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Therapeutic efficacy of voriconazole against a fluconazole-resistant *Candida albicans* isolate in a vaginal model

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***Background*: The purpose of this study was to assess the therapeutic efficacy of oral versus intravaginal voriconazole and compare it with fluconazole for the treatment of experimental vaginitis caused by a fluconazole-resistant *Candida albicans* isolate.**

***Methods*: Mice were treated with voriconazole at 5, 10 and 20 mg/kg once a day and 20 mg/kg twice a day or with fluconazole at 20 mg/kg once or twice a day orally. Intravaginal treatments were evaluated with voriconazole and fluconazole at 0.5, 1 and 5 mg/kg once a day. All treatment regimens were given on days 1–5 post-challenge. One day 6, the vaginas were swabbed to assess treatment effects.**

***Results*: Mice treated orally with voriconazole at ≥10 mg/kg and fluconazole at ≥20 mg/kg showed significantly reduced fungal counts over controls ($P = 0.0002–0.007$). Significant differences were found between the groups that received voriconazole at 20 mg/kg once or twice daily and those that received fluconazole at 20 mg/kg once or twice daily, orally ($P = 0.010$ and 0.001, respectively). Mice treated with voriconazole or fluconazole administered intravaginally at ≥0.5 mg/kg exhibited a reduced fungal burden when compared with the control group ($P = 0.0002–0.007$). There was no statistically significant difference in fungal burden between topical treatment with doses of 0.5, 1 and 5 mg/kg once daily of voriconazole or fluconazole. Sterilization of vaginas was not observed with voriconazole and fluconazole without taking into consideration the therapeutic modality.**

***Conclusions*: Voriconazole could emerge as a new alternative for treatment of vaginal candidosis.**

Keywords: vaginal candidosis, vaginitis, murine model, *C. albicans*

Introduction

Vulvovaginal candidosis is among the most frequent clinical problems in women of childbearing age. *Candida albicans* is the causative agent in ~85%–90% of patients with symptomatic yeast vaginitis.[1] Fluconazole, with its marked *in vitro* activity against *Candida* species and clinical efficacy, accounts for most prescriptions for the treatment of this disease.

Voriconazole possesses a wide spectrum of activity against yeasts (including fluconazole-susceptible and -resistant *Candida* spp. and *Cryptococcus neoformans*) and filamentous and dimorphic fungi.[2,3] Voriconazole was approved by the US Food and Drug Administration (FDA) in 2002 for the treatment of invasive aspergillosis. At present, voriconazole has an extension of this indication to include the treatment of oesophageal candidosis, candidaemia, disseminated *Candida* infections in skin and viscera, and fungal infections caused by *Scedosporium apiospermum* and *Fusarium* spp. The use of voriconazole has been circumscribed to severe systemic conditions and its efficacy as therapy for vulvovaginal candidosis in either animal models or clinical estimation has not been evaluated.

The purpose of this study was to assess the therapeutic efficacy of oral versus intravaginal voriconazole and compare it with fluconazole for the treatment of experimental vaginitis caused by a fluconazole-resistant *C. albicans* isolate.

Materials and methods

Mice

Five-week-old BALB/c mice (weight, 18 g) were purchased from Harlan Mexico. Ten mice were randomly assigned to each treatment or control group. The mice were provided food and water *ad libitum*. All animal research procedures were approved by the

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Ethics Committee of our university. Care, maintenance and handling of the animals were in accordance with Mexican government licensing conditions for animal experimentation. Animal studies were repeated for verification of data.

Drugs

Voriconazole and fluconazole were purchased as Vfend and Diflucan, respectively, and administered orally to mice via a feeding needle. Voriconazole and fluconazole were also obtained as reagent-grade powders from the manufacturer (Pfizer, Inc., New York, NY, USA) and suspended in 0.3% Noble agar. Both antifungal compounds were administered by intravaginal instillation, independently.

Microorganism

C. albicans isolate 03-2718 has been previously used in our laboratory for vaginal candidosis studies.[4] The 48 h MIC of fluconazole is >64 mg/L. The strain was maintained on slopes of Sabouraud dextrose agar and for long-term storage at −70°C in 10% glycerol.

Infection model

A previously described model of vaginal candidosis was utilized.[5] Three days prior to infection and on day 4 post-challenge, mice were given 0.5 mg of oestradiol valerate (Delestrogen, King Pharmaceuticals) subcutaneously to maintain pseudo-oestrus. On infection day, mice were anaesthetized with 80 mg/kg ketamine hydrochloride, intraperitoneally, and then inoculated intravaginally with 20 μL of a suspension of *C. albicans* 2×10^8 cfu/mL. One day after infection, the vaginas were swabbed (prior to treatment) to ensure infection was consistently distributed among animals. The vaginas were also swabbed on day 6 to evaluate early treatment effects. Each alginate swab was placed in 0.9 mL of sterile saline, serial 10-fold dilutions were made and 100 μL aliquots were placed onto Sabouraud dextrose agar supplemented with 0.5% (w/v) chloramphenicol to quantify the cfu/mL.

Treatment schedules

Voriconazole (Vfend) was obtained as an intravenous infusion and reconstituted as per the manufacturer's instructions with water for injection. It was further diluted in 5% dextrose to provide solutions of 1 mg/mL. Voriconazole was given once daily at 5, 10 and 20 mg/kg and twice daily at 20 mg/kg of body weight orally by gavage . Fluconazole (Diflucan) was obtained as an intravenous solution at 2 mg/mL. Fluconazole was administered once or twice daily at 20 mg/kg of body weight orally by gavage. All treatments were administered on days 1–5 post-challenge and in 0.2 mL volumes.

Mice treated orally with voriconazole were also treated orally twice daily with 0.25 mL of grapefruit juice started 3 days before infection and continued for 5 days post-infection. Grapefruit juice was administered 30 min before voriconazole treatment.[6]

Voriconazole and fluconazole powders suspended in 0.3% Noble agar were applied intravaginally once a day at 0.5, 1 and 5 mg/kg in a volume of 20 μL on days 1–5 after infection.

Control mice were infected but received no active treatment. Groups received 5% dextrose and grapefruit juice orally, sterile distilled water orally and 0.3% Noble agar intravaginally.

Statistics

A one-way analysis of variance was applied to compare the control with all groups and, where differences occurred, a Tukey test was performed between paired groups. A P value of <0.05 was considered significant.

Results and discussion

Table 1 shows the results of voriconazole and fluconazole administered orally or intravaginally for the treatment of vaginal infection. Compared with both control groups treated with 5% dextrose and grapefruit juice or sterile distilled water, mice

Table 1. Recovery of *C. albicans* strain 03-2718 from the vagina of mice treated orally or intravaginally with voriconazole and fluconazole

	Post-treatment (day 6), mean $\log_{10}$ cfu (range)/P value	
Treatment	oral	topical
Control (5% d-gfj) twice daily	6.38 (5.39–7.11)	
Control (water) twice daily	6.46 (5.89–7.14)	
VRC (5 mg/kg) once daily	6.12 (5.28–7.04)/0.505	
VRC (10 mg/kg) once daily	4.61 (3.32–5.94)/0.007	
VRC (20 mg/kg) once daily	3.68 (2.41–4.92)/0.0002	
VRC (20 mg/kg) twice daily	2.51 (1.75–3.37)/0.0002	
FLC (20 mg/kg) once daily	4.68 (2.76–6.62)/0.0002	
FLC (20 mg/kg) twice daily	3.61 (2.29–5.01)/0.0002	
Control (0.3% Noble agar) once daily		6.48 (5.16–7.81)
VRC (0.5 mg/kg) once daily		4.90 (4.17–5.61)/0.007
VRC (1 mg/kg) once daily		4.52 (3.91–5.22)/0.007
VRC (5 mg/kg) once daily		3.22 (3.05–3.55)/0.0002
FLC (0.5 mg/kg) once daily		4.85 (4.01–5.67)/0.007
FLC (1 mg/kg) once daily		4.57 (3.90–5.16)/0.007
FLC (5 mg/kg) once daily		4.23 (3.34–5.71)/0.007

VRC, voriconazole; FLC, fluconazole; 5% d-gfj, 5% dextrose/grapefruit juice.

treated orally with voriconazole at $\geq$10 mg/kg and fluconazole at $\geq$20 mg/kg daily showed reduced fungal counts ($P=0.0002-0.007$). When the treatments given orally were compared at equivalent doses (20 mg/kg once or twice daily), voriconazole groups were able to reduce the fungal load significantly with respect to the fluconazole groups ($P=0.010$ and 0.001, respectively). All therapy regimens administered topically for the treatment of vaginal infection significantly reduced the fungal load with respect to the control group treated with 0.3% Noble agar ($P=0.0002-0.007$). On the other hand, when the topical treatments were compared at analogous doses, voriconazole was as effective as fluconazole (0.5, 1 and 5 mg/kg once daily) in reducing the fungal load of vaginas. Nevertheless, neither drug administered orally or topically was able to eradicate this microorganism from the vagina.

Regarding the antifungal effect of topical treatments with voriconazole and fluconazole, it is noteworthy that the results of the treatments administered intravaginally, even though statistically significant with respect to the control, were not as impressive as we expected, in spite of the direct interaction between the antifungal formulation and the *C. albicans* isolate in the vagina. Due to the fact that complex excipients used in different commercial preparations are not available alone, we used Noble agar as the vehicle because it could be used to formulate both drugs, and it also lacks intrinsic antifungal activity. We assume that the different commercial excipients have the advantage of the retention of the drug in the vagina. In this study we noted that considerable amounts of both preparations (voriconazole and fluconazole) leaked from the vagina after application and remained for a relatively short period of time at the target site. This could explain the results obtained regarding the cfu in the vaginas.

Efforts have been made in the past few years to optimize the retention of antifungal products in the vagina and allow prolonged action of active drugs.[7] Nano carriers, such as liposomes, proliposomes or niosomes, may also play a future role in the treatment of vulvovaginal candidosis.[8,9] Recently, Bachhav and Patravale[10] developed and evaluated a microemulsion-based gel for the delivery of fluconazole. The formulation displayed potent bioadhesive activity and did not show any signs of vaginal irritation in rabbits. Further studies indicate that formulation of a microemulsion-based gel of fluconazole could be a viable alternative to the current topical formulations available for the treatment of vulvovaginal candidosis. In spite of this, oral regimens of fluconazole are preferred by physicians and women. This formulation can cause mild side effects. However, these symptoms may cause women to discontinue the treatment. A topical fluconazole formulation could overcome the side effects mentioned and would have the advantage of intravaginal medications: avoiding extensive drug absorption and drug interactions; and safe utilization during pregnancy and breast feeding.[11]

To our knowledge, this is the first study of the efficacy of voriconazole against *C. albicans* in a murine model of vaginal infection. Of particular importance was the activity of voriconazole against this infection caused by a fluconazole-resistant *C. albicans* isolate. The results are consistent with the effects of voriconazole in other animal studies of *Candida* spp. infections, as well as those of other medically important fungi.

The results obtained in this study indicate the possible usefulness of voriconazole administered either orally or topically for the treatment of vulvovaginal candidosis. Voriconazole is available as an intravenous infusion, tablets and oral suspension, but there are no voriconazole formulations for vaginal delivery. This study may favour research of this therapeutic approach together with new strategies of vaginal delivery that could produce a new formulation for the treatment of vulvovaginal candidosis.

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Transparency declarations

We declare that we have no conflicts of interest.

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