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Brief report

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Postantifungal effect of anidulafungin against *Candida albicans*, *Candida dubliniensis*, *Candida africana*, *Candida parapsilosis*, *Candida metapsilosis* and *Candida orthopsilosis*

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ABSTRACT

Objectives. *Candida albicans* remains the most common aetiology of invasive candidiasis, leading to high morbidity and mortality. Nevertheless, the incidence of candidiasis due to non-*C. albicans* species, such as *Candida parapsilosis*, is increasing. Postantifungal effect (PAFE) is relevant for establishing dosage schedules in antifungal therapy, as the frequency of antifungal administration could change depending on PAFE. The aim of this study was to evaluate the PAFE of anidulafungin against *C. albicans*, *Candida dubliniensis*, *Candida africana*, *C. parapsilosis*, *Candida metapsilosis* and *Candida orthopsilosis*.

Material and methods. Twenty-one *Candida* strains were evaluated. Cells were exposed to anidulafungin for 1 h at concentrations ranging from 0.12 to 8 mg/L for PAFE studies. Time-kill experiments (TK) were conducted at the same concentrations. The experiments were performed using an inoculum of 1-5 x 10^5 cells/mL and 48 h incubation. Readings of PAFE and TK were done at 0, 2, 4, 6, 24 and 48 h.

Results. Anidulafungin was fungicidal against 2 out of 14 (14%) strains of *C. albicans* related species in PAFE experiments. Moreover, 2 mg/L of anidulafungin exerted a prolonged PAFE (≥ 33.6 h) against 13 out of 14 (93%) strains. Similarly, fungicidal endpoint was achieved against 1 out of 7 (14%) strains of *C. parapsilosis* complex, being PAFE prolonged (≥ 42 h) against 6 out of 7 (86%) strains.

Conclusions. Anidulafungin induced a significant and prolonged PAFE against *C. albicans* and *C. parapsilosis* and their related species.

Keywords: Postantifungal effect; anidulafungin; *Candida*

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Efecto postantifúngico de anidulafungina contra *Candida albicans, Candida dubliniensis, Candida africana, Candida parapsilosis, Candida metapsilosis* y *Candida orthopsilosis*

RESUMEN

Objetivos. *Candida albicans* continúa siendo la causa más frecuente de candidiasis invasiva; sin embargo, la incidencia de candidiasis causadas por especies diferentes a *C. albicans*, como *Candida parapsilosis*, está aumentando. El efecto postantifúngico (PAFE) es relevante para establecer pautas de dosificación en la terapia antifúngica, ya que la frecuencia de administración de los fármacos antifúngicos podría cambiar dependiendo del PAFE. El objetivo de este estudio fue evaluar el PAFE de anidulafungina contra *C. albicans*, *Candida dubliniensis*, *Candida africana*, *C. parapsilosis*, *Candida metapsilosis* y *Candida orthopsilosis*.

Material y métodos. Se evaluaron 21 cepas de *Candida*. Para llevar a cabo los estudios PAFE, las células se expusieron durante 1 h a concentraciones entre 0,12 y 8 mg/L de anidulafungina. Las curvas de letalidad (TK) se obtuvieron empleando las mismas concentraciones. Los experimentos se realizaron utilizando un inóculo de 1-5 x 10^5 células/mL, durante 48 h de incubación. Las lecturas de PAFE y TK se realizaron a las 0, 2, 4, 6, 24 y 48 h.

Resultados. Anidulafungina, en los experimentos PAFE, fue fungicida contra 2 de 14 (14%) cepas de las especies relacionadas con *C. albicans* y ejerció un PAFE prolongado (≥ 33,6 h) contra 13 de 14 (93%) cepas (2 mg/L). El límite fungicida de anidulafungina se alcanzó contra 1 de 7 (14%) cepas del complejo *C. parapsilosis*, con un PAFE prolongado (≥ 42 h) contra 6 de 7 (86%) cepas.

Conclusiones. Anidulafungina produce un PAFE significativo y prolongado contra *C. albicans* y *C. parapsilosis* y las especies relacionadas con estas.

Palabras clave: Efecto postantifúngico; anidulafungina; *Candida*

INTRODUCTION

Invasive candidiasis is a significant cause of morbidity and mortality, especially among patients suffering from severe immunodeficiency. Although *Candida albicans* remains the most common aetiology, the incidence of candidiasis due to non-*C. albicans* species is increasing. Most *Candida* bloodstream infections are caused by *C. albicans*, *Candida parapsilosis*, *Candida glabrata, Candida tropicalis*, and *Candida krusei* [1,2]. Moreover, *C. albicans* and *C. parapsilosis* have close-related species, such as *Candida dubliniensis* and *Candida africana* (*C. albicans* related species) or *Candida orthopsilosis* and *Candida metapsilosis* (*C. parapsilosis* complex). Differences and variability in the prevalence and antifungal susceptibility of these species have been reported [3,4].

Postantifungal effect (PAFE) describes how long an antifungal drug continues acting after it has been removed. This effect depends on both the fungal species and antifungal drug, and it may be relevant for antifungal therapy, having clinical relevance for establishing dosage schedules. Echinocandins and amphotericin B (fungicidal drugs) exert prolonged PAFE against *C. albicans* while triazoles (fungistatic drugs) possess shorter PAFE. Theoretically, antifungal drugs with long PAFE will require less frequent administration than those with shorter PAFEs [5,6]. The aim of this study has been to evaluate the PAFE of clinically relevant concentrations of anidulafungin against *C. albicans* and *C. parapsilosis* related species.

MATERIAL AND METHODS

Microorganisms. Twenty-one *Candida* clinical isolates and culture collection strains were studied (table 1). The clinical isolates were identified as previously described [7,8].

***In vitro* susceptibility testing.** Anidulafungin (Pfizer SLU, Spain) was dissolved in dimethyl sulfoxide. Further dilutions were done in standard RPMI 1640 medium (Sigma-Aldrich, Spain). Minimum concentrations that produce ≥ 50% growth reductions (MICs) after 24 h of incubation were determined according to the M27-A3, M27-S4 and M60 documents [9,10].

Time-kill procedures. Time-kill studies (TK) were performed out in microtiter plates in a computer-controlled microbiological incubator (BioScreen C MBR, LabSystems, Finland) in RPMI (200 µl) using an inoculum of 1-5 x 10^5 cells/ml, as previously described [11]. The concentrations assayed were 0.125, 0.5 and 2 mg/L for *C. albicans* related species, and 0.25, 2 and 8 mg/L for *C. parapsilosis* complex. Aliquots were removed from each well at 0, 2, 4, 6, 24 and 48 h, after dilution in phosphate buffered saline (PBS), the samples were inoculated onto Sabouraud dextrose agar (SDA) plates. Colonies were counted after incubation of the plates (36 ± 1 ˚C) for 48 h. All experiments were performed in duplicate. The limit of quantification was 20 colony forming units (CFU).

Postantifungal effect. PAFE was evaluated as previously described [12-14]. After an incubation period of 1 h, anidulafungin was removed by serial washing in PBS and centrifuged at 2000 rpm x 10 min. Tested concentrations, incubations, sample collection times and inoculations onto SDA plates were the same as described for TK assay. PAFE was calculated according to the equation PAFE= T - C (T: time required to increase by 1 log the counts in treated culture; C: time required to increase by 1 log the counts following the last washing) [15].

Fungicidal activity was defined as a growth reduction ≥ 3 log (99.9%), and fungistatic activity, as a reduction < 3 log (< 99.9%) in CFU from the starting inoculum. The ratios of the log killing during PAFE assays to the log killing during TK assays were also calculated [16].

Statistical analysis. The differences in PAFEs among the anidulafungin different concentrations and species were evaluated by ANOVA (GraphPad Software, USA). A P value < 0.05 was considered significant.

Table 1 **Anidulafungin MICs against strains from species related with *C. albicans* and *C. parapsilosis***

Isolate	Origin	MIC (mg/L)
Candida albicans NCPF 3153	Reference	0.03
Candida albicans NCPF 3156	Reference	0.03
Candida albicans UPV/EHU 99-101	Blood	0.06
Candida albicans UPV/EHU 99-102	Blood	0.03
Candida albicans UPV/EHU 99-103	Blood	0.03
Candida albicans UPV/EHU 99-104	Blood	0.06
Candida albicans UPV/EHU 99-105	Blood	0.06
Candida dubliniensis NCPF 3949	Reference	0.06
Candida dubliniensis UPV/EHU 00-131	Blood	0.06
Candida dubliniensis UPV/EHU 00-132	Blood	0.06
Candida dubliniensis UPV/EHU 00-133	Blood	0.03
Candida dubliniensis UPV/EHU 00-135	Blood	0.03
Candida africana UPV/EHU 97-135	Vaginal	0.03
Candida africana ATCC 2669	Reference	0.06
Candida parapsilosis ATCC 22019	Reference	1
Candida parapsilosis ATCC 90018	Reference	2
Candida parapsilosis UPV/EHU 09-378	Blood	2
Candida metapsilosis ATCC 96143	Reference	1
Candida metapsilosis UPV/EHU 07-045	Blood	1
Candida orthopsilosis ATCC 96139	Reference	1
Candida orthopsilosis UPV/EHU 07-035	Blood	1

RESULTS

Anidulafungin MICs are summarized in table 1. Anidulafungin (2 mg/L) exhibited a prolonged and significant

Table 2 **Reductions in starting inocula of *Candida* during TK and PAFE experiments and PAFE in hours against fourteen strains of species related with *C. albicans***

Isolate	AND (mg/L)	Killing (log)		PAFE/TK killing[a]	PAFE (h)
		TK	PAFE		
Candida albicans NCPF 3153	0.12	0.27	0.2	85.11	2
	0.5	0.39	0.01	41.69	20
	2	0.05	0.66	100	> 44
Candida albicans NCPF 3156	0.12	1.35	0.15	6.31	0
	0.5	≥ 4	0.04	0.01	0
	2	≥ 4	2.05	1.12	> 42
Candida albicans UPV/EHU 99-101	0.12	2.7	0.36	0.46	0
	0.5	≥ 4	0.51	0.03	0
	2	≥ 4	≥ 4	100	> 43
Candida albicans UPV/EHU 99-102	0.12	0.92	0.30	24	3.2
	0.5	2.02	1.6	38.02	> 39.1
	2	≥ 4	1.3	0.19	> 39.1
Candida albicans UPV/EHU 99-103	0.12	NA[b]	0.1		> 43
	0.5	NA	0.05		19
	2	2.03	1.24	16.22	> 43
Candida albicans UPV/EHU 99-104	0.12	NA	0.35		> 42
	0.5	NA	0.44		> 42
	2	1.1	0.41	20.42	> 42
Candida albicans UPV/EHU 99-105	0.12	≥ 4	0.42	0.02	0
	0.5	≥ 4	0.5	0.03	0
	2	≥ 4	1.82	0.66	> 42
Candida dubliniensis NCPF 3949	0.12	NA	NA		0
	0.5	0.04	0.04	100	0
	2	0.57	0.92	100	> 42
Candida dubliniensis UPV/EHU 00-131	0.12	NA	NA		0
	0.5	NA	NA		2
	2	0.54	1.16	100	> 44
Candida dubliniensis UPV/EHU 00-132	0.12	NA	NA		0
	0.5	NA	0.08		0
	2	0.53	0.03	31.62	> 42
Candida dubliniensis UPV/EHU 00-133	0.12	NA	0.13		0
	0.5	0.39	0.39	100	18
	2	0.72	1.03	100	18
Candida dubliniensis UPV/EHU 00-135	0.12	≥ 4	NA		0
	0.5	≥ 4	≥ 4	100	> 42
	2	≥ 4	1.32	0.21	> 42
Candida africana ATCC 2669	0.12	NA	0.23		2.8
	0.5	0.12	0.25	100	> 37.7
	2	0.15	0.4	100	> 37.7
Candida africana UPV/EHU 97-135	0.12	0.02	0.3	100	0.7
	0.5	0.36	0.34	95.5	2
	2	0.6	0.42	66.07	> 33.6

AND, anidulafungin; TK, time-kill; PAFE, postantifungal effect. [a]Ratio of the log killing during PAFE experiments to the log killing during TK experiments. [b]NA, not applicable (without any reduction in colony counts compared with the starting inoculum)

PAFE (≥ 33.6 h) against most strains of *C. albicans* related species (13 out of 14, 93%) (table 2). Besides, prolonged PAFE (> 37.7 h) with ≤ 0.5 mg/L of anidulafungin was observed against 5 out 14 (36%) of these strains. In TK experiments, anidulafungin (2 mg/L) was fungicidal against 5 out of 14 (36%) strains of *C. albicans* related species. Fungicidal endpoint was achieved against 2 out of 14 (14%) strains of *C. albicans* related species in PAFE experiments (strains *C. albicans* UPV/EHU 99-101 and *C. dubliniensis* UPV/EHU 00-135). This fungicidal effect was even achieved when 0.5 mg/L of anidulafungin was tested against strain *C. dubliniensis* UPV/EHU 00-135. The mean value of PAFE/TK ratio was 52.61 (2 mg/L) for *C. albicans* related species. Although there were no significant differences between the PAFE against *C. albicans*, *C. dubliniensis* and *C. africana*, it could be observed that anidulafungin presented slightly higher PAFE than against *C. dubliniensis* or *C. africana* (table 2).

A significant and prolonged PAFE (≥ 42 h) against 6 out of 7 (86%) strains of *C. parapsilosis* complex was observed with 8 mg/L of anidulafungin ($P < 0.05$) (table 3), but fungicidal endpoint was achieved only against *C. metapsilosis* UPV/EHU 07-045. This concentration was fungicidal against 6 out 7 (86%) strains from the *C. parapsilosis* complex and fungistatic against 1 *C. orthopsilosis*, in TK experiments. The mean value of PAFE/TK ratio was 15.48 (8 mg/L) for *C. parapsilosis* complex. There were no significant differences between the PAFE of anidulafungin against *C. parapsilosis*, *C. metapsilosis* and *C. orthopsilosis* (table 3).

Mean anidulafungin PAFE against *C. albicans* related species (39.6 ± 26.81 h) (2 mg/L) did not differ from that one against *C. parapsilosis* complex (37.6 ± 14.32 h) (8 mg/L) (figure 1 and 2).

DISCUSSION

C. albicans and *C. parapsilosis* are the most frequent aetiological agents of invasive candidiasis in Spain and in many Mediterranean and Latin-American countries [1]. *C. orthopsilosis* and *C. dubliniensis* represent relatively frequent aetiolog-

Table 3 **Reductions in starting inocula of *Candida* isolates during TK and PAFE experiments and PAFE in hours against seven strains of species related with *C. parapsilosis***

Isolate	AND (mg/L)	Killing (log)		PAFE/TK killing[a]	PAFE (h)
		TK	PAFE		
Candida parapsilosis ATCC 22019	0.25	NA[b]	NA		0
	2	0.88	0.11	16.98	0
	8	≥ 4	0.36	0.02	42
Candida parapsilosis ATCC 90018	0.25	NA	NA		0
	2	NA	NA		3.6
	8	≥ 4	0.18	0.02	42
Candida parapsilosis UPV/EHU 09-378	0.25	0.15	NA		0
	2	0.08	0.19	100	5.7
	8	≥ 4	NA		5.2
Candida metapsilosis ATCC 96143	0.25	NA	NA		0
	2	≥ 4	NA		0
	8	≥ 4	2.36	2.29	> 44
Candida metapsilosis UPV/EHU 07-045	0.25	NA	NA		0
	2	1.12	NA		0
	8	≥ 4	≥ 4	100	> 44
Candida orthopsilosis ATCC 96139	0.25	NA	NA		0
	2	3.05	NA		2
	8	≥ 4	2.67	4.68	> 44
Candida orthopsilosis UPV/EHU 07-035	0.25	NA	NA		0
	2	1.73	NA		0
	8	2.06	0.19	1.35	42

AND, anidulafungin; TK, time-kill; PAFE, postantifungal effect. [a]Ratio of the log killing during PAFE experiments to the log killing during TK experiments. [b]NA, not applicable (without any reduction in colony counts compared with the starting inoculum)

ical agents of invasive candidiasis, being in some institutions more prevalent than *C. krusei* [17]. To our knowledge, this is the first study that shows and compares the PAFE of anidulafungin against the emerging species *C. dubliniensis*, *C. africana*, *C. metapsilosis* and *C. orthopsilosis*. PAFE and TK experiments of anidulafungin against *C. albicans* and *C. parapsilosis* have not been widely evaluated and most studies included low numbers of isolates [5,12,15]. Moreover, this study provides a comparison among the in vitro activities of anidulafungin, caspofungin and micafungin [13,14].

Anidulafungin MICs were consistent with those reported in previous studies [8,18]. In the current study, anidulafungin exerted good fungicidal activity against most strains of *C. parapsilosis* complex but this activity was lower against *C. albicans* related species (with 8 mg/L and 2 mg/L, respectively). This discrepancy in the activity of the echinocandins against different species of *Candida* has been reported previously [11,12,16,19]; anidulafungin is considered fungicide against *Candida* but does not achieve this effect against all isolates. This effect depends on isolate, species, antifungal concentration and test conditions [5,11,19]. Similarly, Clancy et al. reported prolonged PAFE of caspofungin against *C. albicans*, *C. parapsilosis* and *C. glabrata*, but fungicidal activity was not observed in TK or PAFE experiments [16]. For this reason, it would be advisable to perform in vitro susceptibly testing, such as TK and PAFE studies, since killing curves are tools that provide much information about the antifungal activity.

Smith et al. [15] described fungicidal activity of anidulafungin in TK and in PAFE against *C. glabrata* and *C. parapsilosis* at similar concentrations. Moreover, Nguyen et al. [12] evaluated the anidulafungin PAFE against several *Candida* species, reporting fungicidal PAFE against the former species. However, we only observed fungicidal activity against *C. parapsilosis* in TK experiments, except for one strain of *C. metapsilosis* with fungicidal PAFE.

In the current study, there were not significant differences in anidulafungin activity among the species related to *C. albicans* and *C. parapsilosis*. However, we have observed previously statistically significant differences between species related to against *C. albicans* and *C. parapsilosis* in the duration of micafungin and caspofungin PAFEs, which were longer against *C. albicans* related species than against *C. parapsilosis* complex [13,14]. Anidulafungin is the echinocandin with greater PAFE against *C. parapsilosis*. Conversely, micafungin was the echinocandin that displayed the lowest PAFE against the *C. parapsilosis* complex. However, all echinocandins showed a similar PAFE against the *C. albicans* related species, with a slightly but non-significant higher PAFE with micafungin [13,14]. In PAFE experiments anidulafungin (≤ 2 mg/L) and micafungin (2 mg/L) achieved the fungicidal endpoint against 2 out 14 (14%) strains of *C. albicans* related species (*C. albicans* UPV/EHU 99-101, *C. dubliniensis* UPV/EHU 00-135 and *C. albicans* UPV/EHU 99-102, and *C. dubliniensis* UPV/EHU 00-135, respectively). Caspofungin (2 mg/L) only achieved this endpoint against 1 out of 14 (7%) strains (*C. albicans* UPV/EHU 99-101). Only anidulafungin (8 mg/L) displayed a fungicidal PAFE against the *C. parapsilosis* complex (1 out 7, 14% strains, *C. metapsilosis* UPV/EHU 07-045) [13,14]. Fungal growth characteristics or binding affinities of each drug could be possible explanations for PAFE differences [12]. PAFE may have clinical relevance to the design of dosing regimens for antifungal agents, as those antifungal drugs with longer PAFEs may be administered less frequently than those ones with shorter PAFEs [5,20].

In conclusion, anidulafungin showed a significant and

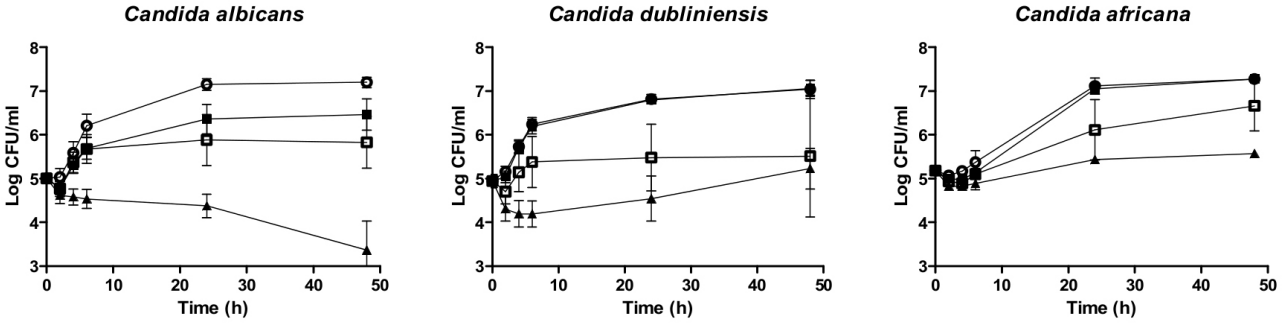

Figure 1 Mean time-kill curves from the PAFE assays against seven *C. albicans*, five *C. dubliniensis* and two *C. africana* strains. Each point represents the mean count ± standard deviation (error bars). Open circles (○): control; filled squares (■): 0.12 mg/L anidulafungin; open squares (□): 0.5 mg/L anidulafungin; and filled triangles (▲): 2 mg/L anidulafungin

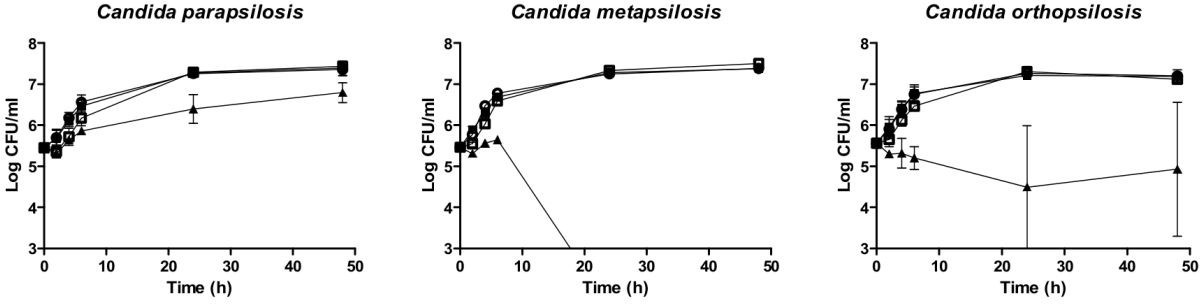

Figure 2 Mean time-kill curves from the PAFE assays against three *C. parapsilosis*, two *C. metapsilosis* and two *C. orthopsilosis* strains. Each point represents the mean count ± standard deviation (error bars). Open circles (○): control; filled squares (■): 0.25 mg/L anidulafungin; open squares (□): 2 mg/L anidulafungin; and filled triangles (▲): 8 mg/L anidulafungin

prolonged PAFE against the species closely related to *C. albicans* and *C. parapsilosis*, being the echinocandin with greater PAFE against *C. parapsilosis* complex. Although the clinical implications of in vitro killing and PAFE need further research, the current findings represent an initial step towards improving dosage regimen in clinical setting.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest related to the current manuscript, but declare the following: G.Q. has received research grants from Astellas Pharma, Pfizer, Merck Sharp & Dohme, and Scynexis. G.Q. has served on advisory/consultant boards for Merck, Sharp & Dohme, and Scynexis, and he has received speaker honoraria from Abbvie, Astellas Pharma, Merck Sharp & Dohme, Pfizer, and Scynexis.

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