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Pulmonary Delivery of Aqueous Voriconazole Solution

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Pulmonary Delivery of Aqueous Voriconazole Solution

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Dedication

To Jodi Ann Oldroyd Tolman

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Pulmonary Delivery of Aqueous Voriconazole Solution

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Invasive Pulmonary Apsergillosis (IPA) is caused by inhalation of fungal conidia to the deep lung followed by germination and invasive hyphal growth in heavily immunosuppressed patients (e.g. those with hematologic malignancies, hematopoietic stem cell transplant recipients, and those undergoing solid organ transplantation). Hyphal growth into pulmonary capillaries often leads to dissemination of the infection and high mortality rates despite current treatment and prophylactic modalities. In addition, systemic antifungal therapy is often limited by drug toxicities, low and variable bioavailability, erratic pharmacokinetics, and drug interactions. Although targeted drug delivery to the lungs has been investigated to reduce adverse events and promote drug efficacy, inconsistent pharmacokinetic properties following inhalation of poorly water soluble antifungals has prompted variable drug efficacy. In this dissertation, inhaled voriconazole was investigated through *in vitro* and *in vivo* testing to evaluate pharmacokinetic properties, characterize drug safety and, determine drug efficacy as prophylaxis against IPA

In Chapter 2, the *in vitro* evaluation of solution properties and aerosol characterization of aqueous voriconazole was evaluated. Subsequent *in vivo* single and multiple dose pharmacokinetic studies demonstrated high drug concentrations were achieved in lung tissue and plasma following inhalation in contrast to previous reports of inhaled antifungals. Inhaled voriconazole was then administered twice daily (BID, at 08:00 and 16:00) in a murine model of IPA as described in Chapter 3 with significant improvements in animal survival over 12 days compared to both positive and negative control groups.

As described in Chapter 4, voriconazole was then chronically administered BID at a high and low dose to rats over 21 days with a 7 day recovery period to assess dose tolerability through laboratory tests and histopathological changes to lung, liver, kidney, and spleen tissues. Inhaled voriconazole was well tolerated through all assessments but with signs of mild acute histiocytosis in lung tissue without other signs of inflammation.

Chapter 5 expanded the single inhaled dose pharmacokinetic profile in lung tissue and plasma with determination of additional pharmacokinetic parameters through compartmental modeling. Peak and trough voriconazole concentrations were also evaluated in mice as well as rats following multiple doses administered over 12 hours (Q12H) as opposed to BID.

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Chapter 1: Advances in the Pulmonary Delivery of Poorly Water Soluble Drugs: Influence of Solubilization on Pharmacokinetic Properties

1.1. INTRODUCTION

Therapeutic administration of active pharmaceutical ingredients (APIs) to the lungs has long historical significance (1). Despite the long term use of therapeutic aerosols, the scientific principles governing the *in vivo* performance of inhaled drugs have only recently been probed. In the modern age of drug research and development focused on pulmonary drug delivery, the fate of inspired aerosols has been correlated to patient specific as well as formulation/device factors. The *in vivo* action of inhaled aerosols can be affected by patients through the control and regulation of the physiologic parameters of breathing; e.g. including respiration rate, tidal volume, inhalation air flow, breath holding, etc. (2). Additionally, the formulation scientist can influence *in vivo* aerosol performance through manipulation of the interrelationships between the formulation and inhalation device, e.g. pressurized metered dose inhaler (pMDI), nebulizer, or dry powder inhaler (DPI). These modifiable relationships govern the aerodynamic particle size distribution, pH, tonicity, and physiologic compatibility of the inspired aerosol.

Traditionally, inhaled APIs have been intended for local drug action in the lungs for treatment of topical conditions in the airways; examples include the treatment of airway inflammation, lung diseases, and lung infections. However, drug delivery to the lungs has recently received increased scientific attention and expansion. This renewed

interest coincides with advances in particle engineering technologies (3, 4), advances in biotechnology-derived therapeutic macromolecules (5), and new APIs with low and/or erratic bioavailability (6-8). Much of the expanded interest in pulmonary drug delivery focuses on systemic drug delivery via the lungs due to the rapid bioavailability and avoidance of the pH, food effects, enzymatic, and first-pass metabolic barriers following oral drug administration. Despite these potential advantages, inhaled drugs must overcome numerous barriers for adequate deposition in the lung.

Several excellent reviews have explained in detail the physiologic barriers to inhaled drug delivery (7, 9-11). Briefly, the lungs are a natural particle filter due to numerous cellular and physiological factors. The majority of lung airway epithelium is ciliated with a mucous layer that can prevent drug from depositing in the deep lungs and promote drug ingestion through the mucociliary escalator. Additionally, the airways in the lung subdivide through a tortuous pathway of bifurcations throughout the lung that allow air communication with the gas-exchange specializing lung structures, the alveoli, also referred to as the deep lung. An inspired particle must therefore, avoid contact with the ciliated and mucous covered epithelium to avoid ingestion, via the mucociliary escalator, as well as traverse numerous potential impaction sites for then deposition along the airways or in the deep lung. The aerodynamic properties and particle behavior of the inspired particle are therefore crucial for drug delivery to the lungs, typically 1-5μm in size (9). The inspired particle must also be physiologically compatible with the lung membranes (i.e. isotonic, iso-pH, non-immunogenic, etc.) to avoid airway hyper-responsiveness, cough or airway spasticity, or inflammation (12). The deposition of particles can also be affected by the increasing relative humidity in the lungs as a particle is inspired into the deep lung (13).

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Once a particle has bypassed these pulmonary barriers and been deposited in the alveolar region, the API must be absorbed for systemic drug action. The ability for APIs to be absorbed across the alveolar membrane has not been investigated to the degree of gastrointestinal (GI) drug absorption. Mechanistic explanations of GI absorption have recently been re-reviewed and form a foundation for explaining pulmonary drug absorption (14, 15). The primary differences between modeling GI and pulmonary drug absorption focus on the fact that the lungs have different physiologic and cellular structures at absorption sites, have a dramatically decreased metabolic capacity, lack the degree of active transport sites, and have a much higher surface area and corresponding blood flow than the GI tract.

An excellent review by Sakagami was published in 2006 and summarized numerous methodological approaches to investigating the mechanisms underlying pulmonary drug absorption and disposition (16). As with any model, control and evaluation of the numerous variables associated with pharmacokinetic profile and properties of a drug following inhalation is very difficult. As a result, researchers have employed *ex vivo*, cellular, *in silico*, and *in vitro* models to isolate and quantify the different variables present in whole animals when investigating the factors affecting drug absorption in the lungs. However, these isolated or simplified models do not adequately simulate the numerous factors involved with pulmonary drug delivery in a living system.

To further complicate the literature describing the pharmacokinetics of inhaled drug delivery, researchers have used whole animal models with varying methods of pulmonary drug administration, i.e. intratracheal instillation of a liquid, orotracheal

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intubation and administration of a liquid spray or powder insufflator, and natural whole-body or nose-only exposure. The method of pulmonary drug administration can affect the reported results due to species-specific differences in the respiratory system between animals. For example, the majority of mammal species are obligate nose breathers with the inability to breathe through the mouth, causing airflow differences and resultant differences in deposition from humans (17). Although these whole animal modeling systems have difficulty isolating the specific contributing factors involved in drug absorption, they are applicable as screening mechanisms for different formulations and can represent a more realistic approach to understanding drug absorption in the lungs. Of the numerous factors that can influence drug absorption from the lungs, the effect of drug solubility, solubility enhancing excipient, and drug solution or solid state for poorly water soluble APIs has not been explained in whole animal or in isolated component systems for pulmonary drug administration.

Poorly water soluble APIs are becoming increasingly common for new chemical entities (18-21). A compound with poor aqueous solubility presents challenges and limitations for formulation development and the clinical utility of a dosage form, particularly in the lungs. The absorption would be limited by the number of dissolved molecules for diffusion through biological membranes. Although there is no unified definition for poorly water soluble drugs, the United States Pharmacopeia (USP) uses descriptive terms related to quantifiable solubility ranges, i.e. very soluble (>1 g mL^{-1}) to insoluble (<0.1 mg mL^{-1}) (22). Instead, the Center for Drug Evaluation and Research (CDER) describes solubility as "high" or "low" based on the ability of 250 mL of dissolution medium to dissolve the dose of drug by *in vitro* methods (23). This categorical classification is intended to describe the impact of solubility on drug

absorption and bioavailability (24). However, the definition of low solubility has little physiologic significance on absorption when applied to pulmonary drug delivery due low masses in inhaled drug doses and a small and dispersed fluid volume within the lungs (7, 25, 26). Therefore, the relationship between low solubility and observed pharmacokinetic properties of drugs when administered to the lungs does not fall into the definitions and testing parameters that are applicable for other routes of drug administration.

Several therapeutic agents with low aqueous solubilities have been investigated for pulmonary drug delivery. These agents include: corticosteroids in the management of asthma and inflammation; anti-infective agents to treat and prevent bacterial, fungal, and viral pneumonias; chemotherapeutic agents for lung cancers and tumors; and numerous other APIs. The low solubility of these APIs can influence the absorption and retention of the drug in the lung tissue and can directly affect drug activity, side effects, and dosing regimens. Accordingly, this article will review the literature available describing the pulmonary drug administration of poorly soluble APIs where some pharmacokinetic data is available. Although drug absorption across membranes in the lungs is the parameter of interest, few researchers directly measure absorption rates across the pulmonary epithelium, e.g. mean absorption times (MAT) or absorption rate constants (k_{abs}). Instead, proxy markers of drug absorption could include other observed pharmacokinetic parameters such as maximal drug concentration in the blood and in the lung tissue if available (C_{max}), the time to reach maximal concentrations (t_{max}), elimination half-life ($t_{1/2}$) and drug exposure (AUC). These proxy markers will allow comparative relationships to be established to evaluate the influence of formulation and solubility enhancements have on drug absorption. Therefore, the influence of solubility and

formulation-based solubility enhancements on pharmacokinetic parameters following inhalation of various classes of poorly water soluble drugs, including corticosteroids, antifungals, oligopeptides, and opioids, will be reviewed.

1.2. INHALED CORTICOSTEROIDS

Inhaled corticosteroids are the most commonly inhaled class of poorly water soluble API. They are therapeutically used to inhibit inflammatory processes in the lungs, primarily in management of asthma. These structurally related agents have a steroid backbone, some with modifications to the steroid ring, and appended functional groups (27). These modifications primarily affect ligand-receptor interactions and lead to varied binding affinities with the glucocorticoid receptor. Because all corticosteroids affect the same receptor, competitive binding assays have allowed the relative potencies of these agents to be stratified as fluticasone propionate > beclomethasone-17-monopropionate > budesonide > beclomethasone dipropionate > triamcinolone acetonide (28). These relative potencies affect drug efficacy as well as the side effect profile and propensity for long-term adverse events. However, many adverse events associated with inhaled corticosteroids result from systemic exposure following absorption. In addition to these structure-based pharmacodynamic properties, most corticosteroids remain poorly water soluble compounds with aqueous solubilities of $21\mu g\ mL^{-1}$ for triamcinolone acetonide, $16\mu g\ mL^{-1}$ for budesonide, $0.14\mu g\ mL^{-1}$ for fluticasone propionate, and $0.13\mu g\ mL^{-1}$ for beclomethasone dipropionate ($15.5\mu g\ mL^{-1}$ for the beclomethasone-17-monopropionate active metabolite) (29). Reported log $P_{o/w}$ values also indicate these agents are very lipophilic with values of 3.4 for triamcinolone acetonide, 3.6 for budesonide, 4.5 for fluticasone propionate, and 4.9 for beclomethasone dipropionate (4.3 for beclomethasone-17-monopropionate). The molecular weights for these compounds

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are 430.5 g mol^{-1} for budesonide, 434.5 g mol^{-1} for triamcinolone acetonide, 500.6 g mol^{-1} for fluticasone propionate, and 521.1 g mol^{-1} for beclomethasone dipropionate. These high log P values and small molecular weights indicate the potential for good passive membrane permeability, leading to dissolution-limited drug absorption following inhalation.

Ideally, an inhaled corticosteroid would have high potency, be retained in the airways and lung tissue for prolonged anti-inflammatory action, and would then have low drug absorption leading to low systemic drug exposure with consequently, low incidence of adverse events. Accordingly, several researchers have investigated the pharmacokinetic properties of inhaled corticosteroids to understand the mechanisms of drug deposition and absorption from the lungs to the systemic circulation (30-35). Some pharmacokinetic profiles of these agents are also influenced by the structural differences between the APIs, specifically the avenues of clearance and metabolic pathways between the various agents (36). The other pharmacokinetic properties of inhaled corticosteroids, including the C_{max}, t_{max}, AUC, and $t_{1/2}$, vary between the agents based, in part, on the physicochemical properties of the API. The interrelationship of pharmacokinetic and pharmacodynamic properties of this drug class defines their clinical utility. For that reason, many researchers have investigated adverse events of these agents through the biomarker of endogenous cortisol secretion suppression and corresponding bioavailabilities between inhaled and other routes of administration (37). However, the utility of a biomarker in this current investigation is limited when correlating the influence of drug solubility and solubilization properties of the formulation on drug absorption following inhalation. Through independent evaluation of corticosteroids with

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reported pharmacokinetic parameters, categorical relationships can describe the influence of solution state and formulation on pulmonary absorption of these inhaled agents.

1.2.1. Fluticasone propionate

(See Table 1.1A) The majority of systemic pharmacokinetic data on inhaled fluticasone propionate is with the dry powder inhaler (DPI) formulation branded as the Flutide®, Flovent®, or Flixotide® administered with the Diskhaler®, Diskus®, or Accuhaler® devices (30, 38-42). These formulations use micronized fluticasone propionate blended with a lactose carrier particle and de-aggregate from the carrier via turbulent airflow through the device. Some pharmacokinetic data is also available with the pressurized metered dose inhaled (pMDI) branded as Flovent®. The pMDI formulation contained a microcrystalline suspension of fluticasone propionate in a propellant mixture of CFC-11 and CFC-12 with soya lecithin as a surfactant and lubricant for the metering valve. Both the DPI and pMDI formulations deliver solid fluticasone propionate particles to the lung and rely on particle size reduction of the API to improve the rate of dissolution for this poorly water soluble drug. Therefore, fluticasone propionate has little data to describe the influence of drug solubilization and solubility enhancement through the formulation on drug absorption from the lungs. However, it does serve as a reference and comparator for the remainder of the inhaled corticosteroids with a moderate aqueous solubility (0.14 µg mL^{-1}), log P value (4.5), and molecular weight (500.6 g mol^{-1}) for this class of poorly water soluble compounds.

Following a single inhalation, maximal concentrations were observed after an average of 0.9 to 1.88 hours (54-118 minutes). Dose normalized maximal concentrations ranged from 0.1 up to 0.3 pg mL^{-1}µg^{-1} while dose normalized AUC values ranged from

0.3 to 3.0 pg hr mL^{-1} µg^{-1} with no real difference between the DPI and pMDI forms. Concentrations and AUC values were not controlled for the influence of oral ingestion of the drug through oral administration of activated charcoal and must be assumed to have been affected by minor, but non-trivial, oral ingestion of fluticasone propionate. However, despite possible oral ingestion of the inhaled product and a 3 to 10 fold difference in maximal drug concentrations and drug exposure, plasma fluticasone concentrations remained very low, in the pg to ng range, following large inhaled doses. The very low systemic fluticasone propionate concentrations indicate very little drug absorption from the inhaled particulate systems.

Several researchers reported the mean residence time (MRT), the average time a molecule resides within the system from absorption to elimination, for fluticasone following inhalation. The reported MRT values were 7.1 to 12 hours for DPIs and 5.3 hours for the pMDI, indicating an prolonged but variable time for the drug to be retained in the studies population. Additionally, Brindley et al. specifically investigated the absorption kinetics of fluticasone propionate following inhalation using both the DPI and pMDI devices (30). Following inhalation from both DPI and pMDI devices, 50% of the bioavailable dose was absorbed within 1.6 to 2.4 hours (95 to 145 minutes) while 90% of the dose was absorbed by 11.4 to 12.3 hours. The average time it takes for a drug molecule to be absorbed, the mean absorption time (MAT), was 4.3 to 4.4 hours. The authors identified that fluticasone propionate is retained in the lungs for an extended period of time with an initial rapid phase of drug absorption followed by a period of prolonged drug absorption.

1.2.2. Budesonide

(See Table 1.1B) Inhaled formulations of budesonide were more diverse than those for fluticasone propionate and included DPI, pMDI, and nebulizer formulations. The branded DPI products included the Pulmicort Turbohaler®, now referred to as the Flexhaler®, with only micronized budesonide in the formulation, and the Giona® Easyhaler® containing budesonide blended with a lactose carrier particle (31, 39-41, 43). The pMDI formulation, Pulmicort® (no longer available in the United States), contained a micronized suspension of budesonide with sorbitan trioleate as a metering valve lubricant , and a propellant mixture of CFC-11, CFC-12, CFC-114 (44). Budesonide suspensions for nebulization were also tested and included the marketed Pulmicort Respules® and two different novel nano-scale suspensions (44-46). The Pulmicort Respules® contained a micronized suspension of budesonide with disodium edetate, sodium chloride, sodium citrate, citric acid, polysorbate 80, in water for injection. The first nano-suspension from Kraft et al. did not contain information on the formulation. However, the second nano-suspension from Shrewsbury et al. contained submicron budesonide in a sterile aqueous formulation containing surface modifiers, possibly including a cyclodextrin (47), and sodium chloride, citric acid, sodium citrate, disodium edetate dehydrate in water. Despite the differences, the DPI, pMDI, and suspension for nebulization formulations all deliver solid budesonide particles to the lung following inhalation and utilize particle size reduction to improve the dissolution rate of the drug. The low aqueous solubility (16 μg mL^{-1}), high log P (3.6), and low molecular weight (430.5 g mol^{-1}) promote a model of solubility limiting drug absorption following inhalation of budesonide particles. However, the novel nano-suspension formulations

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contain excipients that could improve or augment drug solubility in the lung and subsequent drug absorption following inhalation.

Following inhalation of budesonide, t_{max} values were achieved within 0.13 to 0.58 hours (8 to 35 minutes) for the DPI devices, 0.15 to 0.24 hours (9 to 14 minutes) for micronized suspensions, and 0.051 to 0.19 hours (3 to 11 minutes) for nano-sized suspensions with no values reported for the pMDI. Dose normalized C_{max} values for DPI devices, the pMDI, micronized suspensions, and nano-sized suspensions ranged from 0.9 to 1.8 pg mL^{-1}μg^{-1}, 0.6 pg mL^{-1}μg^{-1} (assuming a 2 hr t_{max} due to limited reported data), 0.7 to 1.3 pg mL^{-1}μg^{-1}, and 1.8 to 2.5 pg mL^{-1}μg^{-1}, respectively. These C_{max} ranges indicate approximate equivalence for reported maximal concentrations for all methods of budesonide administrations except a two-fold increase in reported concentrations for nano-scale suspensions. However, no difference was observed for dose normalized AUC values between delivery methods with ranges of 2.5 to 4.5 pg hr mL^{-1} μg^{-1} for DPI devices, 2.1 to 3.3 pg hr mL^{-1} μg^{-1} for micronized suspensions, and 1.1 to 3.3 pg hr mL^{-1} μg^{-1} for nano-sized suspensions with no reported value for the pMDI. As mentioned for inhaled fluticasone propionate, no report was made to control for possible oral ingestion of budesonide following inhalation. In a similar manner, the reported C_{max} and AUC values could have a minor, but non-trivial, contribution of orally absorbed budesonide. The elimination half-life, $t_{1/2}$, for inhaled budesonide also varied by the method of inhalation with DPIs ranging from 2.1 to 3.5 hours, the micronized suspension reporting 2.43 hours, and the nano-scale suspension reporting 1.17 to 2.33 hours. Of note, Kraft and colleagues reported much higher $t_{1/2}$ values, from 5.42 to 6.62 hours for inhaled micro and nano-sized suspensions without corroboration from the other sources, possible indicating a sampling outlier. Some researchers reported MRT values for DPI devices

that ranged from 0.6 to 3.9 hours, indicating varied but relatively rapid drug transit through and low drug retention by the patient.

1.2.3. Beclomethasone dipropionate (Beclomethasone 17-monopropionate)

(See Table 1.1C) Beclomethasone dipropionate is converted in the lungs via epithelial esterases from a functional pro-drug into the active and more potent beclomethasone 17-monopropionate. Therefore, pharmacokinetic studies involving beclomethasone specify the molecule of interest and involve a metabolic process if results are reported for the mono-propionate metabolite. The di- and monopropionate forms have different solubilities (0.13 μg mL^{-1} for the dipropionate and 15.5 μg mL^{-1} for the monopropionate) but similar log P values (4.9 for dipropionate and 4.3 for monopropionate) and molecular weights (521.1 g mol^{-1} for dipropionate and 465.0 g mol^{-1} for monopropionate). Although the active metabolite has a 100-fold improvement in aqueous solubility over the dipropionate form, absorption must take place with the pro-drug dipropionate prior to metabolic conversion. Despite these metabolic complications in assessing systemic pharmacokinetics following inhalation of beclomethasone dipropionate, investigators have administered beclomethasone dipropionate as a nebulized solution in addition to the typical DPI and pMDI devices reported by other researchers.

Specifically, Esposito-Festen et al. generated very low dose mono-disperse particle sized aerosols from an alcoholic solution containing budesonide dipropionate and administered them to healthy volunteers (48). This formulation delivered aerosolized droplets to the lung that contained beclomethasone in solution as a molecular dispersion. In contrast, particle size reduction of the API was utilized for pMDI and DPI

formulations.   A pMDI formulation containing a suspension of micronized beclomethasone dipropionate in CFC-11 and CFC-12 with oleic acid as a valve lubricant, marketed as Beclovent®, was tested in human patients with and without concomitant oral administration of activated charcoal to eliminate oral ingestion and absorption of the API following inhalation (49). Pharmacokinetic values were also evaluated for a DPI device used to administer micronized beclomethasone dipropionate on lactose carrier particles, branded as Becodisks®, to stable human asthma patients (41).

Marked differences were observed for inhaled beclomethasone dipropionate, and the active metabolite beclomethasone 17-monopropionate, based on the formulation. T_{max} values for inhaled particulate formulations of beclomethasone dipropionate from DPI and pMDI devices were 0.8 to 2.5 hours (48 to 150 minutes). In contrast, t_{max} values were much more rapid for inhaled alcoholic solutions with values of 0.17 to 0.33 hours (10 to 20 minutes). Additionally, the dose normalized C_{max} values for DPI and pMDI devices were 0.41 and 0.94 pg mL^{-1}μg^{-1}, respectively, while normalized AUC values with the same devices were 2.13 and 3.85 pg hr mL^{-1} μg^{-1}. However, when patients received oral charcoal to negate gastrointestinal absorption of the drug when administered with the pMDI dose, normalized C_{max} and AUC values were 0.71 and 2.40 pg hr mL^{-1} μg^{-1}, indicating substantial increases in plasma concentrations of beclomethasone 17-monopropionate due to oral ingestion and absorption after normal inhalation with the pMDI.   These findings are in stark contrast with pharmacokinetic results reported following inhalation of a solubilized form of beclomethasone dipropionate.   When administered as a nebulized alcoholic solution, dose normalized C_{max} values ranged from 3.9 to 9.1 pg mL^{-1}μg^{-1}. These values resulted in a 4 to 20 fold increase in maximal concentrations compared to inhaled particulate drug via DPI or pMDI devices.

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Additionally, dose normalized AUC values for the inhaled alcoholic solution ranged from 6.0 to 16.0 pg hr mL^{-1} μg^{-1}, representing a 2.5 to 22.5 fold increase in drug exposure. The administration of an alcoholic solution of beclomethasone dipropionate promoted much more rapid maximal concentrations of the active metabolite as well as markedly elevated drug concentrations and drug exposure compared to inhalation of solid particulate forms of the API.

1.2.4. Triamcinolone acetonide

(See Table 1.1D) Inhaled triamcinolone acetonide was administered to human subjects by both DPI and pMDI devices. The DPI device used was a breath-actuated inhaler, the Ultrahaler®, to optimize lung deposition of the inhaled powder containing micronized triamcinolone acetonide blended with lactose as a carrier particle (50). The pMDI formulations included CFC and HFA formulations of triamcinolone acetonide, marketed as Azmacort® and developed as Azmacort® HFA (43, 51). The CFC-based formulation contained a microcrystalline suspension of triamcinolone acetonide in CFC-12 and 1% w/w dehydrated alcohol to improve drug loading of the API in the propellant. The Azmacort® HFA inhaler contained a microcrystalline suspension of triamcinolone acetonide in HFA 143-a but insufficient detail was provided to identify other excipients if present. Both DPI and pMDI formulations utilized particle size reduction to improve the dissolution rate of the API with an insignificant contribution of the alcohol in the CFC-pMDI formulation to alter solubility of triamcinolone acetonide after dose administration. Additionally, Lim et al. administered oral activated charcoal to some patients to assess the influence of oral ingestion and gut absorption following pMDI and DPI use (50). Triamcinolone acetonide has the highest aqueous solubility (21 μg mL^{-1}) and lowest log P value (3.4) for these poorly water soluble inhaled corticosteroids. However, a log P of

3.4 is still very high and suggests good membrane permeability, particularly with a mid-range molecular weight (434.5 g mol^{-1}).

Following inhalation, t_{max} values for the DPI device was 0.25 hours (15 minutes) while pMDI formulations peaked at 0.66 to 1.74 hours (40 to 104 minutes). Despite these differences in the speed to achieve maximal concentrations, dose normalized C_{max} values were very similar for both DPI and pMDI devices. C_{max} values for the DPI inhaler ranged from 1.77 to 2.25 pg mL^{-1}µg$^-$1 while pMDI values ranged from 0.69 to 2.52 pg mL^{-1}µg^{-1}. In contrast, AUC values were more varied with a range of 6.88 to 8.10 pg hr mL^{-1} µg$^-$1 for the DPI formulation and 2.69 to 12.90 pg hr mL^{-1} µg^{-1} for the pMDI formulation. This variability could be due to oral ingestion of triamcinolone acetonide as demonstrated by C_{max} ratios between DPI and pMDI formulations of 2.44 under typical usage and 1.56 with oral ingestion of charcoal. A similar pattern was reported for AUC ratios between DPI and pMDI formulations without and with charcoal of 1.99 to 1.44, respectively. No change was reported in the elimination half-life based on device and formulation with values ranging from 2.2 to 2.5 hours.

1.2.5. Comparison of Inhaled Corticosteroids

The reduction in inhaled corticosteroid absorption from the lungs is clinically relevant to minimize adverse events associated with systemic drug exposure for all inhaled corticosteroids. All included studies employed a method to enhance drug solubility or improve the rate of drug dissolution including particle size reduction of the API (i.e. micronization or nano-scale particle production) or drug solubilization in a non-aqueous solvent. The methods of solubility enhancement demonstrated that following µg dose masses, normalized plasma drug concentrations were only in the pg mL^{-1} range with

low total drug exposure, as indicated by normalized AUC values, for all the formulations and drug deliver devices. However, the differences in pharmacokinetic parameters within and in-between formulations were illustrative for solubilization effects on pulmonary drug absorption. Specifically, systemic t_{max} values were within two hours, with the majority of reported values within one hour, for all reported drug-formulation combinations. The fastest relative t_{max} values, when compared between different formulations of the same API, were obtained for nano-budesonide suspensions ($\geq$3-times faster than other formulations) and alcoholic solutions of beclomethasone dipropionate ($\geq$4-times faster than other formulations). These values suggest that increasing the velocity of particle dissolution, through administration of a pre-solubilized drug or through extreme particle size reduction into the nano-scale range, promoted the most rapid drug absorption following inhalation of a poorly water soluble API (52-54). However, no consistent differences were observed in dose normalized C_{max} and AUC values for DPI, pMDI, or nebulized suspensions when the formulation contained micro- to nano-meter range particles, suggesting total drug absorption was eventually achieved from the lungs. A striking elevation in drug concentrations and drug exposure were observed for nebulized alcohol solutions suggesting pre-solubilized drug actually can improve the extent of drug absorbed from the lungs (55).

1.3. INHALED ANTIFUNGALS

Most typical fungal infections are found on the skin, genitorurinary, or gastrointestinal tract and involve superficial infiltration of the fungi into the epithelium or mucosal membranes and are readily treated with topical or oral antifungal therapy (56). However, systemic fungal infections can involve numerous organs and systems and are much more difficult to treat with some causative organisms and infections associated with very high rates of mortality (57-59). Many systemic fungal infections begin with the inhalation of fungal spores, or conidia, into the deep lung followed by the establishment of an infection and potential dissemination to the distal organs via the systemic circulation (60). However, systemically administered antifungal agents are limited by poor tissue penetration into lung tissue and associated with high rates of adverse events and the potential for serious drug interactions (61, 62). Therefore, targeted antifungal delivery to the lung could elevate and retain drug concentrations in the lung for improved efficacy and reduce systemic drug exposure to reduce adverse events and drug interactions. Theoretically, an ideal inhaled antifungal would have minimal drug absorption following inhalation for optimum efficacy and minimal adverse events and drug interactions.

Antifungal pharmacology, like that for all anti-infective agents, focuses on selective targeting of microbiological or biochemical differences between the pathogen and host. For fungal infections, the available targets have been difficult to identify and optimize due to the similarities in eukaryotic cellular physiology and biochemical pathways between fungal and animal cells. However, the most commonly used antifungals in systemic fungal infections target ergosterol, a cellular membrane stabilizer

and fungal equivalent to animal cholesterol. Polyene antifungals, including amphotericin B, form drug-ergosterol complexes to create non-selective trans-membrane channels that disrupt cellular integrity. The low aqueous solubility, log P value, and relatively large molecular weight (0.25 μg mL^{-1}, 1.6, and 924 g mol^{-1}, respectively, for amphotericin B) allow the polyene to partition into fungal cell membranes for pharmacologic activity (63). Triazole antifungals, including itraconazole, inhibit ergosterol biosynthesis through reversible antagonism of fungal cell cytochrome P450 isomers (64). Triazoles are also very poorly water soluble but with a much higher log P values indicative of better lipophilicity (approximately 0.001 μg mL^{-1} and 5.7 for itraconazole, respectively) (65, 66). The low solubility and high lipophilicity of triazole antifungals as well as relatively large molecular weight (705.6 g mol^{-1} for itraconazole) allow them to be absorbed into fungal cells and be metabolized by fungal cytochrome P450 enzymes responsible for normal ergosterol biosynthesis. Accordingly, the evaluation of antifungal pharmacokinetic parameters following inhalation will elucidate additional influences of drug solubilization and solubility enhancement on drug absorption.

1.3.1. Amphotericin B

(See Table 1.2A) The medical management of fungal infections was limited by poor pharmacologic selectivity between eukaryotic cellular physiology in both fungal and animal cells until the identification and development of amphotericin B in the mid-twentieth century (67). Amphotericin B preferentially forms non-selective pore or channel complexes with fungal cell membrane ergosterol, a membrane stabilizer analogous to cholesterol in animal cell membranes, to cause a loss of osmotic integrity and ultimately fungal cell death (68, 69). These ergosterol-amphotericin B complexes

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form through nonspecific Van Der Waals forces between the hydrophobic region of the amphiphilic amphotericin B molecule and the lipophilic ergosterol molecule (70). Amphotericin B is a 38-membered cyclic lactone ring composed of a distinct lipophilic region, with seven conjugated ester bonds, and a separate hydrophilic region with ester and ether bonds, a carboxylic acid group, a primary amino group in an attached sugar moiety, and several hydroxyl groups. Amphotericin B has a low aqueous solubility (0.25 µg mL^{-1}), a large molecular weight (924 g mol^{-1}), and lower than expected log P value (1.6) that allow the API to distribute into the membrane to be pharmacologically active.

Accordingly, four commercially available amphotericin B formulations use stabilizers and/or solubilizers to produce pharmaceutically acceptable products. Although all have been administered in an off-label manner via inhalation for analysis of efficacy and tolerability, only reports with the amphotericin B deoxycholate (Fungizone®, hereafter referred to as AmB-d) and liposomal amphotericin B (AmBisome®, hereafter referred to as L-AmB) formulations have associated systemic pharmacokinetic parameters (71, 72). Some investigators have also reported lung tissue or fluid drug concentrations to demonstrate high drug concentrations in the lung following inhalation (72-75). Additionally, Diot et al. reported serum amphotericin B concentrations following nebulization of pure amphotericin B powder and water dispersions without additional excipients (76). AmB-d is a suspension for reconstitution containing deoxycholate as a solubilizer and stabilizer and sodium phosphates as a buffer that forms a colloidal dispersion when reconstituted. L-AmB is suspension for reconstitution containing a bilayered liposome of amphotericin B in lipid membranes of hydrogenated soy phosphatidylcholine, cholesterol, and distearoylphosphatidylglycerol

(2:0.5:0.8 ratio) in a 1:10 ratio. Aerosols of both products have been inhaled using various nebulizers and systemic pharmacokinetic properties have varied widely.

Following inhalation of all formulations, lung t_{max} values were approximately 1 hour while t_{max} values in bronchoalveolar lavage fluid (BAL) following inhalation of AmB-d ranged from 0.5 to 4 hours (30 to 240 minutes). Similarly, serum t_{max} values following inhalation of pure amphotericin B ranged from 0.5 to 3.5 hours (30 to 210 minutes). However, there was great variability in dose normalized C_{max} and AUC values for lung tissue, BAL, and plasma/serum values based on the formulation. For example, inhaled doses of mg masses produced concentration values in serum, lung tissue, and BAL fluid ranged that spanned over 3 orders of magnitude across the $\mu g\ mL^{-1}$ to $ng\ mL^{-1}$ range. Specifically, dose normalized C_{max} values in serum following inhalation of pure amphotericin B ranged from 1.1 to 4.2 $ng\ mL^{-1}\ mg^{-1}$ while plasma C_{max} values ranged from 0.8 to 45 $ng\ mL^{-1}\ mg^{-1}$ following inhalation of AmB-d and were 5 $ng\ mL^{-1}\ mg^{-1}$ for L-AmB. In stark contrast, C_{max} values ranged from 7.3 to 2,625 $ng\ mL^{-1}\ mg^{-1}$ for BAL fluid and from 623 to 987 $ng\ g^{-1}\ mg^{-1}$ for lung tissue following inhalation of AmB-d. The dose normalized lung tissue C_{max} value was also 379 $ng\ g^{-1}\ mg^{-1}$ following inhalation of L-AmB. The dose normalized AUC following inhalation of AmB-d in BAL fluid ranged from 40 to 96 $ng\ hr\ mL^{-1}\ mg^{-1}$. The wide range of observed differences in these pharmacokinetic parameters based on the formulation obfuscated the trends for absorption of inhaled amphotericin B. However, the ratio of lung to plasma concentrations for inhaled AmB-d was over 1000:1, indicating negligible drug absorption following inhalation.

1.3.2. Itraconazole

(See Table 1.2B) Itraconazole must distribute into fungal cells to inhibit the cytochrome P450 enzymes responsible for ergosterol biosynthesis. However, itraconazole has dissolution limited absorption due to the extremely low aqueous solubility (1 ng mL^{-1}). Several particle engineering technologies, including cryogenic-based spray-freeze into liquid (SFL) (77), ultra-rapid freezing (URF) (78), and non-cryogenic evaporative precipitation into aqueous solution (EPAS) (79) have been investigated with itraconazole as a model API (80). These processes have been reviewed elsewhere, but briefly produce amorphous (SFL and URF) or crystalline (EPAS) nano-structured powder agglomerates with enhanced dissolution properties (81). These engineered powders have been nebulized as dispersions to rodent to evaluate the pharmacokinetic parameters following inhalation (80, 82-84). Most of these manuscripts reported lung tissue and plasma drug concentrations allowing better evaluation of drug absorption from the lungs. Additionally, these researchers have provided detailed formulation information allowing a more thorough comparative analysis of the contributing factors involved in solubility and solubilization on pulmonary drug absorption. Specifically, EPAS formulations contained itraconazole and surfactant(s) including polysorbate 20 or polysorbate 80 and poloxamer 407. SFL formulations contained polysorbate 80 with or without poloxamer 407. In contrast, the reported URF formulation contained mannitol and lecithin.

Following inhalation, lung t_{max} values ranged from 0.5 to 1.0 hours (30 to 60 minutes) for all itraconazole formulations while plasma t_{max} were delayed with values of 5.4 hours (342 minutes) for SFL itraconazole and 2.0 hours (120 minutes) for URF

itraconazole. Dose normalized lung C_{max} values were 1.7 μg g^{-1} mg^{-1} for the crystalline EPAS formulation with polysorbate 20. However, normalized maximal lung concentrations increased approximately 3-fold, to 5.4 μg g^{-1} mg^{-1}, when containing polysorbate 80 and poloxamer 407. This elevated lung concentration was associated with a low normalized plasma C_{max} value of 0.44 μg mL^{-1} mg^{-1}. In comparison, the dose normalized lung C_{max} value for amorphous SFL formulations containing only polysorbate 80 was 0.48 μg g^{-1} mg^{-1}. The SFL formulation maximal lung concentrations also increased to 1.1 to 2.4 μg g^{-1} mg^{-1} when poloxamer 407 was added. The corresponding SFL itraconazole, containing polysorbate 80 and poloxamer 407, produced plasma C_{max} values from 0.1 to 0.2 μg mL^{-1} mg^{-1} and were much lower than those reported for the comparable EPAS formulation. In contrast, the amorphous URF formulation contained only mannitol and lecithin but had a high dose normalized lung C_{max} value of 3.0 μg g^{-1} mg^{-1} but low plasma C_{max} value of 0.2 μg mL^{-1} mg^{-1}. Similar trends were observed for dose normalized AUC values. Namely, the addition of poloxamer 407 to EPAS formulations increased normalized lung AUC values from 8.7 μg hr g^{-1} mg^{-1} up to 14.8 μg hr g^{-1} mg^{-1} and SFL formulations from 1.6 μg hr g^{-1} mg^{-1} to a range of 5.8 to 15.1 μg hr g^{-1} mg^{-1}. The normalized lung AUC values for URF itraconazole of 21.1 μg hr g^{-1} mg^{-1} were also the highest reported. Dose normalized plasma AUC values also followed lung AUC trends with a range of 0.1 to 0.3 μg hr g^{-1} mg^{-1} for SFL itraconazole that contained polysorbate and poloxamer achieving while the URF formulation produced a normalized plasma AUC of 0.8 μg hr g^{-1} mg^{-1}. Despite these consistent trends in concentration and AUC values for EPAS, SFL, and URF itraconazole formulations, the lung elimination half-life was variable. The $t_{1/2}$ ranges for itraconazole were 6.7 to 7.2 hr for EPAS, 2.3 to 5.5 hr for SFL, and 7.4 for URF and indicate

variability independent of formulation, crystallization state, and other pharmacokinetic parameters.

In addition to comparison of observable and dose normazlied pharmacokinetic properties, reported itraconazole concentrations and AUC values in lung tissue and plasma from the same study population allow calculation of drug ratio values and distribution coefficients. Specifically, mice with a lung fungal infection had a lung to plasma C_{max} ratio of 59 to 1 for crystalline EPAS itraconazole while mice administered amorphous SFL itraconazole had a ratio of 12 to 1. In comparison, healthy mice administered SFL itraconazole had C_{max} lung to plasma ratios of 112 to 1 while mice that received amorphous URF drug had a ratio of 13 to 1. A lung to blood partition coefficient can also be calculated using a ratio of lung AUC and plasma AUC values. The calculated partition coefficients were 57 for SFL and 21 for URF.

1.3.3. Comparison of Inhaled Antifungals

Inhaled amphotericin B and itraconazole demonstrated more variable pharmacokinetic parameters compared to inhaled corticosteroids due in part to dose differences. These differences can be attributed, in part, to the physicochemical differences between inhaled corticosteroids and inhaled antifungals. Large inhaled antifungal doses, in the mg range, produced plasma concentrations in the $\mu g\ mL^{-1}$ to $ng\ mL^{-1}$ range for amphotericin B and $\mu g\ mL^{-1}$ for itraconazole compared to much smaller doses of inhaled corticosteroids with plasma concentrations in the $ng\ mL^{-1}$ to $pg\ mL^{-1}$ range. Although the scale of dose to effect concentrations was conserved between the agents, the deposition mass of inhaled antifungals was potentially several

orders of magnitude larger than for inhaled corticosteroids and could affect the absorption kinetics of the inhaled API (85).

The incorporation of surface active excipients in the nebulized formulation of amphotericin B elevated the dose normalized plasma C_{max} range from 1.1 to 4.2 µg mL^{-1} for AmB dispersion to 0.8 to 45 µg mL^{-1} for AmB-d. Inhaled AmB-d also produced very high normalized lung tissue C_{max} values from 627 to 987 µg kg mL^{-1} mg^{-1}. The relative ratio of lung to plasma concentrations for inhaled AmB-d of 1000 to 1 suggest very low drug absorption despite the presence of a surface active agent, deoxycholate. Although insufficient data was available for evaluation, L-AmB only elevated plasma concentrations by a factor of 10 and would not significantly improve drug absorption from the lung.

Inhaled itraconazole allows a more thorough analysis of formulation effects and drug solubilization on pulmonary drug absorption. For example, the addition of a second surface active agent, poloxamer 407, increased dose normalized lung concentrations by 2 to 5 times and normalized lung AUC values by 2 to 9 times for both crystalline EPAS and amorphous SFL itraconazole formulations compared with only a polysorbate surfactant. These increases suggest itraconazole improved inhaled particle deposition in the lung or aided in drug wetting and solubilization in lung fluid as has been suggested for other routes of administration (86, 87). Inhaled URF itraconazole contained lecithin instead of poloxamer 407 but produced the highest dose normalized lung AUC values despite consistent lung C_{max} values, suggesting drug wetting by a surface-active agent could be a probable mechanism of improved lung drug exposure and lung concentrations. However, elevated lung concentrations and drug exposure did not correlate to improved

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drug absorption in the lungs. Specifically, lung to plasma concentrations ratios suggested marked drug retention in the lungs with high AUC-based partition coefficients between lung tissue and plasma. In addition, comparison of dose normalized C_{max} and AUC values for formulation-matched crystalline EPAS and amorphous SFL formulations suggest inhalation of crystalline itraconazole dispersions led to higher drug concentrations and AUC values in the lung and plasma. The authors suggest that physiologic factors of mucociliary clearance of amorphous particles or other biopharmaceutical process resulted in lower tissue concentrations of amorphous SFL itraconazole.

1.4. INHALED OLIGOPEPTIDES

Recent trends in biotechnology have led to a surge of protein and peptide candidate drug molecules (88). However, formulation and effective non-invasive delivery of these APIs has been very challenging (89-91). The pulmonary delivery of proteins and peptides as a route for systemic drug delivery is intended to improve systemic bioavailability and reduce the pharmacokinetic variability compared to oral administration. Therefore, goal for most pulmonary peptide administration is typically systemic drug absorption instead of local action in the lungs. However, some therapeutic peptides could exert local action in the lung and targeted delivery could minimize systemic drug exposure. Although several manuscripts have been published that review inhalation of proteins and peptides (5, 6, 8, 92), examples of small molecular weight cyclic peptides with low aqueous solubility are pertinent to an examination of the influence of solubility and solubilization on pulmonary absorption. These agents include the immunosuppressant cyclosporine and an investigational substance P and neurokinin antagonist, FK224. Cyclosporine, a relatively small (1203 g mol^{-1}) cyclic undecapeptide

is very poorly water soluble (0.03 µg mL^{-1}) with a high log P value (2.9). FK224 is also a small cyclic hexapeptide (1041 g mol^{-1}) also has low aqueous solubility (21 µg mL^{-1}) and a lower log P value (1.3).

1.4.1. Cyclosporine

(See Table 1.3A) Cyclosporine is a polypeptide immunosuppressant used primarily to prevent tissue rejection after organ and tissue transplants through inhibition of signaling pathways involved in normal T-cell activation. Although effective following lung transplantation, acute rejection can occur due to delays in drug distribution into lung tissue following systemic drug administration. Additionally, targeting immunosuppressant delivery to the lung can reduce adverse events associated with systemic immunosuppression. Initial pharmacokinetic experiments with inhaled cyclosporine used nebulized alcoholic solutions, associated with poor patient tolerability and high rates of adverse events (93-95), or nebulized propylene glycol solutions (96-98). A nebulized suspension of cyclosporine in multi-lamellar dilauroylphosphatidylcholine liposomes was also investigated in dogs (99, 100). Recently, a nano-scale amorphous dispersion of cyclosporine was produced by controlled precipitation (CP), a stabilized anti-solvent precipitation, and nebulized to mice (101). The use of provided or estimated dose masses for pharmacokinetic parameter normalization produced study-dependent variability in calculated values. Therefore, dose normalization of C_{max} and AUC values were generally performed with the reported mass-based dosing (mg kg^{-1}) rather than the dose mass comparisons used for earlier poorly water soluble APIs.

Following inhalation of an alcoholic solution of cyclosporine, lung and whole blood t_{max} values ranged from 0.5 to 1.0 hours (30 to 60 minutes) while propylene glycol

solutions achieved more variable t_{max} values of 0.1 to 4.6 hours (6 to 276 minutes) in the lung and 0.1 to 2.0 hours (6 to 120 minutes) in the blood. Aerosolization of the liposomal cyclosporine had a t_{max} of 0.5 hours (30 minutes) in lung tissue but was faster in the blood with a value of 0.25 hours (15 minutes), indicating very rapid absorption following inhalation. The nebulized CP nano-scale dispersion also produced a similar lung t_{max} value, 1.0 hours (60 minutes) but with a delayed blood t_{max} value, 3.7 hours

(222 minutes). The nebulized alcoholic cyclosporine solution produced dose normalized C_{max} values from 33 to 35 $\mu g\ kg\ g^{-1}\ mg^{-1}$ in the lung and 0.7 to 0.8 $\mu g\ kg\ mL^{-1}\ mg^{-1}$ in the blood that then decreased to trough concentration ranges from 2.2 to 4.1 $\mu g\ kg\ g^{-1}\ mg^{-1}$ in the lung and 0.1 to 0.2 $\mu g\ kg\ mL^{-1}\ mg^{-1}$ in the blood. In contrast, nebulized propylene glycol solutions produced markedly lower normalized C_{max} values from 1.3 to

6.8 $\mu g\ kg\ g^{-1}\ mg^{-1}$ in the lung and 0.04 to 0.2 $\mu g\ kg\ mL^{-1}\ mg^{-1}$ in the blood. Comparable values were observed for dose normalized C_{max} values for the amorphous CP dispersion in the lung, 3.0 $\mu g\ kg\ g^{-1}\ mg^{-1}$, and blood, 0.1 $\mu g\ kg\ mL^{-1}\ mg^{-1}$. Even lower normalized C_{max} values were observed following inhalation of liposomal cyclosporine in lung tissue, 0.2 to 0.3 $\mu g\ kg\ g^{-1}\ mg^{-1}$, and blood, 0.002 to 0.01 $\mu g\ kg\ mL^{-1}\ mg^{-1}$. Inconsistency in dose normalized AUC values were observed for inhaled alcoholic solutions with values of 96 to 138 $\mu g\ hr\ kg\ g^{-1}\ mg^{-1}$ in the lung and 5.1 to 5.5 $\mu g\ hr\ kg\ mL^{-1}\ mg^{-1}$ in the blood when the dose was 3 to 5 $mg\ kg^{-1}$ but 20 to 24 $\mu g\ hr\ kg\ g^{-1}\ mg^{-1}$ in the lung and 25 to 27 $\mu g\ hr\ kg\ mL^{-1}\ mg^{-1}$ in the blood when the dose was increased to 10 to 25 $mg\ kg^{-1}$. Similar inconsistencies were observed following inhalation of the propylene glycol solution with normalized AUC values of 0.05 to 0.1 $\mu g\ hr\ kg\ mL^{-1}\ mg^{-1}$ in the blood at doses of 4.4 to 9.7 $mg\ kg^{-1}$ (no lung tissue values reported for that dose range) and increasing to 11 to 46 $\mu g\ hr\ kg\ g^{-1}\ mg^{-1}$ in the lung and 0.8 to 1.7 $\mu g\ hr\ kg\ mL^{-1}\ mg^{-1}$ in the blood when the dose was increased to 8.4 to 112.6 $mg\ kg^{-1}$. Comparable normalized

values were calculated for inhaled CP cyclosporine with a lung value of

41 µg hr kg g^{-1} mg^{-1} and blood value of 2.8 µg hr kg mL^{-1} mg^{-1}. The reported pharmacokinetic parameters for both lung and plasma also allow calculation of concentration ratios and partition coefficients for inhaled cyclosporine formulations. Calculated drug concentration ratios were 40-50 to 1 for alcoholic solutions,

30-42 to 1 for propylene glycol solutions, 25-100 to 1 for liposomal suspensions, and 28 to 1 for amorphous CP dispersions. The corresponding partition coefficients were 1-27 for inhaled alcoholic solutions, 14-27 for propylene glycol solutions, and 15 for the CP dispersion.

1.4.2. FK224

(See Table 1.3B) FK224 is an investigational cyclic hexapeptide, (L-Ser-L-Thr-L-Leu-D-Phe-L-allo-Thr-L-Asp- NH2) used as a substance P and neurokinin antagonist with potential utilization in the management of conditions associated with neurotransmitter release, such as depression, analgesia, nociception, inflammation, and nausea and emesis (102-104). However, very low bioavailability was observed following oral administration due to gastrointestinal proteolytic degradation as well as formulation difficulty prompting dose limitations due to the physicochemical properties of the drug (105). Two publications have investigated systemic pharmacokinetic parameters following pulmonary delivery of FK224 with different mechanisms of solubility enhancement (105, 106). Specifically, a micronized co-precipitate of β-cyclodextrin and FK224 was incorporated into a CFC-based pMDI as well as with lactose carrier particles for a DPI formulation.

The addition of β-cyclodextrin decreased plasma t_{max} values in rats to 0.25 hours (15 minutes) compared to a value of 1.0 hr (60 minutes) when no cyclodextrin was present. This value was clearly different for pMDI and DPI administered formulations in humans with values of 2.7 to 3.0 hours (162 to 180 minutes) and 0.7 to 2.2 hours (42 to 132 minutes), respectively. Increasing concentrations of β-cyclodextrin also affected pharmacokinetic parameters in rats when administered drug via a pMDI device with dose normalized plasma C_{max} values increasing from 0.01 µg kg mL^{-1} mg^{-1} to 0.03 µg kg mL^{-1} mg^{-1}, to 0.09 µg kg mL^{-1} mg^{-1} for API to cyclodextrin rations of 1:0, 1:1, and 1:7, respectively with corresponding dose normalized AUC values of 0.06 µg hr kg mL^{-1} mg^{-1}, 0.43 µg hr kg mL^{-1} mg^{-1}, and 1.35 µg hr kg mL^{-1} mg^{-1}. The marked increase in both maximal plasma concentrations and drug exposure from FK224 without cyclodextrin up to a 1:7 mixture of API and cyclodextrin corresponded to an increase in drug solubility from 21 µg mL^{-1} to 1 mg mL^{-1}. When a 1 : 1 :: FK224 : β-cyclodextrin pMDI was administered to humans, dose normalized C_{max} values ranged from 0.07 to 0.09 µg kg mL^{-1} mg^{-1} but considerably increased to 0.34 to 0.37 µg kg mL^{-1} mg^{-1} for the DPI delivered formulation. A similar pattern was observed for normalized AUC values when the same formulation when administered with a pMDI device, 0.13 to 0.79 µg hr kg mL^{-1} mg^{-1} and 3.05 to 3.61 µg hr kg mL^{-1} mg^{-1} with a DPI device.

4.3. Comparison of Inhaled Oligopeptides

Numerous formulation and delivery devises have been investigated for inhaled poorly water soluble oligopeptides including: solutions, suspensions, particle size reduction, solubilizing excipients, nebulizers, DPIs and pMDIs. Inhalation of solubilized cyclosporine in alcohol and propylene glycol solutions produced similar t_{max} values in

lung tissue and plasma but with very different dose normalized C_{max} and AUC ranges, suggesting alcoholic solutions enhanced pulmonary drug absorption compared to propylene glycol solutions, possibly through alterations in hydrodynamics across alveolar membranes (107). In addition, tissue and blood concentration ratios and partition coefficients for pulmonary absorption suggest alcohol solutions promote increased retention of cyclosporine in the lungs following inhalation compared to propylene glycol solutions. Therefore, although alcohol solutions promote improved relative absorption of the oligopeptide, propylene glycol solutions do not promote retention of drug in lung tissue, possibly through non-absorptive lung clearance mechanisms. Further studies are needed to elucidate possible causes of this behavior. Inhalation of a nano-scale dispersion of CP cyclosporine retained drug in the lungs in a similar manner to solutions but had slightly improved drug absorption as evidenced by concentration ratios compared to solutions and could be due to enhanced absorption of nanoparticles (4). Inhalation of liposomal cyclosporine seemed to inhibit systemic drug absorption and could be due to tissue retention of the liposome (108).

The incorporation of β-cyclodextrin into FK224 formulations markedly enhanced the aqueous solubility of the oligopeptide resulting in better pulmonary absorption of the API (109). However, incorporation of solid state micronized FK224-cyclodextrin powders into pMDI and DPI devices prompted divergent pharmacokinetic parameters as evidenced by a 3 to 4-fold increase in dose normalized plasma C_{max} values and 4 to 28-fold increase in normalized plasma AUC values following inhalation of the DPI delivered powder. The authors suggested the DPI produced higher C_{max} and AUC values due to device dependent differences in the delivered dose (106). Ideally, a pMDI and DPI

would produce similar systemic pharmacokinetic parameters for equivalent inhaled doses.

1.5. INHALED OPIOIDS

1.5.1. Fentanyl

(See Table 1.4) Opioid analgesics are based on the prototypical opioid, morphine, but structurally diverse through various ring structures and functional groups to provide consistent binding sites to opioid receptors. As a result of this inconsistency in chemical structures, opioids have varied aqueous solubilities, molecular weights, and log P values. For this review, fentanyl is a poorly water soluble compounds and has been administered via inhalation for the treatment of breakthrough pain. Fentanyl is a small molecule compound (336.5 g mol^{-1}) with low aqueous solubility (200 μg mL^{-1}) and high log P value (3.9) suggesting dissolution limited absorption and good propensity for diffusion controlled absorption. The inhalation of fentanyl gained popular interest when fentanyl derivative was pumped into the ventilation system of a building in Moscow that held terrorists and more than 800 hostages (110). Following the exposure to the inhaled fentanyl derivative and neutralization of the terrorists, a military operation brought the standoff to a close. However, after that incident, over 80% of the hostages required hospitalization with a total of 16% that died as a result to the inhaled fentanyl derivative. Despite these negative results, the controlled and therapeutic use of inhaled fentanyl was investigated as a route of administration for rapid and potentially prolonged systemic drug action using a nebulized suspension of a 50/50 mixture of free and liposome-encapsulated (phospholipon 90-G and cholesterol) fentanyl (FLEF) and as a pMDI containing micronized fentanyl base in a mixture of CFC-11 and CFC-12 propellants

with sorbitan trioleate as a metering valve lubricant (111-113). A DPI formulation of engineered micronized fentanyl on lactose carrier particles was also administered to humans via the Taifun® device (114, 115).

The plasma pharmacokinetic profile following inhalation of the FLEF formulation could be considered the summation of the inhaled encapsulated fentanyl pharmacokinetic profile with the pharmacokinetic profile of the inhaled free fentanyl. However, those two profiles are impossible to isolate based on the available pharmacokinetic data from FLEF. In addition, the pMDI formulation and the DPI fentanyl-lactose system provided different pharmacokinetic profiles, suggesting none of the inhaled fentanyl systems provided an un-modified free liposomal comparator. Specifically, the pMDI formulation used a solution of fentanyl in CFC propellants that volatilized on actuation to deliver particulate fentanyl to the lungs. Administration of the pMDI formulation achieved very rapid plasma t_{max} values of 0.1 to 0.12 hours (6 to 7 minutes) with corresponding dose normalized plasma C_{max} values of 9.5 to 15.0 µg kg mL^{-1} mg^{-1} and a normalized AUC range of 91 to 154 µg hr kg mL^{-1} mg^{-1}. The DPI formulation provided an even quicker t_{max} value, 0.017 hours (1 minute), but with a lower dose normalized C_{max} of 4.7 µg kg mL^{-1} mg^{-1}. However, when compared with the FLEF formulation, plasma t_{max} values were slightly slower and ranged from 0.25 to 0.38 hours (15 to 23 minutes) but with much lower dose normalized C_{max} values of 0.6 to 2.5 µg kg mL^{-1} mg^{-1}. Therefore, a component of both the pMDI and DPI formulations enhanced pulmonary absorption from the lung, or the nebulized liposomal fentanyl suspension behaved in a substantially different manner than suggested by the pMDI formulation. A more thorough analysis was not possible due to incomplete reporting of AUC values for the DPI fentanyl and FLEF. However, fentanyl particle size reduction was the likely mechanism of rapid and

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high maximal drug concentrations for both the DPI and pMDI formulations which occurred either in the particle manufacturing process (DPI) or following volatilization of the CFC propellant from the fentanyl solution which caused precipitation of discrete micro- to nano-sized particles (pMDI). Further studies are needed to better elucidate this possible mechanism of improved drug solubilization for fentanyl.

1.6. SUMMARY

Pulmonary drug delivery is an accepted route of drug administration for lung condition and disease management including: asthma and other inflammatory processes, lung infections, immunosuppression following lung transplantation, and others. The lungs were also investigated as a route of systemic drug administration to bypass oral barriers to absorption and avoid parenteral administration and the pain and inconvenience associated with injections for other APIs. These biopharmaceutical advantages for interest in pulmonary drug delivery have led researchers to administer an increasingly wide variety of APIs to the lungs. Although poorly water soluble drugs pose formulation and drug delivery limitations for typical delivery methods, an evaluation of their impact on pulmonary drug delivery with emphasis on *in vivo* pharmacokinetic effects has not been performed. A sample of poorly water soluble APIs were selected from the literature and included for analysis where a formulation was provided or suggested, the drug was inhaled by an *in vivo* system, and some form of pharmacokinetic evaluation was performed such that drug concentration values were reported. Studies that evaluated a biomarker or physiologic response were not included in the current evaluation. Studies with non-compartmental pharmacokinetic parameters of t_{max}, C_{max}, and AUC were preferentially included and normalized for the drug dose, as an exposure dose instead of a calculated or estimated delivered or inhaled dose, to facilitate inter-API comparison.

Application of particle size reduction to inhaled poorly water soluble agents provided inconsistent effects on pulmonary absorption. Micronized drug formulations had plasma t_{max} values generally less than 2 hours (120 minutes) with some decreases to less than 0.5 hours (30 minutes) and were influenced by the API. Although blood collection procedures limit the earliest reported values, micronized drugs can be rank ordered with the earliest reported value as budesonide (0.13 to 0.58 hours) < beclomethasone 17-monopropionate (0.17 to 2.5 hours) < triamcinolone acetonide (0.25 to 1.75 hours) < amphotericin B (0.5 to 3.5 hours) < fluticasone propionate (0.9 to 1.88 hours). The minimal t_{max} values correlate with aqueous solubilities of the APIs ($R^2 = 0.70$), suggesting the rate of drug absorption from the lungs, as suggested by t_{max} values, is limited by the intrinsic solubility of the API when micronized. However, when the particle size is reduced into the nanometer range, plasma t_{max} values decreased to 0.051 to 0.19 hours for nano-budesonide but were 2.0 hours for URF itraconazole and 5.4 hours for SFL itraconazole. Although insufficient data was available to draw conclusions for t_{max} values for nano-sized poorly water soluble APIs, inhalation of nanoparticles could introduce additional and more variable mechanisms of absorption than affecting micron-sized inhaled drugs (4, 16). The pulmonary administration of alcohol and propylene glycol based beclomethasone 17- monopropionate and cyclosporine solutions generally achieved rapid plasma t_{max} values. Dissolved fentanyl in a propellant mixture also demonstrated very rapid drug absorption with low t_{max} values following inhalation. Incorporation of solubilizing excipients also reduced the t_{max} value as evidenced in the inclusion of cyclodextrin with FK224, surfactants with amphotericin B and itraconazole, and encapsulation of fentanyl, cyclosporine, and amphotericin B into liposomes. The formulation-based inclusion of solubility enhancing excipients did

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appear to improve the rate of drug absorption following inhalation as has been demonstrated for poorly water soluble APIs in other routes of drug delivery (15, 86, 116, 117).

The relationships between drug solubility and solubilization were more complex for dose normalized tissue and systemic drug C_{max} and AUC values than for t_{max} values. This could be due to the fact that pharmacokinetic parameters were adjusted based on the total inhalation exposure dose and not actual deposited doses. The inter-study and intra-study differences in pulmonary deposition based on utilization of different delivery systems, formulations, study populations and species, and physiologic properties following inhalation could not be corrected in the dose normalization due to insufficient and methodologically varied deposition and aerosol aerodynamic information provided by the many authors (16, 118, 119). Additionally, systemic effects were inappropriate to consider in parameter normalization due precisely to the objective of the study to investigate the influence of solubility and solubilization parameters on pulmonary absorption of poorly water soluble APIs. However, normalizing non-compartmental pharmacokinetic parameters based on exposure doses did provide a uniform adjustment for all APIs across varied methodologies and allow for inter-API evaluation.

The most noticeable relationship is the scope of drug concentrations in the systemic circulation following pulmonary absorption, i.e. inhaled corticosteroids and inhaled amphotericin B had dose normalized concentrations in the ng mL^{-1} mg^{-1} range (equivalent to pg mL^{-1} µg^{-1}) while the other APIs had a 1000-fold increase in concentration in the µg mL^{-1} mg^{-1} range. Although this could be an artifact from dose normalization of pharmacokinetic parameters, inhaled corticosteroids and amphotericin B

have very low drug distribution to the plasma from the lungs and suggest mechanistic differences in pulmonary absorption between different APIs. Additional studies are required to control for possible differences in pulmonary deposition and investigate mechanisms of absorption for these agents from the lung.

The differences in tissue and systemic drug concentration scales did not affect trends in drug concentration and drug exposure based on formulation-based solubilization adjustments. Alcoholic solutions prompted higher normalized C_{max} and AUC values, suggesting enhanced drug absorption following inhalation, than propylene glycol solutions. Therefore, the pulmonary administration of pre-dissolved poorly water soluble API does not equate to equivalent rates or extents of drug absorption. Studies have suggested that ethanol could function as a permeation enhancer or disrupt the hydrodynamic balance in tissues to promote drug absorption (107, 120).

Inhalation of nano-scale formulations caused divergent pharmacokinetic findings for nano-budesonide compared to nano-structured compositions of itraconazole and cyclosporine. Inhaled suspensions of nano-budesonide promoted rapid and markedly elevated systemic drug concentrations but with an equivalent dose normalized AUC, suggesting an improved rate of drug absorption without altering the extent of drug absorption. However, for inhaled nano-structured itraconazole and cyclosporine, rapid and extensive tissue concentrations were observed but with very little systemic drug absorption. For those APIs, the rate and extent of systemic drug absorption from the lungs was decreased. The inhaled itraconazole and cyclosporine particles could experience non-absorptive clearance mechanism from the lung tissue, possible alveolar

macrophages or the lymphatic system, that could segregate drug from the systemic circulation (17, 121).

Cyclodextrin also promoted high normalized C_{max} and AUC values following inhalation suggesting similar mechanisms of improved drug absorption as other routes of delivery (109, 122, 123). However, nebulized liposomal formulations promoted relatively low systemic drug concentrations for cyclosporine and fentanyl but elevated concentrations for amphotericin B. Although amphotericin B has been shown to bind to systemically circulating liposomes and cause a high but pharmacologically inactive systemic concentration following IV administration (124), the pulmonary administration of liposomes was suggested to cause enhanced drug retention in the lung and act as a form of drug depot for prolonged action (111, 112). Supplemental AUC values for inhaled liposomal poorly water soluble APIs could resolve this effect.

Although the pharmacokinetic evaluation of select inhaled poorly water soluble APIs demonstrated many drug-dependent and as of yet unexplored effects, drug physicochemical and formulation-based solubility enhancement did affect drug absorption form the lungs. Additional insights will be gained as researchers continue to investigate the delivery of drugs to the lungs and explore the factors that relate drug solubility, formulation-based enhancements to solubility, and local and systemic pharmacokinetics.

1.7. REFERENCES

1.   Sanders, M. 2007. Inhalation therapy: an historical review. Prim. Care Respir. J. 16:71-81.

2.   Derom, E., and L. Thorsson. 2001. Factors Affecting the Clinical Outcome of Aerosol Therapy, p. 143-171. *In* H. Bisgaard, C. O'Callaghan, and G. C. Smaldone (ed.), Drug Delivery to the Lung, vol. 162. Marcel Dekker, Inc., New York, NY.

3.   Chow, A. H. L., H. H. Y. Tong, P. Chattopadhyay, and B. Y. Shekunov. 2007. Particle Engineering for Pulmonary Drug Delivery. Pharm. Res. 24:411-437.

4.   Yang, W., J. I. Peters, and R. O. Williams, III. 2008. Inhaled nanoparticles. A current review. Int. J. Pharm. 356:239-247.

5.   Agu, R. U., M. I. Ugwoke, M. Armand, R. Kinget, and N. Verbeke. 2001. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir. Res. 2:198-209.

6.   Gonda, I. 2006. Systemic Delivery of Drugs to Humans via Inhalation. J. Aerosol Med. 19:47-53.

7.   Patton, J. S., and P. R. Byron. 2007. Inhaling medicines: delivering drugs to the body through the lungs. Nature Reviews Drug Discovery 6:67-74.

8.   Patton, J. S., C. S. Fishburn, and J. G. Weers. 2004. The lungs as a portal of entry for systemic drug delivery. Proceedings of the American Thoracic Society 1:338-344.

9.   Bisgaard, H., C. O'Callaghan, and G. C. Smaldone (ed.). 2002. Drug Delivery to the Lung, vol. 162. Marcel Dekker, Inc., New York, NY.

10.   Cloutier, M. M. 2007. Respiratory Physiology. Mosby, Philadelphia, PA.

11.   Byron, P. R. 1987. Pulmonary targeting with aerosols. Pharm. Technol. 11:42, 44, 46, 48, 50, 52, 54, 56.

12.   Finney, M. J., S. D. Anderson, and J. L. Black. 1987. The effect of non-isotonic solutions on human isolated airway smooth muscle. Respir. Physiol. 69:277-86.

13.   Martin, G. P., A. E. Bell, and C. Marriott. 1988. An in vitro method for assessing particle deposition from metered pressurized aerosols and dry powder inhalers. Int. J. Pharm. 44:57-63.

14.   Bermejo, M., and I. Gonzalez-Alvarez. 2008. How and where are drugs absorbed? Preclinical Development Handbook: ADME and Biopharmaceutical Properties:249-280.

15.   Martinez, M. N., and G. L. Amidon. 2002. A mechanistic approach to understanding the factors affecting drug absorption: A review of fundamentals. J. Clin. Pharmacol. 42:620-643.

16.   Sakagami, M. 2006. In vivo, in vitro and ex vivo models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery. Advanced Drug Delivery Reviews 58:1030-1060.

17.   Miller, F. J. 2000. Dosimetry of particles: critical factors having risk assessment implications. Inhalation Toxicol. 12:389-395.

18.   Pouton, C. W. 2006. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. European Journal of Pharmaceutical Sciences 29:278-287.

19.   Rasenack, N., and B. W. Mueller. 2005. Poorly water-soluble drugs for oral delivery - A challenge for pharmaceutical development: Part I: Physicochemical and biopharmaceutical background / strategies in pharmaceutical development. Pharmazeutische Industrie 67:323-326.

20.   Lipinski, C. A., F. Lombardo, B. W. Dominy, and P. J. Feeney. 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 23:3-25.

21.   Stegemann, S., F. Leveiller, D. Franchi, H. de Jong, and H. Linden. 2007. When poor solubility becomes an issue: from early stage to proof of concept. Eur. J. Pharm. Sci. 31:249-61.

22.   2008. USP 31 - NF 26. The United States Pharmacopeial Convention, Rockville, MD.

23.   Center for Drug Evaluation and Research (CDER). 1997. Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms. *In* Food and Drug Administration (ed.). U.S. Department of Health and Human Services.

24.   Zhou, H. 2008. Water-Insoluble Drugs and Their Pharmacokinetic Behaviors. *In* R. Liu (ed.), Water-Insoluble Drug Formulation, 2nd ed. Taylor & Francis Ltd, Hoboken, NJ.

25.   Drake, R. E., G. A. Laine, S. J. Allen, J. Katz, and J. C. Gabel. 1987. A model of the lung interstitial-lymphatic system. Microvascular Research 34:96-107.

26.   Newhouse, M. T., P. H. Hirst, S. P. Duddu, Y. H. Walter, T. E. Tarara, A. R. Clark, and J. G. Weers. 2003. Inhalation of a dry powder tobramycin PulmoSphere formulation in healthy volunteers. Chest 124:360-366.

27.   Lipworth, B. J., and C. M. Jackson. 2000. Safety of inhaled and intranasal corticosteroids: Lessons for the new millennium. Drug Safety 23:11-33.

28.   Kelly, H. W. 1998. Establishing a therapeutic index for the inhaled corticosteroids: part I. Pharmacokinetic/pharmacodynamic comparison of the inhaled corticosteroids. J. Allergy Clin. Immunol. 102:S36-S51.

29.   Edsbaecker, S. 2001. Uptake, Retention, and Biotransformation of Corticosteroids in the Lung and Airways, p. 213-244. *In* R. P. Schleimer, P. M. O'Byrne, S. J. Szefler, and R. Brattsand (ed.), Inhaled Steroids in Asthma: Oftimizing Effects in the Airways, vol. 163. Marcel Dekker, Inc., New York, NY.

30.   Brindley, C., C. Falcoz, A. E. Mackie, and A. Bye. 2000. Absorption kinetics after inhalation of fluticasone propionate via the Diskhaler, Diskus, and metered-dose inhaler in healthy volunteers. Clin. Pharmacokin. 39:1-8.

31.   Laehelmae, S., M. Kirjavainen, M. Kela, J. Herttuainen, M. Vahteristo, M. Silvasti, and M. Ranki-Pesonen. 2005. Equivalent lung deposition of budesonide in vivo: A comparison of dry powder inhalers using a pharmacokinetic method. British Journal of Clinical Pharmacology 59:167-173.

32.   Kinnarinen, T., P. Jarho, K. Jaervinen, and T. Jaervinen. 2003. The in vitro pulmonary deposition of a budesonide/gamma -cyclodextrin inclusion complex. Journal of Inclusion Phenomena and Macrocyclic Chemistry 44:97-100.

33.   Hochhaus, G. 2007. Pharmacokinetic and pharmacodynamic properties important for inhaled corticosteroids. Annals of Allergy, Asthma, & Immunology 98:S7-S15.

34.   Freiwald, M., A. Valotis, A. Kirschbaum, M. McClellan, T. Murdter, P. Fritz, G. Friedel, M. Thomas, and P. Hogger. 2005. Monitoring the initial pulmonary absorption of two different beclomethasone dipropionate aerosols employing a human lung reperfusion model. Respiratory Research 6:21.

35.   Saari, M., M. T. Vidgren, M. O. Koskinen, V. M. H. Turjanmaa, and M. M. Nieminen. 1999. Pulmonary distribution and clearance of two beclomethasone liposome formulations in healthy volunteers. International Journal of Pharmaceutics 181:1-9.

36. Mollmann, H., S. Balbach, G. Hochhaus, J. Barth, and H. Derendorf. 1995. Pharmacokinetic-Pharmacodynamic Correlations of Corticosteroids. *In* H. Derendorf and G. Hochhaus (ed.), Handbook of Pharmacokinetic/Pharmacodynamic Correlation CRC Press, Boca Raton, FL.

37. Lipworth, B. J. 1996. Pharmacokinetics of inhaled drugs. Br. J. Clin. Pharmacol. 42:697-705.

38. Thorsson, L., K. Dahlstroem, S. Edsbaecker, A. Kaellen, J. Paulson, and J. E. Wiren. 1997. Pharmacokinetics and systemic effects of inhaled fluticasone propionate in healthy subjects. British Journal of Clinical Pharmacology 43:155-161.

39. Thorsson, L., S. Edsbacker, A. Kallen, and C. G. Lofdahl. 2001. Pharmacokinetics and systemic activity of fluticasone via Diskus and pMDI, and of budesonide via Turbuhaler. British Journal of Clinical Pharmacology 52:529-538.

40. Moelmann, H., M. Wagner, S. Krishnaswami, H. Dimova, Y. Tang, C. Falcoz, P. T. Daley-Yates, M. Krieg, R. Stoeckmann, J. Barth, C. Lawlor, A. C. Moelmann, H. Derendorf, and G. Hochhaus. 2001. Single-dose and steady-state pharmacokinetic and pharmacodynamic evaluation of therapeutically clinically equivalent doses of inhaled fluticasone propionate and budesonide, given as Diskus or Turbohaler dry-powder inhalers to healthy subjects. Journal of Clinical Pharmacology 41:1329-1338.

41. Mortimer, K. J., T. W. Harrison, Y. Tang, K. Wu, S. Lewis, S. Sahasranaman, G. Hochhaus, and A. E. Tattersfield. 2006. Plasma concentrations of inhaled corticosteroids in relation to airflow obstruction in asthma. British Journal of Clinical Pharmacology 62:412-419.

42. Whelan, G. J., J. L. Blumer, R. J. Martin, and S. J. Szefler. 2005. Fluticasone propionate plasma concentration and systemic effect: Effect of delivery device and duration of administration. Journal of Allergy and Clinical Immunology 116:525-530.

43. Argenti, D., B. Shah, and D. Heald. 2000. A study comparing the clinical pharmacokinetics, pharmacodynamics, and tolerability of triamcinolone acetonide HFA-134a metered-dose inhaler and budesonide dry-powder inhaler following inhalation administration. J. Clin. Pharmacol. 40:516-526.

44. Bisgaard, H., K. Nikander, and E. Munch. 1998. Comparative study of budesonide as a nebulized suspension vs pressurized metered-dose inhaler in adult asthmatics. Respir. Med. 92:44-9.

45.   Kraft, W. K., B. Steiger, D. Beussink, J. N. Quiring, N. Fitzgerald, H. E. Greenberg, and S. A. Waldman. 2004. The pharmacokinetics of nebulized nanocrystal budesonide suspension in healthy volunteers. Journal of Clinical Pharmacology 44:67-72.

46.   Shrewsbury, S. B., A. P. Bosco, and P. S. Uster. 2009. Pharmacokinetics of a novel submicron budesonide dispersion for nebulized delivery in asthma. Int. J. Pharm. 365:12-17.

47.   Hill, M. Dec. 19, 2006 2007. Systems and methods for the delivery of corticosteroids having an increased lung deposition. US patent US 2007/0197487 A1.

48.   Esposito-Festen, J. E., P. Zanen, H. A. W. M. Tiddens, and J. W. J. Lammers. 2007. Pharmacokinetics of inhaled monodisperse beclomethasone as a function of particle size. British Journal of Clinical Pharmacology 64:328-334.

49.   Daley-Yates, P. T., A. C. Price, J. R. Sisson, A. Pereira, and N. Dallow. 2001. Beclomethasone dipropionate: absolute bioavailability, pharmacokinetics and metabolism following intravenous, oral, intranasal and inhaled administration in man. British Journal of Clinical Pharmacology 51:400-409.

50.   Lim, J. G. P., B. Shah, S. Rohatagi, and A. Bell. 2006. Development of a dry powder inhaler, the Ultrahaler, containing triamcinolone acetonide using in vitro-in vivo relationships. Am J Ther 13:32-42.

51.   Argenti, D., B. Shah, and D. Heald. 1999. A pharmacokinetic study to evaluate the absolute bioavailability of triamcinolone acetonide following inhalation administration. J. Clin. Pharmacol. 39:695-702.

52.   Hintz, R. J., and K. C. Johnson. 1989. The effect of particle size distribution on dissolution rate and oral absorption. Int. J. Pharm. 51:9-17.

53.   Takano, R., K. Furumoto, K. Shiraki, N. Takata, Y. Hayashi, Y. Aso, and S. Yamashita. 2008. Rate-Limiting Steps of Oral Absorption for Poorly Water-Soluble Drugs in Dogs; Prediction from a Miniscale Dissolution Test and a Physiologically-Based Computer Simulation. Pharm. Res. 25:2334-2344.

54.   Johnson, K. C. 2007. Dissolution: fundamentals of in vitro release and the biopharmaceutics classification system. Drugs Pharm. Sci. 165:1-28.

55.   Sigfridsson, K., S. Forssen, P. Hollaender, U. Skantze, and J. de Verdier. 2007. A formulation comparison, using a solution and different nanosuspensions of a poorly soluble compound. Eur. J. Pharm. Biopharm. 67:540-547.

56.   Brown, T. E. R., and T. W. F. Chin. 2008. Superficial Fungal Infections. *In* J. T. DiPiro, R. L. Talbert, G. C. Yee, G. R. Matzke, B. G. Wells, and L. M. Posey (ed.), Pharmacotherapy: A Pathophysiologic Approach, 6th ed. McGraw-Hill, Chicago, IL.

57.   Patterson, T. F. 2005. Advances and challenges in management of invasive mycoses. Lancet 366:1013-25.

58.   Carver, P. L. 2008. Invasive Fungal Infections. *In* J. T. DiPiro, R. L. Talbert, G. C. Yee, G. R. Matzke, B. G. Wells, and L. M. Posey (ed.), Pharmacotherapy: A Pathophysiologic Approach, 6th ed. McGraw-Hill, Chicago, IL.

59.   Kauffman, C. A. 2006. Fungal infections. Proceedings of the American Thoracic Society 3:35-40.

60.   Clemons, K. V., and D. A. Stevens. 2005. The contribution of animal models of aspergillosis to understanding pathogenesis, therapy and virulence. Med. Mycol. 43:S101-S110.

61.   Boucher, H. W., A. H. Groll, C. C. Chiou, and T. J. Walsh. 2004. Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. Drugs 64:1997-2020.

62.   Wiebe, V., and M. Karriker. 2005. Therapy of systemic fungal infections: a pharmacologic perspective. Clinical Techniques in Small Animal Practice 20:250-7.

63.   Yalkowsky, S. H., and Y. He. 2003. Handbook of Aqueous Solubility Data. CRC Press, Boca Raton, FL.

64.   Bennett, J. E. 2006. Antimicrobial Agents: Antifungal Agents. *In* L. L. Brunton, J. S. Lazo, and K. L. Parker (ed.), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed. The McGraw-Hill Companies, Inc. , New York, NY.

65.   Miyake, K., T. Irie, H. Arima, F. Hirayama, K. Uekama, M. Hirano, and Y. Okamaoto. 1999. Characterization of itraconazole/2-hydroxypropyl-beta -cyclodextrin inclusion complex in aqueous propylene glycol solution. Int. J. Pharm. 179:237-245.

66.   Rhee, Y.-S., C.-W. Park, T.-Y. Nam, Y.-S. Shin, S.-C. Chi, and E.-S. Park. 2007. Formulation of parenteral microemulsion containing itraconazole. Archives of Pharmacal Research 30:114-123.

67.   Gold, W., H. A. Stout, J. F. Pagano, and R. Donovick. 1956. Amphotericins A and B, antifungal antibiotics produced by a streptomycete. I. In vitro studies. Antibiotics Annals:579-85.

68. Bennett, J. E. 2001. Antimicrobial Agents: Antifungal Agents, p. 1295-9. *In* J. G. Hardman and L. E. Limbird (ed.), Goodman & Gilman's The Pharamcological Basis of Therapeutics, 10th ed. McGraw-Hill, Chicago, IL.

69. Graybill, J. R., S. G. Revankar, and T. F. Patterson. 1998. Antifungal Agents and Antifungal Susceptibility Testing, p. 163-5. *In* L. Ajello and R. J. Hay (ed.), Med. Mycol., 9th ed, vol. 4. Oxford University Press, Inc., New York, NY.

70. Brajtburg, J., W. G. Powderly, G. S. Kobayashi, and G. Medoff. 1990. Amphotericin B: current understanding of mechanisms of action. Antimicrob. Agents Chemother. 34:183-8.

71. Lowry, C. M., F. M. Marty, S. O. Vargas, J. T. Lee, K. Fiumara, A. Deykin, and L. R. Baden. 2007. Safety of aerosolized liposomal versus deoxycholate amphotericin B formulations for prevention of invasive fungal infections following lung transplantation: a retrospective study. Transpl Infect Dis 9:121-5.

72. Marra, F., N. Partovi, K. M. Wasan, E. H. Kwong, M. H. H. Ensom, S. M. Cassidy, G. Fradet, and R. D. Levy. 2002. Amphotericin B disposition after aerosol inhalation in lung transplant recipients. Ann. Pharmacother. 36:46-51.

73. Koizumi, T., K. Kubo, T. Kaneki, M. Hanaoka, T. Hayano, T. Miyahara, K. Okada, K. Fujimoto, H. Yamamoto, T. Kobayashi, and M. Sekiguchi. 1998. Pharmacokinetic evaluation of amphotericin B in lung tissue: lung lymph distribution after intravenous injection and airspace distribution after aerosolization and inhalation of amphotericin B. Antimicrob. Agents Chemother. 42:1597-1600.

74. Monforte, V., A. Roman, J. Gavalda, R. Lopez, L. Pou, M. Simo, S. Aguade, B. Soriano, C. Bravo, and F. Morell. 2003. Nebulized amphotericin B concentration and distribution in the respiratory tract of lung transplanted patients. Transplantation 75:1571-1574.

75. Ruijgrok, E. J., A. G. Vulto, and E. W. M. Van Etten. 2000. Aerosol delivery of amphotericin B desoxycholate (Fungizone) and liposomal amphotericin B (AmBisome): aerosol characteristics and in-vivo amphotericin B deposition in rats. J. Pharm. Pharmacol. 52:619-627.

76. Diot, P., B. Rivoire, A. Le Pape, E. Lemarie, D. Dire, Y. Furet, M. Breteau, and G. C. Smaldone. 1995. Deposition of amphotericin B aerosols in pulmonary aspergilloma. Eur. Respir. J. 8:1263-8.

77. Rogers, T. L., A. C. Nelsen, M. Sarkari, T. J. Young, K. P. Johnston, and R. O. Williams, III. 2003. Enhanced Aqueous Dissolution of a Poorly Water Soluble Drug by Novel Particle Engineering Technology: Spray-Freezing into Liquid with Atmospheric Freeze-Drying. Pharm. Res. 20:485-493.

78.   Overhoff, K. A., J. D. Engstrom, B. Chen, B. D. Scherzer, T. E. Milner, K. P. Johnston, and R. O. Williams. 2007. Novel ultra-rapid freezing particle engineering process for enhancement of dissolution rates of poorly water-soluble drugs. Eur. J. Pharm. Biopharm. 65:57-67.

79.   Hu, J., K. P. Johnston, and R. O. Williams, III. 2004. Nanoparticle Engineering Processes for Enhancing the Dissolution Rates of Poorly Water Soluble Drugs. Drug Dev. Ind. Pharm. 30:233-245.

80.   Yang, W., J. Tam, D. A. Miller, J. Zhou, J. T. McConville, K. P. Johnston, and R. O. Williams. 2008. High bioavailability from nebulized itraconazole nanoparticle dispersions with biocompatible stabilizers. Int. J. Pharm. 361:177-188.

81.   Purvis, T., M. Vaughn Jason, L. Rogers True, X. Chen, A. Overhoff Kirk, P. Sinswat, J. Hu, T. McConville Jason, P. Johnston Keith, and O. Williams Robert, 3rd. 2006. Cryogenic liquids, nanoparticles, and microencapsulation. Int. J. Pharm. 324:43-50.

82.   Hoeben, B. J., D. S. Burgess, J. T. McConville, L. K. Najvar, R. L. Talbert, J. I. Peters, N. P. Wiederhold, B. L. Frei, J. R. Graybill, R. Bocanegra, K. A. Overhoff, P. Sinswat, K. P. Johnston, and R. O. Williams, III. 2006. In vivo efficacy of aerosolized nanostructured itraconazole formulations for prevention of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. 50:1552-1554.

83.   McConville, J. T., K. A. Overhoff, P. Sinswat, J. M. Vaughn, B. L. Frei, D. S. Burgess, R. L. Talbert, J. I. Peters, K. P. Johnston, and R. O. Williams, III. 2006. Targeted High Lung Concentrations of Itraconazole Using Nebulized Dispersions in a Murine Model. Pharm. Res. 23:901-911.

84.   Vaughn, J. M., J. T. McConville, D. Burgess, J. I. Peters, K. P. Johnston, R. L. Talbert, and R. O. Williams, III. 2006. Single dose and multiple dose studies of itraconazole nanoparticles. Eur. J. Pharm. Biopharm. 63:95-102.

85.   Ewing, P., B. Blomgren, A. Ryrfeldt, and P. Gerde. 2006. Increasing Exposure Levels Cause an Abrupt Change in the Absorption and Metabolism of Acutely Inhaled Benzo(a)pyrene in the Isolated, Ventilated, and Perfused Lung of the Rat. Toxicol. Sci. 91:332-340.

86.   Wong, S. M., I. W. Kellaway, and S. Murdan. 2006. Enhancement of the dissolution rate and oral absorption of a poorly water soluble drug by formation of surfactant-containing microparticles. Int. J. Pharm. 317:61-68.

87.   Bruesewitz, C., A. Schendler, A. Funke, T. Wagner, and R. Lipp. 2007. Novel poloxamer-based nanoemulsions to enhance the intestinal absorption of active compounds. Int. J. Pharm. 329:173-181.

88.   Vermeij, P., and D. Blok. 1996. New peptide and protein drugs. Pharm. World Sci. 18:87-93.

89.   Goldberg, M., and I. Gomez-Orellana. 2003. Challenges for the oral delivery of macromolecules. Nature Reviews Drug Discovery 2:289-295.

90.   Kumar, T. R. S., K. Soppimath, and S. K. Nachaegari. 2006. Novel delivery technologies for protein and peptide therapeutics. Current Pharmaceutical Biotechnology 7:261-276.

91.   Morishita, M., and N. A. Peppas. 2006. Is the oral route possible for peptide and protein drug delivery? Drug Discovery Today 11:905-910.

92.   Shoyele, S. A., and A. Slowey. 2006. Prospects of formulating proteins/peptides as aerosols for pulmonary drug delivery. Int. J. Pharm. 314:1-8.

93.   Blot, F., F. Faurisson, N. Bernard, S. Sellam, S. Friard, R. Tavakoli, C. Carbon, M. Stern, A. Bisson, J. J. Pocidalo, and I. Caubarrere. 1999. Nebulized cyclosporine in the rat: assessment of regional lung and extrapulmonary deposition. Transplantation 68:191-5.

94.   Mitruka, S. N., S. M. Pham, A. Zeevi, S. Li, J. Cai, G. J. Burckart, S. A. Yousem, R. J. Keenan, and B. P. Griffith. 1998. Aerosol cyclosporine prevents acute allograft rejection in experimental lung transplantation. Journal of Thoracic and Cardiovascular Surgery 115:28-37.

95.   Mitruka, S. N., A. Won, K. R. McCurry, A. Zeevi, T. McKaveney, R. Venkataramanan, A. Iacono, B. P. Griffith, and G. J. Burckart. 2000. In the lung aerosol cyclosporine provides a regional concentration advantage over intramuscular cyclosporine. J. Heart Lung Transplant. 19:969-75.

96.   Burckart, G. J., G. C. Smaldone, M. A. Eldon, R. Venkataramanan, J. Dauber, A. Zeevi, K. McCurry, T. P. McKaveney, T. E. Corcoran, B. P. Griffith, and A. T. Iacono. 2003. Lung Deposition and Pharmacokinetics of Cyclosporine After Aerosolization in Lung Transplant Patients. Pharm. Res. 20:252-256.

97.   Keenan, R., A. Iacono, J. Dauber, A. Zeevi, S. Yousem, N. Ohori, G. Burckart, A. Kawai, G. Smaldone, and B. Griffith. 1997. Treatment of refractory acute allograft rejection with aerosolized cyclosporine in lung transplant recipients. Journal of Thoracic and Cardiovascular Surgery 113:335-340.

98.   Wang, T., S. Noonberg, R. Steigerwalt, M. Lynch, R. A. Kovelesky, C. A. Rodriguez, K. Sprugel, and N. Turner. 2007. Preclinical safety evaluation of inhaled cyclosporine in propylene glycol Journal of Aerosol Medicine 20:417-428.

99. Letsou, G., H. Safi, M. Reardon, M. Ergenoglu, Z. Li, C. Klonaris, J. Baldwin, B. Gilbert, and J. Waldrep. 1999. Pharmacokinetics of liposomal aerosolized cyclosporine A for pulmonary immunosuppression. Annals of Thoracic Surgery 68:2044-2048.

100. Waldrep, J. C., J. Arppe, K. A. Jansa, and M. Vidgren. 1998. Experimental pulmonary delivery of cyclosporin A by liposome aerosol. International Journal of Pharamceutics 160:239-249.

101. Tam, J. M., J. T. McConville, R. O. Williams, III, and K. P. Johnston. 2008. Amorphous cyclosporin nanodispersions for enhanced pulmonary deposition and dissolution. J. Pharm. Sci. 97:4915-4933.

102. Adell, A. 2004. Antidepressant properties of substance P antagonists: relationship to monoaminergic mechanisms? Curr. Drug Targets: CNS Neurol. Disord. 3:113-121.

103. Henry, J. L. 1993. Substance P and inflammatory pain: Potential of substance P antagonists as analgesics. Agents Actions Suppl. 41:75-87.

104. Diemunsch, P., and L. Grelot. 2003. Potential of substance P antagonists as antiemetics. Antiemetic Ther.:78-97.

105. Nakate, T., H. Yoshida, A. Ohike, Y. Tokunaga, R. Ibuki, and Y. Kawashima. 2003. Improvement of pulmonary absorption of cyclopeptide FK224 in rats by co-formulating with beta -cyclodextrin. Eur. J. Pharm. Biopharm. 55:147-154.

106. Nakate, T., H. Yoshida, A. Ohike, Y. Tokunaga, R. Ibuki, and Y. Kawashima. 2003. Comparison of the lung absorption of FK224 inhaled from a pressurized metered dose inhaler and a dry powder inhaler by healthy volunteers. Eur. J. Pharm. Biopharm. 56:319-325.

107. Stavchansky, S., A. Martin, and A. Loper. 1979. Solvent system effects on drug absorption. Res. Commun. Chem. Pathol. Pharmacol. 24:77-85.

108. Krishna, R., M. S. Webb, G. St. Onge, and L. D. Mayer. 2001. Liposomal and nonliposomal drug pharmacokinetics after administration of liposome-encapsulated vincristine and their contribution to drug tissue distribution properties. J. Pharmacol. Exp. Ther. 298:1206-1212.

109. Mallick, S., S. Pattnaik, K. Swain, and P. K. De. 2007. Current Perspectives of Solubilization: Potential for Improved Bioavailability. Drug Dev. Ind. Pharm. 33:865-873.

110. Wax, P. M., C. E. Becker, and S. C. Curry. 2003. Unexpected "gas" casualties in Moscow: a medical toxicology perspective. Ann Emerg Med 41:700-5.

111. Hung, O., and D. Pliura. 2008. Comparative phase I PK study of Aerosolized free and Liposome-Encapsulated Fentanyl (AeroLEF™) demonstrates rapid and extended plasma fentanyl concentrations following inhalation, 27th Annual Scientific Meeting of the American Pain Society. YM Biosciences Inc., Biovail Contract Research, Tampa, FL.

112. Hung, O. R., S. C. Whynot, J. R. Varvel, and S. L. Shafer. 1995. Pharmacokinetics of inhaled liposome-encapsulated fentanyl. Anesthesiology 83:277-84.

113. Mather, L. E., A. Woodhouse, M. E. Ward, S. J. Farr, R. A. Rubsamen, and L. G. Eltherington. 1998. Pulmonary administration of aerosolized fentanyl: pharmacokinetic analysis of systemic delivery. British Journal of Clinical Pharmacology 46:37-43.

114. Overhoff, K. A., R. Clayborough, and M. Crowley. 2008. Review of the TAIFUN Multidose Dry Powder Inhaler Technology. Drug Dev. Ind. Pharm. 34:960-965.

115. LAB International Inc. October 26, 2005 2005, posting date. LAB International Announces Positive Preliminary Results from Additional Phase 1 Fentanyl TAIFUN® Trial. [Online.]

116. Miyake, K., H. Arima, T. Irie, F. Hirayama, and K. Uekama. 1999. Enhanced absorption of cyclosporin A by complexation with dimethyl-beta -cyclodextrin in bile duct-cannulated and -noncannulated rats. Biological & Pharmaceutical Bulletin 22:66-72.

117. Porter, C. J. H., C. W. Pouton, J. F. Cuine, and W. N. Charman. 2008. Enhancing intestinal drug solubilization using lipid-based delivery systems. Advanced Drug Delivery Reviews 60:673-691.

118. Winkler, J., G. Hochhaus, and H. Derendorf. 2004. How the lung handles drugs: pharmacokinetics and pharmacodynamics of inhaled corticosteroids. Proc. Am. Thorac. Soc. 1:356-363.

119. Hickey, A. J. 2002. Delivery of drugs by the pulmonary route, p. 479-499. *In* G. S. Banker and C. T. Rhodes (ed.), Drugs and the Pharmaceutical Sciences, 4th ed, vol. 121. Marcel Dekker, New York, NY.

120. Williams, A. C., and B. W. Barry. 2004. Penetration enhancers. Adv. Drug Delivery Rev. 56:603-618.

121. Jarabek, A., B. Asgharian, and F. Miller. 2005. Dosimetric Adjustments for Interspecies Extrapolation of Inhaled Poorly Soluble Particles (PSP). Inhalation Toxicology 17:317-334.

122. Carrier, R. L., L. A. Miller, and I. Ahmed. 2007. The utility of cyclodextrins for enhancing oral bioavailability. J. Controlled Release 123:78-99.

123. Challa, R., A. Ahuja, J. Ali, and R. K. Khar. 2005. Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech 6:E329-57.

124. Bekersky, I., R. M. Fielding, D. E. Dressler, J. W. Lee, D. N. Buell, and T. J. Walsh. 2002. Plasma protein binding of amphotericin B and pharmacokinetics of bound versus unbound amphotericin B after administration of intravenous liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate. Antimicrob. Agents Chemother. 46:834-840.

Chapter 2: Characterization and Pharmacokinetic Analysis of Aerosolized Aqueous Voriconazole Solution

2.1. INTRODUCTION

Invasive fungal infections are increasing in prevalence in immunocompromised patients due to decreased immunity resulting from drug therapy, organ transplantation, and/or various disease states (1). The distribution of causative organisms for invasive fungal infections has been changing with an increase in the prevalence of *Aspergillus spp.* and other invasive molds (2). Systemic fungal infections caused by *Aspergillus spp.* are primarily lung infections due to the inhalation of conidia. The resulting infection, Invasive Aspergillosis (IA), is the cause of serious damage to lung tissue due to invasive hyphal growth (3). Dissemination of IA can also occur to other organ systems and correlates with a poorer prognosis (4). Despite the best therapeutic options, mortality rates for IA remain high (4, 5).

The primary therapy for the treatment of IA is the systemic administration of voriconazole and has led to improved patient outcomes compared to other treatments (6, 7). Voriconazole is a triazole antifungal with broad antifungal activity against numerous pathogenic fungi in addition to its activity against *Aspergillus spp.* (8, 9). Voriconazole has also been reported to distribute to the lungs as measured by tissue and epithelial lining fluid concentrations following systemic administration (10, 11). The commercial voriconazole product, Vfend®, is available as an oral tablet or intravenous formulation. The intravenous product is formulated with Captisol®, sulfobutyl ether-β-cyclodextrin, to form a solubilized drug-cyclodextrin complex due to very slight voriconazole solubility

in water (12). Voriconazole has reported side effects of visual disturbances, hepatic toxicity, and dermatologic reactions as well as serious cytochrome P450 mediated drug interactions (8, 10). Adverse events causing discontinuation of therapy occurred in up to 6% of patients and were primarily due to elevations in liver function tests or rash. Other systemically administered antifungals can be selected as salvage therapy or in patients intolerant to voriconazole but have the potential for other serious side effects as well as drug interactions (6, 13, 14).

The potential side effect profile and drug interactions associated with systemic antifungal administration might be reduced by targeting drug delivery to the lungs, the primary site of IA. Targeted lung delivery of antifungals can also lead to high drug concentrations at the site of infection to improve clinical outcomes. Two antifungals, amphotericin B and itraconazole, have been inhaled with reported pharmacokinetic and outcome measures (13, 15-23). Inhaled amphotericin B formulations include drug solubilized with deoxycholate, drug encapsulated in liposomes, and drug-lipid complexed suspensions. Inhaled itraconazole was formulated as crystalline or amorphous nano-particulate suspensions.

Inhaled amphotericin B has a better but non-optimal side effect profile and significantly improved outcomes compared to the systemically administered formulations (13, 15, 17, 23). However, the pharmacokinetic profiles of inhaled compared to intravenous amphotericin B are substantially different. Lung concentrations of amphotericin B following intravenous administration are initially undetectable followed by low levels despite extensive tissue distribution following multiple doses (24-28). Inhaled amphotericin B has led to much higher lung tissue concentrations but

undetectable plasma levels (16, 29, 30). The high drug concentrations in the lung tissue following amphotericin B inhalation was hypothesized to result in significant outcomes in human patients and animal models of IA compared to intravenous drug administration (15, 17).

Inhaled nano-particulate itraconazole was also well tolerated with normal histological findings and an absence of inflammatory mediators following a chronic, multi-dose study in animals (22). The pharmacokinetic profile of different inhaled formulations following a single inhaled dose demonstrated high and prolonged itraconazole concentrations in the lungs with maximal lung levels achieved 30 to 60 minutes after the completion of nebulization while serum concentrations remained low and peaked after 2 to 5.35 hours in animals (20, 21, 31). The ratio of lung-to-serum AUC values was 25 to 50 and C_{max} ratios ranged from approximately 10 to 100, indicating low drug partitioning out of the lungs. Following multiple doses, lung concentrations remained substantially higher than serum concentrations (21). Inhaled itraconazole demonstrated significantly improved outcomes compared to oral itraconazole and control groups in animal models of IA and was suggested to be due to sufficient drug concentrations in the lungs to inhibit invasive fungal growth at a fraction of the oral dose (18, 19).

Both inhaled amphotericin B and inhaled particulate itraconazole demonstrated substantial drug retention in the lungs, improved survival in animal models of IA, and suggested positive clinical outcomes were associated with favorable lung pharmacokinetic profiles. Gavalda and colleagues reported improved survival in an animal model of IA when both inhaled and intravenous antifungal was administered

concurrently compared to inhaled or intravenous drug administered separately (15). This report suggests near-therapeutic plasma concentrations combined with very high concentrations of antifungal in the lung could improve patient outcomes. However, neither inhaled amphotericin B nor inhaled itraconazole produce blood concentrations that are close to therapeutic levels. Therefore, targeted delivery of an antifungal to the lungs with distribution to the blood producing high drug concentrations in both lung tissue and blood can potentially improve clinical outcomes and be a significant improvement in antifungal therapeutic options.

The poor distribution of amphotericin B and itraconazole to the systemic circulation following inhalation could be due, in part, to the very low aqueous solubilities of those compounds. Inhalation of a solubilized antifungal, the voriconazole-cyclodextrin inclusion complex as Vfend® IV, could lead to better lung concentrations than reported following systemic drug administration as well as systemic drug distribution. In this study, it is hypothesized that an aqueous solution of voriconazole solubilized with sulfobutyl ether-β-cyclodextrin, when inhaled as a single-dose, would produce high lung drug concentrations as well as allow rapid distribution from the lungs to the plasma. Furthermore, following multiple doses, inhaled voriconazole solutions would also produce elevated and consistent trough concentrations in lungs and plasma. Although solubilized voriconazole should distribute to the systemic circulation following inhalation, reductions in the incidence of hepatotoxicity, visual abnormalities, and dermatologic reactions could still occur due to a lower drug burden and dose sparing compared to systemic drug administration.

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2.2. MATERIALS AND METHODS

2.2.1. Materials

Vfend® IV (Pfizer Inc., New York, NY, USA) (Lot # A22278 and A26697), voriconazole standard (Lot number E010000674), and sulfobutyl ether-β-cyclodextrin, Captisol® (Lot # NC-04A-05009) were generously supplied by CyDex Pharmaceuticals, Inc. (Lenexa, KS). Sterile water for injection (SWFI) (Lot # 60-252-DK, Hospira, Inc.) and normal saline (Lot # 58-464-DK) were purchased from Cardinal Health (Dublin, OH). Sodium tetraborate decahydrate (Batch # 057K0070), boric acid (Batch # 097K0063), and sodium acetate trihydrate (Batch # 117K0153) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Acetic acid (Lot # 72270) was purchased from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany). HPLC grade ethyl acetate (Lot # PU0674) was purchased from Spectrum Chemical Manuf. Corp. (Gardena, CA). HPLC grade acetonitrile (Lot # 082967) was purchased from Fisher Scientific (Fair Lawn, NJ). HPLC grade methanol (Lot # 47360) was purchased from EMD Chemicals Inc. (Gibbstown, NJ). Water was obtained from an in-house Milli-Q UV Plus water purification system from the Millipore Corp. (Billerica, MA).

2.2.2. Characterization of *In Vitro* Properties of Voriconazole Solutions

Vfend® IV was reconstituted with SWFI as instructed in the prescribing information to a 10 mg/mL voriconazole concentration, which also contained sulfobutyl ether-β-cyclodextrin at 160 mg/mL. Additional dilutions were prepared with SWFI to voriconazole concentrations from 2.5 to 10 mg/mL . The osmolality of voriconazole solutions were tested (n=10 per concentration) using a μOsmette Micro Osmometer (Precision Systems Inc., Natick, MA). The pH of the 6.25 mg/mL voriconazole dilution

was determined using an Orion 350 PerpHecT® Advanced Benchtop pH Meter (Thermo Fisher Scientific, Waltham, MA).

2.2.3. Particle Size Analysis Using a Cascade Impactor

Voriconazole solutions were diluted to 6.25 mg/mL voriconazole and aerosolized using an Aeroneb® Pro micro pump nebulizer (Nektar Therapeutics, San Carlos, CA) for 20 minutes. Aerodynamic droplet size distributions were determined using a USP Apparatus 1 non-viable eight-stage cascade impactor (Thermo-Anderson, Symrna, GA) to quantify total emitted dose (TED) from the nebulizer output, mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), and percentage droplets with an aerodynamic diameter less than 4.7 μm (defined as the percentage fine particle fraction or FPF). The characterization of aerodynamic droplet size distribution was conducted through modifications to the guidelines described in USP 30 Section 601: Aerosols, Nasal Sprays, Metered-dose Inhalers, and Dry Powder Inhalers (32).

2.2.4. Single-dose Pharmacokinetic Analysis

Male outbred ICR (Institute for Cancer Research) mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and housed with free access to water and food. Prior to dosing, mice were acclimatized to the nose-only dosing animal restraints. Mice received a single inhaled dose of 5 mL aqueous voriconazole solution at 6.25 mg/mL voriconazole using a nose-only dosing apparatus with a drug exposure time of 20 minutes. Single-dose pharmacokinetic profiles were determined in two groups of mice: a high flow-rate group (5 L/min air flow, 32 g average mouse mass) and low flow-rate group (1 L/min, 22 g average mouse mass). Two or more mice were euthanized by

carbon dioxide narcosis at each time point (high flow-rate: 5, 10, 20, 30, 60, 90, 150, 240, 360, 720, and 1440 minutes or low flow-rate mice: 10, 30, 60, 240, 360, and 480 minutes). Whole blood was collected by cardiac puncture into heparinized vials and centrifuged at 9000 RPM for 15 minutes to obtain plasma. Whole lungs were also collected following exsanguination. Plasma samples and whole lungs were frozen and stored at -20°C until assayed. Lungs were thawed and homogenized with 1mL of water using an Omni GLH homogenizer (Omni International, Marietta, GA). Plasma samples and lung homogenates were analyzed individually for each animal sampled for voriconazole concentration by reverse-phase high-performance liquid chromatography (HPLC). Concentration values were then averaged and used to determine the concentration versus time profiles. All animals were handled and maintained in accordance with The University of Texas at Austin Institution Animal Care and Use Committee (IACUC) guidelines and in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines.

2.2.5. Multi-dose Pharmacokinetic Analysis

Male ICR mice weighing, on average, 22.5 g, were administered inhaled voriconazole twice daily using a nose-only dosing apparatus as described above. Doses were administered for 12 consecutive days beginning each day at 08:00 and 16:00 hours. Airflow through the dosing apparatus was constant at 1 L/min throughout the study. The dosing apparatus was disassembled and cleaned between each use. Randomly selected groups of 6 mice were sacrificed by carbon dioxide narcosis on days 3, 5, 10, and 12. For trough concentration determination, lung and plasma samples were collected immediately before the next scheduled morning dose on days 3, 5, 10, and 12 (16 hours after the last dose). For determination of peak voriconazole concentrations (C_{max}), lung and plasma

samples were collected 30 minutes after the completion of the 10th dose on day 5 of administration. Lung and plasma samples were handled and processed as described in the single-dose methodological description.

2.2.6. Chromatographic Analysis

Calibration standards, plasma, and homogenized lung samples were analyzed using similar methods to those previously published (33, 34). Briefly, voriconazole was extracted from plasma samples through the addition of acetonitrile, centrifugation, and supernatant extraction. The supernatant liquid was evaporated under a gentle stream of nitrogen and residual solids, including voriconazole, were re-dispersed with mobile phase and analyzed spectrophotometrically. For lung homogenate, 0.2M borate buffer (pH 9.0) was added followed by three liquid-phase extractions with ethyl acetate. The liquid from collected supernatant fractions were then evaporated under a gentle stream of nitrogen. Any residual solids, including voriconazole, were re-dispersed with mobile phase, centrifuged, and then analyzed spectrophotometrically. Each voriconazole sample was analyzed using a Waters Breeze liquid chromatograph (Waters Corporation, Milford, MA) or Shimadzu LC-10 liquid chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a heated (35°C) Jupiter® C18 (150 mm x 4.6 mm, 5 μm) with a Universal security guard (Widepore C18) guard column (Phenomenex, Torrance, CA). The sample volume was 50 μL with a UV detection wavelength of 254 nm. The mobile phase consisted of a 50:50 mixture of 0.01M pH 5.0 sodium acetate buffer and methanol at 1.0 mL/min.

2.2.7. Pharmacokinetic Analysis

Observed pharmacokinetic parameters were evaluated from the voriconazole concentration versus time profiles in plasma and lung tissue using independent non-compartmental models. The peak concentration (C_{max}) and the time to achieve the C_{max} (t_{max}) were determined. In addition, the area under the curve (AUC) in lung tissue and plasma up to 6 hours after the completion of drug exposure was calculated using the trapezoidal rule.

2.2.8. Statistical Analysis

Maximal concentrations in lung tissue and plasma were compared for statistical analysis by the *t*-test. Single dose C_{max} values were compared by a paired *t*-test with a P-value of <0.05 for significance. Multiple dose C_{max} values were compared using a *t*-test assuming unequal variance with a P-value of <0.05 for significance.

2.3. RESULTS

2.3.1. *In Vitro* Solution Characterization

The osmolality of aqueous solutions of voriconazole and sulfobutyl ether-β-cyclodextrin were correlated with solute concentrations. The osmolality for voriconazole solutions that contained sulfobutyl ether-β-cyclodextrin between 40 and 160 mg/mL were 109.3 to 507.7 mOsm/kg, respectively. A solution containing 6.25 mg/mL voriconazole and 100mg/mL sulfobutyl ether-β-cyclodextrin was isotonic with an osmolality of 293.2 mOsm/kg (Figure 2.1). The pH of the 6.25 mg/mL voriconazole solution ranged from 6.4 to 6.8.

2.3.2. Particle Size Analysis

The aerodynamic particle size distribution of nebulized 6.25 mg/mL voriconazole solution was determined independently in duplicate using an 8-stage non-viable cascade impactor with a spacer and an air-flow rate of 28.3 L/min. Sufficient voriconazole solution was added to the nebulizer medication reservoir such that volume remained after a 20 minute nebulization. The average TED, FPF, MMAD, and GSD were 25.51 ± 6.25 mg, 71.7 ± 2.62 %, 2.98 ± 0.06 μm, and 2.20 ± 0.13, respectively.

2.3.3. Single Dose Pharmacokinetic Studies

Following a single inhaled dose of voriconazole, the observed t_{max} values ranged from 10 to 30 minutes in lung tissue and 20 to 30 minutes in plasma (Table 2.1). Voriconazole concentrations in lung tissue were low to undetectable 6 to 8 hours after a single dose while plasma levels remained quantifiable for up to 24 hours after the completion of nebulization. In mice that received nebulized voriconazole at an air flow rate of 5 L/min, peak lung concentrations were 1.6 μg/g wet lung weight with an AUC_{0-6} value of 205.3 μg min/g wet lung weight (Figurte 2.2A). A peak plasma concentration of 1.2 μg/mL and AUC_{0-6} of 136.4 μg min/mL were achieved in this high air flow rate group (Figure 2.2B). In contrast, markedly, though not statically significant ($P > 0.05$), higher peak lung concentration, 11.0 μg/g, and a AUC_{0-6} value, 2408.0 μg min/g, were achieved in mice that were exposed to nebulized voriconazole at a slower air flow rate of 1 L/min. Similarly, a higher peak plasma concentration, 7.1 μg/mL, and AUC_{0-6} value, 1549.8 μg min/mL, were also achieved in this low air flow rate group.

2.3.4. Multiple Dose Pharmacokinetic Studies

In the multiple dose pharmacokinetic study, 5 mL of 6.25 mg/mL voriconazole solution was administered twice daily, beginning at 08:00 and 16:00, for 12 days to mice at the low air flow rate, 1 L/min. Trough lung and plasma samples were taken immediately before the 08:00 dose on days 3, 5, 10, and 12. Peak lung and plasma samples were taken 30 minutes following the completion of the 08:00 dose on day 5. After 5 days of drug administration, peak voriconazole lung and plasma concentrations were 6.73 µg/g wet lung weight and 2.32 µg/mL, respectively. Peak plasma concentrations were significantly lower (P < 0.05) following multiple doses than following a single inhaled dose. Peak lung concentrations were not statistically significant for single and multiple doses. Trough lung concentrations of voriconazole were not detectable through day 5 but reached 0.11 to 0.19 µg/g wet lung weight on days 10 and 12, respectively. In contrast, trough plasma voriconazole concentrations were quantifiable on each day of sampling and ranged from 0.18 to 0.32 µg/mL (Table 2.2).

2.4. DISCUSSION

The lungs are the primary site of IA due to inhalation of fungal conidia resulting in a focal fungal infection in the lungs that can disseminate to other systems. The site of an IA infection within the lungs is typically in deep alveolar spaces with possible sites of intra-cavity aspergillomas and vascular invasions. Although typical humans are not at risk for IA due to competent immune systems and effective clearance of inhaled spores, immunocompromised patients are at elevated risk for IA and are the focus of prophylactic and treatment regimens with systemic antifungal administration (6, 35). Systemically administered antifungals must distribute to the lungs to be effective

therapeutic options but have high reported rates of mortality, in part, due to poor tissue penetration (7, 9, 10, 14, 28, 36, 37). Targeted antifungal delivery has been reported to cause high drug concentrations at the site of infection in the lung and lead to improved outcomes (20, 21, 29-31, 38). The studies to date, have also demonstrated targeted antifungal delivery to the lungs has not led to distribution of drug to the plasma. Interestingly, Gavalda et al., found that a combination of inhaled and intravenous amphotericin B led to improved survival in a rat model of IA. Therefore, having high antifungal concentrations in lung tissue and plasma can potentially lead to improved outcomes in IA. The current study found that an aerosolized aqueous solution of voriconazole solubilized with sulfobutyl ether-β-cyclodextrin was compatible with inhalation and led to high concentrations in the lung tissue as well as plasma following single and multiple doses in a murine pharmacokinetic model of inhalation.

Inhaled aerosols must be compatible with biological membranes of the respiratory tract to avoid reactive and inflammatory airway side effects, including cough, dyspnea, and irritation (39, 40). Our results demonstrated that an aqueous solution of voriconazole solubilized with sulfobutyl ether-β-cyclodextrin was compatible with aerosol administration. The 6.25 mg/mL voriconazole dilution had an acceptable pH and was the only tested concentration within the isotonic range (Figure 2.1). When this concentration was aerosolized using an Aeroneb® Pro vibrating mesh nebulizer system, the *in vitro* aerodynamic particle size characteristics of MMAD and FPF, 2.98μm and 71.7%, respectively, suggest that the nebulized droplets have a particle size distribution appropriate for inspiration to the deep lung.

Following a single inhaled dose of voriconazole, quantifiable voriconazole concentrations were observed in mice that received low and high doses as determined by the varied air-flow rate from 1 to 5 L/min. At 5 L/min airflow through the dosing chamber, a dilute cloud of voriconazole-containing nebulized droplets was present in the dosing chamber which led to a low dose. Conversely, a more concentrated and stagnant cloud of voriconazole-containing droplets was present at 1 L/min airflow rate through the chamber which led to a higher dose of voriconazole in those mice. This dose difference led to a 7-fold increase in C_{max} values and 11-fold increase in AUC_{0-6} in lung tissue and plasma for mice in the dosing chamber with the slow air flow rate (Table 2.1). Although peak concentrations and drug exposure were affected by the inhaled dose, voriconazole distribution from the lungs to the plasma was unaffected. Possible drug ingestion due to mucociliary clearance is not a contributing factor to plasma voriconazole concentrations due to hyper-metabolism in mice following oral administration (41). An approximation of a partition coefficient, based on the lung to plasma ratios of C_{max} and AUC_{0-6}, ranged from 1.4-1.6 indicating good absorption and distribution of voriconazole across lung mucosal surfaces. In addition, the observed t_{max} values were observed after 10 to 30 minutes in lung tissue and 20 to 30 minutes in plasma after the completion of dosing for all mice tested. Additionally, voriconazole concentrations in lung tissue were low to undetectable 6 to 8 hours while plasma levels remained quantifiable for up to 24 hours after the completion of nebulization. Therefore, voriconazole was rapidly absorbed and distributed to the central plasma with minimal drug retained in lung tissue.

Inhaled voriconazole achieved maximal concentrations within 30 minutes in both lung tissue and plasma. The maximal lung tissue concentration was 1.4 times greater than the maximal plasma concentration value. The rapid distribution of voriconazole

from lung tissue to plasma as well as the extent of distribution differed substantially from previous reports of inhaled antifungal agents. Specifically, amphotericin B has negligible distribution from lung tissue after inhalation. Following nebulized suspensions of pure amphotericin B, very low serum concentrations were achieved with a t_{max} of 30 minutes (29). Inhaled deoxycholate solutions of amphotericin B produced undetectable plasma concentrations up to 24 hours after the dose (27, 30). Additionally, retention of inhaled amphotericin B in lung tissue has been reported to be 15 to 22 days following a single inhaled dose (38). For itraconazole, inhaled suspensions of nanoparticulate formulations had lung t_{max} values of 0.5 to 1 hour after the dose but with delayed plasma t_{max} values of 2 to 5.4 hours (20, 21, 31). For inhaled itraconazole, the reported AUC values were 25 to 50 times greater in lung tissue than in the plasma. Similarly, peak itraconazole concentrations were 12 to 100 greater for lung tissue than plasma. These comparisons indicate negligible distribution of particulate itraconazole from the lungs to the plasma.

The sulfobutyl ether-β-cyclodextrin excipient in inhaled voriconazole solutions prompted the differences in voriconazole distribution compared to inhaled particulate and solubilized amphotericin B as well as inhaled particulate itraconazole. Cyclodextrins have been reported to cause rapid t_{max} and high C_{max} concentrations as well as improved bioavailability of intratracheally administered aerosols or solutions compared to equipotent alternative formulations or routes of administration (42-44). Although Roffey et al. used a 10 mg/kg IV and 30 mg/kg oral dose, direct comparison to pharmacokinetic parameters observed following a single inhaled dose were not directly possible due to dose uncertainties following inhalation in this study (45). Additionally, voriconazole displays non-linear pharmacokinetics in humans and animals which could account for the marked differences observed following inhalation of different doses due to high and low

air flow rate through the dosing chamber. However, the observed C_{max}, t_{max}, and AUC values following a single inhaled dose at 1 L/min air flow rate through the dosing chamber were similar to those reported in mice following intravenous drug administration. Therefore, inhaled voriconazole solubilized with sulfobutyl ether-β-cyclodextrin produced plasma pharmacokinetic parameters similar to intravenous drug administration with additional high lung tissue concentrations.

The multi-dose voriconazole pharmacokinetic profile cannot be extrapolated from a single-dose profile due to non-linear pharmacokinetics resulting from saturable hepatic metabolic pathways (8). Additionally the multi-dose pharmacokinetic profile is complicated in mice due to altered plasma pharmacokinetic profiles following multiple intravenous and oral doses when compared to a single dose (45). The peak plasma voriconazole concentration after 5 days of multiple doses, 2.32 ± 1.52 µg/mL (Table 2.2), was significantly lower than that observed following a single dose, 7.9 ± 0.68 µg/mL (Table 2.1). The peak plasma voriconazole concentration was also lower than the concentration associated with toxicity in human studies (6 to 7 µg/mL) and should therefore correlate with acceptable dose tolerability and side effect profiles following multiple doses (46). However, trough plasma voriconazole concentrations had no discernable trend between day 3 and 12 with concentrations ranging from 0.18 to 0.32 µg/mL (Table 2.2). The trough plasma concentrations observed in this study were affected, in part, by the altered dosing interval and were lower than 1 µg/mL, the trough concentration correlated with improved efficacy in humans (47). Additionally, the influence of altered drug metabolism on plasma concentrations following multiple inhaled doses is uncertain based on this study.

Changes in voriconazole metabolism were not investigated in this current study. There is a very low prevalence of drug metabolizing enzymes in lung tissue (48, 49). Mice livers are also of insufficient size for quantative determination of hepatic metabolic enzyme evaluation. Therefore, altered metabolism was unlikely to affect voriconazole lung pharmacokinetic parameters following multiple doses but could account for changes in plasma voriconazole concentrations. Specifically, the single-dose pharmacokinetic profile of inhaled voriconazole suggested drug accumulation in the lung tissue and plasma would be insignificant following multiple doses. Negligible but quantifiable drug accumulation was evidenced in lung tissue through 12 days of dosing with undetectable trough concentrations on day 5 that increased to values of 0.19 μg/g wet lung weight on day 12 (Table 2.2). The peak lung voriconazole concentration on day 5 was 6.73 ± 3.64 μg/g wet lung weight was not significantly different than the observed value following a single dose (Table 2.1). However, peak plasma voriconazole concentrations were significantly lower following multiple doses compared to a single inhaled dose and could be influenced by altered metabolism.

Mice were selected as the pharmacokinetic model for inhaled voriconazole despite several limitations. Additionally, reports using the Aeroneb® Pro vibrating mesh nebulizer have correlated acceptable *in vitro* aerosol characteristics with low lung deposition in humans (50, 51). Published studies have demonstrated lower lung deposition of inhaled aerosols in rodents compared to humans due to allometric differences between species (52-54). Voriconazole is also hyper-metabolized in mice and rats when administered orally and intravenously (45). Investigators have successfully elevated voriconazole serum concentrations and improved murine survival in a model of fungal infection through the inhibition of voriconazole metabolism by grapefruit juice

administration (41, 55). In the present study, no enzymatic inhibition was undertaken. Despite the limitations of low lung deposition and hyper-metabolism, pharmacokinetic profiles of voriconazole in lung tissue and plasma following single and multiple doses demonstrated high drug concentrations as well as elevated drug exposure levels.

2.5. CONCLUSIONS

An inhaled aqueous solution of voriconazole and sulfobutyl ether-β-cyclodextrin is capable of producing clinically relevant lung tissue and plasma concentrations. Rapid and extensive drug distribution from the lung tissue into the blood was observed leading to potential advantages over contemporary reports of inhaled antifungals. Solubilization of voriconazole by complexation with sulfobutyl ether-β-cyclodextrin potentially contributed to the observed pharmacokinetic properties. High lung tissue as well as plasma concentrations were observed following single and multiple inhaled doses. Further studies are needed to demonstrate the influence of dosing interval on voriconazole concentrations following multiple inhaled doses. However, inhaled voriconazole presents a potentially beneficial improvement in therapeutic options for the treatment of IA.

2.6. REFERENCES

1.   Patterson, T. F. 2005. Advances and challenges in management of invasive mycoses. Lancet 366:1013-25.

2.   Chamilos, G., M. Luna, E. Lewis Russell, P. Bodey Gerald, R. Chemaly, J. Tarrand Jeffrey, A. Safdar, I. Raad Issam, and P. Kontoyiannis Dimitrios. 2006. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003). Haematologica 91:986-9.

3.   Pai, U., R. J. Blinkhorn, Jr., and J. F. Tomashefski, Jr. 1994. Invasive cavitary pulmonary aspergillosis in patients with cancer: a clinicopathologic study. Hum. Pathol. 25:293-303.

4.   Richardson, M. D. 2005. Changing patterns and trends in systemic fungal infections. J. Antimicrob. Chemother. 56:i5-i11.

5.   Cornet, M., L. Fleury, C. Maslo, J. F. Bernard, and G. Brucker. 2002. Epidemiology of invasive aspergillosis in France: a six-year multicentric survey in the Greater Paris area. J. Hosp. Infect. 51:288-96.

6.   Walsh, T. J., E. J. Anaissie, D. W. Denning, R. Herbrecht, D. P. Kontoyiannis, K. A. Marr, V. A. Morrison, B. H. Segal, W. J. Steinbach, D. A. Stevens, J.-A. van Burik, J. R. Wingard, and T. F. Patterson. 2008. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin. Infect. Dis. 46:327-360.

7.   Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J.-W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke, B. de Pauw, R. Allen, M. Aoun, C. Aul, M. Bjorkholm, K. L. Blanchard, M. Boogaerts, E. Bouza, E. J. Bow, H. R. Brodt, J. Brown, D. Buchheidt, J. Y. Cahn, A. Calmaggi, J. M. Cisneros, C. Cordonnier, J. Daly, C. A. Da Cunha, R. De Bock, A. Del Favero, J. Diaz Mediavilla, M. C. Dignani, C. Doyen, J. S. Dummer, B. Dupont, M. Egyed, D. Engelhard, G. Faetkenheuer, R. Feld, D. Fiere, G. Fioritoni, G. Garber, Z. Gasztonyi, K. Godder, D. Graham, A. Gratwohl, R. Greenberg, K. High, F. Jacobs, V. Kremery, P. Kumar, W. Langer, M. Laverdiere, P. Ljungman, H. Lode, A. Louie, D. Maki, J. P. Marie, D. J. E. Marriott, D. S. McKinsey, R. Mertelsmann, M. K. Nair, N. Milpied, A. Nagler, D. Niederwieser, L. Pagano, P. Pappas, J. Perfect, J. Pottage, V. Raina, J. Reinhardt, S. Richardson, L. Rickman, M. Ruhnke, I. Salit, W. M. Scheld, S. Schuler, M. Schuster, R. Schwerdtfeger, S. D. Shafran, B. Simmons, M. Slavin, M. Sokol-Anderson, P. Tebas, C. Tsoukas, A. Ullmann, J. Van Burik, J. W. Van't Wout, E. C. Vinaya Kumar, et al. 2002.

Voriconazole versus amphotericin B for primary therapy on invasive aspergillosis. N. Engl. J. Med. 347:408-415.

8. Scott, L. J., D. Simpson, S. I. Blot, P. H. Chandrasekar, A. H. Groll, R. P. Hobson, V. L. Kan, S. Keady, and T. J. Walsh. 2007. Voriconazole: a review of its use in the management of invasive fungal infections. Drugs 67:269-298.

9. Gabardi, S., D. W. Kubiak, A. K. Chandraker, and S. G. Tullius. 2007. Invasive fungal infections and antifungal therapies in solid organ transplant recipients. Transpl. Int. 20:993-1015.

10. Theuretzbacher, U., F. Ihle, and H. Derendorf. 2006. Pharmacokinetic/pharmacodynamic profile of voriconazole. Clin. Pharmacokin. 45:649-663.

11. Capitano, B., B. A. Potoski, S. Husain, S. Zhang, D. L. Paterson, S. M. Studer, K. R. McCurry, and R. Venkataramanan. 2006. Intrapulmonary penetration of voriconazole in patients receiving an oral prophylactic regimen. Antimicrob. Agents Chemother. 50:1878-1880.

12. Roerig Division of Pfizer Inc. 2006. Vfend Prescribing Information, p. 47. Pfizer Inc., New York, NY.

13. Erjavec, Z., G. M. H. Woolthuis, H. G. De Vries-Hospers, W. J. Sluiter, S. M. G. J. Daenen, B. De Pauw, and M. R. Halie. 1997. Tolerance and efficacy of amphotericin B inhalations for prevention of invasive pulmonary aspergillosis in hematological patients. Eur. J. Clin. Microbiol. 16:364-368.

14. Boucher, H. W., A. H. Groll, C. C. Chiou, and T. J. Walsh. 2004. Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. Drugs 64:1997-2020.

15. Gavalda, J., M.-T. Martin, P. Lopez, X. Gomis, J.-L. Ramirez, D. Rodriguez, O. Len, Y. Puigfel, I. Ruiz, and A. Pahissa. 2005. Efficacy of nebulized liposomal amphotericin B in treatment of experimental pulmonary aspergillosis. Antimicrob. Agents Chemother. 49:3028-3030.

16. Ruijgrok, E. J., A. G. Vulto, and E. W. M. Van Etten. 2000. Aerosol delivery of amphotericin B desoxycholate (Fungizone) and liposomal amphotericin B (AmBisome): aerosol characteristics and in-vivo amphotericin B deposition in rats. J. Pharm. Pharmacol. 52:619-627.

17. Ruijgrok, E. J., A. G. Vulto, and E. W. M. Van Etten. 2001. Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromised rats. J. Antimicrob. Chemother. 48:89-95.

18. Alvarez, C., A., N. P. Wiederhold, J. T. McConville, J. I. Peters, L. K. Najvar, J. R. Graybill, J. J. Coalson, R. L. Talbert, D. S. Burgess, R. Bocanegra, K. P. Johnston, and R. O. Williams, 3rd. 2007. Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis. J. Infect. 55:68-74.

19. Hoeben, B. J., D. S. Burgess, J. T. McConville, L. K. Najvar, R. L. Talbert, J. I. Peters, N. P. Wiederhold, B. L. Frei, J. R. Graybill, R. Bocanegra, K. A. Overhoff, P. Sinswat, K. P. Johnston, and R. O. Williams, III. 2006. In vivo efficacy of aerosolized nanostructured itraconazole formulations for prevention of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. 50:1552-1554.

20. McConville, J. T., K. A. Overhoff, P. Sinswat, J. M. Vaughn, B. L. Frei, D. S. Burgess, R. L. Talbert, J. I. Peters, K. P. Johnston, and R. O. Williams, III. 2006. Targeted High Lung Concentrations of Itraconazole Using Nebulized Dispersions in a Murine Model. Pharm. Res. 23:901-911.

21. Vaughn, J. M., J. T. McConville, D. Burgess, J. I. Peters, K. P. Johnston, R. L. Talbert, and R. O. Williams, III. 2006. Single dose and multiple dose studies of itraconazole nanoparticles. Eur. J. Pharm. Biopharm. 63:95-102.

22. Vaughn, J. M., N. P. Wiederhold, J. T. McConville, J. J. Coalson, R. L. Talbert, D. S. Burgess, K. P. Johnston, R. O. Williams, and J. I. Peters. 2007. Murine airway histology and intracellular uptake of inhaled amorphous itraconazole. Int. J. Pharm. 338:219-224.

23. Drew, R. H., E. Dodds Ashley, D. K. Benjamin, Jr., R. D. Davis, S. M. Palmer, and J. R. Perfect. 2004. Comparative safety of amphotericin B lipid complex and amphotericin B deoxycholate as aerosolized antifungal prophylaxis in lung-transplant recipients. Transplantation 77:232-237.

24. Bekersky, I., G. W. Boswell, R. Hiles, R. M. Fielding, D. Buell, and T. J. Walsh. 2000. Safety, toxicokinetics and tissue distribution of long-term intravenous liposomal amphotericin B (AmBisome): a 91-day study in rats. Pharm. Res. 17:1494-1502.

25. Collette, N., P. van der Auwera, A. P. Lopez, C. Heymans, and F. Meunier. 1989. Tissue concentrations and bioactivity of amphotericin B in cancer patients treated with amphotericin B-deoxycholate. Antimicrob. Agents Chemother. 33:362-8.

26. Christiansen, K. J., E. M. Bernard, J. W. Gold, and D. Armstrong. 1985. Distribution and activity of amphotericin B in humans. J. Infect. Dis. 152:1037-43.

27. Koizumi, T., K. Kubo, T. Kaneki, M. Hanaoka, T. Hayano, T. Miyahara, K. Okada, K. Fujimoto, H. Yamamoto, T. Kobayashi, and M. Sekiguchi. 1998. Pharmacokinetic evaluation of amphotericin B in lung tissue: lung lymph

distribution after intravenous injection and airspace distribution after aerosolization and inhalation of amphotericin B. Antimicrob. Agents Chemother. 42:1597-1600.

28. Wasan, K. M., A. L. Kennedy, S. M. Cassidy, M. Ramaswamy, L. Holtorf, J. W. Chou, and P. H. Pritchard. 1998. Pharmacokinetics, distribution in serum lipoproteins and tissues, and renal toxicities of amphotericin B and amphotericin B lipid complex in a hypercholesterolemic rabbit model: single-dose studies. Antimicrob. Agents Chemother. 42:3146-52.

29. Diot, P., B. Rivoire, A. Le Pape, E. Lemarie, D. Dire, Y. Furet, M. Breteau, and G. C. Smaldone. 1995. Deposition of amphotericin B aerosols in pulmonary aspergilloma. Eur. Respir. J. 8:1263-8.

30. Marra, F., N. Partovi, K. M. Wasan, E. H. Kwong, M. H. H. Ensom, S. M. Cassidy, G. Fradet, and R. D. Levy. 2002. Amphotericin B disposition after aerosol inhalation in lung transplant recipients. Ann. Pharmacother. 36:46-51.

31. Yang, W., J. Tam, D. A. Miller, J. Zhou, J. T. McConville, K. P. Johnston, and R. O. Williams. 2008. High bioavailability from nebulized itraconazole nanoparticle dispersions with biocompatible stabilizers. Int. J. Pharm. 361:177-188.

32. 2008. USP 31 - NF 26. The United States Pharmacopeial Convention, Rockville, MD.

33. Pascual, A., V. Nieth, T. Calandra, J. Bille, S. Bolay, L. A. Decosterd, T. Buclin, P. A. Majcherczyk, D. Sanglard, and O. Marchetti. 2007. Variability of voriconazole plasma levels measured by new high-performance liquid chromatography and bioassay methods. Antimicrob. Agents Chemother. 51:137-143.

34. Lutsar, I., S. Roffey, and P. Troke. 2003. Voriconazole concentrations in the cerebrospinal fluid and brain tissue of guinea pigs and immunocompromised patients. Clin. Infect. Dis. 37:728-32.

35. Maschmeyer, G., A. Haas, and O. A. Cornely. 2007. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs 67:1567-1601.

36. Lewis, R. E., G. Liao, J. Hou, G. Chamilos, R. A. Prince, and D. P. Kontoyiannis. 2007. Comparative analysis of amphotericin B lipid complex and liposomal amphotericin B kinetics of lung accumulation and fungal clearance in a murine model of acute invasive pulmonary aspergillosis. Antimicrob Agents Chemother 51:1253-8.

37.   Lin, S. J., J. Schranz, and S. M. Teutsch. 2001. Aspergillosis case-fatality rate: systematic review of the literature. Clin. Infect. Dis. 32:358-66.

38.   Lambros, M. P., D. A. W. Bourne, S. A. Abbas, and D. L. Johnson. 1997. Disposition of Aerosolized Liposomal Amphotericin B. J. Pharm. Sci. 86:1066-1069.

39.   Suzuki, R., and A. N. Freed. 2000. Hypertonic saline aerosol increases airway reactivity in the canine lung periphery. J. Appl. Physiol. 89:2139-46.

40.   Freed, A. N., K. T. Yiin, and C. E. Stream. 1989. Hyperosmotic-induced bronchoconstriction in the canine lung periphery. J. Appl. Physiol. 67:2571-8.

41.   Sugar, A. M., and X. P. Liu. 2000. Effect of grapefruit juice on serum voriconazole concentrations in the mouse. Med. Mycol. 38:209-12.

42.   Nakate, T., H. Yoshida, A. Ohike, Y. Tokunaga, R. Ibuki, and Y. Kawashima. 2003. Improvement of pulmonary absorption of cyclopeptide FK224 in rats by co-formulating with beta -cyclodextrin. Eur. J. Pharm. Biopharm. 55:147-154.

43.   Koushik, K., D. S. Dhanda, N. P. S. Cheruvu, and U. B. Kompella. 2004. Pulmonary Delivery of Deslorelin: Large-Porous PLGA Particles and HPbeta CD Complexes. Pharm. Res. 21:1119-1126.

44.   Hussain, A., J. J. Arnold, M. A. Khan, and F. Ahsan. 2004. Absorption enhancers in pulmonary protein delivery. J. Controlled Release 94:15-24.

45.   Roffey, S. J., S. Cole, P. Comby, D. Gibson, S. G. Jezequel, A. N. R. Nedderman, D. A. Smith, D. K. Walker, and N. Wood. 2003. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab. Dispos. 31:731-41.

46.   Husain, S., D. L. Paterson, S. Studer, J. Pilewski, M. Crespo, D. Zaldonis, K. Shutt, D. L. Pakstis, A. Zeevi, B. Johnson, E. J. Kwak, and K. R. McCurry. 2007. Voriconazole prophylaxis in lung transplant recipients. Am. J. Transplant. 6:3008-3016.

47.   Brueggemann, R. J. M., J. P. Donnelly, R. E. Aarnoutse, A. Warris, N. M. A. Blijlevens, J. W. Mouton, P. E. Verweij, and D. M. Burger. 2008. Therapeutic Drug Monitoring of Voriconazole. Therap. Drug Monitor. 30:403-411.

48.   Nishimura, M., H. Yaguti, H. Yoshitsugu, S. Naito, and T. Satoh. 2003. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. Yakugaku Zasshi 123:369-75.

49. Zhang, J. Y., Y. Wang, and C. Prakash. 2006. Xenobiotic-metabolizing enzymes in human lung. Curr. Drug Metab. 7:939-948.

50. Dubus, J. C., L. Vecellio, M. De Monte, B. Fink James, D. Grimbert, J. Montharu, C. Valat, N. Behan, and P. Diot. 2005. Aerosol deposition in neonatal ventilation. Pediatr. Res. 58:10-4.

51. Clark, D., M. Pickford, S. Evans, A. Bitonti, A. Bauer, and S. Newman. 2003. Targeting an Inhaled Erythropoietin Fc Fusion Protein (Epo-Fc) to the Human Large Central Airways, International Society for Aerosols in Medicine. Pharmaceutical Profiles, Baltimore, MD.

52. Asgharian, B., R. Wood, and R. B. Schlesinger. 1995. Empirical modeling of particle deposition in the alveolar region of the lungs: a basis for interspecies extrapolation. Fund. Appl. Toxicol. 27:232-8.

53. Nadithe, V., M. Rahamatalla, W. H. Finlay, J. R. Mercer, and J. Samuel. 2003. Evaluation of nose-only aerosol inhalation chamber and comparison of experimental results with mathematical simulation of aerosol deposition in mouse lungs. J. Pharm. Sci. 92:1066-1076.

54. American Institute of Ultrasound in Medicine. 2000. Section 3--Selected biological properties of tissues: potential determinants of susceptibility to ultrasound-induced bioeffects. J. Ultrasound. Med. 19:58-96.

55. Graybill, J. R., L. K. Najvar, G. M. Gonzalez, S. Hernandez, and R. Bocanegra. 2003. Improving the mouse model for studying the efficacy of voriconazole. J. Antimicrob. Chemother. 51:1373-1376.

Chapter 3: Inhaled Voriconazole for the Prevention of Invasive Pulmonary Aspergillosis

3.1. INTRODUCTION

Invasive aspergillosis is a significant cause of morbidity and mortality in heavily immuncompromised patients, including those with hematologic malignancies, hematopoietic stem cell transplant recipients, and those undergoing solid organ transplantation (1, 2). In many large cancer and transplant centers, the incidence of this opportunistic infectious disease has risen over the last two decades (3, 4). Invasive aspergillosis is also associated with significant increases in hospital costs as well as therapy complications in patients with multiple comorbidities, including delays in anti-cancer chemotherapeutic regimens (5). Although the early initiation of appropriate antifungal therapy may be effective in the treatment of this disease, establishing a diagnosis early in the course of therapy is often difficult (6). Thus, preventative strategies against invasive fungal infections, including the administration of antifungal prophylaxis, is often used in high risk patient populations (7, 8).

Antifungal prophylaxis has been shown in some clinical trials to reduce the incidence of invasive mycoses and improve survival rates in high-risk patient groups (9-11). Indeed, prophylactic administration of agents with activity against *Aspergillus* species, i.e. itraconazole, posaconazole, and voriconazole, has been shown to reduce the occurrence of invasive fungal infections, including invasive aspergillosis, in allogeneic stem cell transplant recipients (9, 10, 12-14). However, the systemic administration of antifungals may predispose patients to the adverse effects of these agents and potentially

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deleterious drug interactions (15). Orally administered itraconazole is hampered by the low and erratic bioavailability of the oral capsule formulation and gastrointestinal side effects associated with the oral solution (9, 10). Posaconazole and voriconazole are both associated with significant pharmacokinetic variability, and low plasma concentrations of both agents have been associated with therapy failures (16, 17).

Attention has begun to focus on the pulmonary delivery of antifungals for the prevention of invasive aspergillosis as inhalation of *Aspergillus* conidia into the lungs is the initial step in the pathogenesis of this opportunistic infection. Targeted pulmonary antifungal delivery may lead to high local concentrations within the lungs, the primary site of entry and infection, while avoiding some toxicities associated with systemic administration. Preclinical work using animal models has demonstrated this to be a potentially useful strategy (18-21). Clinically, aerosolization of amphotericin B lipid complex has been shown to be safe in lung transplant recipients and is gaining favor in many transplant centers (22, 23). In addition, a recent study demonstrated that inhaled liposomal amphotericin B was effective in preventing invasive pulmonary aspergillosis in patients with hematologic malignancies (24). Previous work by our group has also demonstrated that aerosolized nanostructure formulations of the poorly water soluble agent itraconazole are effective as prophylaxis in murine models in invasive pulmonary aspergillosis (20, 21).

Voriconazole is a triazole antifungal with potent activity against *Aspergillus* species and has been shown to be effective as primary treatment in patients with proven or probable invasive aspergillosis (25). It is currently available both as an oral and parenteral formulation, the latter containing the excipient sulfobutyl ether-β-

cyclodextrin sodium as a solubilizing agent in aqueous solution. The objective of this study was to assess the utility of aerosolized voriconazole as prophylaxis against invasive pulmonary aspergillosis caused by *Aspergillus fumigatus*. We hypothesized that an inhaled aqueous solution of voriconazole derived from the commercially available intravenous formulation would prevent invasive disease and improve survival in an established murine model of invasive aspergillosis. Secondary endpoints in this study included reductions in pulmonary fungal burden and the degree of invasive disease and lung injury as assessed by histopathology.

3.2. MATERIALS AND METHODS

3.2.1. Isolate

Conidia were harvested from *Aspergillus fumigatus* clinical isolate 293 (AF 293) cultures grown on potato dextrose agar (Hardy Diagnostics, Santa Maria, CA) by washing and scraping agar surfaces with 0.1% Tween 80 in sterile physiological saline and filtering through sterile glass wool. Conidia were re-suspended to achieve a final inoculum of ~1 x 10^9 conidia/mL, as confirmed by hemocytometer counts and serial plating.

3.2.2. Animal Model

An established murine model of invasive pulmonary aspergillosis was used as previously described (26, 27). Female ICR mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were immunosuppressed by intraperitoneal cyclophosphamide (250 mg/kg) and subcutaneous cortisone acetate (250 mg/kg) two days prior to inoculation. Both cyclophosphamide (200 mg/kg intraperitoneal) and cortisone acetate (250 mg/kg

subcutaneously) were re-administered on day 3 post-inoculation, and mice received antibiotic prophylaxis with ceftazidime 50mg/kg/day. This study was approved by the Institutional Animal Care Use Committee at the University of Texas Health Science Center at San Antonio, and all animals were handled in accordance with the American Association for Accreditation of Laboratory Animal Care (28).

To simulate pulmonary pathogenesis, mice were placed inside an acrylic chamber, and *A. fumigatus* conidia were introduced by aerosolizing the conidial suspension with a small particle nebulizer (Hudson Micro Mist, Hudson RCI, Temecula, CA) driven by compressed air (29). A standard exposure time of 1 hour was used to allow for complete aerosolization of the conidial suspension. Starting inocula were assessed by colony forming unit (CFU) enumeration from mice one hour post-inoculation.

3.2.3. Antifungal Therapy

Mice were randomly assigned to three treatment groups: inhaled voriconazole (5 mL of 6.25 mg/mL of the commercially available voriconazole IV formulation containing 100 mg/mL of sulfobutyl ether-β-cyclodextrin sodium via 20 minute aerosolization twice daily; Pfizer, Inc., New York, NY), amphotericin B deoxycholate (1 mg/kg interperitoneally daily; Apothecon, Princeton, NJ), or aerosolized sulfobutyl ether-β-cyclodextrin sodium as control (5mL of 100 mg/mL solution via 20 minute aerosolization twice daily; Captisol®, CyDex Pharmaceuticals, Inc., Lenexa, KS). Voriconazole and control mice received aerosolized solutions in a nose-only dosing chamber by a Aeroneb® Pro micropump nebulizer system with air-flow through the chamber at 1 L/min. Prophylaxis was begun two days prior to pulmonary inoculation and continued for a total of 10 days (day 7 post-inoculation). Amphotericin B was initiated on day 1 following inoculation and continued until day 7 post-inoculation. Mice were

monitored for an additional 5 days following discontinuation of antifungals. Animals that appeared moribund prior to the end of the study were euthanized by halothane and death was recorded as occurring the next day. Twelve mice from each group were randomly selected and euthanized on day 8 and the lung tissue harvested for fungal burden analysis. Additional mice were randomly selected from each group and euthanized on day 8 and day 12 for histopathology.

3.2.4. Pulmonary Fungal Burden

Lungs were homogenized in sterile saline (total volume 2 mL) supplemented with gentamicin and chloramphenicol using a tissue homogenizer (Polytron Dispensing and Mixing Technology PT 2100, Kinematica, Cincinnati, OH). Serial dilutions were prepared in sterile saline and plated in duplicate onto potato dextrose agar. Following 24 hours of incubation at 37°C, colonies were enumerated and colony forming units (CFU) per gram of lung tissue for each animal were calculated.

Pulmonary fungal burden was also quantified by real-time quantitative polymerase chain reaction (qPCR) as previously described (30, 31). Briefly, DNA was extracted from 90 mL of lung homogenate with the use of a commercially available kit (DNeasy Tissue Kit, Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA samples were analyzed in duplicate with the use of the ABI PRISM 7300 sequence-detection system (Applied Biosystems, Foster City, CA) with primers and dual-labeled fluorescent hybridization probes specific for the *A. fumigatus* 1,3-β-glucan synthase (*FKS*) gene (GenBank accession number U79728) (32). The threshold cycle (Ct) of each sample was interpolated from a six-point standard curve generated by spiking naive mouse lungs with known amounts of conidia (10^2 to 10^7). The resulting data was expressed as conidial equivalents (CE).

3.2.5. Histopathological Evaluation

Changes in lung histopathology were evaluated among the different groups on days 8 and 12 post-inoculation. Following sacrifice, 10% v/v formaldehyde was instilled into the lungs via the trachea. Lungs were harvested and placed into 10% v/v formaldehyde. The lungs were then processed and embedding into paraffin wax. Coronal sections of the entire lung were obtained at a thickness of 4-6 μm and mounted on slides. Sections were stained with hematoxylin and eosin and viewed by light microscopy.

3.2.6. Statistics

Survival was plotted by Kaplan-Meier analysis, and differences in median survival and percent survival between prophylaxis groups were analyzed by the log-rank test and chi-square test, respectively, using Prism software version 5 (GraphPad, San Diego, CA). Differences in fungal burden endpoints (CFU/g and CE) were assessed for significance by analysis of variance with Tukey's post-test for multiple comparisons. A p-value of ≤0.05 was considered statistically significant for all comparisons.

3.3. RESULTS

3.3.1. Survival

Mice that received aerosolized voriconazole had a survival advantage compared to controls and those that received amphotericin B. As shown in Figure 3.1, survival while receiving antifungal therapy was 92% for aerosolized voriconazole and was significantly greater than that of control (aerosolized sulfobutyl ether-β-cyclodextrin sodium, 25%; $p < 0.05$) and amphotericin B (31%; $p < 0.05$).   This survival benefit

continued once therapy was discontinued with 67% of animals that received aerosolized voriconazole surviving until day 12 post-inoculation, compared to 17% that received control (aerosolized sulfobutyl ether-β-cyclodextrin sodium; $p < 0.05$) and 23% that received amphotericin B ($p < 0.05$). No significant difference was observed in survival between animals that received control and amphotericin B. The median survival time for mice that received aerosolized voriconazole (>12 days) was also significantly longer than those that received control and amphotericin B deoxycholate, 7.5 and 7 days, respectively ($p < 0.01$).

3.3.2. Pulmonary Fungal Burden

Although survival was improved in animals that received aerosolized voriconazole, this benefit could not be explained by reductions in pulmonary fungal burden. Analysis of lung fungal burden demonstrated no significant differences between the treatment groups as measured by both CFU enumeration or qPCR tests (Table 3.1). The median lung fungal burden 1 hour post-inoculation was 3.99 $\log_{10}$ CFU/g. For all evaluated lungs, including mice from days 8 and 12 post-inoculation, median fungal burden values were 4.43 $\log_{10}$ CFU/g for controls, 4.14 $\log_{10}$ CFU/g for animals that received aerosolized voriconazole, and 4.33 $\log_{10}$ CFU/g for those that received amphotericin B. Similar results were also observed when measured by quantitative PCR. Conidial equivalent values were higher than CFU counts, however, no significant differences in tissue fungal burden were observed with either assay.

3.3.3. Histopathology

Although no differences in tissue fungal burden were observed, marked differences in lung histopathology were found among the different treatment groups (Figure 3.2). Animals that received control (aerosolized sulfobutyl ether-β-cyclodextrin sodium) or amphotericin B were noted to have more severe invasive disease and gross abnormalities within the lungs compared to those that received aerosolized voriconazole. Specifically, lungs from control and amphotericin B animals had more necrotic lesions within the small airways, including epithelial disruption, congestion, necrosis, angioinvasion, and vascular lesions on day 8 post-inoculation. The extent of pulmonary lesions in mice that received amphotericin B was more variable than those that received control or aerosolized voriconazole indicating inconsistent *in vivo* activity. Mice that received aerosolized voriconazole had fewer signs of invasive disease and markedly improved histological findings at this time point. Similar findings among the antifungal groups were also noted on day 12 post-inoculation, supporting the survival results demonstrating that the protective effects of voriconazole were not lost once therapy was discontinued. The differences in histopathology between voriconazole and control could not be explained by the use of sulfobutyl ether-β-cyclodextrin sodium as the control as this solubilization agent was aerosolized to both groups. In addition, preliminary safety studies have shown both aerosolized voriconazole and sulfobutyl ether-β-cyclodextrin sodium to be well-tolerated with no lung injury or inflammatory changes on histology in uninfected mice that have received twice daily dosing for multiple days (data not shown).

3.4. DISCUSSION

The lungs are the primarily site of inoculation and invasive disease in invasive pulmonary aspergillosis (33, 34). Invasive hyphal growth and subsequent angioinvasion

allow dissemination of the organism and invasive disease in other organs, which is associated with a poor prognosis and high mortality despite antifungal therapy in severly immunocompromised patients (35). Because of the high morbidity and mortality associated with invasive aspergillosis, and difficulties in establishing a diagnosis early in the course of disease, many centers utilize preventative strategies, including the administration of antifungal as prophylaxis in patients at high risk.

Antifungal prophylaxis has been shown in clinical trials to reduce the incidence of invasive fungal infections and improve survival. Two open-label studies have reported reductions in the incidence of invasive fungal infections in patients who received itraconazole prophylaxis compared to those who received fluconazole (9, 10). These differences were due to fewer invasive mould infections (primarily invasive aspergillosis) in those randomized to itraconazole. Recently, posaconazole has been shown to be effective in reducing the incidence of invasive fungal infections and invasive fungal infection-related mortality in patients receiving autologous or allogeneic stem cell transplants, and those with neutropenia secondary to chemotherapy (12, 13). Preliminary results from a study comparing voriconazole to fluconazole as prophylaxis in allogeneic blood and marrow transplant recipients also demonstrated a trend towards fewer invasive infections caused by *Aspergillus* species in those randomized to voriconazole (14). However, the systemic exposure of patients to antifungal agents is not without risks as patients are predisposed to the adverse effects and clinically significant drug interactions associated with these agents. The usefulness of orally administered itraconazole solution is hampered by significant gastrointestinal side effects, which were reported to occur in up to 25% of patients in the prophylaxis studies and were a major reason for high patient attrition rates (9, 10). In addition, oral formulations of itraconazole and posaconazole are

hampered by limited and erratic bioavailability that may limit prophylactic efficacy. A small subset analysis of allogeneic stem cell transplant patients who received posaconazole as antifungal prophylaxis reported lower plasma concentrations in patients who developed invasive fungal infections compared to those who did not (17). Similarly, variable pharmacokinetics and therapeutic failures with low plasma concentrations have been reported for voriconazole with systemic administration (36-38).

Targeted drug delivery to the lungs as antifungal prophylaxis is one strategy that is being investigated to overcome the obstacles associated with systemic administration of these agents. Animal studies have reported improved survival with aerosolized administration of amphotericin B deoxycholate and lipid formulations of this polyene (18, 19, 39). Inhaled amphotericin B has also been shown to be safe and effective as prophylaxis in lung transplant recipients and patients with hematologic malignancies (24), and this strategy has begun to gain favor in transplant centers (7, 23, 40-43). However, decreased *in vitro* activity and clinical failures have been reported with the use of amphotericin B against infections caused by non-*fumigatus Aspergillus* species, including *A. flavus* and *A. terreus* (44-46).

Previous studies by our group have demonstrated that aerosolized administration of azoles may be effective as prophylaxis against invasive pulmonary aspergillosis. In a non-neutropenic model of invasive pulmonary aspergillosis caused by *Aspergillus flavus*, a survival rates were significantly improved in mice administered inhaled suspensions of nanoparticulate crystalline and amorphous formulations of itraconazole compared to controls and orally administered itraconazole solution (47). Similarly, survival was also improved in mice administered nanoparticulate amorphous itraconazole compared to

controls in mice with invasive pulmonary aspergillosis caused by *A. fumigatus* with the same neutropenic model used in the current study (27).

In the current study, significant improvements in overall survival rates and median survival times were observed in mice that received inhaled aqueous voriconazole compared to controls and animals administered amphotericin B deoxycholate as treatment. In addition, marked improvements in lung histopathology were observed in animals the received inhaled voriconazole compared to the other therapy groups. These results suggest that aerosolized voriconazole may be an effective strategy for targeted delivery of antifungal prophylaxis to the primary site of infection of this opportunistic infection. These results are encouraging as we adapted the commercially available intravenous formulation with adjustments to ensure that the osmolality (293.2 mOsm/kg) and pH (6.4 to 6.8) were within physiologically acceptable ranges for pulmonary delivery (48).

These encouraging results for inhaled voriconazole are obfuscated by no significant decreases in pulmonary fungal burden measurements among any of the antifungal groups compared to controls. This lack of a reduction in pulmonary fungal burden has been observed in previous work by our and in other studies that have evaluated triazoles for invasive aspergillosis (21, 49, 50). As fungal burden was measured by colony-unit enumeration and quantitative PCR, it is unclear how well these methods differentiate between invasive hyphae versus ungerminated conidia colonizing the airways following inoculation within an aerosol chamber. However, the histopathology results observed in this study suggest that the improved survival may be attributed to reductions in invasive disease following aerosolized delivery of

voriconazole. Another limitation of our study is that the survival rates in mice that received amphotericin B deoxycholate as treatment were low. However, the survival results in this study are consistent with those previously reported after four days of treatment at the same dose (1 mg/kg/day) (51). Furthermore, survival rates have only reached 50% when the dose of amphotericin B deoxycholate has been increased to 3 mg/kg/day or high dose liposomal amphotercin B (10 mg/kg/day) has been used in this animal model (52). It is unclear how well amphotericin B formulations would perform if administered as prophylaxis or by aerosolization in this neutropenic murine model.

3.5. CONCLUSIONS

In conclusion, inhaled voriconazole was effective as prophylaxis in a neutropenic murine of invasive pulmonary aspergillosis. This was evident by improvements in survival and lung histopathology in mice administered voriconazole by aerosolization compared to controls and those treated with amphotericin B deoxycholate. Thus, inhaled voriconazole represents a potential improvement in antifungal prophylaxis. Additional studies are warranted to evaluate the efficacy and tolerability of inhaled voriconazole and further justify its therapeutic implementation.

3.6. ACKNOWLEDGEMENTS

3.7. REFERENCES

1.   Lin, S. J., J. Schranz, and S. M. Teutsch. 2001. Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis 32:358-66.

2.   Denning, D. W. 1998. Invasive aspergillosis. Clin Infect Dis 26:781-803; quiz 804-5.

3.   Baddley, J. W., T. P. Stroud, D. Salzman, and P. G. Pappas. 2001. Invasive mold infections in allogeneic bone marrow transplant recipients. Clin Infect Dis 32:1319-24.

4.   Marr, K. A., R. A. Carter, F. Crippa, A. Wald, and L. Corey. 2002. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 34:909-17.

5.   Slobbe, L., S. Polinder, J. K. Doorduijn, P. J. Lugtenburg, A. El Barzouhi, E. W. Steyerberg, and B. J. Rijnders. 2008. Outcome and Medical Costs of Patients with Invasive Aspergillosis and Acute Myelogenous Leukemia-Myelodysplastic Syndrome Treated with Intensive Chemotherapy: An Observational Study. Clin Infect Dis.

6.   Segal, B. H., N. G. Almyroudis, M. Battiwalla, R. Herbrecht, J. R. Perfect, T. J. Walsh, and J. R. Wingard. 2007. Prevention and early treatment of invasive fungal infection in patients with cancer and neutropenia and in stem cell transplant recipients in the era of newer broad-spectrum antifungal agents and diagnostic adjuncts. Clin Infect Dis 44:402-9.

7.   Dummer, J. S., N. Lazariashvilli, J. Barnes, M. Ninan, and P. Milstone Aaron. 2004. A survey of anti-fungal management in lung transplantation. J Heart Lung Transplant 23:1376-81.

8.   Playford, E. G., A. C. Webster, T. C. Sorrell, and J. C. Craig. 2006. Systematic review and meta-analysis of antifungal agents for preventing fungal infections in liver transplant recipients. Eur. J. Clin. Microbiol. 25:549-561.

9.   Marr, K. A., F. Crippa, W. Leisenring, M. Hoyle, M. Boeckh, S. A. Balajee, W. G. Nichols, B. Musher, and L. Corey. 2004. Itraconazole versus fluconazole for prevention of fungal infections in patients receiving allogeneic stem cell transplants. Blood 103:1527-33.

10.   Winston, D. J., R. T. Maziarz, P. H. Chandrasekar, H. M. Lazarus, M. Goldman, J. L. Blumer, G. J. Leitz, and M. C. Territo. 2003. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal

prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. A multicenter, randomized trial. Ann Intern Med 138:705-13.

11.   Goodman, J. L., D. J. Winston, R. A. Greenfield, P. H. Chandrasekar, B. Fox, H. Kaizer, R. K. Shadduck, T. C. Shea, P. Stiff, D. J. Friedman, and et al. 1992. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. N Engl J Med 326:845-51.

12.   Cornely, O. A., J. Maertens, D. J. Winston, J. Perfect, A. J. Ullmann, T. J. Walsh, D. Helfgott, J. Holowiecki, D. Stockelberg, Y. T. Goh, M. Petrini, C. Hardalo, R. Suresh, and D. Angulo-Gonzalez. 2007. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med 356:348-59.

13.   Ullmann, A. J., J. H. Lipton, D. H. Vesole, P. Chandrasekar, A. Langston, S. R. Tarantolo, H. Greinix, W. Morais de Azevedo, V. Reddy, N. Boparai, L. Pedicone, H. Patino, and S. Durrant. 2007. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med 356:335-47.

14.   Wingard, J. R., S. L. Carter, T. J. Walsh, J. Kurtzberg, T. N. Small, I. D. Gersten, A. M. Mendizabal, H. Leather, D. L. Confer, L. R. Baden, R. T. Maziarz, E. A. Stadtmauer, J. Bolanos-Meade, J. Brown, J. F. DiPersio, M. Boeckh, and K. A. Marr. 2007. Presented at the American Society of Hematology 49th Annual Meeting, Atlanta, GA, December 8-10.

15.   Groll, A. H., S. C. Piscitelli, and T. J. Walsh. 1998. Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. Adv Pharmacol 44:343-500.

16.   Walsh, T. J., I. Raad, T. F. Patterson, P. Chandrasekar, G. R. Donowitz, R. Graybill, R. E. Greene, R. Hachem, S. Hadley, R. Herbrecht, A. Langston, A. Louie, P. Ribaud, B. H. Segal, D. A. Stevens, J. A. van Burik, C. S. White, G. Corcoran, J. Gogate, G. Krishna, L. Pedicone, C. Hardalo, and J. R. Perfect. 2007. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. Clin Infect Dis 44:2-12.

17.   Krishna, G., M. Martinho, P. Chandrasekar, A. J. Ullmann, and H. Patino. 2007. Pharmacokinetics of oral posaconazole in allogeneic hematopoietic stem cell transplant recipients with graft-versus-host disease. Pharmacotherapy 27:1627-36.

18.   Ruijgrok, E. J., A. G. Vulto, and E. W. Van Etten. 2001. Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromised rats. J Antimicrob Chemother 48:89-95.

19.   Ruijgrok, E. J., A. G. Vulto, and E. W. Van Etten. 2000. Aerosol delivery of amphotericin B desoxycholate (Fungizone) and liposomal amphotericin B (AmBisome): aerosol characteristics and in-vivo amphotericin B deposition in rats. J Pharm Pharmacol 52:619-27.

20.   Hoeben, B. J., D. S. Burgess, J. T. McConville, L. K. Najvar, R. L. Talbert, J. I. Peters, N. P. Wiederhold, B. L. Frei, J. R. Graybill, R. Bocanegra, K. A. Overhoff, P. Sinswat, K. P. Johnston, and R. O. Williams, 3rd. 2006. In vivo efficacy of aerosolized nanostructured itraconazole formulations for prevention of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 50:1552-4.

21.   Alvarez, C. A., N. P. Wiederhold, J. T. McConville, J. I. Peters, L. K. Najvar, J. R. Graybill, J. J. Coalson, R. L. Talbert, D. S. Burgess, R. Bocanegra, K. P. Johnston, and R. O. Williams, 3rd. 2007. Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis. J Infect 55:68-74.

22.   Dummer, J. S., N. Lazariashvilli, J. Barnes, M. Ninan, and A. P. Milstone. 2004. A survey of anti-fungal management in lung transplantation. J Heart Lung Transplant 23:1376-81.

23.   Alexander, B. D., E. S. Dodds Ashley, R. M. Addison, J. A. Alspaugh, N. J. Chao, and J. R. Perfect. 2006. Non-comparative evaluation of the safety of aerosolized amphotericin B lipid complex in patients undergoing allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis 8:13-20.

24.   Rijnders, B. J., J. J. Cornelissen, L. Slobbe, M. J. Becker, J. K. Doorduijn, W. C. Hop, E. J. Ruijgrok, B. Lowenberg, A. Vulto, P. J. Lugtenburg, and S. de Marie. 2008. Aerosolized liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis during prolonged neutropenia: a randomized, placebo-controlled trial. Clin Infect Dis 46:1401-8.

25.   Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J. W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke, and B. de Pauw. 2002. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 347:408-15.

26.   Sheppard, D. C., G. Rieg, L. Y. Chiang, S. G. Filler, J. E. Edwards, Jr., and A. S. Ibrahim. 2004. Novel inhalational murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 48:1908-11.

27.   Alvarez, C., A., N. P. Wiederhold, J. T. McConville, J. I. Peters, L. K. Najvar, J. R. Graybill, J. J. Coalson, R. L. Talbert, D. S. Burgess, R. Bocanegra, K. P. Johnston, and R. O. Williams, 3rd. 2007. Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis. J. Infect. 55:68-74.

28. NAS. 1996. Guide for the Care and Use of Laboratory Animals National Academy Press, Washington DC.

29. Sheppard, D. C., G. Rieg, L. Y. Chiang, S. G. Filler, J. E. Edwards, Jr., and A. S. Ibrahim. 2004. Novel inhalational murine model of invasive pulmonary Aspergillosis. Antimicrob. Agents Chemother. 48:1908-1911.

30. Bowman, J. C., G. K. Abruzzo, J. W. Anderson, A. M. Flattery, C. J. Gill, V. B. Pikounis, D. M. Schmatz, P. A. Liberator, and C. M. Douglas. 2001. Quantitative PCR assay to measure Aspergillus fumigatus burden in a murine model of disseminated aspergillosis: demonstration of efficacy of caspofungin acetate. Antimicrob. Agents Chemother. 45:3474-3481.

31. Wiederhold, N. P., V. H. Tam, J. Chi, R. A. Prince, D. P. Kontoyiannis, and R. E. Lewis. 2006. Pharmacodynamic activity of amphotericin B deoxycholate is associated with peak plasma concentrations in a neutropenic murine model of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. 50:469-473.

32. Costa, C., D. Vidaud, M. Olivi, E. Bart-Delabesse, M. Vidaud, and S. Bretagne. 2001. Development of two real-time quantitative TaqMan PCR assays to detect circulating Aspergillus fumigatus DNA in serum. Journal of Microbiological Methods 44:263-269.

33. Kauffman, C. A. 2006. Fungal infections. Proceedings of the American Thoracic Society 3:35-40.

34. Maschmeyer, G., A. Haas, and O. A. Cornely. 2007. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs 67:1567-1601.

35. Lin, S. J., J. Schranz, and S. M. Teutsch. 2001. Aspergillosis case-fatality rate: systematic review of the literature. Clin. Infect. Dis. 32:358-66.

36. Trifilio, S., G. Pennick, J. Pi, J. Zook, M. Golf, K. Kaniecki, S. Singhal, S. Williams, J. Winter, M. Tallman, L. Gordon, O. Frankfurt, A. Evens, and J. Mehta. 2007. Monitoring plasma voriconazole levels may be necessary to avoid subtherapeutic levels in hematopoietic stem cell transplant recipients. Cancer 109:1532-5.

37. Trifilio, S., R. Ortiz, G. Pennick, A. Verma, J. Pi, V. Stosor, T. Zembower, and J. Mehta. 2005. Voriconazole therapeutic drug monitoring in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant 35:509-13.

38.   Pascual, A., T. Calandra, S. Bolay, T. Buclin, J. Bille, and O. Marchetti. 2008. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. Clin Infect Dis 46:201-11.

39.   Gavalda, J., M. T. Martin, P. Lopez, X. Gomis, J. L. Ramirez, D. Rodriguez, O. Len, Y. Puigfel, I. Ruiz, and A. Pahissa. 2005. Efficacy of nebulized liposomal amphotericin B in treatment of experimental pulmonary aspergillosis. Antimicrob Agents Chemother 49:3028-30.

40.   Perfect, J. R., E. Dodds, E. D. Ashley, and R. Drew. 2004. Design of aerosolized amphotericin B formulations for prophylaxis trials among lung transplant recipients. Clin. Infect. Dis. 39:S207-S210.

41.   Monforte, V., A. Roman, J. Gavalda, R. Lopez, L. Pou, M. Simo, S. Aguade, B. Soriano, C. Bravo, and F. Morell. 2003. Nebulized amphotericin B concentration and distribution in the respiratory tract of lung transplanted patients. Transplantation 75:1571-1574.

42.   Drew, R. H., E. Dodds Ashley, D. K. Benjamin, Jr., R. D. Davis, S. M. Palmer, and J. R. Perfect. 2004. Comparative safety of amphotericin B lipid complex and amphotericin B deoxycholate as aerosolized antifungal prophylaxis in lung-transplant recipients. Transplantation 77:232-237.

43.   Corcoran, T. E., R. Venkataramanan, K. M. Mihelc, A. L. Marcinkowski, J. Ou, B. M. McCook, L. Weber, M. E. Carey, D. L. Paterson, J. M. Pilewski, K. R. McCurry, and S. Husain. 2006. Aerosol deposition of lipid complex amphotericin-B (Abelcet) in lung transplant recipients. Am. J. Transplant. 6:2765-2773.

44.   Chamilos, G., M. Luna, R. E. Lewis, G. P. Bodey, R. Chemaly, J. J. Tarrand, A. Safdar, Raad, II, and D. P. Kontoyiannis. 2006. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003). Haematologica 91:986-9.

45.   Steinbach, W. J., D. K. Benjamin, Jr., D. P. Kontoyiannis, J. R. Perfect, I. Lutsar, K. A. Marr, M. S. Lionakis, H. A. Torres, H. Jafri, and T. J. Walsh. 2004. Infections due to Aspergillus terreus: a multicenter retrospective analysis of 83 cases. Clin Infect Dis 39:192-8.

46.   Paterson, P. J., S. Seaton, H. G. Prentice, and C. C. Kibbler. 2003. Treatment failure in invasive aspergillosis: susceptibility of deep tissue isolates following treatment with amphotericin B. J Antimicrob Chemother 52:873-6.

47.   Hoeben, B. J., D. S. Burgess, J. T. McConville, L. K. Najvar, R. L. Talbert, J. I. Peters, N. P. Wiederhold, B. L. Frei, J. R. Graybill, R. Bocanegra, K. A. Overhoff, P. Sinswat, K. P. Johnston, and R. O. Williams, III. 2006. In vivo

efficacy of aerosolized nanostructured itraconazole formulations for prevention of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. 50:1552-1554.

48.   Tolman, J. A., N. A. Nelson, Y. J. Son, S. Bosselmann, N. P. Wiederhold, J. T. McConville, and O. Williams Robert, 3rd. 2008. Aerosol Characterization and Single Dose Pharmacokinetic Analysis of a Nebulized Voriconazole Solution, American Association of Pharmaceutical Scientists Annual Meeting, Atlanta, GA.

49.   MacCallum, D. M., J. A. Whyte, and F. C. Odds. 2005. Efficacy of caspofungin and voriconazole combinations in experimental aspergillosis. Antimicrob Agents Chemother 49:3697-701.

50.   Petraitis, V., R. Petraitiene, A. A. Sarafandi, A. M. Kelaher, C. A. Lyman, H. E. Casler, T. Sein, A. H. Groll, J. Bacher, N. A. Avila, and T. J. Walsh. 2003. Combination therapy in treatment of experimental pulmonary aspergillosis: synergistic interaction between an antifungal triazole and an echinocandin. J Infect Dis 187:1834-43.

51.   Wiederhold, N. P., V. H. Tam, J. Chi, R. A. Prince, D. P. Kontoyiannis, and R. E. Lewis. 2006. Pharmacodynamic activity of amphotericin B deoxycholate is associated with peak plasma concentrations in a neutropenic murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 50:469-73.

52.   Wiederhold, N. P., L. K. Najvar, A. C. Vallor, W. R. Kirkpatrick, R. Bocanegra, D. Molina, M. Olivo, J. R. Graybill, and T. F. Patterson. 2008. Assessment of serum (1->3)-beta-D-glucan concentration as a measure of disease burden in a murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 52:1176-8.

Chapter 4: Dose Tolerability of Chronically Inhaled Voriconazole Solution in Rodents

4.1. INTRODUCTION

Invasive pulmonary aspergillosis (IPA) is an opportunistic infection that primarily affects immuncompromised individuals. The populations with the highest infection rates include patients with hematologic malignancies, hematopoietic stem cell transplant recipients, and those undergoing solid organ transplantation (1, 2). The causative epidemiology of IPA is also changing to more serious Aspergillus spp. with mortality rates up to 75% in some cases and substantial healthcare costs per case (3-6). The serious effects of IPA have prompted investigations to improve therapeutic options for this disease (7, 8).

In 2002, Herbrecht and colleagues published the results from a multi-center randomized controlled clinical trial that established voriconazole as the first-line therapeutic option in the treatment of IPA (9, 10). The study compared the then standard of care, intravenous (IV) amphotericin B deoxycholate, with intravenous voriconazole with the option to switch patients to oral (PO) voriconazole. The authors reported 12 week survival rates of 70.8% and 59.7% for voriconazole and amphotericin B treated patients, respectively. Although this study changed the treatment paradigm for IPA, the mortality rates remain high, prompting researchers to investigate different treatment modalities and approaches to the therapeutic management of IPA (4, 6). Targeted antifungal delivery to the lung was theorized to cause high drug concentrations at the primary site of infection leading to increased efficacy and better patient outcomes but

with lower systemic exposure and subsequent decreased rates of adverse events associated with the antifungal agent.

One of the promising therapeutic approaches included the prophylactic inhalation of antifungals, including aerosolized amphotericin B formulations, in patients at high risk for IPA (11-14). Although no product is approved for inhalation therapy, the various formulations of amphotericin B have been investigated by nebulization, including the deoxycholate (Fungizone®), lipid complex (Abelcet®), or liposomal (Ambisome®) forms. These different formulations, nebulizers, and regimens have led to inconsistent reports of prophylactic efficacy and patient tolerability leading to no unified standard for aerosolized amphotericin B prophylaxis (15). Typically, systemically administered amphotericin B has severe dose limiting adverse effects, including electrolyte abnormalities, nephrotoxicity, and infusion-related toxicities (16). Inhaled amphotericin B has low incidence of these systemic events due to extensive retention of the drug in the lung tissue with very little distribution to the systemic circulation (12, 17). Adverse events associated with inhaled amphotericin B include nausea and vomiting, dysgeusia and taste perversion, dysphagia, coughing and bronchospasm, and decreases in respiratory function with lower incidence rates associated with lipid formulations of amphotericin B (18). Discontinuation of amphotericin B prophylaxis due to poor patient tolerability has been reported in up to 25% of patients receiving the deoxycholate formulation with lower rates for the lipid formulations.

Pre-clinical testing of aerosolized itraconazole in animal models have been investigated and reviewed in efforts to reduce complications and inconsistencies associated with inhaled amphotericin B (8, 19-21). These researchers administered nano-

suspensions of engineered crystalline and amorphous itraconazole particles to mice to establish pharmacokinetic and efficacy profiles for inhaled nanoparticulate itraconazole. Itraconazole was retained in lung tissue for several hours with very little drug distributing to the blood after administration with favorable pharmacokinetic parameters (21-23). In addition, 12-day survival rates in mice infected with A. flavus and A. fumigatus treated daily with inhalations of itraconazole were significantly better than a positive control and were 50-80% and 35%, respectively (24, 25). Inhaled nanoparticulate itraconazole was also well tolerated by evaluation of histopathological lung tissue sections (26).

Although nanoparticulate itraconazole suspensions had encouraging results, voriconazole is the first-line therapeutic agent for the treatment of IPA due to improved survival benefits and with an expanded antifungal activity compared to itraconazole (27). Voriconazole is generally well tolerated with major adverse events that include abnormal vision, fever and chills, rash, nausea and vomiting, and headache (28). Hepatotoxicity as measured by elevations in liver function tests, have also been correlated with peak plasma concentrations (29). Systemic administration of voriconazole has led to variable pharmacokinetic properties and poor lung tissue distribution resulting in-part from non-linear pharmacokinetics in adults resultant from saturable metabolism (28, 30, 31). Through targeted drug delivery to the lungs, inhaled voriconazole could offer higher tissue concentrations than possible following systemic drug delivery and lead to improvements over both inhaled amphotericin B and itraconazole as prophylaxis against IPA.

The IV form of voriconazole, a powder for injection (Vfend® IV), contains voriconazole with sodium sulfobutyl ether-β-cyclodextrin in an inclusion complex to

improve the aqueous solubility of the poorly water soluble active agent. High lung tissue and plasma concentrations with a lung tissue to plasma concentration ratio of 1.4 to 1 were observed in a single and multiple dose pharmacokinetic study of inhaled voriconazole in mice (32). In addition, this inhaled formulation of voriconazole as prophylaxis resulted in improved survival in a murine model of IPA (33). In these studies, phenotypic evaluation of murine behavior and outward appearance suggested inhaled voriconazole was well tolerated. The purpose of this study was to perform a more thorough analysis was performed with inhaled voriconazole administered twice daily (BID) to assess for hepatic, electrolyte, renal, or erythrocyte or histiocyte abnormalities as suggested in the prescribing information for Vfend® IV (34). Organs were also evaluated for histopathological changes at the site of administration and absorption as well as metabolism and elimination. It is hypothesized that inhaled voriconazole is well tolerated and comparable to a negative inhaled control group.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Vfend® IV (Pfizer Inc., New York, NY, USA), voriconazole, and sulfobutyl ether-β-cyclodextrin, Captisol® were generously supplied by CyDex Pharmaceuticals, Inc. (Lenexa, KS). The following items were purchased from the respective suppliers: sterile water for injection (SWFI) and normal saline from Cardinal Health (Dublin, OH); sodium tetraborate decahydrate, boric acid, sodium acetate trihydrate, and neutral buffered formalin 10% solution from Sigma-Aldrich, Inc. (St. Louis, MO); acetic acid from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany); heparin sodium injection, USP from Baxter Healthcare Corporation (Deerfield, IL); Lavender-topped

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(LT) BD Microtainer® Tubes with K2E (K2EDTA) and Red-topped (RT) BD Microtainer® No additive Tubes from Becton, Dickinson and Company (Franklin Lakes, NJ); and HPLC grade solvents including ethyl acetate from Spectrum Chemical Manuf. Corp. (Gardena, CA); acetonitrile from Fisher Scientific (Fair Lawn, NJ); and methanol from EMD Chemicals Inc. (Gibbstown, NJ).

4.2.2. Study Design

Male and female Sprague-Dawley rats, Harlan Sprague Dawley, Inc. (Indianapolis, IN), with an average mass of 250 g at the beginning of the study, were caged separately with free access to food and water. Prior to dosing, animals that would receive any treatment were acclimatized for up to 20 minutes twice daily over 3 days to rodent nose-only restraint systems (Battelle Toxicology Northwest, Richland, WA). All animals were handled and maintained in accordance with The University of Texas at Austin Institution Animal Care and Use Committee (IACUC) guidelines and in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines.

An isotonic drug solution was prepared with Vfend® IV reconstituted and diluted with SWFI to a voriconazole concentration of 6.25 mg/mL as described previously (32). For dose tolerability analysis, 45 male and 45 female rats were randomly divided into three treatment groups: High-dose (HD) that received 5 mL of drug solution nebulized over 20 minutes; Low-dose (LD) that received 2.5 mL of drug solution nebulized over 20 minutes; and Control (C) that received 5 mL of normal saline over 20 minutes. Another group of 20 rats, designated No Treatment (NT), received no treatment but were housed with free access to food and water and used for establishment of normal

laboratory value ranges for statistical comparison. A separate group of 8 male rats was used for pharmacokinetic evaluation of peak and trough voriconazole concentrations in lung tissue and plasma on day 3. Treatments were administered using an in-line Aeroneb® Pro micro pump nebulizer from Aerogen, Inc. (Mountain View, CA) with a nose-only dosing chamber with an air-flow rate of 1 L/min through the chamber. Treatments lasted for up to 20 minutes and all solutions were nebulized to dryness. Treatments were administered BID beginning at 08:00 and 16:00 and continued for up to 21 days with no additional treatments through day 28. Animals were sacrificed on days 7, 14, 21, and 28 by isoflurane narcosis. NT animals were euthanized on day 28 after having received no treatments by isoflurane narcosis. Animals designated for pharmacokinetic analysis on day 3 were euthanized by CO_2 narcosis. Euthanasia was ensured by desanguination via cardiac puncture followed by thoracotomy.

4.2.3. Plasma and Tissue Extraction for Pharmacokinetic Analysis

On day 3, whole blood collected via cardiac puncture was placed into heparinized vials and centrifuged at 9000 RPM for 15 minutes using a Microfuge® 18 Microcentrifuge (Beckman Coulter, Fullerton, CA). Plasma was collected into clean vials and frozen at -20°C until analysis. Lungs were excised and cleaned from external lymphatic, connective, and airway tissue, placed in a clean vial, and frozen at -20°C until analysis.

Calibration standards, plasma, and homogenized lung samples were analyzed using similar methods to those previously published (32, 35, 36). Briefly, voriconazole was extracted from plasma samples through the addition of acetonitrile, centrifugation, and supernatant extraction with fluconazole as an internal standard. The supernatant liquid was evaporated under a gentle stream of nitrogen and residual solids, including

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voriconazole, were re-dispersed with mobile phase and analyzed spectrophotometrically. For lung analysis, lung tissue was homogenized with 2 mL of normal saline per gram wet lung weight, 0.2M borate buffer (pH 9.0) was added, followed by three liquid-phase extractions with ethyl acetate. The liquid from collected supernatant fractions were then evaporated under a gentle stream of nitrogen. Any residual solids, including voriconazole, were reconstituted in 200 µl of acetonitrile and centrifuged. A 4000 Q TRAP mass spectrometer (Applied Biosystems, Foster City, CA) coupled with an online HPLC (Shimadzu, Columbia, MD) was used to analyze the samples. 10 µl samples were injected into Shimadzu Prominence UFLC system equipped with an Restek C18

(4.6 x 50mm, 5 µm, 110 Å) column and eluted with mobile phase A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid) by a gradient of 20%B for

0.5 min, then 20%-50%B over 0.5 min, followed by 50%B for 3 min, at a flow rate of

1 ml/min. Sample was directly eluted from the column into the electrospray ion (ESI) source of 4000 QTRAP.   The heated nebulizer of 4000 QTRAP was set at 700°C, the declustering potential (DP) at 40. The Multiple Reaction Monitoring (MRM) scan experiment with unit resolution for Q1 and low resolution for Q3 was used to quantify voriconazole content. The MRM transitions were set as follow, Q1=350.2, Q3=127.4, CE=40. The limit of detection for voriconazole was 5 pg.

4.2.4. Blood and Tissue Processing and Testing

Following euthanasia and cardiac puncture for HD, LD, C, and NT groups, whole blood was collected into lavender and red-topped sample tubes. Lavender tubes were gently inverted 10 times and stored under refrigeration until a complete blood count with differential (CBC w/ dif.) blood test could be performed within 24 hours from sample collection (IDEXX Laboratories, Inc., Westbrook, ME). Whole blood in red topped

tubes was allowed to coagulate and centrifuged at 3000 RPM for 15 minutes with a Microfuge® 18 Microcentrifuge. Serum was collected into clean vials and stored at 4°C until serum chemistry analysis could be performed within 24 hours from sample collection by IDEXX Laboratories. Whole blood was also collected into heparinized tubes and centrifuged at 9000 RPM for 15 minutes. On days 14 and 21, excess whole blood was collected into heparinized vials and processed in the same manner as described previously for trough voriconazole plasma concentration determination. Serum chemistry samples had the following individual tests performed per sample: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin, total protein, globulin, blood urea nitrogen (BUN), creatinine, cholesterol, glucose, calcium, phosphorous, chloride, potassium, and sodium. The CBC w/diff test included analysis of the following parameters: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), and platelet count.

Following thoracotomy, lungs, liver, kidneys, and spleen were removed from each animal. Prior to preserving in formalin, the lungs were inflated with 10% buffered formalin and tied shut with silk suture, the liver was dissected into three parts, and the kidneys were longitudinally bisected. Following processing, tissues were preserved separately in 40-50mL of 10% buffered formalin for at least 96 hours prior to fixing and sectioning. The post-dissection processing steps ensured penetration of the preservative into the tissues. All tissues were randomly assigned sequential numbers and shipped to IDEXX Laboratories, Inc. for fixing, sectioning, and staining. Pathologic evaluation was independently performed in a blinded manner by both an IDEXX pathologist and a pulmonary pathologist.

4.2.5. Quantitative Analysis of Lung Tissue

Lung tissue slides were digitally scanned at 20x magnification using a ScanScope® CS (Aperio Technologies, Inc., Vista, CA), and analyzed using ImageScope software (Aperio Technologies, Inc., Vista, CA). The Cimolai histopathologic inflammatory score was used by a blinded pulmonary pathologist to assess signs of inflammation in lung airways (37). In this scoring scheme, the histologic response in the lung was graded based on the degree and location of inflammation with possible scores ranging from 0 to 26, least to most severe immunologic response. A second quantative pathological evaluation of lung tissues was developed through assessment of a novel marker of immune response in the lungs, the Respiratory Bronchiole Index (RBI). Inhalation injury occurs at the respiratory bronciole-alveolar interface due to inhalation of particulate and solubilized irritants (38, 39). Therefore, ten randomly chosen respiratory ducts, the site of transition from the respiratory bronchiole to the alveolar space, were marked on the digitized images of all lung sections where two blinded investigators independently counted the number of pulmonary macrophages present at respiratory bronchiole alveolar ducts sites per slide under 20x magnification. The individual scores were then normalized and averaged between evaluators to provide the RBI. Validation of the RBI was not performed against tissues with known or controlled alveolar macrophage responses, but values were instead used to statistically compare lung tissue between treatment groups.

4.2.6. Statistical Analysis

Statistical analysis of results was performed using JMP®-7 (e-academy Inc., Ottawa, Ontario, Canada). Results from blood work were evaluated by analysis of variance (ANOVA) with Dunnett's Control test for the NT group with a p-value ≤0.05

for significance. RBI scores were also compared between treatment groups using JMP®-7 by ANOVA with a p-value ≤0.05 for significance.

4.3. RESULTS

4.3.1. Pharmacokinetics

Peak plasma samples were collected 30 minutes after completion of nebulization due to previous pharmacokinetic findings in mice (32). Following BID dosing for 3 days, peak voriconazole concentrations in lung tissue were 0.85 ± 0.63 µg/g wet lung weight for the high dose group and 0.37 ± 0.01 µg/g wet lung weight for the low dose group with corresponding peak plasma concentrations of 0.58 ± 0.30 and 0.09 ± 0.06 µg/mL, respectively (Table 1). Trough voriconazole concentrations taken on day 3 immediately prior to the 08:00 dose were likewise 0.042 ± 0.002 and 0.044 ± 0.004 µg/g wet lung weight in lung tissue for high and low dose groups and 0.01 ± 0.004 and

0.010 ± 0.003 µg/mL in corresponding plasma samples. After additional days of dosing, trough plasma samples were 0.02 and 0.03 µg/mL for high and low dose groups on day 14 and remained consistent with day 21 trough concentrations of 0.03 ± 0.01 and

0.03 ± 0.003 µg/mL for high and low dose groups.

4.3.2. Serum Chemistries and Complete Blood Counts

When all laboratory values were analyzed by treatment groups, statistically significant increases were observed for components of the hepatic function test compared to the NT group, including ALP, albumin, and total bilirubin (Table 2A-D). However, these increases were observed for both inhaled normal saline control and active treatment groups and would not be deemed clinically significant. Similar patterns of statistical

significance but clinical irrelevance were found for decreases in gamma globulin, BUN, phosphorous, and WBC as well as with elevations in calcium, phosphorous, sodium, chloride, and platelet count. Sub-group evaluation by day of analysis and animal gender provided similar patterns of sporadic statistical but not clinical significance with the absence of trends, except for consistent decreases in RBC count and corresponding drops in Hct from day 21 to day 28 for C, LD, and HD treatment groups (data not shown).

4.3.3. Histopathology

Qualitative pathological descriptions of liver, kidney, and spleen sections suggested that inhalation of high or low dose voriconazole produced no histological differences as compared to inhaled normal saline control (data not shown). However, pathological descriptions of lung tissue suggested inhaled voriconazole promoted an increase in alveolar and respiratory duct macrophages. However, no inflammatory cell infiltrate of neutrophils, eosinophils or lymphoctyes were noted. The lungs were also absent of pathological changes indicative of ulceration of the airway, interstitial changes, or edema (Figure 2). Application of the Cimolai scoring system on lung tissues failed to differentiate between treatment groups with scores of 0 to 1, on a 0 to 26 scale. Therefore, quantative analysis of lung histiocytosis was performed through use of the RBI, which demonstrated significant elevations for both LD and HD groups compared to C group animals for days 7, 14, 21, and 28. In addition, RBI values between LD and HD were not statistically different (p-value >0.05).

4.4. DISCUSSION

The poor prognosis of IPA has prompted investigation of targeted antifungal delivery to the lungs via nebulization. Positive results following initial animal experimentation with inhaled amphotericin B led investigators to early clinical trials with varying results (40, 41). Although initial clinical utilization of inhaled amphotericin B were generally well tolerated with acceptable lung physiological changes given the severity of IPA, large numbers of patients experienced therapy limiting cough, dyspnea, and/or nausea and vomiting (42, 43). Animal studies also suggested positive clinical outcomes could exist for aerosolized suspension of nano-structured itraconazole (24-26). Both aerosolized amphotericin B and itraconazole reported high lung tissue concentrations with very low systemic drug distribution following inhalation. In one study, high tissue concentrations but low systemic drug distribution following targeted antifungal delivery was supplemented with systemic administration of the antifungal and suggested improved survival as treatment in a rodent model of IPA (44). The contributory effect of an inhaled and targeted antifungal with systemic drug distribution could improve survival through prevention of fungal dissemination from alveolar spaces into the pulmonary capillaries as observed following inhalation only (45).

Correlations have been suggested between plasma drug concentrations and adverse events as well as efficacy against IPA for voriconazole in humans (35, 46). However, substantial intra-patient as well as intra-species variability in absorption, distribution, and metabolism associated with oral and intravenous voriconazole limit the utility of human-defined peak or trough voriconazole concentrations associated with efficacy and toxicity with the pharmacokinetic findings in this study (47-50). Thus, the dose of inhaled voriconazole used in the HD group, equivalent to a 31.25 mg exposure

dose, was efficacious in an immunosuppressed murine model of IPA with 67% survival over 12 days. Although voriconazole concentrations were not assessed in infected animals, peak and trough plasma concentrations following multiple doses in healthy mice were 2.32 and 0.28 μg/mL, respectively, that were lower than those associated with efficacy in humans (51). These same healthy mice had peak lung concentrations of 6.73 μg/g wet lung weight following multiple doses which likely contributed to improved murine survival (32, 33). In the present study, HD group rats had an equivalent peak tissue to plasma concentration ratio, 1.5 to 1, following 3 days of BID dosing while a higher ratio was observed in LD group rats, 4.1 to 1, with corresponding trough concentration ratios of 3.8 to 1 and 4.0 to 1. These varied partition factors based on concentration ratios between tissue and plasma are likely due to altered voriconazole metabolism as demonstrated by induction of voriconazole metabolism following oral and IV doses in rodents (49, 52). Nevertheless, moderate trough plasma voriconazole accumulation was observed following BID dosing for up to 21 days, necessitating further pharmacokinetic analysis of voriconazole in lung tissue and plasma following inhalation to verify metabolic induction-based pharmacokinetic variations.

Despite possible changes in voriconazole pharmacokinetics following multiple inhalations in a rodent model, drug distribution to the plasma was markedly improved compared to reports of drug distribution following inhalation of amphotericin B and itraconazole in the literature (21, 23, 25, 53, 54). The relative elevation of voriconazole plasma drug concentrations were likely a result of improved drug solubility due to the cyclodextrin in the formulation leading to improved drug absorption in the lungs (55-58). Although the toxicological profile of inhaled sulfobutyl ether-β-cyclodextrin has not been publicly disclosed, limited data with similar cyclodextrin agents have been shown to be

safe and well tolerated following inhalation (59, 60). Specifically, Evrard et al. demonstrated inhaled cyclodextrins were well tolerated through evaluation of pulmonary epithelium for histological signs of inflammation, morphology and cellular distribution of histiocytes in bronchoalveolar lavage fluid (BAL), bronchial hyperresponsiveness, kidney histology, and BUN values. Thus, the differences between treatment and control groups likely represent responses to inhaled voriconazole and not the cyclodextrin.

Elevated hepatic function values (Table 2A) were observed in all treatment groups and represent artifacts resulting from the method of euthanasia and duration of hypoxia prior to cardiac puncture and blood sampling (61, 62). In addition, observed hyperglycemia (Table 2C), although not statistically significant between NT and treatment groups (HD, LD, and C groups), may be linked to stress imposed through chronic handling and manipulation during animal dosing as well as the use of isoflurane in euthanasia (63-65). Numerous laboratory test values were statistically significant between NT and treatment groups but without clinical significance either due to comparable differences between C and LD or HD groups; e.g. ALP, albumin, bilirubin, globulin, sodium (Table 2A-C); or isolated differences in one group without evidence of trends between groups or throughout treatment; e.g. BUN, creatinine, calcium, phosphorous, WBC, Hgb, and platelet count (Table 2B-D).

Qualitative pathological evaluation of liver, kidney, and spleen tissue samples demonstrated no difference between treatment groups as well as no differences for sub-group analysis based on sample day or gender between HD or LD groups and the control group. Pathologist descriptions of lung tissues, however, suggested inhaled voriconazole high dose and low dose groups could have increased frequency of histiocytes in the

respiratory ducts compared to the inhaled normal saline control group. However, no histologic changes in the airway or interstitial space were noted. Additionally, no inflammatory response (e.g. migration of neutrophils, eosinophils, or lymphocytes) was seen at any time point in either the C or HD and LD groups. Thus, pathologic description of the lungs following inhaled voriconazole suggest isolated elevations in alveolar macrophages that would resolve following cessation of therapy (20, 66).

Quantative evaluation of lung tissue inflammation through the Cimolai scoring system, as previously utilized (26), was not able to differentiate between treatment groups with values of 0 for all groups. Specifically, voriconazole treated lungs had no signs of airway inflammation, peri-vascular inflammation, or signs of pneumonia that are weighted heavily in the Cimolai Score. Therefore, the RBI, was developed to assess the presence of observed alveolar macrophages at some respiratory bronchiole-alveolar spaces. Alveolar macrophages, a non-specific first-line host defense response to inhaled foreign molecules at the respiratory duct, represented an objective comparison of histiocyte presence at the most-likely site for deep-lung tissue damage or irritation. Significant elevations in the RBI were observed for HD and LD groups compared to the control group for all days tested (Figure 1). No significant difference was observed between HD and LD groups. However, substantial inter- and intra-animal variability was observed in the RBI and could be due to regional differences in drug deposition following inhalation within the lung (Figure 2). Although not employed previously as a quantitative measure, the RBI should return to baseline levels after a longer recovery period due to a delay in alveolar macrophage elimination kinetics following a stimulus (67). Additional studies are needed to document the reversibility of RBI elevations after cessation of inhaled voriconazole.

4.5. CONCLUSIONS

Inhaled aqueous solutions of inhaled voriconazole were well tolerated following multiple doses in rats. The pharmacokinetic profile in rats following multiple inhalations demonstrated good drug absorption into the systemic circulation from the lungs due to the sulfobutyl ether-β-cyclodextrin present in the formulation. Some abnormal laboratory test values were statistically significant but did not correlate with clinically importance with hepatotoxicity and hyperglycemia being artifacts associated with methodological procedures in the study. Pathological evaluation of liver, kidney, and spleen tissues demonstrated good inhaled drug tolerability but with acute elevations in alveolar macrophages present at the respiratory duct associated with inhaled voriconazole. Additional studies are needed to further characterize pharmacokinetic and laboratory test parameters and lung histopathology changes before utilization in patient populations.

4.7. REFERENCES

1. Lin, S. J., J. Schranz, and S. M. Teutsch. 2001. Aspergillosis case-fatality rate: systematic review of the literature. Clin. Infect. Dis. 32:358-66.

2. Denning, D. W. 1998. Invasive aspergillosis. Clin. Infect. Dis. 26:781-803; quiz 804-5.

3. Maschmeyer, G. 2006. The changing epidemiology of invasive fungal infections: new threats. International Journal of Antimicrobial Agents 27:3-6.

4. Cornet, M., L. Fleury, C. Maslo, J. F. Bernard, and G. Brucker. 2002. Epidemiology of invasive aspergillosis in France: a six-year multicentric survey in the Greater Paris area. J. Hosp. Infect. 51:288-96.

5. Dixon, S., E. McKeen, M. Tabberer, and S. Paisley. 2004. Economic evaluations of treatments for systemic fungal infections: a systematic review of the literature. Pharmacoeconomics 22:421-33.

6. Maschmeyer, G., A. Haas, and O. A. Cornely. 2007. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs 67:1567-1601.

7. Patterson, T. F. 2005. Advances and challenges in management of invasive mycoses. Lancet 366:1013-25.

8. Yang, W., N. P. Wiederhold, and R. O. Williams, III. 2008. Drug delivery strategies for improved azole antifungal action. Expert Opin. Drug Delivery 5:1199-1216.

9. Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J.-W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke, B. de Pauw, R. Allen, M. Aoun, C. Aul, M. Bjorkholm, K. L. Blanchard, M. Boogaerts, E. Bouza, E. J. Bow, H. R. Brodt, J. Brown, D. Buchheidt, J. Y. Cahn, A. Calmaggi, J. M. Cisneros, C. Cordonnier, J. Daly, C. A. Da Cunha, R. De Bock, A. Del Favero, J. Diaz Mediavilla, M. C. Dignani, C. Doyen, J. S. Dummer, B. Dupont, M. Egyed, D. Engelhard, G. Faetkenheuer, R. Feld, D. Fiere, G. Fioritoni, G. Garber, Z. Gasztonyi, K. Godder, D. Graham, A. Gratwohl, R. Greenberg, K. High, F. Jacobs, V. Kremery, P. Kumar, W. Langer, M. Laverdiere, P. Ljungman, H. Lode, A. Louie, D. Maki, J. P. Marie, D. J. E. Marriott, D. S. McKinsey, R. Mertelsmann, M. K. Nair, N. Milpied, A. Nagler, D. Niederwieser, L. Pagano, P. Pappas, J. Perfect, J. Pottage, V. Raina, J. Reinhardt, S. Richardson, L. Rickman, M. Ruhnke, I. Salit, W. M. Scheld, S. Schuler, M. Schuster, R. Schwerdtfeger, S.

D. Shafran, B. Simmons, M. Slavin, M. Sokol-Anderson, P. Tebas, C. Tsoukas, A. Ullmann, J. Van Burik, J. W. Van't Wout, E. C. Vinaya Kumar, et al. 2002. Voriconazole versus amphotericin B for primary therapy on invasive aspergillosis. N. Engl. J. Med. 347:408-415.

10.   Walsh, T. J., E. J. Anaissie, D. W. Denning, R. Herbrecht, D. P. Kontoyiannis, K. A. Marr, V. A. Morrison, B. H. Segal, W. J. Steinbach, D. A. Stevens, J.-A. van Burik, J. R. Wingard, and T. F. Patterson. 2008. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin. Infect. Dis. 46:327-360.

11.   Schwartz, S., G. Behre, V. Heinemann, H. Wandt, E. Schilling, M. Arning, A. Trittin, W. V. Kern, O. Boenisch, D. Bosse, K. Lenz, W. D. Ludwig, W. Hiddemann, W. Siegert, and J. Beyer. 1999. Aerosolized amphotericin B inhalations as prophylaxis of invasive aspergillus infections during prolonged neutropenia: results of a prospective randomized multicenter trial. Blood 93:3654-3661.

12.   Mohammad, R. A., and K. C. Klein. 2006. Inhaled amphotericin B for prophylaxis against invasive Aspergillus infections. Ann. Pharmacother. 40:2148-2154.

13.   Playford, E. G., A. C. Webster, T. C. Sorrell, and J. C. Craig. 2006. Antifungal agents for preventing fungal infections in non-neutropenic critically ill and surgical patients: systematic review and meta-analysis of randomized clinical trials. J. Antimicrob. Chemother. 57:628-638.

14.   Playford, E. G., A. C. Webster, T. C. Sorrell, and J. C. Craig. 2006. Systematic review and meta-analysis of antifungal agents for preventing fungal infections in liver transplant recipients. Eur. J. Clin. Microbiol. 25:549-561.

15.   Dummer, J. S., N. Lazariashvilli, J. Barnes, M. Ninan, and P. Milstone Aaron. 2004. A survey of anti-fungal management in lung transplantation. J Heart Lung Transplant 23:1376-81.

16.   Cohen, B. E. 1998. Amphotericin B toxicity and lethality. Int. J. Pharm. 162:95-106.

17.   Monforte, V., A. Roman, J. Gavalda, R. Lopez, L. Pou, M. Simo, S. Aguade, B. Soriano, C. Bravo, and F. Morell. 2003. Nebulized amphotericin B concentration and distribution in the respiratory tract of lung transplanted patients. Transplantation 75:1571-1574.

18.   Drew, R. 2006. Potential role of aerosolized amphotericin B formulations in the prevention and adjunctive treatment of invasive fungal infections. International Journal of Antimicrobial Agents 27:36-44.

19.   Purvis, T., M. Vaughn Jason, L. Rogers True, X. Chen, A. Overhoff Kirk, P. Sinswat, J. Hu, T. McConville Jason, P. Johnston Keith, and O. Williams Robert, 3rd. 2006. Cryogenic liquids, nanoparticles, and microencapsulation. Int. J. Pharm. 324:43-50.

20.   Yang, W., J. I. Peters, and R. O. Williams, III. 2008. Inhaled nanoparticles. A current review. Int. J. Pharm. 356:239-247.

21.   Yang, W., J. Tam, D. A. Miller, J. Zhou, J. T. McConville, K. P. Johnston, and R. O. Williams. 2008. High bioavailability from nebulized itraconazole nanoparticle dispersions with biocompatible stabilizers. Int. J. Pharm. 361:177-188.

22.   McConville, J. T., K. A. Overhoff, P. Sinswat, J. M. Vaughn, B. L. Frei, D. S. Burgess, R. L. Talbert, J. I. Peters, K. P. Johnston, and R. O. Williams, III. 2006. Targeted High Lung Concentrations of Itraconazole Using Nebulized Dispersions in a Murine Model. Pharm. Res. 23:901-911.

23.   Vaughn, J. M., J. T. McConville, D. Burgess, J. I. Peters, K. P. Johnston, R. L. Talbert, and R. O. Williams, III. 2006. Single dose and multiple dose studies of itraconazole nanoparticles. Eur. J. Pharm. Biopharm. 63:95-102.

24.   Alvarez, C., A., N. P. Wiederhold, J. T. McConville, J. I. Peters, L. K. Najvar, J. R. Graybill, J. J. Coalson, R. L. Talbert, D. S. Burgess, R. Bocanegra, K. P. Johnston, and R. O. Williams, 3rd. 2007. Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis. J. Infect. 55:68-74.

25.   Hoeben, B. J., D. S. Burgess, J. T. McConville, L. K. Najvar, R. L. Talbert, J. I. Peters, N. P. Wiederhold, B. L. Frei, J. R. Graybill, R. Bocanegra, K. A. Overhoff, P. Sinswat, K. P. Johnston, and R. O. Williams, III. 2006. In vivo efficacy of aerosolized nanostructured itraconazole formulations for prevention of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. 50:1552-1554.

26.   Vaughn, J. M., N. P. Wiederhold, J. T. McConville, J. J. Coalson, R. L. Talbert, D. S. Burgess, K. P. Johnston, R. O. Williams, and J. I. Peters. 2007. Murine airway histology and intracellular uptake of inhaled amorphous itraconazole. Int. J. Pharm. 338:219-224.

27.   Gabardi, S., D. W. Kubiak, A. K. Chandraker, and S. G. Tullius. 2007. Invasive fungal infections and antifungal therapies in solid organ transplant recipients. Transpl. Int. 20:993-1015.

28.   Scott, L. J., D. Simpson, S. I. Blot, P. H. Chandrasekar, A. H. Groll, R. P. Hobson, V. L. Kan, S. Keady, and T. J. Walsh. 2007. Voriconazole: a review of its use in the management of invasive fungal infections. Drugs 67:269-298.

29. Husain, S., D. L. Paterson, S. Studer, J. Pilewski, M. Crespo, D. Zaldonis, K. Shutt, D. L. Pakstis, A. Zeevi, B. Johnson, E. J. Kwak, and K. R. McCurry. 2007. Voriconazole prophylaxis in lung transplant recipients. Am. J. Transplant. 6:3008-3016.

30. Walsh, T. J., M. O. Karlsson, T. Driscoll, A. G. Arguedas, P. Adamson, X. Saez-Llorens, A. J. Vora, A. C. Arrieta, J. Blumer, I. Lutsar, P. Milligan, and N. Wood. 2004. Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. Antimicrob. Agents Chemother. 48:2166-2172.

31. Theuretzbacher, U., F. Ihle, and H. Derendorf. 2006. Pharmacokinetic/pharmacodynamic profile of voriconazole. Clin. Pharmacokin. 45:649-663.

32. Tolman, J. A., N. A. Nelson, Y. J. Son, S. Bosselmann, P. Wiederhold Nathan, J. I. Peters, T. McConville Jason, and R. O. Williams, 3rd. 2009. Characterization and Pharmacokinetic Analysis of Aerosolized Aqueous Voriconazole Solution. Eur. J. Pharm. Biopharm. Accepted.

33. Tolman, J. A., N. P. Wiederhold, J. T. McConville, L. K. Najvar, R. Bocanegra, J. I. Peters, J. J. Coalson, J. R. Graybill, T. F. Patterson, and R. O. Williams, 3rd. 2009. Inhaled Voriconazole for the Prevention of Invasive Pulmonary Aspergillosis. Antimicrob. Agents Chemother. Submitted.

34. Roerig Division of Pfizer Inc. 2006. Vfend Prescribing Information, p. 47. Pfizer Inc., New York, NY.

35. Pascual, A., V. Nieth, T. Calandra, J. Bille, S. Bolay, L. A. Decosterd, T. Buclin, P. A. Majcherczyk, D. Sanglard, and O. Marchetti. 2007. Variability of voriconazole plasma levels measured by new high-performance liquid chromatography and bioassay methods. Antimicrob. Agents Chemother. 51:137-143.

36. Lutsar, I., S. Roffey, and P. Troke. 2003. Voriconazole concentrations in the cerebrospinal fluid and brain tissue of guinea pigs and immunocompromised patients. Clin. Infect. Dis. 37:728-32.

37. Cimolai, N., G. P. Taylor, D. Mah, and B. J. Morrison. 1992. Definition and application of a histopathological scoring scheme for an animal model of acute Mycoplasma pneumoniae pulmonary infection. Microbiol Immunol 36:465-78.

38. Pinkerton, K. E., R. R. Mercer, C. G. Plopper, and J. D. Crapo. 1992. Distribution of Injury and Microdosimetry of Ozone in the Ventilatory Unit of the Rat. Journal of Applied Physiology 73:817-824.

39. Schlage, W. K., H. Bulles, D. Friedrichs, M. Kuhn, A. Teredesai, and P. M. Terpstra. 1998. Cytokeratin expression patterns in the rat respiratory tract as markers of epithelial differentiation in inhalation toxicology. II. Changes in cytokeratin expression patterns following 8-day exposure to room-aged cigarette sidestream smoke. Toxicologic Pathology 26:344-360.

40. Beyer, J., S. Schwartz, G. Barzen, G. Risse, K. Dullenkopf, C. Weyer, and W. Siegert. 1994. Use of amphotericin B aerosols for the prevention of pulmonary aspergillosis. Infection 22:143-8.

41. Schmitt, H. J., E. M. Bernard, M. Haeuser, and D. Armstrong. 1988. Aerosol amphotericin B is effective for prophylaxis and therapy in a rat model of pulmonary aspergillosis. Antimicrob. Agents Chemother. 32:1676-9.

42. Dubois, J., T. Bartter, J. Gryn, and M. R. Pratter. 1995. The physiologic effects of inhaled amphotericin B. Chest 108:750-3.

43. Erjavec, Z., G. M. H. Woolthuis, H. G. De Vries-Hospers, W. J. Sluiter, S. M. G. J. Daenen, B. De Pauw, and M. R. Halie. 1997. Tolerance and efficacy of amphotericin B inhalations for prevention of invasive pulmonary aspergillosis in hematological patients. Eur. J. Clin. Microbiol. 16:364-368.

44. Gavalda, J., M.-T. Martin, P. Lopez, X. Gomis, J.-L. Ramirez, D. Rodriguez, O. Len, Y. Puigfel, I. Ruiz, and A. Pahissa. 2005. Efficacy of nebulized liposomal amphotericin B in treatment of experimental pulmonary aspergillosis. Antimicrob. Agents Chemother. 49:3028-3030.

45. Ruijgrok, E. J., A. G. Vulto, and E. W. M. Van Etten. 2001. Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromised rats. J. Antimicrob. Chemother. 48:89-95.

46. Denning, D. W., P. Ribaud, N. Milpied, D. Caillot, R. Herbrecht, E. Thiel, A. Haas, M. Rubnke, and H. Lode. 2002. Efficacy and safety of voriconazole in the treatment of acute invasive Aspergillosis. Clin. Infect. Dis. 34:563-571.

47. Davis, J. L., J. H. Salmon, and M. G. Papich. 2006. Pharmacokinetics of voriconazole after oral and intravenous administration to horses. Am. J. Vet. Res. 67:1070-1075.

48. Brueggemann, R. J. M., J. P. Donnelly, R. E. Aarnoutse, A. Warris, N. M. A. Blijlevens, J. W. Mouton, P. E. Verweij, and D. M. Burger. 2008. Therapeutic Drug Monitoring of Voriconazole. Therap. Drug Monitor. 30:403-411.

49. Roffey, S. J., S. Cole, P. Comby, D. Gibson, S. G. Jezequel, A. N. R. Nedderman, D. A. Smith, D. K. Walker, and N. Wood. 2003. The disposition of voriconazole

in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab. Dispos. 31:731-41.

50.   Capitano, B., B. A. Potoski, S. Husain, S. Zhang, D. L. Paterson, S. M. Studer, K. R. McCurry, and R. Venkataramanan. 2006. Intrapulmonary penetration of voriconazole in patients receiving an oral prophylactic regimen. Antimicrob. Agents Chemother. 50:1878-1880.

51.   Pascual, A., T. Calandra, S. Bolay, T. Buclin, J. Bille, and O. Marchetti. 2008. Voriconazole therapeutic drug monitoring in patients with invasive mycosis improves efficacy and safety outcomes. Clin. Infect. Dis. 46:201-211.

52.   Sugar, A. M., and X. P. Liu. 2000. Effect of grapefruit juice on serum voriconazole concentrations in the mouse. Med. Mycol. 38:209-12.

53.   Lowry, C. M., F. M. Marty, S. O. Vargas, J. T. Lee, K. Fiumara, A. Deykin, and L. R. Baden. 2007. Safety of aerosolized liposomal versus deoxycholate amphotericin B formulations for prevention of invasive fungal infections following lung transplantation: a retrospective study. Transpl Infect Dis 9:121-5.

54.   Marra, F., N. Partovi, K. M. Wasan, E. H. Kwong, M. H. H. Ensom, S. M. Cassidy, G. Fradet, and R. D. Levy. 2002. Amphotericin B disposition after aerosol inhalation in lung transplant recipients. Ann. Pharmacother. 36:46-51.

55.   Nakate, T., H. Yoshida, A. Ohike, Y. Tokunaga, R. Ibuki, and Y. Kawashima. 2003. Improvement of pulmonary absorption of cyclopeptide FK224 in rats by co-formulating with beta -cyclodextrin. Eur. J. Pharm. Biopharm. 55:147-154.

56.   Miyake, K., H. Arima, T. Irie, F. Hirayama, and K. Uekama. 1999. Enhanced absorption of cyclosporin A by complexation with dimethyl-beta -cyclodextrin in bile duct-cannulated and -noncannulated rats. Biological & Pharmaceutical Bulletin 22:66-72.

57.   Carrier, R. L., L. A. Miller, and I. Ahmed. 2007. The utility of cyclodextrins for enhancing oral bioavailability. J. Controlled Release 123:78-99.

58.   Irie, T., and K. Uekama. 1997. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J. Pharm. Sci. 86:147-162.

59.   Stella, V. J., and Q. He. 2008. Cyclodextrins. Toxicol. Pathol. 36:30-42.

60.   Evrard, B., P. Bertholet, M. Gueders, M. P. Flament, G. Piel, L. Delattre, A. Gayot, P. Leterme, J. M. Foidart, and D. Cataldo. 2004. Cyclodextrins as a potential carrier in drug nebulization. J. Controlled Release 96:403-410.

61.   Hursh, D., S. Gelman, and E. L. Bradley, Jr. 1987. Hepatic oxygen supply during halothane or isoflurane anesthesia in guinea pigs. Anesthesiology 67:701-6.

62.   Gelman, S. 1986. Role of oxygen availability to the liver in anesthesia-induced hepatotoxicity. Mol. Cell. Mech. Anesth.:427-31.

63.   Lattermann, R., T. Schricker, U. Wachter, M. Georgieff, and A. Goertz. 2001. Understanding the mechanisms by which isoflurane modifies the hyperglycemic response to surgery. Anesth. Analg. 93:121-127.

64.   Zardooz, H., S. Zahedi Asl, M. K. Gharib Naseri, and M. Hedayati. 2006. Effect of chronic restraint stress on carbohydrate metabolism in rat. Physiol. Behav. 89:373-378.

65.   Saha, J. K., J. Xia, J. M. Grondin, S. K. Engle, and J. A. Jakubowski. 2005. Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats: Mechanisms and implications for preclinical models. Exp. Biol. Med. 230:777-784.

66.   Fels, A. O., and Z. A. Cohn. 1986. The alveolar macrophage. J Appl Physiol 60:353-69.

67.   Brazil, T. J., M. P. Dagleish, B. C. McGorum, P. M. Dixon, C. Haslett, and E. R. Chilvers. 2005. Kinetics of pulmonary neutrophil recruitment and clearance in a natural and spontaneously resolving model of airway inflammation. Clin. Exp. Allergy 35:854-865.

Chapter 5: Pharmacokinetic Profile of Inhaled Voriconazole Following Single and Multiple Doses in Rodents

5.1. INTRODUCTION

Voriconazole, a triazole antifungal, is a derivative of fluconazole but with a broader spectrum of antifungal activity prompting increased drug utilization in the empiric management of fungal infection (1). The extended spectrum of voriconazole is due to complete inhibition of fungal cytochrome P450 (CYP) mediated ergosterol biosynthesis leading to cell membrane disruption rather than partial inhibition with fluconazole (2). Voriconazole was also demonstrated to be superior to amphotericin B, the gold standard in antifungal agents, in the treatment of a systemic fungal infection, invasive pulmonary aspergillosis (IPA) (3). Systemic fungal infections, including IPA, are a serious source of patient mortality in immunocompromised patients, including those with hematologic malignancies as well as hematopoietic stem cell and solid organ transplant patients (4, 5). This is due to dissemination of fungal hyphae via the circulatory system following inhalation and germination of fungal conidia in the lung that bypass the normal immunologic responses (6-8). Therefore, pharmacologic utilization of voriconazole for the management of systemic fungal infections currently relies on drug distribution to infected lung tissue to prevent fungal growth and dissemination.

Many systemically administered antifungals have low or variable drug penetration into infected organs and tissues leading to poor patient outcomes (9, 10). Some of this variability is due to methodological discrepancies regarding evaluation of drug distribution to lung tissue and lung fluid with some advocating the sampling of

bronchoalveolar lavage fluid or pulmonary secretions as appropriate proxy markers for alveolar drug concentration (11, 12). Another source of inconsistency is due to inter-individual as well as intra-individual variability for plasma voriconazole concentrations following multiple systemic doses (13). This plasma pharmacokinetic variability has been associated with differences in drug absorption following oral administration, genetic polymorphisms in the primary metabolizing isoenzyme for voriconazole, CYP2C19, as well as non-linear pharmacokinetics in adult human patients due to saturable drug elimination. Children, in contrast, demonstrate linear pharmacokinetics following systemic administration due to increased weight-normalized elimination capacities (14). Species variability has also been reported in rodent, lagomorph, canine, and equine animal studies with reported metabolic induction reported by 7 days after single daily oral and intravenous doses (15, 16). In rodents specifically, pre-hepatic voriconazole metabolism also accounted for very low and variable oral drug bioavailability (17). Thus, the clinical utility of reported drug distribution to lung tissue, fluids, or cells following systemically administered triazole antifungals is ambiguous. To overcome this clinical uncertainty of tissue drug concentrations, investigators have correlated pharmacodynamic efficacy with systemic pharmacokinetic measurements. They have advocated optimal voriconazole efficacy in humans with associated random plasma levels greater than 2 µg/mL (18) and trough plasma levels greater than 1 µg/mL (19) with wider concentration ranges in the reported literature (20).

The current therapeutic utilization of voriconazole necessitates the balance of therapeutic trough drug concentrations with adverse events associated with elevated systemic drug exposure, including visual abnormalities, CNS toxicity, and potential hepatotoxicity with associated abnormalities in hepatic function laboratory tests (20-22).

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Researchers have suggested targeted drug delivery to the lungs via inhalation could balance these two optimal criteria for antifungal therapy: therapeutic tissue concentrations with low systemic drug exposure (23, 24). Indeed, numerous transplant centers utilize prophylactic regimens of aerosolized amphotericin B in patients with an elevated risk for IPA (25-29). The pharmacokinetic profile of inhaled amphotericin B demonstrated high lung concentrations with negligible drug distribution to the systemic circulation while conversely, systemically administered amphotericin B demonstrated poor distribution to the lung tissue (30-33). However, a combination of inhaled and IV amphotericin B has demonstrated improved efficacy than targeted delivery alone as treatment in a rodent model of IPA suggesting anti-disseminative effects of both high tissue and moderate plasma concentrations (34).

Similar improvements in efficacy were reported in a murine model of IPA following inhalation of aqueous voriconazole solution with a companion pharmacokinetic profile in healthy mice that described high peak tissue and plasma concentrations following single and multiple doses (35, 36). Substantial and rapid drug distribution to the systemic circulation following inhalation was attributed to drug solubilization by sulfobutyl ether-β-cyclodextrin promoting improved drug absorption across alveolar membranes. Despite, low trough lung and plasma concentrations reported following multiple doses administered twice daily (BID at 08:00 and 16:00), inhaled voriconazole produced 67% survival over 12 days and was significantly improved compared to systemically administered amphotericin B. The current study was designed to expand the pharmacokinetic understanding of inhaled aqueous solutions of voriconazole following a single dose as well as multiple doses administered every 12 hours (Q12H) in mice and compare rat and mice pharmacokinetic parameters through evaluation of peak and trough

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concentrations following Q12H dosing in anticipation of future studies in human subjects. It is hypothesized that a compartmental model of drug absorption from the lungs would characterize inhaled voriconazole pharmacokinetics with the elevation of trough voriconazole concentrations through adjustment of the dosing interval to Q12H from dosing at 08:00 and 16:00 (BID).

5.2. MATERIALS AND METHODS

5.2.1. Materials

Vfend® IV, Pfizer Inc. (New York, NY), voriconazole, and sulfobutyl ether-β-cyclodextrin, Captisol®, were generously supplied by CyDex Pharmaceuticals, Inc. (Lenexa, KS). Sterile water for injection (SWFI) and normal saline were purchased from Cardinal Health (Dublin, OH). Sodium tetraborate decahydrate, boric acid , and sodium acetate trihydrate were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Acetic acid was purchased from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany). HPLC grade ethyl acetate was purchased from Spectrum Chemical Manuf. Corp. (Gardena, CA). HPLC grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ). HPLC grade methanol was purchased from EMD Chemicals Inc. (Gibbstown, NJ). Water was obtained from an in-house Milli-Q UV Plus water purification system from the Millipore Corp. (Billerica, MA).

5.2.2. Single-dose Pharmacokinetic Analysis

Male outbred 20g ICR (Institute for Cancer Research) mice were purchased (Harlan Sprague Dawley, Inc., Indianapolis, IN) and housed with free access to water and food. Prior to dosing, mice were acclimatized for up to 20 minutes twice daily in nose-

only dosing animal restraints (Battelle Toxicology Northwest, Richland, WA). Voriconazole solutions were prepared by reconstitution and dilution of Vfend® IV with SWFI to a solution containing 6.25 mg/mL voriconazole. Mice received a single nebulized dose of 5 mL aqueous voriconazole solution using an Aeroneb® Pro micro pump nebulizer (Nektar Therapeutics, San Carlos, CA) integrated into a customized nose-only dosing apparatus with an airflow rate of 1L/min through the apparatus over 20 minutes. Mice were serially euthanized by carbon dioxide narcosis at 10 and 30 minutes, 1, 4, 6, 8, 12, and 24 hours after the completion of nebulization. Four mice were euthanized at each time point with the exception of only two mice at the 10 minute and 4 hour times. The average mouse mass at the time of euthanasia was 22.5 g. Whole blood was collected by cardiac puncture into heparinized vials and centrifuged at 9000 RPM for 15 minutes to obtain plasma. Whole lungs were also collected following exsanguination. Plasma samples and whole lungs were frozen and stored at -20°C until assayed. All animals were handled and maintained in accordance with The University of Texas at Austin Institution Animal Care and Use Committee (IACUC) guidelines and in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines.

5.2.3. Multi-dose Pharmacokinetic Analysis

Male ICR mice initially weighing 20 g and male Sprague-Dawley rats initially weighing 250 g, (Harlan Sprague Dawley, Inc., Indianapolis, IN), were administered 5mL of 6.25 mg/mL voriconazole with an Aeroneb® Pro nebulizer every 12 hours (Q12H) using a nose-only dosing apparatus as described above for 7 consecutive days. The dosing apparatus was disassembled and cleaned between each use. Randomly selected pairs of mice and rats were sacrificed by carbon dioxide narcosis either 30

minutes after the completion of nebulization for peak values as determined in previous studies (35) or immediately before the next scheduled dose for trough values on days 0, 3, 5, and 7. Lung and plasma samples were handled and processed as described in the single-dose methodological description. Average mouse mass at the time of euthanasia was 23.0 g while average rat mass was 287 g.

5.2.4. Chromatographic Analysis

Plasma and lung samples were thawed prior to analysis. Calibration standards, plasma, and homogenized lung samples were analyzed using similar methods to those previously published (13, 35, 37). Briefly, voriconazole was extracted from mouse plasma samples through the addition of acetonitrile, centrifugation, and supernatant extraction with fluconazole as the internal standard. The supernatant liquid was evaporated under a gentle stream of nitrogen and residual solids, including voriconazole, were re-dispersed with mobile phase and analyzed by HPLC. Mouse lungs were homogenized with 1mL of water using an Omni GLH homogenizer (Omni International, Marietta, GA). 0.2M borate buffer (pH 9.0) was added to mouse lung homogenate followed by three liquid-phase extractions with ethyl acetate. The liquid from collected supernatant fractions were then evaporated under a gentle stream of nitrogen. Any residual solids, including voriconazole, were re-dispersed with mobile phase, centrifuged, and then analyzed by HPLC. Each voriconazole sample from a mouse source was analyzed using a Waters Breeze liquid chromatograph (Waters Corporation, Milford MA) or Shimadzu LC-10 liquid chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a heated (35°C) Jupiter® C18 (150 mm x 4.6 mm, 5 μm) with a Universal security guard (Widepore C18) guard column (Phenomenex, Torrance, CA). The sample volume was 50 μL with a UV detection wavelength of 254 nm. The mobile phase

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consisted of a 50:50 mixture of 0.01M pH 5.0 sodium acetate buffer and methanol at
1.0 mL/min.

Rat plasma and lung samples were extracted in a similar manner to mouse
samples but homogenization was performed with 2 mL normal saline per gram lung
tissue. Following three ethyl acetate extractions of voriconazole from lung homogenate,
solvent was evaporated to give residual solids that were then reconstituted in 200 μl of
acetonitrile and centrifuged. A 4000 Q TRAP mass spectrometer (Applied Biosystems,
Foster City, CA) coupled with an online HPLC (Shimadzu, Columbia, MD) (LC-MS)
was used to analyze all rat samples. 10 μl samples were injected into Shimadzu
Prominence UFLC system equipped with an Restek C18 (4.6 x 50mm, 5 μm, 110 Å)
column and eluted with mobile phase A (water with 0.1% formic acid) and B (acetonitrile
with 0.1% formic acid) by a gradient of 20%B for 0.5 min, then 20%-50%B over
0.5 min, followed by 50%B for 3 min, at a flow rate of 1 ml/min. Sample was directly
eluted from the column into the electrospray ion (ESI) source of 4000 QTRAP.   The
heated nebulizer of 4000 QTRAP was set at 700°C, the declustering potential (DP) at 40.
The Multiple Reaction Monitoring (MRM) scan experiment with unit resolution for Q1
and low resolution for Q3 was used to quantify voriconazole content.   The MRM
transitions were set as follow, Q1=350.2, Q3=127.4, CE=40. The limit of detection for
voriconazole was 5 pg. LC-MS was utilized for analysis of rat samples due to co-elution
of peaks with voriconazole by normal HPLC that was not observed for mouse analysis.
Plasma and lung samples from each animal were analyzed individually for voriconazole
concentration by HPLC or LC-MS. Concentration values were then averaged and used to
determine pharmacokinetic parameters and concentration versus time profiles.

5.2.5. Pharmacokinetic Analysis

Observed pharmacokinetic parameters were evaluated from the voriconazole concentration versus time profiles in mouse plasma and lung tissue. Non-compartmental pharmacokinetic analysis was used to determine peak concentration (C_{max}) and the time to achieve the C_{max} (t_{max}). The trapezoidal rule was used to calculate the area under the concentration versus time curve (AUC_{0-24}). The ratio of lung AUC to plasma AUC values allowed calculation of a partition coefficient following inhalation of voriconazole ($P_{l/p}$). A one-compartment model was fitted to lung concentration data to calculate the lung tissue elimination rate constant (k_e) and half-life ($t_{1/2,\ e}$) A two compartment model was determined by linear regression of plasma concentration versus time values with the method of residuals and used to evaluate absorption and elimination rate coefficients (α, β) and half-lives ($t_{1/2,\ \alpha}$, $t_{1/2,\ \beta}$).

5.2.6. Statistical Analysis

Concentration values following multiple doses were compared between days using the Student's t-test with a p-value of <0.05 for significance. In addition, concentration values were compared on day 3 in mice following Q12H and BID dosing using analysis of variance (ANOVA) with a p-value <0.05 for significance.

5.3. RESULTS

5.3.1. Single Dose Pharmacokinetic Profile

Following a single dose of 31.25 mg voriconazole administered over 20 minutes to 6 mice group in a nose-only dosing chamber, the 24-hour pharmacokinetic profile was determined in lung tissue and plasma (Figure 1). According to non-compartmental

analysis C_{max}, t_{max}, and AUC_{0-24} pharmacokinetic parameters in lung tissue and plasma were determined as 9.98 µg/g and 6.57 µg/mL, 0.17 hours (10 minutes) in both, and 44.4 µg hr/g and 30.2 µg hr/mL, respectively. The ratio of lung and plasma AUC_{0-24} values allowed the determination of a voriconazole partition coefficient, P_{l-p}, following inhalation of 1.47 with the assumption that excised lung density was equal to 1 g/mL. Compartmental analysis of voriconazole pharmacokinetics supported a two compartment model of drug absorption and elimination (Figure 2). The tissue compartment, approximated by lung tissue concentrations and designated "1", was the site of drug administration and subsequent first-order elimination for 12 hours following inhalation and modeled by Equation 1 (R^2=0.944). The central compartment, approximated by plasma concentrations and designated "2" was modeled by a first-order drug absorption phase up to 8 hours after drug administration followed by a first-order drug elimination phase. Equations for the absorption phase (R^2=0.999) and elimination phase (R^2=0.989) were determined by the method of residuals to give A, B, α, and β modeled by Equation 2.

$$1 \qquad C_t = 9.34\frac{\mu g}{g} \times e^{-0.263 hr^{-1} \times t}$$

$$2 \qquad C_p = 6.02\frac{\mu g}{mL} \times e^{-0.274 hr^{-1} \times t} + 0.82\frac{\mu g}{mL} \times e^{-0.057 hr^{-1} \times t}$$

The elimination rate constant (k_e) and associated half-life ($t_{1/2,\ e}$) from the tissue compartment was 0.263 hr^{-1} and 2.63 hr. The central compartment absorption rate constant (α) and half-life ($t_{1/2,\ \alpha}$) were 0.274 hr^{-1} and 2.53 hr with an elimination rate constant (β) and half-life ($t_{1/2,\ \beta}$) of 0.057 hr^{-1} and 12.11 hr.

5.3.2. Multiple Dose Pharmacokinetic Profile

Peak voriconazole concentrations in mouse lung tissue and plasma trended to higher drug concentrations following multiple inhalations over 7 days with only the plasma values for days 0 and 7 showing statistical significance (Figure 3A). Trough concentrations were not statistically significant and demonstrated no evidence of drug accumulation (Figure 3C). In the rat, no voriconazole accumulation or other trend was observed in peak or trough values for lung or plasma voriconazole concentrations over 7 days of Q12H dosing (Figure 3B, D). However, statistically significance differences were observed for day 7 peak lung concentrations from all other days as well as between plasma trough values on days 3 and 7. Lung concentrations remained higher than plasma concentrations for peak and trough values except for mouse trough voriconazole concentrations, where lung concentrations were below plasma levels 12 hours after completion of dosing and were undetectable on days 3, 5, and 7.

5.4. DISCUSSION

Targeted delivery of antifungals has the potential to improve patient outcomes through consistent and high tissue concentrations at the site of systemic fungal infections as prophylaxis or as treatment. Although high tissue concentrations have been reported, inconsistent drug efficacy and poor patient tolerability remain concerns with inhaled amphotericin B due, in part, to variability inherent in prophylaxis trials (38-44). However, the poor drug distribution following inhalation of amphotericin B likely contributed to inconsistent efficacy. Inhalation of solubilized voriconazole has the potential to produce consistent pharmacokinetic responses leading to predictable outcomes.

Non-compartmental pharmacokinetic analysis of voriconazole following a single inhaled dose, demonstrated similarities in drug dose between the current study and previous reports from the authors (Table 1) (35). Specifically, t_{max} values following a single dose were observed after 10 minutes compared to previously reported values of 30 minutes. The associated C_{max} drug concentrations were very similar between studies with values in lung tissue 9.98 ± 0.94 µg/g and plasma 6.57 ± 3.04 µg/mL comparable to reported values of 11.0 ± 1.6 µg/g in the lung and 7.1 ± 0.68 µg/mL in the plasma (35). These values are not statistically significant and represent equivalent drug doses between the current and previously reported study. In addition, the partition coefficient, P_{l-p}, of 1.47 based on AUC_{0-24} values was within the 1.4 to 1.6 range provided in the previous single dose pharmacokinetic study profile based on AUC_{0-6} values, indicative of equivalent absorptive processes and thorough drug distribution to the plasma following inhalation.

The mechanisms governing pulmonary absorption are fundamentally similar to other absorptive sites in the body. Drug is able to pass through biological membranes by active and passive mechanisms with passive drug diffusion being the predominant force. Diffusion across biological membranes is governed by Fick's law of diffusion (Equation 3):

$$3 \qquad \frac{dQ}{dt} = \frac{DKA}{h}\left(C_t - C_p\right)$$

where $\frac{dQ}{dt}$ = rate of drug diffusion from the tissue to the plasma (µg/hr); D= diffusion coefficient (cm^2/hr); K= lipid water partition coefficient in the membrane (unit less); A= surface area of the membrane (cm^2); (C_t-C_p)= difference in tissue and plasma drug concentrations (µg/cm^3); and h= membrane thickness (cm) (45). The concentration

gradient for inhaled voriconazole from tissue to central compartments was low at steady state but provided the driving force for drug absorption based on central compartment sink conditions. In addition, the large alveolar surface area and thin membranes physiologically present in the lungs for gas exchange also promoted a high rate of drug diffusion for the already solubilized voriconazole molecules (46). Reviews have reported numerous models of drug absorption from the lungs utilizing *in vitro*, *ex vivo*, and *in vivo* systems (47-49). These various methodologies have attempted to isolate component processes in the lungs that contribute to the overall pharmacokinetic profile following inhalation, including drug deposition, mechanical clearance, metabolism, diffusion, etc.. Although insufficient information was available for the calculation of D, with the corresponding   in the lung, the rate of drug diffusion was a component of the physiologic rate of drug elimination from the lung as evaluated through the rate constant, k_e. Thus, the numerous rate processes were constituted into pharmacokinetic rate constants k_e, α, and β through the application of compartmental analysis.

Utilization of a one-compartment pharmacokinetic model to evaluate lung tissue concentrations of voriconazole through 12 hours after completion of nebulization described first-order drug elimination from lung tissue with an elimination half-life of 2.53 hours (Table 1). Exclusion of the 24 hour time point was appropriate due to elimination of approximately 96% of the C_0 concentration after 12 hours based on the elimination half-life in the lung. Similarly, two-compartmental evaluation of plasma concentrations correlated with absorption and elimination phases in the central compartment following inhalation of voriconazole. The tissue elimination rate constant ke (0.263 hr^{-1} with 2.63 hr half-life) and central compartment absorption rate constant α

(0.274 hr^{-1} with 2.53 hr half-life) were equivalent and equated to drug absorption from the lung tissue with negligible non-absorptive voriconazole elimination from the lungs.

Elimination of drug from the central compartment was first-order and characterized by β equal to 0.057 hr^{-1} with a half-life of 12.11 hours. This half-life was substantially longer than reported terminal elimination half-lives in other species, including 6 hours in humans, 5.5 hours in guinea pigs, and 1 hour in rabbits (15, 22). Previous studies in mice have not reported elimination half-lives due to very low bioavailability and rapid clearance mechanisms following systemic administration due to drug hypermetabolism (17, 50). Thus, the observed terminal drug elimination from the central compartment demonstrated substantial changes in clearance and elimination mechanisms following the inhalation of voriconazole. These changes were not influenced by prolonged drug distribution from the lungs due to a short absorption half-life but instead could be due to non-linear pharmacokinetics resulting from capacity-limited metabolism as observe d in humans (51). However, plasma concentrations were well within reported values in rodents, lagomorphs, canines, and humans such that the suggested increase in murine metabolic capacity should not have been saturated (15).

Despite prolonged drug elimination from the central compartment, observed trough voriconazole concentrations following multiple doses Q12H were lower than predicted using Equations 1 & 2 as well as half-life elimination from the tissue, $t_{1/2,\ e}$, and central, $t_{1/2,\ \beta}$, compartments. Additionally, no evidence of drug accumulation was observed in lung tissue or plasma. These findings were in accordance with reported values following inhaled voriconazole administered BID (35). Plasma trough concentrations following BID dosing ranged from 0.18 to 0.32 µg/mL while Q12H

dosing increased the concentration range to 0.24 to 0.50 µg/mL but were not significantly different (p-value >0.05). Trough lung concentrations following both BID and Q12H dosing were low to undetectable. These similarities in trough voriconazole concentrations from day 0 through 7 and day 3 through 12 also suggest drug elimination was not affected by a marked induce in drug metabolism following inhalation as reported for other routes of drug administration. Additional studies are required to elucidate possible mechanisms that justify a low elimination rate constant, long elimination half-life, and non-induced drug metabolism following inhalation of voriconazole solutions.

Rats received the same drug exposure as mice, 31.25 mg voriconazole nebulized over 20 minutes, but had much lower drug concentrations in lung tissue and plasma. Peak voriconazole concentrations were approximately 10 to 20 times lower in the lung and 10 to 30 times lower in the plasma compared to mice, while a much larger discrepancy was observed in trough plasma concentrations with rat values 50 to 170 times lower than those observed in mice based on day-matched values. Although the study was not designed to conduct allometric analysis of pharmacokinetic properties between mice and rats, peak values suggest weight-based dilutional effects were observed. The discrepancies between trough values may have been due to metabolic differences between mice and rats as reported following systemic drug administration and would require further study to determine differences in metabolic capacity between the species (15).

The lowering of drug concentration based on animal weight following inhalation would not be expected to translate into low concentrations in humans due to differences in respiratory physiology between rodents and humans. Specifically, rodents are obligate

nose-breathers and have substantial aerosol droplet deposition in the nasal cavity prior to inhalation to the deep lung (52, 53). Humans, in contrast, can inhale through the mouth and have substantially higher deposition fractions in the deep lung when normalized for body weight (54). Thus, the combination of a higher inhaled dose fraction and different metabolic processes for voriconazole between humans and rodents should lead to clinically relevant drug concentrations in lung tissue as well as the systemic circulation.

5.5. CONCLUSION

Following inhalation of aerosolized aqueous voriconazole solution, pharmacokinetic properties were evaluated and compartmental analysis was utilized to determine absorption and elimination rate constants and half-lives. The pharmacokinetic properties of inhaled voriconazole were different from those reported in the literature and represent a possible route of administration to bypass variability reported in the literature. Although Q12H dosing did elevate trough voriconazole concentrations, the increase was not statistically significant and might not correlate to a clinical difference. Although additional studies are needed to further investigate pharmacokinetic processes of drug absorption and elimination, inhaled voriconazole represents a potentially important advancement in the treatment of IPA.

5.6. ACKNOWLEDGEMENTS

5.7. REFERENCES

1.   Herreros, J. M. C., and E. C. Matia. 2006. Therapeutic armamentarium against systemic fungal infections. Clinical Microbiology and Infection 12:53-64.

2.   Sanati, H., P. Belanger, R. Fratti, and M. Ghannoum. 1997. A new triazole, voriconazole (UK-109,496), blocks sterol biosynthesis in Candida albicans and Candida krusei. Antimicrob. Agents Chemother. 41:2492-2496.

3.   Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J.-W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke, B. de Pauw, R. Allen, M. Aoun, C. Aul, M. Bjorkholm, K. L. Blanchard, M. Boogaerts, E. Bouza, E. J. Bow, H. R. Brodt, J. Brown, D. Buchheidt, J. Y. Cahn, A. Calmaggi, J. M. Cisneros, C. Cordonnier, J. Daly, C. A. Da Cunha, R. De Bock, A. Del Favero, J. Diaz Mediavilla, M. C. Dignani, C. Doyen, J. S. Dummer, B. Dupont, M. Egyed, D. Engelhard, G. Faetkenheuer, R. Feld, D. Fiere, G. Fioritoni, G. Garber, Z. Gasztonyi, K. Godder, D. Graham, A. Gratwohl, R. Greenberg, K. High, F. Jacobs, V. Kremery, P. Kumar, W. Langer, M. Laverdiere, P. Ljungman, H. Lode, A. Louie, D. Maki, J. P. Marie, D. J. E. Marriott, D. S. McKinsey, R. Mertelsmann, M. K. Nair, N. Milpied, A. Nagler, D. Niederwieser, L. Pagano, P. Pappas, J. Perfect, J. Pottage, V. Raina, J. Reinhardt, S. Richardson, L. Rickman, M. Ruhnke, I. Salit, W. M. Scheld, S. Schuler, M. Schuster, R. Schwerdtfeger, S. D. Shafran, B. Simmons, M. Slavin, M. Sokol-Anderson, P. Tebas, C. Tsoukas, A. Ullmann, J. Van Burik, J. W. Van't Wout, E. C. Vinaya Kumar, et al. 2002. Voriconazole versus amphotericin B for primary therapy on invasive aspergillosis. N. Engl. J. Med. 347:408-415.

4.   Lin, S. J., J. Schranz, and S. M. Teutsch. 2001. Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis 32:358-66.

5.   Denning, D. W. 1998. Invasive aspergillosis. Clin Infect Dis 26:781-803; quiz 804-5.

6.   Cornet, M., L. Fleury, C. Maslo, J. F. Bernard, and G. Brucker. 2002. Epidemiology of invasive aspergillosis in France: a six-year multicentric survey in the Greater Paris area. J. Hosp. Infect. 51:288-96.

7.   Richardson, M. D. 2005. Changing patterns and trends in systemic fungal infections. J. Antimicrob. Chemother. 56:i5-i11.

8.   Pai, U., R. J. Blinkhorn, Jr., and J. F. Tomashefski, Jr. 1994. Invasive cavitary pulmonary aspergillosis in patients with cancer: a clinicopathologic study. Hum. Pathol. 25:293-303.

9.   Black, K. E., and L. R. Baden. 2007. Fungal infections of the CNS: treatment strategies for the immunocompromised patient. CNS Drugs 21:293-318.

10.   Wiebe, V., and M. Karriker. 2005. Therapy of systemic fungal infections: a pharmacologic perspective. Clinical Techniques in Small Animal Practice 20:250-7.

11.   Prentice, A. G., and A. Glasmacher. 2005. Making sense of itraconazole pharmacokinetics. J. Antimicrob. Chemother. 56:i17-i22.

12.   Capitano, B., B. A. Potoski, S. Husain, S. Zhang, D. L. Paterson, S. M. Studer, K. R. McCurry, and R. Venkataramanan. 2006. Intrapulmonary penetration of voriconazole in patients receiving an oral prophylactic regimen. Antimicrob. Agents Chemother. 50:1878-1880.

13.   Pascual, A., V. Nieth, T. Calandra, J. Bille, S. Bolay, L. A. Decosterd, T. Buclin, P. A. Majcherczyk, D. Sanglard, and O. Marchetti. 2007. Variability of voriconazole plasma levels measured by new high-performance liquid chromatography and bioassay methods. Antimicrob. Agents Chemother. 51:137-143.

14.   Walsh, T. J., M. O. Karlsson, T. Driscoll, A. G. Arguedas, P. Adamson, X. Saez-Llorens, A. J. Vora, A. C. Arrieta, J. Blumer, I. Lutsar, P. Milligan, and N. Wood. 2004. Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. Antimicrob. Agents Chemother. 48:2166-2172.

15.   Roffey, S. J., S. Cole, P. Comby, D. Gibson, S. G. Jezequel, A. N. R. Nedderman, D. A. Smith, D. K. Walker, and N. Wood. 2003. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab. Dispos. 31:731-41.

16.   Davis, J. L., J. H. Salmon, and M. G. Papich. 2006. Pharmacokinetics of voriconazole after oral and intravenous administration to horses. Am. J. Vet. Res. 67:1070-1075.

17.   Sugar, A. M., and X. P. Liu. 2000. Effect of grapefruit juice on serum voriconazole concentrations in the mouse. Med. Mycol. 38:209-12.

18.   Smith, J., N. Safdar, V. Knasinski, W. Simmons, S. M. Bhavnani, P. G. Ambrose, and D. Andes. 2006. Voriconazole therapeutic drug monitoring. Antimicrob. Agents Chemother. 50:1570-1572.

19.   Pascual, A., T. Calandra, S. Bolay, T. Buclin, J. Bille, and O. Marchetti. 2008. Voriconazole therapeutic drug monitoring in patients with invasive mycosis improves efficacy and safety outcomes. Clin. Infect. Dis. 46:201-211.

20.   Brueggemann, R. J. M., J. P. Donnelly, R. E. Aarnoutse, A. Warris, N. M. A. Blijlevens, J. W. Mouton, P. E. Verweij, and D. M. Burger. 2008. Therapeutic Drug Monitoring of Voriconazole. Therap. Drug Monitor. 30:403-411.

21.   Pfizer Global Research & Development. 2001. Voriconazole AC Briefing Document, p. 56, October 4, 2001 ed. Center for Drug Evaluation and Research - Food and Drug Administration.

22.   Theuretzbacher, U., F. Ihle, and H. Derendorf. 2006. Pharmacokinetic/pharmacodynamic profile of voriconazole. Clin. Pharmacokin. 45:649-663.

23.   Conrad, D. J. 2003. The Clinical Use of Aerosolized Antibiotics. Clinical Pulmonary Medicine 10:201-207.

24.   Myers, S. E., S. M. Devine, R. L. Topper, M. Ondrey, C. Chandler, K. O'Toole, S. F. Williams, R. A. Larson, and R. B. Geller. 1992. A pilot study of prophylactic aerosolized amphotericin B in patients at risk for prolonged neutropenia. Leukemia & Lymphoma 8:229-33.

25.   Schwartz, S., G. Behre, V. Heinemann, H. Wandt, E. Schilling, M. Arning, A. Trittin, W. V. Kern, O. Boenisch, D. Bosse, K. Lenz, W. D. Ludwig, W. Hiddemann, W. Siegert, and J. Beyer. 1999. Aerosolized amphotericin B inhalations as prophylaxis of invasive aspergillus infections during prolonged neutropenia: results of a prospective randomized multicenter trial. Blood 93:3654-3661.

26.   Klepser, M. E. 2002. Amphotericin B in lung transplant recipients. Ann. Pharmacother. 36:167-169.

27.   Maschmeyer, G., A. Haas, and O. A. Cornely. 2007. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs 67:1567-1601.

28.   Dummer, J. S., N. Lazariashvilli, J. Barnes, M. Ninan, and P. Milstone Aaron. 2004. A survey of anti-fungal management in lung transplantation. J Heart Lung Transplant 23:1376-81.

29.   Walsh, T. J., E. J. Anaissie, D. W. Denning, R. Herbrecht, D. P. Kontoyiannis, K. A. Marr, V. A. Morrison, B. H. Segal, W. J. Steinbach, D. A. Stevens, J.-A. van Burik, J. R. Wingard, and T. F. Patterson. 2008. Treatment of aspergillosis:

clinical practice guidelines of the Infectious Diseases Society of America. Clin. Infect. Dis. 46:327-360.

30. Bekersky, I., G. W. Boswell, R. Hiles, R. M. Fielding, D. Buell, and T. J. Walsh. 2000. Safety, toxicokinetics and tissue distribution of long-term intravenous liposomal amphotericin B (AmBisome): a 91-day study in rats. Pharm. Res. 17:1494-1502.

31. Christiansen, K. J., E. M. Bernard, J. W. Gold, and D. Armstrong. 1985. Distribution and activity of amphotericin B in humans. J. Infect. Dis. 152:1037-43.

32. Collette, N., P. van der Auwera, A. P. Lopez, C. Heymans, and F. Meunier. 1989. Tissue concentrations and bioactivity of amphotericin B in cancer patients treated with amphotericin B-deoxycholate. Antimicrob. Agents Chemother. 33:362-8.

33. Koizumi, T., K. Kubo, T. Kaneki, M. Hanaoka, T. Hayano, T. Miyahara, K. Okada, K. Fujimoto, H. Yamamoto, T. Kobayashi, and M. Sekiguchi. 1998. Pharmacokinetic evaluation of amphotericin B in lung tissue: lung lymph distribution after intravenous injection and airspace distribution after aerosolization and inhalation of amphotericin B. Antimicrob. Agents Chemother. 42:1597-1600.

34. Gavalda, J., M.-T. Martin, P. Lopez, X. Gomis, J.-L. Ramirez, D. Rodriguez, O. Len, Y. Puigfel, I. Ruiz, and A. Pahissa. 2005. Efficacy of nebulized liposomal amphotericin B in treatment of experimental pulmonary aspergillosis. Antimicrob. Agents Chemother. 49:3028-3030.

35. Tolman, J. A., N. A. Nelson, Y. J. Son, S. Bosselmann, P. Wiederhold Nathan, J. I. Peters, T. McConville Jason, and R. O. Williams, 3rd. 2009. Characterization and Pharmacokinetic Analysis of Aerosolized Aqueous Voriconazole Solution. Eur. J. Pharm. Biopharm. Accepted.

36. Tolman, J. A., N. P. Wiederhold, J. T. McConville, L. K. Najvar, R. Bocanegra, J. I. Peters, J. J. Coalson, J. R. Graybill, T. F. Patterson, and R. O. Williams, 3rd. 2009. Inhaled Voriconazole for the Prevention of Invasive Pulmonary Aspergillosis. Antimicrob. Agents Chemother. Submitted.

37. Lutsar, I., S. Roffey, and P. Troke. 2003. Voriconazole concentrations in the cerebrospinal fluid and brain tissue of guinea pigs and immunocompromised patients. Clin. Infect. Dis. 37:728-32.

38. Erjavec, Z., G. M. H. Woolthuis, H. G. De Vries-Hospers, W. J. Sluiter, S. M. G. J. Daenen, B. De Pauw, and M. R. Halie. 1997. Tolerance and efficacy of amphotericin B inhalations for prevention of invasive pulmonary aspergillosis in hematological patients. Eur. J. Clin. Microbiol. 16:364-368.

39.   Conneally, E., M. T. Cafferkey, P. A. Daly, C. T. Keane, and S. R. McCann. 1990. Nebulized amphotericin B as prophylaxis against invasive aspergillosis in granulocytopenic patients. Bone Marrow Transplant. 5:403-6.

40.   Lowry, C. M., F. M. Marty, S. O. Vargas, J. T. Lee, K. Fiumara, A. Deykin, and L. R. Baden. 2007. Safety of aerosolized liposomal versus deoxycholate amphotericin B formulations for prevention of invasive fungal infections following lung transplantation: a retrospective study. Transpl Infect Dis 9:121-5.

41.   Alexander, B. D., E. S. Dodds Ashley, R. M. Addison, J. A. Alspaugh, N. J. Chao, and J. R. Perfect. 2005. Non-comparative evaluation of the safety of aerosolized amphotericin B lipid complex in patients undergoing allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis 8:13-20.

42.   Drew, R. H., E. Dodds Ashley, D. K. Benjamin, Jr., R. D. Davis, S. M. Palmer, and J. R. Perfect. 2004. Comparative safety of amphotericin B lipid complex and amphotericin B deoxycholate as aerosolized antifungal prophylaxis in lung-transplant recipients. Transplantation 77:232-237.

43.   Lambros, M. P., D. W. Bourne, S. A. Abbas, and D. L. Johnson. 1997. Disposition of aerosolized liposomal amphotericin B. J Pharm Sci 86:1066-9.

44.   Schmitt, H. J., E. M. Bernard, M. Haeuser, and D. Armstrong. 1988. Aerosol amphotericin B is effective for prophylaxis and therapy in a rat model of pulmonary aspergillosis. Antimicrob. Agents Chemother. 32:1676-9.

45.   Martinez, M. N., and G. L. Amidon. 2002. A mechanistic approach to understanding the factors affecting drug absorption: A review of fundamentals. J. Clin. Pharmacol. 42:620-643.

46.   Tronde, A., B. Norden, H. Marchner, A.-K. Wendel, H. Lennernaes, and U. H. Bengtsson. 2003. Pulmonary absorption rate and bioavailability of drugs in vivo in rats: Structure-absorption relationships and physicochemical profiling of inhaled drugs. J. Pharm. Sci. 92:1216-1233.

47.   Cryan, S.-A., N. Sivadas, and L. Garcia-Contreras. 2007. In vivo animal models for drug delivery across the lung mucosal barrier. Advanced Drug Delivery Reviews 59:1133-1151.

48.   Mobley, C., and G. Hochhaus. 2001. Methods used to assess pulmonary deposition and absorption of drugs. Drug Discovery Today 6:367-375.

49.   Sakagami, M. 2006. In vivo, in vitro and ex vivo models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery. Advanced Drug Delivery Reviews 58:1030-1060.

50.   Patterson, B., and P. Coates. 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: disposition in man., p. Abstract F78, 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC.

51.   Roerig Division of Pfizer Inc. 2006. Vfend Prescribing Information, p. 47. Pfizer Inc., New York, NY.

52.   Nadithe, V., M. Rahamatalla, W. H. Finlay, J. R. Mercer, and J. Samuel. 2003. Evaluation of nose-only aerosol inhalation chamber and comparison of experimental results with mathematical simulation of aerosol deposition in mouse lungs. J. Pharm. Sci. 92:1066-1076.

53.   Hsieh, T. H., C. P. Yu, and G. Oberdorster. 1999. Deposition and clearance models of Ni compounds in the mouse lung and comparisons with the rat models. Aerosol Sci. Technol. 31:358-372.

54.   Asgharian, B., R. Wood, and R. B. Schlesinger. 1995. Empirical modeling of particle deposition in the alveolar region of the lungs: a basis for interspecies extrapolation. Fund. Appl. Toxicol. 27:232-8.

Tables

Table 1.1: Properties of Inhaled Corticosteroids

Table 1.1A: Properties of Inhaled Fluticasone Propionate

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Fluticasone propionate						
DPI Administered as Flutide Diskhaler®, Glaxo Ltd Contained microfine fluticasone propionate blended with lactose	1000 µg	t_{max} C_{max} AUC	1.4 ± 1 0.24 ± 0.1 2.44 ± 0.69[a]	hr ng mL^{-1} ng hr mL^{-1}	Healthy human volunteers (Plasma samples)	(38)
DPI Administered as Flovent® Diskus®, GlaxoSmithKline Contained microfine fluticasone propionate blended with lactose	1000 µg	t_{max} C_{max} AUC MRT	1.88 (1.4, 2.38) 0.35 (0.3, 0.45) 2.75 (2.25, 3.45) 7.1 (5.6-8.5)	hr ng mL^{-1} ng hr mL^{-1} hr	Healthy and asthmatic human volunteers (Plasma samples)	(39)
pMDI Administered as Flovent®, GlaxoSmithKline Contained microcrystalline suspension of fluticasone propionate in a mixture of CFC-11 and CFC-12 with soya lecithin	1000 µg	t_{max} C_{max} AUC MRT	1.67 (1.1, 2.25) 0.25 (0.2, 0.3) 1.75 (1.45, 2.15) 5.3 (4.0-6.6)	hr ng mL^{-1} ng hr mL^{-1} hr		
DPI Administered as Flovent® Diskus®, GlaxoSmithKline Contained microfine fluticasone propionate blended with lactose	200 µg	t_{max} C_{max} AUC MRT	1.5 0.037 0.22 7.2	hr ng mL^{-1} ng hr mL^{-1} hr	Healthy human volunteers (Plasma samples)	(40)
	500 µg	t_{max} C_{max} AUC MRT	1.5 0.094 0.79 12	hr ng mL^{-1} ng hr mL^{-1} hr		
DPI Administered as Flixotide® Accuhaler®, GlaxoSmithKline (Marketed as Flovent® Diskus® in the US) Contained microfine fluticasone propionate blended with lactose	1000 µg	t_{max} C_{max} AUC MRT	0.9 (0.68, 1.20) 0.09 (0.07, 0.10) 0.38[b] (0.30, 0.47) 8.46 (6.70, 10.7)	hr ng mL^{-1} ng hr mL^{-1} hr	Stable human asthma patients (Plasma samples)	(41)

Table 1.1A: Properties of Inhaled Fluticasone Propionate (continued)

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Fluticasone propionate						
DPI Administered as Flovent® Diskus®, GlaxoSmithKline Contained microfine fluticasone propionate blended with lactose	800 µg	AUC	0.256	ng hr mL^{-1}	Human asthma patients (Plasma samples)	(42)
pMDI Administered as Flovent®, GlaxoSmithKline Contained microcrystalline suspension of fluticasone propionate in a mixture of CFC-11 and CFC-12 with soya lecithin	704 µg	AUC	0.919	ng hr mL^{-1}		
DPI Administered as Flixotide® Diskhaler®, GlaxoWellcome Contained microfine fluticasone propionate blended with lactose	1000 µg	$T_{10\%}$ $T_{50\%}$ $T_{90\%}$ MAT	0.19 (0.14, 0.26)[d] 1.58 (1.14, 2.20)[d] 12.3 (7.99, 18.9)[d] 4.29 (2.90, 6.34)[d]	hr hr hr hr	Healthy human volunteers (Plasma samples)	(30)
DPI Administered as Flovent® Diskus®, GlaxoSmithKline Contained microfine fluticasone propionate blended with lactose	1000 µg	$T_{10\%}$ $T_{50\%}$ $T_{90\%}$ MAT	0.26 (0.22, 0.30)[d] 2.42 (2.01, 2.91)[d] 12.1 (8.76, 16.8)[d] 4.4 (3.26, 5.95)[d]	hr hr hr hr		
pMDI Administered as Flovent®, GlaxoSmithKline Contained microcrystalline suspension of fluticasone propionate in a mixture of CFC-11 and CFC-12 with soya lecithin	1000 µg	$T_{10\%}$ $T_{50\%}$ $T_{90\%}$ MAT	0.28 (0.20, 0.38)[d] 2.18 (1.77, 2.67)[d] 11.4 (8.15, 16.0)[d] 4.31 (3.17, 5.86)[d]	hr hr hr hr		

Table 1.1B: Properties of Inhaled Budesonide

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Budesonide						

DPI
    Administered as Pulmicort Turbohaler®, AstraZeneca
    Contained micronized budesonide
	400 μg	t_{max}
		C_{max}
		AUC
		MRT
		$t_{1/2}$
			0.17
			0.45
			0.99
			3
			2.1
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				hr
				hr
					Healthy human volunteers
					(Plasma samples)
						(40)

	1000 μg	t_{max} C_{max} AUC MRT $t_{1/2}$	0.17 0.9 2.53 3.9 3.5	hr ng mL^{-1} ng hr mL^{-1} hr hr		

DPI with oral charcoal
    Administered as Giona® Easyhaler®, Orion Pharma
    Contained micronized budesonide blended with lactose
	1000 μg	t_{max}
		C_{max}
		AUC
		MRT
			0.5 ± 0.18
			1.22 ± 0.41
			3.48 ± 0.93
			3.05 ± 0.48
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				hr
					Healthy human volunteers
					(Plasma samples)
						(31)

DPI with oral charcoal
    Administered as Pulmicort Turbohaler®, AstraZeneca
    Contained micronized budesonide
	1000 μg	t_{max}
		C_{max}
		AUC
		MRT
			0.38 ± 0.17
			1.29 ± 0.44
			3.46 ± 1.13
			2.85 ± 0.38
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				hr		

DPI
    Administered as Pulmicort Turbohaler®, AstraZeneca
    Contained micronized budesonide
	1000 μg	t_{max}
		C_{max}
		AUC
		MRT
			0.28 (0.17, 0.4)
			1.64 (1.46, 1.98)
			4.52 (3.66, 5.68)
			0.6 (0.3-0.9)
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				hr
					Human asthma patients
					(Plasma samples)
						(39)

DPI
    Administered as Pulmicort Turbohaler®, AstraZeneca
    Contained micronized budesonide
	800 μg	t_{max}
		C_{max}
		AUC
		MRT
		$t_{1/2}$
			0.13 (0.10, 0.16)
			1.46 (1.18, 1.79)
			3.28 (2.82, 3.81)
			3.47 (3.21, 3.76)
			2.63 (2.46, 2.82)
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				hr
				hr
					Stable human asthmatic patients
					(Plasma samples)
						(41)

DPI
    Administered as Pulmicort Turbohaler®, AstraZeneca
    Contained micronized budesonide
	600 μg	t_{max}
		C_{max}
		AUC
		
		$t_{1/2}$
			0.58 (28.9)[e]
			0.66 (69.8)[e]
			1.97[a] (57.1)[e]
			2.32[c] (47.4)[e]
			2.19
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				ng hr mL^{-1}
				hr
					Healthy human volunteers
					(Plasma samples)
						(43)

Table 1.1B: Properties of Inhaled Budesonide (continued)

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Budesonide						

pMDI
  Administered as Pulmicort®, AstraZeneca
  Contained micronized suspension of budesonide with
  sorbitan trioleate, CFC-11, CFC-12, and CFC-114
	800 µg	C_{2hr}	0.47[g]	ng mL^{-1}	Human asthma patients
					(Plasma samples)
						(44)

Nebulized Suspension
  Administered as Pulmicort Respules®, AstraZeneca
  Contained micronized suspension of budesonide with
  disodium edetate, sodium chloride, sodium citrate, citric
  acid, polysorbate 80, and Water for Injection
	1000 µg	C_{2hr}	0.73[g]	ng mL^{-1}		

	4000 µg	C_{2hr}	2.15[g]	ng mL^{-1}		

Nebulized Suspension
  Administered as Pulmicort Respules®, AstraZeneca
  Contained micronized suspension of budesonide with
  disodium edetate, sodium chloride, sodium citrate, citric
  acid, polysorbate 80, and Water for Injection
	500 µg	t_{max}
		C_{max}
		AUC
		$t_{1/2}$
			0.24 (0.19-0.3)
			0.66 (0.42-0.91)
			1.63 (1.13-2.14)
			5.42
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				hr
					Healthy human volunteers
					(Plasma samples)
						(45)

Nebulized Suspension
  Administered as Nanobudesonide (Smaller particle size
  distribution than Pulmicort Respules®)
  Formulation composition not reported
	500 µg	t_{max}
		C_{max}
		AUC
		$t_{1/2}$
			0.14 (0.09-0.18)
			1.21 (0.75-1.67)
			1.66 (1.28-2.03)
			6.62
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				hr		

	1000 µg	t_{max} C_{max} AUC $t_{1/2}$	0.19 (0.1-0.27) 2.48 (1.24-3.73) 2.89 (2.12-3.67) 5.46	hr ng mL^{-1} ng hr mL^{-1} hr		

Table 1.1B: Properties of Inhaled Budesonide (continued)

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Budesonide						

Nebulized Suspension
  Administered as Pulmicort Respules®, AstraZeneca
  Contained micronized suspension of budesonide with
  disodium edetate, sodium chloride, sodium citrate, citric
  acid, polysorbate 80, and Water for Injection
	250 µg	t_{max}
		C_{max}
		AUC
		
		$t_{1/2}$
			0.15 ± 0.12
			0.30 ± 0.18
			0.48 ± 0.16^{b}
			0.53 ± 0.18^{c}
			2.42 ± 0.68
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				ng hr mL^{-1}
				hr
					Healthy adult
					volunteers
					(Plasma samples)
						(46)

Nebulized Suspension
  Administered as a nano-scale budesonide suspension
  Contains submicron budesonide in a sterile aqueous
  formulation containing surface modifiers sodium
  chloride, citric acid, sodium citrate, and disodium
  edentate dehydrate
  Incomplete report of formulation composition
	60 µg	t_{max}
		C_{max}
		AUC
		
		$t_{1/2}$
			0.075 ± 0.055
			0.11 ± 0.06
			0.066 ± 0.033^{b}
			0.073 ± 0.024^{c}
			1.17 ± 0.56
				hr
				ng mL^{-1}
				ng hr mL^{-1}
				ng hr mL^{-1}
				hr		

	120 µg	t_{max} C_{max} AUC $t_{1/2}$	0.051 ± 0.025 0.24 ± 0.14 0.143 ± 0.070^{b} 0.131 ± 0.061^{c} 1.31 ± 0.45	hr ng mL^{-1} ng hr mL^{-1} ng hr mL^{-1} hr		
	240 µg	t_{max} C_{max} AUC $t_{1/2}$	0.062 ± 0.025 0.43 ± 0.25 0.369 ± 0.161^{b} 0.422 ± 0.196^{c} 2.33 ± 0.90	hr ng mL^{-1} ng hr mL^{-1} ng hr mL^{-1} hr		

Table 1.1C: Properties of Inhaled Beclomethasone Dipropionate (and Beclomethasone 17-Monopropionate)

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Beclomethasone dipropionate (beclomethasone 17-monopropionate)						
pMDI	1000 µg	BDP			Healthy human	(49)
Administered as Beclovent®, GlaxoWellcome		t_{max}	0.3 (0.2, 0.5)[f]	hr	volunteers	
Contained suspension of micronized beclomethasone		C_{max}	0.32[g] (0.18, 0.55)	ng mL^{-1}	(Plasma samples)	
dipropionate in a mixture of CFC-11 and CFC-12 with		AUC	0.15[g] (0.09, 0.27)	ng hr mL^{-1}		
oleic acid		17-BMP				
		t_{max}	1.0 (0.8, 6)[f]	hr		
		C_{max}	0.94[g] (0.67, 1.3)	ng mL^{-1}		
		AUC	3.85[g] (2.8, 5.2)	ng hr mL^{-1}		
		MRT	4.1 (3.5, 4.6)	hr		
		$t_{1/2}$	2.7 (2.1, 3.6)[f]	hr		
pMDI with oral charcoal	1000 µg	BDP			Healthy human	
Administered as Beclovent®, GlaxoWellcome		t_{max}	0.5 (0.2, 0.5)[f]	hr	volunteers	
Contained suspension of micronized beclomethasone		C_{max}	0.46[g] (0.25, 0.72)	ng mL^{-1}	(Plasma samples)	
dipropionate in a mixture of CFC-11 and CFC-12 with		AUC	0.22[g] (0.13, 0.35)	ng hr mL^{-1}		
oleic acid		17-BMP				
		t_{max}	0.8 (0.8, 1)[f]	hr		
		C_{max}	0.71[g] (0.44, 1.1)	ng mL^{-1}		
		AUC	2.4[g] (1.5, 3.7)	ng hr mL^{-1}		
		MRT	3.5 (3, 4)	hr		
		$t_{1/2}$	2.3 (1.7, 5.8)[f]	hr		
DPI	800 µg	17-BMP			Stable human	(41)
Administered as Becodisks ®, Allen & Hanburys		t_{max}	2.5 (1.9, 3.3)	hr	asthma patients	
Contained micronized beclomethasone dipropionate		C_{max}	0.33 (0.28, 0.39)	ng mL^{-1}	(Plasma samples)	
blended with lactose		AUC	1.7[b] (1.5, 2.0)	ng hr mL^{-1}		
		MRT	9.1 (7.1, 11.5)	hr		
		$t_{1/2}$	5.3 (4.1, 7.0)	hr		

Table 1.1C: Properties of Inhaled Beclomethasone Dipropionate (and Beclomethasone 17-Monopropionate) (continued)

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Beclomethasone dipropionate (beclomethasone 17-monopropionate)						

Nebulized Solution
  Administered as a monodisperse aerosol generated by the electrohydrodynamic technique
  Contained 4% beclomethasone dipropionate solubilized in 97% ethanol
	100 µg	17-BMP: 1.5 µm MMAD			Human patients with stable mild asthma (Plasma samples)	(48)

		t_{max}	0.17^g	hr		
		C_{max}	0.39	ng mL^{-1}		
		AUC	0.60	ng hr mL^{-1}		
		17-BMP: 2.5 µm MMAD				
		t_{max}	0.33^g	hr		
		C_{max}	0.91	ng mL^{-1}		
		AUC	1.6	ng hr mL^{-1}		
		17-BMP: 4.5 µm MMAD				
		t_{max}	0.33^g	hr		
		C_{max}	0.74	ng mL^{-1}		
		AUC	1.2	ng hr mL^{-1}		

Table 1.1D: Properties of Inhaled Triamcinolone Acetonide

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Triamcinolone acetonide						
DPI Administered using the breath actuated Ultrahaler®, Aventis Pharma Contains micronized triamcinolone acetonide blended with lactose	200 µg	t_{max} C_{max} AUC $t_{1/2}$	0.25 (0.25–1.00)[e] 0.45 (30.50)[e] 1.62 (20.80)[e] 2.30 (12.61)[e]	hr ng mL^{-1} ng hr mL^{-1} hr	Healthy human volunteers (Plasma samples)	(50)
	450 µg	t_{max} C_{max} AUC $t_{1/2}$	0.25 (0.25–0.50)[e] 0.88 (26.08)[e] 3.13 (15.04)[e] 2.24 (10.12)[e]	hr ng mL^{-1} ng hr mL^{-1} hr		
	900 µg	t_{max} C_{max} AUC $t_{1/2}$	0.25 (0.25–1.00)[e] 1.59 (33.39)[e] 6.19 (27.29)[e] 2.52 (18.55)[e]	hr ng mL^{-1} ng hr mL^{-1} hr		
pMDI Administered as Azmacort®, Aventis Pharma Contained microcrystalline suspension of triamcinolone acetonide in CFC-12 and 1% w/w dehydrated alcohol	800 µg	t_{max} C_{max} AUC $t_{1/2}$	1.74 (44.1)[e] 0.92 (33.4)[e] 4.96 (40.7)[e] 5.12[c] (39.8)[e] 2.52	hr ng mL^{-1} ng hr mL^{-1} ng hr mL^{-1} hr	Healthy human volunteers (Plasma samples)	(51)
pMDI with oral charcoal Administered as Azmacort®, Aventis Pharma Contained microcrystalline suspension of triamcinolone acetonide in CFC-12 and 1% w/w dehydrated alcohol	800 µg	t_{max} C_{max} AUC $t_{1/2}$	0.66 (31.4)[e] 0.55 (57. 1.95 (62.2)[e] 2.15[c] (56.5)[e] 2.47	hr ng mL^{-1} ng hr mL^{-1} ng hr mL^{-1} hr		
pMDI Administered as Azmacort® HFA 225, Aventis Pharma Contained microcrystalline suspension of triamcinolone acetonide in HFA 143-a *(uncertain formulation due to acquisitions)*	675 µg	t_{max} C_{max} AUC $t_{1/2}$	1.59 (57.6)[e] 1.70 (53.2)[e] 8.32[a] (53.7) [e] 8.71[c] (52.1)[e] 2.26	hr ng mL^{-1} ng hr mL^{-1} ng hr mL^{-1} hr	Healthy human volunteers (Plasma samples)	(43)

Table 1.1D: Properties of Inhaled Triamcinolone Acetonide (continued)

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters	Studied Population	Reference
Triamcinolone acetonide				

DPI
    Administered using Ultrahaler®, Aventis Pharma
    Contains micronized triamcinolone acetonide blended
    with lactose
	720 µg for DPI	Ratio of DPI to pMDI C_{max}
		    2.44 (75)[e]
		Ratio of DPI to pMDI AUC
		    1.96 (77)[e]
			Healthy human volunteers (Plasma samples)	(50)

pMDI Administered as Azmacort® HFA 225, Aventis Pharma Contained microcrystalline suspension of triamcinolone acetonide in HFA 143-a	450 µg for pMDI			

DPI
    Administered using Ultrahaler®, Aventis Pharma with
    oral charcoal
    Contains micronized triamcinolone acetonide blended
    with lactose
	720 µg for DPI	Ratio of DPI to pMDI C_{max}
		    1.56 (35)[e]
		Ratio of DPI to pMDI AUC
		    1.44 (42)[e]		

pMDI
    Administered as Azmacort® HFA 225, Aventis Pharma
    with oral charcoal
    Contained microcrystalline suspension of triamcinolone
    acetonide in HFA 143-a
	450 µg for pMDI			

The following caption applied for Tables 1.1A-1.1D:
Values are the geometric mean (95% confidence interval), mean ± standard deviation, or median. Unless otherwise specified, the units are as follows: t_{max} (hr), C_{max} (ng mL^{-1}), AUC (ng hr mL^{-1}), $t_{1/2}$ (hr), MRT (hr). DPI = Dry Powder Inhaler, pMDI = Pressurized Metered-dose Inhaler, MRT = Mean Residence Time, MAT: Mean Absorption Time , $T_{X\%}$ = Absorption time for X% of total absorbed dose.
[a]: AUC_{0-12}, [b]: AUC_{0-8}, [c]: $AUC_{0-\infty}$, [d]: 90% CI, [e]: values are mean (Coefficient of variation %), [f]: values are median (range), [g]: Not expressly reported by the authors. Values inferred from figures, tables, and methodological descriptions.

Table 1.2: Properties of Inhaled Antifungals

Table 1.2A: Properties of Inhaled Amphotericin B

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Amphotericin B						
Nebulized Suspension Administered as a nebulized suspension of pure amphotericin B in sterile water (5 mg in 5 mL)	5 mg	Fisoneb® (Ultrasonic Nebulizer)			Human patients with post-tuberculosis lung aspergilloma (Serum samples)	(76)
		t_{max}	0.5	hr		
		C_{max}	21.0 ± 1.4	ng mL^{-1}		
		DP100® (Ultrasonic Nebulizer)				
		t_{max}	3.5	hr		
		C_{max}	16.8 ± 6.9	ng mL^{-1}		
		Respirgard II® (Air-jet Nebulizer)				
		t_{max}	1.5	hr		
		C_{max}	5.7	ng mL^{-1}		
Nebulized Suspension Administered as a colloidal dispersion of AmB-d, Fungizone®, diluted with 5% glucose Contained amphotericin B, sodium deoxycholate, and sodium phosphates	5 mg	t_{max} C_{max} AUC	0.5 233.8 ± 138.3 481.8 ± 204.1	hr ng mL^{-1} ng hr mL^{-1}	Healthy adult sheep (Bronchial wash fluid samples)	(73)
	30 mg	t_{max} C_{max} AUC	0.5 217.7 ± 53.8 $1,199.0 \pm 163.8$	hr ng mL^{-1} ng hr mL^{-1}		
Nebulized Suspension Administered as a colloidal dispersion of AmB-d, Fungizone® Contained amphotericin B, sodium deoxycholate, and sodium phosphates	10 mg twice daily (Usual dose)	C_{1hr}	>200 to 900 (n=5) *Only 2 patients had detectible levels*	ng mL^{-1}	Long-term prophylaxis in human lung transplant patients (Plasma samples)	(71)
Nebulized Suspension Administered as a nebulized suspension of L-AmB, AmBisome® Contains amphotericin B intercalated into a liposomal membrane (hydrogenated soy phosphatidylcholine, cholesterol, distearoylphosphatidylglycerol, α-tocopherol) with sucrose and disodium succinate hexahydrate.	20 mg twice daily (Usual dose)	C_{1hr}	>200 (n=4)	ng mL^{-1}		

Table 1.2A: Properties of Inhaled Amphotericin B (continued)

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Amphotericin B						
Nebulized Suspension Administered as a colloidal dispersion of AmB-d, Fungizone® Contained amphotericin B, sodium deoxycholate, and sodium phosphates	30 mg	t_{sample} C_{plasma}	0.53 ± 0.17 23 ± 67^a	hr ng g^{-1}	Human lung transplant patients (Plasma, BAL, and lung tissue samples taken sequentially)	(72)
		t_{sample} C_{uBAL}	0.67 ± 0.14 680 ± 360	hr ng g^{-1}		
		t_{sample} C_{lBAL}	0.73 ± 0.13 500 ± 310	hr ng g^{-1}		
		t_{sample} C_{lung}	0.83 ± 0.10 $29,600$ [b]	hr ng g^{-1}		
Nebulized Suspension Administered as a colloidal dispersion of AmB-d, Fungizone® Contained amphotericin B, sodium deoxycholate, and sodium phosphates	6 mg	t_{max} C_{max}	4 15,750 (10,930–20,580)	hr ng mL^{-1}	Human lung transplant patients (BAL fluid sample)	(74)
Nebulized Suspension Administered as a colloidal dispersion of AmB-d, Fungizone® Contained amphotericin B, sodium deoxycholate, and sodium phosphates	35.4 ± 6.2 mg	C_{max}	$22,050 \pm 5,581$	ng g^{-1}	Healthy rats (Lung tissue samples)	(75)
Nebulized Suspension Administered as a nebulized suspension of L-AmB, AmBisome® Contains amphotericin B intercalated into a liposomal membrane (hydrogenated soy phosphatidylcholine, cholesterol, distearoylphosphatidylglycerol, α-tocopherol) with sucrose and disodium succinate hexahydrate.	57.2 ± 10.2 mg	C_{max}	$21,650 \pm 1,741$	ng g^{-1}		

Values are the median or mean ± standard deviation. AmB-d = Amphotericin B deoxycholate, L-AmB = Liposomal amphotericin B, t_{sample}: Time after completion of dose until sample was taken (hr), $C_{plasma, uBAL, lBAL, lung}$: Concentration in the plasma, upper lung bronchoalveolar lavage fluid, lower lung bronchoalveolar lavage fluid, and lung tissue respectively. [a]: Only 1/8 samples had a detectible amphotericin B concentration (value for n=1). [b]: Only 2/6 samples had detectible amphotericin B concentrations (value for n=2)

Table 1.2B: Properties of Inhaled Itraconazole

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters				Studied Population	Reference
Itraconazole							
Nebulized Suspension Administered as a nebulized suspension of EPAS itraconazole Contains nanoparticulate itraconazole with polysorbate 20	10 mg	t_{max} C_{max} AUC $t_{1/2}$	0.5 16.8 86.8 6.7		hr $\mu g\ g^{-1}$ $\mu g\ hr\ g^{-1}$ hr	Healthy mice (Lung tissue samples)	(83)
Nebulized Suspension Administered as a nebulized suspension of SFL itraconazole Contains nanoparticulate itraconazole with polysorbate 80		t_{max} C_{max} AUC $t_{1/2}$	1.0 4.8 15.8 2.3		hr $\mu g\ g^{-1}$ $\mu g\ hr\ g^{-1}$ hr		
Nebulized Suspension Administered as a nebulized suspension of SFL itraconazole Contains nanoparticulate itraconazole with polysorbate 80 and poloxamer 407		t_{max} C_{max} AUC $t_{1/2}$	1.0 13.4 79.8 5.5		hr $\mu g\ g^{-1}$ $\mu g\ hr\ g^{-1}$ hr		
Nebulized Suspension Administered as a nebulized suspension of EPAS itraconazole Contains nanoparticulate itraconazole with polysorbate 80 and poloxamer 407	4.8 mg	t_{max} C_{max} AUC $t_{1/2}$	Lung 0.5 25.9 70.9 7.2	Plasma 0.44 	hr $\mu g\ g^{-1}$ $\mu g\ hr\ g^{-1}$ hr	Aspergillus infected mice (Lung and plasma samples)	(82)
Nebulized Suspension Administered as a nebulized suspension of SFL itraconazole Contains nanoparticulate itraconazole with polysorbate 80 and poloxamer 407	4.8 mg	t_{max} C_{max} AUC $t_{1/2}$	0.5 5.3 28.0 2.9	 0.44 	hr $\mu g\ g^{-1}$ $\mu g\ hr\ g^{-1}$ hr		
Nebulized Suspension Administered as a nebulized suspension of SFL itraconazole Contains nanoparticulate itraconazole with polysorbate 80 and poloxamer 407	5.7 mg	t_{max} C_{max} AUC $t_{1/2}$	Lung 1.0 13.4 85.8 5.5 hr	Plasma 5.4 0.12 1.69 3.7 hr	hr $\mu g\ g^{-1}$ $\mu g\ hr\ g^{-1}$ hr	Healthy mice (Lung and plasma samples)	(84)

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Table 1.2B: Properties of Inhaled Itraconazole (continued)

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters				Studied Population	Reference
Itraconazole							
Nebulized Suspension	7.1 mg		Lung	Plasma		Healthy mice	(80)
Administered as a nebulized suspension of URF itraconazole		t_{max}	0.5	2.0	hr	(Lung and plasma samples)	
		C_{max}	21.1	1.64	$\mu g\ g^{-1}$		
Contains nanoparticulate itraconazole with mannitol and lecithin		AUC	149.9	5.6	$\mu g\ hr\ g^{-1}$		
		$t_{1/2}$	7.4	3.6	hr		

Values are the geometric mean.
EPAS = Evaporative precipitation into aqueous solution (crystalline nanoparticles)
SFL = Spray freezing into liquid (amorphous nanoparticles)
URF = Ultra-rapid freezing (amorphous nanoparticles)

Table 1.3: Properties of Inhaled Oligopeptides

Table 1.3A: Properties of Inhaled Cyclosporine

Delivery Method and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Cyclosporine						
Nebulized Solution	1 mg kg^{-1}	$C_{lung, trough}$	2.56 ± 1.33	µg g^{-1}	Rats having received a lung transplant (Whole blood samples)	(94)
Administered as a solution of cyclosporine in 100% alcohol (40 mg mL^{-1})		$C_{blood, trough}$	0.16 ± 0.08	µg mL^{-1}		
		L/B Ratio[a]	16.0			
No other excipients were used	2 mg kg^{-1}	$C_{lung, trough}$	4.41 ± 1.50	µg g^{-1}		
		$C_{blood, trough}$	0.27 ± 0.10	µg mL^{-1}		
		L/B Ratio[a]	16.6			
	3 mg kg^{-1}	$C_{lung, trough}$	12.35 ± 8.83	µg g^{-1}		
		$C_{blood, trough}$	0.73 ± 0.22	µg mL^{-1}		
		L/B Ratio[a]	17.0			
Nebulized Solution			Lung	Blood	Healthy rats (Lung and blood samples)	(95)
Administered as a solution of cyclosporine in 100% alcohol (40 mg mL^{-1})	3 mg kg^{-1}	t_{max}[c]	0.5	0.5	hr	
		C_{max}[c]	>100	>2.5	µg g^{-1}	
No other excipients were used		AUC	413.32	15.16	µg hr g^{-1}	
		L/B Ratio[b]	27.3			
	5 mg kg^{-1}	t_{max}[c]	1.0	1.0	hr	
		C_{max}[c]	>175	>3.5	µg mL^{-1}	
		AUC	477.96	27.45	µg hr mL^{-1}	
		L/B Ratio[b]	17.4			
Nebulized Solution			Lung	Blood	Healthy rats (Lung and blood samples)	(93)
Administered as a solution of cyclosporine in 95% alcohol (33.3 to 83.3 mg mL^{-1})	10 mg kg^{-1}	AUC	200	253	µg hr g^{-1}	
	25 mg kg^{-1}	AUC	588	684	µg hr g^{-1}	
No other excipients were used						

Table 1.3A: Properties of Inhaled Cyclosporine (Continued)

Delivery Method and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Cyclosporine						

Nebulized Solution
  Administered as a solution of cyclosporine in propylene glycol (62.5 mg mL^{-1})
  No other excipients were used
	300 mg	t_{max}
		C_{max}
		C_{trough}
			1.0
			0.23 ± 0.13
			0.02 ± 0.02
				hr
				µg mL^{-1}
				µg mL^{-1}
					Human lung transplant recipients with persistent acute rejection (Whole blood samples)	(97)

Nebulized Solution
  Administered as a solution of cyclosporine in propylene glycol (62.5 mg mL^{-1})
  No other excipients were used
	300 mg	t_{max}
		C_{max}
		AUC
		$t_{1/2}$
			0.68 ± 0.30
			0.21 ± 0.09
			1.03 ± 0.43
			40.7 ± 17.7
				hr
				µg mL^{-1}
				µg hr mL^{-1}
				hr
					Human lung transplant recipients (Whole blood samples)	(96)

Table 1.3A: Properties of Inhaled Cyclosporine (Continued)

Delivery Method and Formulation	Dose	Pharmacokinetic Parameters				Studied Population	Reference
Cyclosporine							
Nebulized Solution			Lung	Blood		Healthy rats	(98)
Administered as a solution of cyclosporine in propylene glycol (62.5 mg mL^{-1}) No other excipients were used	8.4 mg kg^{-1}	t_{max}	4.6	0.1	hr	(Lung and blood samples)	
		C_{max}	57	1.9	µg g^{-1}		
		AUC	386	14.2	µg hr g^{-1}		
		$t_{1/2}$	2.2	10.0	hr		
	56.2 mg kg^{-1}	t_{max}	0.1	0.3	hr		
		C_{max}	121	2.9	µg g^{-1}		
		AUC	771	48.2	µg hr g^{-1}		
		$t_{1/2}$	5.2	18.5	hr		
	112.6 mg kg^{-1}	t_{max}	0.1	0.6	hr		
		C_{max}	150	5.0	µg g^{-1}		
		AUC	1248	90.3	µg hr g^{-1}		
		$t_{1/2}$	5.8	20.1	hr		
	4.4 mg kg^{-1}	t_{max}	0.1		hr	Healthy beagle dogs	
		C_{max}	0.28		µg mL^{-1}	(Whole blood samples)	
		AUC	59.2		µg hr mL^{-1}		
		$t_{1/2}$	3.6		hr		
	7.7 mg kg^{-1}	t_{max}	0.6		hr		
		C_{max}	0.36		µg mL^{-1}		
		AUC	109.4		µg hr mL^{-1}		
		$t_{1/2}$	4.0		hr		
	9.7 mg kg^{-1}	t_{max}	2.0		hr		
		C_{max}	0.45		µg mL^{-1}		
		AUC	174.0		µg hr mL^{-1}		
		$t_{1/2}$	3.9		hr		
Nebulized Liposomal Suspension	25 mg		Lung	Blood		Healthy Balb/c mice	(100)
Administered as a dilauroylphosphatidylcholine (DLPC) cyclosporine multi-lamellar liposome Contained cyclosporine in a DLPC multi-lamellar liposome of dissolved in ultrapure water		C_{max}	5.0 ± 1.5	0.05 ± 0.05	µg g^{-1}	(Lung and blood samples)	

Table 1.3A: Properties of Inhaled Cyclosporine (Continued)

Delivery Method and Formulation	Dose	Pharmacokinetic Parameters				Studied Population	Reference
Cyclosporine							
Nebulized Liposomal Suspension Administered as a dilauroylphosphatidylcholine (DLPC) cyclosporine multi-lamellar liposome Contained cyclosporine in a DLPC multi-lamellar liposome of dissolved in ultrapure water	25 mg		Lung	Blood		Healthy mongrel dogs (Lung and blood samples)	(99)
		t_{max}	0.5	0.25	hr		
		C_{max}	7.5^c	<0.3	$\mu g\ g^{-1}$		
Nebulized Suspension Administered as a nebulized suspension of CP cyclosporine Contained nano-scale cyclosporine with polysorbate 80	3.5 mg kg^{-1}		Lung	Blood		Healthy mice (Lung and blood samples)	(101)
		t_{max}	1.0	3.7	hr		
		C_{max}	10.5	0.37	$\mu g\ g^{-1}$		
		AUC	144.4	9.7	$\mu g\ hr\ g^{-1}$		
		$t_{1/2}$	9.6	18.2	hr		

Values are the geometric mean or the mean ± standard deviation.

[a]: L/B Ratio = Ratio of lung C_{max} to blood C_{max}

[b]: L/B Ratio = Ratio of lung AUC to blood AUC

[c]: Values not expressly reported by the authors. Values inferred from figures, tables, and methodological descriptions.

Table 1.3B: Properties of Inhaled FK224

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
FK224						
pMDI Administered as a suspension of FK224 and β-cyclodextrin Contains micronized FK224/β-cyclodextrin in various ratios with soybean lecithin in a mixture of CFC-11, CFC-12, and CFC-114	5 mg kg^{-1}	FK224 : β-CD :: 1 : 0			Healthy rats (Plasma samples)	(105)
		t_{max}	1.0 ± 0.3	hr		
		C_{max}	0.05 ± 0.03	µg mL^{-1}		
		AUC	0.32 ± 0.13	µg hr mL^{-1}		
		FK224 : β-CD :: 1 : 1				
		t_{max}	0.25 ± 0.1	hr		
		C_{max}	0.17 ± 0.09	µg mL^{-1}		
		AUC	2.15 ± 0.25	µg hr mL^{-1}		
		FK224 : β-CD :: 1 : 7				
		t_{max}	0.25 ± 0.2	hr		
		C_{max}	0.43 ± 0.22	µg mL^{-1}		
		AUC	6.76 ± 0.92	µg hr mL^{-1}		
pMDI Administered as a suspension of FK224 and β-cyclodextrin in propellant Contains micronized FK224/β-cyclodextrin in a 1:1 ratio with soybean lecithin in a mixture of CFC-11, CFC-12, and CFC-114	1 mg	t_{max} C_{max} AUC	2.7 ± 1.3 0.07 ± 0.02 0.13 ± 0.05	hr ng mL^{-1}ng hr mL^{-1}	Healthy human volunteers (Plasma samples)	(106)
	4 mg	t_{max} C_{max} AUC	3.0 ± 0.8 0.36 ± 0.07 3.16 ± 0.80	hr ng mL^{-1}ng hr mL^{-1}		
	8 mg	t_{max} C_{max} AUC	2.7 ± 0.6 0.55 ± 0.09 5.88 ± 1.57	hr ng mL^{-1}ng hr mL^{-1}		
DPI Administered using filled capsules and with a Spinhaler® Contained micronized FK224/β-cyclodextrin in a 1:1 ratio blended with lactose	4 mg	t_{max} C_{max} AUC	2.2 ± 1.2 1.36 ± 0.17 14.44 ± 2.69	hr ng mL^{-1}ng hr mL^{-1}		
	10 mg	t_{max} C_{max} AUC	0.7 ± 0.1 3.66 ± 0.56 30.51 ± 2.86	hr ng mL^{-1}ng hr mL^{-1}		

Values are the mean ± standard deviation.

Table 1.4: Properties of Inhaled Fentanyl

Delivery Device and Formulation	Dose	Pharmacokinetic Parameters			Studied Population	Reference
Fentanyl						

Nebulized Suspension
  Administered as a mixture of free (50%) and liposome-encapsulated (50%) fentanyl (FLEF)
  Contained free fentanyl and liposomal encapsulated (phospholipon 90-G and cholesterol) fentanyl in sterile water
	2 mg	t_{max}
		C_{max}
			0.38 ± 0.11
			1.2 ± 0.4
				hr
				ng mL^{-1}
					Healthy human volunteers
					(Plasma samples)
						(111, 112)

	$\leq$1 mg	t_{max} C_{max}	0.25 2.53	hr ng mL^{-1}		

pMDI
  Administered as a fentanyl solution in propellant using a pMDI fitted with SmartMist™ (breath actuated adapter)
  Contained fentanyl base solution in a mixture of CFC-11 and CFC-12 with sorbitan trioleate
	100 µg	t_{max}
		C_{max}
		AUC
			0.12 ± 0.08
			1.5 ± 1.5
			15.4 ± 5.57
				hr
				ng mL^{-1}
				ng hr mL^{-1}
					Healthy human volunteers
					(Plasma samples)
						(113)

	200 µg	t_{max} C_{max} AUC	0.12 ± 0.12 1.9 ± 0.9 19.0 ± 7.90	hr ng mL^{-1} ng hr mL^{-1}		
	300 µg	t_{max} C_{max} AUC	0.10 ± 0.07 4.2 ± 2.7 27.4 ± 24.0	hr ng mL^{-1} ng hr mL^{-1}		

DPI
  Administered as fentanyl-lactose blend in Taifun® device
  Contained fentanyl blended with lactose carrier particle
	200 µg	t_{max}
		C_{max}
			0.017
			0.94
				hr
				ng mL^{-1}
					Healthy human volunteers
					(Plasma samples)
						(114, 115)

Values are the mean or mean ± standard deviation.

Table 2.1: Single-dose Pharmacokinetic Parameters for Inhaled Voriconazole

Parameter		High-flow Rate	Low-flow Rate	Units
Air Flow Rate		5.2-5.4	1	(L/min)
Mouse Mass		31.8	21.8	(g)
Lung Mass		0.23	0.17	(g)
Vfend Conc.		6.23	6.9	(mg/mL)
T_{max}	Lung	10	30	(min)
	Plasma	20	30	(min)
C_{max}	Lung	1.6 ± 0.17	11.0 ± 1.6	(μg/g)
	Plasma	1.2 ± 0.25	7.1 ± 0.68	(μg/mL)
AUC_{0-6}	Lung	205.3	2408	(μg min/g)
	Plasma	136.4	1549.8	(μg min/mL)

The inhaled dose was 5 mL of 6.25 mg/mL voriconazole solution nebulized over 20 minutes to mice in a nose-only dosing chamber. All values are the reported average except for the observed t_{max}. The values for C_{max} are the average $\pm$ standard deviation (N=2). The AUC was calculated for concentration versus time profiles from 0-6 hours by the trapezoidal method.

Table 2.2: Multi-dose Pharmacokinetic Parameters for Inhaled Voriconazole

Day	Lung Voriconazole Concentration (µg/g wet lung weight)		Plasma Voriconazole Concentration (µg/mL)	
	Peak	Trough	Peak	Trough
3	-	-*	-	0.22 ± 0.08
5	6.73 ± 3.64	-*	2.32 ± 1.52	0.28 ± 0.14
10	-	0.11 ± 0.09	-	0.18 ± 0.09
12	-	0.19 ± 0.23	-	0.32 ± 0.08

Inhaled voriconazole was administered at 08:00 and 16:00 for 12 days. The inhaled dose was 5mL of 6.25mg/mL voriconazole solution nebulized over 20 minutes to mice in a nose-only dosing chamber. Trough values were determined from samples taken immediately before the 08:00 dose while peak samples were taken 30 minutes after the 08:00 dose. Average values are reported ± standard deviation for N=6 mice per value.
* = Values were below the lower limit of quantification.

Table 3.1: Pulmonary Fungal Burden in Infected Mice

Group	1 hr SAC	Control	Voriconazole	Amphotericin
Median $\log_{10}$ CFU/g (range)	3.99 (3.55 – 4.45)	4.41 (3.56 – 4.91)	4.21 (3.62 – 4.68)	4.33 (3.59 – 5.07)
Median $\log_{10}$ CE/g (range)	---	5.66 (4.47 – 5.95)	5.24 (4.45 – 5.98)	5.56 (5.08 – 5.89)

Pulmonary fungal burden for mice that received aerosolized voriconazole (6.25 mg/mL twice daily), amphotericin B deoxycholate, or control (aerosolized sulfobutyl ether-β-cyclodextrin sodium 100 mg/mL twice daily) and challenged by pulmonary inoculation with *A. fumigatus*. (1 hr SAC = Animals sacrificed 1 hour after inoculation)

Table 4.1: Multiple Dose Pharmacokinetic Profile of Inhaled Vorciconazole in Rats (BID Dosing)

Day	Group	Voriconazole Peak		Voriconazole Trough	
		Lung (µg/g)	Plasma (µg/ml)	Lung (µg/g)	Plasma (µg/ml)
3	High dose	0.85 ± 0.63	0.58 ± 0.30	0.042 ± 0.002	0.011 ± 0.004
	Low dose	0.38 ± 0.01	0.09 ± 0.06	0.040 ± 0.004	0.010 ± 0.003
14	High dose				0.023[a]
	Low dose				0.029[a]
21	High dose				0.030 ± 0.011
	Low dose				0.027 ± 0.003

Values are the mean ± standard deviation.
[a]: Value represents the observed concentration (N=1)

Table 4.2: Laboratory Values Following Multiple Doses of Inhaled Voriconazole in Rats

Table 4.2A: Hepatic Function

	NT	C	LD	HD
ALP (U/L)	188 ± 40	216* ± 52	218* ± 51	213* ± 41
ALT (U/L)	80 ± 54	79 ± 53	96 ± 78	72 ± 31
AST (U/L)	147 ± 123	132 ± 130	164 ± 160	110 ± 58
Albumin (g/dL)	3.2 ± 0.1	3.3* ± 0.2	3.3* ± 0.2	3.3* ± 0.2
Bilirubin (mg/dL)	0.1 ± 0	0.1* ± 0	0.1* ± 0	0.1* ± 0

Table 4.2B: Renal Function

	NT	C	LD	HD
Protein (g/dL)	6.2 ± 0.3	6.1 ± 0.4	6.2 ± 0.3	6.2 ± 0.4
Globulin (g/dL)	3.1 ± 0.3	2.8* ± 0.3	2.9* ± 0.3	2.9* ± 0.3
BUN (mg/dL)	21 ± 2	19* ± 2	21 ± 5	21 ± 3
Creatinine (mg/dL)	0.5 ± 0	0.5 ± 0.1	0.5 ± 0.1	0.4* ± 0.1

Table 4.2C: Blood Chemistry

	NT	C	LD	HD
Cholesterol (mg/dL)	94 ± 10	95 ± 15	95 ± 13	93 ± 19
Glucose (mg/dL)	319 ± 101	329 ± 143	334 ± 154	291 ± 116
Calcium (mg/dL)	11.2 ± 0.5	11.5* ± 0.6	11.4 ± 0.6	11.3 ± 0.6
Phosphorous (mg/dL)	9.0 ± 1.3	9.6 ± 2.1	8.9 ± 1.9	8.0* ± 1.3
Chloride (mg/dL)	99 ± 2	100 ± 2	101* ± 2	101* ± 2
Potassium (mg/dL)	6 ± 1	6 ± 1	7 ± 2	6 ± 1
Sodium (mg/dL)	142 ± 2	144* ± 2	144* ± 3	145* ± 2

Table 4.2: Laboratory Values Following Multiple Doses of Inhaled Voriconazole in Rats (continued)

Table 4.2D: CBC

	NT	C	LD	HD
WBC (10^3/μL)	5.3 ± 2.3	4.1* ± 1.6	4.7 ± 2	5.2 ± 1.9
RBC (10^6/μL)	8.2 ± 1.3	8.0 ± 0.8	7.9 ± 0.8	8.0 ± 0.7
HGB (g/dL)	15.8 ± 2.3	14.7* ± 1.5	14.7* ± 1.2	15.0 ± 1.0
HCT (%)	50.2 ± 7.2	49.5 ± 5.1	49.1 ± 5.3	48.2 ± 7.6
Platelet (10^3/μL)	657 ± 240	767 ± 235	685 ± 264	842* ± 182

Values are the mean ± standard deviation.
NT = No Treatment Group (N=20), C = Inhaled Normal Saline Group (N=30), LD = Inhaled Low Dose Voriconazole Group (N=30), HD = Inhaled High Dose Voriconazole Group (N=30)

*: Statistically significant compared to NT (p-value <0.05).

Table 5.1: Pharmacokientic Parameters of Voriconaozle in Lung Tissue and Plasma
Following a Single Inhaled Dose

Pharmacokinetic Parameter	Lung	Plasma
t_{max}	0.17 hr	0.17 hr
C_{max}	9.98 ± 0.94 µg/g	6.57 ± 3.04 µg/mL
AUC_{0-24}	44.4 µg hr/g	30.2 µg hr/mL
P_{l-p}	1.47	
k_e *(lung elimination)*	0.263 hr^{-1}	
A *(plasma absorption)*		0.274 hr^{-1}
B *(plasma elimination)*		0.057 hr^{-1}
$t_{1/2,\,e}$	2.63 hr	
$t_{1/2,\,\alpha}$		2.53 hr
$t_{1/2,\,\beta}$		12.11 hr

Figures
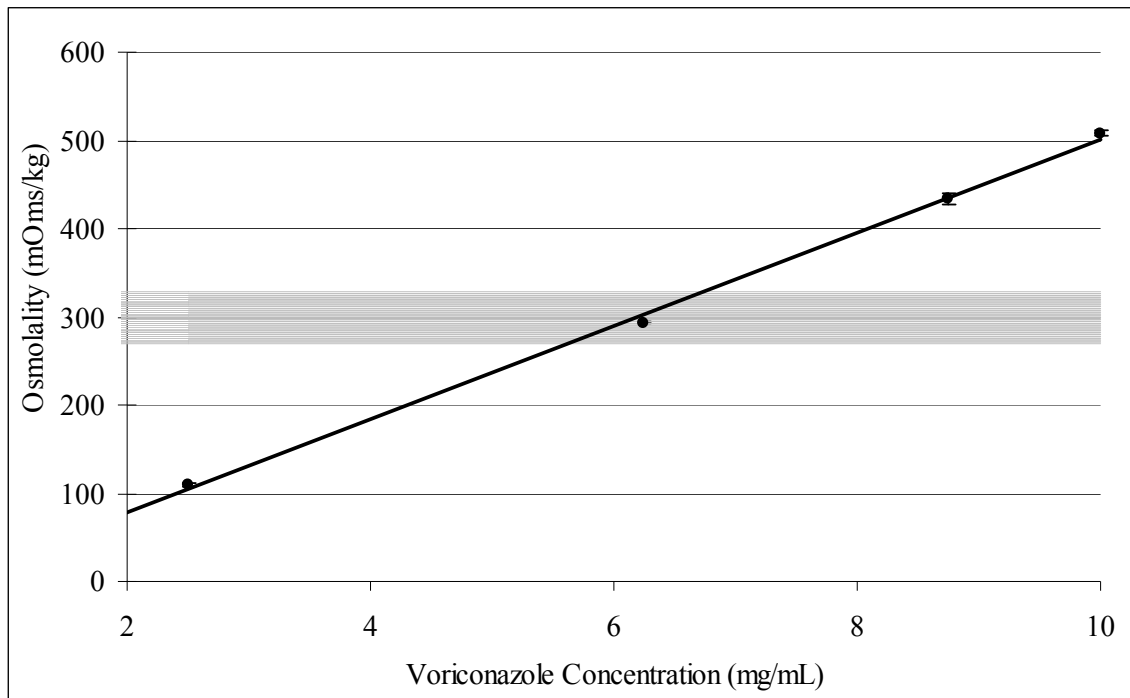
Figure 2.1: Osmolality of Voriconazole and Cyclodextrin Solutions

Osmolality of aqueous solutions in a fixed mass ratio of 1 to 16 of voriconazole and sulfobutyl ether-β-cyclodextrin. The shaded region indicates the limits of isotonicity. Error bars represent one standard deviation (N=10). The fitted line has a correlation coefficient of 0.998.

(A)

(B)
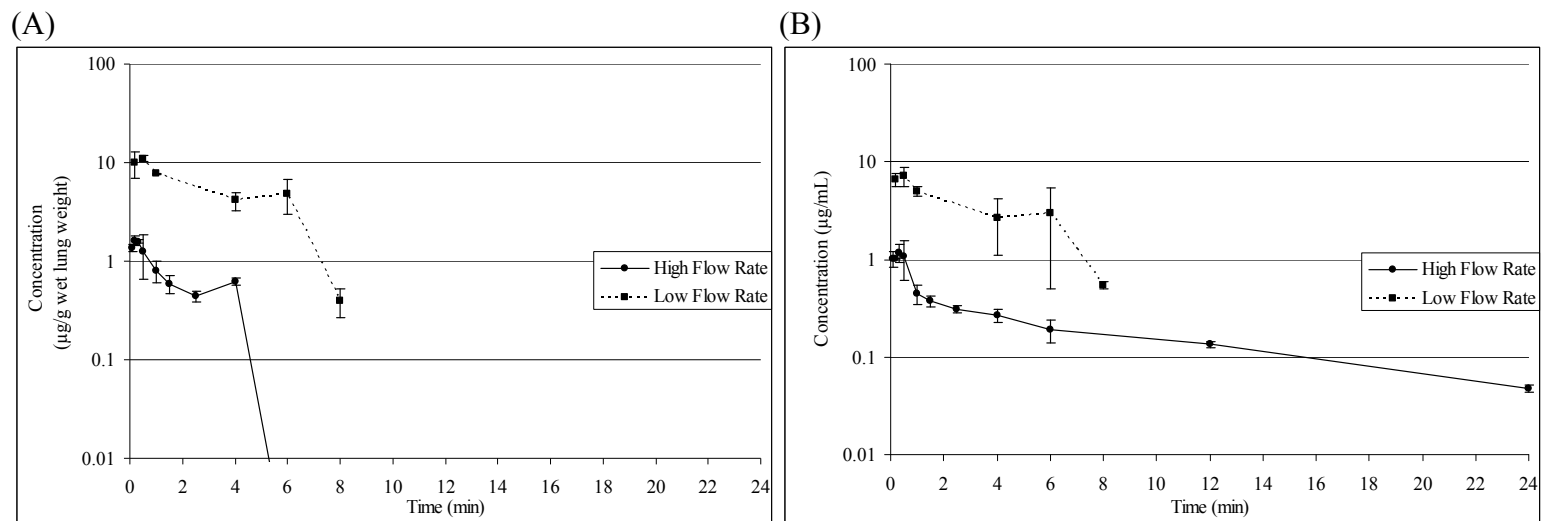
Figure 2.2: Pharmacokinetic Profile of Voriconazole in Lung Tissue and Plasma Following Inhalation

Pharmacokinetic profile of voriconazole in lung tissue (A) and plasma (B) following a single inhaled dose of aqueous voriconazole solution. The inhaled dose was 5 mL of 6.25 mg/mL voriconazole solution nebulized over 20 minutes to mice in a nose-only dosing chamber. Errors bars represent one standard deviation (N=2, except N=4 for 1 hour time point for low flow rate group and N=1 for 10 minute time point for low-flow rate group). Voriconazole was undetectable in lung tissue 6, 12, and 24 hours after the completion of nebulization.
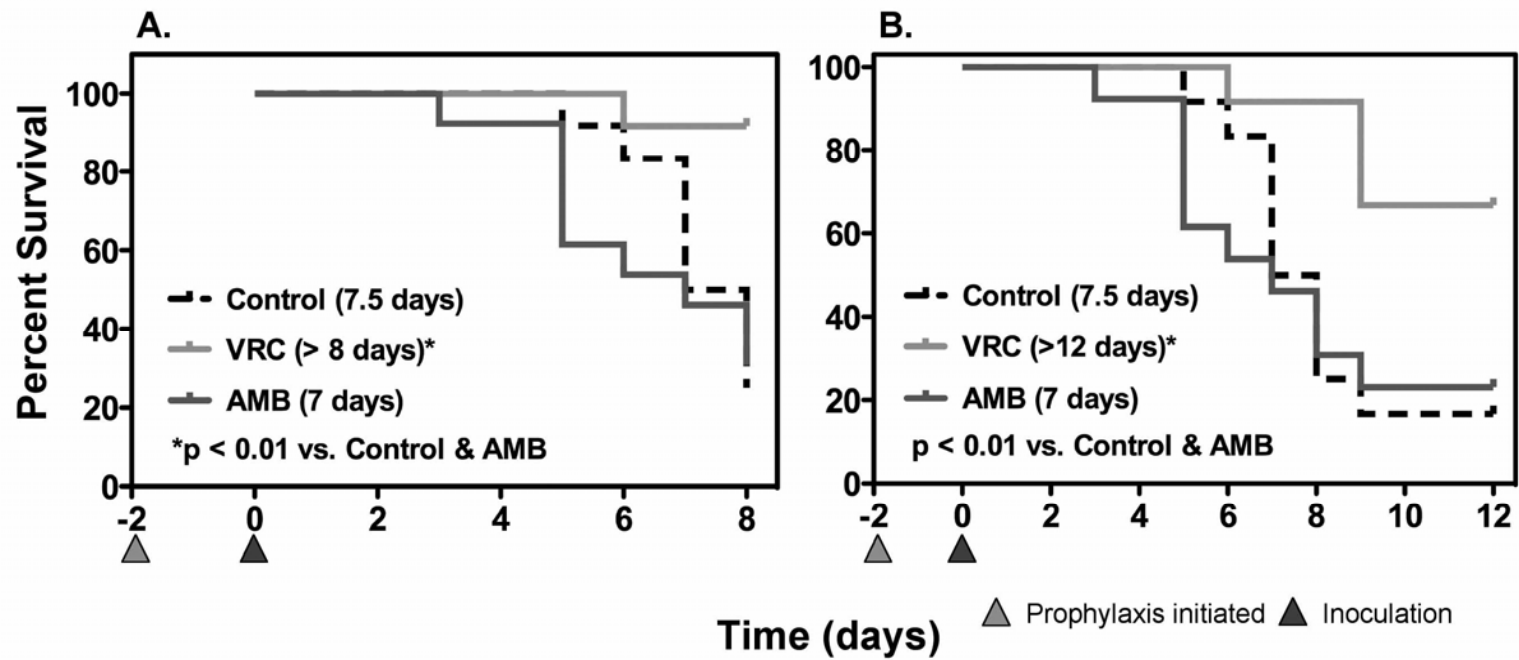
Figure 3.1: Survival in Infected Mice

Survival curves for immunosuppressed mice that received aerosolized voriconazole (6.25 mg/mL twice daily), amphotericin B deoxycholate, or control (aerosolized sulfobutyl ether-β-cyclodextrin sodium 100 mg/mL twice daily) and challenged by pulmonary inoculation with *A. fumigatus*. (A) Survival on therapy (day 7; N = 24 per study group). (B) Survival after therapy discontinued (N = 12 per study group).

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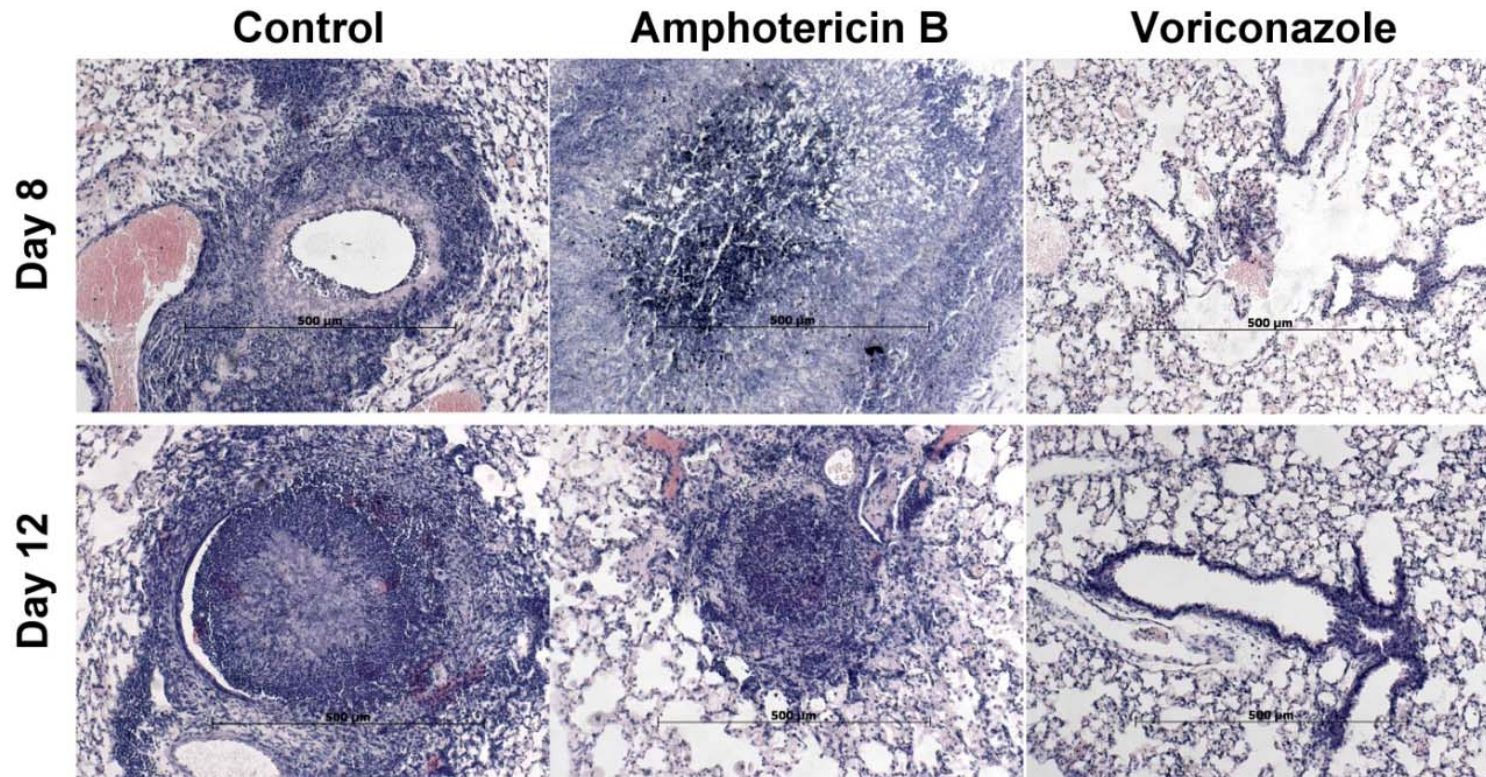
Figure 3.2: Pulmonary Histopathology Images from Infected Mice

Histopathology of lungs from mice on days 8 (A, B, and C) and 12 (E, F, and G) post-inoculation that received aerosolized aerosolized sulfobutyl ether-β-cyclodextrin sodium (A & D), intraperitoneal amphotericin B deoxycholate (B & E), or aerozolied voriconazole (C & F). Lung sections were stained with hemotoxylin and eosin and viewed by light microscopy at 20X magnification.
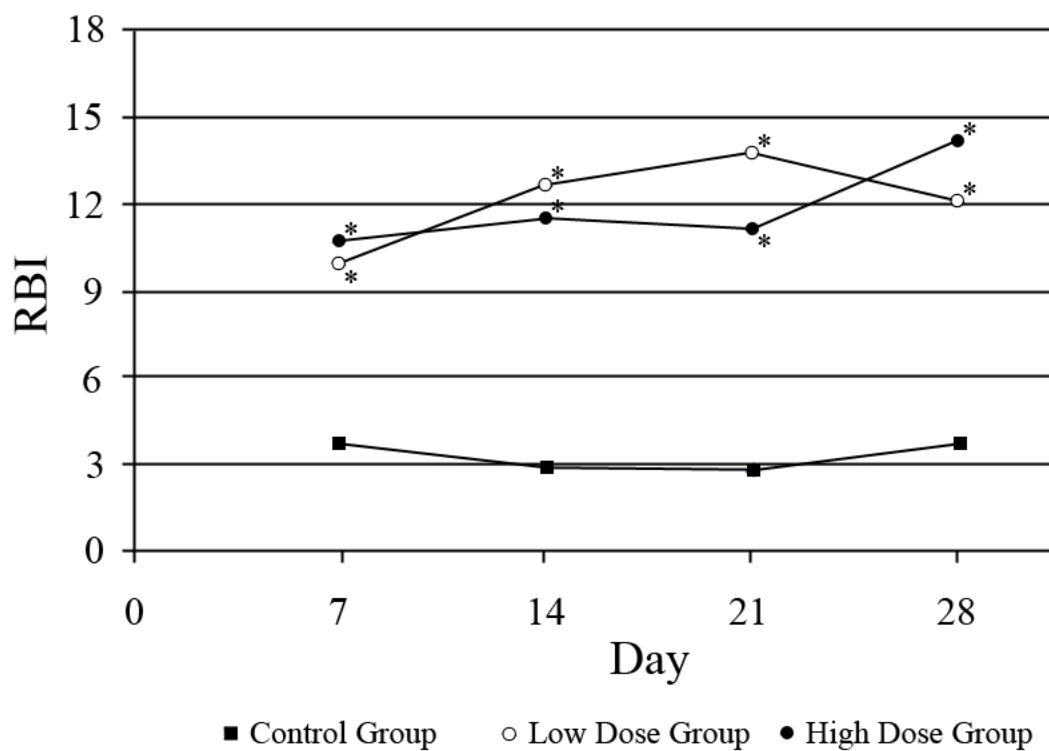
Figure 4.1: Respiratory Bronchiole Index

Rats received inhaled voriconazole BID for 21 days followed by a 7-day recovery period for a total of 28 days. RBI = Respiratory Bronchiole Index. *: Statistically significant difference compared to Control Group (p-value <0.05). Error bars omitted due for clarity in interpreting the figure.
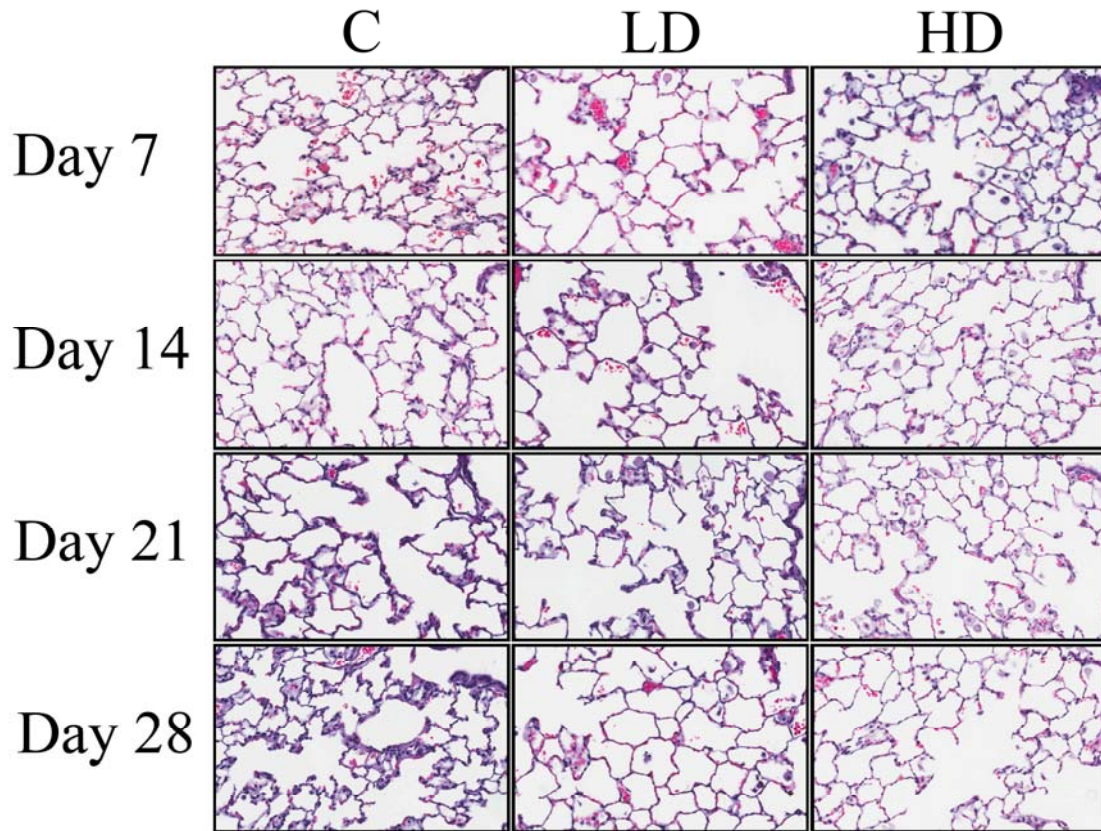
Figure 4.2: Representative Lung Histopathology Images Following Inhalation of
Voriconazole

C = Control Group, LD = Low Dose Group, HD = High Dose Group.
Representative images are of alveolar regions near the respiratory bronchiole
duct. Free alveolar macrophages are visible in several images and demonstrate
the variability observed between treatment groups as well as the duration of
treatment or recovery.
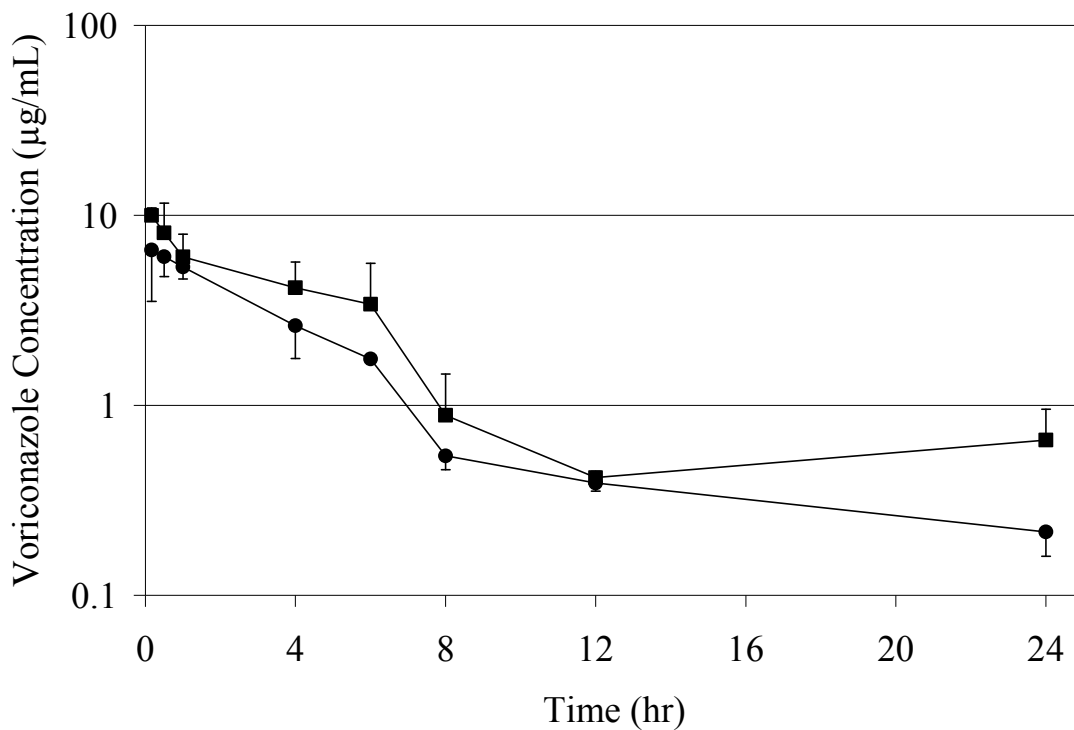
Figure 5.1: Single Dose Pharamcokinetic Profile in Lung Tissue and Plasma of Mice
Following a Single Inhaled Dose

Pharmacokinetic profile following a single dose of inhaled voriconazole in lung
tissue and plasma. Log-scaled drug concentrations are represented in units of
µg/g wet lung weight for lung tissue (■ = Mouse Lung) and µg/mL for plasma (●
= Mouse Plasma). One-tailed error bars represent one standard deviation and
were used for simplicity in interpreting the figure.

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Tissue Compartment   Central Compartment

1    $k_e \approx \alpha$ 2 β
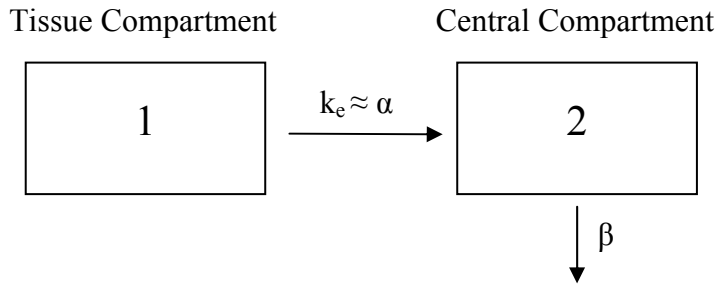
Figure 5.2: Two-compartment Pharmacokientic Model

Two-compartment pharmacokinetic model used to evaluate voriconazole in lung tissue and plasma. Lung tissue was assumed to correspond to the tissue compartment (1) and was the site of drug administration. Drug diffusion from the tissue compartment was assumed to be unidirectional elimination with rate constant k_e. Plasma was assumed to represent the central compartment (2) and was the site of drug absorption, α, and elimination, β.
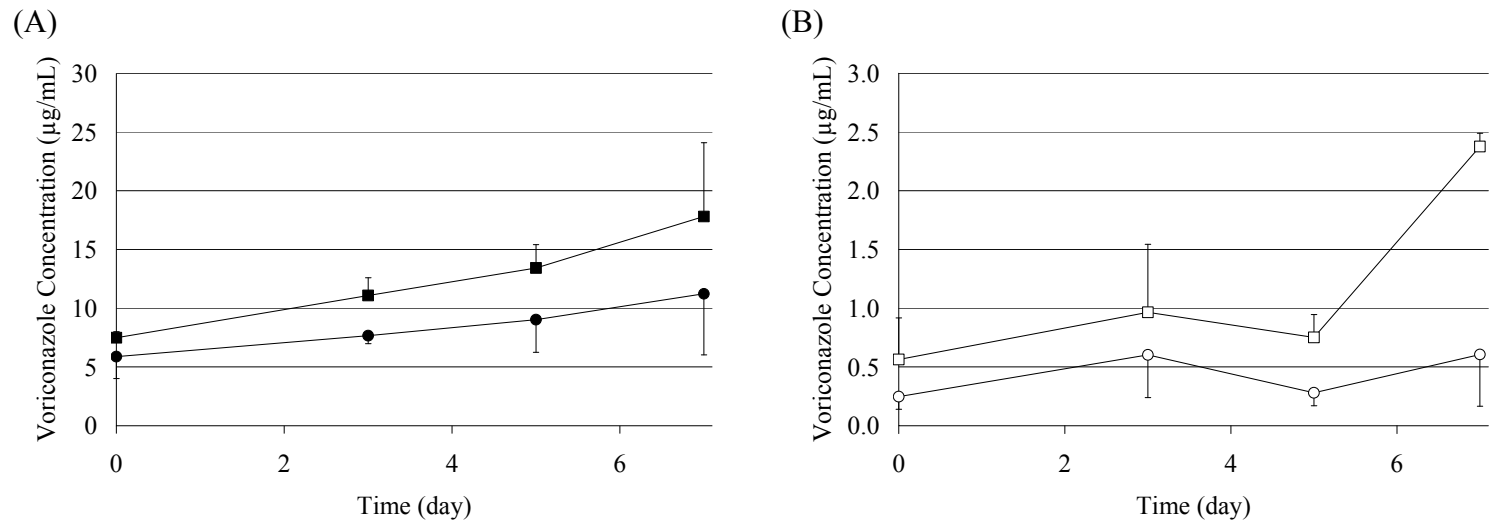
(A)

(B)

Figure 5.3: Peak and Trough Lung and Plasma Voriconazole Concentrations in Mice and Rats Following Multiple Inhaled Doses

Pharmacokinetic profiles of voriconazole in mouse and rat lung tissue and plasma following multiple doses administered every 12 hours. Figure 3A represents peak drug concentrations in mice while Figure 3C represents trough concentrations (■ = Mouse Lung, ● = Mouse Plasma). Figures 3B and 3D represent peak and trough voriconazole concentrations, respectively, in rats (□ = Rat Lung, ○ = Rat Plasma). One-tailed error bars represent one standard deviation and were used for simplicity in interpreting the figure.

(C)
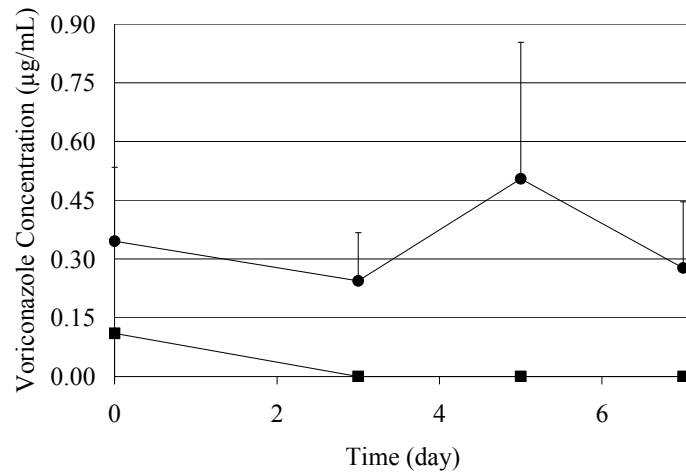
(D)
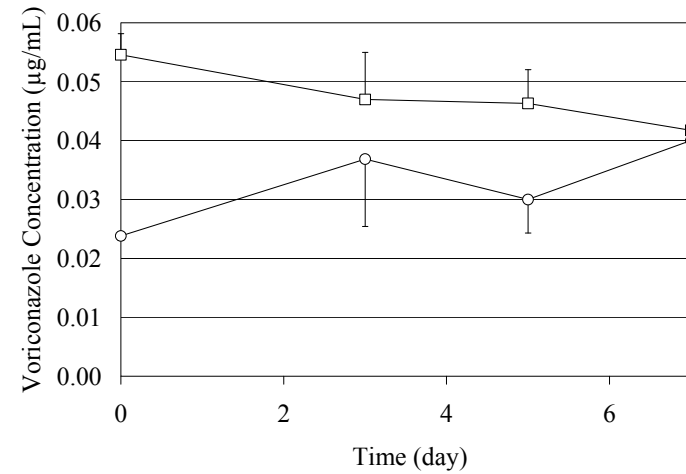
Figure 5.3: Peak and Trough Lung and Plasma Voriconazole Concentrations in Mice and Rats Following Multiple Inhaled
Doses (continued)

Pharmacokinetic profiles of voriconazole in mouse and rat lung tissue and plasma following multiple doses administered every 12 hours. Figure 3A represents peak drug concentrations in mice while Figure 3C represents trough concentrations (■ = Mouse Lung, ● = Mouse Plasma). Figures 3B and 3D represent peak and trough voriconazole concentrations, respectively, in rats (□ = Rat Lung, ○ = Rat Plasma). One-tailed error bars represent one standard deviation and were used for simplicity in interpreting the figure.

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Appendices

APPENDIX A: VALIDATION OF A 6-PORT NOSE-ONLY DOSING CHAMBER

A.1. History

Previous to the studies in Chapter 2, only 4 mice had been exposed in the dosing chamber to the aerosolized droplets.

A.2. Methods

Construction of a 6-port chamber: Contracted with the Chemistry & Biochemistry machine shop (WEL 2.140) to construct a 6-port chamber.

In each port, placed a #6 rubber stopper with a small hole drilled down the center.

Nebulized theophylline in tap-water solution over 20 minutes.

Using a 19G needle on a 5mL syringe at each port, serially withdrew 5mL samples of the nebulized aerosol over 2 seconds per port.

Slowly expelled and rinsed each syringe into and with 5mL water

Analyzed drug concentration using UV-vis

A.3. Results

The 6-port chamber has low concentration variability between ports

The 20 minute concentration value was low likely due to saturated chamber humidity
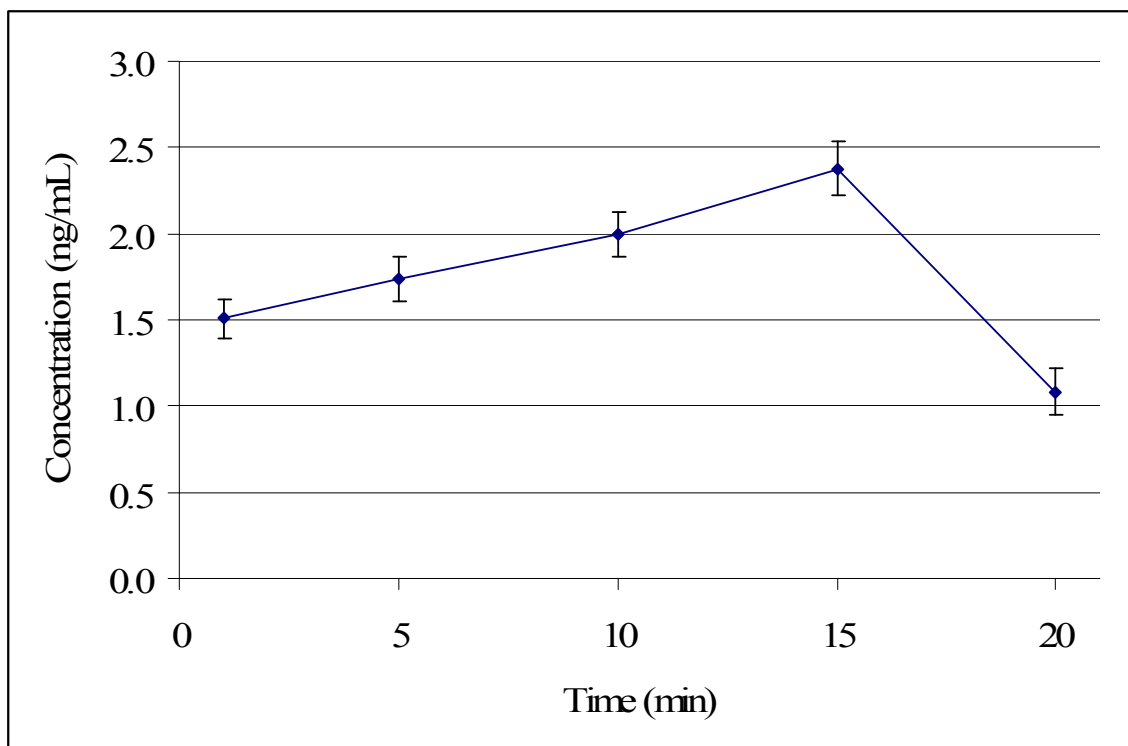
Figure A.1: Validation of a 6-Port Dosing Chamber

Error bars represent the percent relative standard deviation.

APPENDIX B: VISCOSITY OF VFEND® IV DILUTIONS

B.1. History

Very little solution was nebulized when the reconstituted Vfend® IV solution was nebulized (10mg/mL voriconazole concentration). This was thought to be due to increased viscosity of the cyclodextrin-containing solution.

B.2. Methods

Vfend® IV was diluted with sterile water for injection to varying concentrations.

Viscosity was measured using a Physica MCR 300 Cone on Plate Viscometer by Anton Paar USA Inc., Ashland, VA, at 1000RPM and 25°C.

B.3. Results

Viscosity was affected by cyclodextrin concentration within the Vfend® IV dilutions. The 16% cyclodextrin solution corresponded with a 10mg/mL voriconazole solution.
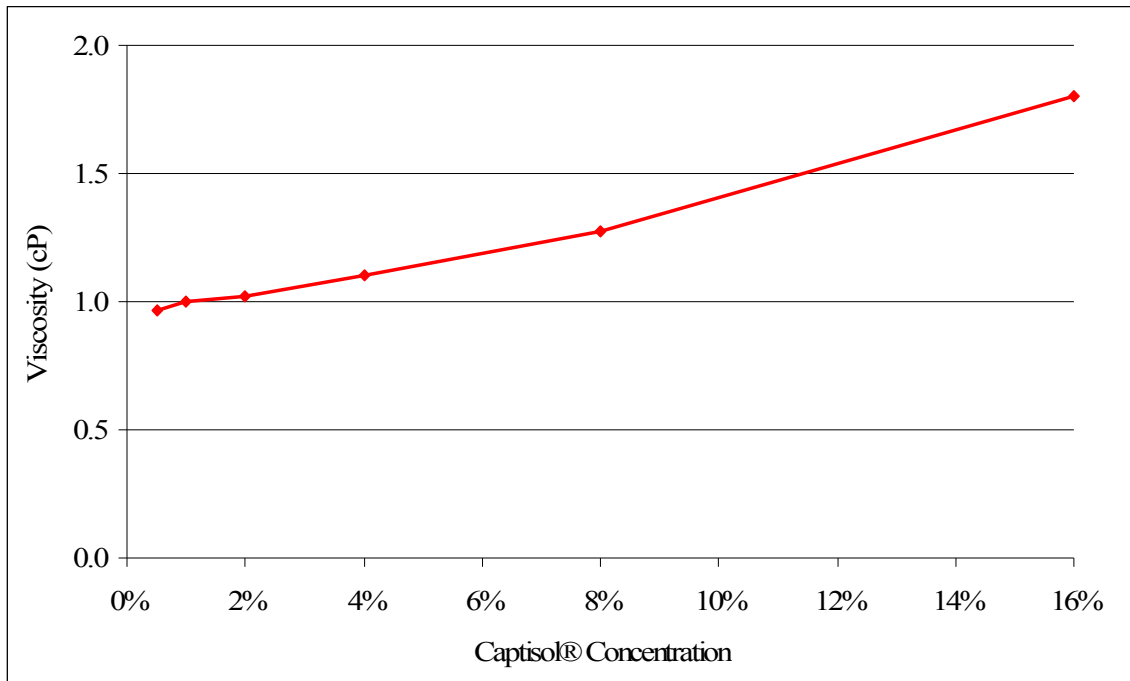
Figure B.1: Viscosity of Vfend® IV Dilutions

C.1. History

Yoen Ju Son adapted published methods for voriconazole (VRC) concentration determination to the equipment and materials in the McConville Laboratory. Following difficulty in reproducing her methods in the Williams's Laboratory, we achieved correlation in voriconazole concentration determination through a clarified method.

C.2. Procedures for Plasma Analysis
- Thaw/defrost plasma samples
- Spike empty Eppendorf tube (E-tube) with VRC spiking solution (mobile phase)
- Quickly transfer 250μL of the plasma sample to E-tube
- Vortex for 30 seconds
- Add 400μL acetonitrile
- Vortex for 30 seconds
- Stored at 4°C for 10 min
- Vortex for 10 seconds
- Centrifuge at 15,000 rpm for 5 mins
- Transfer supernatant to E-tube
- Place samples in the aluminum heating block at R.T.
- Dry under nitrogen stream (~60 min)
- Redisperse with 250μL of mobile phase
- Centrifuge at 15,000 rpm for 3 mins
- Transfer supernatant to HPLC vial

Reference: Pascual et al., Antimicrobial Agents and Chemotherapy , Jan 2007

C.3. Procedures for Lung Tissue Analysis
- Thaw/defrost lung samples
- Collect ice in a large beaker
- Add lung to glass vial
- Add 1mL DI water
- Homogenize the lung tissue with a homogenizer (keep vial on ice) for 3 mins
- Spike empty Eppendorf tube (E-tube) with VRC spiking solution (mobile phase)
- Quickly transfer 200μL of the homogenated lung to the E-tube
- Vortex for 30 seconds
- Add 500μL of 0.2M borate buffer (pH 9.0)

- Vortex for 30 seconds
- Stored at 4°C for 10 min
- Vortex for 10 seconds
- Add 500 µL of ethyl acetate
- Vortex for 30 seconds
- Transfer supernatant to E-tube (Extraction- 3 times)
- Place samples in the aluminum heating block at R.Temp.
- Dry under nitrogen stream (~60 min)
- Redisperse with 200µL of mobile phase
- Centrifuge at 15,000 rpm for 3 mins
- Transfer supernatant to HPLC vial

Reference: Lutsar et al., Clinical Infectious Disease, 2003, 37

C.4. HPLC Parameters

- HPLC : Waters Breeze
- Column: Jupiter® C18 (150mm × 4.6mm, 5µm)
- Guard Column: Universal security guard (Widepore C18)
- Temp: 35 °C
- Flow rate: 1ml/min
- Injection Vol.; 50 µL
- Detect wavelength: UV 255 nm
- Mobile phase (isocratic gradient): Methanol: 0.01M sodium acetate buffer1 (pH 5.0) = 50:50
- Vial 250 µL insert with spring supporter (waters)

Table C.1: Voricoanzole Peak Confirmation

Concentration (µg/mL)	Peak Area	Linearity Parameter
0.125	6553	
0.25	13422	
0.5	26606	
1	53971	RSQ (R^2) : 0.9999
2.5	134757	Slope : 55887
5	269958	Intercept: 2473.1
10	560118	
20	115364	

Table C.2: Extraction Method Validation for Plasma

Concentration (µg/mL)	Recovery Ratio (%) (N=2)
0.25	96.4 ± 0.00
0.5	91.6 ± 0.02
5	105.4 ± 0.04
10	90.0 ± 0.00

Table C.3: Extraction Method Validation for Lung Tissue

Concentration (µg/mL)	Recovery Ratio (%) (N=2)
0.25	96.4 ± 0.00
0.5	96.1 ± 0.03
5	97.1 ± 0.03
10	92.8 ± 0.05
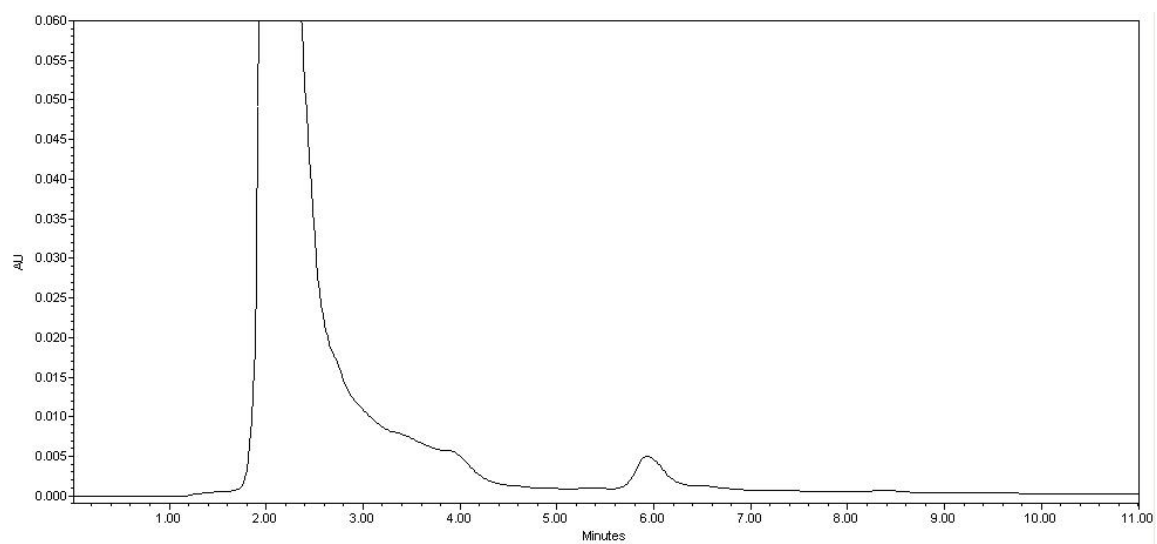
Figure C.1: Blank Plasma Chromatogram
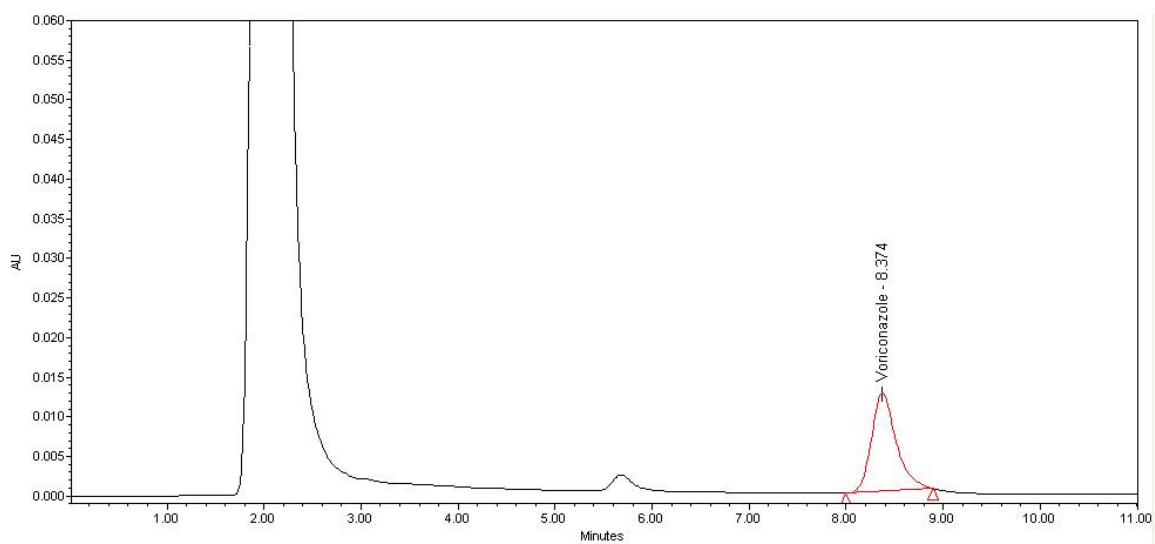
Figure C.2: Voriconazole Spiked Plasma Chromatogram
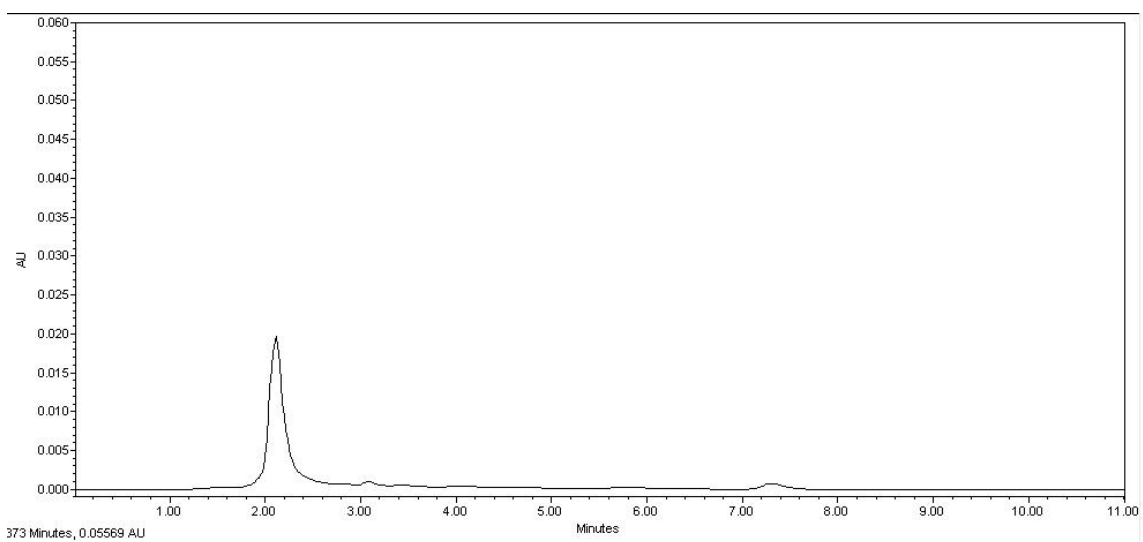
Figure C.3: Blank Lung Homogenate Chromatogram

Figure C.4: Voriconazole Spiked Lung Homogenate Chromatograms
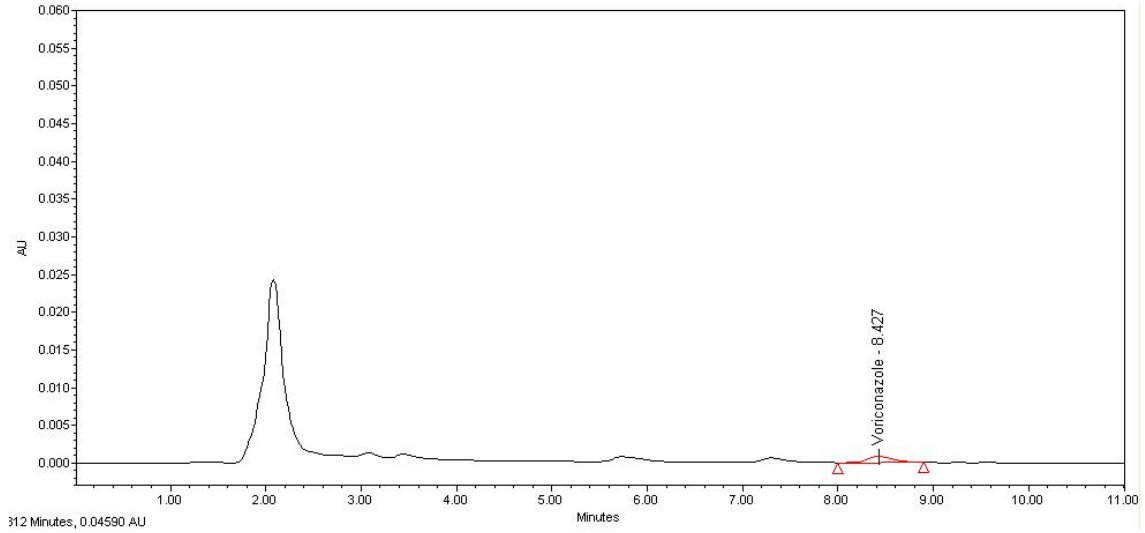
Figure C.4A: Voriconazole Spiked Lung Homogenate (Low Concentration)
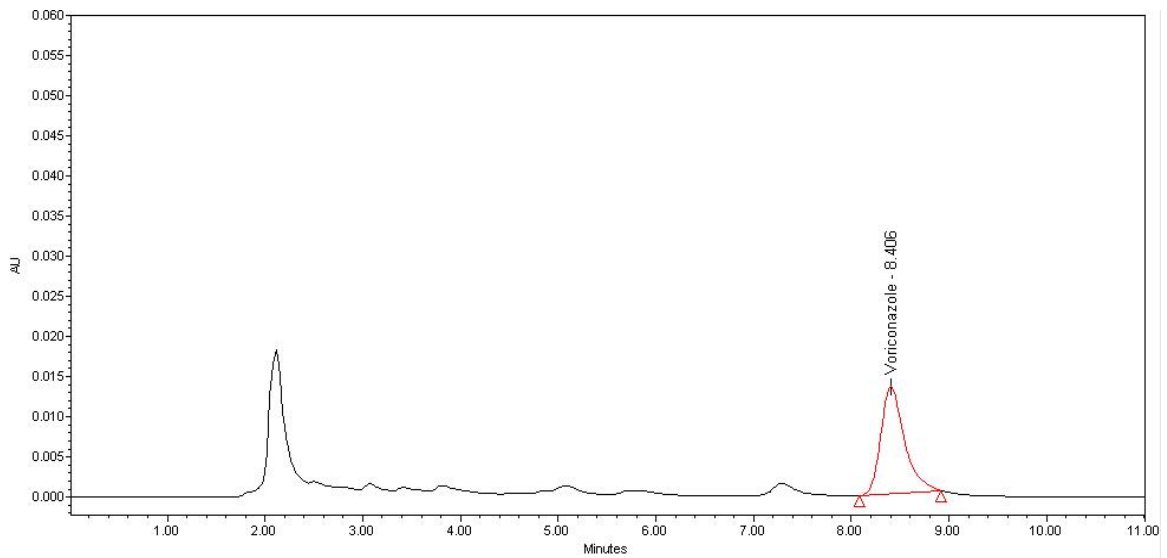
Figure C.4B: Voriconazole Spiked Lung Homogenate (High Concentration)

APPENDIX D: HPLC AND LC-MS ANALYSIS METHODS FOR QUANTITATION OF VORICONAZOLE IN PLASMA AND LUNG HOMOGENATE

D.1. Materials

Voriconazole standard (Lot # E010000674) was generously supplied by CyDex Pharmaceuticals, Inc. (Lenexa, KS). Fluconazole standard (Lot # 43352) was purchased from Hawkins, Inc. (Minneapolis, MN). Sodium tetraborate decahydrate (Batch # 057K0070), boric acid (Batch # 097K0063), sodium acetate trihydrate (Batch # 117K0153), and sodium chloride solution, 0.85% (Batch # 106K6027) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Acetic acid (Lot # 72270) was purchased from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany). HPLC grade ethyl acetate (Lot # PU0674) was purchased from Spectrum Chemical Manuf. Corp. (Gardena, CA). HPLC grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ). HPLC grade methanol was purchased from EMD Chemicals Inc. (Gibbstown, NJ). Water was obtained from an in-house Milli-Q UV Plus water purification system from the Millipore Corp. (Billerica, MA).

D.2. HPLC Mobile Phase

Mobile phase for reverse phase high performance liquid chromatography consisted of a 50:50 mixture of 0.01 M pH 5.0 sodium acetate buffer and methanol. Equal volumes of 0.01 M pH 5.0 sodium acetate buffer and methanol were mixed together, passed through 0.2 μm filter, and degassed with a vacuum, sonication system. 0.01 M pH 5.0 sodium acetate buffer was prepared by adding 0.871 g sodium acetate trihydrate and 200 μL acetic acid to 1 L of water. The pH of the 0.01 M sodium acetate buffer was measured using a pH meter. It was discovered on March, 19, 2008 that the sodium acetate buffer was prepared incorrectly. 0.871 g sodium acetate trihydrate and

200 µL acetic acid was added to 2 L of water, resulting in buffer solution with a different ionic strength which lead to incomplete separation of the plasma peak and voriconazole peak during HPLC analysis.

D.3. Voriconazole Extraction Method for Mouse Plasma

Similar methods were used to those previously published [1]. Validation was performed by spiking voriconazole standard dissolved in mobile phase into 250 µL of blank plasma. Table 1 shows the method validation for the mouse plasma samples spiked with voriconazole solution. Figures 1 and 2 show the chromatrographs of blank plasma and plasma spiked with voriconazole solution, respectively. Voriconazole was extracted from plasma samples through the addition of acetonitrile, centrifugation, and supernatant extraction. 250 µL of plasma was transferred to a 2 mL Eppendorf tube followed by vortex mixing for 30 seconds. 400 µL of acetonitrile was added to the Eppendorf tube followed by vortex mixing for 30 seconds. The Eppendorf tube was stored at 4°C for 10 minutes followed by vortex mixing for 10 seconds and centrifugation at 15,000 rpm for 5 minutes. The supernatant liquid was transferred to a new Eppendorf tube and evaporated under a gentle stream of nitrogen. Residual solids were re-dispersed with 250 µL of mobile phase.

D.4. Voriconazole Extraction Method for Mouse Lung Homogenate

Similar methods were used to those previously published [2]. Lung tissue was thawed and homogenized with 1 mL of water for 3 minutes in an ice bath using an Omni GLH homogenizer from Omni International (Marietta, GA). Validation was performed by spiking voriconazole standard dissolved in mobile phase into 200 µL of lung homogenate. Table 2 shows the method validation for the mouse lung homogenate

samples spiked with voriconazole solution. Figures 3 and 4 show the chromatrographs of blank lung homogenate and lung homogenate spiked with voriconazole solution, respectively. Voriconazole was extracted from lung homogenate samples through the addition of 0.2 M pH 9.0 borate buffer, ethyl acetate, centrifugation, and supernatant extraction. 0.2 M pH 9.0 borate buffer was prepared by by mixing 50 mL of 12.4 g/L boric acid solution with 59 mL of 19.05 g/L sodium tetraborate decahydrate solution. The 0.2 M borate buffer was passed through 0.2 μm filter and the pH was measured. 200 μL of lung homogenate was transferred to a 2 mL Eppendorf tube followed by vortex mixing for 30 seconds. 500 μL of 0.2 M pH 9.0 borate buffer was added to the Eppendorf tube followed by vortex mixing for 30 seconds. The Eppendorf tube was stored at 4°C for 10 minutes followed by vortex mixing for 10 seconds. 500 μL of ethyl acetate was added to the Eppendorf tube followed by vortex mixing for 30 seconds and centrifugation at 15,000 rpm for 1 minute. The supernatant liquid was transferred to a new Eppendorf tube and the ethyl acetate extraction procedure was repeated two more times. The total volume of extraction supernatant from the 3 iterations was approximately 1.4 mL. The supernatant liquid was evaporated under a gentle stream of nitrogen and the residual solids were re-dispersed with 200 μL of mobile phase.

D.5. HPLC Analysis Method for Mouse Plasma and Lung Homogenate Samples

Voriconazole standards were prepared by dissolving voriconazole in mobile phase followed by serial dilution with mobile phase. A standard curve was generated with a minimum of 5 concentrations. Each extracted and reconstituted sample was transferred to a polypropylene conical insert and placed in the appropriate HPLC vial. The samples were analyzed using a Waters Breeze liquid chromatograph (Waters Corporation, Milford MA) or Shimadzu LC-10 liquid chromatograph (Shimadzu Corporation, Columbia, MD)

equipped with a heated (35°C) Jupiter® C18 (150 mm x 4.6 mm, 5 µm, 300 Å) with a security guard cartridge (Widepore C18, 4 x 3.0 mm) (Phenomenex, Torrance, CA). The sample volume was 50 µL with a UV detection wavelength of 254 nm. The mobile phase consisted of a 50:50 mixture of 0.01 M pH 5.0 sodium acetate buffer and methanol at a flowrate of 1.0 mL/minute. The retention time for voriconazole was approximately 8 minutes. The lower limit of quantitation was 100 ng/mL.

D.6. LC-MS Method Development for Rat Plasma and Lung Homogenate Samples

Due to the low concentrations of voriconazole in rats following inhalation, HPLC with UV detection was not suitable for analysis. The LTQ XL Linear Ion Trap Mass Spectrometer located in Welch 5.336 at The University of Texas at Austin was evaluated. The LC-MS method evaluated was a C18 column at room temperature, 100-1000 m/z, 17 minute run, positive with PDA, 10 µL injection, and 0.5 mL/minute flowrate. A mobile phase gradient of 95% water:5% acetonitrile was used for the first minute followed by equilibration from 95% water to 5% water over the next 4 minutes. Metronidazole and ketoconazole were evaluated as internal standards. Ketoconazole was unstable in the aqueous mobile phase and metronidazole was very polar so that it eluted very quickly with a broad peak. Neither metronidazole nor ketoconazole are suitable were suitable as internal standards. The retention time for voriconazole (m/z 350) was approximately 7.6 minutes. The lower limit of detection (LLOD) for voriconazole was approximately 10 ng/mL and the lower limit of quantitation (LLOQ) was approximately 50 ng/mL. A 20 µL injection volume could be used to yield LLOD = 5 ng/mL and LLOQ = 25 ng/mL.

An inter-day and intra-day validation study on the LTQ XL Linear Ion Trap Mass Spectrometer showed that the amount of voriconazole detected was variable from day to day as well as within intraday with a sample set of approximately 20 samples. It was

concluded that this mass spectrometer is unstable and not suitable for quantitation of voriconazole in plasma and lung homogenate samples.

The Applied Biosystems 4000 Q Trap LC-MS/MS system with ESI, APCI and nanospray sources coupled with Shimadzu LC-20AD HPLC system located in the Analytical Instrumentation Facility Core (PHR 1.110) at The University of Texas at Austin was evaluated for quantitation of voriconazole and fluconazole (internal standard). The system was found to be variable from day to day but stable during an intraday study. The LC-MSMS method development and the analysis of the extracted samples were performed by Dr. Herng-Hsiang Lo in the CRED Analytical Instrumentation Facility Core supported by NIEHS center grant ES07784. A 4000 Q TRAP mass spectrometer (Applied Biosystems, Foster City, CA) coupled with an online HPLC (Shimadzu, Columbia, MD) was used to analyze the samples. Dried samples were reconstituted in 200 µL of acetonitrile, 10 µL was injected into Shimadzu Prominence UFLC system equipped with an Restek C18 (4.6 x 50mm, 5 µm, 110 Å) column; both fluconazole and voriconazole were eluted with mobile phase A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid) by a gradient of 20% B for 0.5 minutes, then 20%-50% B over 0.5 minutes, followed by 50% B for 3 minutes, at a flow rate of 1 mL/minute. Sample was directly eluted from the column into the electrospray ion (ESI) source of 4000 QTRAP. The heated nebulizer of 4000 QTRAP was set at 700°C, the declustering potential (DP) at 40. The Multiple Reaction Monitoring (MRM) scan experiment with unit resolution for Q1 and low resolution for Q3 was used to quantify fluconazole and voriconazole. The MRM transitions were set as follows, fluconazole: Q1=307.5, Q3=238.4, collision energy (CE) =25; voriconazole: Q1=350.2, Q3=127.4,

CE=40. The lower limit of detection for both fluconazole and voriconazole was 5 pg. The upper limit of detection for both fluconazole and voriconazole was 20 ng.

D.7. Voriconazole Extraction Method for Rat Plasma and Lung Homogenate

A study was performed to compare extraction methods for rat plasma and lung homogenate for mass spectrometry analysis. Both methods described above (acetonitrile method and borate buffer plus ethyl acetate method) were tested for extraction of voriconazole and fluconazole from rat plasma. Only the borate buffer plus ethyl acetate method was tested for extraction of voriconazole and fluconazole from rat lung homogenate. Thawed blank rat plasma was spiked with voriconazole standard dissolved in acetonitrile and fluconazole standard dissolved in acetonitrile. Lung tissue was thawed and homogenized with 1 mL of 0.85% sodium chloride solution for every 0.5 g of lung tissue for 3 minutes in an ice bath using an Omni GLH homogenizer. Lung homogenate was also spiked with the voriconazole and fluconazole standards dissolved in acetonitrile. Only one spiked quantity of each drug was tested: 40.5 ng voriconazole and 64.8 ng fluconazole. The borate buffer plus ethyl acetate method was superior to the acetonitrile for rat plasma for both fluconazole and voriconazole. The extraction efficiencies were 48% compared to 18% for fluconazole and 74% compared to 50% for voriconazole. Similar extraction efficiencies were found for the lung homogenate: 42% for fluconazole and 74% for voriconazole. Therefore, the borate buffer plus ethyl acetate method was chosen for extraction of voriconazole and fluconazole from both rat plasma and lung homogenate samples. Since the extraction efficiencies differed for fluconazole and voriconzole, fluconazole could not be used as an internal extraction standard to adjust the quantity of voriconazole determined in unknown samples. However, fluconazole could still be used as an internal LC-MSMS standard to detect erroneous results. In order to

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validate the quantitation of voriconazole in unknown samples, a calibration curve must be generated each time a sample set is run on the LC-MSMS system.

Voriconazole standards and fluconazole standards were prepared by dissolving voriconazole or fluconazole in acetonitrile followed by serial dilution with acetonitrile. Lung tissue was thawed and homogenized with 1 mL of 0.85% sodium chloride solution for every 0.5 g of lung tissue for 3 minutes in an ice bath using an Omni GLH homogenizer. A calibration curve with a minimum of 4 concentrations (2 ng, 32 ng, 130 ng, and 520 ng) was generated by spiking voriconazole standards into 200 µL of plasma or lung homogenate. Only 1 concentration of fluconazole standard (approximately 200 ng) was spiked into the plasma and lung homogenate samples. 200 µL of plasma or lung homogenate was transferred to a 2 mL Eppendorf tube followed by vortex mixing for 30 seconds. 500 µL of 0.2 M pH 9.0 borate buffer was added to the Eppendorf tube followed by vortex mixing for 30 seconds. The Eppendorf tube was stored at 4°C for 10 minutes followed by vortex mixing for 10 seconds. 500 µL of ethyl acetate was added to the Eppendorf tube followed by vortex mixing for 30 seconds and centrifugation at 15,000 rpm for 1 minute. The supernatant liquid was transferred to a new Eppendorf tube and the ethyl acetate extraction procedure was repeated two more times. The total volume of extraction supernatant from the 3 iterations was approximately 1 mL for plasma and 1.4 mL for lung homogenate. The supernatant liquid was evaporated under a gentle stream of nitrogen and the dried samples were submitted to Dr. Herng-Hsiang Lo in the CRED Analytical Instrumentation Facility Core for analysis. The standard curve and the calibration curve were used to quantify the amount of voriconazole present in unknown plasma and lung homogenate samples.

D.8. References

1.   Pascual, A., et al., Variability of voriconazole plasma levels measured by new high-performance liquid chromatography and bioassay methods. Antimicrobial Agents and Chemotherapy, 2007. 51(1): p. 137-143.

2.   Lutsar, I., S. Roffey, and P. Troke, Voriconazole concentrations in the cerebrospinal fluid and brain tissue of guinea pigs and immunocompromised patients. Clinical Infectious Diseases, 2003. 37(5): p. 728-732.

Table D.1: Extraction Method Validation for Mouse Plasma

Spiked Concentration (µg/mL)	Recovery Ratio (%) (N=2)
0.25	96.4 ± 0.00
0.5	91.6 ± 0.02
5	105.4 ± 0.04
10	90.0 ± 0.00

Table D.2: Extraction Method Validation for Mouse Lung Homogenate

Spiked Concentration (µg/mL)	Recovery Ratio (%) (N=2)
0.25	96.4 ± 0.00
0.5	96.1 ± 0.03
5	97.1 ± 0.03
10	92.8 ± 0.05
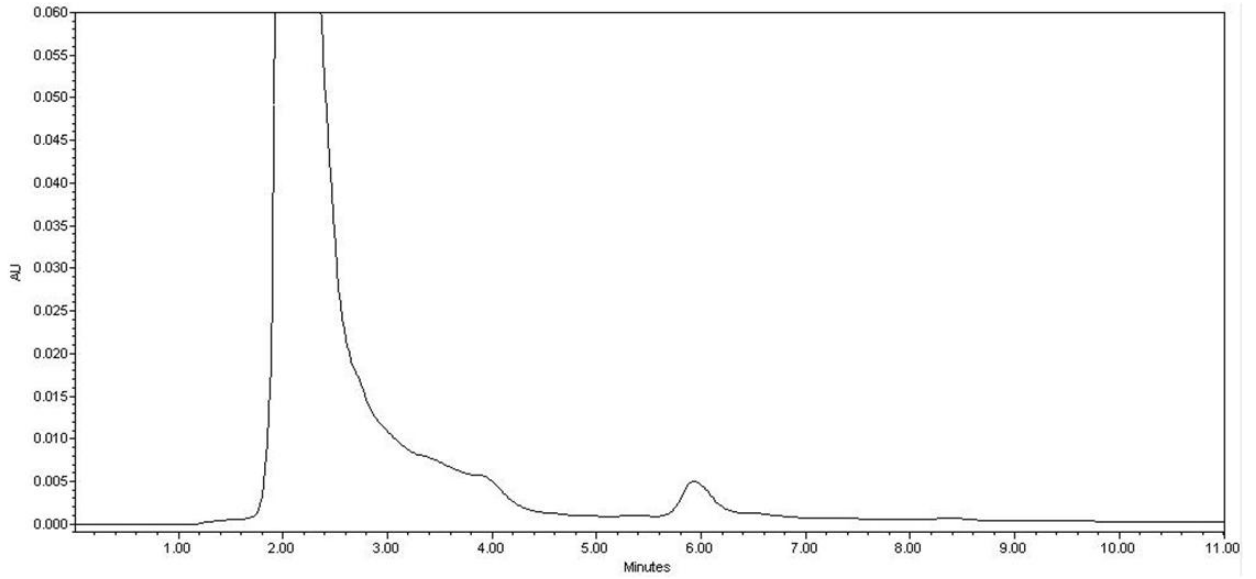
Figure D.1: Chromatograph of Blank Mouse Plasma
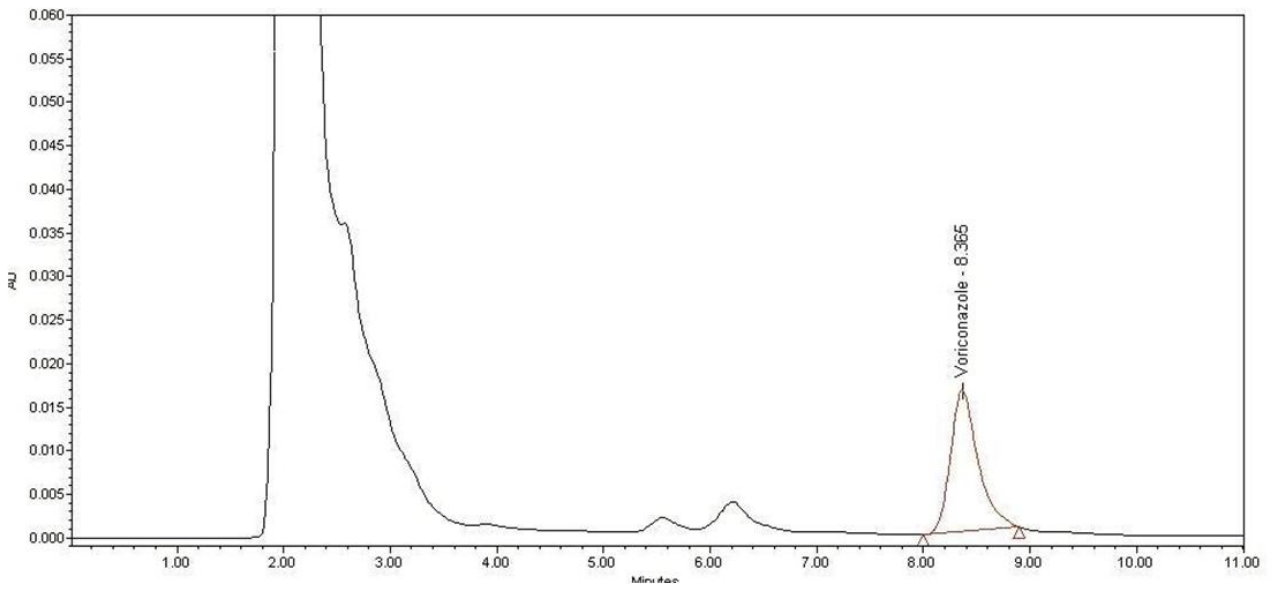
Figure D.2: Chromatograph of Mouse Plasma Spiked with Voriconzole Solution
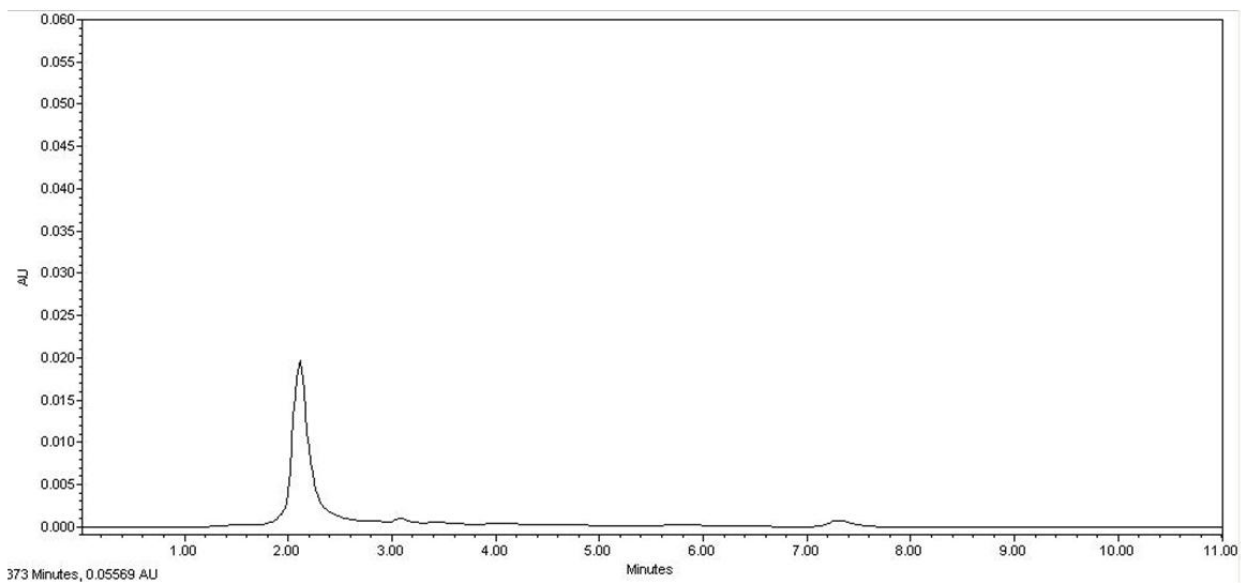
Figure D.3: Chromatograph of Blank Mouse Lung Homogenate
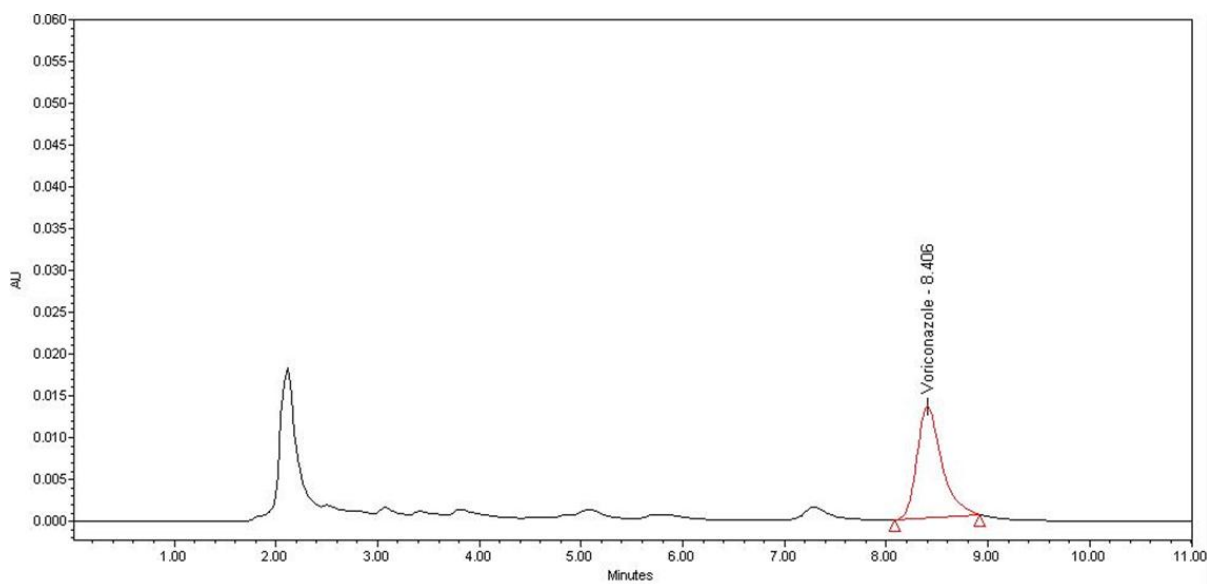
Figure D.4: Chromatograph of Mouse Lung Homogenate Spiked with Voriconzole
        Solution

APPENDIX E: NEBULIZATION PROCEDURES EMPLOYED IN CHAPTER 3

E.1. Acclimatization of Mice to the Animal Restraint Tubes

Mice will be placed in the restraint tubes to acclimatize them to the dosing procedure. Specificlly, mice should be restrained in the tubes for 5 minutes. Time in the tube should be graudually increased so that the mice will tolerate 20 minutes.
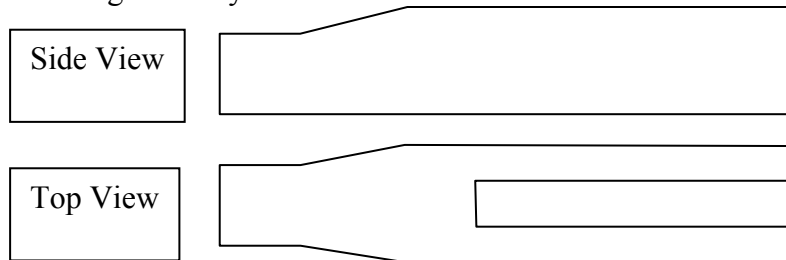
Figure E.1. Schematic of the Mouse Restraint Tube

E.2. Reconstitution and Dilution of Injectable Vfend

The vials of injectable Vfend must be reconstituted and diluted prior to nebulization. The Vfend should be reconstituted to a total volume of 32mL with Sterile Water for Injection (SWFI). This is accomplished by reconstituting per vial instructions by the addition of 19mL SWFI. Then, an additional 12mL should be added before the solution is nebulized. The dilution step (addition of 12mL SWFI) should be done in a separate container than the manufacturer's vial. The final concentration of voriconazole should be 6.25 mg/mL. Remaining solution after nebulization could be refrigerated and used at a later time. The solution should return to room temperature before nebulization however.

E.3. Preparation of the Inhaled Control Solution

Captisol® (β-cyclodextrin sulfobutyl ethers, sodium salt) should be dissolved in SWFI at a concentration of 100mg/mL. This should be done by adding sufficient SWFI to 5000mg Captisol to achieve a total volume of 50mL. Store the Captisol under vacuum and with excess desiccant

E.4. Assembly of the Nose-only Dosing Chamber

The nose-only dosing chamber includes the following components:
- 6-port dosing tube
- 6 animal restraint tubes (including 6 tube restrictors and 6 mouse pushers)
- Fan assembly
- Power adapter and switch
- 4 silicone seals
- Upstream air-flow restrictor
- Downstream exhaust tube
- Nebulizer medication reservoir
- Nebulizer T-type adapter
- Nebulizer controller unit with cord
- Nebulizer power cord and
- The nose-only dosing chamber should be assembled as diagrammed below:
- Fan
- Nebulizer reservoir and t-type adapter
- Exhaust tube
- 6-port dosing tube
- Animal restraint tubes
- Nebulizer controller unit
- Silicone Seals
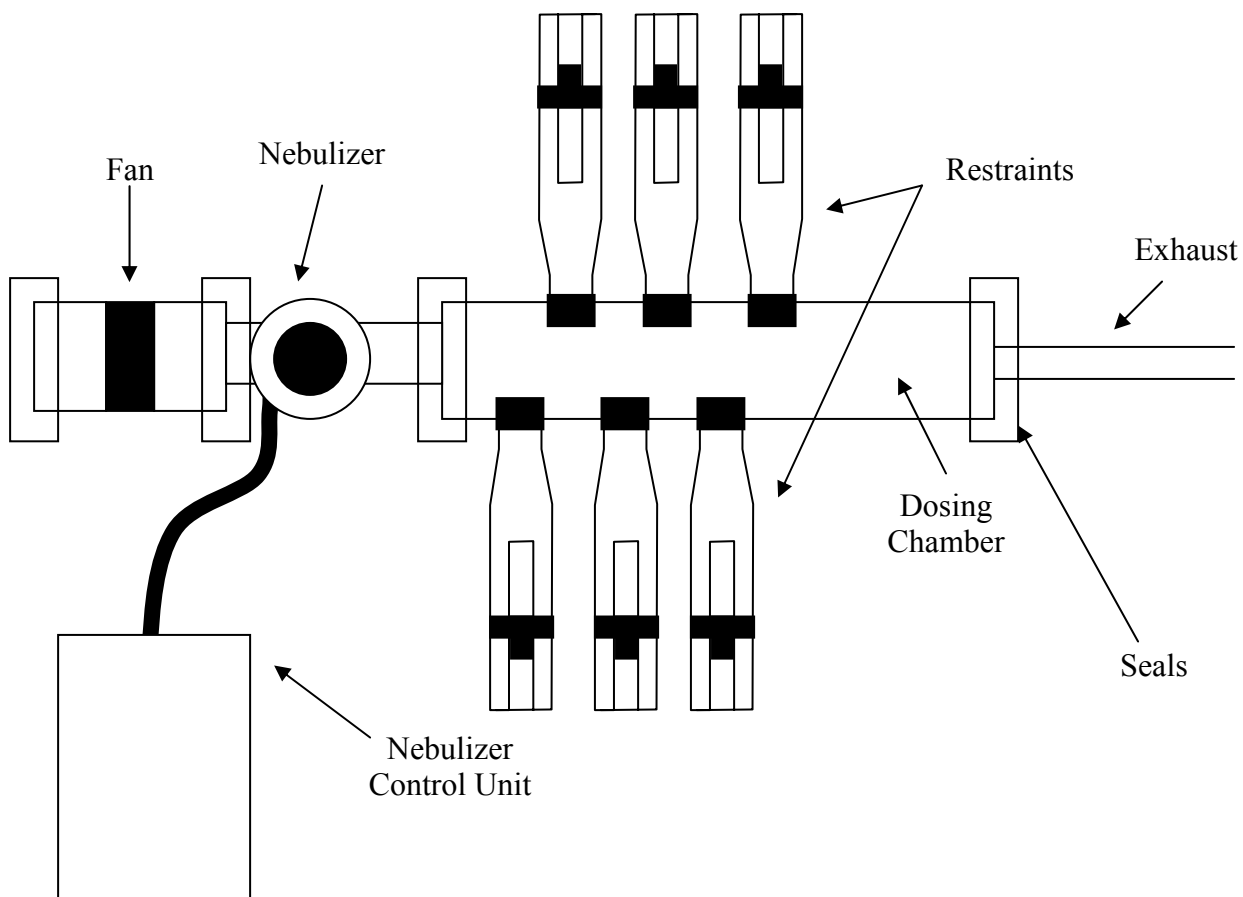
Figure E.2: Schematic of the Dosing Apparatus

E.5. Dosing Procedure

Assemble the dosing apparatus. Turn on fan and verify air flow rate is 1mL/min. Place 5-6mL of the solution into the medication reservoir. Turn on the nebulizer by holding down the ON/OFF button until the 30 minute light is lit. Nebulize the solution for 20 minutes.

When done, collect sample of residual Vfend for concentration analysis. Dry the 6-port dosing tube and t-type nebulizer adapter of any condensation. Clean the

restraining tubes if necessary. Sonicate nebulizer reservoir in detergent solution for 5 minutes. Rinse nebulizer with tap water. Nebulize tap water for 5 minutes and then dry the reservoir. If necessary, measure residual volumes of the tap water to determine decreases in nebulizer performance. Reassemble the nose-only dosing chamber if necessary.

APPENDIX F: EXPANDED METHODS EMPLOYED FOR CHAPTER 3

F.1. Reconstitution and Dilution of Injectable Vfend

- Add 19mL SWFI and allow to dissolve
- Transfer to new container
- Add additional 12mL SWFI
- Final concentration should be 6.25mg/mL
- Preparation of the Inhaled Control Solution
- Weigh 5000mg Captisol
- QS 50mL with SWFI

F.2. Dosing Procedure

- Assemble nose-only dosing chanber
- Turn on fan and verify 1L/min flow rate
- Add solution to medication reservoir
- Hold down ON/OFF button until 30 minute timer is lit
- Nebulize solution for 20 minutes
- Collect sample of residual volume in medication reservoir
- Clean nose-only dosing chamber from condensation if necessary
- Sonicate medication reservoir in detergent solution for 5 minutes
- Rinse medication reservoir in tap water
- Nebulize tap water through medication reservoir for 5 minutes
- If necessary, measure residual volume of tap water to assess nebulizer output
- Reassemble the nose-only dosing chamber
- Aerosolized Voriconazole as Prophylaxis Against Invasive Pulmonary Aspergillosis

F.3. Animal Numbers

Groups:

1 hr SAC	5
Uninfect.Control	5
AMB (1 mg/kg)	29 (4 not immunosuppressed)
Aero. Control	28 (4 not immunosuppressed)
Aero. VOR	28 (4 not immunosuppressed)
	95

56 mice will be acclimatized to the chambers beginning on Friday, July 26
Friday – 7 minutes
Saturday – 14 minutes
Sunday – 21 minutes

F.4. Disposition of Groups

F.4.1. AMB (1 mg/kg)

*4 mice will begin receiving IP AMB on day -2 and continue until through the morning of day 0. These 4 mice will not be infected or receive immunosuppression. The other 25 mice in this group will begin receiving AMB on day +1.

Day 0 (using 4 mice dosed with AMB beginning on day -2)
2 mice will have blood drawn and placed in heparinized tubes for PK analysis
2 mice will have BALs performed with PBS for ELISA analysis

Day 8 (using 12 mice dosed with AMB beginning on day +1)
3 mice will have lungs filled with formalin and removed for histopathology
9 mice will have lungs removed for tissue burden analysis
2 mice from this group will also have blood drawn and placed in heparinized tubes for PK analsysis, and 2 x 200 mcL aliquots from lung hemogenates removed for PK tissue analysis

Day 12 (using 13 mice dosed with AMB beginning on day +1)
Survival analysis
If enough animals remain, 4 mice will have lungs filled with formalin and removed for histopathology, and 2 mice will have blood drawn and lungs removed for PK tissue analysis

F.4.2. Control

Day 0 (4 mice that are not immunosuppressed)
2 mice will have blood drawn and placed in heparinized tubes for PK analysis
2 mice will have BALs performed with PBS for ELISA analysis

Day 8 (using 12 mice that are immunosuppressed and infected)
3 mice will have lungs filled with formalin and removed for histopathology
9 mice will have lungs removed for tissue burden analysis
2 mice from this group will also have blood drawn and placed in heparinized tubes for PK analsysis, and 2 x 200 mcL aliquots from lung hemogenates removed for PK tissue analysis

Day 12 (using 12 mice that are immunosuppressed and infected)

Survival analysis
If enough animals remain, 4 mice will have lungs filled with formalin and removed for histopathology, and 2 mice will have blood drawn and lungs removed for PK tissue analysis

F.4.3. Voriconazole

Day 0 (4 mice that are not immunosuppressed)
2 mice will have blood drawn and placed in heparinized tubes for PK analysis
2 mice will have BALs performed with PBS for ELISA analysis

Day 8 (using 12 mice that are immunosuppressed and infected)
3 mice will have lungs filled with formalin and removed for histopathology
9 mice will have lungs removed for tissue burden analysis
2 mice from this group will also have blood drawn and placed in heparinized tubes for PK analsysis, and 2 x 200 mcL aliquots from lung hemogenates removed for PK tissue analysis

Day 12 (using 12 mice that are immunosuppressed and infected)
Survival analysis
If enough animals remain, 4 mice will have lungs filled with formalin and removed for histopathology, and 2 mice will have blood drawn and lungs removed for PK tissue analysis

APPENDIX G: SAMPLE COLLECTION PROCEDURES EMPLOYED IN CHAPTERS 2-5

G.1. Blood for Voriconazole Concentration Determination

G.1.1. Material preparation
1) Extract 0.5-1 mL of 10,000 Unit/mL heparin and place in a clean 1.5 mL conical vial. This vial is designated the HEPARIN VIAL.
2) From the HERAPIN VIAL, add 40-100 µL (1-2 drops) of heparin to a sufficient number of 1.5 mL conical vials. These vials are designated BLOOD VIALS.
3) From the HEPARIN VIAL, coat the necessary number of 3mL 21 G needles/syringes to be used for blood collection with heparin by drawing 0.1-0.5 mL heparin into the syringe and expel the heparin back into the HEPARIN VIAL. These syringes are designated HEPARINIZED SYRINGES.

G.1.2. Sample collection
1) Immediately after sacrifice*, collect as much blood as possible by cardiac puncture using HEPARINIZED SYRINGES
   a) A maximum of 1 mL of suction could be applied to the HEPARINIZED SYRINGE when performing cardiac puncture.
2) Remove the needle from the HEPARINIZED SYRINGE and fill whole blood into BLOOD VIALS.
3) Invert and gently shake the BLOOD VIALS in order to disperse the heparin throughout the whole blood.
4) Whole blood in BLOOD VIALS should be maintained at room temperature until centrifugation.
5) Whole blood in BLOOD VIALS should be centrifuged as soon as possible after collection

G.1.3. Sample processing
1) Prepare clean 1.5 mL conical vials to collect the plasma that will be obtained after centrifugation. These vials are designated PLASMA VIALS.
2) Centrifuge the whole blood in the BLOOD VIALS to obtain plasma
   a) Centrifugation of BLOOD VIALS should be performed at 9000 rpm (approximately 75 G) for 15 minutes using the Beckman Coulter Microfuge 18 centrifuge.
3) Extract the supernatant (plasma) into PLASMA VIALS using one pipet per vial.
4) Plasma in PLASMA VIALS can be frozen at -5 to -2°C until voriconazole quantification by HPLC.

G.2. Blood for CBC/Serum Chemistry

G.2.1. Material Preperation
1) Fill out the online forms with VetConnect.com (IDEXX) to request sample analysis approximately 24 hours before whole blood and tissue sample collection.
    a) Print the completed requisition(s) and include in samples sent for analysis.
    b) Appendix A includes a completed requisition form as an example.
2) Immediately after sacrifice*, collect as much blood as possible by cardiac puncture using clean (no additive) 3 mL 21 G syringe/needles.
    a) Apply gentle suction when collecting blood to minimize hemolysis.
        i) A maximum of 1 mL of suction could be applied to the syringe when performing cardiac puncture.
    b) A minimum of 1 mL of whole blood is necessary to be able to run both CBC and serum chemistry.

G.2.2. Sample Collection
1) Collect as much blood as possible by cardiac puncture.
2) For CBC Analysis on Whole Blood:
    a) Whole blood processing must be performed at room temperature.
    b) Remove the needle after blood draw and fill 250-500 µL whole blood into Lavender-topped (LT) tubes for CBC analysis.
        i) Anticoagulant coats the inside of the LT tubes
    c) Immediately invert the LT tube 10 times gently to ensure anticoagulation.
        i) DO NOT SHAKE.
        ii) Rat whole blood contains a very large number of platelets and will clot easily and rapidly.
        iii) Immediately disperse anticoagulant by gentle tube inversion after blood collection.
    d) Keep LT tubes at room temperature for 30 minutes before refrigeration at 2-5°C.
        i) Do not freeze whole blood in LT tubes.
3) For Serum Chemistry Analysis:
    a) Whole blood collected for chemistry analysis should be maintained at room temperature.
    b) Whole blood will ultimately be clotted and centrifuged, so anticoagulant should not be used.
    c) Any whole blood remaining after CBC analysis should be filled into clean 1.5 mL conical vials.
        i) If possible, >2 mL of whole blood should be collected for serum chemistry analysis
        ii) Whole blood that pools in the chest cavity can also be collected (even if clotted).

d) Store collected blood at room temperature for 15-20 minutes to allow for clotting (coagulation).
e) Centrifuge coagulated blood at 2500 RPM (approximately 6 G) for 10-15 minutes.**
f) Transfer supernatant (serum) to Red-topped (RT) tubes for serum chemistry analysis.
g) Refrigerate serum-filled RT tubes until analysis at 2-5°C.
h) If excess serum is available after RT tubes are filled, transfer any remaining serum to clean 1.5 mL conical vials.
i) Freeze excess serum at -60 to -80°C until analysis.

G.2.3. Shipping and Handling of Whole Blood for CBC Analysis and Serum for Chemistry Analysis

1) Place LT (for CBC) and RT (for chemistry) tubes in zip-lock bag(s) of an appropriate size.
2) Place the completed requisition from in the appropriate zip-lock bag(s).
3) Place the zip-lock bags, containing samples and requisitions, in the provided IDEXX drop box.
4) Place a frozen ice-pack in the IDEXX drop box without samples coming in contact with the ice pack.
   a) The ice pack maintains a cool environment within the IDEXX drop box until the IDEXX courier is able to collect samples.
   b) Do not freeze CBC or serum chemistry samples.
5) The IDEXX courier will typically pick up samples in the evening (6-7PM) as the last stop before samples are flown to the lab in Dallas.
   a) The courier will refrigerate samples once they are collected.
6) Both CBC and serum chemistry samples should be run within 12 hr of collection.

G.3. Procedure for Tissue Collection

G.3.1. Lung Collection for Voriconazole Concentration Determination

1) After blood is collected by cardiac puncture, extract lungs.
2) Remove adipose and connective tissue.
3) Place lungs in an appropriate vial and freeze until analysis.

G.3.2. Lung, Liver, Kidney, and Spleen Collection for Histological Analysis

1) Place 10-40 mL of 10% formalin in 50 mL sputum vials.
   a) A sufficient volume of formalin should be added to have approximately 10-20x the organ volume.
   b) Excised organs should be immediately placed in 10% formalin

    c) All 10% formalin, as well as organs in 10% formalin, should be stored at room temperature
2) Excision and Fixing of Lungs:
    a) Fill a syringe with 3-5mL of 10% formalin
    b) Attach a filter straw to the filled 5mL syringe
    c) After blood is collected by cardiac puncture, make a small incision in the trachea without cutting through the trachea.
    d) Insert the tip of the filter straw into the incision in the trachea and slowly infuse the lungs with sufficient 10% formalin to inflate the lungs over 1 minute
    e) With the filter straw still in place, tie the trachea shut with suture and withdraw the filter straw
    f) Tighten the suture sufficiently to retain 10% formalin in the lungs.
    g) The inflated whole lungs and heart should then be excised.
    h) Place the inflated lung into 10% formalin
        i) A sufficient volume of formalin should be added to have approximately 10-20x the organ volume.
3) Excision and Fixing of Liver, Kidneys, and Spleen
    a) Liver should be excised and cut into smaller segments (3-5) to allow better penetration of 10% formalin into the tissue
        i) Liver pieces could be placed in multiple 10% formalin vials to allow for adequate tissue fixing
    b) Spleen should be excised and placed in 10% formalin
    c) Kidneys should be excised, cut along the coronal axis (in half), and placed in 10% formalin
4) Allow organs to remain in 10% formalin for at least 24-48 hours before further processing.

*If isoflurane is used for anesthesia prior to sacrifice, elevated glucose levels are likely to occur. (see Saha et al. Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats. Exp. Biol Med (2005) 230:777-784)

**The following formula may be used to calculate revolutions per minute (rpm) from g. $g = (1.12 \times 10\text{-}5) \times r \times (rpm)2$ where r is the distance from the center of the centrifuge head to the base of the tube.

G.4. Chemicals and Supplies

Heparin Sodium Injection, USP, NDC 0641-0410-02, 10,000 UNITS/mL, 25 x 1mL vials, Baxter Healthcare Corporation – Henry Schein Distributor, Item #1105666

Formalin Solution, Neutral buffered, 10%, 4L per box, Sigma Aldrich, Item #HT501128

Lavender-topped (LT) Tubes, BD Microtainer® Tubes with K2E (K2EDTA), 50 per pack, BD, Item #365974

Red-topped (RT) Tubes, BD Microtainer® No additive Tubes, 50 per pack, BD, Item #365957

Syringe – 3mL 21G1, BD Syringe, Sterile, Single Use, Latex Free, Luer-lok, (0.8 mm x 25 mm), 100 per box, BD Medical Systems, Item #309575

Filter Straw, FS-5000: 5 micron – 4 inch, 100 per box, B. Braun Medical Inc., Item #415020

APPENDIX H: ESTABLISHMENT OF AN IN-HOUSE CONTROL FOR BLOOD WORK AND LABORATORY TESTING

H.1. History

For the experiments included in Chapter 4, elevations in critical laboratory test values, including those hepatic function tests and serum glucose, were noted following inhalation of voriconazole as well as inhaled normal saline control compared to the reference values provided by IDEXX laboratories and Harlan. It was suggested that these laboratory abnormalities might be artifacts resulting from the method of euthanasia or blood collection.

H.2. Methods

10 male and 10 female Sprague-Dawley rats, Harlan Sprague Dawley, Inc. (Indianapolis, IN), with an average mass of 250 g at the beginning of the study, were caged separately with free access to food and water. The animals were not handled or manipulated by the investigators. After 28 days, the rats were transferred to the Necropsy Room in the ARC and allowed to return to normal resting behavior. They were euthanized by isoflurane narcosis followed immediately by exsanguination by cardiac puncture and thoracotomy. Whole blood was handled and processed in the same manner described in 4.2.4. Blood and Tissue Processing and Testing. All animals were handled and maintained in accordance with The University of Texas at Austin Institution Animal Care and Use Committee (IACUC) guidelines and in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines.

Table H.1: Raw Data for In-House No Treatment Control Group

ID	Day	Group	Cage	Gender	Sub-Designation	ALK. PHOSPHATASE	ALT (SGPT)	AST (SGOT)	ALBUMIN	TOTAL PROTEIN	GLOBULIN	TOTAL BILIRUBIN	DIRECT BILIRUBIN	BUN	CREATININE
28NDM1	28	ND	1	M	1	200	70	110	3.2	5.7	2.5	0.1	0.1	22	0.5
28NDM2	28	ND	1	M	2	234	133	271	3.4	6.2	2.8	0.1	0.1	22	0.5
28NDF1	28	ND	2	F	1	208	274	510	3.3	5.8	2.5	0.1	0.1	23	0.5
28NDF2	28	ND	2	F	2	243	58	78	3.3	5.7	2.4	0.1	0.1	22	0.5
28ND3M1	28	ND	3	M	1	247	60	79	2.9	6.1	3.2	0.0	0.0	24	0.5
28ND3M2	28	ND	3	M	2	207	62	71	3.2	6.3	3.1	0.1	0.1	24	0.5
28ND4F1	28	ND	4	F	1	174	61	83	3.4	6.7	3.3	0.1	0.1	20	0.4
28ND4F2	28	ND	4	F	2	127	49	73	3.2	6.9	3.7	0.1	0.1	19	0.5
28ND5M1	28	ND	5	M	1	220	141	364	3.1	6.3	3.2	0.0	0.0	23	0.5
28ND5M2	28	ND	5	M	2	188	125	297	3.1	6.3	3.2	0.1	0.1	23	0.4
28ND6F1	28	ND	6	F	1	177	62	101	3.3	6.6	3.3	0.1	0.1	18	0.5
28ND6F2	28	ND	6	F	2	156	66	159	3.0	6.3	3.3	0.1	0.1	20	0.5
28ND7M1	28	ND	7	M	1	186	47	60	3.2	6.4	3.2	0.1	0.1	20	0.4
28ND7M2	28	ND	7	M	2	191	48	111	3.0	6.1	3.1	0.0	0.0	20	0.5
28ND8F1	28	ND	8	F	1	186	73	214	3.2	6.3	3.1	0.0	0.0	18	0.5
28ND8F2	28	ND	8	F	2	139	68	74	3.1	6.1	3.0	0.1	0.1	21	0.5
28ND9M1	28	ND	9	M	1	225	64	63	2.9	5.8	2.9	0.1	0.1	24	0.5
28ND9M2	28	ND	9	M	2	210	48	69	3.1	5.8	2.7	0.1	0.1	20	0.5
28ND10F1	28	ND	10	F	1	113	56	54	3.2	6.5	3.3	0.1	0.1	22	0.4
28ND10F2	28	ND	10	F	2	121	39	108	3.3	6.5	3.2	0.1	0.1	16	0.5
IDEXX REFERENCE					High		40	75	4.8	7.6	3	0.5		21	0.8
IDEXX REFERENCE					Low		30	45	3.8	5.6	1.8	0.2		15	0.2
HSD REFERENCE					High	196	45	104	3.8	6.6	3	0.13		22	0.4
HSD REFERENCE					Low	92	26	68	3.3	5.5	2	0		7	0.2

Table H.1: Raw Data for In-House No Treatment Control Group (continued)

ID	Day	Group	Cage	Gender	Sub-Designation	CHOLESTEROL	GLUCOSE	CALCIUM	PHOSPHORUS	CHLORIDE	POTASSIUM	SODIUM	A/G RATIO	B/C RATIO	INDIRECT BILIRUBIN	NA/K RATIO	HEMOLYSIS INDEX	LIPEMIA INDEX
28NDM1	28	ND	1	M	1	84	486	12.0	9.7	98	7.2	145	1.3	44.0	0	20	+	N
28NDM2	28	ND	1	M	2	94	285	11.6	9.7	100	7.0	146	1.2	44.0	0	21	+	N
28NDF1	28	ND	2	F	1	91	361	11.3	10.3	102	9.4	141	1.3	46.0	0	15	++	N
28NDF2	28	ND	2	F	2	102	357	11.6	10.2	102	5.9	145	1.4	44.0	0	25	+	N
28ND3M1	28	ND	3	M	1	100	478	11.3	10.2	96	5.2	140	0.9	48.0	0	27	++	N
28ND3M2	28	ND	3	M	2	78	237	10.7	9.1	98	5.5	143	1.0	48.0	0	26	N	N
28ND4F1	28	ND	4	F	1	100	305	11.0	6.4	99	5.3	141	1.0	50.0	0	27	N	N
28ND4F2	28	ND	4	F	2	105	376	11.7	8.3	96	6.9	142	0.9	38.0	0	21	N	N
28ND5M1	28	ND	5	M	1	85	526	12.1	11.5	98	9.2	139	1.0	46.0	0	15	++	N
28ND5M2	28	ND	5	M	2	97	237	10.8	9.5	99	6.6	143	1.0	57.5	0	22	++	N
28ND6F1	28	ND	6	F	1	105	230	11.5	8.1	102	5.5	144	1.0	36.0	0	26	N	N
28ND6F2	28	ND	6	F	2	91	378	11.2	8.8	98	6.1	140	0.9	40.0	0	23	+	N
28ND7M1	28	ND	7	M	1	87	275	11.1	8.7	100	5.3	141	1.0	50.0	0	27	N	N
28ND7M2	28	ND	7	M	2	94	303	11.2	9.0	99	6.4	143	1.0	40.0	0	22	+	N
28ND8F1	28	ND	8	F	1	101	226	10.8	9.1	99	5.7	139	1.0	36.0	0	24	++	N
28ND8F2	28	ND	8	F	2	109	264	10.7	7.4	99	5.4	140	1.0	42.0	0	26	N	N
28ND9M1	28	ND	9	M	1	81	259	10.7	8.3	100	4.9	140	1.0	48.0	0	29	N	N
28ND9M2	28	ND	9	M	2	77	424	10.5	10.7	100	6.1	140	1.1	40.0	0	23	+	N
28ND10F1	28	ND	10	F	1	113	206	11.5	8.9	99	5.5	142	1.0	55.0	0	26	N	N
28ND10F2	28	ND	10	F	2	89	163	10.4	7.0	101	5.2	140	1.0	32.0	0	27	+	N
IDEXX REFERENCE				High		130	135	13	8.3		5.8	140						
IDEXX REFERENCE				Low		40	50	5	5.3		3.7	127						
HSD REFERENCE				High			112	11.5	9.2	107	6.6	147						
HSD REFERENCE				Low			45	10.1	7.4	99	5.6	141						

Table H.1: Raw Data for In-House No Treatment Control Group (continued)

ID	Day	Group	Cage	Gender	Sub-Designation	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	NEUTROPHIL SEG	LYMPHOCYTES	MONOCYTES	EOSINOPHIL	BASOPHIL	AUTO PLATELET
28NDM1	28	ND	1	M	1	5.6	8.5	15.8	52.5	62	18.6	30.1	13	81	1	3	2	810
28NDM2	28	ND	1	M	2	7.7	9.3	16.6	56.2	61	17.9	29.5	12	83	1	2	2	896
28NDF1	28	ND	2	F	1	5.2	7.1	13.5	44.6	63	19.0	30.2	9	86	1	4	0	414
28NDF2	28	ND	2	F	2	3.2	7.4	14.4	46.3	63	19.6	31.1	14	83	3	0	0	988
28ND3M1	28	ND	3	M	1	8.9	8.5	16.1	52.4	62	19.0	30.7	16	79	2	3	1	825
28ND3M2	28	ND	3	M	2	7.8	9.4	17.6	56.5	60	18.8	31.2	8	89	2	1	0	873
28ND4F1	28	ND	4	F	1	4.8	8.8	16.0	51.3	58	18.2	31.2	19	79	1	1	0	490
28ND4F2	28	ND	4	F	2	4.0	7.5	15.3	46.9	63	20.4	32.6	17	70	1	5	7	358
28ND5M1	28	ND	5	M	1	3.9	8.9	16.4	51.8	58	18.4	31.7	15	78	5	2	0	807
28ND5M2	28	ND	5	M	2	4.8	8.1	15.9	49.8	62	19.7	31.9	7	90	2	1	0	223
28ND6F1	28	ND	6	F	1	1.1	5.2	9.7	32.2	63	18.8	30.1	12	74	6	5	4	A
28ND6F2	28	ND	6	F	2	4.7	9.1	17.7	52.5	57	19.4	33.7	6	90	3	1	0	643
28ND7M1	28	ND	7	M	1	6.4	9.0	16.7	53.9	60	18.7	31.0	14	83	2	1	1	804
28ND7M2	28	ND	7	M	2	7.9	8.5	17.0	53.5	63	20.0	31.8	12	86	1	1	0	488
28ND8F1	28	ND	8	F	1	5.4	9.8	19.3	59.0	60	19.8	32.7	19	77	3	1	0	352
28ND8F2	28	ND	8	F	2	4.8	8.6	16.3	53.0	61	18.9	30.8	6	93	0	1	0	470
28ND9M1	28	ND	9	M	1	10.2	8.7	16.0	51.2	59	18.5	31.3	11	84	4	1	1	866
28ND9M2	28	ND	9	M	2	4.4	9.1	18.4	56.8	63	20.3	32.4	13	84	2	2	0	A
28ND10F1	28	ND	10	F	1	4.4	8.5	16.0	52.1	62	18.9	30.7	7	85	5	1	1	870
28ND10F2	28	ND	10	F	2	1.1	5.0	10.3	31.4	63	20.6	32.8	13	67	10	7	4	A
IDEXX REFERENCE				High			10		48				34	85	5	6	2	
				Low			7		36				9	68	0	0	0	
HSD REFERENCE				High		16.1	9.0	17.4	52.4	67	21.2	35.3	25	71	5			1655
				Low		2.9	6.9	13.9	43.2	54	18.2	30.1	1	69	1			580

Table H.1: Raw Data for In-House No Treatment Control Group (continued)

ID	Day	Group	Cage	Gender	Sub-Designation	ABSOLUTE NEUTROPHIL SEG	ABSOLUTE LYMPHOCYTE	ABSOLUTE MONOCYTE	ABSOLUTE EOSINOPHIL	ABSOLUTE BASOPHIL	POLYCHROMASIA	ANISOCYTOSIS	CLUMPED PLATELETS	Remarks
28NDM1	28	ND	1	M	1	728	4536	56	168	112	+	+		
28NDM2	28	ND	1	M	2	924	6391	77	154	154			Y	B
28NDF1	28	ND	2	F	1	468	4472	52	208	0	+	+	Y	B
28NDF2	28	ND	2	F	2	448	2656	96	0	0				B
28ND3M1	28	ND	3	M	1	1424	7031	178	267	89			Y	
28ND3M2	28	ND	3	M	2	624	6942	156	78	0	+	+		
28ND4F1	28	ND	4	F	1	912	3792	48	48	0			Y	B
28ND4F2	28	ND	4	F	2	680	2800	40	200	280	+	+		D
28ND5M1	28	ND	5	M	1	585	3042	195	78	0			Y	
28ND5M2	28	ND	5	M	2	336	4320	96	48	0	+	+	Y	D
28ND6F1	28	ND	6	F	1	132	814	66	55	44	+	+		C D
28ND6F2	28	ND	6	F	2	282	4230	141	47	0	+	+	Y	
28ND7M1	28	ND	7	M	1	896	5312	128	64	64			Y	
28ND7M2	28	ND	7	M	2	948	6794	79	79	0	+	+	Y	B
28ND8F1	28	ND	8	F	1	1026	4158	162	54	0			Y	B
28ND8F2	28	ND	8	F	2	288	4464	0	48	0	+	+	Y	B
28ND9M1	28	ND	9	M	1	1122	8568	408	102	102	+	+		
28ND9M2	28	ND	9	M	2	572	3696	88	88	0	+	+		C D
28ND10F1	28	ND	10	F	1	308	3740	220	44	44			Y	
28ND10F2	28	ND	10	F	2	143	737	110	77	44	+	+		C D
IDEXX REFERENCE					High	5400	14100	540						
IDEXX REFERENCE					Low	100	2000	0						
HSD REFERENCE					High	8800	11560	980						
HSD REFERENCE					Low	0	3190	0						

A   Decreased
B   PLATELET COUNT REFLECTS MINIMUM VALUE.
C   PLATELET ESTIMATE APPEARS TO BE <10,000
D   ***BLOOD COUNT AND PLATELET EVALUATION AFFECTED BY PRESENCE OF CLOT IN TUBE***

ND = No dose (equivalent to No Treatment), F= Female, M=Male

Works Cited

2008. USP 31 - NF 26. The United States Pharmacopeial Convention, Rockville, MD.

Adell, A. 2004. Antidepressant properties of substance P antagonists: relationship to monoaminergic mechanisms? Curr. Drug Targets: CNS Neurol. Disord. 3:113-121.

Agu, R. U., M. I. Ugwoke, M. Armand, R. Kinget, and N. Verbeke. 2001. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir. Res. 2:198-209.

Alexander, B. D., E. S. Dodds Ashley, R. M. Addison, J. A. Alspaugh, N. J. Chao, and J. R. Perfect. 2005. Non-comparative evaluation of the safety of aerosolized amphotericin B lipid complex in patients undergoing allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis 8:13-20.

Alvarez, C. A., N. P. Wiederhold, J. T. McConville, J. I. Peters, L. K. Najvar, J. R. Graybill, J. J. Coalson, R. L. Talbert, D. S. Burgess, R. Bocanegra, K. P. Johnston, and R. O. Williams, 3rd. 2007. Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis. J Infect 55:68-74.

American Institute of Ultrasound in Medicine. 2000. Section 3--Selected biological properties of tissues: potential determinants of susceptibility to ultrasound-induced bioeffects. J. Ultrasound. Med. 19:58-96.

Argenti, D., B. Shah, and D. Heald. 1999. A pharmacokinetic study to evaluate the absolute bioavailability of triamcinolone acetonide following inhalation administration. J. Clin. Pharmacol. 39:695-702.

Argenti, D., B. Shah, and D. Heald. 2000. A study comparing the clinical pharmacokinetics, pharmacodynamics, and tolerability of triamcinolone acetonide HFA-134a metered-dose inhaler and budesonide dry-powder inhaler following inhalation administration. J. Clin. Pharmacol. 40:516-526.

Asgharian, B., R. Wood, and R. B. Schlesinger. 1995. Empirical modeling of particle deposition in the alveolar region of the lungs: a basis for interspecies extrapolation. Fund. Appl. Toxicol. 27:232-8.

Baddley, J. W., T. P. Stroud, D. Salzman, and P. G. Pappas. 2001. Invasive mold infections in allogeneic bone marrow transplant recipients. Clin Infect Dis 32:1319-24.

Bekersky, I., G. W. Boswell, R. Hiles, R. M. Fielding, D. Buell, and T. J. Walsh. 2000. Safety, toxicokinetics and tissue distribution of long-term intravenous liposomal amphotericin B (AmBisome): a 91-day study in rats. Pharm. Res. 17:1494-1502.

Bekersky, I., R. M. Fielding, D. E. Dressler, J. W. Lee, D. N. Buell, and T. J. Walsh. 2002. Plasma protein binding of amphotericin B and pharmacokinetics of bound versus unbound amphotericin B after administration of intravenous liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate. Antimicrob. Agents Chemother. 46:834-840.

Bennett, J. E. 2001. Antimicrobial Agents: Antifungal Agents, p. 1295-9. In J. G. Hardman and L. E. Limbird (ed.), Goodman & Gilman's The Pharamcological Basis of Therapeutics, 10th ed. McGraw-Hill, Chicago, IL.

Bennett, J. E. 2006. Antimicrobial Agents: Antifungal Agents. In L. L. Brunton, J. S. Lazo, and K. L. Parker (ed.), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed. The McGraw-Hill Companies, Inc. , New York, NY.

Bermejo, M., and I. Gonzalez-Alvarez. 2008. How and where are drugs absorbed? Preclinical Development Handbook: ADME and Biopharmaceutical Properties:249-280.

Beyer, J., S. Schwartz, G. Barzen, G. Risse, K. Dullenkopf, C. Weyer, and W. Siegert. 1994. Use of amphotericin B aerosols for the prevention of pulmonary aspergillosis. Infection 22:143-8.

Bisgaard, H., C. O'Callaghan, and G. C. Smaldone (ed.). 2002. Drug Delivery to the Lung, vol. 162. Marcel Dekker, Inc., New York, NY.

Bisgaard, H., K. Nikander, and E. Munch. 1998. Comparative study of budesonide as a nebulized suspension vs pressurized metered-dose inhaler in adult asthmatics. Respir. Med. 92:44-9.

Black, K. E., and L. R. Baden. 2007. Fungal infections of the CNS: treatment strategies for the immunocompromised patient. CNS Drugs 21:293-318.

Blot, F., F. Faurisson, N. Bernard, S. Sellam, S. Friard, R. Tavakoli, C. Carbon, M. Stern, A. Bisson, J. J. Pocidalo, and I. Caubarrere. 1999. Nebulized cyclosporine in the rat: assessment of regional lung and extrapulmonary deposition. Transplantation 68:191-5.

Boucher, H. W., A. H. Groll, C. C. Chiou, and T. J. Walsh. 2004. Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. Drugs 64:1997-2020.

Bowman, J. C., G. K. Abruzzo, J. W. Anderson, A. M. Flattery, C. J. Gill, V. B. Pikounis, D. M. Schmatz, P. A. Liberator, and C. M. Douglas. 2001. Quantitative PCR

assay to measure Aspergillus fumigatus burden in a murine model of disseminated aspergillosis: demonstration of efficacy of caspofungin acetate. Antimicrob. Agents Chemother. 45:3474-3481.

Brajtburg, J., W. G. Powderly, G. S. Kobayashi, and G. Medoff. 1990. Amphotericin B: current understanding of mechanisms of action. Antimicrob. Agents Chemother. 34:183-8.

Brazil, T. J., M. P. Dagleish, B. C. McGorum, P. M. Dixon, C. Haslett, and E. R. Chilvers. 2005. Kinetics of pulmonary neutrophil recruitment and clearance in a natural and spontaneously resolving model of airway inflammation. Clin. Exp. Allergy 35:854-865.

Brindley, C., C. Falcoz, A. E. Mackie, and A. Bye. 2000. Absorption kinetics after inhalation of fluticasone propionate via the Diskhaler, Diskus, and metered-dose inhaler in healthy volunteers. Clin. Pharmacokin. 39:1-8.

Brown, T. E. R., and T. W. F. Chin. 2008. Superficial Fungal Infections. In J. T. DiPiro, R. L. Talbert, G. C. Yee, G. R. Matzke, B. G. Wells, and L. M. Posey (ed.), Pharmacotherapy: A Pathophysiologic Approach, 6th ed. McGraw-Hill, Chicago, IL.

Brueggemann, R. J. M., J. P. Donnelly, R. E. Aarnoutse, A. Warris, N. M. A. Blijlevens, J. W. Mouton, P. E. Verweij, and D. M. Burger. 2008. Therapeutic Drug Monitoring of Voriconazole. Therap. Drug Monitor. 30:403-411.

Bruesewitz, C., A. Schendler, A. Funke, T. Wagner, and R. Lipp. 2007. Novel poloxamer-based nanoemulsions to enhance the intestinal absorption of active compounds. Int. J. Pharm. 329:173-181.

Burckart, G. J., G. C. Smaldone, M. A. Eldon, R. Venkataramanan, J. Dauber, A. Zeevi, K. McCurry, T. P. McKaveney, T. E. Corcoran, B. P. Griffith, and A. T. Iacono. 2003. Lung Deposition and Pharmacokinetics of Cyclosporine After Aerosolization in Lung Transplant Patients. Pharm. Res. 20:252-256.

Byron, P. R. 1987. Pulmonary targeting with aerosols. Pharm. Technol. 11:42, 44, 46, 48, 50, 52, 54, 56.

Capitano, B., B. A. Potoski, S. Husain, S. Zhang, D. L. Paterson, S. M. Studer, K. R. McCurry, and R. Venkataramanan. 2006. Intrapulmonary penetration of voriconazole in patients receiving an oral prophylactic regimen. Antimicrob. Agents Chemother. 50:1878-1880.

Carrier, R. L., L. A. Miller, and I. Ahmed. 2007. The utility of cyclodextrins for enhancing oral bioavailability. J. Controlled Release 123:78-99.

Carver, P. L. 2008. Invasive Fungal Infections. In J. T. DiPiro, R. L. Talbert, G. C. Yee, G. R. Matzke, B. G. Wells, and L. M. Posey (ed.), Pharmacotherapy: A Pathophysiologic Approach, 6th ed. McGraw-Hill, Chicago, IL.

Center for Drug Evaluation and Research (CDER). 1997. Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms. In Food and Drug Administration (ed.). U.S. Department of Health and Human Services.

Challa, R., A. Ahuja, J. Ali, and R. K. Khar. 2005. Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech 6:E329-57.

Chamilos, G., M. Luna, E. Lewis Russell, P. Bodey Gerald, R. Chemaly, J. Tarrand Jeffrey, A. Safdar, I. Raad Issam, and P. Kontoyiannis Dimitrios. 2006. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003). Haematologica 91:986-9.

Chamilos, G., M. Luna, R. E. Lewis, G. P. Bodey, R. Chemaly, J. J. Tarrand, A. Safdar, Raad, II, and D. P. Kontoyiannis. 2006. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003). Haematologica 91:986-9.

Chow, A. H. L., H. H. Y. Tong, P. Chattopadhyay, and B. Y. Shekunov. 2007. Particle Engineering for Pulmonary Drug Delivery. Pharm. Res. 24:411-437.

Christiansen, K. J., E. M. Bernard, J. W. Gold, and D. Armstrong. 1985. Distribution and activity of amphotericin B in humans. J. Infect. Dis. 152:1037-43.

Clark, D., M. Pickford, S. Evans, A. Bitonti, A. Bauer, and S. Newman. 2003. Targeting an Inhaled Erythropoietin Fc Fusion Protein (Epo-Fc) to the Human Large Central Airways, International Society for Aerosols in Medicine. Pharmaceutical Profiles, Baltimore, MD.

Clemons, K. V., and D. A. Stevens. 2005. The contribution of animal models of aspergillosis to understanding pathogenesis, therapy and virulence. Med. Mycol. 43:S101-S110.

Cloutier, M. M. 2007. Respiratory Physiology. Mosby, Philadelphia, PA.

Cohen, B. E. 1998. Amphotericin B toxicity and lethality. Int. J. Pharm. 162:95-106.

Collette, N., P. van der Auwera, A. P. Lopez, C. Heymans, and F. Meunier. 1989. Tissue concentrations and bioactivity of amphotericin B in cancer patients treated with amphotericin B-deoxycholate. Antimicrob. Agents Chemother. 33:362-8.

Conneally, E., M. T. Cafferkey, P. A. Daly, C. T. Keane, and S. R. McCann. 1990. Nebulized amphotericin B as prophylaxis against invasive aspergillosis in granulocytopenic patients. Bone Marrow Transplant. 5:403-6.

Conrad, D. J. 2003. The Clinical Use of Aerosolized Antibiotics. Clinical Pulmonary Medicine 10:201-207.

Corcoran, T. E., R. Venkataramanan, K. M. Mihelc, A. L. Marcinkowski, J. Ou, B. M. McCook, L. Weber, M. E. Carey, D. L. Paterson, J. M. Pilewski, K. R. McCurry, and S. Husain. 2006. Aerosol deposition of lipid complex amphotericin-B (Abelcet) in lung transplant recipients. Am. J. Transplant. 6:2765-2773.

Cornely, O. A., J. Maertens, D. J. Winston, J. Perfect, A. J. Ullmann, T. J. Walsh, D. Helfgott, J. Holowiecki, D. Stockelberg, Y. T. Goh, M. Petrini, C. Hardalo, R. Suresh, and D. Angulo-Gonzalez. 2007. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med 356:348-59.

Cornet, M., L. Fleury, C. Maslo, J. F. Bernard, and G. Brucker. 2002. Epidemiology of invasive aspergillosis in France: a six-year multicentric survey in the Greater Paris area. J. Hosp. Infect. 51:288-96.

Costa, C., D. Vidaud, M. Olivi, E. Bart-Delabesse, M. Vidaud, and S. Bretagne. 2001. Development of two real-time quantitative TaqMan PCR assays to detect circulating Aspergillus fumigatus DNA in serum. Journal of Microbiological Methods 44:263-269.

Cryan, S.-A., N. Sivadas, and L. Garcia-Contreras. 2007. In vivo animal models for drug delivery across the lung mucosal barrier. Advanced Drug Delivery Reviews 59:1133-1151.

Daley-Yates, P. T., A. C. Price, J. R. Sisson, A. Pereira, and N. Dallow. 2001. Beclomethasone dipropionate: absolute bioavailability, pharmacokinetics and metabolism following intravenous, oral, intranasal and inhaled administration in man. British Journal of Clinical Pharmacology 51:400-409.

Davis, J. L., J. H. Salmon, and M. G. Papich. 2006. Pharmacokinetics of voriconazole after oral and intravenous administration to horses. Am. J. Vet. Res. 67:1070-1075.

Denning, D. W. 1998. Invasive aspergillosis. Clin Infect Dis 26:781-803; quiz 804-5.

Denning, D. W. 1998. Invasive aspergillosis. Clin. Infect. Dis. 26:781-803; quiz 804-5.

Denning, D. W., P. Ribaud, N. Milpied, D. Caillot, R. Herbrecht, E. Thiel, A. Haas, M. Rubnke, and H. Lode. 2002. Efficacy and safety of voriconazole in the treatment of acute invasive Aspergillosis. Clin. Infect. Dis. 34:563-571.

Derom, E., and L. Thorsson. 2001. Factors Affecting the Clinical Outcome of Aerosol Therapy, p. 143-171. In H. Bisgaard, C. O'Callaghan, and G. C. Smaldone (ed.), Drug Delivery to the Lung, vol. 162. Marcel Dekker, Inc., New York, NY.

Diemunsch, P., and L. Grelot. 2003. Potential of substance P antagonists as antiemetics. Antiemetic Ther.:78-97.

Diot, P., B. Rivoire, A. Le Pape, E. Lemarie, D. Dire, Y. Furet, M. Breteau, and G. C. Smaldone. 1995. Deposition of amphotericin B aerosols in pulmonary aspergilloma. Eur. Respir. J. 8:1263-8.

Dixon, S., E. McKeen, M. Tabberer, and S. Paisley. 2004. Economic evaluations of treatments for systemic fungal infections: a systematic review of the literature. Pharmacoeconomics 22:421-33.

Drake, R. E., G. A. Laine, S. J. Allen, J. Katz, and J. C. Gabel. 1987. A model of the lung interstitial-lymphatic system. Microvascular Research 34:96-107.

Drew, R. 2006. Potential role of aerosolized amphotericin B formulations in the prevention and adjunctive treatment of invasive fungal infections. International Journal of Antimicrobial Agents 27:36-44.

Drew, R. H., E. Dodds Ashley, D. K. Benjamin, Jr., R. D. Davis, S. M. Palmer, and J. R. Perfect. 2004. Comparative safety of amphotericin B lipid complex and amphotericin B deoxycholate as aerosolized antifungal prophylaxis in lung-transplant recipients. Transplantation 77:232-237.

Dubois, J., T. Bartter, J. Gryn, and M. R. Pratter. 1995. The physiologic effects of inhaled amphotericin B. Chest 108:750-3.

Dubus, J. C., L. Vecellio, M. De Monte, B. Fink James, D. Grimbert, J. Montharu, C. Valat, N. Behan, and P. Diot. 2005. Aerosol deposition in neonatal ventilation. Pediatr. Res. 58:10-4.

Dummer, J. S., N. Lazariashvilli, J. Barnes, M. Ninan, and A. P. Milstone. 2004. A survey of anti-fungal management in lung transplantation. J Heart Lung Transplant 23:1376-81.

Dummer, J. S., N. Lazariashvilli, J. Barnes, M. Ninan, and P. Milstone Aaron. 2004. A survey of anti-fungal management in lung transplantation. J Heart Lung Transplant 23:1376-81.

Edsbaecker, S. 2001. Uptake, Retention, and Biotransformation of Corticosteroids in the Lung and Airways, p. 213-244. In R. P. Schleimer, P. M. O'Byrne, S. J. Szefler, and R. Brattsand (ed.), Inhaled Steroids in Asthma: Oftimizing Effects in the Airways, vol. 163. Marcel Dekker, Inc., New York, NY.

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Erjavec, Z., G. M. H. Woolthuis, H. G. De Vries-Hospers, W. J. Sluiter, S. M. G. J. Daenen, B. De Pauw, and M. R. Halie. 1997. Tolerance and efficacy of amphotericin B inhalations for prevention of invasive pulmonary aspergillosis in hematological patients. Eur. J. Clin. Microbiol. 16:364-368.

Esposito-Festen, J. E., P. Zanen, H. A. W. M. Tiddens, and J. W. J. Lammers. 2007. Pharmacokinetics of inhaled monodisperse beclomethasone as a function of particle size. British Journal of Clinical Pharmacology 64:328-334.

Evrard, B., P. Bertholet, M. Gueders, M. P. Flament, G. Piel, L. Delattre, A. Gayot, P. Leterme, J. M. Foidart, and D. Cataldo. 2004. Cyclodextrins as a potential carrier in drug nebulization. J. Controlled Release 96:403-410.

Ewing, P., B. Blomgren, A. Ryrfeldt, and P. Gerde. 2006. Increasing Exposure Levels Cause an Abrupt Change in the Absorption and Metabolism of Acutely Inhaled Benzo(a)pyrene in the Isolated, Ventilated, and Perfused Lung of the Rat. Toxicol. Sci. 91:332-340.

Fels, A. O., and Z. A. Cohn. 1986. The alveolar macrophage. J Appl Physiol 60:353-69.

Finney, M. J., S. D. Anderson, and J. L. Black. 1987. The effect of non-isotonic solutions on human isolated airway smooth muscle. Respir. Physiol. 69:277-86.

Freed, A. N., K. T. Yiin, and C. E. Stream. 1989. Hyperosmotic-induced bronchoconstriction in the canine lung periphery. J. Appl. Physiol. 67:2571-8.

Freiwald, M., A. Valotis, A. Kirschbaum, M. McClellan, T. Murdter, P. Fritz, G. Friedel, M. Thomas, and P. Hogger. 2005. Monitoring the initial pulmonary absorption of two different beclomethasone dipropionate aerosols employing a human lung reperfusion model. Respiratory Research 6:21.

Gabardi, S., D. W. Kubiak, A. K. Chandraker, and S. G. Tullius. 2007. Invasive fungal infections and antifungal therapies in solid organ transplant recipients. Transpl. Int. 20:993-1015.

Gavalda, J., M.-T. Martin, P. Lopez, X. Gomis, J.-L. Ramirez, D. Rodriguez, O. Len, Y. Puigfel, I. Ruiz, and A. Pahissa. 2005. Efficacy of nebulized liposomal amphotericin B in treatment of experimental pulmonary aspergillosis. Antimicrob. Agents Chemother. 49:3028-3030.

Gelman, S. 1986. Role of oxygen availability to the liver in anesthesia-induced hepatotoxicity. Mol. Cell. Mech. Anesth.:427-31.

Gold, W., H. A. Stout, J. F. Pagano, and R. Donovick. 1956. Amphotericins A and B, antifungal antibiotics produced by a streptomycete. I. In vitro studies. Antibiotics Annals:579-85.

Goldberg, M., and I. Gomez-Orellana. 2003. Challenges for the oral delivery of macromolecules. Nature Reviews Drug Discovery 2:289-295.

Gonda, I. 2006. Systemic Delivery of Drugs to Humans via Inhalation. J. Aerosol Med. 19:47-53.

Goodman, J. L., D. J. Winston, R. A. Greenfield, P. H. Chandrasekar, B. Fox, H. Kaizer, R. K. Shadduck, T. C. Shea, P. Stiff, D. J. Friedman, and et al. 1992. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. N Engl J Med 326:845-51.

Graybill, J. R., L. K. Najvar, G. M. Gonzalez, S. Hernandez, and R. Bocanegra. 2003. Improving the mouse model for studying the efficacy of voriconazole. J. Antimicrob. Chemother. 51:1373-1376.

Graybill, J. R., S. G. Revankar, and T. F. Patterson. 1998. Antifungal Agents and Antifungal Susceptibility Testing, p. 163-5. In L. Ajello and R. J. Hay (ed.), Med. Mycol., 9th ed, vol. 4. Oxford University Press, Inc., New York, NY.

Groll, A. H., S. C. Piscitelli, and T. J. Walsh. 1998. Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. Adv Pharmacol 44:343-500.

Henry, J. L. 1993. Substance P and inflammatory pain: Potential of substance P antagonists as analgesics. Agents Actions Suppl. 41:75-87.

Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J. W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke, and B. de Pauw. 2002. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 347:408-15.

Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J.-W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke, B. de Pauw, R. Allen, M. Aoun, C. Aul, M. Bjorkholm, K. L. Blanchard, M. Boogaerts, E. Bouza, E. J. Bow, H. R. Brodt, J. Brown, D. Buchheidt, J. Y. Cahn, A. Calmaggi, J. M. Cisneros, C. Cordonnier, J. Daly, C. A. Da Cunha, R. De Bock, A. Del Favero, J. Diaz Mediavilla, M. C. Dignani, C. Doyen, J. S. Dummer, B. Dupont, M. Egyed, D. Engelhard, G. Faetkenheuer, R. Feld, D. Fiere, G. Fioritoni, G. Garber, Z. Gasztonyi, K. Godder, D. Graham, A. Gratwohl, R. Greenberg, K. High, F. Jacobs, V. Kremery, P. Kumar, W. Langer, M. Laverdiere, P. Ljungman, H. Lode, A. Louie, D. Maki, J. P. Marie, D. J. E. Marriott, D. S. McKinsey, R.

Mertelsmann, M. K. Nair, N. Milpied, A. Nagler, D. Niederwieser, L. Pagano, P. Pappas, J. Perfect, J. Pottage, V. Raina, J. Reinhardt, S. Richardson, L. Rickman, M. Ruhnke, I. Salit, W. M. Scheld, S. Schuler, M. Schuster, R. Schwerdtfeger, S. D. Shafran, B. Simmons, M. Slavin, M. Sokol-Anderson, P. Tebas, C. Tsoukas, A. Ullmann, J. Van Burik, J. W. Van't Wout, E. C. Vinaya Kumar, et al. 2002. Voriconazole versus amphotericin B for primary therapy on invasive aspergillosis. N. Engl. J. Med. 347:408-415.

Herreros, J. M. C., and E. C. Matia. 2006. Therapeutic armamentarium against systemic fungal infections. Clinical Microbiology and Infection 12:53-64.

Hickey, A. J. 2002. Delivery of drugs by the pulmonary route, p. 479-499. In G. S. Banker and C. T. Rhodes (ed.), Drugs and the Pharmaceutical Sciences, 4th ed, vol. 121. Marcel Dekker, New York, NY.

Hill, M. Dec. 19, 2006 2007. Systems and methods for the delivery of corticosteroids having an increased lung deposition. US patent US 2007/0197487 A1.

Hintz, R. J., and K. C. Johnson. 1989. The effect of particle size distribution on dissolution rate and oral absorption. Int. J. Pharm. 51:9-17.

Hochhaus, G. 2007. Pharmacokinetic and pharmacodynamic properties important for inhaled corticosteroids. Annals of Allergy, Asthma, & Immunology 98:S7-S15.

Hoeben, B. J., D. S. Burgess, J. T. McConville, L. K. Najvar, R. L. Talbert, J. I. Peters, N. P. Wiederhold, B. L. Frei, J. R. Graybill, R. Bocanegra, K. A. Overhoff, P. Sinswat, K. P. Johnston, and R. O. Williams, III. 2006. In vivo efficacy of aerosolized nanostructured itraconazole formulations for prevention of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. 50:1552-1554.

Hsieh, T. H., C. P. Yu, and G. Oberdorster. 1999. Deposition and clearance models of Ni compounds in the mouse lung and comparisons with the rat models. Aerosol Sci. Technol. 31:358-372.

Hu, J., K. P. Johnston, and R. O. Williams, III. 2004. Nanoparticle Engineering Processes for Enhancing the Dissolution Rates of Poorly Water Soluble Drugs. Drug Dev. Ind. Pharm. 30:233-245.

Hung, O. R., S. C. Whynot, J. R. Varvel, and S. L. Shafer. 1995. Pharmacokinetics of inhaled liposome-encapsulated fentanyl. Anesthesiology 83:277-84.

Hung, O., and D. Pliura. 2008. Comparative phase I PK study of Aerosolized free and Liposome-Encapsulated Fentanyl (AeroLEF™) demonstrates rapid and extended plasma fentanyl concentrations following inhalation, 27th Annual Scientific Meeting of the American Pain Society. YM Biosciences Inc., Biovail Contract Research, Tampa, FL.

Hursh, D., S. Gelman, and E. L. Bradley, Jr. 1987. Hepatic oxygen supply during halothane or isoflurane anesthesia in guinea pigs. Anesthesiology 67:701-6.

Husain, S., D. L. Paterson, S. Studer, J. Pilewski, M. Crespo, D. Zaldonis, K. Shutt, D. L. Pakstis, A. Zeevi, B. Johnson, E. J. Kwak, and K. R. McCurry. 2007. Voriconazole prophylaxis in lung transplant recipients. Am. J. Transplant. 6:3008-3016.

Hussain, A., J. J. Arnold, M. A. Khan, and F. Ahsan. 2004. Absorption enhancers in pulmonary protein delivery. J. Controlled Release 94:15-24.

Irie, T., and K. Uekama. 1997. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J. Pharm. Sci. 86:147-162.

Jarabek, A., B. Asgharian, and F. Miller. 2005. Dosimetric Adjustments for Interspecies Extrapolation of Inhaled Poorly Soluble Particles (PSP). Inhalation Toxicology 17:317-334.

Johnson, K. C. 2007. Dissolution: fundamentals of in vitro release and the biopharmaceutics classification system. Drugs Pharm. Sci. 165:1-28.

Kauffman, C. A. 2006. Fungal infections. Proceedings of the American Thoracic Society 3:35-40.

Keenan, R., A. Iacono, J. Dauber, A. Zeevi, S. Yousem, N. Ohori, G. Burckart, A. Kawai, G. Smaldone, and B. Griffith. 1997. Treatment of refractory acute allograft rejection with aerosolized cyclosporine in lung transplant recipients. Journal of Thoracic and Cardiovascular Surgery 113:335-340.

Kelly, H. W. 1998. Establishing a therapeutic index for the inhaled corticosteroids: part I. Pharmacokinetic/pharmacodynamic comparison of the inhaled corticosteroids. J. Allergy Clin. Immunol. 102:S36-S51.

Kinnarinen, T., P. Jarho, K. Jaervinen, and T. Jaervinen. 2003. The in vitro pulmonary deposition of a budesonide/gamma -cyclodextrin inclusion complex. Journal of Inclusion Phenomena and Macrocyclic Chemistry 44:97-100.

Klepser, M. E. 2002. Amphotericin B in lung transplant recipients. Ann. Pharmacother. 36:167-169.

Koizumi, T., K. Kubo, T. Kaneki, M. Hanaoka, T. Hayano, T. Miyahara, K. Okada, K. Fujimoto, H. Yamamoto, T. Kobayashi, and M. Sekiguchi. 1998. Pharmacokinetic evaluation of amphotericin B in lung tissue: lung lymph distribution after intravenous injection and airspace distribution after aerosolization and inhalation of amphotericin B. Antimicrob. Agents Chemother. 42:1597-1600.

Koushik, K., D. S. Dhanda, N. P. S. Cheruvu, and U. B. Kompella. 2004. Pulmonary Delivery of Deslorelin: Large-Porous PLGA Particles and HPbeta CD Complexes. Pharm. Res. 21:1119-1126.

Kraft, W. K., B. Steiger, D. Beussink, J. N. Quiring, N. Fitzgerald, H. E. Greenberg, and S. A. Waldman. 2004. The pharmacokinetics of nebulized nanocrystal budesonide suspension in healthy volunteers. Journal of Clinical Pharmacology 44:67-72.

Krishna, G., M. Martinho, P. Chandrasekar, A. J. Ullmann, and H. Patino. 2007. Pharmacokinetics of oral posaconazole in allogeneic hematopoietic stem cell transplant recipients with graft-versus-host disease. Pharmacotherapy 27:1627-36.

Krishna, R., M. S. Webb, G. St. Onge, and L. D. Mayer. 2001. Liposomal and nonliposomal drug pharmacokinetics after administration of liposome-encapsulated vincristine and their contribution to drug tissue distribution properties. J. Pharmacol. Exp. Ther. 298:1206-1212.

Kumar, T. R. S., K. Soppimath, and S. K. Nachaegari. 2006. Novel delivery technologies for protein and peptide therapeutics. Current Pharmaceutical Biotechnology 7:261-276.

LAB International Inc. October 26, 2005 2005, posting date. LAB International Announces Positive Preliminary Results from Additional Phase 1 Fentanyl TAIFUN® Trial. [Online.]

Laehelmae, S., M. Kirjavainen, M. Kela, J. Herttuainen, M. Vahteristo, M. Silvasti, and M. Ranki-Pesonen. 2005. Equivalent lung deposition of budesonide in vivo: A comparison of dry powder inhalers using a pharmacokinetic method. British Journal of Clinical Pharmacology 59:167-173.

Lambros, M. P., D. A. W. Bourne, S. A. Abbas, and D. L. Johnson. 1997. Disposition of Aerosolized Liposomal Amphotericin B. J. Pharm. Sci. 86:1066-1069.

Lambros, M. P., D. W. Bourne, S. A. Abbas, and D. L. Johnson. 1997. Disposition of aerosolized liposomal amphotericin B. J Pharm Sci 86:1066-9.

Lattermann, R., T. Schricker, U. Wachter, M. Georgieff, and A. Goertz. 2001. Understanding the mechanisms by which isoflurane modifies the hyperglycemic response to surgery. Anesth. Analg. 93:121-127.

Letsou, G., H. Safi, M. Reardon, M. Ergenoglu, Z. Li, C. Klonaris, J. Baldwin, B. Gilbert, and J. Waldrep. 1999. Pharmacokinetics of liposomal aerosolized cyclosporine A for pulmonary immunosuppression. Annals of Thoracic Surgery 68:2044-2048.

Lewis, R. E., G. Liao, J. Hou, G. Chamilos, R. A. Prince, and D. P. Kontoyiannis. 2007. Comparative analysis of amphotericin B lipid complex and liposomal

amphotericin B kinetics of lung accumulation and fungal clearance in a murine model of acute invasive pulmonary aspergillosis. Antimicrob Agents Chemother 51:1253-8.

Lim, J. G. P., B. Shah, S. Rohatagi, and A. Bell. 2006. Development of a dry powder inhaler, the Ultrahaler, containing triamcinolone acetonide using in vitro-in vivo relationships. Am J Ther 13:32-42.

Lin, S. J., J. Schranz, and S. M. Teutsch. 2001. Aspergillosis case-fatality rate: systematic review of the literature. Clin. Infect. Dis. 32:358-66.

Lipinski, C. A., F. Lombardo, B. W. Dominy, and P. J. Feeney. 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 23:3-25.

Lipworth, B. J. 1996. Pharmacokinetics of inhaled drugs. Br. J. Clin. Pharmacol. 42:697-705.

Lipworth, B. J., and C. M. Jackson. 2000. Safety of inhaled and intranasal corticosteroids: Lessons for the new millennium. Drug Safety 23:11-33.

Lowry, C. M., F. M. Marty, S. O. Vargas, J. T. Lee, K. Fiumara, A. Deykin, and L. R. Baden. 2007. Safety of aerosolized liposomal versus deoxycholate amphotericin B formulations for prevention of invasive fungal infections following lung transplantation: a retrospective study. Transpl Infect Dis 9:121-5.

Lutsar, I., S. Roffey, and P. Troke. 2003. Voriconazole concentrations in the cerebrospinal fluid and brain tissue of guinea pigs and immunocompromised patients. Clin. Infect. Dis. 37:728-32.

MacCallum, D. M., J. A. Whyte, and F. C. Odds. 2005. Efficacy of caspofungin and voriconazole combinations in experimental aspergillosis. Antimicrob Agents Chemother 49:3697-701.

Mallick, S., S. Pattnaik, K. Swain, and P. K. De. 2007. Current Perspectives of Solubilization: Potential for Improved Bioavailability. Drug Dev. Ind. Pharm. 33:865-873.

Marr, K. A., F. Crippa, W. Leisenring, M. Hoyle, M. Boeckh, S. A. Balajee, W. G. Nichols, B. Musher, and L. Corey. 2004. Itraconazole versus fluconazole for prevention of fungal infections in patients receiving allogeneic stem cell transplants. Blood 103:1527-33.

Marr, K. A., R. A. Carter, F. Crippa, A. Wald, and L. Corey. 2002. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 34:909-17.

Marra, F., N. Partovi, K. M. Wasan, E. H. Kwong, M. H. H. Ensom, S. M. Cassidy, G. Fradet, and R. D. Levy. 2002. Amphotericin B disposition after aerosol inhalation in lung transplant recipients. Ann. Pharmacother. 36:46-51.

Martin, G. P., A. E. Bell, and C. Marriott. 1988. An in vitro method for assessing particle deposition from metered pressurized aerosols and dry powder inhalers. Int. J. Pharm. 44:57-63.

Martinez, M. N., and G. L. Amidon. 2002. A mechanistic approach to understanding the factors affecting drug absorption: A review of fundamentals. J. Clin. Pharmacol. 42:620-643.

Maschmeyer, G. 2006. The changing epidemiology of invasive fungal infections: new threats. International Journal of Antimicrobial Agents 27:3-6.

Maschmeyer, G., A. Haas, and O. A. Cornely. 2007. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs 67:1567-1601.

Mather, L. E., A. Woodhouse, M. E. Ward, S. J. Farr, R. A. Rubsamen, and L. G. Eltherington. 1998. Pulmonary administration of aerosolized fentanyl: pharmacokinetic analysis of systemic delivery. British Journal of Clinical Pharmacology 46:37-43.

McConville, J. T., K. A. Overhoff, P. Sinswat, J. M. Vaughn, B. L. Frei, D. S. Burgess, R. L. Talbert, J. I. Peters, K. P. Johnston, and R. O. Williams, III. 2006. Targeted High Lung Concentrations of Itraconazole Using Nebulized Dispersions in a Murine Model. Pharm. Res. 23:901-911.

Miller, F. J. 2000. Dosimetry of particles: critical factors having risk assessment implications. Inhalation Toxicol. 12:389-395.

Mitruka, S. N., A. Won, K. R. McCurry, A. Zeevi, T. McKaveney, R. Venkataramanan, A. Iacono, B. P. Griffith, and G. J. Burckart. 2000. In the lung aerosol cyclosporine provides a regional concentration advantage over intramuscular cyclosporine. J. Heart Lung Transplant. 19:969-75.

Mitruka, S. N., S. M. Pham, A. Zeevi, S. Li, J. Cai, G. J. Burckart, S. A. Yousem, R. J. Keenan, and B. P. Griffith. 1998. Aerosol cyclosporine prevents acute allograft rejection in experimental lung transplantation. Journal of Thoracic and Cardiovascular Surgery 115:28-37.

Miyake, K., H. Arima, T. Irie, F. Hirayama, and K. Uekama. 1999. Enhanced absorption of cyclosporin A by complexation with dimethyl-beta -cyclodextrin in bile duct-cannulated and -noncannulated rats. Biological & Pharmaceutical Bulletin 22:66-72.

Miyake, K., T. Irie, H. Arima, F. Hirayama, K. Uekama, M. Hirano, and Y. Okamaoto. 1999. Characterization of itraconazole/2-hydroxypropyl-beta -cyclodextrin inclusion complex in aqueous propylene glycol solution. Int. J. Pharm. 179:237-245.

Mobley, C., and G. Hochhaus. 2001. Methods used to assess pulmonary deposition and absorption of drugs. Drug Discovery Today 6:367-375.

Moelmann, H., M. Wagner, S. Krishnaswami, H. Dimova, Y. Tang, C. Falcoz, P. T. Daley-Yates, M. Krieg, R. Stoeckmann, J. Barth, C. Lawlor, A. C. Moelmann, H. Derendorf, and G. Hochhaus. 2001. Single-dose and steady-state pharmacokinetic and pharmacodynamic evaluation of therapeutically clinically equivalent doses of inhaled fluticasone propionate and budesonide, given as Diskus or Turbohaler dry-powder inhalers to healthy subjects. Journal of Clinical Pharmacology 41:1329-1338.

Mohammad, R. A., and K. C. Klein. 2006. Inhaled amphotericin B for prophylaxis against invasive Aspergillus infections. Ann. Pharmacother. 40:2148-2154.

Mollmann, H., S. Balbach, G. Hochhaus, J. Barth, and H. Derendorf. 1995. Pharmacokinetic-Pharmacodynamic Correlations of Corticosteroids. In H. Derendorf and G. Hochhaus (ed.), Handbook of Pharmacokinetic/Pharmacodynamic Correlation CRC Press, Boca Raton, FL.

Monforte, V., A. Roman, J. Gavalda, R. Lopez, L. Pou, M. Simo, S. Aguade, B. Soriano, C. Bravo, and F. Morell. 2003. Nebulized amphotericin B concentration and distribution in the respiratory tract of lung transplanted patients. Transplantation 75:1571-1574.

Morishita, M., and N. A. Peppas. 2006. Is the oral route possible for peptide and protein drug delivery? Drug Discovery Today 11:905-910.

Mortimer, K. J., T. W. Harrison, Y. Tang, K. Wu, S. Lewis, S. Sahasranaman, G. Hochhaus, and A. E. Tattersfield. 2006. Plasma concentrations of inhaled corticosteroids in relation to airflow obstruction in asthma. British Journal of Clinical Pharmacology 62:412-419.

Myers, S. E., S. M. Devine, R. L. Topper, M. Ondrey, C. Chandler, K. O'Toole, S. F. Williams, R. A. Larson, and R. B. Geller. 1992. A pilot study of prophylactic aerosolized amphotericin B in patients at risk for prolonged neutropenia. Leukemia & Lymphoma 8:229-33.

Nadithe, V., M. Rahamatalla, W. H. Finlay, J. R. Mercer, and J. Samuel. 2003. Evaluation of nose-only aerosol inhalation chamber and comparison of experimental results with mathematical simulation of aerosol deposition in mouse lungs. J. Pharm. Sci. 92:1066-1076.

Nakate, T., H. Yoshida, A. Ohike, Y. Tokunaga, R. Ibuki, and Y. Kawashima. 2003. Comparison of the lung absorption of FK224 inhaled from a pressurized metered dose inhaler and a dry powder inhaler by healthy volunteers. Eur. J. Pharm. Biopharm. 56:319-325.

Nakate, T., H. Yoshida, A. Ohike, Y. Tokunaga, R. Ibuki, and Y. Kawashima. 2003. Improvement of pulmonary absorption of cyclopeptide FK224 in rats by co-formulating with beta -cyclodextrin. Eur. J. Pharm. Biopharm. 55:147-154.

NAS. 1996. Guide for the Care and Use of Laboratory Animals National Academy Press, Washington DC.

Newhouse, M. T., P. H. Hirst, S. P. Duddu, Y. H. Walter, T. E. Tarara, A. R. Clark, and J. G. Weers. 2003. Inhalation of a dry powder tobramycin PulmoSphere formulation in healthy volunteers. Chest 124:360-366.

Nishimura, M., H. Yaguti, H. Yoshitsugu, S. Naito, and T. Satoh. 2003. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. Yakugaku Zasshi 123:369-75.

Overhoff, K. A., J. D. Engstrom, B. Chen, B. D. Scherzer, T. E. Milner, K. P. Johnston, and R. O. Williams. 2007. Novel ultra-rapid freezing particle engineering process for enhancement of dissolution rates of poorly water-soluble drugs. Eur. J. Pharm. Biopharm. 65:57-67.

Overhoff, K. A., R. Clayborough, and M. Crowley. 2008. Review of the TAIFUN Multidose Dry Powder Inhaler Technology. Drug Dev. Ind. Pharm. 34:960-965.

Pai, U., R. J. Blinkhorn, Jr., and J. F. Tomashefski, Jr. 1994. Invasive cavitary pulmonary aspergillosis in patients with cancer: a clinicopathologic study. Hum. Pathol. 25:293-303.

Pascual, A., T. Calandra, S. Bolay, T. Buclin, J. Bille, and O. Marchetti. 2008. Voriconazole therapeutic drug monitoring in patients with invasive mycosis improves efficacy and safety outcomes. Clin. Infect. Dis. 46:201-211.

Pascual, A., V. Nieth, T. Calandra, J. Bille, S. Bolay, L. A. Decosterd, T. Buclin, P. A. Majcherczyk, D. Sanglard, and O. Marchetti. 2007. Variability of voriconazole plasma levels measured by new high-performance liquid chromatography and bioassay methods. Antimicrob. Agents Chemother. 51:137-143.

Paterson, P. J., S. Seaton, H. G. Prentice, and C. C. Kibbler. 2003. Treatment failure in invasive aspergillosis: susceptibility of deep tissue isolates following treatment with amphotericin B. J Antimicrob Chemother 52:873-6.

Patterson, B., and P. Coates. 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: disposition in man., p. Abstract F78, 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC.

Patterson, T. F. 2005. Advances and challenges in management of invasive mycoses. Lancet 366:1013-25.

Patton, J. S., and P. R. Byron. 2007. Inhaling medicines: delivering drugs to the body through the lungs. Nature Reviews Drug Discovery 6:67-74.

Patton, J. S., C. S. Fishburn, and J. G. Weers. 2004. The lungs as a portal of entry for systemic drug delivery. Proceedings of the American Thoracic Society 1:338-344.

Perfect, J. R., E. Dodds, E. D. Ashley, and R. Drew. 2004. Design of aerosolized amphotericin B formulations for prophylaxis trials among lung transplant recipients. Clin. Infect. Dis. 39:S207-S210.

Petraitis, V., R. Petraitiene, A. A. Sarafandi, A. M. Kelaher, C. A. Lyman, H. E. Casler, T. Sein, A. H. Groll, J. Bacher, N. A. Avila, and T. J. Walsh. 2003. Combination therapy in treatment of experimental pulmonary aspergillosis: synergistic interaction between an antifungal triazole and an echinocandin. J Infect Dis 187:1834-43.

Pfizer Global Research & Development. 2001. Voriconazole AC Briefing Document, p. 56, October 4, 2001 ed. Center for Drug Evaluation and Research - Food and Drug Administration.

Pinkerton, K. E., R. R. Mercer, C. G. Plopper, and J. D. Crapo. 1992. Distribution of Injury and Microdosimetry of Ozone in the Ventilatory Unit of the Rat. Journal of Applied Physiology 73:817-824.

Playford, E. G., A. C. Webster, T. C. Sorrell, and J. C. Craig. 2006. Antifungal agents for preventing fungal infections in non-neutropenic critically ill and surgical patients: systematic review and meta-analysis of randomized clinical trials. J. Antimicrob. Chemother. 57:628-638.

Playford, E. G., A. C. Webster, T. C. Sorrell, and J. C. Craig. 2006. Systematic review and meta-analysis of antifungal agents for preventing fungal infections in liver transplant recipients. Eur. J. Clin. Microbiol. 25:549-561.

Porter, C. J. H., C. W. Pouton, J. F. Cuine, and W. N. Charman. 2008. Enhancing intestinal drug solubilization using lipid-based delivery systems. Advanced Drug Delivery Reviews 60:673-691.

Pouton, C. W. 2006. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. European Journal of Pharmaceutical Sciences 29:278-287.

Prentice, A. G., and A. Glasmacher. 2005. Making sense of itraconazole pharmacokinetics. J. Antimicrob. Chemother. 56:i17-i22.

Purvis, T., M. Vaughn Jason, L. Rogers True, X. Chen, A. Overhoff Kirk, P. Sinswat, J. Hu, T. McConville Jason, P. Johnston Keith, and O. Williams Robert, 3rd. 2006. Cryogenic liquids, nanoparticles, and microencapsulation. Int. J. Pharm. 324:43-50.

Rasenack, N., and B. W. Mueller. 2005. Poorly water-soluble drugs for oral delivery - A challenge for pharmaceutical development: Part I: Physicochemical and biopharmaceutical background / strategies in pharmaceutical development. Pharmazeutische Industrie 67:323-326.

Rhee, Y.-S., C.-W. Park, T.-Y. Nam, Y.-S. Shin, S.-C. Chi, and E.-S. Park. 2007. Formulation of parenteral microemulsion containing itraconazole. Archives of Pharmacal Research 30:114-123.

Richardson, M. D. 2005. Changing patterns and trends in systemic fungal infections. J. Antimicrob. Chemother. 56:i5-i11.

Rijnders, B. J., J. J. Cornelissen, L. Slobbe, M. J. Becker, J. K. Doorduijn, W. C. Hop, E. J. Ruijgrok, B. Lowenberg, A. Vulto, P. J. Lugtenburg, and S. de Marie. 2008. Aerosolized liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis during prolonged neutropenia: a randomized, placebo-controlled trial. Clin Infect Dis 46:1401-8.

Roerig Division of Pfizer Inc. 2006. Vfend Prescribing Information, p. 47. Pfizer Inc., New York, NY.

Roffey, S. J., S. Cole, P. Comby, D. Gibson, S. G. Jezequel, A. N. R. Nedderman, D. A. Smith, D. K. Walker, and N. Wood. 2003. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab. Dispos. 31:731-41.

Rogers, T. L., A. C. Nelsen, M. Sarkari, T. J. Young, K. P. Johnston, and R. O. Williams, III. 2003. Enhanced Aqueous Dissolution of a Poorly Water Soluble Drug by Novel Particle Engineering Technology: Spray-Freezing into Liquid with Atmospheric Freeze-Drying. Pharm. Res. 20:485-493.

Ruijgrok, E. J., A. G. Vulto, and E. W. M. Van Etten. 2000. Aerosol delivery of amphotericin B desoxycholate (Fungizone) and liposomal amphotericin B (AmBisome): aerosol characteristics and in-vivo amphotericin B deposition in rats. J. Pharm. Pharmacol. 52:619-627.

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Ruijgrok, E. J., A. G. Vulto, and E. W. M. Van Etten. 2001. Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromised rats. J. Antimicrob. Chemother. 48:89-95.

Saari, M., M. T. Vidgren, M. O. Koskinen, V. M. H. Turjanmaa, and M. M. Nieminen. 1999. Pulmonary distribution and clearance of two beclomethasone liposome formulations in healthy volunteers. International Journal of Pharmaceutics 181:1-9.

Saha, J. K., J. Xia, J. M. Grondin, S. K. Engle, and J. A. Jakubowski. 2005. Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats: Mechanisms and implications for preclinical models. Exp. Biol. Med. 230:777-784.

Sakagami, M. 2006. In vivo, in vitro and ex vivo models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery. Advanced Drug Delivery Reviews 58:1030-1060.

Sanati, H., P. Belanger, R. Fratti, and M. Ghannoum. 1997. A new triazole, voriconazole (UK-109,496), blocks sterol biosynthesis in Candida albicans and Candida krusei. Antimicrob. Agents Chemother. 41:2492-2496.

Sanders, M. 2007. Inhalation therapy: an historical review. Prim. Care Respir. J. 16:71-81.

Schlage, W. K., H. Bulles, D. Friedrichs, M. Kuhn, A. Teredesai, and P. M. Terpstra. 1998. Cytokeratin expression patterns in the rat respiratory tract as markers of epithelial differentiation in inhalation toxicology. II. Changes in cytokeratin expression patterns following 8-day exposure to room-aged cigarette sidestream smoke. Toxicologic Pathology 26:344-360.

Schmitt, H. J., E. M. Bernard, M. Haeuser, and D. Armstrong. 1988. Aerosol amphotericin B is effective for prophylaxis and therapy in a rat model of pulmonary aspergillosis. Antimicrob. Agents Chemother. 32:1676-9.

Schwartz, S., G. Behre, V. Heinemann, H. Wandt, E. Schilling, M. Arning, A. Trittin, W. V. Kern, O. Boenisch, D. Bosse, K. Lenz, W. D. Ludwig, W. Hiddemann, W. Siegert, and J. Beyer. 1999. Aerosolized amphotericin B inhalations as prophylaxis of invasive aspergillus infections during prolonged neutropenia: results of a prospective randomized multicenter trial. Blood 93:3654-3661.

Scott, L. J., D. Simpson, S. I. Blot, P. H. Chandrasekar, A. H. Groll, R. P. Hobson, V. L. Kan, S. Keady, and T. J. Walsh. 2007. Voriconazole: a review of its use in the management of invasive fungal infections. Drugs 67:269-298.

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Segal, B. H., N. G. Almyroudis, M. Battiwalla, R. Herbrecht, J. R. Perfect, T. J. Walsh, and J. R. Wingard. 2007. Prevention and early treatment of invasive fungal infection in patients with cancer and neutropenia and in stem cell transplant recipients in the era of newer broad-spectrum antifungal agents and diagnostic adjuncts. Clin Infect Dis 44:402-9.

Sheppard, D. C., G. Rieg, L. Y. Chiang, S. G. Filler, J. E. Edwards, Jr., and A. S. Ibrahim. 2004. Novel inhalational murine model of invasive pulmonary Aspergillosis. Antimicrob. Agents Chemother. 48:1908-1911.

Shoyele, S. A., and A. Slowey. 2006. Prospects of formulating proteins/peptides as aerosols for pulmonary drug delivery. Int. J. Pharm. 314:1-8.

Shrewsbury, S. B., A. P. Bosco, and P. S. Uster. 2009. Pharmacokinetics of a novel submicron budesonide dispersion for nebulized delivery in asthma. Int. J. Pharm. 365:12-17.

Sigfridsson, K., S. Forssen, P. Hollaender, U. Skantze, and J. de Verdier. 2007. A formulation comparison, using a solution and different nanosuspensions of a poorly soluble compound. Eur. J. Pharm. Biopharm. 67:540-547.

Slobbe, L., S. Polinder, J. K. Doorduijn, P. J. Lugtenburg, A. El Barzouhi, E. W. Steyerberg, and B. J. Rijnders. 2008. Outcome and Medical Costs of Patients with Invasive Aspergillosis and Acute Myelogenous Leukemia-Myelodysplastic Syndrome Treated with Intensive Chemotherapy: An Observational Study. Clin Infect Dis.

Smith, J., N. Safdar, V. Knasinski, W. Simmons, S. M. Bhavnani, P. G. Ambrose, and D. Andes. 2006. Voriconazole therapeutic drug monitoring. Antimicrob. Agents Chemother. 50:1570-1572.

Stavchansky, S., A. Martin, and A. Loper. 1979. Solvent system effects on drug absorption. Res. Commun. Chem. Pathol. Pharmacol. 24:77-85.

Stegemann, S., F. Leveiller, D. Franchi, H. de Jong, and H. Linden. 2007. When poor solubility becomes an issue: from early stage to proof of concept. Eur. J. Pharm. Sci. 31:249-61.

Steinbach, W. J., D. K. Benjamin, Jr., D. P. Kontoyiannis, J. R. Perfect, I. Lutsar, K. A. Marr, M. S. Lionakis, H. A. Torres, H. Jafri, and T. J. Walsh. 2004. Infections due to Aspergillus terreus: a multicenter retrospective analysis of 83 cases. Clin Infect Dis 39:192-8.

Stella, V. J., and Q. He. 2008. Cyclodextrins. Toxicol. Pathol. 36:30-42.

Sugar, A. M., and X. P. Liu. 2000. Effect of grapefruit juice on serum voriconazole concentrations in the mouse. Med. Mycol. 38:209-12.

Suzuki, R., and A. N. Freed. 2000. Hypertonic saline aerosol increases airway reactivity in the canine lung periphery. J. Appl. Physiol. 89:2139-46.

Takano, R., K. Furumoto, K. Shiraki, N. Takata, Y. Hayashi, Y. Aso, and S. Yamashita. 2008. Rate-Limiting Steps of Oral Absorption for Poorly Water-Soluble Drugs in Dogs; Prediction from a Miniscale Dissolution Test and a Physiologically-Based Computer Simulation. Pharm. Res. 25:2334-2344.

Tam, J. M., J. T. McConville, R. O. Williams, III, and K. P. Johnston. 2008. Amorphous cyclosporin nanodispersions for enhanced pulmonary deposition and dissolution. J. Pharm. Sci. 97:4915-4933.

Theuretzbacher, U., F. Ihle, and H. Derendorf. 2006. Pharmacokinetic/pharmacodynamic profile of voriconazole. Clin. Pharmacokin. 45:649-663.

Thorsson, L., K. Dahlstroem, S. Edsbaecker, A. Kaellen, J. Paulson, and J. E. Wiren. 1997. Pharmacokinetics and systemic effects of inhaled fluticasone propionate in healthy subjects. British Journal of Clinical Pharmacology 43:155-161.

Thorsson, L., S. Edsbacker, A. Kallen, and C. G. Lofdahl. 2001. Pharmacokinetics and systemic activity of fluticasone via Diskus and pMDI, and of budesonide via Turbuhaler. British Journal of Clinical Pharmacology 52:529-538.

Tolman, J. A., N. A. Nelson, Y. J. Son, S. Bosselmann, N. P. Wiederhold, J. T. McConville, and O. Williams Robert, 3rd. 2008. Aerosol Characterization and Single Dose Pharmacokinetic Analysis of a Nebulized Voriconazole Solution, American Association of Pharmaceutical Scientists Annual Meeting, Atlanta, GA.

Tolman, J. A., N. A. Nelson, Y. J. Son, S. Bosselmann, P. Wiederhold Nathan, J. I. Peters, T. McConville Jason, and R. O. Williams, 3rd. 2009. Characterization and Pharmacokinetic Analysis of Aerosolized Aqueous Voriconazole Solution. Eur. J. Pharm. Biopharm. Accepted.

Tolman, J. A., N. P. Wiederhold, J. T. McConville, L. K. Najvar, R. Bocanegra, J. I. Peters, J. J. Coalson, J. R. Graybill, T. F. Patterson, and R. O. Williams, 3rd. 2009. Inhaled Voriconazole for the Prevention of Invasive Pulmonary Aspergillosis. Antimicrob. Agents Chemother. Submitted.

Trifilio, S., G. Pennick, J. Pi, J. Zook, M. Golf, K. Kaniecki, S. Singhal, S. Williams, J. Winter, M. Tallman, L. Gordon, O. Frankfurt, A. Evens, and J. Mehta. 2007. Monitoring plasma voriconazole levels may be necessary to avoid subtherapeutic levels in hematopoietic stem cell transplant recipients. Cancer 109:1532-5.

Trifilio, S., R. Ortiz, G. Pennick, A. Verma, J. Pi, V. Stosor, T. Zembower, and J. Mehta. 2005. Voriconazole therapeutic drug monitoring in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant 35:509-13.

Tronde, A., B. Norden, H. Marchner, A.-K. Wendel, H. Lennernaes, and U. H. Bengtsson. 2003. Pulmonary absorption rate and bioavailability of drugs in vivo in rats: Structure-absorption relationships and physicochemical profiling of inhaled drugs. J. Pharm. Sci. 92:1216-1233.

Ullmann, A. J., J. H. Lipton, D. H. Vesole, P. Chandrasekar, A. Langston, S. R. Tarantolo, H. Greinix, W. Morais de Azevedo, V. Reddy, N. Boparai, L. Pedicone, H. Patino, and S. Durrant. 2007. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med 356:335-47.

Vaughn, J. M., J. T. McConville, D. Burgess, J. I. Peters, K. P. Johnston, R. L. Talbert, and R. O. Williams, III. 2006. Single dose and multiple dose studies of itraconazole nanoparticles. Eur. J. Pharm. Biopharm. 63:95-102.

Vaughn, J. M., N. P. Wiederhold, J. T. McConville, J. J. Coalson, R. L. Talbert, D. S. Burgess, K. P. Johnston, R. O. Williams, and J. I. Peters. 2007. Murine airway histology and intracellular uptake of inhaled amorphous itraconazole. Int. J. Pharm. 338:219-224.

Vermeij, P., and D. Blok. 1996. New peptide and protein drugs. Pharm. World Sci. 18:87-93.

Waldrep, J. C., J. Arppe, K. A. Jansa, and M. Vidgren. 1998. Experimental pulmonary delivery of cyclosporin A by liposome aerosol. International Journal of Pharamceutics 160:239-249.

Walsh, T. J., E. J. Anaissie, D. W. Denning, R. Herbrecht, D. P. Kontoyiannis, K. A. Marr, V. A. Morrison, B. H. Segal, W. J. Steinbach, D. A. Stevens, J.-A. van Burik, J. R. Wingard, and T. F. Patterson. 2008. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin. Infect. Dis. 46:327-360.

Walsh, T. J., I. Raad, T. F. Patterson, P. Chandrasekar, G. R. Donowitz, R. Graybill, R. E. Greene, R. Hachem, S. Hadley, R. Herbrecht, A. Langston, A. Louie, P. Ribaud, B. H. Segal, D. A. Stevens, J. A. van Burik, C. S. White, G. Corcoran, J. Gogate, G. Krishna, L. Pedicone, C. Hardalo, and J. R. Perfect. 2007. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. Clin Infect Dis 44:2-12.

Walsh, T. J., M. O. Karlsson, T. Driscoll, A. G. Arguedas, P. Adamson, X. Saez-Llorens, A. J. Vora, A. C. Arrieta, J. Blumer, I. Lutsar, P. Milligan, and N. Wood. 2004.

Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. Antimicrob. Agents Chemother. 48:2166-2172.

Wang, T., S. Noonberg, R. Steigerwalt, M. Lynch, R. A. Kovelesky, C. A. Rodriguez, K. Sprugel, and N. Turner. 2007. Preclinical safety evaluation of inhaled cyclosporine in propylene glycol Journal of Aerosol Medicine 20:417-428.

Wasan, K. M., A. L. Kennedy, S. M. Cassidy, M. Ramaswamy, L. Holtorf, J. W. Chou, and P. H. Pritchard. 1998. Pharmacokinetics, distribution in serum lipoproteins and tissues, and renal toxicities of amphotericin B and amphotericin B lipid complex in a hypercholesterolemic rabbit model: single-dose studies. Antimicrob. Agents Chemother. 42:3146-52.

Wax, P. M., C. E. Becker, and S. C. Curry. 2003. Unexpected "gas" casualties in Moscow: a medical toxicology perspective. Ann Emerg Med 41:700-5.

Whelan, G. J., J. L. Blumer, R. J. Martin, and S. J. Szefler. 2005. Fluticasone propionate plasma concentration and systemic effect: Effect of delivery device and duration of administration. Journal of Allergy and Clinical Immunology 116:525-530.

Wiebe, V., and M. Karriker. 2005. Therapy of systemic fungal infections: a pharmacologic perspective. Clinical Techniques in Small Animal Practice 20:250-7.

Wiederhold, N. P., L. K. Najvar, A. C. Vallor, W. R. Kirkpatrick, R. Bocanegra, D. Molina, M. Olivo, J. R. Graybill, and T. F. Patterson. 2008. Assessment of serum (1->3)-beta-D-glucan concentration as a measure of disease burden in a murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 52:1176-8.

Wiederhold, N. P., V. H. Tam, J. Chi, R. A. Prince, D. P. Kontoyiannis, and R. E. Lewis. 2006. Pharmacodynamic activity of amphotericin B deoxycholate is associated with peak plasma concentrations in a neutropenic murine model of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. 50:469-473.

Williams, A. C., and B. W. Barry. 2004. Penetration enhancers. Adv. Drug Delivery Rev. 56:603-618.

Wingard, J. R., S. L. Carter, T. J. Walsh, J. Kurtzberg, T. N. Small, I. D. Gersten, A. M. Mendizabal, H. Leather, D. L. Confer, L. R. Baden, R. T. Maziarz, E. A. Stadtmauer, J. Bolanos-Meade, J. Brown, J. F. DiPersio, M. Boeckh, and K. A. Marr. 2007. Presented at the American Society of Hematology 49th Annual Meeting, Atlanta, GA, December 8-10.

Winkler, J., G. Hochhaus, and H. Derendorf. 2004. How the lung handles drugs: pharmacokinetics and pharmacodynamics of inhaled corticosteroids. Proc. Am. Thorac. Soc. 1:356-363.

Winston, D. J., R. T. Maziarz, P. H. Chandrasekar, H. M. Lazarus, M. Goldman, J. L. Blumer, G. J. Leitz, and M. C. Territo. 2003. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. A multicenter, randomized trial. Ann Intern Med 138:705-13.

Wong, S. M., I. W. Kellaway, and S. Murdan. 2006. Enhancement of the dissolution rate and oral absorption of a poorly water soluble drug by formation of surfactant-containing microparticles. Int. J. Pharm. 317:61-68.

Yalkowsky, S. H., and Y. He. 2003. Handbook of Aqueous Solubility Data. CRC Press, Boca Raton, FL.

Yang, W., J. I. Peters, and R. O. Williams, III. 2008. Inhaled nanoparticles. A current review. Int. J. Pharm. 356:239-247.

Yang, W., J. Tam, D. A. Miller, J. Zhou, J. T. McConville, K. P. Johnston, and R. O. Williams. 2008. High bioavailability from nebulized itraconazole nanoparticle dispersions with biocompatible stabilizers. Int. J. Pharm. 361:177-188.

Yang, W., N. P. Wiederhold, and R. O. Williams, III. 2008. Drug delivery strategies for improved azole antifungal action. Expert Opin. Drug Delivery 5:1199-1216.

Zardooz, H., S. Zahedi Asl, M. K. Gharib Naseri, and M. Hedayati. 2006. Effect of chronic restraint stress on carbohydrate metabolism in rat. Physiol. Behav. 89:373-378.

Zhang, J. Y., Y. Wang, and C. Prakash. 2006. Xenobiotic-metabolizing enzymes in human lung. Curr. Drug Metab. 7:939-948.

Zhou, H. 2008. Water-Insoluble Drugs and Their Pharmacokinetic Behaviors. In R. Liu (ed.), Water-Insoluble Drug Formulation, 2nd ed. Taylor & Francis Ltd, Hoboken, NJ.

Vita

Justin Andrew Tolman was born on March 12, 1978 in Little Rock, Pulaski County, Arkansas to Russell Kent and Lizbeth Anne Sexton Tolman. From the age of 3, Justin was raised in Fort Worth, Texas. In 1996, he graduated Sumna Cum Laude from R.L. Paschal High School in Fort Worth, Texas and entered Brigham Young University in Provo, Utah as a chemistry major. Following his freshman year, he served a proselytizing mission for The Church of Jesus Christ of Latter-day Saints in the Philippines, Cabanatuan Mission, later renamed the Philippines, Angeles Mission, under President A. Burnell Hunt. In 1999, Justin returned to Brigham Young University to complete pre-pharmacy requirements preparatory to applying to pharmacy school. He married Jodi Ann Oldroyd in Bountiful, Utah on June 2, 2000. Justin was admitted to and began the Doctor of Pharmacy Program at the University of Texas at Austin in 2001. While in pharmacy school, two daughters were born to Justin and Jodi; Annie Michelle Tolman, born May 24, 2002 with Aicardi Syndrome, and Molly Lyn Tolman, born March 21, 2005. During his professional pharmacy education at the University of Texas at Austin, Justin engaged in undergraduate research in the laboratory of Dr. Robert O. Williams III. He graduated in 2005 with a Doctor of Pharmacy with Special Honors and immediately entered the graduate program under the supervision of Dr. Robert O. Williams III. Lexie Jane Tolman was born to Jodi and Justin on December 10, 2007. At the completion of his dissertation, Justin will begin as an Assistant Professor in the Division of Pharmaceutical Sciences at the Creighton University School of Pharmacy and Health Professions in Omaha, Nebraska.

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