



Original article

Efficacy of ravuconazole in a murine model of vaginitis by *Candida albicans*

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ABSTRACT

Background: The incidence of vulvovaginal candidiasis, a common infection among healthy women primarily caused by the yeast *Candida albicans*, has increased significantly in recent years.

Aims: The purpose of this study was to compare the efficacy of ravuconazole (RVC) and fluconazole (FLC) in the treatment of experimental *C. albicans* vaginitis.

Methods: Forty isolates of *C. albicans* were screened for their *in vitro* susceptibility to RVC and FLC. A strain of *C. albicans* that was resistant to FLC (minimum inhibitory concentration [MIC] of >64 µg/ml) was selected for the *in vivo* study. Treatment regimens for the murine vaginal infection model were (1) 1, 5, 10, and 20 mg/kg RVC once daily, (2) 20 mg/kg RVC twice daily, (3) 20 mg/kg FLC once daily, and (4) 20 mg/kg FLC twice daily.

Results: The geometric means of the MIC values at 48 h for all isolates tested were 0.05 and 0.5 µg/ml for RVC and FLC, respectively. Regimens of either RVC or FLC at 20 mg/kg twice daily were more effective to reduce the load of FLC-resistant *C. albicans* than single dose administration.

Conclusions: Complete eradication of *C. albicans* from the vagina was not observed with RVC or FLC treatment in the animal model, although RVC treatment showed a lower fungal concentration 14 days after drug administration.

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Eficacia del ravuconazol en un modelo murino de vaginitis por *Candida albicans*

RESUMEN

Antecedentes: En los últimos años, ha aumentado sustancialmente la incidencia de candidiasis vulvovaginal, una infección frecuente entre mujeres sanas, causada sobre todo por la levadura *Candida albicans*.

Objetivos: El objetivo del presente estudio fue comparar la eficacia del ravuconazol (RVC) y del fluconazol (FLC) en el tratamiento de la vaginitis experimental inducida por *C. albicans*.

Métodos: Se examinó la sensibilidad *in vitro* de 40 aislamientos de *C. albicans* frente a RVC y FLC. Para el estudio *in vivo* se seleccionó una cepa de *C. albicans* que fue resistente a FLC (concentración inhibitoria mínima [CIM] >64 µg/ml). Las pautas de tratamiento para el modelo murino de infección vaginal fueron 1) 1, 5, 10 y 20 mg/kg de RVC una vez al día, 2) 20 mg/kg de RVC dos veces al día, 3) 20 mg/de FLC una vez al día, y 4) 20 mg/kg de FLC dos veces al día.

Resultados: Para todos los aislamientos las medias geométricas de los valores de la CIM a las 48 h fueron de 0,05 y 0,5 µg/ml para RVC y FLC, respectivamente. Las pautas de 20 mg/kg de RVC o FLC dos veces al día fueron más eficaces para reducir la carga infectiva de *C. albicans* resistente a FLC que las administradas una vez al día.

Palabras clave:

Vaginitis

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Conclusiones: En el modelo animal no se eliminó completamente *C. albicans* del tracto vaginal estéril mediante tratamiento con RVC o FLC. Sin embargo, el tratamiento con RVC derivó en concentraciones fúngicas más bajas 14 días después de su administración.

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Vulvovaginal candidiasis is an infection that affects women frequently, particularly during their childbearing years. It is caused by yeasts of the genus *Candida*, usually *Candida albicans*. *C. albicans* is the causative agent of vulvovaginal candidiasis in 90% of symptomatic patients, and vulvovaginal candidiasis is the second most common infection among all vaginal infections.^{11,31} Current treatments for *Candida* vulvovaginitis include a wide range of intravaginal azole preparations that are typically administered over several days,¹ but many patients prefer the convenience of oral medications. Oral fluconazole (FLC) is a triazole with marked *in vitro* activity against *Candida* species. Due to its clinical efficacy and ease of administration, it is the treatment most often prescribed for this disease.^{30,32}

However, with the increasing incidence of vulvovaginal candidiasis caused by *C. albicans* and the growing resistance of infections to conventional antifungals, especially FLC, there is a need to evaluate new therapeutic agents. Ravuconazole (RVC) is a novel triazole antifungal molecule developed by Eisai Co. Ltd. (Tokyo, Japan) that has a half-life of over 100 h, exhibits broad spectrum activity, and has a good safety profile.^{3,12,17} *In vitro* studies have demonstrated potent RVC activity against *Candida* spp., *Cryptococcus neoformans*, and other yeast species, including some strains that are not susceptible to FLC.^{8,23,26,28,33} Additional studies have demonstrated that RVC has *in vitro* activity against clinical isolates of the filamentous fungi *Aspergillus*, *Paecilomyces*, *Fonsecaea pedrosoi*, *Cladophialophora carionii*, the dimorphic fungi *Coccidioides immitis*, and *Histoplasma capsulatum*.^{9,13,21,27}

Martinez *et al.*² developed a murine model of vulvovaginal candidiasis to evaluate the therapeutic efficacy of novel antifungals for recurrent infections caused by species of *Candida* resistant to conventional antifungals. RVC has been evaluated in murine and guinea pig models of infection, and has been found to be effective for the treatment of mucosal candidiasis, disseminated aspergillosis, and systemic histoplasmosis.^{6,7,18,25} In this study, we compared the effectiveness of RVC and FLC for the treatment of experimental *C. albicans* vaginitis.

Materials and methods

Strains and *in vitro* susceptibility testing

Forty isolates of *C. albicans* from patients with vaginal infections were sent for identification to the Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Mexico. These clinical isolates were identified by standard biochemical (API 20C AUX, Biomerieux) and microbiological procedures.²⁴ They were stored in water at room temperature until use.

RVC (Eisai Co. Ltd. Tokyo, Japan) and FLC (Pfizer Inc., New York, USA) were obtained as reagent grade powders from their manufacturers. Isolates were evaluated for their *in vitro* susceptibilities by the broth macrodilution method described in the Clinical and Laboratory Standards Institute reference document M27-A3.²² *Candida parapsilosis* ATCC 22019 and *Candida krusei* 6258 were included as control organisms. *C. albicans* isolate 03-2718 has been used in our laboratory for previous vaginal candidiasis studies.^{14,15,16} The minimum inhibitory concentrations (MICs) of FLC and RVC for this strain were >64 µg/ml and 0.25 µg/ml, respectively. Strains were

maintained on Sabouraud dextrose agar slants for short-term storage and kept in 10% glycerol at –70 °C for long-term storage.

Animal infection model

Five week-old BALB/c mice (weight, 18 g) were purchased from Harlan Mexico. Ten mice were randomly assigned to a treatment or control group and were housed in cages containing five mice each. Food and water were provided *ad libitum*. All animal research procedures were approved by the University Ethics Committee. Care, maintenance, and handling of the animals followed Mexican government licensing requirements for animal experimentation, and studies were performed in duplicate.

A previously described model of recurrent vaginal candidiasis was used.² Three days prior to infection and on days 4, 11, and 18 post-challenge, mice were given 0.5 mg estradiol valerate (Delesgrogen, King Pharmaceuticals) subcutaneously to maintain pseudoestrus during the entire experiment. On the day of infection, mice were anaesthetized with 80 mg/kg ketamine hydrochloride intraperitoneally and were inoculated intravaginally with 20 µl of a 2×10^8 colony-forming units (CFU)/ml suspension of *C. albicans* isolate 03-2718. One day after infection, the vaginal cavity of each mouse was swabbed (prior to treatment) to ensure that the infection was consistently distributed among animals.²⁹ The same procedure was repeated on days 6 and 20 to evaluate treatment efficacy. Each alginate swab was placed in 0.9 ml of sterile saline, 10-fold serial dilutions were made, and 100 µl aliquots were plated onto Sabouraud dextrose agar plates supplemented with 0.5% (w/v) chloramphenicol to determine the CFU/ml.

Drugs were administered orally on days 1 to 5 after infection. RVC was prepared fresh daily and dissolved in 0.5% carboxymethylcellulose with 10% dimethyl sulfoxide. RVC was administered in 0.2-ml doses of 1, 5, 10, or 20 mg/kg once a day. One group of animals received a dose of 20 mg/kg twice a day. FLC was dissolved in distilled water and was administered once or twice a day in 0.2 ml doses of 20 mg/kg of body weight. Control mice were infected but received no active treatment; they received the drug vehicle containing 0.5% carboxymethylcellulose and 10% dimethyl sulfoxide orally. On day 21, mice were sacrificed by cervical dislocation.

Statistical analysis

Comparisons were performed using the Mann–Whitney *U*-test, with significance set at a *P*-value < 0.05.

Results

Antifungal susceptibility

The 40 *C. albicans* clinical isolates were inhibited *in vitro* by 0.015–8 µg/ml of RVC and 0.125 to >64 µg/ml of FLC. The geometric means were 0.05 mg/ml and 0.5 µg/ml for RVC and FLC, respectively (Table 1). The concentrations that inhibited 50% of the isolates were 0.03 µg/ml for RVC and 0.25 µg/ml for FLC. RVC displayed stronger *in vitro* antifungal activity at lower concentrations than FLC. The MICs of the control strains were within the acceptable ranges for the drugs tested.⁴

Table 1
In vitro activity of RVC and FLC against 40 vaginal strains of *C. albicans*.

Drug	48 h MIC ($\mu\text{g/ml}$)			
	Range	GM	50% ^a	90% ^b
RVC	0.015–8	0.05	0.03	2
FLC	0.125 to >64	0.5	0.25	2

RVC, ravuconazole; FLC, fluconazole; GM, geometric mean.

^a MIC at which 50% of isolates were inhibited.

^b MIC at which 90% of isolates were inhibited.

Table 2
Recovery of *C. albicans* strain 03-2718 from vaginas of mice treated orally with RVC and FLC.

Treatment (dose [mg/kg] and frequency) ^a	Mean log ₁₀ CFU on day post-inoculation ^b		
	1	6	20
Control [DMSO/CMC] QD	5.673	5.674	6.597
RVC (1) QD	5.609	4.767*	3.749*
RVC (5) QD	5.656	4.532*	3.565*
RVC (10) QD	5.467	4.464*	3.471*
RVC (20) QD	5.389	4.169*	3.205*
RVC (20) BID	5.779	3.744*	3.000*
FLC (20) QD	5.608	3.866*	3.874*
FLC (20) BID	5.829	3.173*	3.441*

Each group had 10 mice. The mice were monitored on days 1, 6 and 20 after inoculation.

^a 10% DMSO/0.5% CMC; QD, once a day; BID, twice a day.

^b *, $P < 0.05$ with regard to the control group.

Animal model

The effects of RVC were compared with those of FLC for the treatment of experimental vaginitis induced by a *C. albicans* isolate (Table 2). Compared with the vehicle-treated control, mice treated with RVC at ≥ 1 mg/kg once daily had reduced loads of vaginal *C. albicans* ($P = 0.006$ to < 0.001) on days 6 and 20 postinoculation. In addition, twice-daily administration of 20 mg/kg RVC resulted in larger reduction of *C. albicans* loads compared to single-dose administration ($P = 0.022$). In contrast, RVC concentration did not affect *C. albicans* loads on days 6 and 20 postinoculation when administered at ≥ 1 mg/kg once daily ($P = 0.63$ to > 0.87). Once- and twice-daily oral administrations of 20 mg/kg FLC reduced the vaginal load of *C. albicans* ($P \leq 0.001$) on days 6 and 20 postinoculation.

C. albicans loads at days 6 and 20 postinoculation were compared between mice treated with 20 mg/kg RVC and FLC twice daily. RVC showed better *in vivo* activity 15 days after treatment (day 6, $P = 0.003$; day 20, $P = 0.03$). However, there was no difference in the fungal burden at days 6 and 20 postinoculation when 20 mg/kg of RVC or FLC were given once daily ($P = 0.27$ – 0.67 , respectively). Complete eradication of *C. albicans* from the vaginal cavity was not observed with either RVC or FLC regimens.

Discussion

RVC is a novel triazole that has shown considerable activity against various fungi.²⁰ In this study, RVC exhibited improved *in vitro* antifungal activity compared to FLC, which is the most widely used drug for vulvovaginal candidiasis. The antifungal activity of RVC has been demonstrated against strains of *Cryptococcus neoformans* isolated from HIV patients, where 100% of the isolates were susceptible to the drug.¹⁹ Although the antifungal activity of RVC against mucormycetes, such as *Rhizopus*, was recently confirmed, further experimentation using an animal model is required to obtain a wider perspective of its efficacy.¹⁰

For the animal model, we used a mouse reference strain for vaginal infection.⁵ We evaluated the *in vitro* activity of RVC against a

C. albicans FLC-resistant isolate, as well as its efficacy during 20 days of postinfection pseudoestrus. BALB/c mice in the control group did not show a significant decrease in CFUs on days 1, 6, and 20 postinfection, confirming persistent infection in the murine model. When 20 mg/kg RVC and FLC were administered twice daily, the FLC-resistant *C. albicans* microbial burden was significantly reduced. RVC had a prolonged duration of efficacy because it decreased the fungal burden 15 days after treatment ended. This result can be attributed to the long half-life (> 100 h) of RVC. In contrast, FLC has a short half-life of 20–50 h. The longevity of RVC makes it a viable alternative for persistent *Candida* infections.

RVC is currently being evaluated in clinical trials, but the results of these trials have not yet been published. To the best of our knowledge, this is the first study evaluating the efficacy of RVC against *C. albicans* in a murine model of vaginal infection. Our results are particularly important because they demonstrate the efficacy of RVC against candidiasis caused by a FLC-resistant *C. albicans* isolate. Our results also show the usefulness of orally administered RVC for the treatment of vulvovaginal candidiasis.

Conflicts of interest

All authors declare no conflict of interest.

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