

## Effects of itraconazole and terbinafine on *Leishmania major* lesions in BALB/c mice

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Received 12 November 1999, Revised and accepted 20 September 2000

The effects of two antifungal compounds, the azole itraconazole and the allylamine terbinafine, on *Leishmania major* infections in mice are reported. Sixty BALB/c mice were each inoculated subcutaneously with metacyclic promastigotes of *L. major* at the base of the tail. From 4 weeks post-inoculation, 40 of the mice were treated for 4 weeks (20 with itraconazole and 20 with terbinafine) and the rest were left untreated. Lesion sizes were estimated weekly for 10 weeks post-infection. Both drugs appeared effective in treating the cutaneous lesions but response to itraconazole was faster and, at the end of the experiment, the mean size of the lesions on the mice treated with itraconazole was smaller than that of the lesions on the terbinafine-treated mice.

*Leishmania* spp. cause a wide range of human diseases, from localized, self-healing, cutaneous lesions to fatal, visceral disease. The leishmaniasis constitute a major public-health problem on a global scale, 2-3 million having clinical leishmaniasis and 350 million people, in 80 countries, being at risk (Iwu *et al.*, 1994). Since the 1940s, pentavalent antimonials have been the most widely used treatments for leishmaniasis, although they are not always effective, require long-term administration, and are associated with drug toxicity, including severe kidney, heart and liver disorders (WHO, 1990; Balzan and Fenech, 1992; Giacchino *et al.*, 1993). Little progress has been made in improving the chemotherapy of *Leishmania* infections since the discovery that some antifungal agents had potent leishmanicidal activity (Chance, 1995). There have been several studies on the effect of antifungal agents on experimental visceral leishmaniasis (New *et al.*, 1981; Berman *et al.*, 1992;

Haughan *et al.*, 1992, 1993; Negre *et al.*, 1992; Gebre-Hiwot and Frommel, 1993; Dietze *et al.*, 1995) and human cutaneous leishmaniasis (Larbi *et al.*, 1995; Bahamdan *et al.*, 1997). Two such agents, the azole ketoconazole and the allylamine terbinafine, had a suppressive effect on cell growth when tested against *Leishmania* promastigotes and amastigotes *in vitro*, inducing the appearance of large multivesicular bodies in the parasites (Vannier-Santos *et al.*, 1995).

When Larbi *et al.* (1995) performed a randomized, double-blind, clinical trial, to compare the efficacies of clotrimazole and miconazole in the treatment of cutaneous leishmaniasis (CL) in the eastern province of Saudi Arabia, they found that clotrimazole was the more effective. However, the *Leishmania* species involved was never identified and only 16% of the lesions treated with clotrimazole healed fully after 30 days. When, in a pilot, clinical trial, Bahamdan *et al.* (1997) tested terbinafine on 27 patients with *L. major* infections, the overall clinical response in the

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10 patients who completed treatment and follow-up was estimated to be 71.5%. There appears to have been no other previous study on the effect of itraconazole on confirmed *L. major* infections *in vivo*. The aim of the present study was to investigate the effect of itraconazole and terbinafine on the cutaneous lesions caused by *L. major* in BALB/c mice.

## MATERIALS AND METHODS

### The Drugs

The itraconazole [cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazole-1-yl)-methyl]-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 M. Jansen of the Janssen Research Foundation, Beerse, Belgium. The terbinafine [(E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalenemethanamine], provided by Dr B. Willi of Novartis Pharma AG, Basel, Switzerland, had to be pre-dissolved in dimethyl sulphoxide (DMSO) but the final concentration of DMSO in the inoculum never exceeded 1% (vol/vol).

### The Parasite

Freshly isolated promastigotes of *L. major* clone FV-1 (MHOM/IL/80/Friedlin) were maintained in Schneider's *Drosophila* medium supplemented with 10% (v/v) heat-inactivated foetal calf serum (FCS), pH 7.0, at 26°C in 25-cm<sup>2</sup> tissue-culture flasks, using air as the gas phase. The development of metacyclic promastigotes in culture was induced as described by Zakai *et al.* (1998). Briefly, exponential-phase promastigotes were cultured at 26–27°C for 6 days, in Schneider's *Drosophila* medium supplemented with 20% (v/v) heat-inactivated FCS, pH 5.5 (adjusted using 1 M HCl or 1 M NaOH), using 10 ml culture/25-cm<sup>2</sup> culture flask and with air as the gas phase. The parasites were then harvested, washed three times with Hanks balanced salt solution (HBSS), and finally resuspended in HBSS at a density of  $2 \times 10^7$  cells/ml to produce the suspension of metacyclic promastigotes used as the inoculum.

### Infection of BALB/c Mice

Each of 60 BALB/c mice was inoculated subcutaneously, in the shaved base of its tail, with 0.05 ml of the parasite suspension (i.e.  $10^6$  promastigotes). The diameters of the resulting lesions were measured and monitored weekly for a total of 10 weeks. From 4 weeks post-infection, 40 of the mice were treated daily for 4 weeks with either 0.1 mg itraconazole/day (20 mice) or 0.2 mg terbinafine/day (20 mice), from a gastric syringe, the remaining 20 mice being left untreated, as controls. Student's *t*-tests were used to compare mean lesion diameters for each treatment group and the control group, at various time-points.

### Parasite Viability at the End of the Experiment

All the mice were killed 10 weeks post-infection, at the end of the experiment. A necropsy sample was aseptically removed from one lesion on each mouse and placed in a 25-cm<sup>2</sup> tissue-culture flask containing Schneider's *Drosophila* medium supplemented with 20% FCS and 25 µg gentamicin sulphate/ml. Each flask was incubated at 27°C and checked for promastigotes, daily for 21 days, on an inverted microscope.

## RESULTS

Lesions of CL appeared on the second week post-infection, in all of the mice. Each lesion was first noticed as a patch of rough hairless skin, each measuring approximately 2 mm in greatest diameter. Each patch developed into a lesion with a raised edge and sunken centre. Some of the lesions had centrally ulcerated nodules during the last 2 weeks of the experiment. Up to the initiation of treatment on week 4, lesion size increased with time post-infection (see Fig.).

### Itraconazole

One week after starting oral treatment with itraconazole, mean lesion size decreased and this trend continued even after treatment stopped at the end of week 8, with lesions among the itraconazole-treated animals being

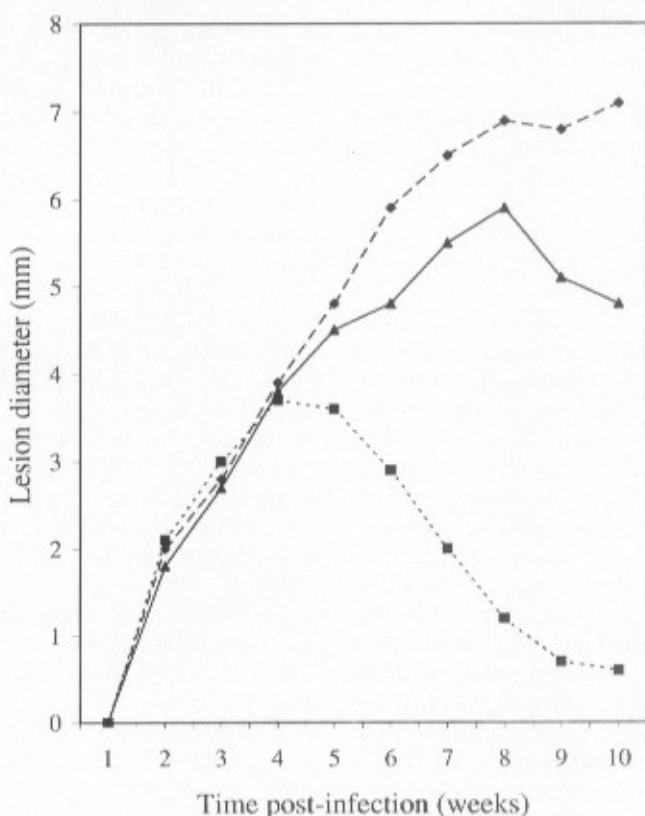


Fig. Changes in mean lesion diameter in BALB/c mice infected with *Leishmania major*. The mice were left untreated (◆) or treated daily for 4 weeks, from week 4 post-infection, with oral itraconazole (■) or oral terbinafine (▲).

significantly smaller than those in the controls whenever they were measured between weeks 5 and 10 ( $P < 0.001$  for each comparison). By the end of the experiment, mean lesion size in the mice treated with itraconazole was  $< 1$  mm.

#### Terbinafine

Treatment with terbinafine did not have as dramatic an effect on lesion size as treatment with itraconazole, although the lesions seen post-treatment were still smaller than in the controls. As with the itraconazole-treated, the lesions among the terbinafine-treated animals were significantly smaller than those in the controls whenever they were measured be-

tween weeks 5 and 10 ( $P < 0.001$  for each comparison). However, the lesions on the terbinafine-treated animals only began to decrease in size towards the end of the 4 weeks of treatment, being largest in week 8, and fell to a minimum diameter of 4.8 mm by week 10 (see Fig.).

#### Parasite Viability

All of the necropsy samples from the control group of mice produced promastigotes when cultured, promastigotes first being detected 10–15 days after the cultures were set up. None of the samples from the mice treated with itraconazole produced promastigotes. Although promastigotes were detected in one of

the 20 samples from the terbinafine-treated mice, on day 19 post-initiation, they were in such low numbers that their presence was confirmed by centrifugation of a subsample of the culture and examination of the pellet.

## DISCUSSION

Several studies have focused on the potential of inhibitors of sterol biosynthesis as useful therapeutic agents for leishmaniasis. Itraconazole and terbinafine both inhibit the synthesis of ergosterol, a sterol which is usually found in large quantities in the cell wall of *Leishmania* parasites (Beach *et al.*, 1988). Although oral itraconazole has been used in the treatment of human CL, the sample sizes in such clinical trials have been small (Al-Fouzan *et al.*, 1991; Enden *et al.*, 1994). Terbinafine has been shown to be effective in the treatment of *Trypanosoma cruzi* infections in mice (Maldonado *et al.*, 1993) but the only reported use of terbinafine to treat CL appears to be in the pilot, clinical study of Bahamdan *et al.* (1997). The sample size in the latter study was also small, however, and only half the patients completed the scheduled follow-up.

In the present study, the response of the cutaneous lesions of *L. major* to two widely used antifungal compounds—itraconazole and terbinafine—was assessed in mice. Four weeks' treatment with either drug caused significant reductions in lesion size and generally cleared the lesions of viable parasites. However, the response to itraconazole was relatively fast and much more marked, in terms of reduced lesion size, compared with

the response to terbinafine. The difference in efficacy probably reflects the relatively short half-life of terbinafine in plasma (Ahonen *et al.*, 1995). The present results are in agreement with those of Dogra and Saxena (1996) who, in a randomized, double-blind clinical trial, used itraconazole for the treatment of human CL, and declared 70% of the patients who received itraconazole to be cured (based on clinical and parasitological criteria). In an earlier study, 67% of patients with human CL were considered to be cured following treatment with itraconazole (Dogra *et al.*, 1990).

*Leishmania* spp. appear to vary in their susceptibility to sterol-biosynthesis inhibitors (Urbina, 1997). The promastigotes of *L. brasiliensis* are naturally resistant to ketokonazole but are susceptible to a combination of ketokonazole and terbinafine (Rangel *et al.*, 1996).

Terbinafine and particularly itraconazole may offer potential new treatments for human CL. Further clinical trials are required to study the efficacy of each in the treatment of CL in the Old and New Worlds. The efficacy of a combination of the two drugs and the effect of different doses also need further investigation.

**ACKNOWLEDGMENTS.** The authors thank Dr M. Saimaldahr of the Medical Technology Program, King Abdulaziz University, for his help with the statistical analysis. Our thanks also go to the director of King Fahad Medical Research Center and the staff of the Center's animal unit for their help. This study was financially supported by King Abdulaziz University (grant 016/418).

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