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In Vitro Activity of a Novel Broad-spectrum Antifungal Agent, E1210, and Comparators Tested against *Candida* spp.

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Abstract

Background: E1210 is a first-in-class broad-spectrum antifungal that suppresses hyphal growth by inhibiting fungal glycophosphatidylinositol (GPI) biosynthesis. We evaluated the activity of E1210 and comparator agents against *Candida* spp., including azole- and echinocandin-resistant strains.

Methods: 90 clinical isolates of *Candida* were tested by CLSI broth microdilution method: 21 *C. albicans* (CA), 20 *C. glabrata* (CG), 25 *C. parapsilosis* (CP), and 24 *C. tropicalis* (CT), comprising 20 fluconazole (FLU)-resistant strains (8 CA, 1 CG, 5 CP, and 6 CT) and 15 caspofungin (CSF)-resistant (R) strains (5 each of CA, CG and CT). CSF-R strains contained mutations in *fks1* or *fks2* hotspots. Comparators included CSF, FLU, posaconazole (PSC) and voriconazole (VRC). MIC results were read after 24-h incubation and 50% growth inhibition.

Results: E1210 was highly active against all species tested and was more potent than all of the comparators. The MIC_{90} (µg/mL) for E1210, CSF, FLU, PSC, and VRC, respectively were as follows by species: CA (0.06, 4, 64, 0.5, 0.5), CG (0.06, 2, 32, 1, 1,), CP (0.06, 4, 16, 0.12, 0.25) and CT (0.06, 4, ≥64, 0.5, 2). E1210 was the most active agent against FLU-R strains of CA (MIC range, 0.015-0.12 µg/mL), CG (0.06 µg/mL), CP (MIC range, 0.06-0.5 µg/mL) and CT (MIC range, 0.008-0.06 µg/mL). E1210 was also the most potent agent against CSF-R strains of CA (MIC range, 0.008-0.12 µg/mL), CG (MIC range, 0.03-0.06 µg/mL) and CT (MIC range, 0.015-0.06 µg/mL).

Conclusions: E1210 is a very potent and broad-spectrum antifungal agent with excellent activity against azole- and echinocandin-R *Candida* spp. Clearly, further in vitro and in vivo studies are warranted for this novel agent.

Species (no. tested)	Agent	Occurrences (cumulative %) at each MIC (µg/mL): ≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	≥1
C. albicans (21)	E1210	8 (38.1)	4 (57.1)	6 (85.7)	2 (95.2)	1 (100.0)			
	VRC[a]	10 (47.6)	2 (57.1)	0 (57.1)	3 (71.4)	1 (76.2)	0 (76.2)	3 (90.5)	2 (100.0)
C. glabrata (20)	E1210	4 (20.0)	4 (40.0)	5 (65.0)	6 (95.0)	0 (95.0)	1 (100.0)		
	VRC	1 (5.0)	1 (10.0)	4 (30.0)	3 (45.0)	3 (60.0)	1 (65.0)	3 (80.0)	4 (100.0)
C. parapsilosis (25)	E1210	1 (4.0)	8 (36.0)	10 (76.0)	4 (92.0)	1 (96.0)	0 (96.0)	1 (100.0)	
	VRC	3 (12.0)	11 (56.0)	3 (68.0)	2 (76.0)	3 (88.0)	0 (88.0)	2 (96.0)	1 (100.0)
C. tropicalis (24)	E1210	6 (25.0)	5 (45.8)	8 (79.2)	4 (95.8)	1 (100.0)			
	VRC	0 (0.0)	6 (25.0)	9 (62.5)	2 (70.8)	0 (70.8)	1 (75.0)	0 (75.0)	6 (100.0)

a. Voriconazole (VRC) data shown as the most potent comparison agent.

Introduction

New formulations and classes of antifungal agents have dramatically expanded the options for the treatment of invasive candidiasis. Despite these advances, the steady emergence of strains with intrinsic and acquired resistance to both new and established antifungal agents continues to be a concern prompting an expanded search for new antifungal agents with novel mechanisms of action.

E1210 (Eisai Co., Japan) is a novel first-in-class broad-spectrum antifungal agent that inhibits the inositol acylation step in fungal glycophosphatidylinositol (GPI) biosynthesis resulting in defects in various steps in cell wall biosynthesis leading to the inhibition of cell growth, hyphal elongation, and attachment of fungal cells to biological substrates. Differences in the inositol acylation of GPI in yeast and human cells suggest that this could be a good target for drugs directed against yeasts that do not impair inositol acylation in human cells. 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.

In this study, we extend these findings by examining the activity of E1210 and comparator agents against recent clinical isolates of *Candida* spp., including azole- and echinocandin-resistant strains.

Methods

Organisms. A total of 90 clinical isolates of *Candida* spp. were tested including 21 isolates of *C. albicans*, 20 of *C. glabrata*, 25 of *C. parapsilosis* and 24 of *C. tropicalis*. The collection included 20 fluconazole-resistant strains (8 *C. albicans* [MIC, ≥8 µg/mL], 1 *C. glabrata* [MIC, 64 µg/mL], 5 *C. parapsilosis* [MIC, ≥8 µg/mL] and 6 *C. tropicalis* [MIC, ≥8 µg/mL]) and 15 caspofungin-resistant (MIC, ≥1 µg/mL) strains (5 each of *C. albicans, C. glabrata* and *C. tropicalis*). The caspofungin-resistant strains all contained mutations in the *fks1* or *fks2* hotspot regions. All isolates were sent to JMI Laboratories (North Liberty, Iowa, USA) for identification and susceptibility testing as described previously. The isolates were identified by standard methods and stored as water suspensions until used in the study. Prior to testing, each isolate was passaged at least twice on Sabouraud dextrose agar (Remel, Lenexa, Kansas, USA) and CHROMagar™ *Candida* (Becton Dickinson and Company, Sparks, Maryland, USA) to ensure purity and viability.

Antifungal susceptibility testing. BMD testing was performed in accordance with the guidelines in CLSI document M27-A3 by using RPMI 1640 medium with 0.2% glucose, and inoculums of 0.5 X 10^3 to 2.5 X 10^3 cells/mL and incubation at 35°C. MIC values for all five antifungal agents were determined visually after 24-h of incubation, as the lowest concentration of drug that caused a significant diminution (≥50% inhibition) of growth below that of the drug-free control. Recently revised CLSI breakpoints to identify strains as susceptible (S), susceptible-dose-dependent (SDD), intermediate (I) or resistant (R) to caspofungin, fluconazole, and voriconazole were used. Caspofungin MIC values of ≤0.25 µg/mL (S), 0.5 µg/mL (I) and ≥1 µg/mL (R) were used for *C. albicans* and *C. tropicalis*; MIC values of ≤2 µg/mL (S), 4 µg/mL (I), and ≥8 µg/mL (R) were used for *C. parapsilosis;* and MIC values of ≤0.12 µg/mL (S), 0.25 µg/mL (I) and ≥0.5 µg/mL (R) were used for *C. glabrata.* Fluconazole MIC results of ≤2 µg/mL (S), 4 µg/mL (SDD) and ≥8 µg/mL (R) were used to classify isolates of *C. albicans, C. tropicalis* and *C. parapsilosis;* and MIC values of ≤32 µg/mL (SDD) and ≥64 µg/mL (R) were used for *C. glabrata*. For voriconazole, MIC results of ≤0.12 µg/mL (S), 0.25-0.5 µg/mL (I), and ≥1 µg/mL (R) were used for *C. albicans, C. tropicalis*, and *C. parapsilosis;* and MIC values of ≤0.5 µg/mL (wild-type [WT]) and >0.5 µg/mL (non-WT) were used for *C. glabrata*.

Quality control was performed by testing the CLSI-recommended strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Results

- E1210 was highly active against all species tested (MIC_{90} of 0.06 µg/mL for all four species; Table 1) and was more potent than all comparators against the *Candida* species.
- E1210 was not active against *C. krusei* (MIC_{50}, >16 µg/mL; data not shown).
- Among comparator agents caspofungin, posaconazole and voriconazole showed acceptable activity against *Candida* strains, including resistant subsets, and MIC_{50} results for these agents by species were: 0.25, 0.03, 0.015 µg/mL for *C. albicans*; 0.25, 0.5 and 0.12 µg/mL for *C. glabrata*; 1, 0.12 and 0.015 µg/mL for *C. parapsilosis*; and 0.25, 0.06 and 0.03 µg/mL for *C. tropicalis*, respectively.
- E1210 was the most active agent against fluconazole-resistant strains (Table 2) of *C. albicans* (MIC range, 0.015-0.12 µg/mL), *C. glabrata* (0.06 µg/mL), *C. parapsilosis* (MIC range, 0.06-0.5 µg/mL), and *C. tropicalis* (MIC range, 0.008-0.06 µg/mL).
- Cross-resistance between fluconazole and voriconazole, and to a lesser extent posaconazole, was evident among isolates of *C. albicans* (25% voriconazole-resistant), *C. glabrata* (100%) and *C. tropicalis* (83%). E1210 was also the most potent agent tested against caspofungin-resistant strains (Table 3) of *C. albicans* (MIC range, ≤0.008-0.12 µg/mL), *C. glabrata* (MIC range, 0.03-0.06 µg/mL) and *C. tropicalis* (MIC range, 0.015-0.06 µg/mL).
- Three strains of *C. albicans* with resistance to both caspofungin and fluconazole were inhibited by E1210 at ≤0.12 µg/mL.

Table 1. In vitro activity of a novel broad-spectrum antifungal, E1210, and comparator agents tested against *Candida* spp. as determined by CLSI broth microdilution methods.

Species (no. tested)	Antifungal agent	MIC (µg/mL) Range	50%	90%	% by category[a,b] S	SDD/I	R
C. albicans (21)	E1210	0.008 - 0.12	0.015	0.06	NA	NA	NA
	Caspofungin	0.12 - >16	0.25	4	71.4	0.0	23.8
	Fluconazole	0.12 - >128	0.25	64	57.1	4.8	38.1
	Posaconazole	0.015 - 1	0.03	0.5	NA	NA	NA
	Voriconazole	0.008 - 2	0.015	0.5	76.2	14.3	9.5
C. glabrata (20)	E1210	≤0.008 - 0.25	0.03	0.06	NA	NA	NA
	Caspofungin	0.12 - 4	0.25	2	60.0	15.0	25.0
	Fluconazole	0.5 - 128	8	32	NA	94.4	5.6
	Posaconazole	0.06 - 2	0.5	1	NA	NA	NA
	Voriconazole	≤0.008 - 1	0.12	1	60.0	NA	20.0
C. parapsilosis (25)	E1210	≤0.008 - 0.5	0.03	0.06	NA	NA	NA
	Caspofungin	0.5 - 4	1	4	87.5	12.5	0.0
	Fluconazole	0.5-≥64	1	16	75.0	4.2	21.7
	Posaconazole	0.03-0.5	0.12	0.12	NA	NA	NA
	Voriconazole	0.008-1	0.015	0.25	87.5	8.3	4.2
C. tropicalis (24)	E1210	0.008-0.06	0.03	0.06	NA	NA	NA
	Caspofungin	0.12-4	0.25	4	73.9	4.8	33.8
	Fluconazole	0.25-≥64	0.5	≥64	73.9	0.0	26.1
	Posaconzole	0.015-≥8	0.06	0.5	NA	NA	NA
	Voriconazole	0.015-≥8	0.03	2	73.9	4.4	21.7

a. S, susceptible; SDD, susceptible-dose-dependent; I, intermediate; R, resistant; NA, not available
b. Breakpoints for each antifungal and species: fluconazole and *C. albicans, C. parapsilosis* and *C. tropicalis* (S, ≤2 µg/mL; SDD, 4 µg/mL; R ≥8 µg/mL); fluconazole and *C. glabrata* (SDD, ≤32 µg/mL; R, ≥64 µg/mL); caspofungin and *C. albicans* and *C. tropicalis* (S, ≤0.25 µg/mL; I, 0.5 µg/mL; R, ≥1 µg/mL); caspofungin and *C. glabrata* (S, ≤0.12 µg/mL; I, 0.25 µg/mL; R, ≥0.5 µg/mL); caspofungin and *C. parapsilosis* (S, ≤2 µg/mL; I, 4 µg/mL; R, ≥8 µg/mL); voriconazole and *C. albicans, C. tropicalis,* and *C. parapsilosis* (S, ≤0.12 µg/mL; I, 0.25-0.5 µg/mL; R, ≥1 µg/mL); voriconazole and *C. glabrata* (S, ≤0.5 µg/mL; R, >0.5 µg/mL).

Table 2. In vitro activity of E1210 and comparator agents tested against fluconazole-resistant *Candida* spp. as determined by CLSI broth microdilution methods.

Species	Isolate #	MIC (µg/mL)[a] FLU	PSC	VRC	CSF	E1210
C. ablicans	373	64	0.25	0.12	0.12	0.015
	28	32	0.12	0.06	0.25	0.03
	8676	32	1	2	0.25	0.03
	8677	32	0.5	0.5	0.25	0.06
	8679	32	1	0.5	0.25	0.03
	8650	>64	0.12	2	1	0.06
	8651	32	0.12	0.06	1	0.015
	8667	64	0.25	0.5	>8	0.12
C. glabrata	3472	>64	2	1	0.25	0.06
C. parapsilosis	2626	16	0.12	0.12	2	0.06
	666	8	0.12	0.25	2	0.06
	147	8	0.12	0.06	1	0.06
	676	32	0.12	0.5	1	0.06
	730	64	0.5	1	4	0.5
C. tropicalis	10873	8	0.5	0.25	0.12	0.06
	16245	>64	4	8	0.25	≤0.008
	410	64	0.25	2	0.12	0.06
	540	>64	0.25	2	0.12	0.03
	1628	>64	>8	>8	0.5	≤0.008
	699	16	0.5	1	0.25	0.06

Abbreviations: Fluconazole (FLU), posaconazole (PSC), voriconazole (VRC) and caspofungin (CSF).

Table 3. In vitro activity of E1210 and comparator agents tested against caspofungin-resistant *Candida* spp. as determined by the CLSI broth microdilution method.

Species	Isolate #	MIC (µg/mL) CSF	FLU	PSC	VRC	E1210
C. albicans	8647	8	0.25	0.03	0.015	0.03
	8649	4	0.12	0.03	≤0.008	≤0.008
	8650	1	>64	0.12	2	0.06
	8651	1	32	0.12	0.06	0.015
	8667	>8	64	0.25	0.5	0.12
C. glabrata	8653	4	8	0.5	0.25	0.03
	8655	4	32	1	0.5	0.03
	8658	2	1	0.25	0.03	0.03
	8659	2	16	1	0.5	0.03
	8661	1	32	1	1	0.06
C. tropicalis	8662	2	0.25	0.03	0.015	0.015
	8663	4	0.25	0.03	0.03	0.015
	8664	1	0.5	0.03	0.03	0.03
	8684	4	0.5	0.03	0.015	0.03
	8685	4	0.25	0.03	0.015	0.06

Abbreviations: Caspofungin (CSF), fluconazole (FLU), posaconazole (PSC), voriconazole (VRC).

Conclusions

- Although cross-resistance between echinocandins and azoles is unusual, *C. albicans* with resistance to both caspofungin and fluconazole were inhibited by very low concentrations (0.015-0.12 µg/mL) of E1210.
- Despite the introduction of expanded-spectrum triazoles and the echinocandin class of antifungal agents, there remains a need for additional agents with novel mechanisms of action in order to combat the steady emergence of antifungal resistance among the various species of *Candida.* E1210 was identified as a potent, novel antifungal agent with impressive activity against both azole- and echinocandin-resistant strains of *Candida*.

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