Clinical Microbiology and Infection 27 (2021) 915.e5–915.e8

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Research Note

In vitro activity of ibrexafungerp and comparators against *Candida albicans* genotypes from vaginal samples and blood cultures

Aina Mesquida [1, 2], Teresa Vicente [1], Elena Reigadas [1], María Palomo [1], Carlos Sánchez-Carrillo [1, 2, 3], Patricia Muñoz [1, 2, 3, 4], Jesús Guinea [1, 2, 3, *], Pilar Escribano [1, 2]

[1] *Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain*
[2] *Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain*
[3] *CIBER Enfermedades Respiratorias-CIBERES (CB06/06/0058), Madrid, Spain*
[4] *Department of Medicine, Faculty of Medicine, Universidad Complutense de Madrid, Spain*

ARTICLE INFO

Article history:
Received 16 October 2020
Received in revised form
19 January 2021
Accepted 6 February 2021
Available online 16 February 2021

Editor: E. Roilides

Keywords:
Antifungal susceptibility
Candida albicans
EUCAST
Genotyping
Ibrexafungerp
Microsatellites
Vulvovaginal candidiasis

ABSTRACT

Objectives: Emergence of azole resistance may contribute to recurrences of vulvovaginal candidiasis. Thus, new drugs are needed to improve the therapeutic options. We studied the *in vitro* activity of ibrexafungerp and comparators against *Candida albicans* isolates from vaginal samples and blood cultures. Furthermore, isolates were genotyped to study compartmentalization of genotypes and the relationship between genotype and antifungal susceptibility.

Methods: Candida albicans unique patient isolates (n = 144) from patients with clinical suspicion of vulvovaginal candidiasis (n = 72 isolates) and from patients with candidaemia (n = 72) were studied. Antifungal susceptibility to amphotericin B, fluconazole, voriconazole, posaconazole, isavuconazole, clotrimazole, miconazole, micafungin, anidulafungin and ibrexafungerp was tested (EUCAST 7.3.2). Mutations in the *erg11* gene were analysed and isolates genotyped.

Results: Ibrexafungerp showed high activity (MICs from 0.03 mg/L to 0.25 mg/L) against the isolates, including those with reduced azole susceptibility, and regardless of their clinical source. Fluconazole resistance rate was 7% (n = 5/72) and 1.4% (n = 1/72) in vaginal and blood isolates, respectively. Some amino acid substitutions in the Erg11 protein were observed exclusively in phenotypically fluconazole non-wild type. Population structure analysis suggested two genotype populations, one mostly involving isolates from blood samples (66.3%) and the mostly from vaginal samples (69.8%). The latter group hosted all fluconazole non-wild-type isolates.

Discussion: Ibrexafungerp shows good *in vitro* activity against *Candida albicans* from vaginal samples including phenotypically fluconazole non-wild-type isolates. Furthermore, we found a certain population structure where some genotypes show reduced susceptibility to fluconazole. **Aina Mesquida, Clin Microbiol Infect 2021;27:915.e5–915.e8**

Introduction

Oral or topical azoles are recommended for the treatment of vulvovaginal candidiasis [1]. Reported fluconazole resistance rates in *Candida albicans* vaginal isolates are considerably higher (42%) than for blood isolates [2]; some vaginal *C. albicans* genotypes may show high fluconazole MICs [3]. The emergence of azole resistance has prompted the development of new drugs such as ibrexafungerp, an orally and intravenously available beta-d-glucan synthase inhibitor currently under clinical development for treating vulvovaginal candidiasis. Ibrexafungerp has shown good *in vitro* activity against *C. albicans* [4–7], but studies focusing on vaginal isolates are limited [8,9]. We assessed the *in vitro* activity of ibrexafungerp, and comparators, against *C. albicans* vaginal and blood isolates. We also genotyped the isolates to study potential

* **Corresponding author:** Jesús Guinea, Servicio de Microbiología Clínica y Enfermedades Infecciosas, Hospital General Universitario Gregorio Marañón, C/ Dr. Esquerdo, 46, 28007, Madrid, Spain.
E-mail address: jguineaortega@yahoo.es (J. Guinea).

https://doi.org/10.1016/j.cmi.2021.02.006
1198-743X/

compartmentalization and the relationship between genotypes and antifungal susceptibility.

Material and methods

Samples and patients

One hundred and forty-four molecularly proven *C. albicans* patient-unique isolates (vaginal isolates ($n = 72$) of women with clinical suspicion of vulvovaginal candidiasis] and blood isolates ($n = 72$) from patients with candidaemia) collected between 2017 and 2019 at the Clinical Microbiology Department, Hospital Gregorio Marañón (Madrid, Spain), were studied.

Antifungal susceptibility testing and erg11 sequencing

Antifungal susceptibility to amphotericin B, fluconazole, voriconazole, posaconazole, clotrimazole and miconazole (the last two only against vaginal isolates) (Sigma-Aldrich, Madrid, Spain), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), micafungin (Astellas Pharma, Inc., Tokyo, Japan), anidulafungin (Pfizer Pharmaceutical Group, New York, NY, USA) and ibrexafungerp (Scynexis, Inc., Jersey City, NJ, USA) was studied (EUCAST E.Def 7.3.2) [10]. Inoculated tissue-treated trays (CELLSTAR® Ref. 655 180, Greiner bio-one, Frickenhausen, Germany) were used to obtain MIC values, defined as the lowest concentration of the drug that inhibits ≥50% of growth in comparison with a drug-free growth control well for all drugs with the exception of amphotericin B (≥90%). Isolates were classified as resistant/phenotypically non-wild type (non-wild type from now on) according to the updated 2020 EUCAST clinical breakpoints/epidemiological cut-off values (ECOFFs) (Fluconazole ECOFF and breakpoints are 0.5 mg/L and >4 mg/L, respectively) [11] or as isavuconazole non-wild type (MIC >0.03 mg/L) [12]. Resistant/non-wild-type isolates were retested at least three times.

A phylogenetic tree was constructed using *erg11* gene sequences from fluconazole non-wild-type isolates and in a set ($n = 18$) of wild type control isolates from blood and vaginal isolates [13].

Genotyping and population structure

Isolates were genotyped and defined as either singletons (genotypes found only once) or genotypically identical (showing the same alleles with all markers) [14]. Moreover, identical genotypes found in two or more patients were further defined as a cluster. Population composition estimates the log probability of the data for each K value (number of populations) and it was analysed using the Structure version 2.3.4 [15].

This study was approved by the Ethics Committee of Hospital Gregorio Marañón (CEIm; study no. MICRO.HGUGM.2020-010).

Results

Ibrexafungerp showed good *in vitro* activity against the tested isolates ($n = 144$), regardless of their source, with all MICs within a range of four twofold dilution concentrations (from 0.03 mg/L to 0.25 mg/L) (Table 1). Ibrexafungerp MICs of all fluconazole non-wild-type isolates ranged between 0.03 mg/L and 0.125 mg/L. No statistical differences (p=0.431) from ibrexafungerp mean MICs against fluconazole wild-type isolates (0.059 mg/L) and fluconazole non-wild-type isolates (0.063 mg/L) were detected.

The percentage of fluconazole non-wild-type isolates was higher in vaginal isolates ($n = 17/72$, 23.6%) than in blood isolates ($n = 1/72$, 1.4%) ($p < 0.001$) (Table 1). Regarding vaginal isolates, 17/72 isolates were fluconazole non-wild type (12 isolates were susceptible (MIC of 1 mg/L, 2 mg/L or 4 mg/L) and five resistant). In contrast, only one blood isolate was fluconazole-resistant. All voriconazole, posaconazole or isavuconazole non-wild-type isolates were also fluconazole non-wild type. Interestingly, miconazole clearly separated fluconazole wild-type isolates (miconazole MICs between 0.004 mg/L and 0.125 mg/L) from fluconazole non-wild-type isolates (miconazole MICs between 0.5 mg/L and 2 mg/L).

Table 1
Minimal inhibitory concentration distributions of drugs tested against the studied *Candida albicans* from blood and incident vaginal isolates

Antifungal drug	Sample	MIC distributions (number of isolates for each MIC, in mg/L)																No. of isolates (%)	
		≤0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Resistant	Non-wild type
Amphotericin B	Vaginal	—	—	0	0	0	0	3	42	26	1	0	**0**	—	—	—	—	—	0 (0)	0 (0)
	Blood	—	—	0	0	0	0	6	32	26	8	0	**0**	—	—	—	—	—	0 (0)	0 (0)
Fluconazole	Vaginal	—	—	—	—	0	0	16	35	2	2	8	3	1	**4**	**1**	**0**	**0**	5 (7)	17 (23.6)
	Blood	—	—	—	—	0	0	10	29	28	4	0	0	0	**0**	**0**	**0**	**1**	1 (1.4)	1 (1.4)
Voriconazole	Vaginal	0	4	44	5	0	2	8	2	5	**1**	**0**	**1**	**0**	**0**	—	—	—	2 (2.8)	17 (23.6)
	Blood	0	7	47	13	4	0	0	0	0	**0**	**0**	**0**	**0**	**1**	—	—	—	1 (1.4)	1 (1.4)
Posaconazole	Vaginal	0	0	1	7	28	31	2	**3**	**0**	**0**	**0**	**0**	**0**	**0**	—	—	—	3 (4.2)	3 (4.2)
	Blood	1	0	7	38	25	0	0	**0**	**0**	**0**	**0**	**0**	**0**	**1**	—	—	—	1 (1.4)	1 (1.4)
Isavuconazole	Vaginal	1	24	29	3	4	7	3	1	0	0	0	0	0	0	—	—	—	NA	4 (5.6)
	Blood	8	42	16	4	1	0	0	0	0	0	0	0	0	1	—	—	—	NA	1 (1.4)
Micafungin	Vaginal	2	3	28	33	6	**0**	**0**	**0**	**0**	**0**	—	—	—	—	—	—	—	0 (0)	0 (0)
	Blood	3	12	43	6	8	**0**	**0**	**0**	**0**	**0**	—	—	—	—	—	—	—	0 (0)	0 (0)
Anidulafungin	Vaginal	0	17	45	10	0	0	**0**	**0**	**0**	**0**	—	—	—	—	—	—	—	0 (0)	0 (0)
	Blood	1	15	44	6	6	0	**0**	**0**	**0**	**0**	—	—	—	—	—	—	—	0 (0)	0 (0)
Ibrexafungerp	Vaginal	—	—	—	—	0	3	53	13	3	0	0	0	0	0	—	—	—	NA	NA
	Blood	—	—	—	—	0	26	40	6	0	0	0	0	0	0	—	—	—	NA	NA
Clotrimazole	Vaginal	—	—	3	37	14	6	7	1	4	0	0	0	—	—	—	—	—	NA	NA
	ATCC 6258	—	—	0	0	1	4	10	0	0	0	0	0	—	—	—	—	—	NA	NA
	ATCC 22019	—	—	0	0	0	1	14	0	0	0	0	0	—	—	—	—	—	NA	NA
Miconazole	Vaginal	—	—	1	5	32	14	2	1	0	10	3	4	—	—	—	—	—	NA	NA
	ATCC 6258	—	—	0	0	0	0	0	0	1	3	8	3	—	—	—	—	—	NA	NA
	ATCC 22019	—	—	0	0	0	0	0	0	1	14	0	0	—	—	—	—	—	NA	NA

Hyphens indicate untested drug concentrations. Underlined values indicate non-wild-type isolates according to tentative ECOFFs; values in bold indicate resistant isolates according to EUCAST breakpoints table v 10.0 [11]. MIC, minimal inhibitory concentration; NA, not applicable due to the absence of clinical breakpoints or ECOFFs.

The *Erg11* gene from fluconazole non-wild type vaginal isolates ($n = 17$) and a blood isolate ($n = 1$) was sequenced. Owing to the low number of fluconazole non-wild-type isolates from blood, two available blood isolates (fluconazole MIC of 1 mg/L) collected outside the study period were also analysed. Thus, 18 fluconazole wild-type isolates were used as controls. Several amino acid substitutions were only found in fluconazole wild-type isolates, most of them in heterozygosis. Four combinations of amino acid substitutions in homozygosis (A114S/Y257H; T123I/Y132H; Y132H/G450E; D116E/K128T/Y132H/G465S) were detected exclusively in fluconazole non-wild-type isolates (Fig. S1). No resistance to either amphotericin B or echinocandin was found.

One hundred and thirty-three genotypes were found (144 isolates and two fluconazole non-wild-type blood isolates from outside the study period). Some degree of genotype compartmentalization in blood and vagina was found given that there was only one cluster of isolates from both compartments. Genotyping and population structure analysis suggested two different genotypes populations: one with 83 isolates involving mostly blood isolates (66.3%) and another with 63 isolates involving mostly vaginal isolates (69.8%), including all fluconazole non-wild-type isolates (Fig. S2). Ibrexafungerp was uniformly active against all identified genotypes, including fluconazole non-wild-type genotypes.

Discussion

Azole resistance has prompted the development of new drugs for the treatment of patients with vulvovaginitis, being ibrexafungerp one of the most promising ones. Our results strengthen previous studies reporting good *in vitro* activity of ibrexafungerp (modal MIC 0.06 mg/L) against both azole-susceptible and azole-resistant *C. albicans* strains [7,11].

The percentage of fluconazole non-wild-type isolates found in blood isolates is lower than in vaginal isolates (1.4% vs. 23.6%). We confirm the presence of some homozygous mutations only in fluconazole non-wild-type isolates and heterozygous mutations in wild-type isolates. Loss of heterozygosity is a second step in azoles resistance mechanisms [3].

Although a neat compartmentalization between vaginal and blood isolates is not seen, we observe one population involving isolates from vagina that includes all identified fluconazole non-wild-type isolates. It is unknown if the genotypes in that population are prone to develop azole resistance when isolates are exposed to azoles. We only found one cluster (CA-071) involving isolates from blood and vaginal samples; that genotype had been previously reported to cause candidaemia in different countries [14]. Our study is limited by the fact that we were unable to collect clinical information from patients, including the use of azoles prior to their participation in the study.

In conclusion, ibrexafungerp is a promising agent for the treatment of vulvovaginal candidiasis with good *in vitro* activity against *C. albicans* isolates from vaginal and blood samples including fluconazole non-wild-type isolates. Furthermore, we found a population structure in which some genotypes show reduced phenotypical fluconazole susceptibility.

Transparency declaration

Dr. Guinea reports grants from Basilea Ltd, grants from, Scynexis, grants from F2G, personal fees from Pfizer, personal fees from Gilead, personal fees from United Medical, outside the submitted work. The remaining authors have nothing to declare.

This study was supported by grants PI16/01012, PI18/01155, and PI19/00074 from Fondo de Investigación Sanitaria (FIS. Instituto de Salud Carlos III. Plan Nacional de I+D+I 2013-2016). The study was co-funded by the European Regional Development Fund (FEDER) 'A way of making Europe.' This study was partially funded by SCYNEXIS Inc., Jersey City, NJ, USA. The funders had no role in the study design, data collection, analysis, decision to publish, or preparation/content of the manuscript. P.E. (CPI15/00115) is a recipient of a Miguel Servet contract supported by the FIS. J.G. is a steady researcher contracted by Fundación para Investigación Sanitaria del Hospital Gregorio Marañón. A.M. is supported by a grant from the Ministerio de Ciencia, Innovación y Universidades (PEJ2018-004609-A) and from Fondo de Investigación Sanitaria (FI20/00089).

Author contributions

Aina Mesquida: methodology; formal analysis; writing – original draft preparation and review and editing, visualization. Teresa Vicente, Elena Reigadas, María Palomo, Carlos Sánchez-Carrillo: data collection; resources (samples); writing – review and editing. Patricia Muñoz: data collection; writing – review and editing. Pilar Escribano, Jesús Guinea: conceptualization; project administration; data collection; supervision; validation; visualization; writing – original draft preparation and review and editing.

Acknowledgements

The authors are grateful to Dainora Jaloveckas for editing assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.02.006.

References

[1] Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the infectious diseases society of America. Clin Infect Dis 2016;62:e1–50.

[2] Brandolt TM, Klafke GB, Goncalves CV, Bitencourt LR, Martinez AM, Mendes JF, et al. Prevalence of *Candida* spp. in cervical-vaginal samples and the *in vitro* susceptibility of isolates. Braz J Microbiol 2017;48:145–50.

[3] Wu Y, Li C, Wang Z, Gao J, Tang Z, Chen H, et al. Clonal spread and azole-resistant mechanisms of non-susceptible *Candida albicans* isolates from vulvovaginal candidiasis patients in three Shanghai maternity hospitals. Med Mycol 2018;56:687–94.

[4] Pfaller MA, Messer SA, Rhomberg PR, Borroto-Esoda K, Castanheira M. Differential Activity of the oral glucan synthase inhibitor scy-078 against wild-type and echinocandin-resistant strains of *Candida* Species. Antimicrob Agents Chemother 2017;61. e00161–17.

[5] Davis MR, Donnelley MA, Thompson GR. Ibrexafungerp: a novel oral glucan synthase inhibitor. Med Mycol 2020;58:579–92.

[6] Arendrup MC, Jorgensen KM, Hare RK, Chowdhary A. In Vitro Activity of Ibrexafungerp (SCY-078) against *Candida auris* Isolates as determined by EUCAST methodology and comparison with activity against *C. albicans* and *C. glabrata* and with the activities of six comparator agents. Antimicrob Agents Chemother 2020;64. e02136–19.

[7] Marcos-Zambrano LJ, Gomez-Perosanz M, Escribano P, Bouza E, Guinea J. The novel oral glucan synthase inhibitor SCY-078 shows in vitro activity against sessile and planktonic *Candida* spp. J Antimicrob Chemother 2017;72:1969–76.

[8] Larkin EL, Long L, Isham N, Borroto-Esoda K, Barat S, Angulo D, et al. A novel 1,3-beta-d-glucan inhibitor, ibrexafungerp (Formerly SCY-078), shows potent activity in the lower pH environment of vulvovaginitis. Antimicrob Agents Chemother 2019;63:e02611–8.

[9] Azie N, Angulo D, Dehn B, Sobel JD. Oral Ibrexafungerp: an investigational agent for the treatment of vulvovaginal candidiasis. Expert Opin Invest Drugs 2020;29:893–900.

[10] Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope W. EUCAST-AFST EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). Clin Microbiol Infect 2012;18:E246–7.

[11] Arendrup MC, Friberg N, Mares M, Kahlmeter G, Meletiadis J, Guinea J. How to interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European committee on antimicrobial susceptibility testing (EUCAST). Clin Microbiol Infect 2020;26:1464–72.

[12] Howard SJ, Lass-Florl C, Cuenca-Estrella M, Gomez-Lopez A, Arendrup MC. Determination of isavuconazole susceptibility of *Aspergillus* and *Candida* species by the EUCAST method. Antimicrob Agents Chemother 2013;57:5426–31.

[13] Liu JY, Shi C, Wang Y, Li WJ, Zhao Y, Xiang MJ. Mechanisms of azole resistance in *Candida albicans* clinical isolates from Shanghai, China. Res Microbiol 2015;166:153–61.

[14] Guinea J, Arendrup MC, Canton R, Canton E, Garcia-Rodriguez J, Gomez A, et al. Genotyping reveals high clonal diversity and widespread genotypes of *Candida* Causing Candidemia at distant geographical areas. Front Cell Infect Microbiol 2020;10:166.

[15] Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000;155:945–59.