A Randomized Trial to Compare Topical MJ1 with Routine Care for the Treatment of Cutaneous Leishmaniasis

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ABSTRACT

The objective of this study was to assess the relative efficacy and tolerability of topical MJ1 compared to routine care in the treatment of histologically proven CL. A total of 150 patients with 297 lesions were randomly allocated to receive either topical MJ1 agent as a paste to applied to the lesions without cover three times a day for 20 days or routine care (intramuscular injection of meglumine antimonate 20 mg/kg/day for 2 weeks or intralesional 0.5-1CC for a total of four injection with one week interval). The primary end point of this study was the clinical cure of the lesion. Response to treatment was assessed at 1, 2, 3 and 8 weeks after start of therapy. Of the 132 lesions treated with MJ1, the mean size of lesions decreased from 423.9 to 30.4 mm^2, 111 (84.1%) were reduced in size and 21 (15.9%) not responded. Correspondingly, in the 165 lesions treated with routine care, the mean size of lesions slightly increased from 295.8 to 330.5 mm^2, 82 (49.7%) were reduced in size and 83 (50.3%) not responded. The differences were statistically significant ($P<0.001$). No sever adverse effect occurred. The findings highlight that topical treatment with MJ1 agent was much more effective than routine care and is safe and well tolerated.

Key words: Cutaneous Leishmaniasis, Meglumine Antimonate, MJ1 Agent, Iran

INTRODUCTION

Cutaneous leishmaniasis (CL) is prevalent in the tropical and semitropical regions of the world with high disease burden at both individual and social levels [1]. It is a major public health problem in Iran and is endemic in many provinces, and almost all CL cases in Iran are caused by L. tropica and L. major [2]. CL can progress to formation of papules, nodules, plaques and especially ulcers. Although all the patients do not need treatment and many lesions heal spontaneously, the disease is treated to shorten the duration of lesions, decrease the morbidity associated with large, chronic ulcer and prevent ugly scarring [3].

Although there is no optimal treatment, many therapeutic modalities have been suggested in the treatment of CL, and pentavalent antimony compounds are considered as the most effective drug of choice for it so far and could be associated with severe side effects and significant discomfort [4]. Currently available treatment is not fully effective, safe, cheap, requiring systemic or intralesional injection, and the large daily volume required is difficult to administer intramuscularly [3-5]. An ideal treatment for CL should be rapidly effective, easily administered, cheap, available at all times and should have no side effects. However, new safe and economical treatments for it are still needed. Several topical therapies have been tested and found to be effective [2, 6-9]. MJ1 (standing for the first version of the drug developed by Mohsen Janghorbani) is a non-synthetic dairy origin agent. It is cheap, easily administered, available at any time and has no side effects. Although antileishmanial properties of MJ1 have been shown in traditional medicines a clinical trial has not previously been conducted. A pilot study showed that MJ1 caused immediate death of Leishmania parasites when added either directly on a slide or to a culture of the parasite. Also, MJ1 was pre-tested in few candidate patients with CL as a pilot study and was very effective and safe treatment.

The aim of this study therefore was to undertake a randomized trial of patients with histologically proven CL comparing routine care and topical MJ1 to determine the best regimen for treatment of CL in Iran and to widen therapeutic options.

PATIENTS AND METHODS

Patients

Patients who sought treatment for suspected CL at our local primary health clinics (Shahinshar and Natanz, Iran), between September 2013 and October 2014 and not received any treatment were evaluated and direct smears for leishmaniasis were prepared. Patients were eligible for the study if they had CL that was proved parasitologically for a duration of less than three months, no previous treatment, no serious concomitant medical problems such as heart, kidney, liver, endocrinologic, hemato-logic disease, or serious infection other than CL- that were indicated by the medical history, willing to participate and availability for follow-up for two months, and gave informed consent (by the patient or his/her parent/guardian in cases younger than 18 years) to participate in the study. Patients were excluded if they were pregnant or lactating women, had duration of lesion more than three months and history of receiving treatment or allergy to meglumine antimonite (Meglusan), presence of secondary bacterial infection of the lesion according to clinical appearance and occurrence of a serious adverse event and presence of > 6 lesions. The study was reviewed and approved by the Ethical Review Committee of Isfahan University of Medical Sciences. The nature of the trial was explained to the patient or parents/legal guadians of minors (younger than 18 years old) and his/her informed consent obtained. The study complied with the Declaration of Helsinki. This trial is registered on Iranian Registry of Clinical Trials (www.irct.ir) IRCT-2013092414746N1.

Randomization scheme. A total of 150 participants who met the inclusion criteria were assigned randomly and equally to one of the two treatment groups. Patients were randomized according to a preexisting list produced by a computer program that differed from a random number generator only in that it assigned equal numbers of patients into each treatment group.

The first treatment group received daily intra-muscular injection of meglumine antimoniate (Meglusan; 5 ml, registered trade name of Sterop, Pharmaceutical Company, Belgium), at a dose of 20mg/kg, for a total of 2 weeks (112 lesions) or intralesional 0.5-1CC for a total of four injection with one week interval (53 lesions), according to the Iranian Ministry of Health and Medical Education guidelines. The second group received topical MJ1 agent as a paste applied to the lesions without cover three times a day for 20 days and were monitored closely by trained medical doctors during the course of the study. The MJ1 is a non-synthetic dairy origin agent that used as a traditional medicine in some part of Iran and contain lactic acid, NaCl, calcium, and some protein stabilized in a base using 1% carbopol 924 polymer, 25% methyl paraben, 5% glycerin, 0.5% triethanol amine and 68.5% deionized distilled water. All patients had a pre-treatment evaluation that consisted of obtaining demographic data, duration of symptoms and previous treatment, and examination of lesion(s) that included their location, number of lesions, duration, diameters, and type of lesion such as nodule, papule, plaque, ulcer and scaling and the lesions were photographically recorded.

Parasitological diagnosis

One lesion on each patient was parasitologically examined. It is important to note that the diagnosis of leishmaniasis was made by the visualization of amastigotes in smears of lesion material. The organisms were not routinely cultured from the material and as almost all CL observed in this province is due to L. major, polymerase chain reaction tests were not done.

Patient evaluation: The trial was discerning in that both patient and doctor were aware that the patient was receiving treatment. Patients admitted into the trial were given either unlabeled boxes containing MJ1 paste and instructed to apply them on the lesions without cover three times a day for 20 consecutive days or administered intramuscularlly 20mg/kg of Meglusan for a total of 2 weeks or intralesional 0.5-1CC for a total of four injection with one week interval. Patients were evaluated at 1, 2, 3, or 8 weeks after the start of therapy. The lesions were remeasured at the baseline and at these follow-up visits (lesions were measured by two dimensions in mm, and their area was calculated as if they were rectangles). The size was assessed by marking the lesion and measuring its diameter. Clinical evaluation of all lesions was made by physicians who know which patients had received which treatment.

Determination of response

The primary end point of this study was the clinical cure of the lesion. The definition of

initial healing and definitive cure was based on clinical criteria only. Complete healing defined as complete re-epithelialization and relief of induration, partial improvement defined as more than 50% re-epithelialization and decrease of induration and size of the lesion, and no response to treatment defined as less than 50% decrease of induration and size of the lesion or worsening of lesion compared with baseline.

Patients who developed secondary infections during treatment were excluded. Patients who were removed from the study were treated with Meglusan at doses of 20mg/kg/day for 2 weeks.

Statistical analysis

The sample size was calculated when the study was designed and was based on the comparison of two proportions. We calculated that 80 patients per treatment group would be required to provide the study with 80 percent power to detect (with a two-sided alpha of 0.05) a significant difference in the expected cure rate of 80% in patients who received MJ1 and the desire cure rate of 60% in those who received the routine care at week 8. Appropriate statistical analyses were done; independent Student's t-test (for normally distributed variables), The Mann-Whitney U test (for not normally distributed variables) and chi-square or Fisher's exact test were used to determine the significance. All statistical tests were two-sided, and $P<0.05$ was considered statistically significant. The analyses were undertaken using SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 176 consecutive patients were recruited. Thirteen patients refused to participate, and 3 patients did not meet our study criteria. Thus, 160 patients were randomized: 80 in the MJ1 and 80 in the routine care. Ten patients were not included in the intention-to-treat analysis: 5 patients lost to follow-up and 2 patients discontinue treatment in the routine care group and 3 patients lost to follow-up in MJ1 group (Fig. 1).

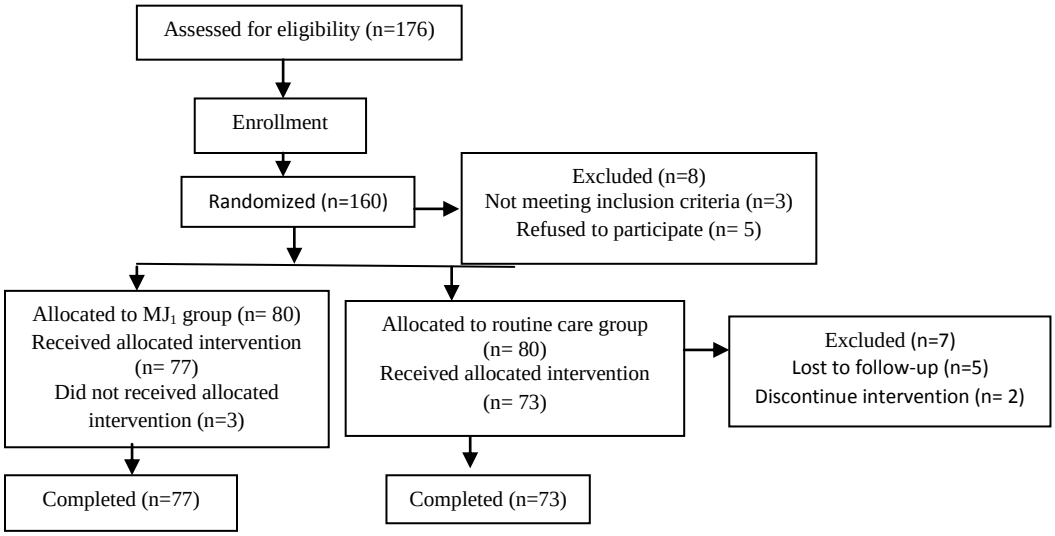

Fig. 1:Design of the study.

Of the remaining 150 patients, 89 (59.3%) were male with 178 lesions and 61 (40.7%) were female with 119 lesions. Differences in distribution of several characteristics among 77 (43 males and 34 females) are patients in MJ1 group and 73 (46 male and 27 female) patients in routine care group with 297 lesions are shown in Table 1. There was no difference between treatment groups regarding gender, age, number of lesions per patients, and distribution of lesions and baseline size of lesions. Routine care group had more popular lesions while MJ1 group had more lesions in form of ulcer ($P<0.05$). Patients in the MJ1 group had lower total number of lesions than patients in routine care group ($P<0.05$). All of the 150 patients completed their treatment without interruption and were available for follow-up at 1, 2, 3, or 8 weeks. Mean (SD) ages of MJ1 and routine care groups were 28.2

(19.7) and 29.5 (17.7) years, respectively. The average numbers of lesions per patient in MJ1 and routine care groups were 1.9 (1.2) and 2.3 (2.0) respectively. The duration of the disease in all cases was less than three months. Most patients in MJ1 (89.6%) and routine care (82.2%) groups had less than three lesions. The most common sites of involvement were the face and the extremities.

Table 1: Characteristics of patients with cutaneous leshmaniasis by treatment group.

Characteristics	Treatment group MJ1 N= 77 Mean (SD)	 Routine care N=73 Mean (SD)	 Differences (95% CI)
Age (years)	28.2 (19.7)	29.5 (17.7)	-1.2 (-7.3, 4.8)
No. of lesions/patients	1.9 (1.2)	2.3 (2.0)	-0.4 (-0.9, 0.2)
Lesion size (mm^2) at baseline	423.9 (713.9)	295.8 (512.2)	128.1 (-17.4, 273.5)
	No. (%)	**No. (%)**	
Total no. of lesions	132 (44.4)	165 (55.6)	-11.2 (-19.1, -3.1)*
Gender			
Male	43 (55.8)	46 (63.0)	-7.2 (-22.8, 8.5)
Female	34 (44.2)	27 (37.0)	-
Distribution of lesions			
Upper limbs	64 (48.5)	77 (46.7)	1.8 (-9.6, 13.2)
Lower limbs	51 (38.6)	66 (40.0)	-1.4 (-12.5, 9.8)
Face	11 (8.3)	9 (5.5)	2.8 (-3.8, 8.7)
Trunk	6 (4.6)	13 (7.9)	-3.3 (-8.8, 2.1)
Type of lesions			
Nodule	12 (9.1)	13 (7.9)	1.2 (-5.2, 7.6)
Papule	98 (74.2)	141 (85.5)	-11.3 (-20.4, -2.0)*
Ulcer	22 (16.7)	11 (6.7)	10.0 (2.6, 17.4)*

CI =Confidence interval. *P<0.05, **P<0.01, **P<0.001 for the difference in the mean and proportion of the variables between MJ_1 and routine care.

Of the 132 lesions treated with MJ1 after 8 weeks of follow-up, the mean size of lesions decreased from 423.9 (95% confidences interval (CI): 301.0, 547.0) mm^2 to 30.4 (95% CI: 21.7, 39.1) mm^2. Correspondingly, in the 165 lesions treated with routine care, the mean size of lesions increased from 295.8 (95% CI: 217.0, 375.0) to 330.5 (95% CI: 289.0, 372.0) mm^2. Mean lesion size at baseline and after the first week did not differ between the MJ1 and routine care groups, but after the second week, mean lesion size was significantly lower in MJ1 group (Table 2).

Table 2: Comparison of lesion size in 150 patients with 297 cutaneous leishmaniasis lesions before and after treatment with MJ1 and Meglusan.

	Treatment group MJ1 Mean (SD)	 Routine care Mean (SD)	Differences (95% CI)
Number of lesions at baseline	132 (44.4)	165 (55.6)	-
Lesion size (mm^2) at baseline	423.9 (713.9)	295.8 (512.2)	128.1 (-17.4, 273.5)
Lesion size (mm^2) at 1st week	310.4 (565.6)	271.2 (463.3)	39.2 (-81.0, 159.4)
Differences (95% CI)	113.5 (66.3, 160.8)***	24.6 (-1.1, 50.5)	-
Lesion size (mm^2) at 1st week	310.4 (565.6)	271.2 (463.3)	-
Lesion size (mm^2) at 2nd weeks	164.7 (418.1)	266.3 (341.9)	-101.6 (-189.0, -14.2)*
Differences (95% CI)	145.7 (89.5, 202.9)***	4.9 (-31.9, 41.7)	-
Lesion size (mm^2) at 2nd weeks	164.7 (418.1)	266.3 (341.9)	-
Lesion size (mm^2) at 3rd weeks	88.0 (174.3)	254.3 (372.7)	-166.3 (-244.9, -87.7)***
Differences (95% CI)	76.7 (52.3, 101.0)***	12.0 (-33.3, 57.3)	-
Lesion size (mm^2) at 3rd weeks	88.0 (174.3)	254.3 (372.7)	-
Lesion size (mm^2) at 8th weeks	30.4 (50.6)	330.5 (268.0)	-300.1 (-434.5, -165.6)***
Differences (95% CI)	57.6 (-5.4, 121.0)	76.2 (-154.0, 1.3)-	-

*P<0.05, **P<0.01, ***P<0.001. CI= confidence interval.

A reduction in lesion size was observed in 91.7% (121/132) of patients during MJ1 treatment and in 83.0% (137/165) of patients during routine care treatment ($\chi 2$=4.8, P<0.05) after the first week. After the second week, a reduction in the lesion size was observed in

93.9% (124/132) of patients during MJ1 treatment and in 68.9% (111/161) of patients during routine care treatment ($\chi 2$ test =28.5, P<0.001). After the third week, a reduction in the lesion size was observed in 89.7% (96/107) of patients during MJ1 treatment and in 67.3% (72/107) of patients during routine care treatment ($\chi 2$ test =15.9, P<0.001). After the eight week, a reduction in the lesion size was observed in 100.0% (26/26) of patients during MJ1 treatment and in 66.7% (12/18) of patients during routine care treatment ($\chi 2$ test=10.0, P<0.01) (Table 2).

Eight weeks after start of treatment, the size of 77 of the 165 lesions (46.7%; 95% CI: 39.1, 54.3) treated with routine care was reduced and it not responsed in 88 (53.3%; 95% CI: 45.7, 60.9). Correspondingly, the size of 110 of 132 lesions (83.3%; 95% CI: 77.0, 89.7) treated with MJ1 was reduced and 22 (16.7%; 95% CI: 10.3, 23.0) not responsed. The differences were statistically significant (P<0.001). Complete response was seen in 40.9% and 9.1%, partial response in 42.4% and 37.6%, and no response in 16.7% and 53.3% of the patients in the MJ1 group vs. routine care group, respectively. Therefore, the response rate in MJ1 group was much superior to that of the routine care group (P<0.001; Table 3).

Table 3: Response of lesions to treatment 8 weeks after initiation of the treatment.

Response	*MJ1* *No. (%)*	**Routine care** *No. (%)*	*Difference* *(95% CI)*
Total lesions			
Number of lesions	132 (44.4)	165 (55.6)	-
Complete recovery	54 (40.9)	15 (9.1)	31.8 (22.4, 41.3)*
Partial recovery	56 (42.4)	62 (37.6)	4.8 (-6.4, 16.1)
No response	22 (16.7)	88 (53.3)	-36.7 (-46.6, -26.7)*
Non-ulcerated lesions			
Number of lesions	110 (83.3)	154 (93.3)	-
Complete recovery	44 (40.0)	14 (9.1)	30.9 (20.7, 41.1)*
Partial recovery	44 (40.0)	54 (35.1)	4.9 (-6.9, 16.4)
No response	22 (20.0)	86 (56.8)	-35.8 (-46.7, -25.0)*
Ulcerated lesions			
Number of lesions	22 (16.7)	11 (6.6)	-
Complete recovery	10 (45.5)	1 (9.1)	36.4 (9.5, 63.2)*
Partial recovery	12 (54.4)	8 (72.7)	-18.2 (-51.7, 15.4)
No response	0 (0.0)	2 (18.2)	-18.2 (-41.0, 4.6)

*P<0.001, CI=confidence interval

Eight weeks after start of treatment, complete response in non-ulcerated lesions was seen in 40.0% and 9.1%, partial response in 40.0% and 35.1%, and no response in 20.0% and 56.8% of the patients in the MJ1 group vs. routine care group, respectively. The difference in response rates was statistically significant between two treatment groups in non-ulcerated lesions (P<0.001).

A total of 33 patients (22 in MJ1 group and 11 in routine care group) had ulcerated lesions and complete response in ulcerated lesions was seen in 45.5% and 9.1%, partial response in 54.4% and 72.7%, and no response in 0.0% and 18.2% of the patients in the MJ1 group vs. routine care group, respectively. The difference in complete recovery was statistically significant between two treatment groups in ulcerated lesions (P<0.001), but the difference in partial recovery and not respond was not statistically significant between two treatment groups in ulcerated lesions (P>0.05), probably due to the small number of ulcerated lesions.

MJ1 treatment was tolerated well and did not lead to discontinuation of treatment. The only observed adverse events were slight burning, which occurred in about 50% of patients with ulcerated lesions.

DISCUSSION

In Iran, during the study period, the standard first line treatment for CL was meglumine antimoniate (Meglusan). During the last two decades clinical trials in the search of new treatments for CL have been developed [10-14], but some of them have not been effective and there is not enough evidence about the effectiveness for others. In different parts of Iran, various combinations of herbal and animal origin medicine have been used for the treatment of CL [7]. Treatment regimens that involve parenteral and interalesional injection and that exposed patients to untoward side effects may not only be inconvenient but also unnecessary. Topical treatment of CL is thus

desirable. In the present study, we assessed response to early healing of lesions and found that MJ1 agent was more effective than intramuscular or intralesional injection of meglumine antimoniate. This trial is the first clinical evidence on efficacy of MJ1 in patients infected with CL. No unusual or unexpected safety risks were found with MJ1 therapy in our study population.

The efficacy of intralesional versus intramuscular administration of Glucantime was studied in Saudi Arabia and no statistically significant differences was noted between the two treatment groups [15]. The efficacy of interalesional injection of meglumine antimoniate alone in the treatment of lesions of CL due to L.major, 6 weeks after initiation of this intervention, was different from 26.0% to 86.7% in previous studies [16-21]. In the present study, response to meglumine antimoniate was relatively low and was not effective because of their adverse effects, high cost, and emergence of drug resistance. Meglumine antimonate has been associated with several disadvantages such as need for parenteral route, discomfort, and the presence of various side effects (fatigue, vomiting, anorexia, muscle and abdominal pain, cardiac abnormalities, increased hepatic aminotransferase, pancreatitis, renal toxicities) [10, 11]. For special populations such as pregnant women, children, or patients to whom pentavalent antimony compounds is contraindicated, the development of a new safer therapeutic options are needed

The dairy extract used in this study was in the form of a crude paste. The possible mechanisms of action of MJ1 agent in CL is unknown. Further studies must be undertaken to determine the possible effective ingredients which are important in the recovery of the lesions.

Although, this study is only controlled trials to date of effect of MJ1 agent on the CL the trial was carried out in primary care clinics with referral system. Patients could drop out at any time and seek another treatment in any other public or private health institution. In such a setting, patients usually do not come for follow-up if they feel that their condition is not improving, or sometimes even their condition improved. For these reasons although a much longer follow-up period was needed to adequately assess treatment efficacy of MJ1, it was not possible in this study. So, selection and volunteer bias cannot be ruled out. Patient compliance was not a problem in this study since almost all patients presented for follow-up. CL is a self-healing disease and, in most cases, heals spontaneously in less than one year. The follow-up period in present study was 8 weeks and the duration of disease in all cases was less than three months. Therefore, the total time for the trial was well below the time needed for self-healing for both species of CL in our country.

Although the value of the double blind, controlled trial is widely recognized, this design is not always appropriate or indicated. Because of the different side-effect profile of pentavalent antimony compounds and MJ1 administered as a paste and meglumine antimoniate intramuscularly or intralesionally, it would have been impossible to keep patients blinded in a study of this nature. Similarly, treating physicians dealing with clinical and laboratory adverse events can easily become discerning. However, Schultz and co-workers reported that, to avoid bias in clinical trials, careful randomization is more important than a double-blind design [22].

As this study indicated that MJ1 was superior to meglumine antimoniate, we did a pilot study before this study to assess the applicability of those results to CL [23]. The encouraging results obtained in this trial warrant further studies, a larger scale, probably blinded, trial is needed.

We conclude that MJ1 compound was more effective than intramuscular or intralesional injection of meglumine antimoniate and was safe and well tolerated. Since it was rapidly effective, easily administered, painless, cheap, available at all times and had no side effects, it is recommended as an alternative treatment option, especially in patients with history of cardiac, renal, hepatic disease and in patients not tolerating meglumine antimoniate for any reason. MJ1 is much less expensive than meglumine antimoniate and more available in developing countries where leishmaniasis is prevalent. Another advantage of MJ1 over alternative treatments is the route of administration of this drug, which is topically administered.

ETHICAL ISSUES

The study was reviewed and approved by the Ethical Review Committee of Isfahan University of Medical Sciences. The nature of the trial was explained to the patient or parents/legal guardians of minors (younger than

18 years old) and his/her informed consent obtained. The study complied with the Declaration of Helsinki. This trial is registered on Iranian Registry of Clinical Trials (www.irct.ir) IRCT2013092414746N1.

AUTHORS' CONTRIBUTIONS

Janghorbani M conceived and designed the study, analyzed the data and wrote the manuscript; Faraji M and Ramaznpour J contributed to data collection and revised the manuscript; Fadaei R, contributed to the discussion and revision of the manuscript. All authors have given final approval of the version to be published.

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