EFFECT OF RIFAMPIN AND CIPROFLOXACIN ON THE GROWTH OF PROMASTIGOTES OF *LEISHMANIA MAJOR* IN VITRO

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ABSTRACT
Different concentration of rifampin and Ciprofloxacin were tested for their activities in vitro against promastigote of *L. major*.

**LD**$_{50}$ (lethal dose) for rifampin and ciprofloxacin were 0.794mg/ml and 3.55mg/ml respectively. The results show that rifampin has better leishmanicidal effect as low concentration is required to kill 50% of the parasite cells.

INTRODUCTION
Over 20 different species of the genus *Leishmania* are known to be pathogenic for humans.

The leishmaniasis are divided into three general clinical patterns according to the form of the disease: Cutaneous, visceral and mucocutaneous. The cutaneous leishmaniasis (CL), the abundant, at an estimated 1.5 million new cases per year, is caused by over a dozen different species of *Leishmania* in different parts of the world$^{(1)}$. CL is a self-healing disease and in most cases heals spontaneously in less than a year. However, if treatment does not offer any risk and discomfort (e.g. topical ointment), it would be recommended for all cases$^{(2)}$.

There are many old and new treatment modalities suggested for CL. But many of the treatments in the past were introduced without proper evaluation in controlled trails, and most of them have not passed the test of time. The spread of drug resistance combined with other shortcomings of the available antileishmanial drugs emphasizes the importance of the development of new, effective, and safe drugs against leishmaniasis$^{(3)}$.

The present study was done to test the activities of Rifampin and Ciprofloxacin against the promastigotes of *L. major* in vitro.
MATERIALS AND METHODS

The parasite was isolated from 30 years old patient from Baji, North of Baghdad, with multiple wet cutaneous lesions in his left hand.

The parasite, which is suspected to be L.major, is maintained by continuous passage in diphasic media consisting of a solid phase and liquid phase, lock's overlay and in laboratory Swiss strain Albino mice.

Drug efficacy:

The drug rifampin was used in concentration of 2, 1, 0.5 and 0.1 mg/ml while ciprofloxacin was used in concentration of 6, 5, 4, 3, and 2 mg/ml, by dissolving the drug powder in lock's solution, using magnetic stirrer for one hour and then sterilized by 45 μ millipore filters.

The promastigotes were harvested at the logarithmic growth phase; they were adjusted to 5x10^6 parasite/ml of each drug concentration. The liquid phase which contain the drug and the parasites were added to the solid phase in proportion of 1:5. The parasites were cultivated at 26-28°C and counted once daily for the following three days and the effect of the drug on the parasite, growth index (GI) was determined according to the following formula:

\[
\text{Mean No. of the treated promastigotes} \times 100
\]

\[
\text{Mean No. of untreated promastigotes (control)}
\]

The 50% lethal dose (LD50) values were calculated by linear regression analysis.

RESULTS

The present study showed that both of these drugs had good leishmanicidal effect at different concentrations. The morphology of the promastigote as seen by light microscope showed that the treated parasites became smaller and rounded, slow or loss their motility as compared to the normal spindle-shaped flagellated one. These changes were more evident at higher concentrations of each drug.
Table (1) shows the density of the parasite cells in control untreated group compared with that treated with rifampin during four days of the experiment. The results reflect the high sensitivity of *L. major* promastigote to the highest concentration of this drug.

Table (1): The effect of various concentration of rifampin on *in vitro* culture of *L. major* promastigotes.

<table>
<thead>
<tr>
<th>Days after exposure</th>
<th>Drug concentration (mg/ml)</th>
<th>Total No. of parasite cells/ml (X10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.51</td>
<td>2</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.85</td>
<td>2.6</td>
</tr>
<tr>
<td>Day 3</td>
<td>3.18</td>
<td>4.65</td>
</tr>
<tr>
<td>Day 4</td>
<td>3.89</td>
<td>5.98</td>
</tr>
</tbody>
</table>

Also the percentage of growth index (GI%) was decreased from 100% at zero time and zero concentration to 27.39, 42.11, 65.5% and 87.39% at concentration of 2, 1, 0.5 and 0.01 mg/ml at the fourth day of the experiment respectively. The (LD₅₀) was 0.794 mg/ml (Fig.1).
Figure (1): The effect of rifampin on the growth of *L. major* promastigote

![Graph showing the effect of rifampin on the growth of *L. major* promastigote.](image)

The effect of various concentrations of ciprofloxacin was shown in Table (2). Also the untreated control group showed a higher density of parasite cells/ml compared with treated groups.

**Table (2): The effect of various concentrations of ciprofloxacin on *in vitro* culture of *L. major* promastigotes.**

<table>
<thead>
<tr>
<th>Days after exposure</th>
<th>Total No. of parasite cells/ml (X10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug concentration (mg/ml)</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Day 1</td>
<td>0.75</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.23</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.2</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.77</td>
</tr>
</tbody>
</table>

The growth index was decreased to 18.96, 20.69, 45.69, 53.03 and 66.28% at 6, 5, 4, 3, and 2 mg/ml of ciprofloxacin respectively. The LD_{50} was 3.55 mg/ml. (Fig.2).
DISCUSSION

Treatment of patients with leishmaniasis still poses a serious problem. Most of the commonly used antileishmanial drugs, such as pentavalent antimonial agents (SbV), exhibit considerable toxicity, and there are reports of large-scale clinical drug resistance among the organism\(^8\). Second-line drugs, such as pentamidine and amphotericin B, do not have a therapeutic index as favorable as that of SbV, long-term therapy is often required, and they often induce toxic effect\(^9\).

A number of investigations to explore potential antileishmanial drugs have been carried out. It has been reported that chlorpromazine\(^1\), methylenedioxamine chloride\(^6\), inosine analogs\(^12\), sulfonamides\(^13\), belomycin\(^14\) have antileishmanial activities.

The present study was designated to examine the potential antileishmanial activity of rifampin and ciprofloxacin in vitro.

Rifampin is a macrocyclic antibiotic, it is a semisynthetic derivative of rifamycin. It active in vitro against some gram-positive and gram-negative cocci, some enteric bacteria, mycobacteria, Chlamydiae and poxviruses, Rifampin bind strongly to DNA-dependent RNA polymerase thus inhibits RNA synthesis in bacteria. It blocks a late stage in assembly of poxviruses. It penetrates phagocytic cells well and can kill intracellular organisms\(^15\).
The results showed that the rifampin exhibited a strongest antileishmanial activity on culture of the parasite. The growth of the promastigotes was rapidly inhibited. Killing of the parasites may be due to the fact that this drug impairs rRNA synthesis\textsuperscript{(15)}.

This drug was also reported by Zueherman and Lainson\textsuperscript{(16)} to has antileishmanial activity against CL while EL-On et al.,\textsuperscript{(17)} reported that rifampin as well as the standard drugs (SbV, Pentamidine and amphotericin B) showed a slight effect on parasite in experimental animals when used as topical ointment or cream. In other study, combination of rifampin and isoniazid were found ineffective in vitro against \textit{L. tropica} although these two drugs have been reported as efficacious orally in certain human studies\textsuperscript{(18)}.

The ciprofloxacin showed also a good antileishmanial activity but at lesser extent. The mode of action of this antibiotic involve inhibition of bacterial DNA synthesis by blocking the DNA gyrase\textsuperscript{(15)}. Jarallah\textsuperscript{(19)} showed that the ciprofloxacin had a good leishmanicidal effect in vivo. A highly difference was found between the density of \textit{Leishmania} amastigote in cutaneous stained smears of infected control and infected treated mice.

The response of different \textit{Leishmania} species parasite to the drugs varies, and without laboratory analysis in each case of the disease, it is uncertain which parasite involved. It is, therefore crucial to identify the causative agent in any chemotherapeutic trial\textsuperscript{(20)}. The dose and the method of application of the drug also have great effect on the activity of that drug.

The results obtained from this study, suggest that antileishmanial activity of rifampin and ciprofloxacin should further investigated and their potential as drugs for leishmaniasis warrant more study.
تأثیر استخدام عقار الريفامیسین والساباروفلوکساسین على نمو النشمانيّا قبیلیة السوط خارج الجسم الحي

عبد المحسن حمید جاسم
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الخلاصة

أُستخدمت تراكیز مختلفة لعقار الريفامیسین والساباروفلوکساسین لتقیيم درجه النشاط على النشمانيّا قبیلیة السوط خارج الجسم الحي. كانت الجرعة القاتلة لعقاري الريفامیسین والساباروفلوکساسین: 0.794 ملجم/ل و 0.03 ملجم/ل على التوالي.

أظهرت النتایج أن الريفامیسین أكثر فعالية عند التركیز الوافی مقارنة مع السباروفلوکساسین

لتقلل ٥٠% من النشاط الطفیلیة

REFERENCES


