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In Vivo Pharmacodynamic Correlates of Success for E1210 Treatment of Disseminated Candidiasis

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Abstract

Background

E1210 is a new, broad-spectrum antifungal that inhibits the biosynthesis of fungal glycosylphosphatidylinositol (GPI). In order to determine the optimal clinical trial dose, antimicrobial pharmacodynamic characterization is important. It correlates plasma drug exposures with the probability of treatment efficacy. In this study, we examined the relationship between E1210 drug dose, MIC, and efficacy in a murine disseminated candidiasis model. The pos-tantifungal effects (PAFEs) against *Candida albicans* were also examined *in vivo* and *in vitro*.

Methods

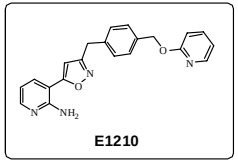
To determine the best pharmacodynamic property that correlated with outcome, three pharmacodynamic parameters (AUC/MIC, C_{max}/MIC, and time above MIC) were assessed using mouse pharmacokinetics and the in vitro antifungal activity of E1210. In a murine disseminated candidiasis model, orally administrated E1210 was studied using several drug doses and dosing intervals. The correlation between fungal burden in murine kidneys and pharmacodynamic parameters were examined. The in vivo PAFE was determined using the time to fungal regrowth in kidneys post E1210-treatment serum drug levels falling below the MIC, and compared to that of untreated animals. In vitro PAFEs were also defined by the number of CFU in liquid culture after E1210 removal.

Results

E1210 treatment decreased the renal fungal burden in a dose-dependent manner. Among the 3 parameters tested, AUC/MIC and time>MIC correlated better with in vivo efficacy than C_{max}/MIC. In vivo PAFEs in mice were observed about 10 hours after the serum drug levels fell below the MIC. In vitro PAFEs at concentrations 4 × MIC, 16 × MIC, and 64 × MIC were 1.8, 3.9, and 4.6 hours, respectively.

Conclusion

The in vivo efficacy of E1210 correlated best with AUC/MIC and T>MIC. E1210 showed significant PAFEs both *in vivo* and *in vitro*. These results will be used to select the E1210 target serum level for clinical trials.



E1210

Introduction

Invasive fungal infections have become increasingly more common among immunocompromised or immunosuppressed patients, including solid-organ or hematopoietic stem-cell transplant recipients and individuals who are 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 adverse effects, pharmacokinetics, and mechanisms 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. Hence, there is a critical need for new antifungal compounds that have a broad spectrum of activity and have fewer adverse effects.

When a new antifungal is planned to be introduced into clinical trials, it is important to predict a clinically effective dosing regimen. Antimicrobial pharmacodynamic characterizations have provided insight into the link between drug exposures and treatment efficacy [1, 2]. Therapeutic outcome predictions based upon these pharmacodynamic relationships have correlated well with treatment outcomes for infections due to both susceptible and resistant pathogens. This has proven useful for the design of optimal dosing regimens and the development of susceptibility breakpoint determinations.

E1210 is a new antifungal compound, discovered by Tsukuba Research Laboratories, Eisai Co., Ltd., with a novel mechanism of action: glycosylphosphatidylinositol-biosynthesis inhibition [3, 4]. This compound showed potent in vitro activity and was effective in murine infection models after oral administration [5–7]. The purpose of this study was to examine the antifungal pharmacodynamics and the post-antifungal effects (PAFEs) of E1210 in a murine candidiasis model to provide insight into the correlations between drug exposure, in vitro susceptibility and treatment efficacy. The PAFEs against *C. albicans* were also examined *in vitro*.

Methods

Organisms

The IFM49971strain of *Candida albicans* used in these studies was obtained from the Medical Mycology Research Center, Chiba University (Chiba, Japan).

Disseminated candidiasis model

All mice were rendered neutropenic by subcutaneous injection of 5-FU (200 mg/kg), 6 days prior to infection (day -6). *C. albicans* was cultured on a Sabouraud dextrose agar (SDA) plate after incubation at 35ºC overnight. The *C. albicans* cells obtained from the surface of the SDA plate were suspended in sterile saline and counted using a hemocytometer. The final inoculum was adjusted to the required density with sterile saline. Infection was induced in the neutropenic mice by the intravenous inoculation of 0.2 mL of a *C. albicans* cell suspension (an inoculum of 1 × 10^4 CFU/mouse) into the lateral tail vein (day 0).

Total plasma level and pharmacodynamic parameters of E1210 in infected mice

Infection was induced in the neutropenic mice as above. Oral administration of E1210 (2.5, 10 and 40 mg/kg) was given 1 hour after the inoculation. Whole blood was obtained at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hours post-dose, and plasma was obtained by centrifugation. Plasma concentrations at each time point were calculated at Mitsubishi Chemical Medience Corporation (Tokyo, Japan). Pharmacodynamic parameters were calculated using the serum C_{max}, T_{max} and AUC values and the MIC value for E1210 of 0.5 μg/mL for *C. albicans* in the presence of 90% mouse serum. The AUC was calculated using the trapezoidal rule. C_{max} was obtained directly from the simulated plasma concentration data. Time above MIC (T>MIC) was calculated using the total time that the plasma concentration was above the target MIC value over 48 hours based on analysis of the concentration over time graph.

Correlation analysis of pharmacodynamic parameters and in vivo efficacies

Correlation analyses were performed for two endpoints of in vivo efficacy; that is, fungal burden in the kidneys and survival rate. To evaluate the fungal burden, antifungal therapy was initiated 1 hour after infection. E1210 (at doses ranging between 0.313 to 40 mg/kg) was given based on a pre-planned dosing schedule. It was orally administered q12h, q24h or q48h, and given for a total of two consecutive days (day 0-1). Forty-nine hours after the infection (day 2), all living mice were sacrificed and the kidneys of each mouse were immediately removed. Homogenates of the kidneys were prepared using glass homogenizers and were serially diluted with sterile saline. Aliquots from each sample were plated on SDA plates and incubated overnight at 35ºC. After incubation, viable fungal colonies were counted. Fungal burden in each mouse was logged and then averaged for each group. These values were calculated as changes in $\log_{10}$ CFU/renal homogenate by subtracting the values of the control group. To evaluate the survival rate, antifungal therapy was initiated 2 hours after infection and was continued for three consecutive days (days 0-2). E1210 (1.25 to 40 mg/kg) was orally administered q12h, q24h or q36h, based on a pre-planned schedule. The mortality rate for the mice was recorded daily for 14 days after infection, and results obtained on the final day were used for the correlation analyses. Three pharmacodynamic parameters and two measures of in vivo efficacy (changes in $\log_{10}$ CFU/renal homogenate and survival rate at Day 14) were plotted, and fit to curves and R^2 values were calculated using SAS 8.2 software package (SAS Institute Japan Ltd., Tokyo, Japan).

In vivo PAFE

Two hours after the infection, mice were treated with either a single oral dose of E1210 (either 10 or 40 mg/kg) or vehicle. Mice were sacrificed at sampling time intervals ranging from 4 to 34 hours. Mice in one group did not receive any treatment and were sacrificed 2 hours after infection to measure the fungal burden at the start of therapy. Control growth was determined based on five sampling points (0, 4, 8, 12 and 22 h), and E1210-treated mice were sampled at six sampling points (at 4, 8, 12, 22, 28 and 34 h). After sacrifice, the kidneys were removed and the fungal burden was determined as above. Results were expressed as the mean $\log_{10}$ CFU/renal homogenate. The total time that elapsed from the start of E1210 dosing (10 or 40 mg/kg) until the plasma levels of E1210 fell below the target MIC for the *C. albicans* strain studied was calculated from the concentration over time graph data. Plasma drug concentrations were utilized for the kinetic calculations and the *C. albicans* MIC determined in the presence of 90% mouse serum (0.5 μg/mL) was used for the target MIC that the plasma concentration must be above. The PAFEs were calculated by determining the time it took for *C. albicans* to grow to a concentration of 1 $\log_{10}$ CFU/renal homogenate in control animals and subtracting this from the amount of time it took *C. albicans* to grow to a concentration of 1 $\log_{10}$ CFU/renal homogenate in E1210 treated animals after E1210 plasma levels fell below the target MIC for the organism.

In vitro PAFEs

C. albicans cells cultured after overnight incubation were resuspended in warmed Yeast Peptone Dextrose (YPD) broth to achieve a final fungal density of 1 × 10^4 cells/mL. The suspension was incubated at 35ºC for 1 hour, and E1210 was then added to aliquots containing concentrations of: 0.03 (4 × MIC), 0.13 (16 × MIC) or 0.5 μg/mL (64 × MIC). These aliquot suspensions were incubated again at 35ºC for 3 hours, centrifuged and washed twice in PBS. Warmed YPD was then added, and quantitative subcultures were obtained from each culture immediately and then hourly for 8 h. A further aliquot of each sample was plated on SDA plates and incubated overnight at 35ºC. After incubation, numbers of viable cells were determined by counting fungal cell colonies and results were expressed in CFUs. The PAFEs were defined as the difference in the time required for E1210 exposed *C. albicans* subculture regrowth to increase in density by 1 $\log_{10}$ CFU/mL after E1210 removal relative to the time taken for the untreated control aliquot to grow by 1 $\log_{10}$ CFU/mL.

Results

In vivo pharmacodynamic correlates

Correlation between pharmacodynamic parameters and in vivo efficacies were analyzed using fungal burden (Fig. 1, A-C) and survival rate (Fig. 1, D-F) as endpoints. Efficacy of E1210 best correlated with AUC/MIC and T>MIC.

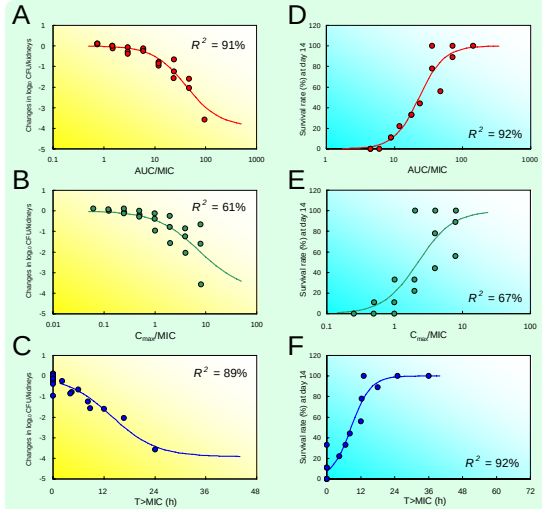


FIG. 1. Relationship between pharmacodynamic parameters and in vivo efficacies

(A-C) Correlation between pharmacodynamic parameters and fungal burden in kidneys homogenates. (D-F) Correlation between pharmacodynamic parameters and survival rates at day 14. (A and D) AUC/MIC, (B and E) C_{max}/MIC, (C and F) T>MIC

In vivo PAFEs

Single-dose post-antifungal effects (PAFEs) studies demonstrated prolonged suppression of organism regrowth after plasma E1210 levels had fallen below the MIC. E1210 produced in vivo *C. albicans* PAFEs of 14.4 and 11.0 hours after oral doses of 10 and 40 mg/kg, respectively (Fig. 2). These prolonged PAFEs raise the possibility that wider dosing intervals can be considered when determining the E1210 clinical dosing regimen.

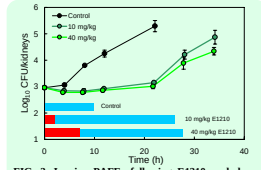


FIG. 2. In vivo PAFEs following E1210 oral doses against *C. albicans* in neutropenic infected mice

Each symbol represents the mean ± S.E.M. of four mice. The widths of the blue horizontal bars at the bottom of the graph represent the time that it took organisms to regrow to 1 $\log_{10}$ CFU/renal homogenates after E1210 levels in plasma fell below the target MIC for the organism. The widths of the red horizontal bars represent the total time duration that plasma E1210 levels exceeded the target MIC.

In vitro PAFEs

The in vitro post-antifungal effects (PAFEs) of E1210 on *C. albicans* growth were evaluated after *C. albicans* was exposed to concentration of 4 × MIC, 16 × MIC and 64 × MIC of E1210 at 35ºC for 3 h (Fig. 3). At a concentration of 4 × MIC, the PAFE of E1210 was 1.8 h, and at concentrations 16 × MIC and 64 × MIC, the PAFEs were 3.9 and 4.6 hours, respectively.

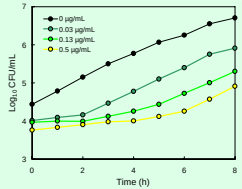


FIG. 3. In vitro PAFEs following E1210 removal against *C. albicans*

C. albicans growth after E1210 removal was shown.

Pharmacokinetics of E1210 in infected mice

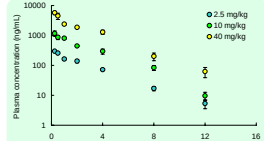


FIG. 4. Plasma concentrations of E1210 after single oral dose administration in a disseminated candidiasis model

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

TABLE 1. Pharmacokinetic parameters of E1210 after single oral dose administration to candidiasis model mice

Parameters		2.5 mg/kg	10 mg/kg	40 mg/kg
t_{max}	(h)	0.25	0.25	0.25
C_{max}	(ng/mL)	296.6	1169.8	5655.56
$t_{1/2}$	(h)	2.13	1.62	1.83
$AUC_{(0-inf)}$	(ng·h/mL)	769.7	2971.58	11809.16
$MRT_{(0-inf)}$	(h)	3.1	2.95	2.85

Each value represents the mean. Pharmacokinetic parameters of E1210 were calculated by fitting a 1-compartment model with first order absorption to the plasma concentrations after single oral administrations using WinNonlin® program version 6.1 (Pharsight Corporation, CA, USA). Since the pharmacokinetic parameters obtained showed linearity within the dosing range, the plasma concentration-time profile of E1210 for each dosing regimen was simulated using the mean value of the kinetic parameters obtained from the above three doses.

Conclusions

- **E1210 decreased the total fungal burden in murine kidney homogenates in a disseminated candidiasis model. This in vivo efficacy correlated best with AUC/MIC and T>MIC (R^2 = 91% and 89%, respectively).**
- **E1210 lowered the mortality rate in this disseminated candidiasis model. This efficacy also correlated best with AUC/MIC and T>MIC (R^2 = 92%).**
- **E1210 showed significant concentration dependent PAFEs *in vivo*.**
- **E1210 showed significant concentration dependent PAFEs *in vitro*.**

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