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Efficacy of Oral E1210, a New Broad-Spectrum Antifungal, in Murine Models of Oropharyngeal Candidiasis, Disseminated Candidiasis, and Pulmonary Aspergillosis

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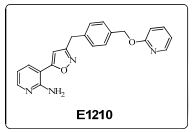
Abstract

Background: E1210 is a first-in-class, broad-spectrum antifungal with a novel mechanism of action – inhibition of fungal GPI biosynthesis. In this study, the efficacies of E1210 and reference antifungals were evaluated in murine models of oropharyngeal candidiasis, disseminated candidiasis and pulmonary aspergillosis.

Methods: All mice were immunosuppressed by a subcutaneous injection of cortisone acetate (4 mg/mouse) 1 day before infection and 3 days after infection or by 5-florouracil (200 mg/kg) 5 or 6 days before infection. Mice were infected orally or intravenously with *C. albicans* or intranasally with *A. flavus* under anesthesia. In the oropharyngeal candidiasis model, oral treatment was initiated 3 days post-infection and was continued for 3 consecutive days. *C. albicans* cells in the oral cavity were recovered by oral swabbing on the day after the final treatment and were subsequently cultured. In the disseminated candidiasis and pulmonary aspergillosis models, oral treatment was initiated 1 h after infection and was continued for 3 or 4 consecutive days. The survival rate and survival period were determined over 14 days.

Results: In the oropharyngeal candidiasis model, the treatment of E1210 at 5 and 10 mg/kg (bid) significantly reduced the number of CFU in the oral cavity in comparison to the control treatment. In the disseminated candidiasis model, the mice treated with E1210 at 5 and 12.5 mg/kg (BID) showed significantly improved survival in comparison to the control mice, and the 50% effective dose (ED_{50}) of E1210 was 4.8 mg/kg at day 14 after infection. In the pulmonary aspergillosis model, the mice treated with E1210 at 10 and 25 mg/kg (BID) showed significantly improved survival in comparison to the control mice, and the ED50 of E1210 was 10 mg/kg at day 14 after infection.

Conclusion: E1210 was effective in murine models of oropharyngeal candidiasis, disseminated candidiasis and pulmonary aspergillosis.



E1210

Introduction

Invasive fungal infections have become increasingly more common among immunocompromised and immunosuppressed patients, including solid-organ and hematopoietic stem-cell transplant recipients and individuals on immunosuppressive drug regimens. There is still a high rate of morbidity and mortality associated with invasive fungal infections, because the currently available antifungal drugs are limited in terms of their side effects and mode of action. In addition, there has been an increase in resistance to commonly used antifungal compounds and an epidemiological shift towards more drug-resistant strains. Thus, there is a critical need for new antifungal compounds that have a broad spectrum of activity, more favorable pharmacokinetics, and fewer side effects.

E1210 is a new, first-in-class, antifungal compound that was discovered by the Tsukuba Research Laboratories, Eisai Co., Ltd. (Ibaraki, Japan). It has potent, broad-spectrum, antifungal activity and a novel mechanism of action – inhibition of fungal GPI biosynthesis (1, 2). E1210 dosed as an oral solution was rapidly absorbed, and oral bioavailability ranged between 58-73% in animals (3, 4). In the current studies, the efficacies of oral E1210 and reference drugs, such as fluconazole, voriconazole, caspofungin and liposomal amphotericin B, were evaluated in murine models of oropharyngeal candidiasis, disseminated candidiasis and fusariosis, and pulmonary aspergillosis.

Methods

Organisms: Strains IFM49971 and IFM49738 of *Candida albicans*, IFM50915 of *Aspergillus flavus*, IFM51126 of *Aspergillus fumigatus* and IFM50956 of *Fusarium solani* used in the current studies were obtained from the Medical Mycology Research Center, Chiba University (Chiba, Japan), and were stored in glycerol stocks at -80° C.

Oropharyngeal Candidiasis model: ICR mice were immunosuppressed with 4 mg cortisone acetate administered subcutaneously 1 day before and 3 days after infection. The mice were given 1 mg/mL tetracycline hydrochloride in their drinking water, starting on the day of cortisone acetate administration and continuing throughout the experiment, in order to prevent bacterial infection. *C. albicans* IFM49971 was grown on Sabouraud dextrose agar (SDA) at 35° C for 2 days. The cells were suspended in sterile physiological saline. The cell number was counted using a hemocytometer, and adjusted to the required density with sterile physiological saline. Using a micropipette, aliquots (10 μL) of *C. albicans* suspension were inoculated into the oral cavity of mice that had been anesthetized subcutaneously with chlorpromazine hydrochloride (0.5 mg/mouse). The challenge dose was 4×10^5 colony-forming units (CFU)/mouse. Either E1210 orally administered BID or fluconazole orally administered QD was given for 3 consecutive days starting 3 days post-infection. The control group also received the equivalent oral volume of 5% glucose BID. *C. albicans* cells in the oral cavity were collected the day after the final administration, diluted and cultured. The number of cells was counted and the results were expressed as the number of CFUs.

Disseminated Candidiasis and Fusariosis models: ICR mice (for candidiasis) or DBA/2N mice (for fusariosis) were immunosuppressed with 200 mg/kg of 5-FU administered subcutaneously 6 days prior to infection. The mice were orally administered 0.1 mg/mL ciprofloxacin in their drinking water, from 2-3 days prior to infection up until 5-7 days after infection, in order to prevent bacterial infections. *C. albicans* IFM49971 and IFM49738 were cultured on a SDA plate at 35°C for 2 days. *F. solani* IFM50956 was cultured on a potato dextrose agar (PDA) plate at 30°C for 7 days. The cells from the surface of the agar plate were suspended in sterile physiological saline (containing 0.05% Tween 80 for *F. solani* cell preparation), and the number of cells was counted using a hemocytometer. The final inoculum was adjusted to the required density using sterile physiological saline (containing 0.05% Tween 80 for the *F. solani* cell preparation). Infection was induced in the neutropenic mice by the intravenous inoculation of a 0.2 mL *C. albicans* cell suspension (0.8×10^4 CFU/mouse) or a 0.2 mL *F. solani* cell suspension (5.0×10^3 cells/mouse) into the lateral tail vein. Antifungal therapy was initiated 1 h or 24 h after infection and was continued for 3 consecutive days in the candidiasis models (days 0-2 or 1-3) or for 5 consecutive days in the fusariosis model (days 0-4). E1210 was orally administered BID or TID, fluconazole was orally administered QD and liposomal amphotericin B or caspofungin was intravenously administered QD. The control group received an equivalent volume of 5% glucose orally BID or TID. The survival rate and survival period were determined over 14 days.

Pulmonary Aspergillosis model: DBA/2N mice were immunosuppressed with 200 mg/kg of 5-FU administered subcutaneously 5-6 days prior to infection. The mice were orally administered 0.1 mg/mL ciprofloxacin in their drinking water, from 3-4 days prior to infection up until 7 days after infection. *A. flavus* IFM50915 and *A. fumigatus* IFM51126 were cultured on a PDA plate at 35°C for 7 days. The conidia from the surface of the agar plate were suspended in sterile physiological saline containing 0.05% Tween 80, and the cell number was counted using a hemocytometer. The final inoculum was adjusted to the required density using sterile physiological saline containing 0.05% Tween 80. The mice were anesthetized intravenously with 0.1 mL ketamine hydrochloride (4.17 mg/mL). Infection was induced in the neutropenic mice by the intranasal inoculation of a 0.05 mL *A. flavus* conidia suspension (3.0×10^4 conidia/mouse) or a 0.05 mL *A. fumigatus* conidia suspension (6.0×10^6 conidia/mouse). Antifungal therapy was initiated 1 h after infection and was continued for four or 7 consecutive days (days 0-3 or 0-7). E1210 was administered orally BID or TID, voriconazole was administered orally BID, and caspofungin or liposomal amphotericin B was administered intraperitoneally QD. The control group received an equivalent volume of 5% glucose orally BID or TID. The survival rate and survival period were determined over 14 days.

Results

In Vitro Antifungal Activity

TABLE 1. In vitro antifungal activities of E1210 and reference compounds against strains causing experimental infections in mice.

Organism	Strain No.	MIC (μg/mL)				
		E1210	FLCZ	VRCZ	CSFG	AMPH
C. albicans	IFM49971	0.004	0.13	0.002	0.06	0.5
C. albicans	IFM49738	0.016	>32	>32	0.06	0.5
A. flavus	IFM50915	0.03	>32	0.5	0.13	1
A. fumigatus	IFM51126	0.03	>32	0.25	0.13	0.25
F. solani	IFM50956	0.06	>32	4	>16	1

The minimum inhibitory concentrations (MICs) were determined with the broth-microdilution method developed by the Clinical and Laboratory Standards Institute (CLSI) in documents M27-A3 (5) and M38-A2 (6). Each experiment was repeated three times.

Abbreviations: FLCZ, fluconazole; VRCZ, voriconazole; CSFG, caspofungin; AMPH, amphotericin B; L-AmB, liposomal amphotericin B.

Efficacy in the Disseminated Candidiasis model

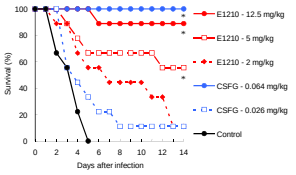


FIG. 2 (A). Efficacies of E1210 and CSFG in a murine model of disseminated candidiasis caused by *C. albicans*.
Strain: *C. albicans* IFM49971, Mice: ICR F 5W (n=9), Therapy: BID (E1210) or QD (CSFG) for 3 days. * p<0.05 versus control group (log-rank test with Bonferroni adjustment). [See TABLE 2.]

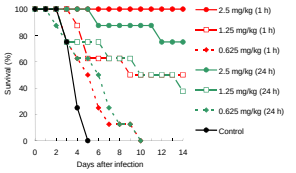


FIG. 2 (C). Effect of treatment delay on the efficacy of E1210 in a murine model of disseminated candidiasis.
Strain: *C. albicans* IFM49971, Mice: ICR F 5W (n=8), Therapy: TID for 3 days starting 1 h or 24 h post-infection.

Efficacy in the Oropharyngeal Candidiasis model

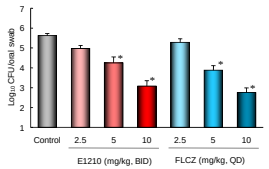


FIG. 1. Efficacy of E1210 and FLCZ in a murine model of oropharyngeal candidiasis
Strain: *C. albicans* IFM49971, Mice: ICR F 5W (n=6), Therapy: bid for 3 days starting 3 days post-infection. * p<0.05 versus control (one-way ANOVA with the Dunnett multiple-comparison test).

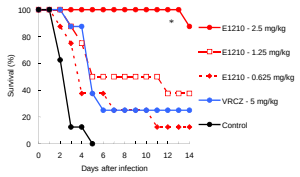


FIG. 2 (B). Efficacies of E1210 and VRCZ in a murine model of disseminated candidiasis caused by azole-resistant *C. albicans*.
Strain: *C. albicans* IFM49738, Mice: ICR F 5W (n=8), Therapy: TID for 3 days. * p<0.05 versus control group (log-rank test with Bonferroni adjustment).

Efficacy in the Disseminated Fusariosis model

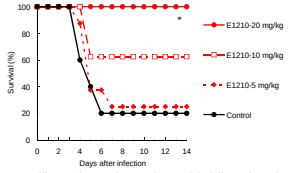


FIG. 3. Efficacy of E1210 in a murine model of disseminated fusariosis caused by *F. solani*.
Strain: *F. solani* IFM50956, Mice: DBA/2N F 8W (n=8-10), Therapy: TID for 5 days. * p<0.05 versus control group (log-rank test with Bonferroni adjustment).

Efficacy in the Pulmonary Aspergillosis model

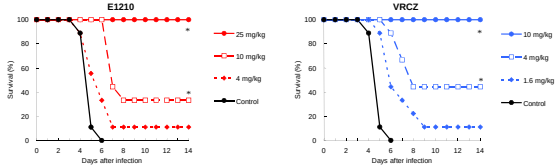


FIG. 4 (A). Efficacies of E1210 and VRCZ in a murine model of pulmonary aspergillosis caused by *A. flavus*.
Strain: *A. flavus* IFM50915, Mice: DBA/2N F 8W (n=9), Therapy: BID for 4 days. * p<0.05 versus control group (log-rank test with Bonferroni adjustment). [See TABLE 2.]

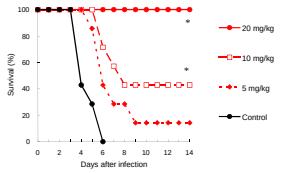


FIG. 4 (B). Efficacy of E1210 in a murine model of pulmonary aspergillosis caused by *A. fumigatus*.
Strain: *A. fumigatus* IFM51126, Mice: DBA/2N F 8W (n=7), Therapy: TID for 7 days. * p<0.05 versus control group (log-rank test with Bonferroni adjustment).

ED_{50} value

TABLE 2. ED_{50s} of E1210 and reference compounds based on survival at day 14

Disseminated Candidiasis caused by *C. albicans* IFM49971

Compound	Route	Dose	ED_{50} (mg/kg)	(95% CI)
E1210	p.o.	BID	4.8	(3.0 - 7.9)
FLCZ	p.o.	QD	1.9	(0.80 - 5.0)
CSFG	i.v.	QD	0.03	(0.026 - 0.064)
L-AmB	i.v.	QD	0.31	(0.20 - 0.49)

Pulmonary Aspergillosis caused by *A. flavus* IFM50915

Compound	Route	Dose	ED_{50} (mg/kg)	(95% CI)
E1210	p.o.	BID	10	(6.8 - 16)
VRCZ	p.o.	BID	3.7	(2.5 - 5.9)
CSFG	i.p.	QD	0.41	(0.21 - 1.3)

The 50% effective dose (ED_{50}) value and 95% confidence interval (CI) were estimated with a probit method. [See FIG.2 (A). and FIG. 4 (A).]

Conclusions

- E1210 dosed orally increased survival duration in a dose dependent manner in mice infected with *C. albicans* (including azole-resistant strains), *A. flavus*, *A. fumigatus* or *F. solani*.
- E1210 (≥5 mg/kg BID) effectively treated oropharyngeal candidiasis in mice.
- E1210 effectively treated disseminated candidiasis caused by azole-susceptible *C. albicans* (≥5 mg/kg BID) and azole-resistant *C. albicans* (≥2.5 mg/kg TID) in mice.
- The efficacy of E1210 in treating disseminated candidiasis was minimally affected by a treatment delay of 24 h.
- E1210 (≥ 20 mg/kg TID) was effective in treating disseminated fusariosis caused by *F. solani* in mice.
- E1210 (≥10 mg/kg BID) was effective in treating pulmonary aspergillosis caused by *A. flavus* and *A. fumigatus* in mice.

References

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