Antifungal activity of posaconazole against *Candida* spp. and non-*Candida* clinical yeasts isolates

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ABSTRACT

The *in vitro* antifungal activity of posaconazole was tested against 315 yeast clinical isolates and 11 ATCC reference strains by means an agar diffusion method (Neosensitabs, Rosco, Denmark) based in CLSI M44-A2 document. Posaconazole activity was excellent against *Cryptococcus* and *Rhodotorula* species studied and showed very good activity against most species of *Candida* tested. A total of 13 clinical isolates (4.1%) were resistant: *Candida albicans* (n=5), *Candida glabrata* (n=5), *Candida tropicalis* (n=1), *Geotrichum australiensis* (n=1) and *Geotrichum capitatum* (n=1). Our results suggest posaconazole is an effective antifungal agent against the most clinically important yeasts species (92.7% of susceptibility). Agar diffusion method provides good conditions for the posaconazole susceptibility study in the routine laboratory.

Keywords: Antifungal agent, susceptibility, posaconazole, yeasts, *Candida, Cryptococcus, Rhodotorula*

Actividad antifúngica de posaconazol frente a aislamientos de levaduras del género *Candida* y otros de interés clinico

RESUMEN

Se ha determinado la actividad antifúngica *in vitro* de posaconazol frente a 315 aislamientos clínicos de levaduras y 11 cepas ATCC por medio de un método de difusión en agar (Neosensitabs, Rosco, Dinamarca) basado en el documento CLSI M44-A2. Posaconazol presentó una excelente actividad frente a las especies de *Cryptococcus* y *Rhodotorula*, como así también, frente a la mayoría de los aislamientos de *Candida* estudiados. Un total de 13 aislamientos (4,1%) resultaron resistentes: *Candida albicans* (n=5), *Candida glabrata* (n=5), *Candida tropicalis* (n=1), *Geotrichum australiensis* (n=1) y *Geotrichum capitatum* (n=1). Nuestros resultados sugieren que posaconazol es un efectivo agente antifúngico frente a las especies de levaduras de mayor relevancia clínica (92,7% de sensibilidad). La técnica de difusión en agar aporta buenas condiciones para la realización de estudios de sensibilidad al posaconazol en la rutina del laboratorio.

Palabras clave: Antifúngico, sensibilidad, posaconazol, levadura, *Candida, Cryptococcus, Rhodotorula*.

INTRODUCTION

Posaconazole is a third generation triazole antifungal agents designed to improve clinical profiles of fluconazole or itraconazole against *Candida* and *Aspergillus* spp. Posaconazole mode of action is directly based in the inhibition of lanosterol 14-α-demethylase activity[1], resulting in a high *in vitro* activity against a wide spectrum of pathogenic yeast-like and filamentous fungi and also protozoans[1,2]. Posaconazole activity was demonstrated in fungal infections of different immunocompromised animal models and also in clinical trials against usual and unusual fungal infections. Posaconazole was effective in candidiasis, disseminated aspergillosis and zygomycoses, pulmonary histoplasmosis, coccidioidomycosis and disseminated fusariosis and also is useful for the treatment of refractory mycoses, trypanosomiasis and leishmaniasis[2]. The purpose of this study was to determine the *in vitro* antifungal activity of posaconazole against common and uncommon yeasts and yeast-like clinical isolates by means an agar diffusion method that could be more reliable for routine laboratory work[3].

MATERIAL AND METHODS

Strains. A total of 326 clinical isolates and type culture collection strains of pathogenic fungi were studied, including *Candida albicans* (*C. albicans*) (n=129), *C. colliculosa* (n=1), *C. dubliniensis* (n=25), *C. famata* (n=10), *C. glabrata* (n=59), *C. guilliermondii* (n=9), *C. intermedia* (n=2), *C. kefyr* (n=3), *C.*

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krusei (n=2), *C. lusitaniae* (n=9), *C. lipolytica* (n=2), *C. parapsilosis* (n=16), *C. pulcherrima* (n=2), *C. tropicalis* (n=31), *Cryptococcus laurentii* (n=1), *Cryptococcus neoformans* (n=6), *Geotrichum australiensis* (n=1), *Geotrichum capitatum* (n=2), *Pichia etchells* (n=1), *Rhodotorula minuta* (n=2) and *Rhodotorula rubra* (n=2). Yeasts were isolated from superficial or invasive fungal infections in human patients and stored in sterile distilled water less than 3 months. To ensure the inoculum purity and viability, isolates were subcultured on Sabouraud glucose agar at 35ºC for 24 h (*Candida* spp.) and 48 to 72 h (*Cryptococcus spp.*, *Geotrichum* spp and *Rhodotoula rubra*). *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019), *C. albicans* (ATCC 90028) and *C. tropicalis* (ATCC 750) were used as quality controls (QC)[3-5] and also other ATCC strains (*C. albicans* n=2, *C. guilliermondii* n=1, *C. krusei* n=1 and *C. lusitaniae* n=5) were tested.

Susceptibility test. Antifungal susceptibility test was performed as described by CLSI M44-A2 document and following manufacturer guidelines[4,5]. Supplemented Mueller-Hinton agar with 2% (p/v) glucose and methylene blue (0.5 mg/L) was used at a depth of 4.0 mm[4,5]. The agar surface was inoculated using a swab dipped in a cell suspension adjusted to a 0.5 McFarland standard (1-5x10^6 CFU/ml) turbidity[4,5]. Posaconazole Neosensitabs tablets (Rosco Diagnostica, Taastrup, Denmark) of 5 µg were dispensed onto the inoculated agar surface[4,5]. Only posaconazole tablets were used to avoid interactions with other antifungal agents. Plates were incubated at 36ºC (±1ºC) and read at 24 and 48 h. Zone diameter endpoints was measured (mm) using a calliper. Following CLSI and the manufacturer[5] criteria, the diameter of inhibition areas were interpreted as follows: susceptible (S) zone diameter of ≥17 mm (MIC≤1 mg/L); resistant (R), zone diameter of ≤13 mm (MIC≥4 mg/L) and intermediate (I), zone diameter of 14-16 mm[5].

RESULTS

Quality control strains showed *in vitro* susceptibility values for posaconazole inside to the reported ranges, for *C. krusei* ATCC 6252 was 23-28mm, for *C. parapsilosis* ATCC 22019 was 25-30mm and for *C. parapsilosis* ATCC 90028 was 26-32mm[3-5]. Table 1 shows the *in vitro* susceptibility values obtained with the 327 yeasts and yeast-like fungi studied. Most clinical isolates (92.7%) were susceptible to posaconazole. Resistance was observed in 5/129 *C. albicans* isolates (3.1%) and also in 5/59 *C. glabrata* (8.4%), 1/31 *C. tropicalis* (3.2%), 1/1 *G. australiensis* (100%) and in 1/2 *G. capitatum* (50%). Susceptibility interpreted as intermediate was observed only in 7/59 (11.8%) of *C. glabrata*, and in only one strain of *C. albicans*, *C. guilliermondii* and *C. krusei*.

C. albicans resistant strains were isolated from oropharyngeal lesion (n=4) and vaginal candidiasis (n=1). *C. glabrata* resistant to posaconazole strains were isolated from vagina (n=3), oesophagus (n=1) and urine (n=1). Resistant *C. tropicalis* (n=1) was isolated from urine and *G. capitatum* (n=1) from skin. *G. australiensis* (n=1) was recovered from oropharyngeal lesion in HIV positive patient. All yeasts isolated from blood samples (1 *C. albicans*, 2 *C. glabrata*, 1 *C. guilliermondii*, 1 *C. krusei* and 5 *C. tropicalis*), were posaconazole susceptible (n=10).

DISCUSSION

The *in vitro* susceptibility tests give us useful information for management of invasive fungal infections. The detection of a resistant isolate can be a warning for the clinician and an important data for the therapeutic[6]. Posaconazole has a good *in vitro* activity profile against many yeast and filamentous fungi with low resistant isolates percentages[2-25]. Resistance percentages for posaconazole observed in this study was 3.9%. Using the same agar diffusion method and microdilution methods, resistance percentages for fluconazole (10%), itraconazole (18%) and amphotericin B (2-3%) reported for another authors, show the high activity of posaconazole against clinical yeasts isolates[2,6,11,26]. In addition, our results agree with those obtained by microdilution methods showing posaconazole ranges of activity between 0.03-0.125 mg/L for most isolates[6].

As describe some authors in other countries, the *in vitro* antifungal activity of posaconazole seems to be influenced by the origin of the clinical sample and the geographical factors, even in the same geographical area. This reason can be explain the differences between our results and published data[15-22].

In the current study, an excellent activity of posaconazole against *C. tropicalis* was observed in comparison with data from Ostrosky et al[15]. These authors reported an increased MIC values due to the trail effect and the observation of an even greater susceptibility of 366 isolates MICs under 0.5 mg/L. The agar diffusion method could solve the trail effect simplifying the reading intrepretation.

A reduced susceptibility for *C. glabrata* (5 resistant and 7 intermediate isolates) (table 1) was observed in this study agreeing with data described by Ostrosky et al.[15] who compared posaconazole antifungal activity with other promising new antifungal drugs like the echinocandins. In contrast, we observed a high activity of posaconazole against species less susceptible echinocandins such as *C. parapsilosis*, *C. neoformans* and *G. cutaneum*. Also, *in vitro* antifungal activity of posaconazole was obtained against all *C. dubliniensis* isolates

In vitro antifungal activity of posaconazole covers most aetiological agents involved in moderate and severe mycoses in the Western world. The fact that posaconazole shows a good activity against most *C. glabrata* and *C. krusei* isolates, allows considered this drug as an excellent alternative to fluconazole for disseminated candidemia and invasive candidiasis treatment caused by these species.

Moreover, the broad spectrum antifungal *in vitro* action observed for posaconazole against usual and unusual yeast-

Tabla 1 **Antifungal activity of posoconazole against common and uncommon clinical yeast isolates.**

Species	Susceptible	Intermediate	Resistant
Candida albicans (n=129)	123	1	5
C. glabrata (n=59)	47	7	5
C. tropicalis (n=31)	30	-	1
C. dubliniensis (n=25)	25	-	-
C. parapsilosis (n=16)	16	-	-
C. lusitaniae (n=9)	9	-	-
C. guilliermondii (n=9)	8	1	-
C. famata (n=10)	10	-	-
C. kefyr (n=3)	3	-	-
C. krusei (n=2)	1	1	-
C. intermedia (n=2)	2	-	-
C. lipolytica (n=2)	2	-	-
C. pulcherrima (n=2)	2	-	-
C. colliculosa (n=1)	1	-	-
Cryptococcus neoformans (n=6)	6	-	-
Geotrichum australiensis (n=1)	-	-	1
G. capitatum (n=2)	1	-	1
Pichia etchells (n=1)	1	-	-
Rhodotorula minuta (n=2)	2	-	-
R. rubra (n=2)	2	-	-
TOTAL (n=315)	292 (92.7%)	10 (3.2%)	13 (4.1%)

like microorganisms, converts this drug in a useful alternative to amphotericin B or fluconazole for the treatment of severe fungal infections. This agar diffusion method allows the antifungal susceptibility testing determination and the detection of resistant strains in a routine laboratory, reduces the experimental procedure of microdilution methods and avoids some problems such as the trailing or Eagle effect described for dilution tests[15,27,28].

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