51st ICAAC
F1-1376

Physicochemical properties and Nonclinical Pharmacokinetics of E1211, a Water-Soluble Prodrug of E1210

M. OKUBO, E. YAMAMOTO and K. HATA*

Eisai Product Creation Systems, Eisai Co., Ltd., Tsukuba, Japan

Katsura Hata, PhD
Eisai Product Creation Systems, Eisai Co., Ltd.
Tsukuba, Japan
Phone: 81 29 847 5742
Fax: 81 29 847 2037
K-hata@hhc.eisai.co.jp

Abstract

Background: E1211 is a water-soluble prodrug of E1210, a new antifungal compound with a novel mechanism of action - fungal GPI-biosynthesis inhibition. In this study, the physicochemical properties of E1211 were demonstrated, and the pharmacokinetic (PK) profiles of E1210 and E1211 were assessed after administration of E1211 to mice, rats, dogs, and monkeys.
Methods: Solubility of E1211 in JP-1 fluid (HCl/KCl), 100 mmol/L phosphate buffer, and 10 and 50 mmol/L sodium hydroxide were each evaluated. E1210 or E1211 was administered intravenously or orally to male mice, rats, dogs, and monkeys. Plasma levels of E1210 and E1211 were each determined by an LC/MS/MS method. PK parameters of E1210 and E1211 were calculated using model-independent analyses. The percentage conversion of E1211 to E1210 in vivo was evaluated using the AUC ratio of E1210 after intravenous (i.v.) administration of E1211 and E1210. The relative bioavailability (BA) of E1211 was determined by calculating the AUC ratio of E1210 after oral administration of E1211 and E1210 after i.v. administration of E1210. In addition, the conversion of E1211 to E1210 in human plasma and in S9 tissue fractions was evaluated.
Results: Above pH 6.5, the solubility of E1211 increased with increasing pH and the solubility at pH 7.6 was more than 24 mg/mL. The percentage conversion of E1211 to E1210 was approximately 100%, 100%, 60% and 100% in mice, rats, dogs, and monkeys, respectively. The relative BAs of E1210 after E1211 oral administrations were 57%, 53%, 47%, and 41% in mice, rats, dogs, and monkeys, respectively. These relative BAs after E1211 administrations were similar to the BAs of E1210 in all species tested. E1211 was efficiently converted to E1210 in the human liver and intestine S9 fraction and to some extent in the plasma.
Conclusion: E1211 was shown to be soluble at pH levels suitable for an intravenous formulation. E1211 was efficiently converted to E1210 in animals, and in human S9 tissue fractions prepared in vitro. E1211 showed acceptable physicochemical and pharmacokinetic properties that allow for potential future clinical development as an E1210 prodrug.

Introduction

The expanding population of immunocompromised patients receiving immunosuppressive or anticancer therapy has resulted in an increased incidence of opportunistic mycoses. Invasive fungal infections have become increasingly common among such immunocompromised and immunosuppressed patients, including solid-organ and hematopoietic stem-cell transplant recipients and individuals on immunosuppressive drug regimens. In response, the guidelines for treatment of invasive fungal infections previously established have been recently updated. There is, however, still a high rate of morbidity and mortality associated with invasive fungal infections, because the currently available antifungal drugs, such as polyenes, azoles and echinocandins, are limited in terms of their antifungal spectrum, side effects and mode of action. In addition, there has been an increase in resistance to commonly used antifungal compounds, especially azoles, and an epidemiological shift towards more drug-resistant strains. Thus, there is a critical need for new antifungal compounds with a novel mechanism of action that have a broad spectrum of activity and fewer side effects, although the development of such new antifungals is just starting to be reported.
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, 3, 5] and demonstrated therapeutic efficacy in the various mouse model as reported at 50th ICAAC last year [2].
E1211 is a water-soluble prodrug of E1210, and demonstrated therapeutic efficacy comparable to E1210 in the various mouse models [4]. In this study, the physicochemical properties of E1211 were demonstrated, and the pharmacokinetic profiles of E1210 and E1211 were assessed after administration of E1211 to mice, rats, dogs, and monkeys.

Methods

Physicochemical properties

Lipophilicity

E1211 was dissolved in PBS-acetonitrile (60:40, v/v) at 100 μg/mL. Ten μL aliquot of the sample was injected to high-performance liquid chromatograph (HPLC) with UV detector. An polymer based octadecyl-poly(vinyl alcohol) column (4.6 mm I.D. x 30 mm) was employed as an HPLC column. A mixture of PBS and acetonitrile (60: 40, v/v) was delivered at 1 mL/min as HPLC mobile phase. The logarithm of retention factor (log kw) was determined from retention time of E1211. The log P scale lipophilicity was determined from linear regression curve of logP values and logkw of standard compounds.

Solubility

Equilibrium solubility of E1211 crystal in aqueous solution was determined. The JP-1 fluid (HCl/KCl, pH 1.2), 100 mmol/L phosphate buffer, and 10 and 50 mmol/L sodium hydroxide was employed as solvents. The solubility of E1211 in each solvent was determined by HPLC.

Pharmacokinetics / ADME

E1210 or E1211 was administered intravenously or orally to male mice, rats, dogs, and monkeys. Dosage of E1211 was set to be equivalent to that of E1210 using molecular weight ratio of 1.3 (M.W. of E1211/M.W. of E1210). Plasma levels of E1210 and E1211 were each determined by an LC/MS/MS method. PK parameters of E1210 and E1211 were calculated using model-independent analyses. The conversion rate of E1211 to E1210 in vivo was evaluated based on the plasma concentration-time course and calculated by the following equation;

$$\text{Conversion rate} = \frac{\text{AUC of E1210 after i.v. administration of E1211}}{\text{AUC of E1210 after i.v. administration of E1210}}$$

The relative bioavailability (BA) of E1211 was determined as follows;

$$\text{Relative bioavailability} = \frac{\text{AUC of E1210 after p.o. administration of E1211}}{\text{AUC of E1210 after i.v. administration of E1210}}$$

In addition, the conversion of E1211 to E1210 in human plasma and in S9 tissue fractions was evaluated.

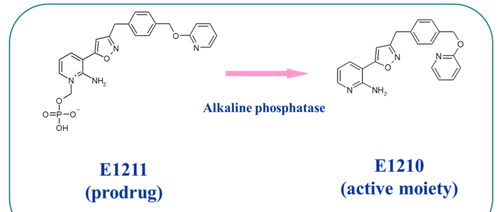

FIG. 1. Conversion of E1211 to E1210.

Results

Physicochemical properties of E1211 DS

MW E1210: 358.39, E1211: 468.40

Lipophilicity

The value for E1211 was not more than -0.07.

Solubility in buffer

Relationship between resultant pH and the solubility is shown in FIG.2. Above pH 6.5, the compound solubility increased with pH and the solubility at pH 7.6 was more than 24 mg/mL. The apparent pKa value of the compound was calculated as 6.32 from the obtained pH-solubility profile.

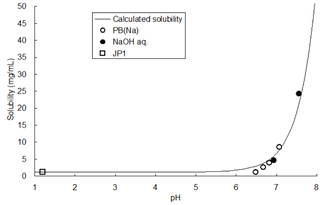

FIG. 2. Solubility of E1211 in JP-1 fluid, phosphate buffer and sodium hydroxide at 25 ºC.
Open square; JP-1 fluid, Open circle; phosphate buffer, closed circle; sodium hydroxide, solid line; calculated values by Henderson-Hasselbalch equation as mono-acid.

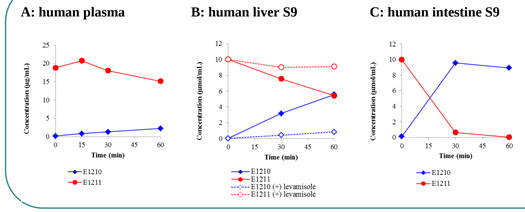

FIG. 3. Conversions of E1211 to E1210 in human plasma, liver and intestine S9 fraction.
Levamisole is an inhibitor of liver alkaline phosphatase. S9 fractions were suspended in 100 mmol/L Tris-HCl buffer, pH 7.4 to be 0.2 mg protein/mL. Incubations were performed at 37ºC.

Table 1. Pharmacokinetic parameters of E1210 after Single Intravenous Administration of E1210 or E1211, and conversion rate of E1211 to E1210 in mice, rats, dogs and monkeys.

Species	Mouse		Rat		Dog		Monkey	
Administration	E1210	E1211	E1210	E1211	E1210	E1211	E1210	E1211
Route and Dose	i.v. 10 mg/kg	i.v. 13 mg/kg	i.v. 1 mg/kg	i.v. 1.3 mg/kg	i.v. 1 mg/kg	i.v. 1.3 mg/kg	i.v. 1 mg/kg	i.v. 1.3 mg/kg
Analyte	E1210	E1210	E1210	E1210	E1210	E1210	E1210	E1210
C_{max} (ng/mL)	–	5050.3	–	560.1	–	1133.8	–	2961.7
t_{max} (h)	–	0.1	–	0.1	–	0.1	–	0.1
$t_{1/2}$ (h)	4.4	3.2	2.7	2.8	4.9	5.2	3.8	3.8
$AUC_{(0\text{-}inf)}$ (ng ·h/mL)	5822.2	6120.1	1390.4	1485.4	4763.6	2886.7	5784.4	5930.8
CL_{total} (mL/h/kg)	1717.6	–	729.4	–	215.7	–	179.4	–
Vd_{ss} (mL/kg)	1995.0	–	2525.8	–	1229.2	–	744.4	–
Conversion rate	–	1.05	–	1.07	–	0.6	–	1.05

Each parameter represents the mean of two animals in mice, four animals in rats, dogs, and monkeys.
–: Not applicable.

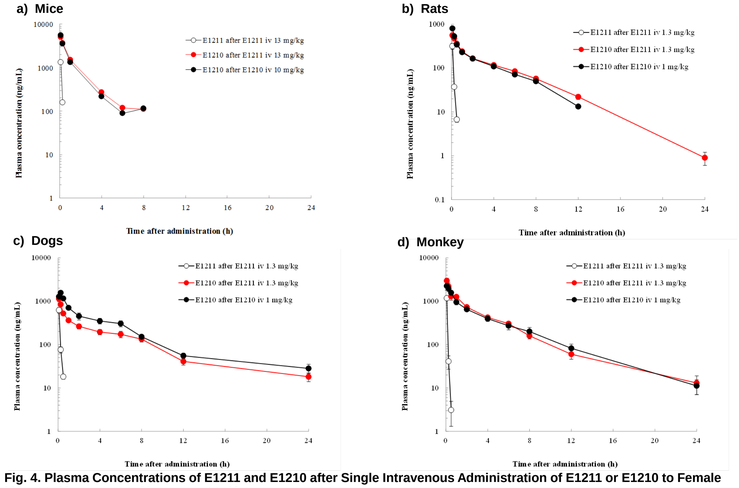

Fig. 4. Plasma Concentrations of E1211 and E1210 after Single Intravenous Administration of E1211 or E1210 to Female Mice (a), Male rats (b), Male dogs (c), Male monkeys (d). Each point represents the mean of two animals in mice, the mean ± SEM of four animals in rats, dogs, and monkeys

Table 2. Pharmacokinetic parameters of E1210 after Single OralAdministration of E1210 or E1211, and conversion rate of E1211 to E1210 in mice, rats, dogs and monkeys.

	Mouse		Rat		Dog		Monkey	
Administration	E1210	E1211	E1210	E1211	E1210	E1211	E1210	E1211
Route and Dose	p.o. 1 mg/kg	p.o. 1.3 mg/kg	p.o. 1 mg/kg	p.o. 1.3 mg/kg	p.o. 1 mg/kg	p.o. 1.3 mg/kg	p.o. 1 mg/kg	p.o. 1.3 mg/kg
Analyte	E1210	E1210	E1210	E1210	E1210	E1210	E1210	E1210
C_{max} (ng/mL)	1135.7	1108.7	207.1	267.1	624.2	675.1	501.1	349.4
t_{max} (h)	0.25	0.5	0.4	0.4	0.4	0.4	2.3	1.5
$t_{1/2}$ (h)	1.5	1.5	2.8	2.5	4.4	5.2	3.7	3.9
$AUC_{(0\text{-}inf)}$ (ng ·h/mL)	3735.0	3332.8	625.6	734.8	2057	2289.4	3586.5	2388.2
BA (%)	64.2	–	45.0	–	42.9	–	62.9	–
Relative BA (%)	–	57.2	–	52.9	–	47.2	–	41.2

Each parameter represents the mean of two animals in mice, four animals in rats, dogs, and monkeys.
–: Not applicable.

Conclusion

- E1211 was shown to be soluble at pH levels suitable for an intravenous formulation.
- E1211 was efficiently converted to E1210 in animals, and in human S9 tissue fractions prepared *in vitro*.
- In addition, E1211 showed good oral absorption as oral formulation.
- Totally, E1211 showed acceptable physicochemical and pharmacokinetic properties that allow for potential future clinical development as an E1210 prodrug.
- Furthermore, the above data would support the pharmacologic efficacies of E1211 observed in murine infection models (F1-1377).

References

1. Miyazaki, M. *et al*. 2011. In vitro antifungal activity of E1210, a novel antifungal, against clinically important yeasts and moulds. Antimicrob. Agents Chemother., in press.
2. Hata, K. *et al*. 2011. Efficacy of Oral E1210, a New Broad-Spectrum Antifungal with a Novel Mechanism of Action, in Murine Models of Candidiasis, Aspergillosis, and Fusariosis. . Antimicrob. Agents Chemother., in press.
3. Pfaller, M. A. *et al*. 51st ICAAC abstr. F1-1373, F1-1374, F1-1375 (2011)
4. Hata, M. *et al*. 51st ICAAC abstr. F1-1377 (2011)
5. Watanabe, N. *et al*. 51st ICAAC abstr. F1-1378 (2011)