

EFFECT OF ITRACONAZOLE AND TERBINAFINE ON *LEISHMANIA* PROMASTIGOTES

By

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Abstract

The antiproliferative effect induced in vitro by two antifungal compounds, the azole itraconazole and the allylamine terbinafine on *Leishmania major*, *L.donovani* and *L.mexicana* promastigotes are reported. Treatment of promastigotes cultures with itraconazole or with terbinafine induced growth arrest with *L.major* but neither with *L.donovani* nor with *L.mexicana*. Concentrations of 0.75 µl/l or more of itraconazole induced cell lysis after 72 hours with *L.major*. However, even relatively large concentrations of terbinafine (2.0 µl/l) did not induce cell lysis. For *L.major*, the IC 50 for itraconazole and terbinafine were 0.31 µl/l and 3.3 µl/l respectively.

Introduction

Leishmania parasites cause a wide range of human diseases from localized self-healing cutaneous lesions to fatal visceral disease (Morsy, 1996). It is a major global public health problem since 2-3 million people are affected by the disease and

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350 million people in 80 countries are at risk (Iwu *et al.*, 1994). Pentavalent antimonials are the most widely used antileishmanial agents since the 1940s. This drug is not always effective and require long term administration which is associated with drug toxicity and sever kidney, heart and liver disorder (Balzan and Fenech, 1992; Giacchino *et al.*, 1993; WHO, 1990). Many investigators are therefore currently involved in seeking alternatives with improved activity and safety. Although there are several compounds under various stages of development, no drug has yet been discovered which is definitively effective in achieving a complete cure of the infections (Iwu, *et al.*, 1994). Since the discovery that some antifungal agents had a potent leishmanicidal activity, the chemotherapy of *Leishmania* sp. appeared to stagnate (Chance, 1995). Several studies examined the effect of antifungal agents on visceral leishmaniasis (New *et al.*, 1981; Negre *et al.*, 1992; Berman *et al.*, 1992; Dietze *et al.*, 1995; Haughan *et al.*, 1992; 1993; Gebre-Hiwot and Formmel, 1993). However, only few studies had reported on the treatment of cutaneous leshmaniasis with antifungal agents (El-On *et al.*, 1993; Larbi *et al.*, 1995; Bahamdan *et al.*, 1997). The azole ketoconazole and the allylamine terbinafine, when tested against *Leishmania* promastigotes and amastigotes *in vitro*, had a suppressive effect on cell growth. Furthermore, both compounds induced ultrastructural changes by inducing appearance of large multivesicular bodies in both amastigotes and promastigotes (Vannier-Santos *et al.*, 1995). By literature review, there is no study on the effect of itraconazole and terbinafine on *L.major*, *L. mexicana*, or *L.donovani* *in vitro*. A pilot clinical trial have tested terbinafine on 27 patients and the overall clinical response in 10 patients, who continued the study, was estimated to be 71.5% (Bahamdan *et al.*, 1997).

The aim of this study is to investigate the effect of itraconazole and terbinafine on the cultured promastigotes of *L.major*, *L.mexicana* and *L.donovani*

Material and Methods

Freshly isolated promastigotes of *L.major* clone FV-1 (MHOM/IL/80/Friedlin), *L.mexicana* (MNYC/BZ/62/M379),

and *L. donovani* (MHOM/ET/67/HU3;LV9) were maintained in Schneider's *Drosophila* medium supplemented with 10% (v/v) heat-inactivated foetal calf serum pH 7.0 at 26°C in 25 cm² tissue culture flasks using air as the gas phase. The promastigotes were used to start new cultures at a density of 5x10⁵ cells/ml.

Itraconazole: (cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazole-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]-phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methyl-propyl)-3h-1,2,4-triazol-3-one) was provided by Dr. Marcel Jansen, Janssen Research Foundation, Belgium. The drug was added as an aqueous solution titrated to pH 2.4 with HCl and sterilized by filtration. The initial stock solution concentration was 0.5 mg/ml.

Terbinafine: ((E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalenemethanamine) was provided by Dr. Barbra Willi of Novartis Pharma AG, Basel, Switzerland. It was added as dimethyl sulfoxide (DMSO). The final concentration of DMSO never exceeded 1% (vol/vol) and it had no effect by itself on the growth and multiplication of promastigotes. The initial stock solution concentration was 0.5 mg/ml.

Schneider's *Drosophila* medium supplemented with 10% FCS with different concentrations of itraconazole and terbinafine (0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 µl/l) were prepared and all culture flasks were started with the original promastigotes concentration of 5x10⁵. Cell densities were estimated daily using an Improved Neubauer haemocytometer under phase contrast microscopy.

Data analysis. All results were entered in a PC. Plots were made using MS Excel and analysed with "Dose effect analysis with microcomputers, Biosoft" software and the IC 50 of each drug was estimated.

Results

Promastigotes of *L. major*, *L. mexicana*, & *L. donovani* were cultured in Schneider's *Drosophila* medium, supplemented with 10% FCS, containing different concentrations of itraconazole and terbinafine and cell densities were estimated daily as described above. The data shown in table (1) and figures (1 – 6).

Table 1. Population doubling time of *L. major*, *L. donovani* & *L. mexicana* promastigotes cultured in Schneider's *Drosophila* medium supplemented with 10% FCS in different concentrations of itraconazole and terbinafine.

Concentration	Population Doubling Time					
	<i>L. major</i>		<i>L. donovani</i>		<i>L. mexicana</i>	
	Itraconazole	Terbinafine	Itraconazole	Terbinafine	Itraconazole	Terbinafine
Control	15.6	15.6	18.1	18.1	18.1	18.1
0.25 µg/ml	26.8	17.2	19.1	18.3	18.3	18.2
0.50 µg/ml	48.4	18.7	18.2	18.3	18.8	18.8
0.75 µg/ml	57.8	19.7	19.0	19.2	18.7	18.5
1.00 µg/ml	80.3	20.8	18.8	18.8	19.0	19.0
1.50 µg/ml	138.9	21.9	19.3	19.7	18.5	18.3
2.00 µg/ml	173.4	27.9	19.7	20.2	18.7	18.8

Itraconazole. Incubation of promastigotes in cell cultures containing different concentrations produced growth arrest with *L. major* only, as judged by the decrease in cell densities as the culture progress. Cell lysis of *L. major* promastigotes was observed with concentrations of 0.75 µl/l or more. The IC 50 was estimated to be 0.3 µl/l and less than 0.5 µl/l concentrations were enough to double the population doubling time. *L. donovani* and *L. mexicana* promastigotes were not affected as judged by the insignificant change in population doubling time.

Terbinafine. Incubation of promastigotes in cultures with different concentrations produced growth arrest as judged by the decrease in cell densities as the culture progress with *L. major* promastigotes. Unlike itraconazole, even high concentrations of terbinafine (2 µl/l) did not induce cell lysis and was not enough to double the population doubling time. The IC 50 was found to be 3.3 µl/l. Terbinafine had no effect on growth rate of *L. donovani* nor *L. mexicana* promastigotes as judged by the insignificant change in population doubling time.

Discussion

The effect of antifungal compounds on *Leishmania* have been previously tested. In cases of visceral leishmaniasis (V.L), amphotericin B proved well in disease curity (New *et al.*, 1981; Berman *et al.*, 1992; Dietze *et al.*, 1995). Some antifungal agents such as the lovastatin and the micronazole have inhibited the promastigote proliferation by inhibiting sterol biosynthesis (Haughan *et al.*, 1992,

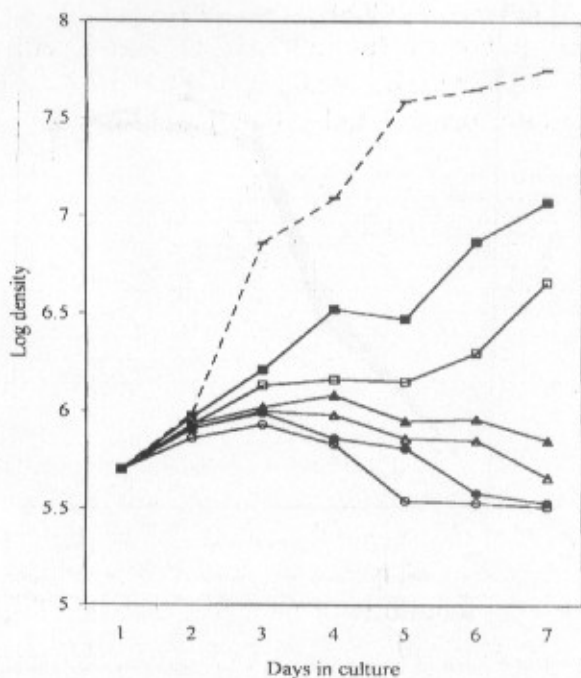


Figure 1. Growth of *L. major* promastigotes in Schneider's *Drosophila* medium supplemented with 10% FCS in different concentrations of itraconazole.

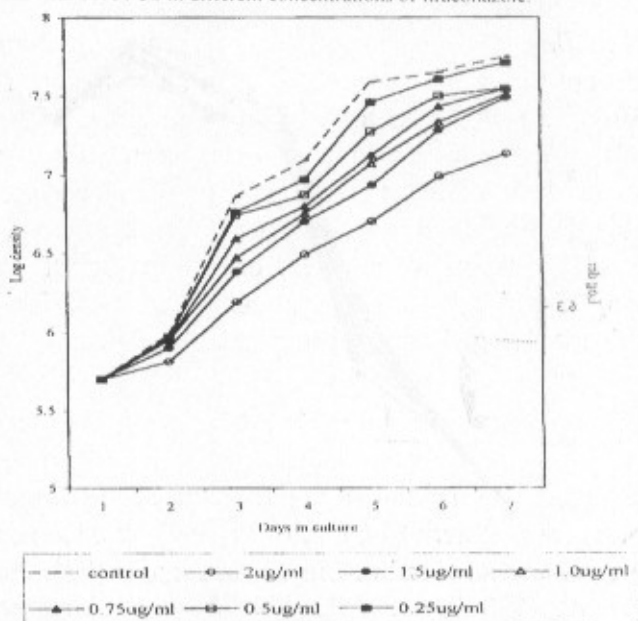


Figure 2. Growth of *L. major* promastigotes in Schneider's *Drosophila* medium supplemented with 10% FCS in different concentrations of terbinafine.

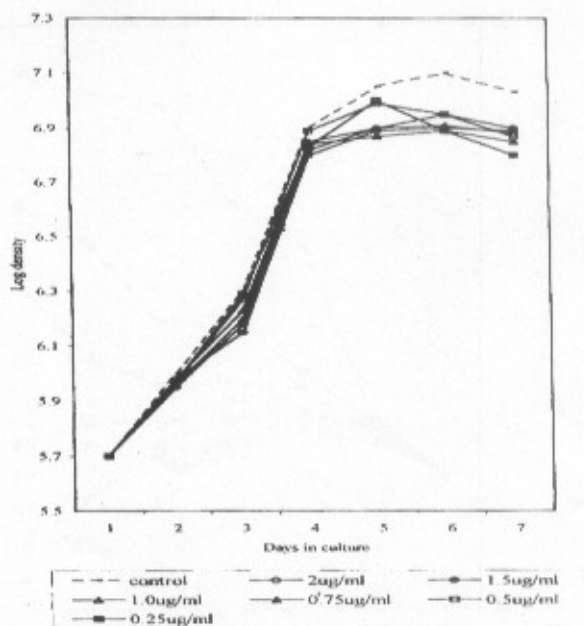


Figure 3. Growth of *L. donovani* promastigotes in Schneider's *Drasophila* medium supplemented with 10% FCS in different concentrations of itraconazole.

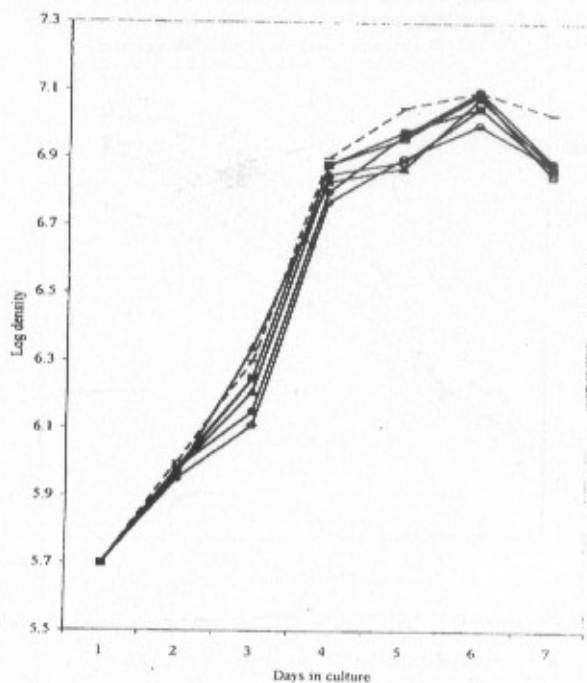


Figure 4. Growth of *L. donovani* promastigotes in Schneider's *Drasophila* medium supplemented with 10% FCS in different concentrations of terbinafine.

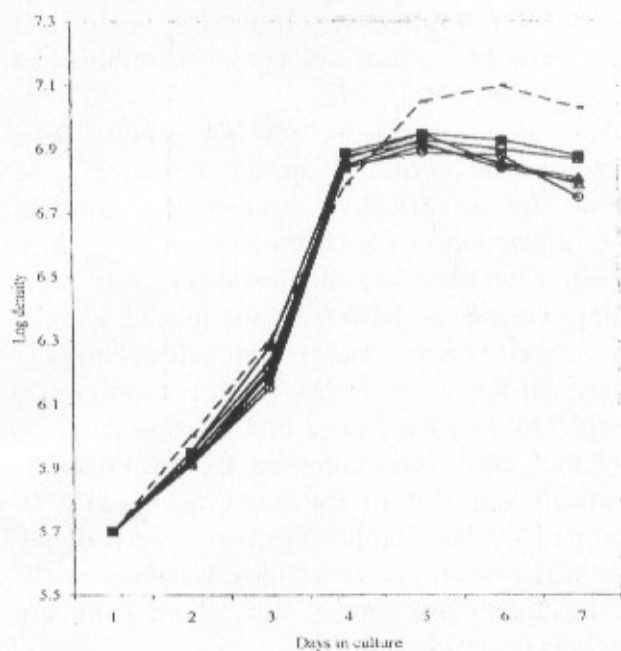


Figure 5. Growth of *L. mexicana* promastigotes in Schneider's *Drosophila* medium supplemented with 10% FCS in different concentrations of itraconazole.

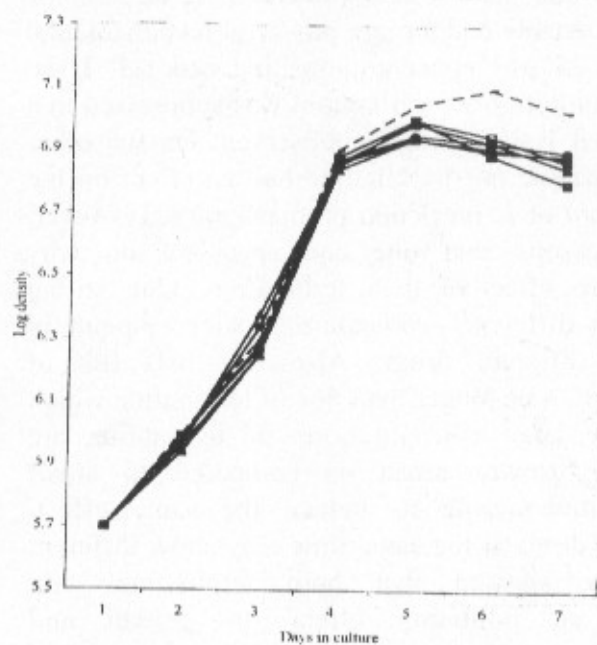


Figure 6. Growth of *L. mexicana* promastigotes in Schneider's *Drosophila* medium supplemented with 10% FCS in different concentrations of terbinafine.

Gebre-Hiwot *et al.*, 1993), while others (e.g. sinefungin) had limited effect on the sterol production and hence on inhibition of proliferation (Haughan *et al.*, 1993).

In cases of cutaneous leishmaniasis, only few studies have reported on treatment with antifungal agents. Poromomycine sulphate was found to be effective against the cultured promastigotes of *L. mexicana* at concentrations of 100 µg/ml (El-On *et al.*, 1993). Clotrimazole and miconazole were also effective in treating cutaneous leishmaniasis in the eastern province of Saudi Arabia, where *L. major* dominates (Peters *et al.*, 1985; Morsy and Al-Seghayer, 1992). However, only 16% of lesions treated with clotrimazole healed fully after 30 days.

The only study that used Terbinafine in the treatment of cutaneous leishmaniasis was that of Bahamdan *et al.* (1997). Ten out of fourteen patients had complete or partial cure after 4 weeks treatment with. However, the sample size was very small, the duration of the treatment course was short, and the *Leishmania* species was never identified.

In this study, both itraconazole and terbinafine had an inhibitory effect on the growth and proliferation of *L. major* promastigotes. Itraconazole had a more powerful leishmanicidal effect since only 0.75 µl/l concentrations induced cell lysis. With terbinafine, promastigote proliferation was suppressed to a lesser extent and cell lysis was never observed. On the other hand, neither itraconazole nor terbinafine had an effect on the growth of *L. donovani* or *L. mexicana* promastigotes. However, there are many reasons that one can speculate on why itraconazole is more effective than terbinafine. One strong reason may be that different *Leishmania* species respond in different ways to different drugs. Also, the half life of itraconazole is known to be longer than that of terbinafine which would explain why large concentrations of terbinafine are required to induce growth arrest as compared to small concentrations of itraconazole to induce the same effect. Combination of two drugs at the same time may show different results. One study showed that both terbinafine with ketoconazole had an inhibitory effect on growth and proliferation of *L. amazonensis* promastigotes. Combination of

the two drugs had an additive effect on promastigote proliferation (Vannier-Santos *et al.*, 1995).

The promastigotes (culture form) and amastigotes (mammalian form) of *Leishmania* react differently to different drugs (Coombs *et al.*, 1983; Dutta, 1983). So, the ease in obtaining and culturing promastigotes makes it an ideal stage studying drug effects on such parasite before in vitro or in vivo studies on amastigotes. The results of this study proved that single drug can not cure leishmaniasis caused by different species.

To examine the effect of both itraconazole and terbinafine together with lovastatin, a hypocholesterolaemic agent, on *Leishmania* amastigotes in vitro and in vivo is ongoing and will be published in due time elsewhere. This is because *Leishmania* parasites have the ability to utilize some cholesterol from the host and not rely only on ergosterol. Besides, miconazole was more effective in inhibiting *Leishmania* parasites when used with lovastatin (Haughan *et al.*, 1992; Olliaro *et al.*, 1993).

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