51st ICAAC
F1-1375

In Vitro Activity of a Novel Broad-spectrum Antifungal, E1210, Tested against *Aspergillus* spp. by CLSI and EUCAST Broth Microdilution (BMD) Methods

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Abstract

Background: E1210 is a new broad-spectrum antifungal agent that suppresses hyphal growth by inhibiting fungal glycophosphatidylinositol biosynthesis. We examined the activity of E1210 and comparator antifungal agents against *Aspergillus* spp. using CLSI and EUCAST BMD methods to test wild-type as well as amphotericin B (AMB)- and azole-resistant (R) strains.

Methods: 78 *Aspergillus* clinical isolates were tested by CLSI and EUCAST methods: 20 isolates of *A. flavus* (AFL), 22 *A. fumigatus* (AFM), 13 *A. niger* (ANG) and 23 *A. terreus* (ATR), including 15 AMB-R ATR (MIC, ≥2 µg/mL) and 10 itraconazole (ITR)-R AFM (7 isolates; MIC, ≥4 µg/mL), ANG (2) and ATR (1). Comparators included anidulafungin (ANF), caspofungin (CSF), ITR, posaconazole (PSC) and voriconazole (VRC). All isolates were read visually after 48-h incubation. For E1210, ANF and CSF the minimum effective concentration (MEC) was determined as described by CLSI (M38-A2). For ITR, PSC, and VRC, an MIC endpoint of complete growth inhibition was applied for both methods.

Results: CLSI and EUCAST methods were highly concordant for E1210 and comparators (essential agreement [EA; ± 2 $\log_2$ dilutions] 100%) with the exception of PSC and ATR (EA = 91.3%). E1210 was highly active against tested isolates. The MEC/MIC_{90} values (µg/mL) for E1210, ANF, CSF, ITR, PSC, and VRC, respectively, were as follows for each species: AFL (0.03, ≤0.008, 0.12, 1, 1, 1), AFM (0.06, 0.015, 0.12, >8, 1, 4), ANG (0.015, 0.03, 0.12, 4, 1, 2) and ATR (0.06, 0.015, 0.12, 1, 0.5, 1). E1210 was very active against AMB-R strains of ATR (MEC range, 0.015-0.06 µg/mL) and ITR-R strains of AFM (MEC range, 0.03-0.12 µg/mL), ANG (MEC, 0.008 µg/mL) and ATR (MEC, 0.015 µg/mL).

Conclusions: E1210 was documented to be a very potent and broad-spectrum novel antifungal agent with excellent activity against AMB- and ITR-R strains of *Aspergillus* spp. In vitro susceptibility testing of E1210 and other mould-active antifungal agents may be accomplished by either CLSI or EUCAST BMD methods, producing comparable results.

Introduction

The introduction of mould-active antifungal agents of the triazole (itraconazole, posaconazole, voriconazole) and echinocandin (anidulafungin, caspofungin, micafungin) classes has dramatically increased the options for prophylaxis and empirical, or directed therapy for the prevention and treatment of invasive aspergillosis (IA). Although resistance to these agents among clinical isolates of *Aspergillus* has been considered uncommon, both increased resistance and breakthrough infections have been increasingly reported among patients with long-term exposure to these agents. These observations have prompted a call for an expanded search for new antifungal agents with novel mechanisms of action, as well as an expanded role for antifungal susceptibility testing of *Aspergillus* spp.

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 with accompanying inhibition of cell wall growth, hyphal elongation, and attachment of fungal cells to biological substrates. Differences in the inositol acylation of GPI in fungi and in human cells suggest that this may prove to be a good target for drugs directed against fungi (yeasts and moulds) that do not impair inositol acylation in human cells.

In this presented study, we have used a collection of *Aspergillus* spp. isolates selected to represent both polyene- and triazole-resistant strains to examine the activity of E1210 and comparator agents as determined by CLSI and EUCAST reference broth microdilution (BMD) methods.

Methods

Organisms. A total of 78 clinical isolates of *Aspergillus* spp. obtained from centers participating in the 2008-2009 ARTEMIS and SENTRY Antimicrobial Surveillance Programs, were tested against E1210, amphotericin B, anidulafungin, caspofungin, itraconazole, posaconazole and voriconazole. The collection included 20 isolates of *A. flavus* species complex (SC), 22 *A. fumigatus* SC, 13 *A. niger* SC, and 23 *A. terreus* SC. The phenotypically resistant isolates (as determined by CLSI methods) included 15 amphotericin B-resistant (MIC, ≥2 µg/mL) isolates of *A. terreus* SC and 10 itraconazole-resistant (MIC, ≥4 µg/mL) isolates of *A. fumigatus* SC (7 isolates), *A. niger* SC (2 isolates), and *A. terreus* SC (1 isolate). Before testing, each isolate was subcultured at least twice on potato dextrose agar (Remel, Lenexa, Kansas, USA) to ensure viability and purity.

Antifungal susceptibility testing. All isolates were tested for in vitro susceptibility to E1210 and comparators using the CLSI and EUCAST BMD methods. CLSI MIC values for the triazoles were determined visually as the lowest concentration of drug that caused complete inhibition of growth (first clear well) relative to that of the growth control. MEC values for E1210, anidulafungin, and caspofungin were determined as described previously by the CLSI. The MEC endpoint was chosen for E1210 due to the fact that, similar to the echinocandins, E1210 inhibits cell wall synthesis and hyphal extension of *Aspergillus* spp. resulting in aberrant hyphal growth (short stubby, highly branched hyphae), but not complete growth inhibition. EUCAST MIC results (triazoles) and MECs (E1210, echinocandins) were determined visually after 48-h as described above. Quality control was assured by testing *Candida parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, and *A. flavus* ATCC 204304.

Analysis. The MIC/MEC results for each antifungal agent obtained with the EUCAST method were compared to those of the CLSI BMD method. High off-scale MIC/MEC results were converted to the next highest concentration and low off-scale MIC/MEC results were left unchanged. Discrepancies of more than ± 2 $\log_2$ dilution steps among MIC results were used to calculate the level of essential agreement (EA) between the two methods with a target of ≥95% EA at ≤2 doubling dilutions.

Results

- Table 1 summarizes the in vitro susceptibilities of tested *Aspergillus* spp. to E1210 and five comparators as determined by the CLSI and EUCAST BMD methods. All *Aspergillus* spp. were inhibited by ≤0.06 µg/mL of E1210 as determined by both reference methods.
- The echinocandins (anidulafungin and caspofungin), displayed the lowest MEC_{50}/MIC_{50} values among comparator agents (MEC_{50} , ≤0.008 µg/mL for anidulafungin against all species; MEC_{50} range from 0.06 to 0.12 µg/mL for caspofungin) followed by posaconazole (MIC_{50} of 0.5 µg/mL for all *Aspergillus* spp.).
- The EA between the two reference methods was 100.0% for E1210, anidulafungin, caspofungin, itraconazole, and voriconazole, across all four *Aspergillus* spp. groups, but was 97.4% (76 of 78 results) for posaconazole (Table 1)
- E1210 and the echinocandins were the most active agents tested against amphotericin B-resistant strains of *A. terreus* SC as determined by the CLSI method (Table 2).

Table 2. In vitro activity of E1210 and comparators against amphotericin B-resistant *Aspergillus terreus* SC as determined by CLSI broth microdilution methods[a].

	MIC/MEC (µg/mL)[b,c]						
Isolate #	AMB	ITR	PSC	VRC	ANF	CSF	E1210
446	4	0.5	0.5	0.5	0.008	0.12	0.015
618	4	0.5	0.25	0.25	0.008	0.12	0.03
8691	2	1	0.5	1	0.015	0.25	0.06
8693	2	1	0.5	0.5	0.015	0.25	0.06
8694	2	0.5	0.25	0.5	0.008	0.12	0.03
8695	2	1	0.5	1	0.008	0.12	0.06
8696	2	0.25	0.25	0.25	0.008	0.12	0.015
8722	2	0.5	0.5	1	0.015	0.12	0.03
8723	2	0.5	0.25	0.5	0.008	0.12	0.03
8727	2	0.5	0.5	1	0.008	0.12	0.03
8728	2	0.5	0.5	0.5	0.008	0.12	0.03
8729	2	1	0.5	1	0.008	0.12	0.03
8730	2	0.5	0.5	1	0.008	0.12	0.03
8731	2	4	2	>8	0.008	0.12	0.015
8733	2	0.5	0.5	1	0.015	0.12	0.015

a. The minimum inhibitory concentrations (MIC) and minimum effective concentrations (MEC) were determined after 48-h incubation.
b. The MICs for AMB (amphotericin B), ITR (itraconazole), PSC (posaconazole), and VRC (voriconazole) were read at complete (100%) inhibition.
c. The MECs for ANF (anidulafungin), CSF (caspofungin) and E1210 were read as described in CLSI document M38-A2 .

Table 3. In vitro activity of E1210 and comparators against itraconazole-resistant *Aspergillus* spp. as determined by CLSI broth microdilution methods[a].

		MIC/MEC (µg/mL)[a]						
Species	Isolate #	ITR	PSC	VRC	AMB	ANF	CSF	E1210
A. fumigatus SC	8686	>8	1	2	1	0.015	0.12	0.12
	8687	>8	1	2	1	0.015	0.12	0.06
	8688	>8	1	1	1	0.008	0.12	0.03
	8689	>8	1	2	1	0.015	0.12	0.06
	8690	>8	2	8	1	0.008	0.12	0.06
	8737	4	1	2	1	0.015	0.12	0.03
	8737	>8	1	0.25	1	0.008	0.12	0.03
A. niger SC	8698	4	1	2	1	0.008	0.12	0.008
	301	4	1	2	0.5	0.008	0.12	0.008
A. terreus SC	8731	4	2	>8	2	0.008	0.12	0.015

a. Definitions for MIC and MEC and all abbreviations as described in Table 2. Resistance to itraconazole defined as MIC at ≥4 µg/mL.

- The rank order of tested antifungal activity against itraconazole-resistant isolates was: anidulafungin ($MEC_{90,}$ 0.015 µg/mL) > E1210 ($MEC_{90,}$ 0.06 µg/mL) > caspofungin ($MEC_{90,}$ 0.12 µg/mL) > amphotericin B ($MIC_{90,}$ 1 µg/mL) > posaconazole ($MIC_{90,}$ 2 µg/mL) > voriconazole ($MIC_{90,}$ 8 µg/mL) > itraconazole ($MIC_{90,}$ >8 µg/mL), see Table 3.

Table 1. In vitro susceptibilities of *Aspergillus* spp. to E1210 and comparators as determined by CLSI and EUCAST broth microdilution methods.

Species (no. tested)	Antifungal agent	Test method	No. of isolates at MIC/MEC (µg/mL): ≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8	%EA[a]
A. flavus SC[b] (20)	E1210	EUCAST		4	14	2								100.0
		CLSI	1	14	4	1								
	Anidulafungin	EUCAST	19	1										100.0
		CLSI	20											
	Caspofungin	EUCAST				4	16							100.0
		CLSI				1	15	4						
	Itraconazole	EUCAST						7	13					100.0
		CLSI							7	13				
	Posaconazole	EUCAST					2	17	1					100.0
		CLSI						1	16	3				
	Voriconazole	EUCAST							1	12	7			100.0
		CLSI							4	14	1	1		
A. fumigatus SC (22)	E1210	EUCAST		5	13	4								100.0
		CLSI	1	1	16	4								
	Anidulafungin	EUCAST	17	5										100.0
		CLSI	14	8										
	Caspofungin	EUCAST				3	15	4						100.0
		CLSI				4	17	1						
	Itraconazole	EUCAST						2	13				7	100.0
		CLSI							1	14		1	6	
	Posaconazole	EUCAST					13	1	2	5	1			100.0
		CLSI						8	7	6	1			
	Voriconazole	EUCAST						1	12	4	4	1		100.0
		CLSI						4	10	3	4		1	
A. niger SC (13)	E1210	EUCAST		5	6	2								100.0
		CLSI	8	5										
	Anidulafungin	EUCAST	13											100.0
		CLSI	12		1									
	Caspofungin	EUCAST					13							100.0
		CLSI				4	9							
	Itraconazole	EUCAST						1	3	6	3			100.0
		CLSI								6	5	2		
	Posaconazole	EUCAST					2	4	7					100.0
		CLSI						1	8	4				
	Voriconazole	EUCAST							4	5	4			100.0
		CLSI							4	5	4			
A. terreus SC (23)	E1210	EUCAST		2	17	4								100.0
		CLSI	1	11	8	3								
	Anidulafungin	EUCAST	20	3										100.0
		CLSI	15	8										
	Caspofungin	EUCAST				1	18	4						100.0
		CLSI					21	2						
	Itraconazole	EUCAST						15	7		1			100.0
		CLSI						1	15	6		1		
	Posaconazole	EUCAST				1	14	7		1				91.3
		CLSI						7	14	1	1			
	Voriconazole	EUCAST							11	11			1	100.0
		CLSI						4	13	5			1	

a. Definitions for MIC and MEC and all abbreviations as described in Table 2. Resistance to itraconazole defined as MIC at ≥4 µg/mL.

Conclusions

- E1210 displayed excellent in vitro potency against contemporary isolates of *Aspergillus* spp., including polyene- and triazole-resistant strains.
- Whereas cross-resistance was apparent between itraconazole, posaconazole, and voriconazole, the different mechanisms of action represented by E1210, amphotericin B, and the echinocandins results in sustained activity of these antifungal agents against this subset of *Aspergillus* species isolates.
- A high-level of agreement between the CLSI and EUCAST methods for testing E1210 and the echinocandins against *Aspergillus* spp. was observed. Results also confirm the comparability of the CLSI and EUCAST BMD methods when testing the triazoles against *Aspergillus* spp. strains.
- E1210 is a potent, novel antifungal agent with impressive activity against both wild-type and antifungal-resistant strains of *Aspergillus* spp. Further development of E1210 appears warranted.

Acknowledgements

This work was supported by an educational/research grant from Eisai Co. (Japan).

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