The effect of different concentrations of topical podophyllin on cutaneous leishmaniasis

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Conflict of interest: None to declare

Received: December 3, 2010
Accepted: May 24, 2011

Introduction: Cutaneous Leishmaniasis (CL) is a parasitic disease caused by Leishmania species. Currently accessible treatments remain insufficient, and there is pressure to develop suitable and effectual options. In this study, we used different concentrations of podophyllin in vitro on *leishmania* parasites and then on leishmaniasis lesions in mice and compared their efficacy.

Method: We used podophyllin (14.3 µg/ml) in vitro against leishmania major parasites, then in experimental animals in different concentrations.

Results: Podophyllin (14.3 µg/ml) that used in vitro eradicated leishmania major parasites, but, in mice after four weeks was not effective and the diameter of the lesions increase with use of topical podophyllin.

Conclusion: Despite the lethal effect on leishmania in vitro, treatment with different doses of podophyllin could not accelerate the healing process of the leishmaniasis lesions of the experimental rats.

Keywords: leishmaniasis, podophyllin, treatment, in vitro

Iran J Dermatol 2011; 14: 48-51

INTRODUCTION

Leishmaniasis is an infectious disease [1] commonly caused by different kinds of *leishmania* like *leishmania tropica* (in urban areas) and *leishmania major* (in rural areas) in the old world. The phlebotomine insect vector bite transmits leishmaniasis to humans [2].

Leishmaniasis is characterized by a clinical, immunological and histopathological range that is associated with immunological capability of the host, the species and virulence of the parasite and inadequately defined environmental factors. Clinical manifestations of leishmaniasis are cutaneous, mucosal and visceral lesions. A rare form is diffuse cutaneous leishmaniasis (DCL) that is related to the imperfect cell-mediated immune response to the *leishmania* parasite [3].

The first line treatment for Leishmaniasis is still pentavalent antimonials, but other drugs such as amphotericin B, paromomycin, imidazoquinoline derivatives, and miltefosine have been studied in other reviews for treatment of leishmaniasis [4,5].

Although many treatment modalities are present, there is no perfect therapy for leishmaniasis.

Some treatments such as glucantime are painful and costly [4,5], other treatments have side effects such as headache, vomiting, skin reaction and abdominal pain [5]. As a result, development of more efficient and easily tolerated medications that can be suitably administered are of important advantages. Podophyllin is a plant alkaloid used for the treatment of genital warts. It interferes with cell replication, crosses cell membranes and works as a keratolytic that causes arrest of cell mitosis in metaphase [6].

In this study, we postulated podophyllin could eradicate *leishmania* parasite by arresting cell mitosis; therefore, we used different concentrations of podopoyllin in vitro on *leishmania* parasites and then on leishmaniasis lesions in mice and compared their efficacy.

MATERIAL AND METHODS:

Cell culture

Leishmania major (MRHO/IR/75/ER) promastigotes were cultured at 26ºC in RPMI 1640 containing 10% fetal calf serum (FCS) and antibiotics. The log-phase promastigotes were washed in phosphate-buffered saline (PBS), adjusted to a concentration of 1×10^6 cell/ml into 98 wells plate in a fresh medium. Podophyllin was added to the promastigotes (14.3 µg /ml). After incubation at 26ºC for 1h, 2h and 24 h at 5% CO_2, the promastigotes were counted and checked for viability using a light microscope.

Mouse infection

Fifty four BALB/c mice (4- to 6-week-old female) were purchased from Pasteur Institute, Karaj, Iran. The animal experiments were carried out according to Ethical Committee Acts of the Shahid Beheshti and Tehran University of Medical Sciences. Mice were inoculated subcutaneously at the base with amastigote forms obtained from the spleen and the liver of mice infected with *leishmania major* (MRHO/IR/75/ER). Four groups were tested by podophyllin 5%, podophyllin 10%, podophyllin 20% and control group (infected but untreated mice). Podophyllin was applied using a sterile tip directly onto the skin to form a thin layer once weekly for 4 weeks. The treated area was then left open without dressing. Images were taken at baseline and then weekly. The lesion size measurement (mm) was done weekly with a caliper. Ethical committee of the Skin Research Center of Shahid Beheshti Medical University approved the study protocol in March 2009 (Certificate number: 1112MTB).

Statistical analysis

The Kruskal-Wallis test was used to compare groups at similar sessions and in case of any difference, the Mann-Whitney-U-test with Bonferroni correction was applied for pair-wise comparisons. For each group, Friedman test was used to evaluate the differences of lesions' diameters among the five time-points (baseline, and after 1, 2, 3 and 4 weeks) and if there were any differences, the Wilcoxon tests with Bonferroni corrections were conducted to compare the lesions' diameters at each week with the previous week and baseline. Statistical analysis was performed using the statistical software SPSS 16 (SPSS Inc. Chicago, IL, U.S.A.). P values less than 0.05 were considered significant.

RESULT

Podophyllin, 14.3 µg /ml used in vitro, eradicated *leishmania major* parasites.The first group (13 mice), second group (13 mice) and third group (12 mice) were treated with podophyllin 5%, 10% and 20% respectively. The control group (13 mice) did not receive any treatment.

The four groups were compared according to lesions' diameters at similar sessions. There were no statistical significant differences in lesions' diameter among the groups at baseline ($p=0.105$).

At first week, a significant difference in lesions' diameter was observed in all groups ($p=0.004$) and pair-wise comparisons showed a significant difference between podophyllin 10% and the control group, and between podophyllin 20% and the control group ($p=0.006$ and $p<0.001$, respectively). Mean lesions' diameter of the podophyllin 10% and 20% groups were more than control group one week after beginning of the treatment (Table 1).

At second week, there was a significant difference in lesions' size among four groups ($p<0.001$) and with pair-wise comparisons, significant differences between podophyllin 10% and the

Table 1. Mean (standard deviation) diameters of lesions by groups over time.

Group	baseline	1th week	2th week	3th week	4th week
Podophyllin 5%	4.74 (1.87)	6.19 (1.93)	7.64 (1.73)	7.04 (3.29)	10.10 (2.57)
Podophyllin 10%	4.65 (0.98)	6.78 (1.58)	10.57 (3.02)	8.03 (3.10)	9.42 (3.73)
Podophyllin 20%	4.56 (1.79)	7.15 (1.34)	8.92 (1.32)	9.28 (1.79)	11.01 (2.26)
Control	5.72 (2.46)	5.21 (1.06)	6.96 (1.84)	8.80 (2.13)	10.08 (2.27)

control group, podophyllin 20% and the control group, and between podophyllin 5% and 10% groups were demonstrated (p=001, p=005 and p=0.002, respectively). According to Table 1, the mean lesions' diameter at the second week after beginning of the treatment in podophyllin 10% group was more than podophyllin 5% and the control group.

At weeks 3 and 4, no significant difference was observed among four groups (Table 1). There were significant increases in lesions' diameters of control group at second week in comparison to first week and at third and fourth weeks in comparison to baseline (p=0.005,p=0.001 and p=0.002, respectively) (Figure 1).

In the podophyllin 5% group, there were significant increases in lesions' diameter at the second and fourth week in comparison with the baseline and also at fourth week in comparison with the third week (p=0.004, p=0.002 and p=0.002, respectively) (Figure 1).

Significant increases in lesions' diameters of second, third and fourth weeks in comparison with baseline and of second week in comparison to first week were observed in podophyllin 10% group (p-values at most 0.004) (Figure 1).

Finally, there were significant increases in lesions' diameter of the podophyllin 20% group at the first, second, third and fourth weeks in comparison with baseline, and the fourth week compared to the third week (p-values at most 0.005) (Figure 1).

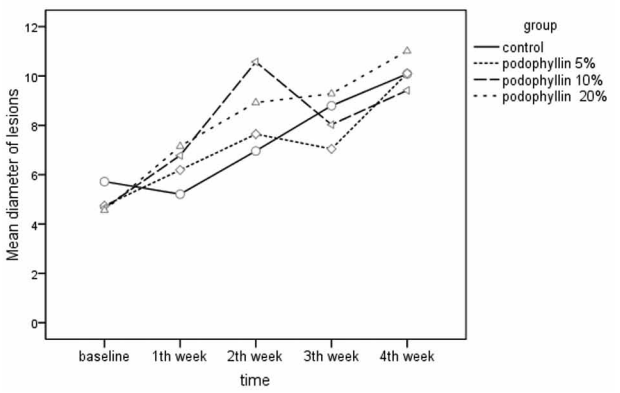

Figure 1. Mean diameters of lesions by groups over time.

DISCUSSION

Leishmaniasis has a big range of clinico-pathological forms and therefore needs different treatments. The most common type of cutaneous leishmaniasis (CL) treatment is antimonials. Intralesional injection of antimonials can prevent side-effects resulting from systemic administration [7] and numerous studies have confirmed their efficacy in the treatment of CL [8-12]. Various other drugs have also been used but all have limitations concerning ease of use and financial aspects [4,5]. Podophyllin is a plant alkaloid [6], commonly used in topical forms such as a chemotherapeutic agent [13]. It interferes with cell replication, crosses cell membranes and works as a keratolytic that causes arrest of cell mitosis in metaphase [6]. Podophyllin has been applied in many disease e.g oral hairy leukoplakia [14-16], measles and herpes simplex virus type I infection [17], condyloma acuminatum [18,19], cytomegalovirus and Sindbis virus infections [20]. Also, it has an anti-tumor and anti-rheumatoid arthritis activity [21] and has been traditionally used for the treatment of genital warts [6].

Despite the evident beneficial effects of podophyllin, no study has been conducted to assess its effects on leishmaniasis. In this regard, in the present study, the efficacy of podophyllin was investigated on leishmaniasis. The present survey postulated that podophyllin could eradicate *leishmania* parasite by arresting cell mitosis. Therefore, we used different concentrations of podopoyllin in vitro on *leishmania* parasites and then on leishmaniasis lesions in mice and compared their efficacy.

Our study demonstrated that podophyllin could eradicate *leishmania* parasite in vitro although in mice, after four weeks of treatment, the mean lesion size increased significantly in podophyllin groups with different concentrations and also in the

control group over time. Our findings suggested that podophyllin was not an appropriate drug for the treatment of CL. A possible explanation could be the anti mitotic effect of podophyllin, which prevents the proliferation of cells near the lesion thus resulting in the expansion of the wound. This effect was more prominent especially when we used podophyllin 20%.

The reason we did not achieve proper results from our study may refer to the fact that the dose of podophyllin was not adjusted appropriately according to the weight of the mice, which led to disorganization in cellular proliferation. Another limitation of our study was that we did not check the parasite burden by smear from the lesions, and podophyllin might have been injected into a non-healing ulcer without any parasites in some cases. In conclusion, treatment with different doses of podophyllin could not accelerate the healing process of leishmaniasis lesions. However, furthur structured studies could be more beneficial to elucidate the possible role of podophyllin in the treatment of cutaneous leishmaniasis.

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