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The Investigational Agent E1210 is Effective for the Treatment of Experimental Invasive Candidiasis Caused by Resistant *Candida albicans*

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ABSTRACT

Background: Treatment options against resistant *C. albicans* are limited. The inositol acyltransferase inhibitor E1210 has *in vitro* and *in vivo* activity against different pathogenic fungi, including *Candida*, *Aspergillus*, *Fusarium*, and *Scedosporium* spp. Our objective was to evaluate the *in vitro* potency and *in vivo* activity of E1210 against echinocandin-resistant *C. albicans*. **Methods:** Antifungal susceptibility testing was performed against 29 *C. albicans* clinical isolates, including 16 echinocandin-resistant strains by CLSI methods. Immunocompetent ICR mice (N = 20/treatment group) were inoculated intravenously with *C. albicans* 43001 (E1210, fluconazole [FLU], and caspofungin [CAS] MICs ≤ 0.03, >64, and 1 µg/ml, respectively). Therapy with placebo control, E1210 (2.5, 10, or 40 mg/kg PO BID), FLU (20 mg/kg PO BID) or CAS (10 mg/kg IP QD) began 1 day post-challenge and continued for 7 days. Mice were followed off therapy until day 21 to assess survival. Kidneys were collected on day 8, and fungal burden was assessed by colony-forming units (CFU). **Results:** E1210 demonstrated potent *in vitro* activity against *C. albicans*, as the MICs ranged between ≤ 0.03 – 0.25 µg/ml against all isolates, including echinocandin-resistant isolates. *In vivo*, survival was significantly longer in mice treated with E1210 10 and 40 mg/kg (>21 days) compared to placebo and CAS (8 and 13.5 days, respectively; $p < 0.01$). Percent survival was also significantly longer in mice treated with E1210 10 and 40 mg/kg (55% and 60%, respectively) compared to placebo (20%; $p < 0.05$). Fungal burden was also significantly lower with each dose of E1210 (mean range $\log_{10}$ CFU/g 4.19 - 4.79 $\log_{10}$ CFU/g) compared to placebo (5.60 $\log_{10}$ CFU/g; $p < 0.05$) and CAS (5.88 $\log_{10}$ CFU/g; $p < 0.01$). FLU also resulted in significant improvements in survival (>21 days and 70% survival) and reductions in fungal burden (3.52 $\log_{10}$ CFU/g) compared to placebo and CAS ($p < 0.05$). **Conclusions:** E1210 demonstrated potent *in vitro* and *in vivo* activity against echinocandin-resistant *C. albicans* in this study. These results demonstrate the potential for E1210 for the treatment of invasive candidiasis caused by resistant *C. albicans*.

BACKGROUND

- Invasive candidiasis remains a significant challenge to clinicians, and infections caused by echinocandin-resistant isolates may be especially difficult to treat due to limited therapeutic options.
- Glycosylphosphatidylinositol (GPI)-anchored proteins are known to serve as adhesins and some fungal adhesins, as well as other virulence factors, are derived from GPI-anchored proteins
- E1210 (Figure 1) is a broad-spectrum investigational agent that inhibits inositol acyltransferase, thereby preventing GPI-anchored protein maturation.
- This investigational antifungal has been shown to have potent *in vitro* activity against *Candida* spp. and *Cryptococcus neoformans*, and works by causing the collapse of the fungal mitochondrial membrane potential.

OBJECTIVE

Our objective was to evaluate the *in vitro* and *in vivo* effectiveness of E1210 against invasive candidiasis caused by resistant *C. albicans*.

- Broth microdilution susceptibility testing was used to evaluate the *in vitro* potency of E1210.
- An established murine model of this infection was used to evaluate survival and fungal burden within the kidney tissues.

MATERIALS AND METHODS

***Candida albicans* Isolates**

- 29 *C. albicans* clinical isolates from the Fungus Testing Laboratory at the UT Health Science Center at San Antonio were used for *in vitro* susceptibility testing. These included 16 echinocandin-resistant isolates.
- *Candida albicans* clinical isolate 43001 (MICs E1210 ≤ 0.03 µg/ml, fluconazole ≥64 µg/ml, caspofungin 1 µg/ml) was used in the *in vivo* experiments. This isolate has a F641S point mutation in hot spot 1 of the gene encoding Fks1p.

Antifungal Agents

- For *in vitro* studies, stock solutions of E1210, fluconazole, and caspofungin were prepared in DMSO prior to further dilutions in RPMI (0.165M MOPS, pH 7.0).
- For *in vivo* studies, E1210 was dissolved 400 mM HCl to a concentration of 40 mg/ml followed by further dilutions in 5% glucose, and commercially available formulations of fluconazole and caspofungin were used.

Susceptibility Testing

- Microdilution broth susceptibility testing was performed as described by CLSI M27-A3 methodology.
- Minimum inhibitory concentrations (MIC) for E1210, fluconazole, caspofungin were read at 24 hours as the lowest concentration that caused a significant decrease (>50%) in turbidity compared to that of the growth control. The E1210 100% inhibition MIC endpoint was also assessed.

Murine Model of Invasive Candidiasis

- Mice were infected intravenously with a 0.2 mL volume with *C. albicans* at an inoculum of ~10^6 CFU/mouse on day 0.
- Treatment began 1 day post-inoculation and consisted of the following groups:
  1) Placebo Control (5% glucose by oral gavage twice daily)
  2) E1210 at doses of 2.5, 10, or 40 mg/kg by oral gavage twice daily
  3) Fluconazole 20 mg/kg by oral gavage twice daily
  4) Caspofungin 10 mg/kg once daily by intraperitoneal injection
- In the fungal burden arm, treatment continued through day 7 post-inoculation. Kidneys from each animal were homogenized in sterile saline supplemented with antibiotics, and serial dilutions were prepared and plated in duplicate onto Sabouraud dextrose agar. After 24 hours of incubation at 37°C, the colonies were counted and the numbers of colony forming units (CFU) per gram of tissue calculated.
- In survival studies, treatment continued through day 7. Mice were then followed off therapy until day 21 post-inoculation.

Statistical Analysis

- MIC concentrations at which 50% and 90% of the isolates were inhibited, and geometric mean (GM) MICs were determined. Differences in the GM MIC values were assessed for significance by ANOVA with Tukey's post-test for multiple comparisons.
- Survival was plotted by Kaplan-Meier analysis, and differences in median survival and percent survival between groups were analyzed by the log rank and Fischer's exact test, respectively.
- Differences in kidney tissue fungal burden were assessed for significance by ANOVA with Tukey's post-test for multiple comparisons.
- A p-value of ≤ 0.05 was considered statistically significant for all comparisons.
- Each dose group within the survival and fungal burden arms was tested in duplicate to evaluate the reproducibility of the results (N = 20 mice total per dosage group per study arm).

RESULTS

Figure 1. Chemical structure of E1210.

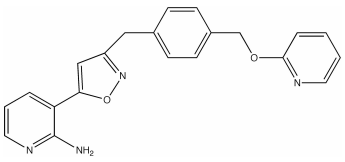


Table 1. Minimum inhibitory concentrations (MIC, µg/ml) for E1210, fluconazole and caspofungin against all *C. albicans* isolates.

All Isolates (N = 29)	E1210 50%	E1210 100%	Fluconazole	Caspofungin
MIC Range	≤ 0.03	≤ 0.03-0.25	≤ 0.12->64	≤0.015-4
MIC50	≤ 0.03	≤ 0.03	0.25	1
MIC90	≤ 0.03	0.25	>64	2
GM MIC	0.03*,¶,§	0.0438*,¶,§	1.414	0.3074

*p-value vs. Control; ¶p-value vs. Fluconazole; §p-value vs. Caspofungin

RESULTS (cont.)

Table 2. Minimum inhibitory concentrations (MIC, µg/ml) for E1210, fluconazole and caspofungin against susceptible and resistant *C. albicans* isolates.

All Isolates (N = 29)	E1210 50%	E1210 100%	Fluconazole	Caspofungin	E1210 50%	E1210 100%	Fluconazole	Caspofungin
Group	Echinocandin-Susceptible Isolates (N = 13)				Echinocandin-Resistant Isolates (N = 16)			
MIC Range	≤ 0.03	≤ 0.03-0.25	≤ 0.125-8	≤0.015-0.12	≤ 0.03	≤ 0.03-0.25	≤ 0.125->64	1-4
MIC50	≤ 0.03	≤ 0.03	≤ 0.12	0.06	≤ 0.03	≤ 0.03	16	1
MIC90	≤ 0.03	0.25	8	0.12	≤ 0.03	0.25	>64	4
GM MIC	0.03*,¶	0.0353*,¶	0.213	0.0574	0.03*,¶,§	0.0528*,¶,§	8	1.320

*p-value vs. Control; ¶p-value vs. Fluconazole; §p-value vs. Caspofungin

Figure 2. Survival data for E1210, fluconazole (FLU), and caspofungin (CAS) to day 21 post-inoculation in mice infected intravenously with *C. albicans* 43001.
*p-value vs. Control
§p-value vs. Caspofungin

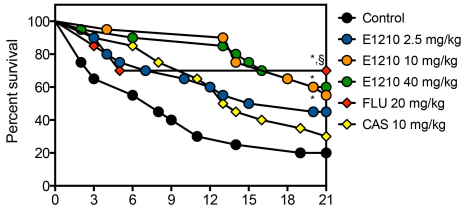
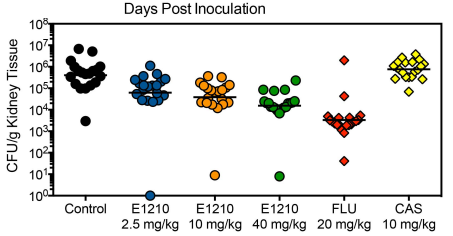


Figure 3 & Table 3. Kidney fungal burden (CFU/g) for E1210, fluconazole (FLU) and caspofungin (CAS) on day 8 post-inoculation in mice infected intravenously with *C. albicans* 43001.



Group	Control	E1210 2.5 mg/kg	E1210 10 mg/kg	E1210 40 mg/kg	Fluconazole 20 mg/kg	Caspofungin 10 mg/kg
Mean (SD) log CFU/g Day 8	5.60 (0.73)	4.79 (1.22) *p=0.0498 §p=0.0019	4.59 (0.06) *p=0.0053 §p<0.0001	4.19 (0.86) *p<0.0001 §p<0.0001	3.52 (0.85) *p<0.0001 §p<0.0001	5.88 (0.42)

*p-value vs. Control; §p-value vs. Caspofungin; lines in figure represent mean values

CONCLUSIONS

E1210 demonstrated potent *in vitro* and *in vivo* activity against echinocandin-resistant strains of *C. albicans*. Improvements in survival and reductions in fungal burden were significantly greater than those observed with caspofungin. These data demonstrate the potential utility of E1210 as therapy against echinocandin-resistant *Candida* infections.

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