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In Vitro and In Vivo Antifungal Activities of E1211, a Water-Soluble Prodrug of E1210

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Abstract

Background: E1211 is a water-soluble prodrug of E1210 which is a broad-spectrum antifungal with a novel mechanism of action – inhibition of fungal GPI biosynthesis. In this study, in vitro antifungal activity of E1211 and in vivo efficacy of E1211 in murine models of oropharyngeal and disseminated candidiasis, and in pulmonary aspergillosis were each evaluated.
Methods: Growth inhibition was assayed by the broth microdilution method. Synergy was assessed between E1211 and E1210 in *C. albicans*, *C. glabrata* and *A. fumigatus* utilizing the checkerboard method. In vivo studies of E1211 utilized mice immunosuppressed by subcutaneous injection of cortisone acetate or 5-florouracil and infected with *C. albicans* or *A. flavus* under anesthesia. In the oropharyngeal candidiasis model, treatment was initiated 3 days post-infection and given for 3 consecutive days. *C. albicans* was cultured from the oral cavity on the day after the final E1211 dose. In the disseminated candidiasis and pulmonary aspergillosis models, treatment was initiated 1 h after infection and continued for 3 or 4 consecutive days. The 50% effective dose (ED_{50}) was determined at day 14 after infection.
Results: The E1211 MIC range was 2 to 32 μg/mL against *C. albicans*, *C. glabrata* and *A. fumigatus*. This activity was much less than that of its parent, E1210 (MIC range of, 0.004 to 0.06 μg/mL). No antagonism was found between E1211 and E1210. In oropharyngeal candidiasis, 5 and 10 mg/kg (bid) of intravenous E1211 significantly reduced the number of CFU in the oral cavity in comparison to control treatment. In disseminated candidiasis, the ED_{50} was 7.1 and 5.5 mg/kg for intravenous and oral E1211, respectively. In pulmonary aspergillosis, the ED_{50} was 5.9 and 11 mg/kg of intraperitoneal and oral E1210, respectively.
Conclusion: The in vitro antifungal activity of E1211 was about 500-fold lower than that of its parent E1210, and no antagonism was found between E1211 and E1210. E1211 was effective in murine models of oropharyngeal candidiasis, disseminated candidiasis and pulmonary aspergillosis.

Introduction

E1210 is a first-in-class, new antifungal compound that was discovered by the Tsukuba Research Laboratories, Eisai Co., Ltd. (Ibaraki, Japan). It has strong broad-spectrum antifungal activity with a novel mechanism of action – inhibition of fungal GPI biosynthesis [1, 5, 6, 7, 9] and demonstrated therapeutic efficacy in the various mouse models as reported at 50th ICAAC last year [2].

E1211, a water-soluble prodrug of E1210, is efficiently converted to E1210 in animals [8]. In the current studies, the efficacies of E1211 and reference drugs, such as fluconazole, voriconazole, caspofungin and liposomal amphotericin B, were evaluated in murine models of oropharyngeal and disseminated candidiasis, disseminated fusariosis, pulmonary aspergillosis and pulmonary scedosporiosis.

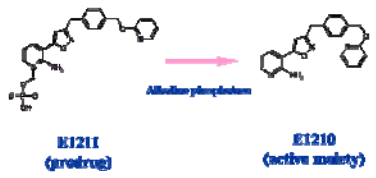

FIG. 1. Chemical structures of E1210 and E1211

Methods

In vitro Study

Organisms: The strains of *Candida* and *Aspergillus spp.* used in this study were obtained from the Medical Mycology Research Center, Chiba University (Chiba, Japan). They were stored as glycerin stock cultures at –80°C. The cell suspensions were diluted with RPMI 1640 medium to obtain an inoculum size of 2×10^3 cells/mL for *Candida* strains or 2×10^4 cells/mL for *Aspergillus* strains.

In vitro Antifungal Activity: The broth microdilution method was employed in accordance with the guidelines presented in the Clinical and Laboratory Standards Institute (CLSI) documents M27-A3 [3] and M38-A2 [4] for the determination of minimum inhibitory concentrations (MICs) of E1211 and E1210 and of the combination effect of E1211 plus E1210 against *C. albicans, C. glabrata* and *A. fumigatus*. The fractional inhibitory concentration (FIC) indices were calculated.

In vivo Study

Organisms: Strains IFM49971 of *Candida albicans*, IFM50915 of *Aspergillus flavus*, IFM51126 of *Aspergillus fumigatus*, IFM49205 of *Scedosporium prolificans* and IFM49271 of *Fusarium solani* used in current studies were obtained from the Medical Mycology Research Center, Chiba University (Chiba, Japan), and were stored as glycerin stock 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 and given 1 mg/mL tetracycline hydrochloride via their drinking water. *C. albicans* was grown on Sabouraud dextrose agar (SDA) at 35°C for 2 days. The cell number was adjusted to the required density with sterile normal saline. Aliquots (10 μL) of *C. albicans* suspension were inoculated into the oral cavity of mice that were anesthetized subcutaneously with chlorpromazine hydrochloride (0.5 mg/mouse). The challenge dose was 4×10^5 colony-forming units (CFU)/mouse. E1211 was intravenously or orally administered twice daily, and fluconazole (FLCZ) was intravenously administered once daily for three consecutive days starting 3 days post-infection. The *C. albicans* cells in the oral cavity were collected the day after the final administration and the number of cells was counted as the number of CFUs.

Disseminated Candidiasis and Fusariosis models: ICR or DBA/2N mice were immunosuppressed with 200 mg/kg of 5-FU administered subcutaneously 6 days prior to infection and given 0.1 mg/mL ciprofloxacin via their drinking water. *C. albicans* were cultured on a SDA plate at 35°C for 2 days. *F. solani* was cultured on a potato dextrose agar (PDA) plate at 30°C for 7 days. The cells were suspended in sterile normal saline (containing 0.05% Tween 80 for *F. solani* cell preparation), and the cell number was counted. The final inoculum was adjusted to the required density in sterile normal saline (containing 0.05% Tween 80 for *F. solani* cell preparation). Infection was induced by the intravenous inoculation of 0.2 mL suspension of *C. albicans* cells (0.8×10^4 CFU/mouse) or *F. solani* conidia (8.0×10^3 cells/mouse) into the lateral tail vein. Antifungal therapy was initiated 1 h after infection and was continued for three consecutive days for candidiasis model or for five consecutive days for fusariosis model. E1211 was intravenously, orally or intraperitoneally administered twice or 3 times daily, FLCZ and liposomal amphotericin B (L-AmB) were intravenously or intraperitoneally administered once daily. The survival rate and survival period were determined over 14 days.

Pulmonary Aspergillosis and Scedosporiosis models: DBA/2N mice were immunosuppressed with 200 mg/kg of 5-FU administered subcutaneously 5-6 days prior to infection and given 0.1 mg/mL ciplofloxacin via their drinking water. *A. flavus*, *A. fumigatus* and *S. prolificans* were cultured on a PDA plate at 35°C for 7 days. The conidia were suspended in sterile normal saline containing 0.05% Tween 80, and the cell number was counted. The final inoculum was adjusted to the required density in sterile normal saline containing 0.05% Tween 80. The mice were anesthetized intravenously with 0.1 mL ketamine hydrochloride (4.17 mg/mL). Infection was induced by the intranasal inoculation of 0.05 mL suspension of *A. flavus* conidia (3.0×10^4 conidia/mouse), *A. fumigatus* conidia (6.0×10^4 conidia/mouse) or *S. prolificans* conidia (2.5×10^4 conidia/mouse). Antifungal therapy was initiated 1 h after infection and was continued for four to seven consecutive days. E1210 or voriconazole (VRCZ) was administered intraperitoneally twice or 3 times daily, and L-AmB was administered intraperitoneally once daily. 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 E1211

Fungal strain		MIC (μg/mL)		MIC ratio	FIC index[a]
		E1211	E1210	(E1211/E1210)	
C. albicans	IFM49686	8	0.016	512	0.625
C. albicans	IFM49741	4	0.008	512	0.750
C. albicans	IFM49971	2	0.004	512	1.000
C. glabrata	IFM5864	16	0.06	256	0.625
C. glabrata	IFM46887	32	0.06	512	0.750
C. glabrata	IFM46888	32	0.06	512	0.750
A. fumigatus	IFM51126	16	0.03	512	1.000
A. fumigatus	IFM51357	16	0.03	512	0.750
A. fumigatus	IFM52108	16	0.03	512	1.000

[a] FIC, fractional inhibitory concentration; FIC index ≤0.5, synergistic; 0.5<FIC index≤4, indifferent; 4<FIC index, antagonistic.

Efficacy in the Oropharyngeal Candidiasis model

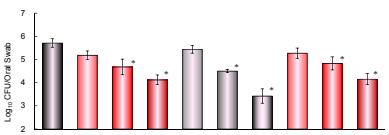

FIG. 2. Efficacy of intravenously or orally administered E1211 in a murine model of oropharyngeal candidiasis. Strain: *C. albicans* IFM49971, Mice: ICR F 5W (n=6), Therapy: bid for 3 days starting 3 days post-infection. * p<0.05 versus control (one-way ANOVA with the Dunnett multiple-comparison test).

Efficacy in the Disseminated Candidiasis model

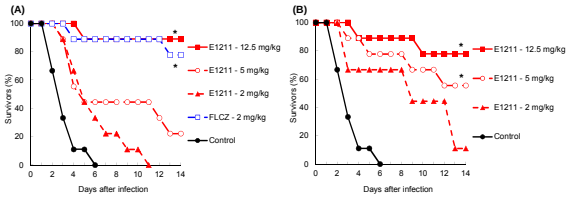

FIG. 3. Efficacy of E1211 administered intravenously (A) or orally (B) in a murine model of disseminated candidiasis caused by *C. albicans*. Strain: *C. albicans* IFM49971, Mice: ICR F 5W (n=9), Therapy: bid (E1210) or qd (FLCZ) for 3 days. E1211 was administered as equivalent to E1210. * p<0.05 versus control group (log-rank test with Bonferroni adjustment).

Efficacy in the Disseminated Fusariosis model

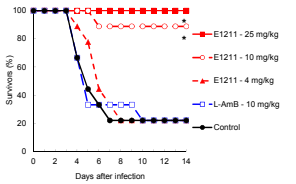

FIG. 4. Efficacy of E1211 administered intraperitoneally in a murine model of disseminated fusariosis caused by *F. solani*. Strain: *F. solani* IFM49271, Mice: DBA/2N F 8W (n=9), Therapy: tid (E1211) or qd (L-AmB) for 5 days. E1211 was administered as equivalent to E1210. * p<0.05 versus control group (log-rank test with Bonferroni adjustment).

Efficacy in the Pulmonary Aspergillosis model

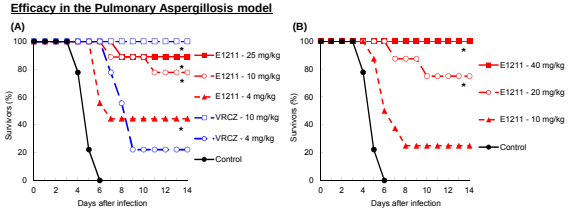

FIG. 5. Efficacy of E1211 administered intraperitoneally in a murine model of pulmonary aspergillosis caused by *A. flavus* (A) or *A. fumigatus* (B). Strain: *A. flavus* IFM50915, *A. fumigatus* IFM51126, Mice: DBA/2N F 8W (n=8 - 9), Therapy: bid for 5 days in *A. flavus* infection model or tid for 5 days in *A. fmigatus* infection model. E1211 was administered as equivalent to E1210. * p<0.05 versus control group (log-rank test with Bonferroni adjustment).

Efficacy in the Pulmonary Scedosporiosis model

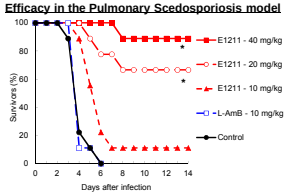

FIG. 6. Efficacy of E1211 administered intraperitoneally in a murine model of pulmonary scedosporiosis caused by *S. prolificans*. Strain: *S. prolificans* IFM49205, Mice: DBA/2N F 8W (n=9), Therapy: tid (E1211) or qd (L-AmB) for 5 days. E1211 was administered as equivalent to E1210. * p<0.05 versus control group (log-rank test with Bonferroni adjustment).

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

Disseminated Candidiasis caused by *C. albicans* IFM49971

Compound	Route	Dose	ED_{50} (mg/kg)	(95% CI)
E1211	i.v.	BID	7.1	(4.8 – 11)
E1211	p.o.	BID	5.5	(3.3 – 11)
FLCZ	i.v.	QD	1.4	(0.94 – 2.1)
MCFG	i.v.	QD	0.35	(0.16 – 0.4)
L-AmB	i.v.	QD	0.19	(0.12 – 0.28)

Pulmonary Aspergillosis caused by *A. flavus* IFM50915

Compound	Route	Dose	ED_{50} (mg/kg)	(95% CI)
E1211	i.p.	BID	5.9	(3.3 – 9.8)
E1211	p.o.	BID	11	(4.0 – 25)
VRCZ	i.p.	BID	4.4	(1.6 – 10)
MCFG	i.p.	QD	0.42	(0.28 – 0.66)
L-AmB	i.p.	QD	NE[a]	-

[a] NE, not estimated.

Conclusion

- The in vitro antifungal activity of E1211 was about 500-fold lower than that of its parent E1210.
- No antagonism was found between E1211 and E1210.
- E1211 increased survival time in a dose dependent manner in mice infected with *C. albicans*, *A. flavus*, *A. fumigatus*, *F. solani* or *S. prolificans*.
- E1211 dosed intravenously or orally (≥5 mg/kg BID) showed efficacy in treating oropharyngeal candidiasis in mice.
- E1211 dosed intravenously (≥12.5 mg/kg BID) or orally (≥5 mg/kg BID) was effective against disseminated candidiasis in mice.
- E1211 dosed intraperitoneally (≥ 10 mg/kg TID) was effective against disseminated fusariosis caused by *F. solani* in mice.
- E1211 dosed intraperitoneally was effective against pulmonary aspergillosis caused by *A. flavus* (≥4 mg/kg BID) and *A. fumigatus* (≥20 mg/kg TID) in mice.
- E1211 dosed intraperitoneally (≥ 20 mg/kg TID) was effective against pulmonary scedosporiosis caused by *S. prolificans* in mice.

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