*, 2004; Firdous *et al*, 2009), which may be due to difference in *Leishmania* species, subspecies and severity of disease. The results of our study are close of a study conducted in Peru on CL caused by different

Table 1
Demographic, epidemiologic and clinical factors of cutaneous leishmaniasis patients and clinical outcomes after meglumine treatment.

Factor	Treatment outcome		*p*-value	Method
	Failure (n = 15)	Cure (n= 28)		
Age, years				
Mean ± SD	25.4 ± 11.36	31.1 ± 14.53	0.19	*t*-test
Median (IQR)	23 (13-56)	27 (13-70)		
0-20	4 (26.7)	7 (25)		
>20	11 (73.3)	21 (75)	0.9	Fisher's exact test
Sex				
Male	12 (80)	18 (64.3)		
Female	3 (20)	10 (35.7)	0.48	Fisher's exact test
Occupation				
Employed	9 (60)	11 (39.3)		
Student	4 (26.7)	5 (17.9)		
Housekeeper	1 (6.7)	9 (32.1)		
Unemployed	1 (6.7)	3 (10.7)	0.24	Chi-square test
Duration of disease, months				
0-1	6 (40)	11 (39.3)		
>1	9 (60)	17 (60.7)	0.9	Fisher's exact test
Mean ± SD	2.33 ± 1.87	1.94 ± 1.01		
Median (IQR)	2 (1-7)	2 (1-5)	0.95	Mann-Whitney test
Lesions, number				
0-3	3 (20)	10 (35.7)		
>3	12 (80)	18 (64.3)	0.48	Fisher's exact test
Mean ± SD	8.6 ± 5.9	6.46 ± 4.88		
Median (IQR)	9 (3-26)	5 (3-25)	0.1	Mann-Whitney test
Lesion diameter, mm				
0-20	12 (80)	19 (67.9)		
>20	3 (20)	9 (32.1)	0.49	Fisher's exact test
Mean±SD	15.73 ± 14.29	22.22 ± 21.89		
Median (IQR)	10 (2-50)	12.5 (2-100)	0.21	Mann-Whitney test
Lesion type				
Ulcer	11 (73)	21 (75)		
Nonulcer	4 (26.7)	7 (25)	0.9	Fisher's exact test

species of *Leishmania* in which a failure rate of 24.4% at 6 months was reported (Lianos-Cuentas *et al*, 2008).

Follow-up of patients varied from 1 to 6 months in different studies. We evaluated the patients after 6 weeks; in patients who were unresponsive either the treatment regimen was changed or an additional course of the drug was given according to the physician's decision. Time required for spontaneous healing of the disease varies considerably depending on the *Leishmania*

species, amount of parasite present, host immune status and other unexplained factors of the parasite and host (Dowlati, 1996). Spontaneous resolution of *L. major* is reported in 60-70% of cases by 3 months and 100% by 12 months (Dowlati, 1996; Bailey and Lockwood, 2007). Based on these data evaluation should be done six weeks after treatment.

The need for daily injection of antimonials for at least 2 weeks is a limitation of these drugs (Frézard *et al*, 2009). They are associated with focal pain at the site of intramuscular injection and may cause systemic side effects which necessitate administration of the drug under close medical observation. Fifteen of the patients in our study refused to continue treatment and were replaced with patients who completed therapy. Given these limitations, investigating new anti-*Leishmania* drugs is important and needs to be aided by the World Health Organization (Frézard *et al*, 2009). Some patients may self treat which can lead to emergence of drug resistant species (Lucumi *et al*, 1998; Frézard *et al*, 2009). There were no subjects with elevated liver enzymes, BUN, creatinine, or injection site skin reactions in our study.

We observed no statistically significant associations in occupation, gender, chronicity of the disease before starting treatment, number of lesions, and age between glucantime sensitive and resistant patients. In other studies, age was an important factor affecting treatment results with sodium stibogluconate (Decuypere *et al*, 2005; Bailey and Lockwood, 2007; Lianos-Cuentas *et al*, 2008). This difference may be due to drug type, different species of parasite or host-related differences.

The high prevalence and large distribution of *L. major* resistant to treatment is alarming. Additional studies regarding correct usage of the drug and investigating alternative medications should be conducted.

ACKNOWLEDGEMENTS

The authors would like to thank the vice chancellery of research affairs of Shiraz University of Medical Sciences for financial support, the physicians and staff of Valfajr Health Center for helping with sample collection, and Mr Amir Almasi for his help with statistical analysis. We would also like to thank Dr M Motazedian for editing the English manuscript.

REFERENCES

Aransay AM, Scoulica E, Tselentis Y. Detection and identification of *Leishmania* DNA within naturally infected sand flies by PCR on minicircle kinetoplastic DNA. *Appl Environ Microbiol* 2000; 66: 1933-8.

Beheshti M, Ghotbi Sh, Amirizade S. Therapeutic and adverse effects of glucantime used for treatment of cutaneous Leishmaniasis. *Shiraz E-Medical J* 2007; 8: 155-61.

Bailey MS, Lockwood DNJ. Cutaneous leishmaniasis. *Clin Dermatol* 2007; 25: 203-11.

Blum JA, Hatz CF. Treatment of cutaneous leishmaniasis in travelers. *J Travel Med* 2009; 16: 123-31.

Boggild AK, Miranda-Verastegui C, Espinosa D, *et al*. Evaluation of a microculture method for isolation of *Leishmania* parasites from cutaneous lesions of patients in Peru. *J Clin Microbiol* 2007; 45: 3680-4.

Decuypere S, Rijal S, Yardley V, *et al*. Gene expression analysis of the mechanism of natural Sb (V) resistance in *Leishmania donovani* isolates from Nepal. *Antimicrob Agents Chemother* 2005; 49: 4616-21.

Dowlati Y. Cutaneous leishmaniasis: clinical aspects. *Clin Dermatol* 1996; 14: 425-31.

Firdous R. Yasinzai M. Ranja K. Efficacy of glucantime in the treatment of old World

cutaneous leishmaniasis. *Int J Dermatol* 2009; 48: 758-62. 21.

Frézard F, Demicheli C, Ribeiro RR. Pentavalent antimonials: new perspectives for old drugs. *Molecules* 2009; 14: 2317-36.

Hadighi R, Mohebali M, Boucher P, Hajjaran H, Khamesipour A, Ouellette M. Unresponsiveness to glucantime treatment in Iranian cutaneous leishmaniasis due to drug-resistant *Leishmania tropica* parasites. *PLoS Med* 2006; 3: e162.

Hatam, G R. Hosseini, SM H, Ardehali S. Isoenzyme studies in characterization of *Leishmania* isolated in Iran. *IJMS* 1999; 24: 110-5.

Lianos-Cuentas A, Tulliano G, Araujo-Castillo R, *et al*. Clinical and parasite species risk factors for pentavalent antimonial treatment failure in cutaneous leishmaniasis in Peru. *Clin Infect Dis* 2008; 46: 223-31.

Lucumi A, Robledo S, Gama V, Saravia NG. Sensitivity of *Leishmania viannia panamensis* to pentavalent antimony is correlated with the formation of cleavable DNA-protein complexes. *Antimicrob Agents Chemother* 1998; 42: 1990-5.

MohebaliA M, Fotouhi B, Hooshmand Z, *et al*. Comparison of miltefosine and meglumine antimoniate for the treatment of zoonotic cutaneous leishmaniasis (ZCL) by a randomized clinical trial in Iran. *Acta Trop* 2007; 103: 33-40.

Motazedian H, Noamanpoor B, Ardehali S. Characterization of *Leishmania* parasites isolated from provinces of the Islamic Republic of Iran. *East Mediterr Health J*. 2002; 8: 338-44.

Nilforoushzadeh MA, Jaffary F, Ansari N, Siadat AH, Nilforoushan Z, Firouz A. A comparative study between the efficacy of systemic meglumine antimoniate therapy with standard or low dose plus oral omeprazole in the treatment of cutaneous leishmaniasis. *J Vector Borne Dis* 2008; 45: 287-91.

Ouellette M, Drummelsmith J, Papadopoulou B. Leishmaniasis: drugs in the clinic, resistance and new developments. *Drug Resist Updat* 2004; 7: 257-66.

Papadopoulou B, Kundig C, Singh A, Ouellette M. Drug resistance in *Leishmania*: similarities and differences to other organisms. *Drug Resist Updat* 1998; 1: 266-78.

Pouran L, Fakhrozaman P, Amir HS, Parisa N, Toktam M. Efficacy of cryotherapy versus intralesional meglumine antimoniate (Glucantime) for treatment of cutaneous leishmaniasis in children. *Am J Trop Med Hyg* 2009; 80: 172-5.

Pourmohammadi B, Motazedian MH, Kalantari M. Rodent infection with *Leishmania* in a new focus of human cutaneous leishmaniasis, in northern Iran. *Ann Trop Med Parasitol* 2008; 102: 127-33.

Santos DO, Coutinho CER, Madeira MF, *et al*. Leishmaniasis treatment- a challenge that remains: a review. *Parasitol Res* 2008; 103: 1-10.

Sereno D, Da Silva AC, Mathieu-Daude F, Ouaissi A. Advances and perspectives in *Leishmania* cell based drug-screening procedures. *Parasitol Int* 2007; 56: 3-7.

Soto J, Valda-Rodriquez L, Toledo J, *et al*. Comparison of generic to branded pentavalent antimony for treatment of new world cutaneous leishmaniasis. *Am J Trop Med Hyg* 2004; 71: 577-81.

Zerehsaz F, Salmanpour R, Handjani F, *et al*. A double-blind randomized clinical trial of a topical herbal extract (Z-HE) vs. systemic meglumine antimoniate for the treatment of cutaneous leishmaniasis in Iran. *Int J Dermatol* 1999; 38: 610-2.