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Pre-clinical Development of Susceptibility Testing Methods for the Novel Antifungal E1210 Tested against *Candida*: Comparison of CLSI and EUCAST Methods

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Abstract

Background: E1210, a first-in-class broad-spectrum antifungal agent, suppresses hyphal growth by inhibiting fungal glycophosphatidylinositol biosynthesis. Initial studies using CLSI broth microdilution (BMD) demonstrated excellent activities for E1210 against *Candida* spp.; however, specific parameters such as duration of incubation, MIC endpoint (EP) determination, and the level of agreement between the CLSI and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) BMD methods must be investigated.

Methods: 102 *Candida* clinical isolates were tested by CLSI and EUCAST methods: 21 *C. albicans* (CA), 20 *C. glabrata* (CG), 25 *C. parapsilosis* (CP), 24 *C. tropicalis* (CT), and 12 *C. krusei* (CK), including echinocandin- and azole-resistant isolates. CLSI MIC EPs of 50% and 100% inhibition were determined using visual reading at 24- and 48-h incubation. EUCAST MICs were read spectrophotometrically at 24-h incubation and at 50% and 100% inhibition.

Results: E1210 CLSI MIC results ranged from 0.008 to only 1 µg/mL depending on the species, the duration of incubation and EP criteria (C). E1210 was not active against CK (MIC_{50},>16 µg/ml). Overall essential agreement (EA; ± 2 doubling dilutions) between the 24- and 48-h CLSI readings was 100 and 97.6%, using the 50% and 100% inhibition EPC, respectively. Slightly more trailing growth at 48-h was observed with the 100% inhibition EPC. Comparison of the 50% and 100% of EPC at 24-h incubation showed an overall EA of 100%. Comparison of CLSI and EUCAST read at 24-h incubation and either 50% or 100% inhibition revealed an EA of 97.8% using the 50% inhibition EPC and 88.9% using the 100% inhibition EPC.

Conclusions: E1210 had potent in vitro activity against *Candida* spp. when tested by CLSI and EUCAST methods. Optimal conditions for both methods included 24-h incubation and 50% inhibition MIC EPC. When these conditions were employed for E1210 testing, one can achieve a high level of intermethod agreement between the two standardized test results.

Introduction

E1210 (Eisai Co., Japan) is a first-in-class broad-spectrum antifungal agent that suppresses hyphal growth by inhibiting fungal glycophosphatidylinositol (GPI) biosynthesis. Preliminary data using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution (BMD) method has demonstrated the excellent potency and spectrum of E1210 against *Candida* spp.; however, specific methodological parameters such as the duration of incubation, MIC endpoint determination, and the level of agreement between the CLSI and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) BMD methods must be investigated.

Given the important role that both international susceptibility testing methods currently play in antifungal resistance surveillance, it is important to demonstrate the comparability of the results in the pre-clinical studies of new antifungal agents. In the present study, we have used a panel of *Candida* spp. isolates, selected to represent phenotypically and genotypically antifungal-resistant strains, to initially examine the effect of incubation time and MIC endpoint criterion on the results of the CLSI method and subsequently to determine the essential agreement (EA; MIC ± two log_2 dilutions) between CLSI and EUCAST BMD results.

Methods

Organisms. A total of 102 clinical isolates of *Candida* spp. were tested including 21 isolates of *C. albicans,* 20 of *C. glabrata*, 25 of *C. parapsilosis*, 24 of *C. tropicalis*, and 12 of *C. krusei*. The collection consisted of 20 fluconazole-resistant strains (8 *C. albicans*, 1 *C. glabrata*, 5 *C. parapsilosis* and 6 *C. tropicalis* [supplied in part by D.J. Diekema, University of Iowa, Iowa City, Iowa]) and 15 caspofungin-resistant strains (5 each of *C. albicans*, *C. glabrata,* and *C. tropicalis* [kindly supplied by D.S. Perlin, Public Health Research Institute, New Jersey Medical School – UMDNJ]). All wild-type strains were from the SENTRY Antimicrobial Surveillance Program collection. Prior to testing, each isolate was passaged at least twice onto Sabouraud dextrose agar (Remel Lenexa, Kansas, USA) and CHROMagar[TM] *Candida* medium (Becton Dickinson and Company, Sparks, Maryland, USA) to ensure purity and viability.

Antifungal susceptibility. All isolates were tested for E1210 and fluconazole by BMD using CLSI guidelines exactly as outlined in document M27-A3 and EUCAST according to the document EDef. 7.1. Reference powder of E1210 was obtained from the manufacturer. Stock solutions were prepared in dimethyl sulfoxide and the final range of E1210 concentrations tested was 0.008-16 µg/mL. CLSI MIC endpoint criteria was determined at 24- and 48-h and included the lowest concentration of drug that caused a significant diminution (≥50% inhibition) as well as complete (100%) inhibition of growth relative to that of the growth control. EUCAST MIC values were determined spectrophotometrically (at 450 nm), after 24-h incubation, as the lowest concentration of drug that resulted in both ≥50% inhibition and 100% inhibition of growth relative to that of the growth control.

Quality control (QC) was ensured by testing *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019. Fluconazole was included in each MIC panel for QC purposes. All fluconazole results were within the acceptable QC limits for both methods. E1210 MIC values for *C. krusei* ATCC 6258 were all >16 µg/mL and for *C. parapsilosis* ATCC 22019 were 0.015 or 0.03 µg/mL.

Method agreement analysis. The MIC results for E1210 obtained with the CLSI method using both partial (≥50% inhibition) and complete (100% inhibition) were compared at each reading time in order to determine the EA between MIC values obtained at 24- and 48-h with each endpoint criterion and subsequently to determine the EA at 24-h between MIC values determined using partial versus complete endpoint criteria. The MIC results for E1210 obtained with the EUCAST method were compared with those of the CLSI method at 24-h incubation using both complete and partial inhibition endpoint criteria for both methods. High off-scale MIC results were converted to the next highest concentration and low off-scale MIC results were left unchanged. Discrepancies of more than ± two log_2 dilutions among MIC results were used to calculate the EA.

Results

- Table 1 summarizes the in vitro susceptibilities of 90 isolates of *Candida* spp. (*C. krusei* [12 strains] was not included) to E1210 determined by the CLSI BMD method. The MIC results determined by the CLSI method ranged from 0.008 to 1 µg/mL depending on the species, duration of incubation, and MIC endpoint criteria.
- E1210 was not active against *C. krusei* ($MIC_{50,}$ ≥16 µg/mL; data not shown).
- Overall, the EA between the 24- and 48-h CLSI readings was 100.0% (76 % within ± 1 doubling dilution) using the partial inhibition (≥50%) endpoint criterion and 97.6% (91% within ± 1 doubling dilution) using the complete (100%) inhibition endpoint criterion.
- Comparison of the MIC results obtained with the CLSI method at 24-h incubation and using the two different MIC endpoints showed an overall EA of 100% (83% within ± 1 doubling dilution; Table 2).
- Comparison of CLSI and EUCAST methods read at 24-h incubation and either partial or complete inhibition revealed an EA of 97.8% using the partial inhibition endpoint criterion and 88.9% using the complete inhibition endpoint criterion (Table 3).
- Among the few discrepancies (≥3 doubling dilutions) noted, the CLSI method tended to produce higher values using the partial inhibition criterion, whereas the EUCAST method tended to give higher values using the complete inhibition endpoint criterion.

Table 1. In vitro susceptibility of *Candida* spp. to E1210 by CLSI broth microdilution methods: assessment by incubation time and MIC endpoint criteria.

Species (no. tested)	Incubation time (h)	% inhibition[a]	No. of isolates at MIC (µg/mL): ≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	%EA[b]
C. albicans (21)	24	50	8	4	6	2	1				100.0
	48	50	4	5	7	4	1				
	24	100	4	3	8	4	2				100.0
	48	100	1	3	9	4	4				
C. glabrata (20)	24	50	4	4	5	6		1			100.0
	48	50	1		5	7	4	3			
	24	100		4	4	5	5	2			95.0
	48	100			4	4	4	6	1	1	
C. parapsilosis (25)	24	50	1	8	10	4	1		1		100.0
	48	50		6	12	4	2		1		
	24	100		4	11	7	2		1		100.0
	48	100		1	10	9	4			1	
C. tropicalis (24)	24	50	6	5	8	4	1				100.0
	48	50		3	13	3	3	2			
	24	100	2	1	8	6	4	3			95.8
	48	100			4	9	4	4	3		

a. % inhibition relative to growth control.
b. %EA, % essential agreement (± two log_2 dilution steps).

Table 2. In vitro susceptibility of *Candida* spp. at 24-h incubation using CLSI broth microdilution methods and partial and complete inhibition MIC endpoint criteria.

Species (no. tested)	Incubation time (h)	% inhibition[a]	No. of isolates at MIC (µg/mL): ≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	%EA[b]
C. albicans (21)	24	50	8	4	6	2	1				100.0
	24	100	4	3	8	4	2				
C. glabrata (20)	24	50	4	4	5	6		1			100.0
	24	100		4	4	5	5	2			
C. parapsilosis (25)	24	50	1	8	10	4	1		1		100.0
	24	100		4	11	7	2		1		
C. tropicalis (24)	24	50	6	5	8	4	1				100.0
	24	100	2	1	8	6	4	3			

a. % inhibition relative to growth control.
b. %EA, % essential agreement (± two log_2 dilution steps).

Table 3. In vitro susceptibilities of *Candida* spp. to E1210 as determined by the 24-h CLSI and EUCAST broth microdilution methods using partial (≥50%) and complete (100%) inhibition MIC endpoint criteria.

Species (no. tested)	Test method	% inhib.[a]	No. of isolates at MIC (µg/mL) ≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	>16	%EA[b]
C. albicans (21)	EUCAST	50	14	2	4	1							95.2
	CLSI	50	8	4	6	2	1						
	EUCAST	100	1	1	9	5	3	2					100.0
	CLSI	100	4	3	8	4	2						
C. glabrata (20)	EUCAST	50		1	6	6	7						95.0
	CLSI	50	4	4	5	6		1					
	EUCAST	100				3	5	6	3		1	2	80.0
	CLSI	100		4	4	5	5	2					
C. parapsilosis (25)	EUCAST	50	10	2	11	1			1				100.0
	CLSI	50	1	8	10	4	1		1				
	EUCAST	100			3	7	8	4	2	1			84.0
	CLSI	100		4	11	7	2		1				
C. tropicalis (24)	EUCAST	50	10	5	5	3	1						95.8
	CLSI	50	6	5	8	4	1						
	EUCAST	100	2		4	7	5	3	3				95.8
	CLSI	100	2	1	8	6	4	3					

a. % inhibition relative to growth control.
b. %EA, % essential agreement (± two log_2 dilution steps).

Conclusions

- These results indicate that E1210 may be tested against *Candida* spp. using either the CLSI or EUCAST BMD method and an incubation time of 24-h.
- Although either partial or complete inhibition MIC endpoints give comparable results, the highest level of inter- and intra-method agreement was observed when the partial inhibition (≥50%) endpoint was used for both methods.
- In summary, E1210 has potent in vitro activity against *Candida* spp. when tested by both reference BMD methods. Optimal testing conditions using CLSI and EUCAST methods include 24-h incubation and ≥50% inhibition MIC endpoint criterion. When these conditions are employed, one may achieve a high-level of intermethod agreement for testing E1210 against *Candida* spp.

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