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Comparison between the efficacy of ivermectin and other drugs in treatment of cutaneous leishmaniasis

M. A. Kadir, H. S. Aswad, A. M. Al-Samarai and G. A. Al-Mula

College of Medicine, Tikrit University, Tikrit, Iraq

Abstract

The study was conducted to compare the efficacy of ivermectin with some drugs in treatment of cutaneous leishmaniasis in vitro and in Balb/ C mice. The cultivation of Leishmania tropica was done on Nove MacNeal-Nicole medium (NNN). Assessment of the efficacy of ivermectin and other drugs was performed in vitro and in vivo (Balb/C mice). Ivermectin lead to sharp decrease in viable promastigotes in vitro. The efficacy of ivermectin was highest followed by rifampicin amphotericin B. and nystatin. In subcutaneous injection of the drugs in mice, one month post treatment, it was found that the MLS was reached zero in ivermectin treated group, while in berenil, amphotericin B, metronidazole, pentostam and control groups were 3.5, 3.1, 4, 3.6 and 4 respectively. The cure rate one month after treatment with ivermectin was 100%, followed by pentostam 70%, berenil 60%, amphotericin B 50%, and metronidazole 50%. One month after topical treatment, the MLS was reached to 1 in rifampicin treated group, while in ivermectin, nystatin, erythromycin and control groups were 3.1, 3, 4 and 4 respectively. The cure rate with ivermectin was 40% while it was 80% for rifampicin and 60% for nystatin. The results concluded that the efficacy of ivermectin was highest than er drugs in killing the parasites in vitro and by subcutaneous inoculation, while the efficacy of rifampicin was highest by topical treatment.

Keywords: Ivermectin, Cutaneous leishmaniasis.
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مقارنة بين كفاءة الايفرمكتين و العقاقير الأخرى في علاج اللشمانيا الجلديه

محمد عبد العزيز قادر، حسين ساهر اسود، عبد الغني محمد السامرائي و غانم عبود الملا

كلية الطب، جامعة تكريت، تكريت، العراق

الخلاصة

أجريت الدراسة لمقارنة كفاءة الايفرمكتين و العقاقير الأخرى لعلاج اللشمانيا الجلدية في الزجاج وفي الفئران من النوع BalbC. تم زرع طفيلي اللشمانيا الجلدية على وسط NNN وتم تقدير كفاءة الايفرمكتين و العقاقير الأخرى في الزجاج و في الفئران. ادى عقار الايفرمكتين الى تقليل حيوية الطفيلي في طور (Promastigotes) في الزجاج.كانت كفاءة الايفرمكتين هي الاعلى يتبعها ريفامپسين وامفوترسين B ونيستاتين. عند حقن العقاقير تحت الجلد في الفئران وجدت ان معدل حجم الافة (MLS) وصل الى صفر بعد شهر من العلاج بالايفر مكتين بينما بقيت ٤,٣,٦,٤,٣,١,٣,٥ في البرنيل امفوتريسين, الميترو نيدازول و البينتوستام ومجموعة السيطرة على التوالي. وكانت نسبة الشفاء ١٠٠% في مجموعة الايفر مكتين, بينما كانت ٧٠%.٦٠%.٥٠%.٥٠% و صفر في مجاميع البنتوستام، البرنيل، الامفوترسين، الميترو نيدازول ومجموعة السيطرة على التوالي. عند استعمال العلاج الموضعي للعقاقير وجد ان معدل حجم الافة بعد شهر من العلاج بالريفامبسين وصل الى ١ بينما بقيت ٤,٤,٣,٣,١ لمجاميع الايفر مكتين ونيستاتين و ارثرومايسين ومجوعة السيطرة على التوالي، وان نسبة الشفاء بعد ٣٠ يوم من العلاج الموضعي بايفر مكتين كان ٤٠% بينما كان ٨٠% للريفامبسين و ٦٠% للنستاتين. نستنتج من نتائج الدراسة بان كفاءة الايفر مكتين كانت الاعلى من بقية العقاقير في قتل الطفيلي في الزجاج وبعد الحقن تحت الجلد في الفئران بينما كانت كفاءة الريفامبسيين أعلى بعد العلاج الموضعي.

Introduction

Leishmaniasis are a group of diseases with a broad range of clinical manifestations caused by several species of parasites belonging to the genus Leishmania. The organism has been found to be a complex grouping of species at least 20 of which cause infection in humans (l). There are an estimated 12 million cases worldwide, with 1.5 to 2 million new cases each year (2). There are three clinical forms of leishmaniasis: visceral, cutaneous and mucocutaneous. Leishmania has two stages in its life cycle, amastigote stage in animal host and promastigote stage in vector (3).

If it is not extensive, cutaneous leishmaniasis (CL) may clear by itself and it does not necessarily require treatment (4). The treatment is difficult and cure rates decrease with advanced disease condition. In extensive and disfiguring disease or when multiple lesions are present, treatment with antimony is successful (5).

Treatment of leishmanial diseases involves systemic or local use of pentavalent antimony and aromatic diamidines. The most commonly used compounds are the two pentavalent antimonials, sodium stibogluconate (Pentostam) and neglumine antimonite (Glucantime). If these drugs are not effective, pentamidine and amphotericin B are used. Stibogluconate is the drug of choice for treatment of leishmaniasis but it is expensive and toxic (6,7).

Ivermectin is chemically related to the insecticide avermectin, they are derived from the bacterium Streptomyces avermectin and it acts by interfering with the target animals nervous system. Although highly poisonus to insects, mammals are not generally affected by therapeutic doses of avermectin formulation (8).

Rasheid and Morsy (9) studied the effect of ivermection on the infectivity of Leishmania major promastigotes in Syrian golden hamsters. They showed that hamsters infected with promastigotes treated with a single dose of 100 mg/ ml ivermectin in cultured medium did not develop skin lesions at the site of infection. Promastigotes died or lost their infectivity when treated for two days with 90 mg/ ml.

The present study was planned to compare the efficacy of ivermectin with some drugs for treatment of cutaneous leishmaniasis.

Materials and methods

Leishmania tropica promastigotes were obtained from Leishmania unit at the Research Center/Nahrin College of Medicine. They were maintained on semi-solid medium and subcultured every 21 days (5).

For cultivation of the parasite, biphasic medium Nove-MacNeal-Nicole (NNN) was used. The solid and liquid phase were prepared according to the method described by Al-Bashir et al. (10).

On day of experiment, heavy growth of parasites were harvested and diluted at 1x 10^6 promastigotes per ml. in liquid medium in screw-capped vials to which different concentration of drugs under study were added. Each run also included a control and every concentration was done in duplicate. The vials were then incubated at 26ºC for the next five days. The promastigotes in culture were counted in Neubauer haemocytometer, using 1:10 dilution of normal saline and 0.4% Trypan blue dye.

The growth index was estimated by the mean number of treated promastigotes divided by the mean number of untreated (control) promastigotes multiplied by 100 (11).

Assessment of the effect of treatments: Balb/ C mice were assessed for development of lesion at weekly intervals beginning from the second week till the nine week post-infection. The development of the lesion was followed macroscopically and the presence of parasites in aspirated materials was monitored microscopically in both smears and cultures. Materials aspirated with a fine glass pipette through a small lesion made at the margin of the lesion with a sterile surgical blade, stained with giemsa and cultured in NNN media. Cultures were considered negative only after 20 days without growth. The lesions were scored according to the scale of Peters et al. (12).

To study the effect of drugs injected subcutaneously on L. tropica, 60 Balb/ C mice were used. They were divided in to 6 groups of 10 mice. Each mouse had an area 1 cm above the base of tail, shaved, sterilized and injected with 5X 10^6 promastigotes subcutaneously.

Group 1 was treated with ivermectin (Al-Sark Co. Syria) in a dose of 200 µg/Kg/day for 5 days. Group 2 was treated with diminazine aceturate (Hoechst, Germany) 100mg/Kg/day for 5days. Group 3 was treated with metronidazole (Samara Drug Industry, Iraq, SDI), 200 mg/kg/day for 5 days. Group 4 was treated with amphotericin B (Welcome, England), 50 mg/kg/day for 5 days. Group 5 was treated with pentostam Welcome, England), 400 mg/kg/day. Group 6 was received distilled water and used as negative control.

To study the effect of topically administered drugs, 50 mice were included in this experiment. They were inoculated subcutaneously with 0.15 ml of promastigotes suspension containing 5X10^6 organisms. Mice were divided into groups, 10 mice in each and treated topically as follows: Group 1 was treated with rifampicin (SDI), 15% for 10 days. Group 2 was treated with erythromycin (SDI), 15% for 10 days. Group 3 was treated with nystatin (lifepharma-Italfarma Co. Italy), 100.000 IU/Kg for 10 days. Group 4 was treated with ivermectin 20% for 10 days. Group 5 received distilled water and used as control. The drugs were dissolved in distilled water.

Statistical analysis

Analysis of variance and L.S.D. (Least significant difference tests were used to show significant difference between groups).

Results

The effect of different concentration of ivermectin, nystatin, rifampicin and amphotericin B on the total number of L.tropica promastigotes in vitro is indicated in table 1.

The exposure of promastigotes to different concentration of ivermectin, revealed that increasing the concentration of drug had led to decrease in the number of live promastigotes and complete disappearance of promastigotes occurred in 16 µg /ml after 5 days.

In nystatin treatment, using 1000 IU/ml the number of live promastigotes decreased to the lowest number (10.7 $X10^6$) after 5 days.

Treatment of promastigotes with rifampicin, showed that, the total number of live promastigotes decreased to 4.75X 10^6 after 5 day, using 1000 µg /ml.

Using different concentration of amphotericin B, it was found that the number of live promastigotes decreased to 7.95$X10^6$ after five days of exposure using 32 µg /ml.

In control group, the number of live promastigotes was 2.5X 10^6 after one day, and became 19.2X 10^6 after five days of incubation.

Statistical analysis revealed that, there was significant difference between the effect of different concentration of drugs on promastigotes of L. tropica (P< 0.01). It is obvious that the effect of ivermectin was highest followed by rifampicin, amphotericin B. and nystatin.

Table 1. The effect of different concentration of drugs on the number of promastigotes in vitro. (No. live promastigotes $X10^6$).

Drugs	Days	1	2	3	4	Control
Ivermectin	1	2.14	1.88	1.70	1.21	2.5
µg/ml	5	10.50	4.88	0.25	0	19.2
Nystatin	1	2.1	2.1	1.9	1.5	=
IU	5	17.4	16.1	12.1	10.7	
Rifampicin	1	2.25	2.01	2.14	1.93	=
µg/ml	5	15.66	10.13	9.88	4.75	
Amphotericin	1	2.88	2.6	2.55	2.50	=
B µg/ml	5	20.3	14.6	11.2	7.95	

P=<0.01, Ivermectin= 2, 4, 8, 16 µg/ml. Nystatin= 1, 10, 100, 1000 IU/ml. Rifampicin= 1, 10, 100, 1000 µg/ml. Amphotericin B= 4, 8, 16, 32 µg/ml

Table (2), reveals the growth index (GI) of parasites, using different concentrations of drugs. After exposure to ivermectin, the growth index of promastigotes reached 18.22% after five days at concentrations of 2 µg/ml, while it reached to zero at the concentration of 16µg/ml.

The growth index of the parasite after 5 days of treatment with nystatin was 89.58% at the concentration of 1 I.U./ml, while at the concentration of 1000 µg/ml reached 55.72%.

The growth index after 5 days exposure to 1 and 1000 µg/ml rifampicin were 81.56% and 24.73% respectively.

It is indicated that the growth index of promastigotes after exposure to amphotericin B for five days reached to 69.95% at exposure to 4 µg/ml, while it reached to 27.2% at 32 µg/ml.

Table 2. The effect of different concentration of drugs on the growth index of L. tropica promastigotes in vitro at 5 days post inoculation. Percentage (%).

Drugs	1	2	3	4
Ivermectin µg/ml	18.22	15.3	11.2	0
Nystatin IU	89.58	83.85	63.02	55.72
Rifampicin µg/ml	81.56	52.76	51.45	24.73
Amphotericin B µg/ml	69.5	50.0	42.5	27.2

Ivermectin= 2, 4, 8, 16 µg/ml. Nystatin= 1, 10, 100, 1000 IU/ml. Rifampicin= 1, 10, 100, 1000 µg/ml. Amphotericin B= 4, 8, 16, 32 µg/ml.

Table 3 shows the mean lesion score (MLS) and cure rate of the infected mice pre-treatment and one month post-treatment. It was found that the MLS after one month post-treatment was reached to zero in ivermectin treated group, while the MLS were 3.5, 3.1, 4, 3.6 and 4 in berenil, amphotericin, metronidazole, pentostam and control groups respectively. Statistical analysis revealed that the effect of ivermectin was higher than others (P<0.01). No significant differences were found between other drugs and control.

The cure rate after 5 days post treatment with ivermectin was 30% while the cure rate was zero in other treatments. While after 10 days post treatment was 80% in ivermectin treated group, while cure rates were 20, 30, 20, 30 and 0 in berenil, amphotericin B, metronidazole, pentostam and control groups respectively.

The cure rate after one month post treatment with ivermectin was 100%, while with berenil, amphotericin B, metronidazole, pentostam and control were 60, 50, 50, 70 and 0 % respectively.

Table 3. Mean lesion scores and cure rate of Balb/ C mice infected with L. tropica and treated with different drugs subcutaneously.

Treatment	MLS		Cure rate %		
	Pre-treatment	One month Post treatment	5 days	10 days	30 days
Ivermectin 200mg/kg B.W.	3.37	0	30	80	100
Berenil 300mg/kg B.W.	3.6	3.5	0	20	60
Amphotericin B 50mg/kg B.W.	3.4	3.1	0	30	50
Metronidazole 200mg/kg B.W.	3.6	4	0	20	50
Pentostam 400mg/kg B.W.	3.7	3.6	0	30	70
Control	3.5	4	0	0	0

(P<0.01).

Table 4, represent the MLS and cure rate of infected mice before and after treatments with different drugs topically. It was found that the MLS before treatment was 3.33, 3.86, 2.95 and 3.21 while one month after treatments it was 3.1, 1, 3, 4 and 4 for ivermectin, rifampicin, nystatin, erythromycin and control groups respectively. Statistically it is shown that the efficacy of rifampicin was higher than those of other drugs (P<0.05), followed by nystatin and ivermectin, while no significant difference found between erythromycin treated group and the control one.

The cure rate of mice after 5 days post treatment with rifampicin was 30%, while with ivermectin, nystatin, erythromycin treated and control groups were 10, 20, 0 and 0 % respectively.

The cure rate of mice 10 days post treatment was 70% in rifampicin treated group, while it was 30, 20, 0 and 0 % in nystatin, ivermectin, erythromycin treated and control groups respectively.

After 30 days post treatment, the cure rate in mice treated with rifampicin was 80%, while with nystatin, ivermectin, erythromycin and control groups were 60, 40, 0 and 0 % respectively.

Discussion

The study revealed that the most effective drug in vitro was ivermectin. There are no reports in the published literature on the activity of ivermectin on leishmania for comparison. Ivermectin is a synthetic derivative of a macrocyclic lactone produced by an actinomycetes Streptomyces avermitilis, it has a broad spectrum antiparasitic activity against many types of parasites. Lariviere et al. (13) found that a single oral dose of 200Mg/kg of ivermectin reduces the dermal microfilaria population to nearly zero.

Table 4. Mean lesion scores and cure rate of Balb/C mice infected with L. tropica and treated with different drugs topically.

Treatment	MLS		Cure rate %		
	Pre-treatment	One month Post treatment	5 days	10 days	30 days
Ivermectin 20%	3.33	3.1	10	20	40
Rifampicin 15%	3.86	1	30	70	80
Nystatin 100.000IU/kg	2.95	3	20	30	60
Erythromycin 15%	3.21	4	0	0	0
Control	3.31	4	0	0	0

(P<0.05).

It was found that amphotericin B and nystatin inhibits the growth of L. tropica promastigotes and decreases the number of viable parasites in vitro. The mechanism of action of amphotericin and nystatin might be based on the peculiar metabolism of sterols of Leishmania species. In contrast to mammalian host, 24-ergosterol is the main sterol synthesized and existing in fungal and Leishmania membranes. Polyene macrolides bind to these molecules, creating pores that leak ions (14). Amphotericin B changes the sterol membrane composition, changing its permeability and killing the parasite which is in agreement with that found by Mattock et al. (15).

In agreement with El-On et al. (16), high concentration of rifampicin decreased the viable counts of Leishmania promastigotes. Rifampicin is a macrocyclic antibiotics produced by streptomyces mediterranei. It inhibits DNA-dependent RNA polymerase leading to suppression of initiation of chain formation in RNA synthesis.

High concentrations of rifampicin lead to decrease in the viable counts of promastigotes. This is in agreement with that found by El-On et al (16). Rifampicin is a macrocyclic antibiotics produced by Streptomyces mediterranei, It inhibits DNA-dependent RNA polymerase

leading to suppression of initiation of chain formation in RNA synthesis.

The 100% cure rate of mice treated with ivermectin reflects the highest efficacy of ivermectin in treatment of CL. There are no data available regarding the effect of ivermectin on CL for comparison.

Berenil (Diminazene aceturate, PP-diamidinazoamino-benzene diaceturate tetrahydrate) showed 60% cure rate in mice infected with CL. This finding is in agreement with that found by Lynen et al (17) but in contrast to the finding of Peter et al. (12) who showed that 100 mg/kg berenil per day X5 subcutaneously to NMRI mice infected with L. infantum had resulted in activity level of grade 1.the differences might be due to differences in the daily doses and Leishmania species used.

Berenil is a chemical compound related to aromatic diamidines group. The mode of action is controversial and not completely understood (18). The cationic structure of this molecule is due to its strong cationic nature at physiological pH, thus being responsible for its multi-target interaction with the parasite. The multi-target nature of the pentamidine include inhibition of ATP-consuming topoisomerase 11 (19), inhibition of spermidine biosynthetic pathway (20) and the regulation of thiol/disulfide redox balance can be affected by cationic diamidines (21).

In amphotericin B treated group, the cure rate was 50%. This result is in agreement with that found by Peter et al. (12). Amphotericin B is a polyne antibiotic used mainly as antifungal agent.

The cure rate of metronidazole treated group was 50% only. Peter et al. (12) found the activity level of metronidazole administered subcutaneously was 1 (actively level grade from 0-3) on their study on L. infantum in NMRI mice.

To study the effect of topical administration of drugs on cutaneous leishmaniasis, four types of drugs were used. Erythromycin, nystatin and ivermectin were commercially prepared as ointment, while rifampicin was prepared by mixing the pure powder of rifampicin with white soft paraffin. It was found that rifampicin was highly effective in both healing the lesion and eliminating the parasites with total elimination of the parasites was achieved after 10 days of treatment. Furthermore, this duration of treatment was sufficient to clear the lesion from both the leishmanial parasites and the secondary bacterial infections accompanying this disease. The present data showed that 80% of infected mice were healed completely within four weeks after the treatment. This finding is in agreement with that found by Joshi et al (22) who found that 80% of patients healed completely within four weeks of topically treatment with rifampicin. In contrast, El-On et al (16) found that only 30% of lesions were healed completely after four weeks of treatment with rifampicin. Rifampicin is a lipid soluble drug has the ability to penetrate cell membrane and attacks intracellular bacteria. It kills bacteria by inhibiting RNA synthesis by binding to DNA-dependent RNA polymerase. In vitro studies have demonstrated antileishmania activity of rifampicin against L. mexicana, L. major and L. donovani (23,24).

In nystatin treated group, only 60% of skin lesion in infected mice were completely healed within four weeks after treatment.

Ivermectin is widely used as anti-filarial drugs, orally or parentally.In this study it was applied as a topical treatment. It was found that only 40% of lesions were cured within four weeks after treatment. This result might be due to poor absorption of drugs and inability to penetrate the skin to reach to the intracellular parasites.

In erythromycin treated group, no lesion was cured and this may explain that erythromycin has poor or no anti-leishmanial activity. In contrast, Peter et al. (12) found that erythromycin is effective when injected subcutaneously for 5 days. The differences in these two results may be due to the differences in the dose of drug and the route of administration.

It is concluded from this study that, ivermectin had high efficacy in vitro killing the parasite in comparison with other drugs (rifampicin, amphotericin B and nystatin).

Subcutaneous inoculation of mice with different drugs, showed that the efficacy of ivermectin was higher than pentostam, berenil, amphotericin B and metronidazole.

Treatment of mice locally with different drugs, showed that the efficacy of rifampicin was highest, followed by nystatin and ivermectin.

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