POSACONAZOLE PHARMACOKINETICS IN LUNG TRANSPLANT RECEIPIENTS WITH AND WITHOUT CYSTIC FIBROSIS

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The incidence of invasive fungal infections (IFIs) is significantly high in patients following lung transplantation. There have been no investigations on the pharmacokinetics of posaconazole in patients with CF. In this study, 7 patients with CF and 13 patients without CF were enrolled in a pharmacokinetic study upon initiation of posaconazole prophylaxis following lung transplantation. We established a sensitive HPLC-fluorescence assay method to measure plasma concentration of posaconazole. After a minimum five days of treatment, maximum plasma concentration $C_{ss,\,max}$ (0.311 μg/mL) in CF patients was 56% lower compared to $C_{ss,\,max}$ (0.699 μg/mL) in non-CF patients; the minimum plasma concentration $C_{ss,\,min}$ (0.189·μg/mL) in CF patients was lower by 60% compared to $C_{ss,\,min}$ (0.474 μg/mL) in non-CF patients; the average plasma concentration $C_{ss,\,av}$ (0.233 μg/mL) in CF patients was lower by 61% compared to $C_{ss,\,av}$ (0.594 μg/mL) in non-CF patients, the dose normalized plasma area under curve $AUC_{0\text{-}24}$ (0.007 h*μg/mL) in CF patients was 65% lower compared to dose normalized $AUC_{0\text{-}24}$ (0.02 h*μg/mL) in non-CF patients, and the apparent oral clearance of 2.51 L/h/kg in CF patients was 3.4 times higher compared to 0.74 L/h/kg in non-CF patients. Moreover, a steady state average concentration of 0.7 μg/mL that is considered to be essentials for prophylaxis was only achieved in 4 out of 20 patients. A good correlation between C_{trough} and $AUC_{0\text{-}\tau}$ demonstrates C_{trough} is a good surrogate marker to monitor systemic exposure of posaconazole in LTRs.

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PREFACE

First and foremost, I would like to express gratitude to my advisor, Dr. Raman Venkataramanan for all his support. He always provided me with not only professional guidance in my research, but also personal life lessons. I appreciate his diligence, generosity and kindness. I am forever grateful to have been his student.

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I thank my mother, father, sister, husband, and sons (Nash Wei and Spence Wei) for their love and endorsement. Words cannot express my love and gratitude to them. This work is dedicated to them.

1.0 INTRODUCTION

1.1 LUNG TRANSPLANTATION

1.1.1 History of Lung Transplantation

The foundation for clinical organ transplantation was laid by Alexis Carrel (1873-1944). In 1912, Dr. Carrel was awarded the first Nobel Prize in physiology and medicine in recognition of his groundbreaking contribution to vascular suture, and the transplantation of blood vessels and organs (*1*). In 1963, Dr. James Hardy conducted the first human lung transplantation, but the transplant recipient died after 18 days (*2*). Further, clinical lung transplantation was hampered by lack of effective immunosuppressive agents and suboptimal surgical techniques. Twenty years later, in 1982, the first successful human heart-lung transplantation was performed (*3*), and in the following year, Dr. Joel Cooper carried out the first successful human single lung transplantation with prolonged postoperative survival (*4*).

Lung transplantation has become a treatment option for patients with end-stage lung disease over the past three decades. According to the 31st adult lung and heart-lung transplant report from the Registry of the International Society for Heart and Lung Transplantation, 47, 647 adult lung transplants have been performed worldwide in 136 participating transplant centers from 1985 to 2013 (*5*).

1.1.2 Types of Lung Transplantation

There are various types of lung transplantations including single lung transplantation, bilateral lung transplantation, heart-lung transplantation and lobar lung transplantation. Both single and bilateral lung transplantation are widely used in a variety of end-stage lung diseases. Single lung transplantation could be applied to all end-stage lung diseases except for patients with septic lung disease, which is an indication for bilateral lung transplantation. Although both single and bilateral lung transplantation have continued to increase since the 1980s, the growth of bilateral lung transplantation has exceeded single lung transplantation and has become the main form of lung transplantation performed currently. According to the 29th report of the Registry of the International Society for Heart and Lung Transplantation, conditional median survival (transplant half-life) of single lung transplantation is 6.5 years, while it is 9.4 years for bilateral lung transplantation (6). The more favorable median survival rate may be the reason that has led to the increased numbers of bilateral lungs transplanted.

The indications for heart-lung transplantation are pulmonary vascular diseases, like congenital heart disease and primary pulmonary hypertension. After a decline in 2003, the number of heart-lung transplantation has stayed relatively stable. Around 100 cases are carried out worldwide every year (6).

Living lobar lung transplantation is an alternative to cadaveric lung transplantation in which right and left lower lobes from two separate donors are removed and implanted in a recipient in place of the entire right and left lungs. Lobar lung transplantation remains a small part of the total number of lung transplantations performed currently (*7*).

1.1.3 Median Survival of Lung Transplantation

In 2009, the conditional half-life of heart transplantation recipients was 14 years (*8*), while the conditional half-lives of liver and kidney transplantation were over 12 years (*9*) and over 10 years (*10*) respectively. As of June 2010 overall median survival of single and bilateral lung transplantation was 5.5 years, and conditional median survival was 7.7 years (*7*). Currently, the median survival of lung transplantation is significantly lower compared with other solid organ transplantations.

1.2 COMPLICATIONS AFTER LUNG TRANSPLANTATION

1.2.1 Causes of Death in LTRs

After lung transplantation, recipients may experience bronchiolitis, acute rejection, graft failure, malignancy, infections and cardiovascular diseases that can cause recipients' death.

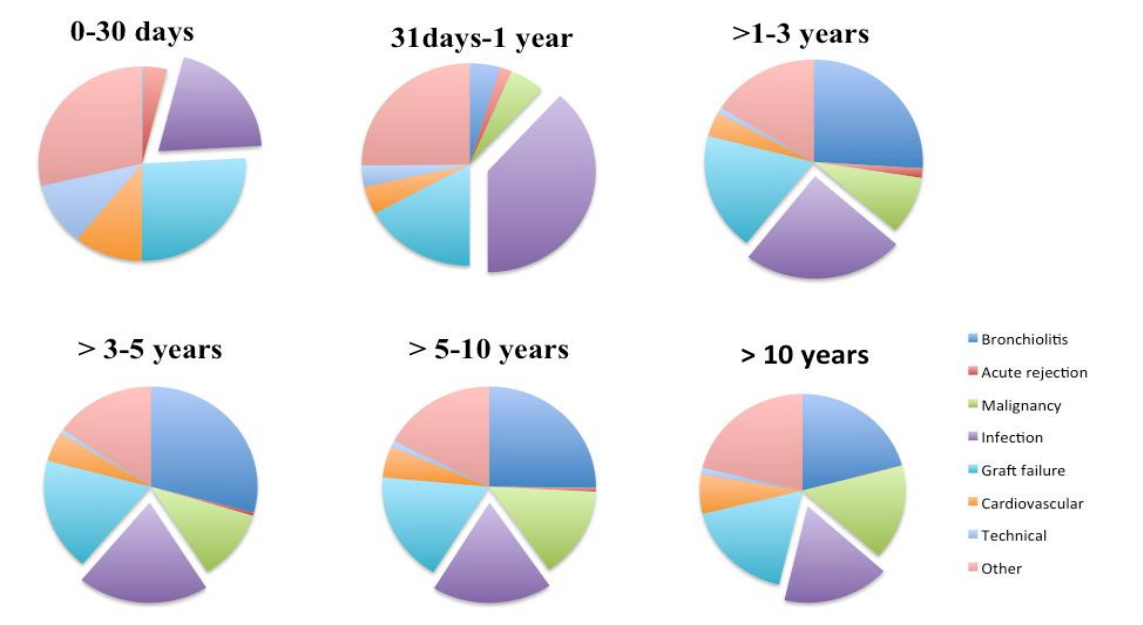


Figure 1. Allocation of causes of death in a time period following lung transplantation from 1992 to 2011

Figure 1 demonstrates that infections are one of the main reasons for the mortality of the LTRs and infections can result in death immediately after transplantation or over several years (*6*). Between 1992 and 2011, during the first year after lung transplantation, almost 40% of patients with infections died as a result of those infections (*6*).

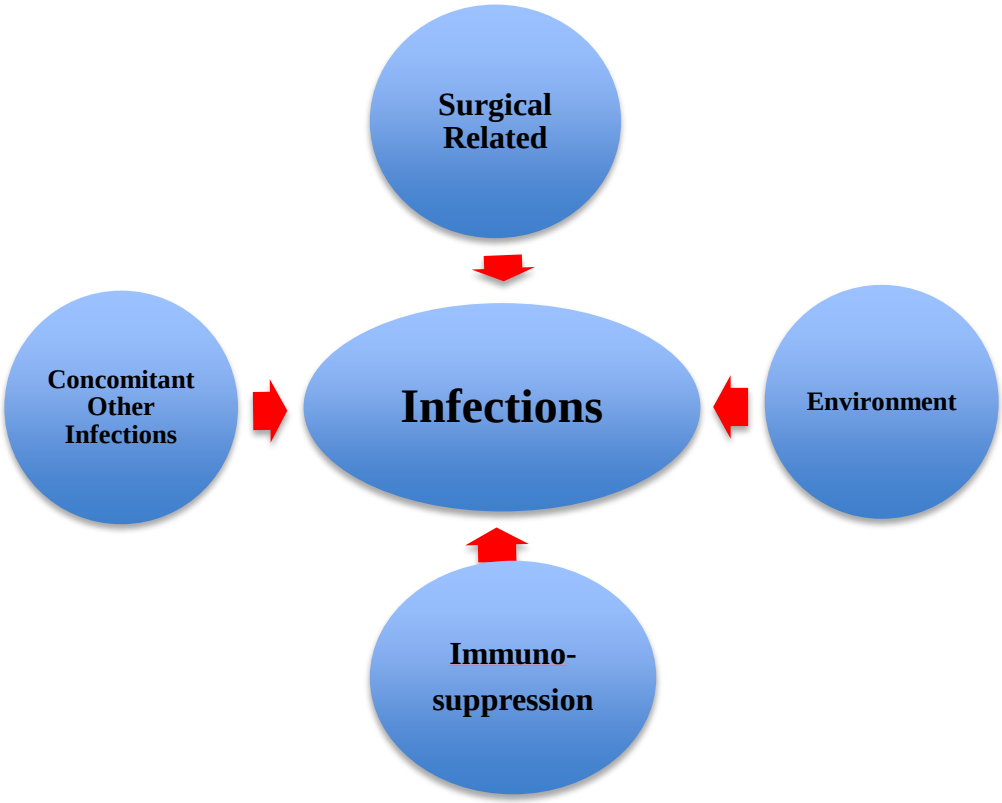


Figure 2. Risk factors for infections following lung transplantation

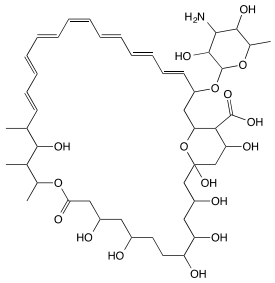
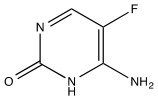
As figure 2 shows, several reasons make infections more serious after lung transplantation than after other solid organ transplantations. For example, the body may be contaminated by the process of opening of the recipient's airway during the lung transplant surgery. Also, lungs are the only organs that are under a constant exposure to environmental pathogens, which increases the chances of infections. Third, after lung transplantation, immunosuppression must be well maintained in the recipients. Infection is a direct result of the immunocompromised state of the LTRs. Moreover, lung infections can be caused by other concomitant infections in the recipients.

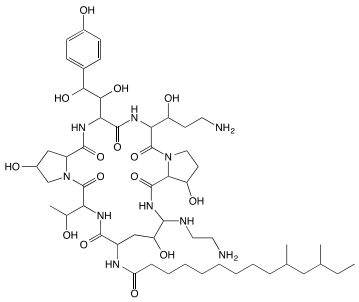
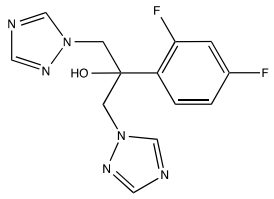
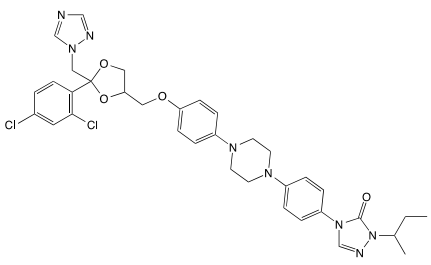
The incidence of IFIs is especially high in LTRs. IFIs are a direct consequence of immunosuppression after solid organ transplantation. An investigation showed that fungal infections occur in 15-35% of lung transplantations with a 60% overall mortality (*11*).

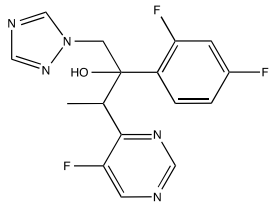
1.3 CURRENT THERAPIES FOR INVASIVE FUNGAL INFECTIONS

A few medications have been approved by the FDA for prophylaxis or treatment of lung infections, including echinocandins, amphotericin, flucytosine and azoles. Voriconazole and liposomal amphotericin B are recommended as the primary therapy for IFIs currently (*12*). These drugs have certain limitations as summarized in Table 1. For example, voriconazole is involved is metabolized by CYP2C19, CYP2C9 and CYP3A4 and involved in a number of drug interactions. Amphotericin B is well known for its severe and potentially lethal side effects and low response rate of 35%. Flucytosine has to be used with other antifungal agents due to preexisting or emerging resistance. Echinocandins are only available for intravenous administration (*13*).

Table 1**. Disadvantages of current antifungal agents**

Agent	Profiles
Polyenes	
Amphotericin B	 Physiochemical: MW: 924, LogP: 0.8, pka[1]: 5.5 Only available for intravenous administration. PK parameters: different formulations possess unique PK profiles. Well known for its severe nephrotoxicity and infusion related reactions.
Pyrimidine analogues	
Flucytosine	 Physiochemical: MW: 129 , LogP: -1.1, pKa: 3.26 Only available for oral formulation. PK parameters: half-life 2.4 to 4.8 hours, linear correlation between the elimination rate constant of flucytosine and creatinine clearance, widely distributes in body water (volumes of distribution from 0.6 to 0.9 L/kg Preexisting or emerging resistance is common, must be combined with another antifungal.
Echinocandins	
Caspofungin	

	 Physiochemical: MW: 1093, LogP: -4.82 Only available for intravenous administration. PK parameters: half-life 9-11 hours, clearance: 12 mL/min, volume of distribution: 9.67L Significant acquisition costs.
Azole	
Fluconazole	 Physiochemical: MW: 306, LogP: 0.4, pKa^2: 1.76 Available for intravenous and oral administration. PK parameters: half-life 30 hours, clearance: 15 mL/min, volume of distribution: 50 L Absence of activity against *Aspergillus spp.*; frequently reduced or absent activity against *Candida glabrata*; inherent resistance of *Candida krusei*.
Itraconazole	 Physiochemical: MW: 706 , LogP: 5.66, pKa: 3.7

	Available for intravenous and oral administration. PK parameters: half-life 21hours, clearance: 381 mL/min, volume of distribution: 796 L FDA black box warning for heart failure; Capsule formulation highly variable; Acid-dependant oral bioavailability leads to subtherapeutic; concentrations in critically ill patients with elevated gastric pH; solution improves bioavailability, but associated with gastrointestinal intolerance.
Voriconazole	 Physiochemical: MW: 349 , LogP: 1, pKa: 2.27 Available for intravenous and oral administration. PK parameters: half-life 21hours, clearance: 338 mL/min, volume of distribution: 4.6 L/kg Voriconazole is metabolized by CYP2C19, 2C9, 3A4). Highly variable pharmacokinetics Involved in a number of drug interactions.

Note: information of the table is from package insert of individual drug and www.drugbank.ca, except other expcification.

[1]https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/6/a9528dat.pdf

[2] http://www.pfizer.ca/sites/g/files/g10017036/f/201410/DIFLUCAN%282%29.pdf

1.4 POSACONAZOLE

1.4.1 Posaconazole: a Promising First-line Prophylaxis and Treatment for Invasive Fungal Infections

Posaconazole was initially approved by the FDA as a suspension in 2006 for prophylaxis of invasive *Aspergillus* and *Candida* infections in patients who are at high risk of developing these infections due to the immunocompromised status of the patients. Posaconazole is also a treatment for oropharyngeal candidiasis (OPC), including OPC refractory (rOPC) to itraconazole and/or fluconazole. Posaconazole is a potent and broad-spectrum triazole antifungal agent. It is effective against fungi that are refractory to other antifungal drugs. Posaconazole tablets and injection were approved in 2013 and 2014, respectively (Table 2).

Table 2. FDA approved formulations of posaconazole

Formulation	Strength	Approval date
Suspension	40 mg/mL	Sep. 2006
Tablet, Delayed- release	100 mg	Nov. 2013
Injection	300mg per 16.7 mL	Mar. 2014

1.4.2 Posaconazole Property and Mechanism of Its Antifungal Action

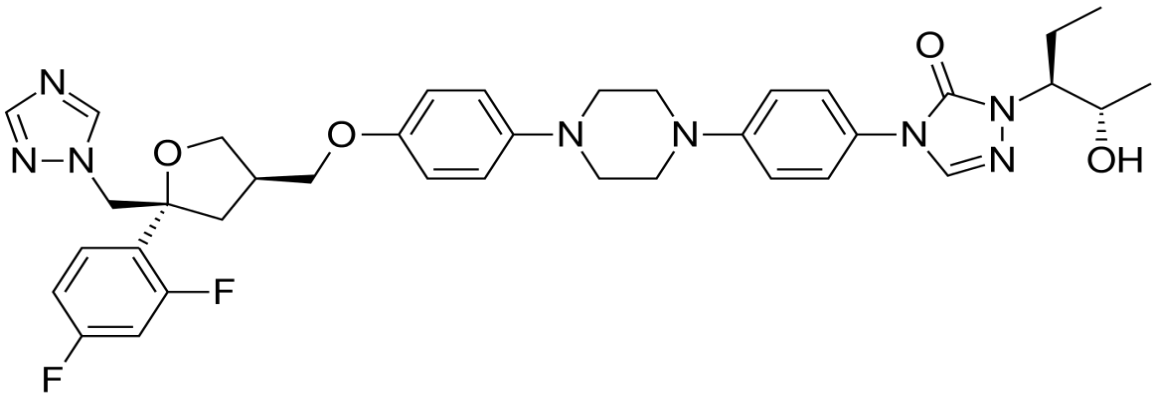


Figure 3. Structure of posaconazole

Posaconazole is a lipophilic drug with a logP of 5.4, pKa_1 of 3.6 (piperazine) and pKa_2 of 4.6 (triazole) (*14*). It belongs to class II in the biopharmaceutical drug classification system, which means posaconazole has low solubility and high permeability. The molecular structure is shown in Figure 4.

As with other triazole antifungal agents, posaconazole works by binding to the heme cofactor located on the active site of the cytochrome P450 (CYP)-dependent 14**α**-demethylase, an enzyme responsible for the conversion of lanosterol to 14**α**-dimethyl lanosterol in the ergosterol biosynthetic pathway (Fig. 5). Ergosterol is an essential component of the fungal cell membrane; therefore, inhibition of synthesis of ergosterol leads to disruption of the integrity and function of the fungal cell membrane, as well as inhibition of fungal growth (*15*).

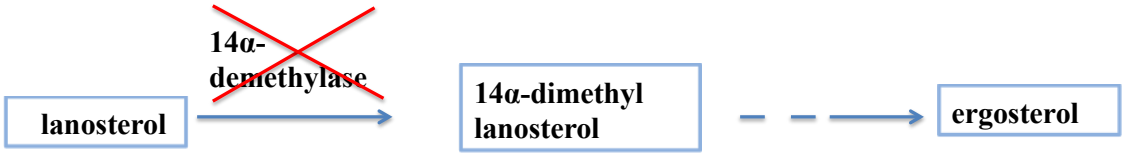


Figure 4. Mechanism of antifungal action of posaconazole

1.4.3 Pharmacokinetic Profiles of Posaconazole

The pharmacokinetics properties of posaconazole are listed in Table 3.

Table 3. Summary of posaconazole pharmacokinetic profiles

Parameter	Pharmacokinetic Profile
Absorption	The peak of plasma concentration was observed around 3-5 hours following administration of the oral suspension. Absorption is significantly improved when administered with food.
Distribution	Large apparent volume distribution (261 L). Posaconazole is highly protein bound (98.2%), mainly to albumin.
Metabolism	Primarily by glucuronidation; P-gp substrate. CYP3A4 inhibitor.
Elimination	In healthy volunteers, after intravenous injection, the clearance was 6.5 L/h with a half-life of 24 h. Mainly eliminated in feces with the parent drug as the major component.

1.4.3.1 Absorption

The peak of plasma concentration of posaconazole (C_{max}) is normally observed around 3-5 hours following administration of an oral suspension. Both C_{max} and area under curve (AUC) are significantly higher when posaconazole is administrated with food, therefore whenever possible, posacoazole suspension should be taken after a full meal (*16*). Because posaconazole AUC can be markedly improved by smaller multiple daily doses (*17*), two or three doses per day are recommended, although posaconazole has a long half-life. Besides food and dosing frequency,

posaconazole absorption can be affected by gastric pH. For example, proton pump inhibitors can decrease posaconazole absorption (*18*). Given that gastric acid suppression therapy is common among patients undergoing transplantation, we would anticipate poor absorption of posaconazole in this patient population.

There is no data available regarding to the relative bioavailability of posaconazole suspension, however, we predict that posaconazole suspension has a low bioavailability due to its low solubility, being a substrate of P-gp efflux, and due to gut metabolism by UGT phase II enzymes.

Recently a delayed-release tablet was developed with improved absorption and bioavailability of posaconazole. The pharmacokinetics of tablet has been evaluated in healthy volunteers. Under fasting condition, the AUC and C_{max} for the tablet were slightly higher than the suspension. However, under fed condition, the AUC and C_{max} for the tablet were 3 times higher than the suspension, and tablet showed less variability in exposure compared to the suspension (*19*). The absolute bioavailability of the delayed-release tablet is 54% under fasting conditions (*16*).

1.4.3.2 Distribution

Posaconazole has a large volume distribution and is also highly bound to albumin (*20*). The average volume distribution is 261 L, after intravenous administration. Posaconazole is distributed to pulmonary tissue and alveolar cells (*21*).

1.4.3.3 Metabolism

In healthy subjects, posaconazole has a mean half-life about 24 hours, but no half-life is reported in patients following intravenous injection. Posaconazole mainly circulates in the blood as the parent compound. Unlike other triazole antifungal agents, posaconazole is not metabolized by

cytochrome P450 enzyme. Only about 17% of the administered posaconazole is metabolized by UDP-glucuronosyl transferase (UGT) 1A4 *(22, 23),* and its glucuronide metabolites have no antifungal activities (*24*). Compared with other triazole antifungal agents, posacoazole has fewer drug-drug interactions so its pharmacokinetic parameters are less influenced when co-administrated with other drugs. However, posaconazole is a substrate of P-glycoprotein (P-gp) mediated efflux (*16*), and when P-gp function is altered by either endogenous or exogenous compounds, posaconazole pharmacokinetic parameters will be altered. In healthy volunteers, after a single intravenous injection of 200 mg, the total body clearance was 6.5 L/h (*16*). But clearance of posaconazole after intravenous injection has not been reported in patients.

1.4.3.4 Excretion

In one study, after administration of a radiolabeled suspension dosage form, 76.9% of administrated posaconazole was excreted in feces (66.3% was posaconazole). Meanwhile, the urinary excretion of posaconazole was negligible (*24*).

1.4.4 Safety of posaconazole

The most frequently observed adverse events in patients using posaconazole were diarrhea, hypokalemia, pyrexia and nausea in clinical trials. Mild to moderate elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have also been reported in clinical trials. Therefore patient's liver function should be assessed at the onset of posaconazole therapy and monitored during treatment (*16*). Like other azole antifungal agents, posaconazole can prolong QT interval and can lead to arrhythmias (*16*).

1.4.5 A Large Variation Is Observed in The Systemic Exposure of Posaconazole in LTRs

Recent clinical studies have demonstrated an association between plasma concentrations of posaconazole and clinical response (*25-27*). According to the FDA pharmacological review, a steady state average concentration below 700 ng/mL is expected to result in breakthrough invasive fungal infections of more than 25% of patients. Our lab routinely measures posaconazole plasma concentrations in transplant patients as part of a therapeutic drug monitoring program. Over a period of ten months, we have observed posaconazole concentrations to be below 700 ng/mL in more than 60% of the patient samples (Fig. 6). Lung transplant patients have a high risk for invasive fungal infections with subtherapeutic posaconazole concentrations (*28*).

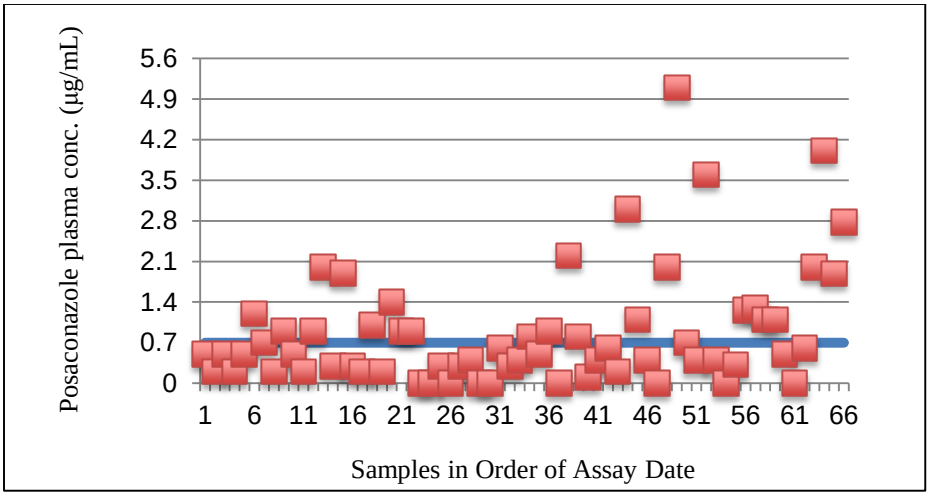


Figure 5. Distribution of posaconazole plasma trough concentrations measured at our lab

The above distribution pattern of posaconazole concentrations was observed in 66 patient samples over a period of ten months. Posaconazole plasma trough concentrations were < 0.7 µg/mL (The blue line, therapeutic threshold) in more than 60% of LTRs. The distribution

demonstrates posaconazole suspension didn't produce sufficient plasma concentration, which puts patients at high risk for invasive fungal infection after transplantation.

1.5 CYSTIC FIBROSIS

Cystic fibrosis is an autosomal recessive genetic disorder that primarily affects the lungs and digestive system (Fig. 3). In patients with CF, a defective gene causes the body to produce unusually thick, sticky mucus that clogs the lungs and leads to life-threatening lung infections. Lung transplantation is a treatment for patients with advanced CF. Between 1995 and 2011, CF was the third most common reason for lung transplant, with an overall rate of 16.7% (*6*).

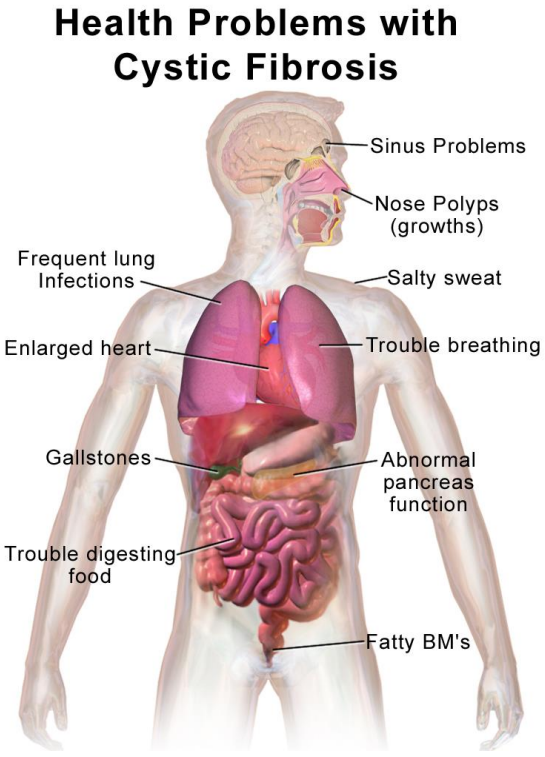


Figure 6. Health problem of cystic fibrosis

Resource: http://en.wikipedia.org/wiki/Cystic_fibrosis

1.5.1 Pharmacokinetic Studies in CF Patients

Lower plasma concentrations of many drugs have been reported in patients with CF. The altered pharmacokinetics of drugs in CF patients are the results of multiple factors. It is well-known that CF patients are malnutrished due to malabsorption in the intestinal tract (*29*). The main cause of malabsorption is pancreatic insufficiency, so CF patients normally receive pancreatic enzyme supplement to facilitate absorption of nutrients (*30*). Decreased absorption of drugs in CF patients similar to malabsorption of nutrients has been speculated. In fact, there are many pathological changes in CF patients that can affect drug absorption, such as a more acidic circumstance in the duodenum, insufficient pancreatic enzymes secretion, altered bile acid turnover and a prolonged intestinal mobility (*31*). Therefore, drugs with different physiochemical property may demonstrate variable absorption in CF patients.

Poor absorption, larger volume distribution and high total body clearance of certain drugs in patients with CF may explain the observed lower plasma concentrations of several drugs in patients with CF (*32-34*). However, the mechanism behind the altered pharmacokinetic profiles of many drugs in CF patients has not been completely evaluated. Many studies have investigated pharmacokinetics changes in CF patients, but the results are conflicting.

Pharmacokinetics of oral ciprofloxacin has been studied in six CF patients and six healthy control subjects, closely matched with age, sex and weight. There was no statistical differences in dose normalized plasma area under curve (AUC), maximum plasma concentration (C_{max}), half-life, total body clearance, renal clearance, and volume of distribution of ciprofloxacin between CF and the control group (*35*).

One study demonstrated a significantly lower serum concentration of cloxacillin in CF patients accompanied by dramatically increased total body clearance. However, no significant difference in the bioavailability of cloxacillin was observed in CF group compared with non-CF group after patients intravenous and oral administeration of cloxacillin (*36*).

1.5.2 Pharmacokinetic Studies in LTRs With CF

To date, limited information is available regarding the pharmacokinetics of drugs in LTRs with CF. Three pharmacokinetic studies have been conducted in this patient population but only one study compared the pharmacokinetics to a group of control subjects (*37*-*39*). That study was designed to assess the pharmacokinetics of tacrolimus in 22 LTRs (11 with CF and 11 without CF) (*39*). In the study, the CF group required a higher dose of tacrolimus to achieve similar drug exposure compared to non-CF group, and after dose normalization, AUC and C_{max} were significantly lower in CF group compared to the non-CF group. Higher apparent total body clearance and larger apparent volume of distribution were reported in the CF group.

1.5.3 Pharmacokinetics of Antifungal Agents in CF Patients

Although there is a high incidence of IFIs in CF patients, there are very few studies that have investigated pharmacokinetic profiles of antifungal agents in this patient population. Only three studies have been performed, but none of them compared the pharmacokinetics of antifungal drugs in CF patients with a control group.

The pharmacokinetics and safety of oral itraconazole was evaluated in 17 patients with CF. Despite receiving the recommended dosages of the oral solution, 11 of the patients did not

reach the target therapeutic threshold at steady state (*40*). The pharmacokinetics itraconazole after administration of capsule and oral solution were assessed in 30 CF patients. Although the patients received standard dosage of itraconazole, one half of the plasma samples had concentrations below the limit of detection (0.04 mg/L) (*41*). The recommended breakpoint of C_{trough} for itraconazole is 0.5 mg/L (*42*).

A retrospective study reported the plasma trough concentration of voriconazole in CF patients after lung transplantation. Following the standard dosing regimen (400 mg/day), in 30% of the 35 CF patients the plasma concentration was lower than 0.5 mg/L (therapeutic concentration is 1-2 mg/L). Even after increasing the dose (570 ± 160 mg/day), 40% of these patients did not reach target plasma concentrations 16 days later (*38*).

No study has been performed to investigate the pharmacokinetic alterations of antifungal agents in LTRs with CF. Our goal is to compare the pharmacokinetics of posaconazole in LTRs with and without CF in order to improve treatment outcome with posaconazole. Because of the physiochemical property of posaconazole, a lipophilic drug with low solubility, we anticipated poor absorption of posaconazole in LTRs with CF, which exposes these patients to higher risk for IFIs.

We hypothesize that LTRs with CF will have a significantly lower systemic exposure compared with LTRs without CF following oral administration of posaconazole suspension due to pathophysiological changes observed in CF. Our aims were as follows,

Specific aim 1 was to develop and validate a simple and sensitive analytical method to quantify plasma concentrations of posaconazole in clinical samples.

Specific aim 2 was to investigate the pharmacokinetic difference in LTRs with and without CF.

2.0 MATERIALS AND METHODS

2.1 CHEMICALS

Posaconazole was a gift from the Schering-Plough (Wicklow, Ireland). The itraconazole that was used as an internal standard (IS) was a gift from Janssen-Cilag (Beerse, Belgium). Acetonitrile, methanol, and water, all HPLC Grade, were purchased from Sigma-Aldrich. Formic acid, reagent grade, was also purchased from Sigma-Aldrich. Pooled human plasma was obtained from the central lab at the University of Pittsburgh Medical Center (Pennsylvania, USA). Ultrapure water was obtained from Milli-Q water purification system.

A Symmetry C_{18} column (4.6×250 mm) was purchased from Waters; solid phase extraction (SPE) cartridges (Oasis, Hydrophilic-Lipophilic-Balanced, reverse-phase sorbent, 1cc/ 30 mg) were also purchased from Waters.

2.2 METHOD FOR POSACONAZOLE ASSAY

2.2.1 Sample Preparation

Posaconazole stock solution with a concentration of 10 mg/mL was prepared in methanol and was diluted with methanol to obtain 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, and 3.0 mg/mL as working

solutions. Posaconazole working solutions were spiked into pooled human plasma to obtain samples at concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, and 3.0 μg/mL as calibration standards. Stock solution for quality controls (QCs) with a concentration of 4.9 mg/mL was prepared in methanol and was diluted with methanol afterwards to obtain 0.049, 0.49, and 2.94 mg/mL as working solutions. These working solutions were spiked into pooled human plasma to obtain samples with the concentrations of 0.049, 0.49, and 2.94 μg/mL as QCs. IS stock solution 400 μg/mL in methanol was diluted to 8 μg/mL with 50% methanol. In safe-lock tubes, these stock solutions, working solutions, calibration standards, and QCs were frozen at –20°C.

Plasma posaconazole concentrations were determined by a reverse-phase HPLC with a fluorescence detector. Standard curves were prepared by spiking blank human plasma with posaconazole working solutions. Internal standard 25μL was added to each sample. Solid phase extraction cartridges loaded with 200μL plasma samples, were washed independently with 50% methanol and then the posaconazole and IS were eluted with 100% methanol. The eluent was dried under a stream of air. The dried residues were reconstituted into 100 μL with acetonitrile and water (60:40, v: v).

2.2.2 HPLC Analysis Conditions

The system consisted of Waters 2695 HPLC separation module and Waster 2475 fluorescence detector. Excitation and emission wavelengths were set as 258 and 350 nm, respectively. A Symmetry C_{18} column was used together with a 0.5-mm precolumn filter. The column oven was set to 40°C, the injection volume was 20 μL, and the autosampler tray was set to 10°C. The compounds were separated with 25% of A consisting of water: acetonitrile (95:5, v:v) with

formic acid at a pH of 2, and 75% of B consisting acetonitrile: formic acid (100: 0.1, v: v), as mobile phase. The mobile phase flow rate was 1.2 mL/min.

2.2.3 Validation Procedures

The HPLC-fluorescence method was optimized for emission and excitation wavelengths, and pH, and validated by selectivity, linearity, precision, accuracy, recovery, and stability.

Emission, excitation wavelengths, and pH optimization were performed by comparing the signal intensity of posaconazole and IS by changing different combinations of emission, excitation wavelengths, and pH. Samples for study of emission, excitation wavelengths, and pH optimization were prepared by spiking posaconazole and IS working solutions into reconstitution medium. Selectivity was performed by analyzing pooled human blank plasma from six individuals in order to exclude potential interference from any endogenous substances in the assay. (What about other drug interference?)

Linearity was studied by assessing linear regression of HPLC response and posaconazole concentration in a range of 0.02-3.0 μg/mL. Samples for linearity study were prepared by spiking IS into calibration standards at series of concentrations (0.02, 0.05, 0.1, 0.2, 0.5, 1, and 3 μg/mL) and samples were processed as described in the sample preparation section. The peak area ratios of the posaconazole against the IS were plotted against posaconazole concentration. Weighting of 1/response was applied during the regression analysis.

The lowest limit of quantification (LLOQ) was evaluated by using five samples from the calibration standards at 0.02 μg/mL to analyze if the back-calculated concentrations have the precision that are within 20% of CV and have the accuracy within 20% of 0.02 μg/mL.

Precision included intra- and inter-day reproducibility. QCs of three different

concentrations (0.049, 0.49, and 2.94 μg/mL) were tested. For intra-day precision, three samples of each concentration were assayed on a single day; for inter-day precisions, six samples of each concentration were measured on three consecutive days. The intra-day and inter-day coefficient of variations were within 15%.

Accuracy included intra- and inter-day accuracy. QCs of three different concentrations (0.049, 0.49, and 2.94 μg/mL) were tested. For intra-day accuracy, three samples of each concentration were assayed on a single day; for inter-day accuracy, six samples of each concentration were measured on three consecutive days. The intra-day and inter-day accuracy were within 85% to 115% of the nominal values.

Recovery experiments (extraction efficiency) were performed by comparing the responses of extracted QC samples (0.049, 0.49, and 2.94 μg/mL) with the response of corresponding concentrations of posaconazole in eluent obtained from extracted blank human plasma that represents 100% recovery.

The stability of posaconazole QCs at low and high concentrations were assessed under three freeze-thaw cycles, placing samples on room temperature for 24 hours and remaining processed samples in the autosampler for 16 hours.

2.3 SYNOPSIS OF CLINICAL PROTOCOL

Title of Study	Pharmacokinetic Analysis of Posaconazole in LTRs
Number of Planned Subjects	A total enrollment of 20 patients 10 patients in each of the following groups will be enrolled:

	1) LTRs with CF 2) LTRs without CF as controls
Study Model	A single-center, two-cohort, prospective and observational study.
Primary Objectives	To characterize the PK of posaconazole in LTRs on a fixed-dose regimen in order to determine the extent of interpatient variability and factors that lead to interpatient variability in plasma levels.
Subject Selection Criteria	<u>Primary Inclusion criteria:</u> • Inform consented; • ≥18 years old; • Initiation of posaconazole prophylaxis following lung transplantation. <u>Primary Exclusion criteria:</u> • Received posaconazole within the previous 30 days.
Study Therapies	Posaconazole suspension (NOXAFIL®) 400 mg b.i.d.
Blood sampling	Serial blood samples (3 mL) will be collected in heparinized tubes from each patient just prior to (0 h) and at 2, 4, 6, 8, and 12 h following administration of a minimum of five days of treatment.
Blood Samples Processing	The exact time of the blood draw will be recorded, and blood samples will be placed in ice and subsequently centrifuged at 1,000g for 10 min, and the plasma stored at − 80°C until they are assayed.

2.4 POSACONAZOLE PHARMACOKINETIC AND STATISTICAL ANALYSES

Posaconazole pharmacokinetic analyses Pharmacokinetic analyses were performed using Phoenix WinNonlin® 6.4. Maximum plasma concentration at steady state ($C_{ss, max}$), time to $C_{ss, max}$ (T_{max}), minimum plasma concentration at steady state ($C_{ss, min}$), and elimination rate constant k were obtained from Phoenix WinNonlin®. Posaconazole plasma concentrations were used to determine the pharmacokinetic parameters using a non-compartment model. Maximum plasma concentration at steady state ($C_{ss, max}$), time to $C_{ss, max}$ (T_{max}), minimum plasma concentration at steady state ($C_{ss, min}$) after five doses were obtained from each patient's plasma concentration-time profile. C_{trough} was defined as concentration at time zero. Area under plasma concentration versus time curve (AUC) was determined from time zero (0 h) to 12 h or to 8 h using the trapezoidal method. Average concentration at steady state ($C_{ss, av}$) was calculated by $AUC_{0-24}/24$. There was some deviation from the protocol regarding dosing and blood sampling time, therefore, sometimes plasma concentrations at 12 h or 8 h were extrapolated using the elimination rate constant k; plasma concentrations at 0 h were assumed to be identical to the concentrations at 12 h or 8 h at steady state whenever necessary. Oral clearance (CL/F) was calculated according to the equation CL/F = Daily Dose/AUC_{0-24}.

Statistic analyses Statistic analyses were conducted using GraphPad Prism 6.0. Mann-Whitney non-parametric test was applied to compare the difference in pharmacokinetic parameters between LTRs with or without CF. A P value of < 0.05 was considered statistically significant.

3.0 RESULTS

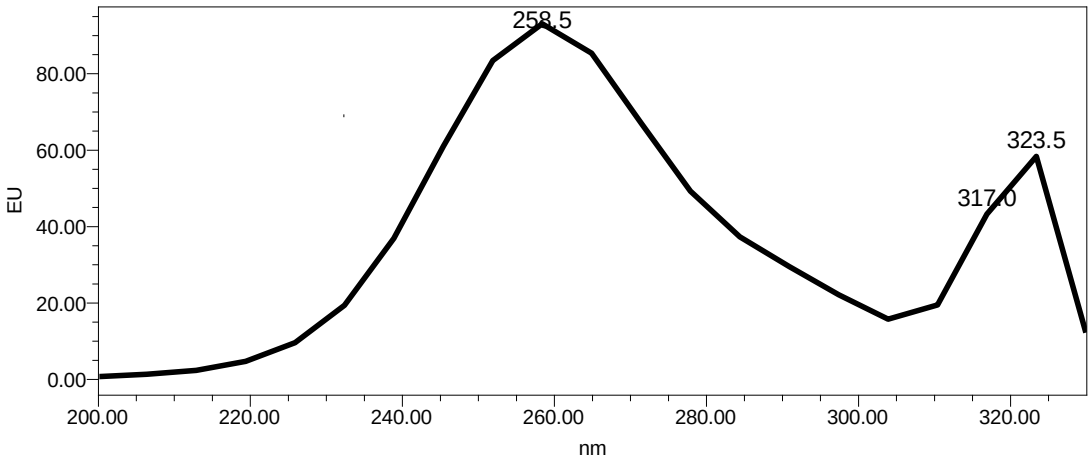
3.1 DETERMINATION OF EMISSION, EXCITATION AND OPTIMAL PH

Optimal emission, excitation and pH were evaluated by spiking posaconazole and IS working solutions into reconstitution solution to obtain posaconazole at 6 μg/mL and IS at 4μg/mL. Injection volume was 10 μL.

We observed excitation and emission peaks at 258 nm and 321 nm, respectively, after wavelength scans for excitation and emission (Fig. 7). To the best of our knowledge, there are two publications using HPLC with fluorescence detection for posaconazole quantification. They reported using excitation 260 nm, emission 350 nm, and excitation wavelength 240 nm, emission wavelength 385 nm, respectively (*43*, *44*). We optimized the emission wavelength and pH to get a good response from both posaconazole and IS from a fluorescence detector.

When pH of the mobile phase was greater than 3.5, it was difficult to detect the peaks of posaconazole and IS. In this experiment, we fixed the excitation wavelength at 258 nm to optimize emission wavelength and pH. The results showed when excitation was fixed at 258nm, the response of posaconazole and IS were at the maximum with an emission wavelength of 350 nm and a pH of 2 (Table 4).

Excitation Wavelength Scan



Emission Wavelength Scan

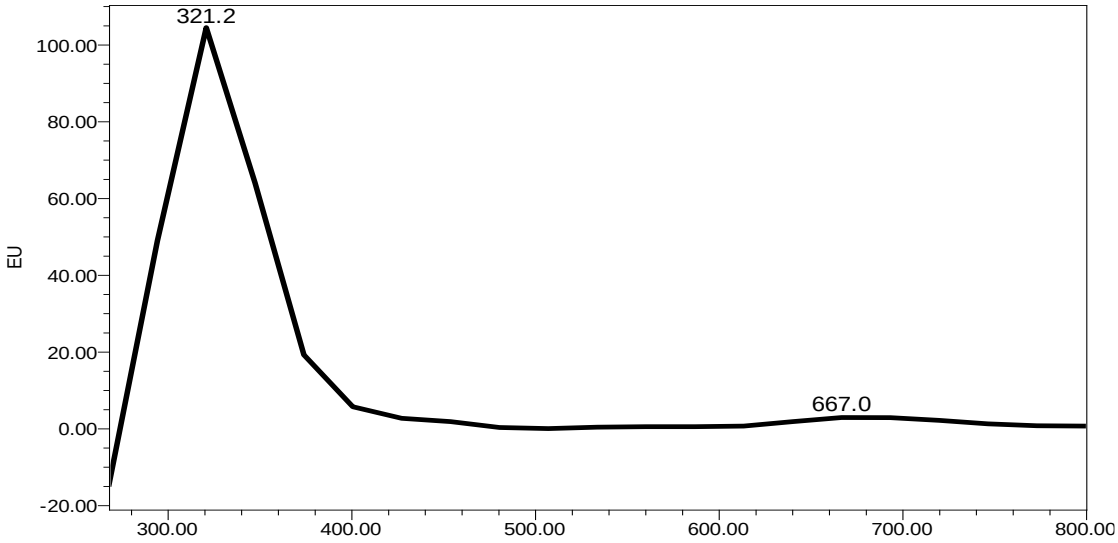


Figure 7. Excitation and emission wavelength scan

Table 4. PH and emission optimization

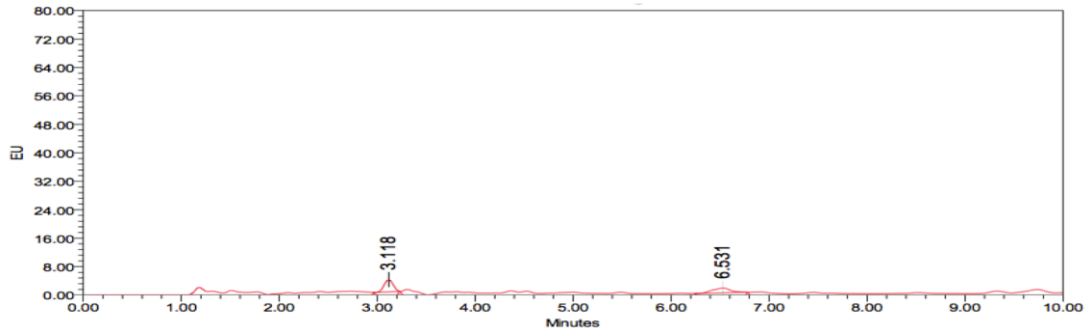
PH	Wavelength (nm)	Posaconazole Area	IS Area
PH=3.5	Ex258/Em321	76611	Hard to detect peak
	Ex258/Em385	3174348	5372943
	Ex258/Em350	3568617	4406309
PH=3	Ex258/Em321	334689	Hard to detect peak
	Ex258/Em385	5301558	5528465
	Ex258/Em350	8929110	9580873
PH=2.5	Ex258/Em321	Hard to detect peak	Hard to detect peak
	Ex258/Em385	4923988	5068319
	Ex258/Em350	8685634	9731927
PH=2	Ex258/Em321	9825702	138057161
	Ex258/Em385	108036	Hard to detect peak
	Ex258/Em350	11299093	10237234

3.2 METHOD VALIDATION

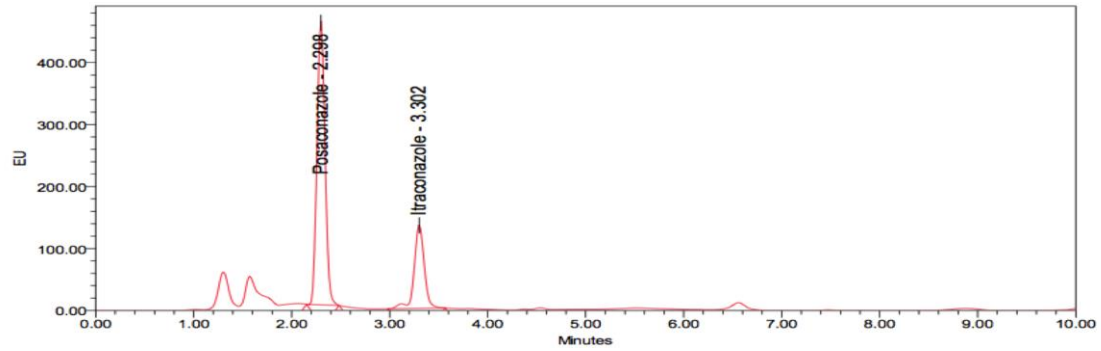
3.2.1 Selectivity

Posaconazole and IS were eluted at 2.3 min and 3.3 min, respectively with sharp and symmetric peaks using the HPLC method described above. No interference peaks were found at the retention time of posaconazole and IS in pooled blank human plasma (Fig. 8).

A



B



C

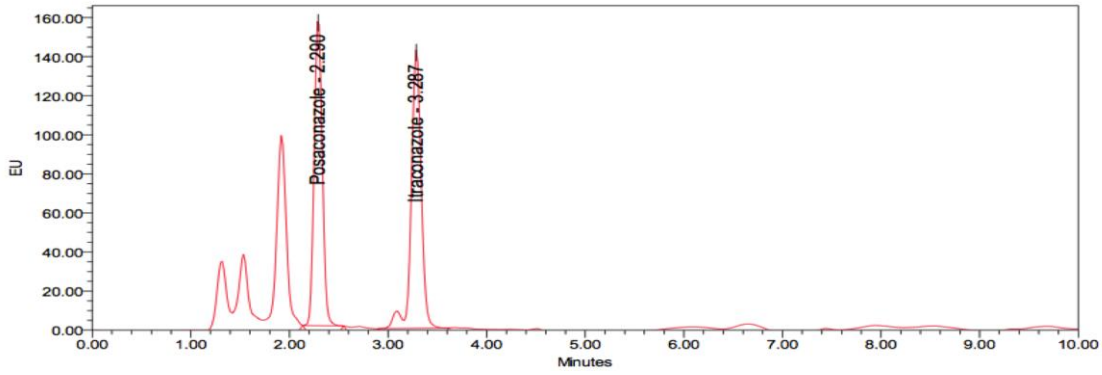


Figure 8. Representative chromatogram (A) Pooled blank human plasma; (B) Standard calibration sample (3.0 µg/mL of posaconazole); (C) One patient sample

3.2.2 Linearity

Linearity was tested by spiking IS into calibration standards at series of concentrations (0.02, 0.05, 0.1, 0.2, 0.5, 1, 3 μg/mL) and samples were processed as described in method section. The peak area ratios of the posaconazole to the IS were plotted against analyte concentration (least squares linear regression). A weighting factor of 1/response was applied. A linear response was observed over the calibration range ($r^2 > 0.99$) (Table 5).

Table 5. Linearity data of posaconazole in pooled human plasma

Nominal Conc. (μg/mL)	POSA/IS	POSA/ IS	POSA/ IS	POSA/IS	POSA/IS
0.02	0.028	0.024	0.027	0.025	0.030
0.05	0.066	0.066	0.068	0.052	0.059
0.1	0.113	0.104	0.110	0.109	0.114
0.2	0.192	0.188	0.179	0.194	0.204
0.5	0.519	0.511	0.465	0.493	0.502
1	1.092	1.073	1.162	1.090	1.109
3	2.938	2.841	3.034	2.993	3.101
Weighting: 1/y					
Equation	Y=1.001X+ 0.01039	Y=0.9745+ 0.008597	Y=1.026X+ 0.00703	Y=1.012X+ 0.003738	Y=1.040X+ 0.007888
r^2	0.9974	0.9965	0.9932	0.9982	0.9987

3.2.3 Precision

The intra-day and inter-day precisions were within 15% of the coefficient of variation (CV) (Table 6, 7).

Table 6. Intra-day precision of posaconazole in pooled human plasma (expressed as CV%, n=3)

Nominal Conc. (μg/mL)	Calculated Conc. (μg/mL)	Mean (μg/mL)	SD (μg/mL)	CV (%)
0.049	0.054 0.054 0.052	0.054	0.001	2.0
0.49	0.550 0.517 0.496	0.521	0.028	5.3
2.94	3.003 2.806 2.768	2.859	0.126	4.4

CV%=standard deviation/mean×100%;

Table 7. Inter-day precision of posaconazole in pooled human plasma (expressed as CV%, n = 6)

Nominal Conc. (μg/mL)	Calculated Conc. (μg/mL)	Mean (μg/mL)	SD (μg/mL)	CV (%)
0.049	0.054 0.054 0.052 0.045 0.060 0.045	0.052	0.006	10.8
0.49	0.550 0.517 0.496 0.472 0.478 0.472	0.498	0.031	6.3
2.94	3.003 2.806 2.768 2.749 2.551 2.791	2.778	0.144	5.2

CV%=standard deviation/mean×100%;

3.2.4 Accuracy

The intra-day and inter-day accuracy were within 85% to 115% of the nominal values of QC samples (Table 8, 9).

Table 8. Intra-day accuracy of posaconazole in pooled human plasma (expressed as percent of nominal concentration, n = 3)

Nominal Conc. (μg/mL)	Calculated Conc. (μg/mL)	% of Nominal Conc.	Mean of % of Nominal Conc.	SD (%)
0.049	0.054 0.054 0.052	111.0 109.8 106.8	109.2	2.2
0.49	0.550 0.517 0.496	112.3 105.6 101.2	106.4	5.6
2.94	3.003 2.806 2.768	102.1 95.5 94.2	97.2	4.3

Table 9. Inter-day accuracy of posaconazole in pooled human plasma (expressed as percent of nominal concentration, n = 6)

Nominal Conc. (μg/mL)	Calculated Conc. (μg/mL)	% of Nominal Conc.	Mean of % of Nominal Conc.	SD (%)
0.049	0.054	111.0	105.6	11.4
	0.054	109.8		
	0.052	106.8		
	0.045	92.1		
	0.060	121.4		
	0.045	92.6		
0.49	0.550	112.3	101.6	6.4
	0.517	105.6		
	0.496	101.2		
	0.472	96.2		
	0.478	97.6		
	0.472	96.4		
2.94	3.003	102.1	94.5	4.9
	2.806	95.5		
	2.768	94.1		
	2.749	93.5		
	2.551	86.8		
	2.791	95.0		

3.2.5 Recovery

The mean recovery of posaconazole in three QC concentrations were in the range 86.21–105.96% (Table 10).

Table 10. Recovery data of posaconazole

	Conc. (µg/mL)	POSA AREA	IS AREA	RATIO	Mean RATIO	Recovery (%)
After extraction of QC samples, spike IS into eluent, evaporate and reconstitute as described in method section.	0.049	455657 396451 494735	10480984 10433095 10390469	0.0435 0.0380 0.0476	0.043	106
	0.49	4642214 4595010 4583174	10823417 10964174 11087297	0.4289 0.4191 0.4134	0.421	86
	2.94	25888591 26004189 24258448	10699642 10888182 10412266	2.4196 2.3883 2.3298	2.379	89
Spike posaconazole and IS into extracted blank plasma, evaporate and reconstitute as described in method section	0.049	404749 470954 432906	10546872 10830538 10830538	0.0384 0.0435 0.0400	0.041	
	0.49	5213640 5171392 5270551	10438718 10777765 10893464	0.4995 0.4798 0.4838	0.488	
	2.94	29682732 29791933 29872087	11132868 10838863 11448286	2.6662 2.7486 2.6093	2.675	

3.2.6 Lower Limit of Quantification (LLOQ)

The LLOQ was measured using five samples. The back-calculated concentrations have precision and accuracy within 20% of the nominal concentration (Table 11).

Table 11. Accuracy and precision of LLOQ (n=5)

Nominal Conc. (μg/mL)	Calculated Conc. (μg/mL)	% of Nominal Conc. ±SD	CV (%)
0.02	0.021	109.4±2.3	2.1
0.02	0.022		
0.02	0.022		
0.02	0.022		
0.02	0.022		

CV%=standard deviation/mean×100%;

3.2.7 Stability

Stability was assessed through three cycles of freeze and thaw stability test, as well as a bench-top stability test, and a processed sample stability test. The changes in the QC samples of posaconazole concentrations were within 15% of nominal concentrations (Table 12).

Table 12. Stability of posaconazole QC samples

	Nominal Conc. (μg/mL)	Calculated Conc. (μg/mL)	RE (%)
3 cycles of Freeze/ thaw	0.049	0.046	-6.1
		0.048	-1.5
		0.045	-7.7
	2.94	2.753	-6.4
		2.872	-2.3
		2.971	1.0
24 hours room temperature	0.049	0.044	-10.6
		0.044	-10.6
		0.048	-2.4
	2.94	2.857	-2.8
		2.871	-2.4
		2.875	-2.2
Re-injection after 16 hours	0.049	0.054	10.9
		0.053	8.7
		0.053	7.3
	2.94	2.942	0.1
		2.936	-0.2
		2.965	0.9

RE%= ((calculated concentration−nominal concentration)/(nominal concentration))×100%

3.2.8 Quality Controls

The samples below were applied as quality controls when measuring patient samples (Table 13).

Table 13. Accuracy of QCs

Nominal Conc. (μg/mL)	Calculated Conc. (μg/mL)	RE(%)
Day 1		
0.049	0.063	28.1
0.49	0.564	15.1
2.94	2.703	-8.1
Day 2		
0.049	0.0575	17.3
0.49	0.5163	5.4
2.94	2.7063	-8.0
Day 3		
0.049	0.0517	5.6
0.049	0.0518	5.7
0.49	0.5401	10.2
0.49	0.5118	4.5
2.94	2.8920	-1.6
2.94	2.7761	-5.6
Day 4		
0.049	0.053	8.2
0.049	0.052	6.4
0.49	0.538	9.7
0.49	0.540	10.2
2.94	2.88	-2.0
2.94	2.92	-0.6

3.3 POSACONAZOLE PLASMA CONCENTRATIONS IN PATIENT SAMPLES

3.3.1 Patient Demographic Data

A total of 20 patients, including 7 LTRs with CF disease and 13 LTRs without CF disease were enrolled into the study. The summarized patient demographic data lists in Table 14. The mean age and weight of CF group are smaller than non CF group because patients with CF are under malnourished (*45*).

Table 14. Patients demographic data

Characteristic	Value			
	CF Group (n=7)	Non-CF Group (n=13)	Total (n=20)	P value
Mean Age [yr (range)]	36.1 (25-51)	60.7 (28-77)	52.1 (25-77)	0.0006
Age Range				0.04
≥ 18 to < 65 [n (%)]	7 (100%)	7 (54%)	14 (70%)	
≥ 65 [n (%)]	0	6 (46%)	6 (30%)	
Sex [n (%)]				0.07
Female	5 (71%)	3 (23%)	8 (40%)	
Male	2 (29%)	10 (76%)	12 (60%)	
Race [n (%)]				0.25
Caucasian	7 (100%)	10 (76%)	7 (85%)	
Others	0	3 (23%)	3 (15%)	
Mean Weight [kg(range)]	56.66 (41.6-68)	78.15 (48.3-110)	70.63 (41.6-110)	0.01

3.3.2 Posaconaozle Plasma Concentrations in Lung Transplant Patients

Posaconazole plasma concentrations-time profiles for 20 enrolled patients are listed in Figure 9.

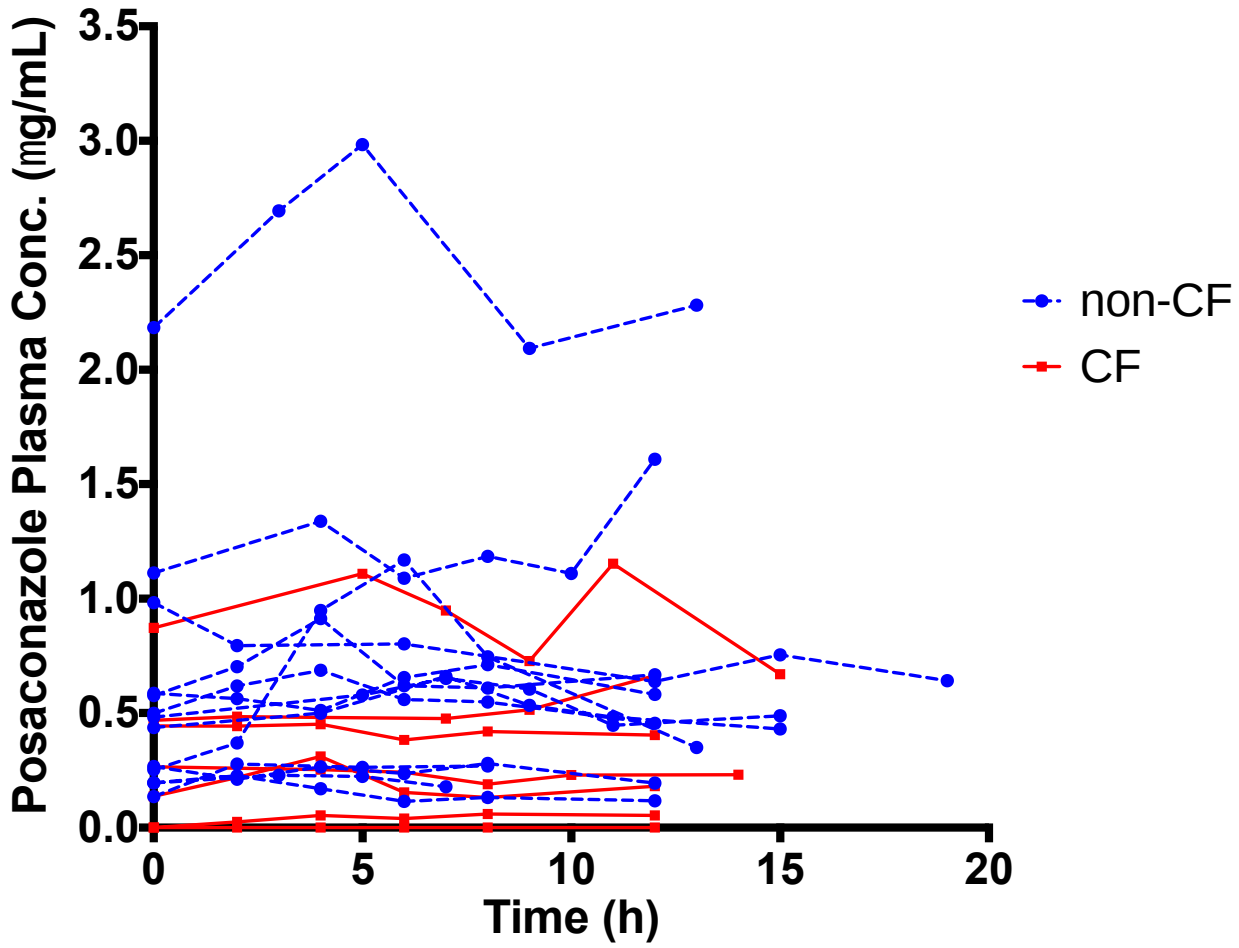


Figure 9. Posaconazole concentration - time profiles in 20 patients

3.4 PHARMACOKINETIC PARAMETERS OF POSACONAZOLE

One patient from the non-CF cohort was excluded from the pharmacokinetic analysis due to administration of a second dose within 6 hours after the first dose. The typical dosing frequency of posaconazole suspension is twice daily, but 4 patients in CF group and 4 patients in non-CF group received a second dose of posaconazole around 8 hours after the first dose. To measure the area under the posaconazole plasma concentration-time curve from 0 to 12 hours or 0 to 8 hours at steady state, plasma concentrations at 12 hours (5 patients), and plasma concentrations at 8 hours (5 patients) were extrapolated from last observed data and the elimination rate constant k. Plasma concentrations at 0 hour were assumed to be the same as the concentrations at 12 hours or 8 hours at steady state in 6 patients. After a minimum of five days treatment of posaconazole maximum plasma concentration $C_{ss,\ max}$ (0.311 μg/mL) in CF patients was 56% lower compared to $C_{ss,\ max}$ (0.699 μg/mL) in non-CF patients; the minimum plasma concentration $C_{ss,\ min}$ (0.189·μg/mL) in CF patients was 60% lower compared to $C_{ss,\ min}$ (0.474 μg/mL) in non-CF patients; the average plasma concentration $C_{ss,\ av}$ (0.233 μg/mL) in CF patients was 61% lower compared to $C_{ss,\ av}$ (0.594 μg/mL) in non-CF patients, the dose normalized plasma area under curve $AUC_{0\text{-}24}$ (0.007 h*μg/mL) in CF patients was 65% lower compared to dose normalized $AUC_{0\text{-}24}$ (0.02 h*μg/mL) in non-CF patient, and the apparent oral clearance of 2.51 L/h/kg in CF patients was 3.4 times higher compared to 0.74 L/h/kg in non-CF patients. We observed no significant difference of T_{max} and apparent oral clearance (Table 15). Weight normalized apparent clearance however was significantly higher in CF patients.

Table 15. Pharmacokinetic parameters of posaconazole in CF and non-CF group (expressed as median (range))

Parameter	CF cohort N= 7	Non-CF cohort N= 12	P value
T_{max} (h)	4.4 (0-7.8)	4 (0-11.8)	0.4
$C_{ss,\ max}$ (µg/mL)	0.311 (0.021-0.968)	0.699 (0.227-2.983)	NA*
$C_{ss,\ min}$ (µg/mL)	0.189 (0-0.619)	0.474 (0.115-2.094)	NA*
$C_{ss,\ av}$ (µg/mL)	0.233 (0.01-0.772)	0.594 (0.154-2.455)	0.03
AUC_{0-24} / Daily dose (h*µg/mL)	0.007 (0.0003-0.019)	0.02 (0.005-0.074)	0.02
Oral clearance (L/h)	143.16 (32.26-3278.69)	51.83 (13.58-216.33)	> 0.05
Weight normalized oral clearance (L/h/kg)	2.51 (0.76-48.22)	0.74 (0.25-2.66)	0.005

*NA: Not Applicable. Statistic analyses are not applicable to $C_{ss,\ max}$, and $C_{ss,\ min}$, because the enrolled patients didn't follow same dose interval.

3.5 A GOOD CORRELATION BETWEEN C_{TROUGH} AND AUC_{0-T}

C_{trough} and $AUC_{0-\tau}$ were well correlated ($r^2 = 0.91$, $P < 0.0001$) (Fig. 10).

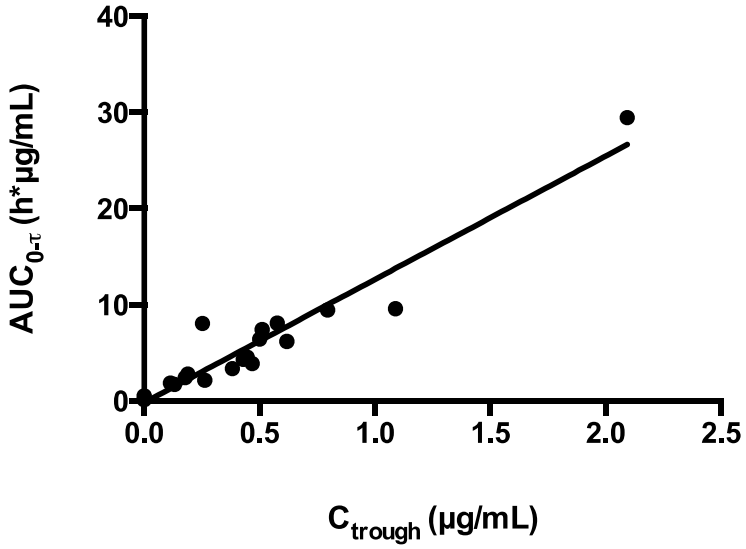


Figure 10. Correlation between C_{trough} and AUC0-τ

A good correlation between C_{trough} and $AUC_{0-\tau}$ demonstrates that measurement of posaconazole plasma concentration at trough when reaching steady state can predict posaconazole systemic exposure. It documents that C_{trough} can be used as surrogate for therapeutic monitoring of posaconazole.

4.0 DISCUSSION

Lung transplant recipients have a high risk for invasive fungal infections. At the University of Pittsburgh Medical Center, all LTRs receive routine antifungal prophylaxis with a triazole agent (voriconazole or posaconazole) for at least four to six months after transplantation. Posaconazole is an important antifungal agent for prophylaxis and treatment of invasive fungal infections. On one hand, posaconazole has potent and broad-spectrum activity against yeast and molds; on the other hand, posaconazole has fewer drug-drug interactions because it is not metabolized by cytochrome P450 enzymes. Now posaconazole is extensively used after transplantation for prophylaxis of invasive fungal infections and it is promising to become the first-line of therapy.

According to pharmacology review of two phase 3 clinical trials by the FDA, the plasma exposure of posaconazole is associated with clinical response. A steady-state C_{av} of below 700 ng/mL is expected to result in a 25% invasive fungal infection breakthrough rate (*28*). Posaconazole plasma exposure was proportionally increased when healthy volunteers were given 50 mg to 400 mg b.i.d. in a multiple-dose study. But plasma exposure of posaconazole was not further increased following multiple doses from 400 mg to 600 mg b.i.d. in patients (*16*). The absorption limitation of posaconazole suspension is probably due to its poor solubility. The FDA suggests that if plasma concentration of posaconazole is below 0.7 μg /mL after patients receive a posaconazole suspension of 400 mg t.i.d. for 7 days, the regimen should be switched to other antifungal agents.

Besides posaconazole's poor solubility, the absorption from a suspension can be affected by a number of variables. Firstly, high-fat meals and non-fat meals significantly increase plasma exposure of posaconazole. When patients are not allowed or not able to have a full meal while on posaconazole, they are at high risk for invasive fungal infection breakthrough due to low drug exposure. Secondly, drug-drug interactions can lead to low plasma exposure of posaconazole. Posaconazole is a substrate of P-gp efflux and is metabolized by UGT 1A4. Drugs that induce either UGT enzyme or P-gp efflux can decrease plasma exposure of posaconazole. The third factor is the pH of gastrointestinal tract in patients. When patients undergo acid-suppressive therapy, their plasma exposure of posaconazole is decreased. Given the unpredictable plasma exposure of posaconazole, therapeutic drug monitoring should be applied to posaconazole to individualize its dosing regimen (*46-48*).

Patients with CF have increased invasive fungal infections after lung transplantation, but they are suspected to experience poor absorption of posaconazole. Most patients with CF have exocrine pancreatic insufficiency and altered bile acid turnover, which may affect the absorption of lipophilic drugs such as posaconazole (*31*). Due to the reasons above, it is imperative to understand pharmacokinetic profiles of posaconazole in lung transplant recipients with CF. This study is the first one to investigate pharmacokinetics of posaconazole suspension in lung transplant recipients with CF.

In this study, we established a HPLC-fluorescence method to analyze plasma concentrations of posaconazole. The method is very sensitive. HPLC–ultraviolet (UV) and HPLC tandem mass spectrometer (MS) have been used to analyzed posaconazole (*49-51*). The HPLC–UV was not sensitive to measure posaconazole in patient samples. One study reported using HPLC-fluorescence to analyze posaconazole and itraconazole following liquid-liquid

extraction of its plasma samples (*43*). But an analytical interference was observed with this method for posaconazole in pooled human blank plasma. We evaluated different extraction reagents such as terbutylmethylether, hexane-dichloromethane and ethyl ether, but none of them solved the analytical problem. Finally, we developed solid phase extraction for sample extraction, and no interfering peaks appeared with the same retention time of posaconazole. In this method, out of 100 μL of reconstitution volume, the injection volume used is only 20 μL. This makes it possible to re-inject sample, if necessary. Since this assay requires only a small volume of plasma, it is favorable to both pharmacokinetic studies and therapeutic drug monitoring of posaconazole. There was no interfering endogenous materials in human plasma.. The probability for other drugs used with posaconazole to elute at same retention time and to interfere with posaconazole and itraconazole at the specific excitation and emission wavelengths and pH 2 used is rare. We tested chromatographic peaks of ketoconazole, fluconazole and voriconazole at the described chromatographic method, and these three drugs didn't show any interfering peaks.

In the label of posaconazole, it is recommended to avoid concomitant use of cimetidine (an H_2-receptor antagonist) and esomeprazole (a proton pump inhibitor) with posaconazole oral suspension, because lower plasma concentrations have been observed when patients concomitantly used the two drugs with posaconazole suspension. In this study, none of the twenty patients were taking either of the two drugs during the pharmacokinetic study.

In the study, we observed large variation in posaconazole pharmacokinetic parameters and lower $C_{ss,\ max}$, $C_{ss,\ min}$, $C_{ss,\ av}$, and dose normalized $AUC_{0\text{-}24}$ in CF group. Among the CF patients, one patient did not receive any oral feeding, two patients were receiving tube feeding and the other four patients were normally feed during the pharmacokinetic study. In the non-CF

group, among 13 patients, 2 patients were tube fed and the other patients were normally feed. We observed a lower plasma concentration of posaconazole in the most of these patients not receiving normal feeding, but lower plasma concentration of posaconazole was also observed in patients with normal feeding. Because of the small sample size of patients, we can't find association between feeding and plasma concentration of posaconazole.

There was no statistic difference of total apparent oral clearance between the CF and non-CF group, but we found significantly higher weight normalized total apparent oral clearance in the CF patients. Currently, there is no published literature reporting association between weight and total body clearance of posaconazole. Considering posaconazole is a highly lipophilic drug, a patient with greater weight may have a larger volume distribution. From this study, we believe weight may be a potential viability that could affect the total body exposure of posaconazole. But additional studies need to be performed to understand the impact of weight of the patient on the total body clearance of posaconazole.

Several factors could result in a lower systemic exposure of posaconazole in patients with CF including absorption, glucuronidation, and reabsorption. Posaconazole is a lipophilic drug and the decreased pancreatic enzyme secretion and altered bile acid turnover in CF can lead to reduced absorption of posaconazole (*24*). Secondly, an enhanced activity of UGT1A4 in patients with CF may be one of the reasons behind a lower plasma concentration of posaconazole. Some studies reported the hepatic glucuronosyltransferase activity was increased in CF disease (*52, 53*), but more data are needed to prove the observation and no study specifically assessed the alteration of UGT1A4 in CF. If UGT1A4 exhibits enhanced activity in CF, more posaconazole would be metabolized into its inactive glucuronide conjugates, leading to decreased plasma concentration of posaconazole. There are multiple factors that could affect posaconazole

reabsorption in CF patients. For example, a decreased activity of multidrug resistant protein 2, a major transporter responsible for the biliary excretion of glucuronides, could decrease the secretion of glucuronide conjugates from bile to intestine. Considering the altered microflora in the intestine, patient with CF may have a lower glucuronidase activity in the gut to convert posaconazole glucuronide to posaconazole for reabsorption. The alteration of glucuronidation, and reabsorption can be confirmed by quantifying glucuronide conjugates of posaconazole in plasma, but standard posaconazole glucouronide is not commercially available, currently.

Out of 20 patients, a steady state average concentration of 0.7 μg/mL was achieved only in one CF patient and three non-CF patients. The findings supports therapeutic drug monitoring of posaconazole is very important to LTRs, especially in LTRs with CF. Moreover, a good correlation between C_{trough} and $AUC_{0-\tau}$ demonstrated that C_{trough} is a good surrogate marker to monitor systemic exposure of posaconazole.

In conclusion, therapeutic drug monitoring of posaconazole should be applied to LTRs. If increasing the dosage of posaconazole suspension doesn't help patients achieve desired target therapeutic level in patients, using a posaconazole tablet or switching to other antifungal agents should be considered.

There are some limitations to this clinical study. First, for pharmacokinetic study, the time period of blood sampling should be 3 to 5 terminal elimination half-lives following the last dose. The half-life of posaconazole is 24 hours, so ideally blood samples should be collected at least 72 hours after the final dose. In this study, blood samples were only collected for 8 to 12 hours after a dose. This is the reason that we observed flat time-concentration profiles, because concentration time profiles for posaconazole didn't reach true terminal phase. This limitation was due to clinical practice of administrating of drug b.i.d or t.i.d. Second, the clearance assessed

from suspension is oral clearance, not true systemic clearance. Based on the oral clearance we could not conclude that CF patients have higher total body clearance for posaconazole or poor oral absorption. Now, posaoncaozle injection has been approved by the FDA. In the future, we could use the intravenous formulation to investigate true total body clearance of posaconazole in CF patients. Furthermore, if we had measured the glucuronide metabolite of posaconazole and analyzed the ratio of systemic exposure of metabolite against the parent drug, we would be able to understand if there are changes to posaconazole metabolism in LTRs with CF. Alterations to metabolic pathway could be one possible cause of low systemic exposure of posaconazole in LTRs with CF.

In the past two decades, pulmonary drug delivery has been become a popular route because 70-140 m^2 of lung surface could be an ideal drug absorption site in addition to small intestinal tract (*54*). From this study, we observed a low systemic exposure in LTRs. In the future, we could develop inhaled posaconazole to improve prophylaxis of IFIs in LTRs because localized posaconazole delivery by inhalation would increase intrapulmonary concentration of posaconazole and bypass gastrointestinal absorption. Due to bypassing gastrointestinal absorption, inhaled posaconazole will result in high drug concentration in the respiratory tract with low systemic exposure, which will enable posaconazole to have better therapeutic effect with fewer side effects. Moreover, an inhaled formulation would have fewer drug-drug interactions that are very common observed with triazole antifungal drugs due to their metabolism by cytochrome P450 enzyme.

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