Short Communication

The topical treatment of old world cutaneous leishmaniasis with gentian violet along with cryotherapy: a pilot single-blind randomized controlled clinical trial

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Abstract

Introduction: The promising non-clinical antileishmanial effects of gentian violet (GV) encouraged us to evaluate the additive effect of GV on cryotherapy. Methods: For 8 weeks, 59/161 cutaneous leishmaniasis patients/lesions underwent cryotherapy alone (group 1) or cryotherapy accompanied by 1% GV application (group 2). The primary endpoint was clinical response. Results: Ultimately, 54.7% and 45.3% of the significantly cured lesions belonged to groups 1 and 2, respectively, which was not statistically significant. The clinical response was significantly different between the two groups at the end of the fourth week. Conclusions: Although the clinical response of the two groups was significantly different at the end of the fourth week, application of GV did not increase the efficacy of cryotherapy.

Keywords: Leishmaniasis. Treatment. Topical. Gentian violet. Cryotherapy.
Patients with clinically infected lesions were recruited after a complete course of oral antibiotic therapy.

Sample size: The sample size calculation was based on the confidence interval approach of Cocks and Torgerson for pilot randomized trials. Considering the proportion of patients with significant cure approximately equal to 30% among those treated with cryotherapy combined with gentian violet, a power of 80% and a significance level of 5%, we would require a pilot sample of 60 participants (30 in each group) in order to detect a minimum difference of 10% between the treatment groups.

Randomization: The patients were randomly allocated to two groups [MEDCALC software version 8 (Ostend, Belgium)] by permuted block randomization (in blocks of size 4). Thirty-three (33) patients were allocated to each group, but some patients did not complete the treatment after allocation. Thus, during follow-up, there were 30 patients in GV and cryotherapy combination group, whereas 29 patients completed the study in cryotherapy group. The details are shown in Figure 1.

Interventions: Patients in both groups underwent weekly liquid nitrogen cryotherapy using cryospray (Sarmadarman, Tehran, Iran) for 8 weeks. Liquid nitrogen was sprayed approximately 10 cm away from the lesion for 15 s with a double freeze-thaw cycle. In addition to cryotherapy, patients in one of the groups applied 1% gentian violet (GV) ointment twice daily over the lesions for 8 weeks at their home without supervision. The ointment was prepared by dissolving 1 g of GV (Merck, Darmstadt, Germany) in 100 g of Eucerin (Abidayaco, Isfahan, Iran). The patients were assessed at the beginning of

*Number of lesions

FIGURE 1: CONSORT flow diagram of participants through enrollment, allocation, and follow-up stages of the study
allocation, and also at the end of 4th and 8th weeks of treatment by an investigator who was unaware of the treatment. The patients in the GV group were recommended to wash the lesion(s) with water and soap or cleanse with alcohol to wash out the purple color of GV before the 4th and 8th week visits.

Outcome measures: The primary endpoint of this study was defined as clinical cure⁹ as shown below.

Significant cure: more than 75% reduction in the size of lesion (largest indurated diameter multiplied by the shortest indurated diameter of the lesion, measured by a ruler).

Partial cure: marked by 50–75% reduction in lesion size.

Failure to respond: less than 50% reduction in the size of lesion or increase in lesion size.

The clinical cure was reported at the end of the 4th and 8th weeks of treatment.

Ethical considerations: The protocol was approved by the ethical committee of the Shiraz University of Medical Sciences (Ethical code: IR.SUMS.med.REC.1394.29). The ethical principles of the 1975 Declaration of Helsinki were followed. The patients (and parents or legal guardian for patients younger than 18 years) were informed about the study and asked to complete the written consent form.

Data analysis: The data were analyzed using SPSS software version 18 (Chicago, IL, United States). Data of the groups were compared using Chi-square test. The significance level was set at 0.05.

This study lasted from October 2015 to February 2016 and a total of 68 cases were screened. Sixty-six patients with 182 lesions were recruited into the study. After allocation and during follow-up, the cases declined to 59 with 161 lesions (Figure 1).

The baseline characteristics of the patients and lesions recruited into the study groups are shown in Table 1.

Table 2 presents a comparison of the treatment with cryotherapy combined with GV and the treatment with cryotherapy alone.

At the end of the study, the rate of significant clinical cure was not different between the two groups (P = 0.549).

In the 4th week follow-up, 14 (70.0%) and 6 (30.0%) partially cured lesions were treated with cryotherapy combined with GV and cryotherapy alone, respectively. In the same time, 18 (33.3%) of GV-administered group and 34 (66.7%) of the patients treated with cryotherapy alone healed significantly. The therapeutic responses of the two groups were significantly different in the 4th week follow-up (P = 0.02).

No side effect was reported in either group except the transient purple staining of the skin in GV-treated patients.

Despite the variable clinical responses in the 4th week of follow-up, adding gentian violet did not increase the efficacy of cryotherapy in the treatment of cutaneous leishmaniasis.

Investigating topical regimen is an expanding field in pharmacological studies due to their convenience and fewer side effects. Various topical medications have been studied for CL treatment. Few of these topical treatments could be strongly recommended based on qualified studies².

Gentian violet, also known as crystal violet, is a triphenylmethane dye used for the Gram staining of bacteria.

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**Table 1**: Baseline characteristics of patients and lesions in cryotherapy combined with gentian violet (GV) and cryotherapy alone groups

| Variant                      | Cryotherapy / GV group | Cryotherapy group |
|------------------------------|------------------------|-------------------|
| **Number of patients**       | 30                     | 29                |
| **Number of lesions**        | 71                     | 90                |
| **Methods of diagnosis**     |                        |                   |
| Direct smear                 | 24                     | 25                |
| PCR*                         | 1                      | 0                 |
| Clinical                     | 5                      | 4                 |
| **Mean age ± SD** (years)    | 29.4 ± 2.8             | 27.3 ± 3.2        |
| **Sex**                      |                        |                   |
| Male                         | 18 (60%)               | 14 (48%)          |
| Female                       | 12 (40%)               | 15 (52%)          |
| **Site of involvement**      |                        |                   |
| Lower extremities            | 42 (60%)               | 39 (44%)          |
| Upper extremities            | 18 (25%)               | 45 (50%)          |
| Trunk                        | 11 (15%)               | 3 (3%)            |
| Head and neck                | 0 (0%)                 | 3 (3%)            |
| **Mean duration of disease ± SD** (months) | 2.08 ± 0.16 | 2.10 ± 0.19 |

*Polymerase chain reaction; **Standard deviation.
It has also been used clinically for treatment of various infections caused by various Gram-positive and Gram-negative bacteria including methicillin-resistant Staphylococcus aureus, fungi such as Candida, parasitic protozoa such as Trypanosoma Cruzi, parasitic roundworms such as Strongyloides and Enterobius. In addition, anti-angiogenic and antitumor activity of GV has also been mentioned6.

de Souza Pietra et al.7 tested 9 synthetic triphenylmethane derivatives along with GV on Leishmania (L.) amazonensis, Leishmania (V.) braziliensis, and Leishmania major in vitro. GV was the most effective agent in this study. In BALB/c mice infected with Leishmania (L.) amazonensis and subsequently treated with 1% GV gel twice daily, no parasite was detected after 20 days of treatment7.

However, these promising results were not reproduced in our clinical trial, which may, at least in part, be explained by the difference in the preparation of the GV (ointment versus gel). To the best of our knowledge, our study is the first clinical trial to evaluate the clinical efficacy of topical GV in the treatment of cutaneous leishmaniasis.

The mechanism of action of GV is not clear exactly. Different hypotheses have been proposed to explain the effects of GV, especially the antimicrobial effects10. Among these, two hypotheses are mostly emphasized: 1) inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and 2) formation of a covalent complex between GV and thioredoxin reductase 2 (TrxR2) in mitochondria. The latter mechanism was considered to be more admissible explanation for the role of GV in the treatment of leishmaniasis6. TrxR2 is also considered as the target for GV in treatment of cancer and another parasitic infection, malaria11.

Although gastrointestinal and hematological side effects as well as carcinogenicity have been reported in rodents following the systemic use of GV, there is no evidence of significant systemic toxicity following external topical application of GV12.

Limited on the parameters essential for ideal efficacy of topical formulations may explain the discrepancy between the outcomes of in vitro and animal model studies on antileishmanial effect of GV and clinical outcome in our study. Designing more efficient formulations by emerging delivery systems like liposomes, microsponges, lipid nanoparticles, polymeric particles, dendrimers, dendritic-core multishell nanotransporters or even appropriately designing conventional formulations may improve clinical efficacy of topical GV in treating CL13,14.

Besides this limitation in topical medication formulation, our study results may be limited by the small number of patients and lack of follow-up after cessation of treatment. Additionally, we did not determine the parasite species in our study; however, the most common species in our province causing leishmaniasis is Leishmania major15.

In conclusion, despite the variable therapeutic effects of GV-added cryotherapy and cryotherapy alone in the early stages of treatment, topical gentian violet ointment did not increase the efficacy of cryotherapy in the treatment of CL.

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