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Preclinical Pharmacokinetics and Toxicology of E1210, a New Broad-Spectrum Antifungal

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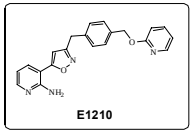
Abstract

Background: E1210 is a new antifungal compound with a novel mechanism of action, glycosylphosphatidylinositol-biosynthesis inhibition. In this study, the pharmacokinetic profile of E1210 was characterized in mice, rats, dogs, and monkeys. Toxicological findings of E1210 after 7-day oral administration in rats are also presented.

Methods: Pharmacokinetics: E1210 was intravenously or orally administered to male CD-1 mice, Sprague-Dawley (SD) rats, beagle dogs, and cynomolgus monkeys. Plasma levels of E1210 were determined by an LC-MS/MS method. Pharmacokinetic parameters of E1210 were calculated by model-independent analyses.
Toxicology: E1210 was administered orally once a day for 7 days to male and female SD rats at doses of 100, 300, or 1000 mg/kg. The following were evaluated: mortality, clinical signs, body weights, food consumption, hematology, blood chemistry, toxicokinetics, hepatic drug metabolizing enzymes, and macroscopic- and microscopic-pathology.

Results: Pharmacokinetics: The pharmacokinetic profile of E1210 in animals was characterized by low to moderate plasma clearance and a moderate volume of distribution. E1210 dosed as an oral solution was rapidly absorbed and achieved maximum concentration at 30-60 min after administration in mice and rats. When dosed as an oral powder, a delay in absorption was observed in dogs and monkeys. Oral bioavailability ranged between 58-73% in animals.
Toxicology: Mortality/morbidity associated with anorexia and gastrointestinal lesions was noted at 1000 mg/kg. Adaptive hepatocellular hypertrophy resulting from liver enzyme induction was observed at 300 mg/kg and higher. No test article-related change was noted at 100 mg/kg.

Conclusion: Pharmacokinetics: E1210 showed an acceptable pharmacokinetic profile in all species allowing for future clinical development.
Toxicology: E1210 did not show any unfavorable toxicity in this study and support further studies being conducted.



E1210

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 potent, broad-spectrum, antifungal activity with a novel mechanism of action – inhibition of fungal GPI biosynthesis.[1,2] E1210 dosed orally showed efficacy in murine models of oropharyngeal candidiasis, disseminated candidiasis and pulmonary aspergillosis[3] and the in vivo efficacy of E1210 correlated well with pharmacokinetic-pharmacodynamic parameters such as AUC/MIC and Time above MIC.[4] In this study, the pharmacokinetic profile of E1210 was characterized in mice, rats, dogs, and monkeys. Toxicological findings of E1210 after 7-day oral administration in rats are also presented.

Methods

Pharmacokinetics

Strain and sex of studied animal, dosing route, dosage, and formulation are summarized below.

Species	Strain	Sex	Dosing route		Dosage (mg/kg)	Formulation
Mouse	CD-1	male	iv	via tail vein	1	1mM HCl in 5% glucose
			po	by gastric gavage	1	1mM HCl in 5% glucose
Rat	Sprague-Dawley	male	iv	via jugular vein	3	30 mM HCl in 5% glucose
			po	by gastric gavage	30	40 mM HCl in 5% glucose
Dog	Beagle	male	iv	via cephalic vein	1	100 mM HCl in 5% glucose
			po	by capsule	10	Crystalline powder 4-fold diluted with lactose
Monkey	Cynomolgus	male	iv	via cephalic vein	0.4	10 mM HCl in 5% glucose
			po	by capsule	10	Crystalline powder

After administration of E1210 as designed above, blood samples were drawn from the vena cava, jugular vein, cephalic vein, and cephalic vein of mice, rats, dogs, and monkeys, respectively at designated timepoints. Plasma samples were obtained by centrifuging blood (8000xg, for 5min). After deproteinization with methanol, the extracted sample was analyzed by LC/MS(/MS). The concentrations of E1210 in plasma were determined by an internal standard method using MassLynxTM (Waters, Milford, MA, US). Pharmacokinetic parameters of E1210 were calculated using model independent analyses.

Toxicology

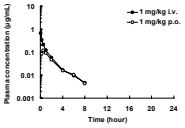
E1210 was administered orally by gavage once a day for 7 days to male and female Sprague-Dawley rats (3 animals/group/gender) at doses of 100, 300, or 1000 mg/kg. A control group received an equivalent volume (10 mL/kg) of vehicle (0.4 mol/L hydrochloric acid). All rats found dead or moribund were necropsied as soon as they were discovered, and all surviving animals were necropsied after 7-days of E1210 administration. Additional rats (3 animals/group/gender, excluding control group) were included and dosed with E1210 in the same manner for toxicokinetics. The following were evaluated: mortality, clinical signs, body weights, food consumption, hematology, blood chemistry, toxicokinetics, hepatic drug metabolizing enzymes, and macroscopic- and microscopic-pathology.

Results

Pharmacokinetics

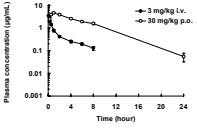
Mean plasma concentrations of E1210 after oral and intravenous administration to mice, rats, dogs, and monkeys are shown Figure 1. Pharmacokinetic parameters are summarized in Table 1.
In mice, after intravenous administration, E1210 exhibited moderate clearance and volume of distribution and the elimination half-life was 2.2 hours. E1210 dosed as an oral solution was rapidly absorbed and achieved maximum concentration at 0.5 hours post-dose. Oral bioavailability was 57.5%.
In rats, after intravenous administration, E1210 exhibited low clearance and volume of distribution, and the elimination half-life was 3.7 hours. E1210 dosed as an oral solution was rapidly absorbed and achieved maximum concentration at 1.13 hours post-dose. Oral bioavailability was 65.3%.
In dogs, after intravenous administration, E1210 exhibited low clearance and moderate volume of distribution, resulting in the elimination half-life of 3.2 hours. E1210 dosed as an oral powder showed a delay in absorption and achieved maximum concentration at 2.7 hours post-dose. Oral bioavailability was 72.8%.
In monkeys, after intravenous administration, E1210 exhibited low clearance and moderate volume of distribution, resulting in a relatively long elimination half-life (5.2 hours). E1210 dosed as an oral powder showed a further delay in absorption compared to dogs and tmax was 7.0 hours. Oral bioavailability was 68.9%.

a. Mice



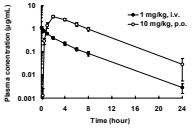
Each value represents the mean of two animals.

b. Rats



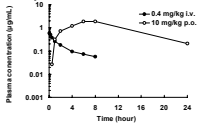
Each value represents the mean±S.E.M of four animals

c. Dogs



Each value represents the mean±S.E.M of three animals

d. Monkeys



Each value represents the mean of two animals.

FIGURE 1 Time-Plasma Concentration Profiles of E1210 after Oral and Intravenous Administrations to Mice (a), Rats (b),Dogs (c), and Monkeys (d)

TABLE 1 Pharmacokinetic Parameters of E1210 after Oral and Intravenous Administrations to Mice, Rats, Dogs, and Monkey

Parameters		mouse		rat		dog		monkey	
		i.v.	p.o.	i.v.	p.o.	i.v.	p.o.	i.v.	p.o.
		1 mg/kg	1 mg/kg	3 mg/kg	30 mg/kg	1 mg/kg	10 mg/kg	0.4 mg/kg	10 mg/kg
t_{max}	(h)	-	0.50	N.A.	1.13	-	2.67	-	7.00
C_{max}	(μg/mL)	-	0.12	N.A.	4.55	-	3.52	-	2.22
$t_{1/2}$	(h)	2.15	2.14	3.65	3.31	3.17	3.14	5.15	5.44
$AUC_{(0-inf)}$	(μg·h/mL)	0.48	0.28	4.37	28.55	3.22	23.44	1.63	27.99
$MRT_{(0-inf)}$	(hr)	1.61	2.47	3.87	5.72	3.78	5.42	5.75	9.73
CL	(L/h/kg)	2.06	-	0.71	-	0.32	-	0.25	-
Vd	(L/kg)	3.33	-	2.65	-	0.52	-	1.42	-
F	(%)	-	57.5	-	65.3	-	72.8	-	68.9

Each parameter represents the mean of two, four, three, and four animals in mice, rats, dogs, and monkeys, respectively.
-: not applicable or not calculated.

Toxicology

Toxicologic findings and toxicokinetics of E1210 after 7-Days of oral administration to rats are summarized in Table 2 and Table 3, respectively. At 1000 mg/kg, mortality (1 male) and morbidity (1 male) were observed, which were thought to be caused by anorexia and/or gastrointestinal lesions. Adaptive hepatocellular hypertrophy resulting from liver enzyme induction was observed at 300 mg/kg and higher. No test article related change was observed at 100 mg/kg.. Apparent gender differences in C_{max} or AUC were not observed.

TABLE 2 Toxicologic Findings of E1210 after 7-Day Oral Administration in Rats

Dosage	Findings
≥ 100 mg/kg	Increases in activity of hepatic CYP1A (both genders) and CYP3A (female)
≥ 300 mg/kg	Increases in total cholesterol, AST and liver weight *
	Hypertrophy of hepatocytes *
≥ 1000 mg/kg	Death (1 male) and moribundity (1 male)
	Increases in ALT * and total bilirubin
	Decreases in creatinine and ALP
	Decreases in body weight and food consumption associated with anorexia
	Distended stomach
	Epithelial hyperplasia in the forestomach

*: considered to be adaptive changes due to CYP1A induction in the liver

TABLE 3 Toxicokinetics of E1210 in 7-day Oral Dose Range Toxicity Study in Rats.

Sex	Dose (mg/kg)	Day	C_{max} (mg/mL)	AUC_{0-24hr} (mg×hr/mL)
Male	100	1	9.31 ± 0.95	93.55 ± 21.19
		7	11.51 ± 2.48	132.75 ± 28.74
	300	1	17.37 ± 2.45	200.87 ± 46.69
		7	26.11 ± 7.01	418.27 ± 92.5
	1000	1	23.5 ± 1.21	316.53 ± 47.32
		7	47.50*	807.76*
Female	100	1	15.17 ± 3.48	154.37 ± 18.43
		7	18.50 ± 9.56	199.49 ± 85.43
	300	1	20.14 ± 3.67	290.49 ± 34.85
		7	30.57 ± 5.93	510.57 ± 118.7
	1000	1	25.26 ± 2.05	383.52 ± 74.3
		7	52.37 ± 8.82	943.78 ± 223.06

C_{max} and AUC_{0-24hr} represent the mean ± S.D. of 3 animals (*: the value of 1 anima, two animals had no data due to euthanasia or death).

Conclusions

Pharmacokinetics:

- The pharmacokinetic profile of E1210 in animals was characterized by low to moderate plasma clearance and a moderate volume of distribution. Bioavailability ranged between 58-73% in animals.
- E1210 showed an acceptable pharmacokinetic profile in all species allowing for future clinical development.

Toxicology:

- Mortality/morbidity was observed at 1000 mg/kg and was thought to be caused by anorexia and/or gastrointestinal lesions.
- Adaptive hepatocellular hypertrophy resulting from liver enzyme induction was observed at 300 mg/kg and higher.
- No test article related changes were observed at 100 mg/kg.
- E1210 did not show any unfavorable toxicity in this study and support further studies being conducted.

References

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