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The Investigational Agent E1210 Is Effective in Treatment of Experimental Invasive Candidiasis Caused by Resistant *Candida albicans*

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The *in vitro* and *in vivo* activity of the inositol acyltransferase inhibitor E1210 was evaluated against echinocandin-resistant *Candida albicans*. E1210 demonstrated potent *in vitro* activity, and in mice with invasive candidiasis caused by echinocandin-resistant *C. albicans*, oral doses of 10 and 40 mg E1210/kg of body weight twice daily significantly improved survival and reduced fungal burden compared to those of controls and mice treated with caspofungin (10 mg/kg/day). These results demonstrate the potential use of E1210 against resistant *C. albicans* infections.

Microorganisms must attach to host cell surfaces prior to colonization, replication, and penetration through mucosal barriers. Glycosylphosphatidylinositol (GPI)-anchored proteins are known to serve as adhesins (1), and some fungal adhesins are derived from GPI-anchored proteins (2–5). E1210 is a broad-spectrum investigational antifungal agent that inhibits inositol acyltransferase, thereby preventing GPI-anchored protein maturation (6). This agent has potent *in vitro* activity against different pathogenic fungi, including *Candida*, *Aspergillus*, *Fusarium*, and *Scedosporium* species (6–10), and inhibition of inositol acyltransferase by E1210 appears to be fungus specific (11). Animal models have also demonstrated *in vivo* efficacy of this agent against invasive candidiasis, invasive aspergillosis, and fusariosis (12). Our objective was to evaluate the *in vitro* potency and *in vivo* activity of this agent against *Candida albicans*, including echinocandin-resistant isolates.

In vitro susceptibility testing was performed according to the CLSI M27-A3 methods against 29 *C. albicans* clinical isolates, including 16 echinocandin-resistant isolates and 10 isolates with known *FKS1* hot spot mutations (13, 14). The MICs for E1210 were read at both 50% and 100% inhibition of growth compared to the growth controls, while those of fluconazole and caspofungin were read at 50% inhibition. In *in vivo* studies, immunocompetent outbred male ICR mice (Harlan) were used in all experiments (http://www.sacmm.org/pdf/SOP-murine-model-candida-albicans.pdf) (15). On day 0, animals were infected intravenously with *C. albicans* 43001 (~1×10^6 cells/mouse; E1210 MIC of ≤0.03 μg/ml; fluconazole MIC of >64 μg/ml; caspofungin MIC of 1 μg/ml; F641S amino acid change in Fks1p) (14, 15). Mice were then randomly placed into six groups: placebo control (5% glucose by oral gavage twice daily), E1210 at doses of 2.5, 10, or 40 mg/kg of body weight by oral gavage twice daily, fluconazole at 20 mg/kg by oral gavage twice daily, or caspofungin at 10 mg/kg by intraperitoneal injection once daily. Treatment started 1 day after inoculation and continued for 7 days. In the survival arm, mice were monitored off therapy until day 21. Any animal that appeared moribund was euthanized, with death recorded as occurring the next day. In the fungal burden arm, kidneys were collected on day 8. Kidneys were weighed and homogenized in sterile saline. Serial dilutions were prepared and plated, and following 24 h of

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TABLE 1 MICs for E1210, fluconazole, and caspofungin against 29 *C. albicans* isolates, including echinocandin-susceptible and -resistant isolates

	MIC (μg/ml)[a]											
	All *Candida albicans* isolates (n = 29)				Echinocandin-susceptible isolates (n = 13)				Echinocandin-resistant isolates (n = 16)			
Antifungal agent	Range	50%	90%	GM	Range	50%	90%	GM	Range	50%	90%	GM
E1210, 50% inhibition	≤0.03	≤0.03	≤0.03	0.03	≤0.03	≤0.03	≤0.03	0.03	≤0.03	≤0.03	≤0.03	0.03
E1210, 100% inhibition	≤0.03–0.25	≤0.03	0.25	0.0438	≤0.03–0.25	≤0.03	0.25	0.0353	≤0.03–0.25	≤0.03	0.25	0.0528
Fluconazole	≤0.125–>64	0.25	>64	1.414	≤0.125–8	≤0.125	8	0.213	≤0.125–>64	16	>64	8
Caspofungin	≤0.015–4	1	2	0.3074	≤0.015–0.125	0.06	0.125	0.0574	1–4	1	4	1.32

[a] MICs were read after 24 h of incubation at 35°C. The E1210 MICs were read at the 50% and 100% growth inhibition endpoints, while fluconazole and caspofungin MICs were read using the 50% inhibition endpoint. GM, geometric mean.

TABLE 2 MICs for E1210, fluconazole, and caspofungin against individual *C. albicans* isolates with known *FKS1* point mutations and those resistant to fluconazole (MICs of ≥8 μg/ml)

Isolate no.	MIC (μg/ml)[a]			
	E1210, 50% inhibition	E1210, 100% inhibition	Fluconazole	Caspofungin
FKS1 T1922C (F641S amino acid change)				
2	≤0.03	≤0.03	8	1
4	≤0.03	≤0.03	≤0.125	1
5	≤0.03	0.25	64	1
8	≤0.03	0.125	64	1
9	≤0.03	≤0.03	32	1
10	≤0.03	≤0.03	>64	1
FKS1 T1933C (S645P amino acid change)				
6	≤0.03	0.06	4	1
7	≤0.03	≤0.03	16	2
11	≤0.03	≤0.03	16	1
12	≤0.03	≤0.03	≤0.125	4
Fluconazole-resistant strains				
1	≤0.03	0.06	>64	2
5	≤0.03	0.25	64	1
7	≤0.03	≤0.03	16	2
8	≤0.03	0.125	64	1
9	≤0.03	≤0.03	32	1
10	≤0.03	≤0.03	>64	1
11	≤0.03	≤0.03	16	1
19	≤0.03	0.25	8	0.125
27	≤0.03	0.25	16	1
28	≤0.03	0.125	>64	1

[a] MICs were read after 24 h of incubation at 35°C. The E1210 MICs were read at the 50% and 100% growth inhibition endpoints, while fluconazole and caspofungin MICs were read using the 50% inhibition endpoint.

incubation at 37°C, fungal burden (CFU/g) was determined. Each group in both the survival and fungal burden arms consisted of 10 mice, and both arms were conducted in duplicate to evaluate the reproducibility of the results ($n = 20$ mice total per dosage group per study arm). This study was approved by the Institutional Animal Care and Use Committee at the UT Health Science Center San Antonio.

E1210 demonstrated potent *in vitro* activity against *C. albicans*, including the echinocandin-resistant isolates (Tables 1 and 2). The E1210 MICs, using either the 50% or 100% growth inhibition endpoint, were low for all isolates and did not differ between the fluconazole- or caspofungin-susceptible and -resistant strains. Treatment with E1210 also resulted in improvements in survival compared to both the placebo control and caspofungin groups (Fig. 1). Median survival was significantly longer in mice that received 10 and 40 mg E1210/kg twice daily (>21 days for each) than in controls and mice treated with caspofungin (8 and 13.5 days, respectively; $P < 0.01$). Percent survival at day 21,14 days after therapy was stopped, was also significantly higher in mice treated with E1210 at doses of 10 and 40 mg/kg twice daily (55% and 60%, respectively) than in controls (20%; $P < 0.05$). In contrast, there was no difference in percent survival between controls and mice treated with caspofungin (30%). Fungal burden within the kidneys on day 8 postinoculation was also significantly lower with each dosage group of E1210 (means ± standard deviations [SD] in $\log_{10}$ CFU/g: 4.79 ± 1.22, 4.59 ± 0.06, and 4.19 ± 0.86 for the groups treated with 2.5, 10, and 40 mg/kg, respectively) than in controls (5.60 ± 0.73 $\log_{10}$ CFU/g; $P < 0.05$) and mice treated with caspofungin (5.88 ± 0.42 $\log_{10}$ CFU/g; $P < 0.01$) (Fig. 2). When the outliers with very low fungal burden observed in each E1210 dosage group were excluded from the analysis, fungal burdens in each of the E1210 dosage groups remained significantly lower (5.05, 4.78, and 4.36 $\log_{10}$ CFU/g; $P < 0.05$) than those in the control and caspofungin groups. Interestingly, despite reduced *in vitro* potency, the dose of fluconazole used in this study, 20 mg/kg twice daily, also resulted in significant improvements in survival (>21 days and 70% survival) and reductions in fungal burden (3.52 ± 0.85 $\log_{10}$ CFU/g) compared to those in controls and mice treated with caspofungin ($P < 0.05$ for all comparisons). It is known that *in vitro* fluconazole resistance in *C. albicans* does not necessarily predict *in vivo* failure, especially when higher doses are used (16, 17). As the mechanism of fluconazole resistance in this strain is unknown, it is possible that this isolate may not have had the full complement of mechanisms that would also translate into clinical resistance.

These results demonstrate the potential for E1210 for the treatment of invasive candidiasis caused by echinocandin-resistant *C. albicans*. E1210 demonstrated potent *in vitro* activity against *C. albicans* tested in this study, including the 16 echinocandin-resistant isolates. This *in vitro* potency translated into *in vivo* efficacy in the murine model of invasive candidiasis caused by one of the resistant isolates. These results are consistent with previous reports that demonstrated both *in vitro* potency and *in vivo* efficacy for E1210 against different pathogenic fungi, including *Candida*,

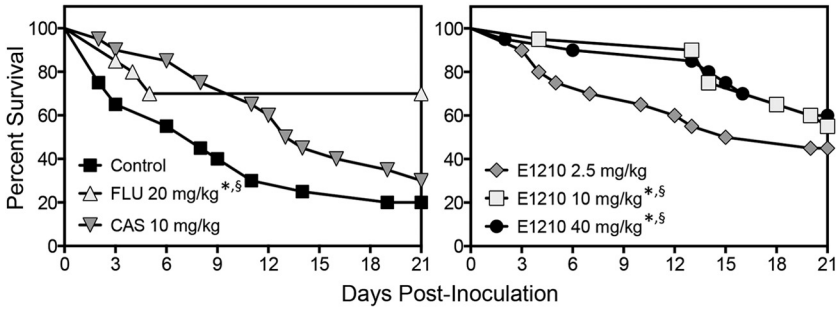

FIG 1 Survival curves in mice infected with *C. albicans* 43001 and treated with placebo by oral gavage twice daily (5% glucose twice daily by oral gavage), E1210 at doses of 2.5 mg/kg, 10 mg/kg, or 40 mg/kg by oral gavage twice daily, fluconazole (FLU) at 20 mg/kg by oral gavage twice daily, or caspofungin (CAS) at 10 mg/kg by intraperitoneal injection once daily. Treatment began 1 day postinoculation and continued for 7 days. Mice were then monitored off therapy until day 21. $n = 20$ mice per group. Survival was plotted by Kaplan-Meier analysis, and differences in median survival time and the percent survival among groups were analyzed by the log-rank test and Fischer's exact test, respectively. *, $P < 0.05$ versus control; §, $P < 0.05$ versus the caspofungin group.

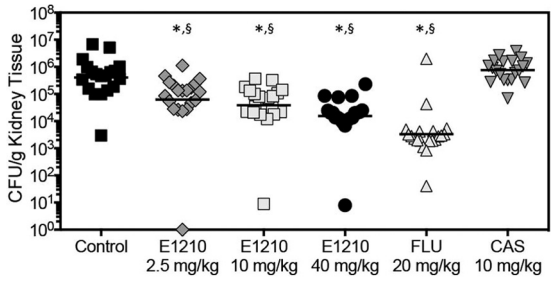

FIG 2 Kidney fungal burden (CFU/g of tissue) on day 8 in mice infected with *C. albicans* 43001 and treated with placebo (5% glucose twice daily by oral gavage), E1210 (2.5, 10, or 40 mg/kg by oral gavage twice daily), fluconazole (FLU; 20 mg/kg by oral gavage twice daily), or caspofungin (CAS; 10 mg/kg by intraperitoneal injection once daily) beginning 1 day postinoculation and continuing for 7 days. $n = 20$ mice per group. Differences in kidney fungal burden (reported as mean CFU/g ± standard deviation) among the groups were assessed for significance by analysis of variance (ANOVA) with Tukey's posttest for multiple comparisons. *, $P < 0.05$ versus control; §, $P < 0.05$ versus caspofungin.

Aspergillus, and *Fusarium* species (6–12). Interestingly, the *in vivo* activity of E1210 was similar to that of fluconazole in our animal model despite the *in vitro* resistance of this isolate against fluconazole. However, we did not evaluate the pharmacokinetics of either agent, and the pharmacokinetic/pharmacodynamic parameters of E1210 are unknown. Notably, efficacy of E1210 was significantly better than that of caspofungin, which was administered in a dose and route previously shown to have excellent activity in this model against wild-type *Candida* isolates (15, 18). E1210 may have other advantages in the treatment of invasive fungal infections, as it appears to inhibit inositol acylation in the biosynthetic pathway of glycosylphosphatidylinositol in fungi but not in humans (11). Thus, toxicities and drug interactions due to cross-reactivity with mammalian enzymes and cell membrane components observed with other antifungals may be avoided with E1210. Further studies, including pharmacokinetic/pharmacodynamic analysis and clinical trials, are needed to truly assess the potential for E1210 for the treatment of invasive infections caused by resistant fungi.

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