PHARMACOKINETICS OF VORICONAZOLE IN HORSES AND ALPACAS

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PHARMACOKINETICS OF VORICONAZOLE IN HORSES AND ALPACAS

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PHARMACOKINETICS OF VORICONAZOLE IN HORSES AND ALPACAS

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Hui Min Chan, daughter of Ling Chan and Cheng-Yi Chan-Chao, was born on April 24, 1978, in Taipei, Taiwan. She attended Edmond North High School in Edmond, Oklahoma. In June 2001, she received her Bachelor of Science degree in Biology, with a minor degree in Classical Civilization, from the University of California, Los Angeles (UCLA). After working as an office assistant at the institutional review board office during the Summer of 2000 and as an undergraduate research assistant at a pharmacology lab at UCLA during the 2000-2001 school year, Hui Min entered the Graduate Program in Pharmaceutics at Auburn University in August 2001. She has served as a graduate teaching and research assistant in the School of Pharmacy while in the program.

DISSERTATION ABSTRACT

PHARMACOKINETICS OF VORICONAZOLE IN HORSES AND ALPACAS

Hui Min Chan

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Voriconazole is a new antifungal drug that has shown effectiveness in treating serious fungal infections and has the potential for being used in large animal veterinary medicine. The objectives of this study were to determine the plasma concentrations and pharmacokinetic parameters of voriconazole after single-dose intravenous (IV) and oral administration to alpacas and horses. In addition, the pharmacokinetics after oral multiple administrations to horses were determined. Voriconazole concentrations were measured by the use of high-performance liquid chromatography throughout this study.

After single 4 mg/kg intravenous and oral administrations of voriconazole to four alpacas, the mean terminal half-life ($t_{1/2\beta}$) following IV and oral administration was 8.011 ± 2.879 and 8.748 ± 4.307 hours, respectively; mean maximum plasma concentrations (C_{max}) were 5.930 ± 1.132 and 1.700 ± 2.707 mg/L, respectively; and area under the curve from time zero extrapolated to infinity ($AUC_{0\text{-}inf}$) was 38.50 ± 11.11 and

9.484 ± 6.983 mg-hr/L, respectively. The mean apparent systemic oral availability (F) was low with a value of 22.74 ± 9.48 %.

Two horses were used for the treatment of 2 mg/kg single dose IV and 3 mg/kg oral administration of a crushed tablet formulation of voriconazole, and four horses were used for the treatment of 4 mg/kg single dose intravenous and oral administration of a powder formulation of voriconazole. The mean $t_{1/2\beta}$ following IV, oral crushed tablet, and oral powder administrations were 12.22 ± 3.54, 9.482 ± 0.899, and 15.284 ± 3.497 hours, respectively. The mean extents of oral absorption for the crushed tablet and the powder formulations were 57.88 ± 8.62% and 103.07 ± 9.15%, respectively.

Two horses were dosed 3 mg/kg orally by a crushed tablet formulation of voriconazole, and four horses were given 4 mg/kg orally as a powder formulation of voriconazole every 24 hours. On day 14/15 of the treatment, the mean $t_{1/2\beta}$ following oral 3 mg/kg/day crushed tablet and 4 mg/kg/day powder administration were 10.046 and 7.141 hours, respectively; mean C_{max} were 1.977 and 1.892 µg/mL, respectively; mean dose-adjusted area under the curve from time zero to 24 hours (AUC_{0-24}/Dose) were 9.433 and 5.944 kg-hr/L, respectively. The mean accumulation factor calculated from the AUC values on day 14/15 compared to that of day 1 were 2.202 and 1.062, respectively, for the crushed tablet and the powder formulations. This difference in accumulation factor suggests a dosage form effect on drug accumulation during multiple dosing, possibly related to completeness of oral absorption.

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1. INTRODUCTION

There has been a steady increase in frequency and severity of fungal infections in human and veterinary medicine in recent years, and the species causing the infections are becoming more diverse (Kauffman, 2006). Among human patients, *Candida* and *Aspergillus* species are the two most common causes of invasive mycoses (Lamagni *et al*, 2001). Deep or generalized mycoses are uncommon but some can be life threatening. Fungal infections are often difficult to eradicate. Timely diagnosis and proper treatment are crucial for the control and cure of local and systemic fungal infections. Although the exact incidence of fungal infections has not been established in veterinary medicine, it is generally accepted that the trend has paralleled that of human medicine, with the increase in mortality from systemic mycoses (Wiebe and Karriker, 2005).

Fungal infections in horses include localized infections of the dermis, guttural pouches, cornea, and respiratory system (Valentine *et al*, 2006; Ludwig *et al*, 2005; Andrew *et al*, 1998; Sweeney and Habecker, 1999). The main site of entry of systemic fungal infections is the respiratory and gastrointestinal tract, where the spores are inhaled or ingested and disseminate via the systemic circulation, causing infections in deep tissues and body cavities. Primary systemic mycoses from pathogenic fungi are capable of establishing infections in apparently normal individuals, resulting in disease such as histoplasmosis, coccioidomycosis, and blastomycosis; whereas opportunistic fungi, including *Aspergillus* and *Cryptococcus*, usually require debilitated or

immunocompromised hosts. The increased frequency of severe and invasive fungal infections appears to be associated with the prolonged use of antibiotics and immunosuppressant agents. Growing resistance to conventional antifungal therapies is also believed to contribute to the rise in incidence (Merial, 2007).

With other large animals, such as the increasingly popular alpaca, those having chronic diseases and receiving prolonged antibiotic or immunosuppressive therapies are also prone to developing yeast and fungal infections. Cryptococcosis, aspergillosis, and coccidioidomycosis have been reported to occur in alpacas (Goodchild *et al*, 1996; Muntz, 1999; Thedford and Johnson, 1989). Despite considerable progress in the pharmaceutical management of invasive mycosis, the majority of antifungal agents are approved for human use only, and pharmacokinetic studies of antifungal drugs in alpacas and horses are limited. Usually the dosages given to large animals are based on study results from other mammals (Hector, 2005).

Since its discovery in 1952, amphotericin B has been the gold standard of therapy for invasive mycoses. Along with other systemic antifungal agents, flucytosine, griseofulvin, ketoconazole, and the newer azoles, fluconazole and itraconazole, these drugs have improved the survival rates of animals with systemic fungal infections (Wiebe and Karriker, 2005). However, the prognosis for many is still poor and guarded, and treatment failure is common. In addition, emergence of resistance of previously susceptible strains to older therapeutic agents has been observed in recent years (Brooks *et al*, 1998). Furthermore, antifungal drugs are associated with some serious adverse effects when administered systemically (Bennett, 1992). Therefore, there is a need for

investigation of new antifungal agents with good bioavailability, reduced toxicity, and improved resistance profile.

Voriconazole is a new broad-spectrum triazole antifungal drug, with *in vitro* activity against a variety of yeasts, filamentous fungi, and dimorphic molds. It was approved by the FDA for clinical use in humans in 2002 for the treatment of invasive aspergillosis and as salvage therapy when patients fail standard treatments for fungal infections caused by *Scedosporium apiospermum* and *Fusarium* species. In 2005, it was also approved for the treatment of invasive candidiasis (Pfizer, 2006). In several large *in vitro* surveillance studies of antifungal drugs, voriconazole demonstrated better activities with lower minimum inhibitory concentrations (MIC) against many isolates of *Candida* and *Aspergillus* species than fluconazole, itraconazole, and amphotericin B (Scott and Simpson, 2007).

Voriconazole is available in both intravenous (IV) and oral formulations. This drug is moderately lipophilic and rapidly absorbed after oral administration to mice, rats, rabbits, guinea pigs, dogs, and humans with high bioavailability (>75%), and it distributes well into various tissues and body fluids in human patients and animal subjects (Roffey *et al*., 2003; Capitano *et al*, 2006; Elter *et al*, 2006). Voriconazole has been used successfully in humans to treat various invasive mycoses involving the central nervous, skeletal, cardiac, and respiratory systems, either as the primary therapy, in combination with other antifungal drugs, or as salvage therapy (Pan *et al*, 2005; Mursch *et al*, 2006; Denes *et al*, 2007). The most commonly reported adverse effects of voriconazole are hepatotoxocity, skin rashes, and visual disturbances. However when compared to some of the older antifungal agents, voriconazole is well-tolerated and has

minimal side effects, and treatment discontinuation is rarely needed (Herbrecht *et al*, 2002). Due to its pharmacokinetic and pharmacological characteristics in human medicine, voriconazole appears to be a promising new candidate in veterinary medicine for the effective treatment of invasive mycoses.

Voriconazole exhibits non-linear elimination in all adult species investigated so far. Therefore, pharmacokinetic parameters are dependant upon dose, and an increase in dose results in superproportional increase in area under the plasma concentration versus time curve (AUC), due to saturable metabolism. Furthermore, after multiple dosing, autoinduction of the liver enzyme, cytochrome P450, have been noted in mouse, rat, and dog, attributing to a decrease in AUC and systemic concentration of the drug, and an increase in clearance values. This autoinduction of metabolism after chronic administration is not observed in the guinea pig, rabbit, or human (Roffey *et al*, 2003). Consequently, due to the discrepancy in the pharmacokinetic values between species and the drug's metabolism and elimination profiles, extrapolation of pharmacokinetic parameters from clinical and preclinical studies for veterinary use is likely to be inaccurate.

Two pharmacokinetic and disposition studies in horses have been recently published. The first study investigated the absorption of multiple dosing of topical and single dosing of oral voriconazole into plasma and aqueous humor. It showed that drug levels reached concentrations above the MIC values for voriconazole reported for many fungi species in the equine ocular fluid after single oral administration (Clode *et al*, 2006). In the study of single administration of IV and oral voriconazole in horses, the investigators demonstrated that voriconazole has excellent oral bioavailability, long

half-life, and no adverse reactions after administration of a single dose. But because of the non-linearity characteristic of this drug and the potential for accumulation, the authors recommended that multiple-dosing studies should be performed in addition to clinical trials (Davis *et al*, 2006).

This thesis is divided into five chapters. Chapter 2 will review the literature regarding invasive fungal infections and their treatment in relation to horses and alpacas. Chapter 3 presents the results from the investigation of single dosing of voriconazole to alpacas via IV and oral routes. Chapter 4 reports our findings of single dosing of IV and oral voriconazole in horses. In Chapter 5, pharmacokinetic effects of repeated oral dosing of voriconazole in horses will be discussed. Lastly, Chapter 6 will summarize the study, with regards to allometric scaling across species for voriconazole, and plans for future studies.

The objectives of this study were to (i) establish and compare the pharmacokinetic parameters after single intravenous and oral administration of voriconazole to horses and alpacas (ii) investigate the changes in elimination, absorption, and pharmacokinetic profiles and the safety of repeated oral dosing of voriconazole in horses (iii) evaluate the plasma concentration correlation of voriconazole when administered to horses as long-term dosing (14 to 15 days) by oral route and (iv) to use the results and data to develop and propose dosing regimens that will result in therapeutic plasma concentrations in horses and alpacas.

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2. REVIEW OF LITERATURE

2.1 Fungal Infections

A fungus is a primitive vegetable that can be found living in air, in soil, on plants, and in water. Some species of fungi live in and on human and other animal bodies. However, only a small fraction of the fungi species are identified to be pathogenic, and most do not cause infections. Some fungi species reproduce through microscopic spores in the air, and the spores can land on an individual or be inhaled. Therefore, a fungal infection often initiates on the skin or in the lungs in immunocompromised patients, or from penetration due to trauma in immunocompetent individuals. In immunocompetent individuals, after exposure to mold spores (conidia), macrophages kill the conidia, preventing them from becoming hyphae that invade the tissues, and polymorphonuclear leukocytes are the primary immune cells that protect against the hyphal form of the mold (Reichenberger *et al*, 2002; Martino *et al*, 2002; Miller, 1996). In immunocompromised patients, those with chemotherapy-induced neutropenia, and transplant recipients treated with immunosuppressants and corticosteroids have decreased or inhibited macrophage functions, which predispose patients to fungal infections (Brenguer *et al*, 1995). Prolonged neutropenia with a granulocyte count less than 500 μL^{-1} for over 20 days is the strongest risk factor for developing invasive fungal infections (Sharma and Chwogule, 1998).

Bacteria are usually the initial cause of fever during a neutropenic episode, but major developments in broad-spectrum antibiotics have significantly improved treatment outcome. However, prior antibiotic therapy puts the patients at subsequent risks for other infectious complications (De Pauw and Meis, 1998). This happens when the bacteria normally present are killed by antibiotic agents, thus causing the microbial flora to become unbalanced and inducing the overgrowth of certain fungi. Fungi are far less commonly the cause of disease as compared to viruses and bacteria. Nevertheless, fungal infections are more frequently the cause of severe diseases because of the greater difficulty of diagnosis and early treatment (Farina *et al*, 2006).

2.2 Clinical Symptoms

While an acute disseminated fungal infection can appear in a matter of hours, the chronic form of the disease develops over several months. Typically, the initial signs and symptoms of chronic disseminated fungal infection are non-specific, with the most common presentation being persistent fever despite empirical broad-spectrum antibiotic therapy (De Pauw and Meis, 1998). Cough, chest pain, hemoptysis, and dyspnea can indicate lower respiratory tract infections (Ascioglu *et al*, 2002).

Host factors also need to be considered. Those who are immunocompromised or have a foreign material, such as intravenous catheter, in the body are prone to being infected. The first year following transplantation is generally the period of highest risk for invasive mold infections, due to the intensification of immunosuppressive therapy to prevent allograft rejection (Segal and Walsh, 2006). In addition, the probability of acquiring fungal infection increases in people who are taking antibiotics. Some fungal infections can cause serious symptoms in even apparently healthy individuals. Delay or

absence of antifungal therapy is associated with poor outcome from invasive fungal infections (Eggimann *et al*, 2005).

2.3 Diagnostic Tools

In cases of invasive fungal infections that were positively identified post mortem, up to 30% of the patients did not receive any prior antifungal therapy, indicating that clinical diagnostic tools for detecting disseminated fungal infection are still largely insufficient (De Pauw and Meis, 1998). A positive culture from a normally sterile body site, such as blood, cerebrospinal fluid, joint aspirate, sterilely drained abscess or other sterile surgical specimen, is the gold standard for the diagnosis of a fungal infection. Culture from sputum, bronchoalveolar lavage fluid, exposed wound, abdominal drain, epithelium, or other mucocutaneous sources can help express the disease as possible or probable, but not diagnostically proven, due to its inability to differentiate colonization from infection (el-Ebiary *et al*, 1997; Cornwell *et al*, 1995). The culture for definitive diagnosis is limited by the slow growth of the organisms (Al-Agha *et al*, 2006). Serological antibody tests are also limited due to the long interval between infection and antibody production, and decreased immune response in immunocompromised individuals. In addition, false positives can occur from previous infections (Wheat *et al*, 1990).

Other laboratory tests include detections of galactomannan and β-glucan, which are fungal cell wall components. The galactomannan test utilizes the enzyme linked immnosorbent assay (ELISA) and is specific for *Aspergillus* species. However, its sensitivity is only 64% in cases of definite invasive aspergillosis (Herbrecht *et al*, 2002a). Polymerase chain reaction (PCR)-based detection of aspergillosis and candidiasis, like

the β-glucan assay, is still in its early stages of development and needs more standardization and validation before they can be widely applied in clinical settings (Ostrosky-Zeichner *et al*, 2005; Raad *et al*, 2002). Currently, bronchoalveolar cultures have only approximately 50% sensitivity, and blood culture results from patients are only positive in 50% of cases of invasive candidiasis and in 10% of cases of invasive aspergillosis (Levine, 1992; Horvath and Dummer, 1996). A positive result is observed usually only when the fungal burden is large enough and when the patient is in a late stage of the disease. In a study of experimental models of *Aspergillus* infection, mice whose blood became PCR positive had a mean fungus load in the lungs 10 times higher than that in mice whose blood remained PCR negative, and *Aspergillus* DNA was only detected in 25% of the blood samples (Loeffler *et al*, 2000). Furthermore, cultures for fungal presence have poor clinical sensitivity due to sampling error, as these organisms are ubiquitous in the environment, such that a culture that yields one of these fungi may merely reflect contamination or colonization (Perfect *et al*, 2001). Computed tomography (CT) scan may facilitate the early diagnosis of pulmonary fungal infections with a positive predictive value of 80%. The "halo sign" is a characteristic of angioinvasive organisms (Kuhlmam *et al*, 1987). With tissues cytology, it is difficult to differentiate certain genus of fungi, such as *Aspergillus*, *Scedosporium*, and *Fusarium*, from each other, but it is important to do so because each of these organisms has different susceptibilities to antifungal agents. Thus, diagnosis should be supported by serologic studies, pathological evaluation of the tissues involved, and radiographic imaging results (Kim *et al*, 1999).

2.4 Epidemiology

There has been a steady increase in frequency and severity of fungal infections in human and veterinary medicine in recent years, and the species causing the infections are becoming more diverse (Kauffman, 2006a). From 1980 to 1987, the increase in mortality from invasive fungal infections was 3.4-fold, from 0.7 to 2.4 deaths per 100,000 people (McNeil *et al*, 2001). *Candida* and *Aspergillus* are the most common causes of invasive fungal infections, accounting for 79-90% and 10-20% of all invasive mycoses, respectively (Fluckiger 2006). The mortality rate involving *Candida* infection is 33-47%, and the overall fatality rate for aspergillosis is 58% (Gudlaugsson *et al*, 2003; Pappas *et al*, 2003; Lin *et al*, 2001).

2.4.1 Candidiasis

Candida represents the fourth leading organism causing septicemia in the United States, Europe, and Australia (Pfaller *et al*, 1998). Candidemia rates vary geographically, but *C. albicans* is still the most common species causing candidemia worldwide (Colombo *et al*, 2006). Other frequent causes of disseminated candidiasis in immunocompromised individuals include *C. glabrata*, *C. parapsilosis*, *C. tropicalis,* and *C. guilliermondii* species (Girmenia *et al*, 2006). Studies that included pediatric patients have reported *C. parapsilosis* as the most common non-*albicans Candida* species, in contrast to adult patients, in whom *C. glabrata* is more common (Pfaller *et al*, 2002a; Pappas *et al*, 2003; Zaoutis *et al*, 2004). Invasive candidiasis is most often associated with translocation across colonized gastrointestinal epithelial surface damaged by surgical trauma, cytotoxic therapy as part of cancer treatment, or graft-versus-host reactions after transplantation (Bow, 2005).

Candida species can infect prosthetic valves, endocardium, myocardium, or pericardium. *Candida* endocarditis represents 48% of the cases of fungal endocarditis, with *C. albicans* accounting for one half of the *Candida* species (Ellis *et al*, 2001). Candidal esophagitis and oropharyngitis are most commonly diagnosed among AIDS patients, transplant recipients, and patients receiving antibiotics, inhaled corticosteroids, or chemotherapy (Morris and Villmann, 2006). Ocular candidasis are often preceded by topical steroid use, local trauma, corneal transplantation, or dissemination from another site (Ainbinder *et al*, 1998; Parke *et al*, 1982; Brooks *et al*, 1989). The most common form of primary abdominal candidal infection is peritonitis. It is caused by bowel perforation, post-operative infection, or peritoneal dialysis. *Candida* infections following abdominal surgery are characterized by mortality rates higher than 50% (Calandra *et al*, 1989; Solomkin *et al*, 1982). Osteomyelitis and septic arthritis caused by *Candida* are usually initiated by inoculation of organisms into the area by trauma, steroid injection into the joint, during surgery, contiguous spread from chronic foot ulcers, or disseminated from another site (Malani *et al*, 2002; Campen *et al*, 1990; Gathe *et al*, 1987). Large joints are most commonly affected, with at least one knee joint being involved in 71% of cases of polyarticular disease (Sims *et al*, 2005).

2.4.2 Aspergillosis

In contrast to candidiasis, invasive aspergillosis is most often acquired from inhalation of airborne-spores (conidia) into the respiratory tract (Bow, 2005). Once inside the respiratory tract, the conidia germinate to produce hyphae for subsequent entry into the lung alveoli to establish disease, particularly in immunocompromised individuals, and disseminate to other body sites (Krishnan *et al*, 2005). Invasive aspergillosis alone

affects 5% of bone marrow transplant recipients, 15% of patients with hematological malignancies, and 10-14% of solid organ transplant cases, with *Aspergillus fumigatus* being the most common cause (Denning *et al*, 1997b; Morrison *et al*, 1993). But in high risk patients, more than one *Aspergillus* isolates or species can be colonized (Chazalet *et al*, 1998). Systemic aspergillosis primarily affects the lungs (>90%) and to a lesser degree other internal organs such as the heart, kidneys, and spleen (Kaiser *et al*, 1998).

Pulmonary aspergillosis can be divided into four categories, depending on the patient's immune status: 1) allergic bronchopulmonary aspergillosis, which is caused by hypersensitivity to fungus, appears most commonly in patients with asthma and cystic fibrosis; 2) saprophytic aspergillosis (aspergilloma), is the most common form and involves the colonization of pre-existing cavities; 3) chronic necrotizing aspergillosis, which is a chronic cavitary pneumonic illness, is often found in patients with pre-existing chronic lung disease or in mildly immunocompromised patients; and 4) angioinvasive pulmonary aspergillosis, which affects immunocompromised patients with a mortality rate near 100%, is the most common form of invasive aspergillosis (Khan *et al*, 2003; Soubani and Chandrasekar, 2002).

2.4.3 Fusariosis

Like *Aspergillus*, *Fusarium* species are hyaline molds. *Fusarium* is an opportunistic fungal pathogen found in soil, water, and decaying debris, but it is emerging as a significant cause of morbidity and mortality in immunocompromised hosts (Fleming *et al*, 2002). Skin is an important port of entry for fusariosis, which can cause localized infections such as onychomycosis, paronychia, and cellulites. Inhalation of *Fusarium* spores can cause sinusitis and pneumonia (Boutati *et al*, 1997). Risk factors

for disseminated fusariosis include profound neutropenia, lymphopenia, graft-versus-host disease, severe burns, immunosuppressive or corticosteroid therapy, and indwelling venous catheters (Walsh *et al*, 2004a; Dignani and Anaissie, 2004). Pneumonia has been reported in 84% of cases of disseminated fusariosis, and *Fusarium* species are the most common cause of fungal keratitis world-wide (Boutati *et al*, 1997; Dignani and Anaissie, 2004). Fusariosis can be localized, locally invasive, or disseminated, when two or more noncontiguous sites are involved. Therapeutic options are scarce (Stanzani *et al*, 2006).

2.4.4 Other Rare Fungal Infections

Other emerging fungi include *Scedosporium apiospermum*, a ubiquitous filamentous fungus and the asexual state of *Pseudallescheria boydii*, is mostly found in stagnant or polluted water. Diseases caused by this fungal species include severe pulmonary infections and osteoarticular infection following trauma, otitis, and endocarditis. The disseminated form of scedosporiosis has a mortality rate >75% (Bennett, 1990).

Cryptococcus neoformans is a relatively frequent cause of serious fungal infection in HIV patients, as a major source of meningo-encephalitis. Patients with AIDS complicated by cryptococcosis often respond poorly to treatment, and they require lifelong maintenance therapy in the setting of continued immunosuppression, because currently available antifungal agents seldom eradicate this pathogen (Zuger *et al*, 1986).

Histoplasma capsulatum is endemic in the Mississippi and Ohio River valley regions. Disseminated histoplasmosis is the most severe form of *Histoplasma capsulatum* infection. Infection develops when microconidia are inhaled into the lungs,

germinate into parasitic yeast, attack macrophages and are capable of spreading throughout the reticuloendothelial system (Hector and Laniado-Laborin, 2005).

Trichosporon is a fungal organism usually found in soil and fresh water, and it is an emerging pathogen in immunocompromised patients. The mortality rate in these patients is estimated to be approximately 78%, despite amphotericin B therapy (Hoy *et al*, 1986).

2.5 Drug Treatments for Invasive Fungal Infections

The success of fungal treatment in invasive fungal infections depends on how early in the course of infection that the treatment has been instituted. The selection of therapy should depend on the presence of complicating organ dysfunction that might affect drug pharmacokinetics, relative drug toxicity, prior exposure to antifungal agents for therapy or prophylaxis, and the clinician's knowledge of the species and potential susceptibility pattern of the isolate (Sims *et al*, 2005). For example, conidia from mold species are generally absent in infected tissues. Thus, it is essential that the chosen antifungal drug should kill the active growing hyphae for a successful outcome in the treatment of invasive aspergillosis (Krishnan *et al*, 2005).

The currently available major antifungals for invasive aspergillosis target four different cell functions: cell membrane integrity (polyenes), DNA synthesis (pyrimidine analogs), ergosterol biosynthesis (azoles), and cell wall integrity (echinocandins) (Steinbach, 2005).

2.5.1 Polyenes

Amphotericin B, nystatin, and natamycin are examples of polyene antifungals. It is believed that drugs from this class of antifungals bind to sterols, preferentially to the

fungal cell membrane sterol, ergosterol, and disrupt the osmotic integrity of the fungal cell membrane, resulting in leakage of intracellular components and leading to cell death. Since its introduction in 1958, amphotericin B has been the gold standard for the treatment of invasive aspergillosis, candidiasis, and cryptococcosis. It is available in intravenous and oral suspension formulations.

Amphotericin B has a distribution half-life of 0.9 to 1.5 hours in mice, rats, dogs, and cynomolgus monkeys, and a elimination half-life of 25 to 28 hours in mice, 16 to 18 hours in rats, 44 to 47 hours in dogs, 35 hours in cynomolgus monkeys, and 15 days in humans (Kim *et al*, 1984; Wingard and Leather, 2004). The long elimination half-life is caused by much of the administered dose depositing in tissues, especially in fat, therefore, a depot effect is notable with a slow release into the blood long after cessation of administration. The large total volume of distribution of 4 L/kg contributes to the long elimination half-life of amphotericin B in humans, and the elimination clearance averages 30 ml/min (Atkinson and Bennett, 1978)

However, significant toxicity and low tolerability with high rates of nephrotoxicity still remain the major reasons for withdrawal of therapy, despite the introduction of the liposomal and colloidal formulations (Bowden *et al*, 2002). Evidence suggests the lipid formulation may be as efficacious as the conventional amphotericin B deoxycholate but with fewer and less severe side effects (Wingard *et al*, 2000). But the current practice is to switch to liposomal amphotericin B only when necessary, due to the higher acquisition cost of the liposomal formulation (Stevens *et al*, 2000).

Furthermore, amphotericin B resistant isolates of *C. glabrata* is becoming more common in Europe (Totorano *et al*, 2003). Approximately 20% of *C. parapsilosis*

isolates are resistant to amphotericin B (Zaoutis *et al*, 2005). It has also failed against infections caused by *Trichosporon* species (Wildfeuer *et al*, 1998). *In vitro* studies have revealed that some *Aspergillus* and most *Fusarium* and *Scedosporium* isolates are only moderately susceptible or resistant to amphotericin B (Pfaller *et al*, 2002b).

2.5.2 Pyrimidine Analogs

Flucytosine is a pyrimidine analog that is transported by cytosine permease into fungi and then deaminated to the active form to interfere with fungal nucleic acid synthesis. It inhibits fungal protein synthesis by replacing uracil in the fungal RNA with 5-flurouracil. Flucytosine also interferes with fungal DNA synthesis by inhibiting thymidylate synthetase action via 5-fluorodeoxy-uridine monophosphate (Schwartz and Thiel, 2003). This drug is water soluble with negligible protein binding (2.9-4%) and readily penetrates the blood-brain barrier, with around 74% of corresponding serum concentration appears in the cerebralspinal fluid (CSF) (Groll *et al*, 1998; Block and Bennett, 1972). Flucytosine is available only in oral formulation.

In humans, 76-89% of flucytosine is bioavailable after oral administration (Cutler *et al*, 1978). This drug is principally eliminated by the kidneys and the plasma clearance is closely related to creatinine clearance (Schonebeck *et al*, 1973; Cutler *et al*, 1978; Vermes *et al*, 2000). In patients with normal renal function, peak concentrations are observed in serum and other body fluids within 1 to 2 hours postdose (Cutler *et al*, 1978; Daneshmend and Warnock, 1983). The elimination half-life of flucytosine is approximately 3 to 4 hours in patients with normal renal function and up to 85 hours in patients with severe renal insufficiency (Frances and Walsh, 1992). Pharmacokinetic data of flucytosine on veterinary species is apparently unavailable.

Candida and *Cryptococcus* species are generally susceptible to flucytosine, but *Aspergillus* species are resistant to the drug. As rapid development of fungal resistance occurs through alterations in cytosine permease or altered metabolism, its role has been mainly used as combination therapy with amphotericin B (Wingard and Leather, 2004).

2.5.3 Azoles

Azoles are five-membered nitrogen heterocyclic ring compounds. The main cellular target of azoles is C14α-demethylase in the ergosterol biosynthetic pathway. Ergosterol is a main component of fungal cell membranes and plays an integral role in its fluidity, proper function of several membrane-bound enzymes, and mitochondrial respiration. When the fungal cytochrome P450 enzyme C14α-demethylase is inhibited by an azole, conversion of lanosterol to ergosterol is prevented. Thus ergosterol is depleted and toxic 14α-methyl-sterol accumulates. These result in an altered structure of the fungal cell membrane, making it more susceptible to further damages and modified functions of membrane-bound enzymes (Lupetti *et al*, 2005). The two major categories of azoles for the treatment of invasive fungal infections are imidazole and triazoles.

2.5.3.1 Miconazole

Miconazole is an imidazole and the first azole available for parenteral administration in 1978. Most *Pseudallescheria boydii* isolates exhibit *in vitro* susceptibility to miconazole, and it has a limited spectrum of activity against certain *Candida* species (Maertens, 2004). However, miconazole has an inconvenient dosing schedule that requires multiple daily intravenous infusions, and it can cause serious side effects and relapse upon discontinuation of treatment (Cuenca-Estrella *et al*, 1999; Johnson *et al*, 1998; Galgiani *et al*, 1984;). Due to its toxicity, the intravenous

formulation of miconazole is no longer available in the United States (Schaenman *et al*, 2005). Miconazole is available as an oral gel formulation for the treatment of oral fungal infections in some countries, but not in the United States.

When micellar and cyclodextrin-containing solutions of miconazole was administered intravenously to sheep, the distribution half-life ranged from 1.42 to 2.33 minutes and the elimination half-life ranged from 51.80 to 57.72 minutes; the mean clearance values were 16.54 mL/min/kg for the micellar solution, and 11.11 and 12.78 mL/min/kg for the two solutions containing cyclodextrin (Piel *et al*, 1999). In humans, the half-life of miconazole ranged from 24.1 to 25.4 hours (Lewi *et al*, 1976),

2.5.3.2 Ketoconazole

Ketoconazole is also an imidazole, approved by the United States Food and Drug Administration (FDA) for human use in 1981, and for almost a decade, it was the only oral drug available for the treatment of systemic fungal infections. It has biphasic pharmacokinetics, with a terminal half-life of 8 hours after single dosing and 3.3 hours after chronic administration (Badcock *et al*, 1987). The mean clearance divided by extent of absorption (Cl/F) of the ketoconazole oral solution was 209 mL/min, and the mean volume of distribution divided by extent of absorption (Vd/F) was 88.31 L. The relative bioavailabilities for the tablet and suspension formulations of ketoconazole were 81.2 and 89.0%, respectively, of that of the solution formulation (Huang *et al*, 1986). However, oral absorption of ketoconazole has high inter-individual variation and is influenced by gastric pH (Maertens, 2004). The pharmacokinetics of ketoconazole also showed dose-dependent characteristics, with an increase in the area under the curve values more than proportional to the increased dose given (Huang *et al*, 1986).

In rats, the mean volume of distribution value of ketoconazole after IV dosing was 655 mL/kg, clearance was observed to be 5.1 mL/min/kg, and the mean elimination half-life value was 35.0 minutes (Remmel *et al*, 1987). The mean bioavailability after oral dosing was 35.8%, with peak times observed between 30 and 60 minutes. In dogs, the mean systemic clearance was 2.74 mL/min/kg, the volume of distribution at steady state was 0.72 L/kg, and the half-life was 2.7 hours. The absolute bioavailability of ketoconazole tablets was 50%, and that of the ketoconazole solution was 56% in dogs (Baxter *et al*, 1986). After single oral administration of ketoconazole to gopher tortoises, the elimination half-life was 11.57 hours and the clearance was 5.02 mL/min/kg (Page *et al*, 1988).

Ketoconazole has been reported to be active against *Pseudallescheria boydii* and certain *Candida* species, but it has poor penetration into the central nervous system (CNS) (Nesky *et al*, 2000). Therefore, it is not recommended for cerebral involvement of invasive fungal infections. Furthermore, use of ketoconazole has been associated with gastrointestinal side effects and hepatitis (Lewis *et al*, 1984).

2.5.3.3 Fluconazole

Fluconazole is a first generation triazole, approved by the FDA for human use in 1990. As a triazole, fluconazole has three nitrogen atoms in the azole ring instead of two as in imidazoles. To date, it is the most widely used triazole, due to its high solubility in water, wide tissue distribution after oral administration, absorption not affected by gastric pH, and excellent tolerability compared to older antifungals (Lortholary and Dupont, 1997; Brammer *et al*, 1990). In humans, it has high oral bioavailability (>60%), low protein binding (11-12%), and a mean half-life of 22 hours. In addition, a volume of

distribution of 0.7 L/kg and a clearance of 0.40 mL/min/kg was found in humans after oral dosing. In animal species, the mean half-life values in mice and dogs after IV dosing were 4.5 and 13 hours, respectively; the mean volume of distribution values were 1.1 and 0.7 L/kg, respectively; and the mean clearance values were 3.9 and 0.65 mL/min/kg, respectively. The mean half-life values after oral administration in mice, rats, and dogs were 5.1, 4.0, and 15 hours, respectively; the mean volume of distribution values were 0.9, 0.8, and 0.8 L/kg, respectively; and the mean clearance values were 2.0, 2.2, and 0.62 mL/min/kg, respectively; and the mean systemic bioavailability values were 100, >80, and 100%, respectively (Humphrey *et al*, 1985).

In veterinary species, fluconazole has a mean elimination half-life value of 41.6 hours, mean volume of distribution at steady state value of 1.21 L/kg, and a mean clearance value of 0.02 L/hr/kg in horses after IV dosing (Latimer *et al*, 2001). Following oral administration in horses, peak plasma concentration was observed at an average of 1.97 hours postdose, and a systemic bioavailability of 101% was obtained. In cats, the mean half-life of 13.8 hours determined after IV dosing was similar to that observed after oral dosing of 12.4 hours. The plasma clearance was 0.9 mL/min/kg after both IV and oral dosing, and the volume of distribution at steady state was 1.1 L/kg after IV and 1.0 L/kg after oral administrations in cats. The peak plasma concentration was reached 2.6 hours after oral dosing and the drug had a systemic bioavailability value of 109% (Craig *et al*, 1994). When various dosage forms of fluconazole was given orally to African grey parrots, the mean peak plasma concentration values were between 5.18 and 9.54 hours; the mean absorption half-life values were between 1.54 and 4.54 hours; and the elimination half-life values ranged from 9.22 to 11.65 hours. The observed mean

volume of distribution divided by extent of absorption values ranged from 564.05 to 897.30 mL/kg, and the mean clearance divided by extent of absorption values were between 38.25 and 64.71 mL/hr/kg (Flammer and Papich, 2006).

Fluconazole is approved for the treatment of *Candida* and *Cryptococcus* infections, and it has good activity against *Coccidioides* species (Maertens, 2004). However, due to its increased use, fungal resistance to fluconazole is becoming more prevalent. Fluconazole has no intrinsic activity against *Aspergillus* and several *Candida* species (Walsh and Lee, 1993). *C. glabrata*, *C. tropicalis*, and *C. krusei* isolates resistance to fluconazole in the United States is well documented (Wispinghoff *et al*, 2004; Pfaller, 2000). Fluconazole also fails against infections caused by *Trichosporon* species (Wildfeuer *et al*, 1998). Therefore, current guidelines suggest that clinicians consider antifungals other than fluconazole as therapy for invasive candidiasis in patients who have had prior azole exposure and who are unstable or infected with species known to have reduced susceptibility to fluconazole (Pappas *et al*, 2004).

2.5.3.4 Itraconazole

Itraconazole is a first generation triazole, approved by the FDA in 1992 for the treatment of blastomycosis and histoplasmosis. It also has good activity against *Pseudallescheria boydii* but appears to have negligible activity against *Fusarium* and *Scedosporium* species, and *Aspergillus fumigatus* are resistant to itraconazole (Pfaller *et al*, 2002b; Denning *et al*, 1997a; Walsh *et al*, 1995). Currently there are both oral and intravenous formulations of itraconazole available. Itraconazole is highly lipid soluble, with high affinity for fat-containing tissues, but poor penetration into the CSF (Heykants *et al*, 1989). Therefore, it is not recommended for the treatment of cerebral involvement

of invasive fungal infections. Itraconazole is primarily metabolized by the liver (Boelaert *et al*, 1988).

With once daily oral administration of 100 mg, 200 mg, and 200 mg twice a day of voriconazole capsules to healthy human volunteers, the mean peak plasma concentrations were observed at 2.8, 3.0, and 3.4 hours, respectively, postdose on day 1, and at 3.0, 4.4, and 6.0 hours, respectively, on day 15; the mean elimination half-life values were 15.0, 20.7, and 25.0 hours, respectively, on day 1, and 34.0, 36.5, and 41.7 hours, respectively, on day 15; and the mean clearance divided by extent of absorption values were 1.511, 0.937, and 0.533 L/hr/kg, respectively, on day 1, and 0.308, 0.233, and 0.167 L/hr/kg, respectively, on day 15 (Hardin *et al*, 1988). The capsule formulation of itraconazole has limited oral bioavailability, but the liquid formulation in cyclodextrin has 60% higher bioavailability than that of capsules in humans (Stevens *et al*, 2003; Barone *et al*, 1998; de Repentigny *et al*, 1998). The absorption of the liquid formulation is enhanced if given under fasting conditions, whereas the capsule formulation requires food for dissolution of the drug from the solid formulation (Pandya *et al*, 2003). Gastrointestinal side effects can be caused by reaction to cyclodextrin, and elevated liver enzyme levels have been observed (Van Peer *et al*, 1989; Foot *et al*, 1999).

In horses, itraconazole has a half-life of 6.52 hours with IV dosing, 11.30 hours with the solution formulation taken orally, and 7.97 hours with the capsules (Davis *et al*, 2005). The clearance of itraconazole is 11.14 mL/min/kg and the volume of distribution at steady state is 4.47 L/kg in horses. The extent of absorption of itraconazole solution in horses is 64.96%, and that of the capsule formulation is 12.18%.

2.5.4 Echinocandins

The echinocandin are limited spectrum antifungal agents and are fungicidal for all *Candida* species, while fungistatic against *Aspergillus* species (Deresinski and Stevens, 2003). Echinocandins inhibit the synthesis of β-1,3-glucan of the fungal cell wall leading to osmotic instability and cell death. Due to the absence of glucan synthesis enzymes in mammalian cells, this class of drugs is selectively toxic for fungi. Approved by the FDA for human use in 2001, caspofungin is the first drug developed in this class (Keating and Jarvis, 2001). Caspofungin is indicated for the treatment of invasive candidiasis and also for invasive aspergillosis in patients refractory to or intolerant of other substances (Merck, 2004).

This drug exhibits linear pharmacokinetics and is mainly eliminated by the liver. Caspofungin has an elimination half-life of 9-10 hours in humans, allowing for once daily administration (Groll *et al*, 2001). Its plasma drug clearance rate averages 10 to 12 mL/min (Stone *et al*, 2002). It has low bioavailability and thus is available only in the intravenous formulation. The drug is also highly protein bound (> 95%), with extensive distribution into various tissues (Letscher-Bru *et al*, 2003; Hajdu *et al*, 1997). When caspofungin was administered to rats, the mean elimination half-life values ranged from 7.0 to 10.9 hours, the mean volume of distribution values were between 470 and 670 mL/kg, and the mean clearance values were approximately 0.7 mL/min/kg (Van Vianen *et al*, 2006). In rabbits, the mean apparent volume of distribution at steady state was observed to be 0.299 L/kg after 1-mg/kg dosing and 0.351 L/kg after 6-mg/kg dosing. Clearance ranged from 0.086 L/kg/hr, after 1-mg/kg dosing, to 0.043 L/kg/hr, after

6-mg/kg dosing, and the mean elimination half-life was between 30 and 34 hours (Groll *et al*, 2001).

Echinocandins have been reported to be extremely safe because they only target the cell wall, which does not exist in the mammalian system. The most common adverse effects are infusion reactions, such as flushing, urticaria, thrombophlebitis at the infusion site, and elevated liver enzymes (Kauffman, 2006b). Echinocandin therapy for endocarditis, endophthalmitis, meningitis, and osteomyelitis is not currently recommended because of the difficulty of reaching therapeutic levels at these sites of infection using currently approved drug doses. In addition, a lack of an oral formulation limits the usefulness of this class of drugs after hospital discharge (Morris and Villmann, 2006). Furthermore, echinocandins are not active against endemic mycoses such as *Histoplasma capsulatum, Blastomyces dermatitidis*, *Coccidioides immitis*, and certain *Candida* species, such as *C. guilliermondii*. Infections caused by *Cryptococcus*, *Trichosporon*, and *Zygomycetes* species are also resistant to echinocandin therapy (Girmenia *et al*, 2006).

2.6 Minimum Inhibitory Concentration (MIC) Determination

There are no standard methods for determining the minimum inhibitory concentrations (MICs) of the drugs for various fungi, and definitions of the extent of decreasing fungal growth differ between investigators, thus making direct comparisons between fungal strains difficult (Rubin *et al*, 2002). In attempt to standardize research studies and clinical reports, the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) published broth microdilution and disk diffusion techniques and guidelines for the determination of MIC

for various antimicrobials (Clinical and Laboratory Standard Institute, 2006; Espinel-Ingroff *et al*, 2005). The CLSI also proposed interpretive breakpoints for *Candida* species (Rex *et al*, 1997). However, the preparation of the trays described by the CLSI methods can be time consuming. Alternatives to the CLSI method include colorimetric assay using reduction of tetrazolium salts to color formazan products by viable cells for antifungal susceptibility testing in filamentous fungi (Lewis *et al*, 2005). Fundamentally, there is concern of extrapolating a mathematical value from a microtiter plate and applying the number towards predicting the results in a patient. The greatest usefulness for *in vitro* combination possibly is to screen antagonistic interactions before investigating animal model or clinical studies (Steinbach, 2005).

2.7 Antifungal Resistance

Failure to achieve drug accumulation during multiple dosing of azole antifungals has been identified as a major cause of resistance in several post-treatment fungal isolates and species that are less sensitive to azole antifungal agents and other ergosterol biosynthesis inhibitors (Vanden Bossche *et al*, 2003). There are different definitions of antifungal resistance in the *in vitro* and clinical settings. *In vitro* resistance involves laboratory measurements and obtaining the range of susceptibility results for particular drugs and fungal species. Clinical resistance is defined as the persistence or progression of the fungal infection despite the administration of appropriate antifungal therapy. Antifungal resistance can be subclassified into primary resistance, where the fungus is resistant prior to drug exposure, or secondary resistance, where the resistance develops in response to drug exposure (Rogers, 2006).

2.8 Treatment Duration

Some fungal infections can be hard to treat or cure, this is especially true if the infection has disseminated to other areas of the body, such as brain or blood. In addition, immunocompromised patients who obtained resolution from an episode of invasive fungal infection are at risk for relapse of infection, unless granulocyte recovery can be achieved (Fukuda *et al*, 2004; Offner *et al*, 1998). A typical feature of systemic fungal infections is that they need long-term therapy. Generally, the antifungal therapy should be continued until the manifestation of invasive infection have completely resolved or are reduced to residual scarring (Bohme *et al*, 2003). Long-term intravenous therapy can cause effusion or phlebitis, due to the need to puncture veins frequently, and high risk of additional infection and/or deterioration of the venous reservoir related to daily use. Therefore, when the antifungal therapy lasts longer than 7-10 days, the need to replace intravenous with oral administration becomes a high priority. Furthermore, oral therapy should mean much lower costs than intravenous therapy, due to the savings in intravenous administration and decreased hospital stay or elimination of visiting an outpatient clinic. However, the switch should not mean a risk of reduced efficacy or an increase in adverse effects (Perea *et al*, 2004).

2.9 Adjunctive Therapies

Understanding the pathophysiology of invasive fungal infection in relation to host factors will be important in designing novel therapeutic strategies, such as immune augmentation (Bhatti *et al*, 2006). Augmentation of the host immune response, whenever feasible, is a critical factor in determining the outcome of immune suppressed patients with fungal infections (Al-Abdely, 2004).

Catheter-related infections are a major cause of nosocomial bloodstream infections in intensive care units. Infection could be initiated by contamination of the catheter hub at the skin, resulting in catheter infection, or by transient bloodstream infection from another source, resulting in secondary catheter colonization or infection (Anaissie *et al*, 1998; Krause *et al*, 1969). *Candida* species are the most common fungal pathogens associated with such infections, with *C. parapsilosis* being the most frequently involved species in catheter-related infections (Abi-Said *et al*, 1997). Many of the pathogens responsible for these line-related or device-related infections exist in an extensive polysaccharide matrix called biofilm (Mukherjee and Chandra, 2004). Biofilms are communities of microorganisms attached to a solid surface enclosed in an exopolymeric matrix (Hall-Stoodley *et al*, 2004; Donlan, 2002). Biofilm formation is associated with persistent infection to many antifungal agents, since biofilms constitute a physical barrier that prevents the efficient penetration of antifungal drugs, thus microorganisms that form biofilms have higher levels of resistance to antifungal activity than their planktonic counterparts (Al-Fattani and Douglas, 2004; Chandra *et al*, 2001).

To date, none of the regimens available demonstrated the ability to completely eradicate catheter-related *Candida* bloodstream, due to the lack of activity against biofilm-encased organisms (Lewis *et al*, 2002). Therefore, catheter removal may be the most important therapy in the management of the line-associated infections. However, prompt removal of an infected central venous catheter is not always possible, especially in a patient who is hemodynamically unstable or in a cytopenic patient who is at high risk for bleeding. In these situations, clinicians are often forced to treat catheter-related

bloodstream fungal infections with systemic antifungal therapy, until the removal or replacement of the catheter is medically feasible (Mermel *et al*, 2001; Pearson, 1996).

Treatments for osteoarticular fungal infection often consist of a combination of pharmacological therapy and surgical procedures. Debridement and excision of the infected site are often required, but surgical interventions are often mutilating, and some patients with complicating illnesses are not recommended to undergo surgery due to the potential risks and mortality involved (Tirado-Miranda *et al*, 2001).

There is no standardized therapy for pulmonary fungal infections, and different strategies have been used, including inhaled, intracavitary, and endobranchial antifungal therapies. Surgery is the treatment of choice of symptomatic aspergilloma. When surgery is not feasible due to patient status, itraconazole has been used for the treatment of various forms of aspergillosis. However, itraconazole resistance has been reported in the clinical setting, and can occur after long-term therapy (Dannaoui *et al*, 2001; Chryssanthou *et al*, 1997).

In the majority of the monotherapy studies using the murine model, efficacies were found, but a cure was not obtained. As newer classes of antifungal agents such as the echinocandins with unique mechanisms of action are being developed, there is more interest for combination therapy, because of the possibility of synergistic activity against various fungi. Synergy generally occurs through three possible mechanisms: sequential inhibition of different steps of a common biochemical pathway, simultaneous inhibition of cell wall and cell membrane targets, or use of a cell wall- or cell membrane- active drug to enhance penetration of a second antifungal (Lewis and Kontoyiannis, 2001). For solid organ transplant recipients diagnosed with *Aspergillus fumigatus*, in which there is

generally a high mortality rate, lower mortality has been observed with combination therapy (Munoz *et al*, 2006). Combination antifungal therapy is still controversial, as some researchers have shown *in vitro* synergism between caspofungin and another class of antifungal, but other results only indicate additive effects (Cuenca-Estrella *et al*, 2005). Furthermore, some researchers believe that combination therapy has not been proven to be more effective by controlled clinical study and that reports of success in case studies depend largely on patient status (MacCallum *et al*, 2005). On the other hand, drug combinations can contribute to increased drug interactions and toxicity. Therefore, combination therapy should be reserved for very special situations in which failure of monotherapy is obvious (Munoz *et al*, 2006).

2.10 Need for Newer Antifungal Agents

The increasing population and life expectancy of immunocompromised patients have increased the number of cases of fungal infections. The reasons for increasing use of antifungal drugs are manifold. Among hospitalized patients, empirical use of antifungals in hematology-oncology and intensive care patients is now common (Gutierrez *et al*, 1995; Alvarez-Lerma *et al*, 2003; Trifilio *et al*, 2004; Gutierrez *et al*, 1996). Also there is emergence of infection from rare organisms (Nucci, 2003). Furthermore, newer antifungal drugs have become available and broadened therapeutic options for clinicians (Gallagher *et al*, 2004). There are several new antifungal drugs introduced in the past few years. The ideal antifungal agent should be available in both oral and intravenous dosage forms, have a broad spectrum of fungicidal activity, display a good pharmacokinetic profile with minimal drug interactions, have little or no potential

toxicity or resistance, and be inexpensive. However, no such drug exists (Golan, 2005; Maertens, 2004). This has created an urgent need for new antifungal agents.

2.11 Fungal Infections and Treatments in Veterinary Medicine

Although the exact incidence of fungal infections in veterinary medicine has not been established, it is believed that the trend has paralleled the increase seen in human medicine (Wiebe *et al*, 2005). For example, fungal infections in horses include localized infections of the dermis, eyes, central nervous system (CNS), guttural pouches, respiratory system, and reproductive tract, as well as disseminated systemic infections involving multiple organs and body cavities (Davidson, 1991; Steckel *et al*, 1982; Rawlinson and Jones, 1978; Sweeney, 1997; Swerczek and Donahue, 1990; Rochette *et al*, 2003). Some of the most commonly reported fungi species found in infected horses include *Aspergillus*, *Fusarium*, *Paecilomyces*, *Acremonium*, *Histoplasma*, *Rhinosporidium*, *Cryptococcus*, *Penicillium*, *Alternaria*, *Cladosporium*, and *Candida* (Blomme *et al*, 1998; Plumlee and Galey, 1994; Foley *et al*, 2002; Latch, 1985; Ajello, 1998; Wallin *et al*, 2001; Steckel *et al*, 1982; Samuelson *et al*, 1984; Brooks, 1999). One of the most frequent clinical manifestations of fungal infections in horses is keratitis, and *Aspergillus* species are the most commonly isolated organisms from horse eyes (Brooks *et al*, 1998).

Successful antifungal therapy for affected horses is limited to only a few drugs (Latimer *et al*, 2001). Amphotericin B was the first available drug for the treatment of systemic fungal infections (Brook, 1982). The disadvantages associated with the use of this drug in horses, as in humans, include required IV administration and resultant phlebitis, poor perfusion into the cerebralspinal fluid (CSF), and renal toxicity with

prolonged administration (Speller, 1979). Griseofulvin is the most commonly used systemic antifungal in horses (Rochette *et al*, 2003). However, its spectrum of activity is limited to *Trichophyton*, *Epidermophyton*, and *Microsporum* species (Vanden Bossche *et al*, 2003). Of the azoles, the most commonly used is miconazole. Miconazole is active against *Aspergillus* species, but poor levels are observed in the CSF (Hamor and Whelan, 1999; Vanden Bossche *et al*, 2003). Ketoconazole is moderately effective against *Aspergillus* species but considerably less effective against *Fusarium* species, and it is not absorbed well from the digestive tract of horses (Prades, 1989; Brooks *et al*, 1998). Although fluconazole has excellent absorption and tissue distribution after oral administration in horses, it is intrinsically resistant to certain *Aspergillus* species (Brooks *et al*, 1998). Another azole, itraconazole, has substantial *in vitro* activity against *Aspergillus* and many other filamentous fungi, but it was found to have variable absorption with the capsule formulation, relatively low extent of absorption (65%) with the improved solution formulation as well as poor penetration into the aqueous humor of horses and inconsistent plasma concentrations for the treatment of fusariosis (Wong-Beringer & Kriengkauykiat, 2003; Davis *et al*, 2005). A few new antifungal drugs were recently approved for human use and could potentially provide effective treatments for horses (Vanden Bossche *et al*, 2003).

2.12 Voriconazole

Voriconazole (2R,3S 2-[2,4-diflurophenyl]-3-[5-fluropyrimidine-4-yl]-1-[1,2,4-triazol-l-yl]butan-2-ol), formerly known as UK-109,496 and produced by Pfizer Pharmaceuticals, is a second generation triazole. It was developed from fluconazole, by a fluoropyrimidine ring substitution for one of the azole groups, and an added α-methyl

group. The molecular weight of voriconazole is 349.3, and it is a weak base with pKa value of 1.76 (Kulemann *et al*, 2005).

2.12.1 Mechanism of Action

Voriconazole was approved by the FDA in 2002 for human use. It is indicated for the treatment of invasive aspergillosis, candidaemia in non-neutropenic patients, disseminated infections caused by *Candida* species, oesophageal candidiasis, and in patients with scedosporiosis and fusariosis who are refractory to or intolerant of other antifungal therapy (Pfizer, 2006). Voriconazole's mode of action is through its inhibition of cytochrome P450-dependent 14α-lanosterol demethylation, which is a critical step in fungal cell membrane ergosterol synthesis. Voriconazole binds to the active site of the cytochrome P450-dependent 14α-demethylase (CYP51 or Erg11p) and ligates the iron heme cofactor through a nitrogen atom. This inhibition leads to the depletion of ergosterol and accumulation of 14α-methyl sterols such as lanosterol, affecting the integrity and function of the fungal membrane (Xiao *et al*, 2004; Podust *et al*, 2001; Sanati *et al*, 1997). The drug is about 250-fold more active against the fungal demethylase enzyme than against the mammalian P450-dependent steroid hormone biosynthesis (Ghannoum and Kuhn, 2002).

2.12.2 Spectrum of Activity

Voriconazole has the broadest antifungal spectrum of all the azoles (Kulemann *et al*, 2005). The *in vitro* activity of voriconazole against some of the common pathogenic fungi has been demonstrated. Against *Candida* species, on a milligram-to-milligram basis, voriconazole is 60 to 100 fold more potent than fluconazole and has activity against *C. krusei*, which are inherently resistant to fluconazole (Boucher *et al*, 2004).

Among *Candida* species, it was found in a large breakpoint interpretive study involving 8,702 clinical isolates, that the overall MIC required to inhibit the growth of 90% of organisms (MIC_{90}) for voriconazole was 0.25 μg/ml, and 99% of the isolates tested were inhibited by concentrations ≤1 μg/ml. Voriconazole has a very low MIC against the most commonly found *Candida* species, *C. albicans*, *C. glabrata*, and *C. krusei*. *C. albicans* was the most susceptible species, and *C. glabrata* was the least susceptible to voriconazole. Despite a strong positive correlation between the fluconazole and voriconazole MICs and possible cross resistance, 82% of the *Candida* isolates that were resistant to fluconazole were inhibited by voriconazole at concentrations ≤1 μg/ml. The authors assigned a MIC breakpoint of ≥4 μg/ml as resistant to voriconazole (Pfaller *et al*, 2006).

The information gathered from MIC values has some limitations, as it provides only a point-in-time, static measurement of antimicrobial effect in a defined medium. On the other hand, time-kill curves can follow microbial reduction and growth as a function of both time and antimicrobial concentration (Theuretzbacher *et al*, 2006). A study using time-kill curve demonstrated that voriconazole exhibits fungistatic activity against *Candida* species and fungicidal against *Aspergillus* species. In general, the fungicidal effect of voriconazole on *A. fumigatus* is slow, and it takes 12-24 hours to obtain >90% killing of the cells (Klepser *et al*, 2000). Researchers theorize the difference in activity may be related to the stronger affinity for lanosterol 14α-demethylase found in mold, which may allow more complete interruption of ergosterol synthesis (Manavathu *et al*, 1998). For voriconazole to be effective as a fungicidal agent, an extended period of fungal cell growth in the presence of voriconazole is required to deplete ergosterol

content completely to bring about a lethal effect on *A. fumigatus* cells (Krishnan *et al*, 2005). Voriconazole also has a very low MIC against the most commonly found *Aspergillus* species, *A. fumigatus*, *A. terreus*, *A. niger*, and *A. flavus* (Theuretzbacher *et al*, 2006). In an *in vitro* comparative study, voriconazole had higher activity than amphotericin B and itraconazole against various *Aspergillus* species (Guinea *et al*, 2006).

With rare fungal species, *in vitro* testing results show voriconazole would seem to offer a new option for the treatment of infections caused by *Fusarium*, *Scedosporium*, *Cryptococcus*, and *Prototheca*, an ubiquitous, unicellular, achlorophyllic algae, generally isolated from tree sap, potato peel, seawater, lakes, marshes, and rivers (Lewis *et al*, 2005; Linares *et al*, 2005; Casal and Solis, 1981). However, voriconazole has no activity against *Zygomycetes* species, such as *Mucor* and *Rhizopus*, and voriconazole used as prophylaxis in stem cell transplant patients has been associated with the emergence of zygomycosis.

Some researchers have reported low incidence of cross resistance to voriconazole in fluconazole resistant *Candida* species, therefore, voriconazole is useful for the treatment of fluconazole resistant fungal infections (Chryssanthou *et al*, 2004). Voriconazole also has good *in vitro* activity against most of the itraconazole-resistant strains of *Aspergillus* (Dannaoui *et al*, 2006). It has been theorized that the absence of cross-resistance between itraconazole and voriconazole is due to their chemical structural differences. Itraconazole has an extended side chain, whereas voriconazole has a relatively compact structure. Therefore, the binding does not involve the same residues on their target enzyme, 14α-demethylase CTP51A (Meneau and Sanglard, 2005).

2.12.3 Pharmacokinetic Properties

Pharmacokinetic and pharmacodynamic characteristics of voriconazole have been well established, especially in humans. The mean elimination half life ($t_{1/2}$) of voriconazole is about 6 hours after single and multiple oral or intravenous administrations (Purkins *et al*, 2003b). Following intravenous administration in healthy volunteers, a 1.7-fold increase in dose resulted in 2.4-fold increase in maximum observed plasma concentration (C_{max}) and 3.1-fold increase in area under the plasma concentration-time curve (AUC). Similarly, following oral administration, a 2-fold increase in dose resulted in 2.8-fold increase in C_{max} and 3.9-fold increase in AUC values (Purkins *et al*, 2002). It is believed that the non-linear characteristic is due to saturable metabolism with respect to dose. The terminal half-life of voriconazole is dose-dependent and is not predictive of its accumulation or elimination (Donnally and De Pauw, 2004).

The elimination of voriconazole is mainly by metabolic clearance, with <2% of the dose excreted unchanged in the urine. Metabolism is hepatic by the CYP isoenzymes CYP2C9, CYP2C19, and CYP3A4 via N-oxidation. The primary metabolizing enzyme is CYP2C19 (Hyland *et al*, 2003). Voriconazole has the potential to be both a substrate and inhibitor of these three enzymes but does not induce CYP3A4 activity (Roffey at al, 2003). The major metabolite, the N-oxide, has no significant antifungal activity. In a radiolabelled drug study, approximately 80% of the radioactivity is recovered in the urine and 20% in the feces (Pfizer, 2006). Since voriconazole is extensively eliminated through metabolism, saturation of metabolism is likely the cause of nonlinearity (Roffey *et al*, 2003). However, inhibition of biotransormation by a metabolite could also be the cause of the dose-dependent pharmacokinetics (Leveque *et al*, 2006.)

When oral voriconazole was administered to 18 patients, the C_{max} tripled from day 1 to day 14, showing accumulation with repeated dosing (Lazarus *et al*, 2002). The mean $t_{1/2}$ values were greater after multiple oral administration than after single oral administration, and were generally associated with a greater accumulation of voriconazole after multiple dosing regimens than predicted from single dosing results. However, because of non-linear pharmacokinetic characteristic of voriconazole, the $t_{1/2}$ is dose-dependent, and therefore, there is limited predictability of its accumulation after multiple dosing. In a pre-clinical study, multiple oral administration of voriconazole results in decrease in AUC in mouse and rat, but this effect was less pronounced in the dog, and not observed in guinea pig and rabbit. Repeated dosing in rats and dogs caused up to a 3-fold induction of liver cytochrome P450 concentration and liver enlargement compared with control animals. These effects are completely reversible with a one-month recovery period. A reduction in AUC following multiple oral and intravenous dosing is observed in rat and dog, and the researchers surmised this effect to be related to the autoinduction of metabolism. Autoinduction is not observed in humans, guinea pigs, or rabbits after chronic voriconazole dosing. Voriconazole is extensively metabolized in all species (Roffey *et al*, 2003). Voriconazole has a large volume of distribution, suggesting extensive distribution into extracellular and intracellular compartments. Published values of the steady-state volume of distribution range from 2 to 4.6 L/kg (Pfizer, 2006).

Voriconazole is rapidly absorbed within 2 hours of single oral administration in healthy volunteers (Purkins *et al*, 2003b). The bioavailability is greater than 90%, and the absorption is not affected by gastric pH, unlike some other triazole antifungals

(Pfizer, 2006). The high bioavailability of the oral formulation offers the distinct advantage of voriconazole over the echinocandins and most of the older antifungals, because patients can easily switch from the intravenous to the oral form. This makes voriconazole more attractive for long-term use. Most hospitalized patients with serious fungal infections require intravenous treatment initially and are then switched to an oral regimen when appropriate.

In a study of the pharmacokinetic profile of intravenous-to-oral dosing, it was found that although the C_{max} value of voriconazole decreased after the switch from intravenous to oral administration, there was no significant changes in volume of distribution at steady state, clearance, or terminal phase elimination rate constant (Purkins *et al*, 2002). On average, oral voriconazole reaches steady state concentration after 5-7 days of multiple oral administration in the absence of a loading dose, and the mean minimum observed plasma concentration (C_{min}) value on day 1 was below the therapeutic level for many common fungal pathogens. Therefore, the recommended dosing regimen of voriconazole for adults is an intravenous loading dose of 6 mg/kg twice daily for 24 hours, followed by a maintenance dose of 3-4 mg/kg twice daily. This allows the peak plasma concentration (C_{max}) to reach that of steady state within the first 24 hours (Purkins *et al*, 2003a).

Voriconazole exhibits a moderate dose- and plasma concentration-dependent protein binding of 58% in adult humans (Pfizer, 2006). With antifungal agents, it has been demonstrated that only the free or unbound fraction of the drug is available for antimycotic activity (Zhanel *et al*, 2001). Therefore, free drug concentrations must be considered when examining the relationship between pharmacokinetic parameters and

clinical efficacy. A study based on the murine candidiasis model showed that the ratio of free drug AUC from 0 to 24 hours to the minimum inhibitory concentration (AUC_{24}/MIC) can be a predictive parameter for effective treatment if the ratio ranges from 20-25. Given the plasma protein binding ratio of voriconazole is 58%, it is necessary to take this characteristic into account when calculating pharmacodynamic targets (Andes *et al*, 2003). Clinical studies with fluconazole support this theory (Lee *et al*, 2000). Even though the exact relationship between voriconazole concentration in plasma and the drug's clinical efficacy is uncertain, infected adults tend to improve when the recommended plasma concentration of 2-5 μg/ml has been achieve (Destino *et al*, 2006).

2.12.4 Drug Administration

Voriconazole is formulated as a lyophilized powder for solution for intravenous infusion, as a film-coated tablet for oral administration, and as a powder for oral suspension. The standard dosing of voriconazole to adult humans is intravenous 6 mg/kg loading dose every 12 hours for 24 hours, then 4 mg/kg every 12 hours as the maintenance dose. Voriconazole must be infused over 1-2 hours at a concentration of 2-5 mg/ml and at a maximum rate of 3mg/kg/hr. Infusion of blood products and any electrolyte supplementation must not occur simultaneously with voriconazole infusion. With oral administration, voriconazole is given 100 mg to patients under 40 kg and 200 mg to those over 40 kg, twice a day (Pfizer, 2006). If response to treatment is inadequate, then oral maintenance dosage may be increased by 50% (Johnson and Kauffman, 2003).

When voriconazole (200-250 mg twice daily) was delivered via a nasogastric tube to eight patients, the C_{max} value (6.4 ± 4.3 μg/ml) appeared to be higher than previously reported after oral administration, adequate concentrations in the plasma were achieved, and the authors suggested that nasogastric administration can be used as an alternative to intravenous injection for mechanically ventilated patients (Mohammedi *et al*, 2005).

When voriconazole was administered as suspension from crushed tablet via jejunostomy tube to a patient with *C. glabrata* infection, the C_{max} values appeared to be slightly lower than the mean C_{max} obtained following oral administration, and there was no C_{max} escalation from day 2 to 28, as did in repeated oral administration (Martinez *et al*, 2003).

Even though an oral suspension is available in the United States, the viscosity of the oral suspension can cause difficulties to administer this preparation via enteral feeding tubes. Therefore, crushing the whole tablet formulation of voriconazole is an alternative approach for oral administration used by clinicians (Dodds Ashley *et al*, 2007). A comparative pharmacokinetic study between crushed and whole tablet administration showed that the bioavailability, AUC, and C_{max} of voriconazole were not significantly different. However, the absorption of voriconazole after administration of the crushed tablets was faster compared to the whole tablet, due to the longer disintegration and dissolution time of the whole tablets in the gastrointestinal tract. The authors noted that the crushed tablets appeared to adhere to plastic medication cups. Therefore, glass mortar and pestle were required and were rinsed serially following drug preparation to ensure complete recovery of the drug product (Dodds Ashley *et al*, 2007).

2.12.5 Tissue Fluid Distribution

There has been limited data presented on tissue distribution of voriconazole. Voriconazole is moderately lipophilic and rapidly absorbed after oral administration to mice, rats, rabbits, guinea pigs, dogs, and humans with high bioavailability (>75%), and it distributes well into various tissues and body fluids in human patients and animal subjects.

Cerebral aspergillosis involves about 10% of cases of invasive aspergillosis, but the mortality rate of CNS aspergillosis is nearly 100% (Boes *et al*, 1994; Walsh *et al*, 1985). The concentration of voriconazole in CSF has been characterized in guinea pigs, showing a linear relationship between the voriconazole dose and CSF concentration (Lutsar *et al*, 2003a). Concentration of voriconazole in CSF collected from patients also showed a linear relationship between the dose and concentration in the CSF samples, and concentration in the CSF is about 50% of the serum concentration (Danaher and Walter, 2004; Pearson *et al*, 2003). Penetration of voriconazole into CSF has been characterized in human patients with proven cerebral aspergillosis, indicating drug level reaching therapeutic range (Elter et. al. 2006; Tattevin *et al*, 2004). Therefore, voriconazole exhibits significant transport across the blood-brain barrier.

With pulmonary involvement of fungal diseases, alveolar macrophages constitute the first line of host defense against aerosolized conidia, whereas neutrophils are the dominant host defense against the invasive hyphal stage (Segal and Walsh, 2006). *Candida* and *Aspergillus* are not only found in extracellular compartments, but are also able to invade endothelial cells. Therefore, it is important to investigate the intracellular penetration also. Polymorphonuclear leukocytes (PMNs) are an important component of

the host's defense mechanism against infection by *Candida* species. An *in vitro* study with human PMNs demonstrated that the uptake of voriconazole by PMNs was rapid and unsaturated. The cellular to extracellular concentration ratio was 8.5, and the release of loaded PMNs appeared to be as rapid as the uptake (Ballesta *et al*, 2005). In a study with uninfected rabbits, it was found that voriconazole distributes prominently into pulmonary epithelial lining fluid. However, lower concentrations were found in the pulmonary alveolar macrophages and total lung tissue sample than in the plasma (Boucher *et al*, 2004).

Voriconazole has been reported to penetrate the pleural cavity in human as well. With 200 mg twice daily oral administration of voriconazole, 0.8 and 1.4 μg/ml of the drug was found 2 hours post dose on days 12 and 22, respectively (Stern *et al*, 2004).

It has been found that voriconazole penetrates well into peritoneal fluid, but peritoneal clearance of voriconazole is minimal. In patients undergoing peritoneal dialysis, the passage of voriconazole into the peritoneal dialysate is less than 1% of the administered dose in 24 hours after dosing. Therefore, no dose adjustment is necessary for these patients (Peng and Lien, 2005).

Voriconazole appears to have high penetration into bone. In a case study of *Aspergillus* osteomyelitis, voriconazole concentration in serum and synovial fluid were 2.41 and 0.76 μg/ml on day 1 and 4.09 and 1.07 μg/ml on day 2, respectively. The researchers measured the bone concentrations to be 20.3 μg/g of tissue in the medullar bone and 1.9 μg/g of tissue in the cortical bone (Denes *et al*, 2007).

The pharmacokinetic data for saliva showed a similar pattern to that observed for plasma. The concentration in saliva was approximately 65% of that in plasma, which is consistent with plasma protein binding of 58% (Purkins *et al*, 2003a).

Voriconazole effectively penetrated the anterior and posterior segments of eyes of rabbits after oral and topical administration (Zhou *et al*, 2002). After oral administration of voriconazole, therapeutic concentrations were achieved in the vitreous and aqueous humor in 14 patients (Hariprasad *et al*, 2004).

2.12.6 Animal Models

The guinea pig is the laboratory animal species best suited for *in vivo* pharmacokinetic studies of voriconazole, as the plasma pharmacokinetics in guinea pigs are closest to those in humans (Boucher *et al*, 2004). Researchers have attempted to improve the murine models of fungal infection for the study of voriconazole because the serum half-life was 1-2 hours after a single dose in mouse, and after 10 days of dosing, only animals dosed with 40 mg/kg had detectable concentrations in the serum. In mice given 50% grapefruit juice in water, the animals consumed the diluted juice readily and the rapid metabolism of voriconazole was inhibited and AUC increased (Clemons *et al*, 2005; Graybill *et al*, 2003; Sugar and Liu, 2000).

Murine model infected with *C. neoformans* showed dramatic increase in survival rate of mice treated with various concentrations of voriconazole (1 to 40 mg/kg/day) versus control. However, the authors theorized that the lack of significant difference in fungal burden observed in mice administered with different drug concentrations might be due to autoinduction of metabolism (Mavrogiorgos *et al*, 2006). In a neutropenic guinea pig model with *Candida krusei*, voriconazole was shown to be more efficacious than

either amphotericin B or fluconazole in eradicating *C. krusei* from brain, liver, and kidney tissues (Ghannoum *et al*, 1999).

When amphotericin B and voriconazole were compared in experimental pulmonary invasive aspergillosis in guinea pigs, no statistically significant difference was observed between the efficacies of voriconazole and amphotericin B, and both were associated with a favorable trend in survival (Chandrasekar *et al*, 2000). However, in animal models of invasive and disseminated aspergillosis, voriconazole was observed to be more efficacious than amphotericin B in increasing survival and decreasing fungal burden in guinea pigs, rats, and rabbits (Chandrasekar *et al*, 2000; Kirkpatrick *et al*, 2000; Murphy *et al*, 1997; George *et al*, 1996). In an experimental model of invasive aspergillosis of rabbits, voriconazole eliminated mortality and reduced antigenemia (George *et al*, 1996). A study of experimental *Aspergillus* endocarditis in guinea pigs showed that voriconazole was more active than itraconazole against established *Aspergillus* endocarditis (Martin *et al*, 1997). In an experiment involving rabbits with infected eyes, voriconazole concentration was found in cornea and resolution of fungal keratitis was achieved (Sponsel *et al*, 2006).

In a comparative study, voriconazole has been evaluated to be more effective than amphotericin B in treating guinea pig model of invasive trichosporonosis by prolonging survival and reducing tissue burden (Serena *et al*, 2006).

2.12.7 Clinical Efficacy

Voriconazole has been used successfully in humans to treat various invasive mycoses involving the central nervous, skeletal, cardiac, and respiratory systems, either as the primary therapy, as salvage therapy, or in combination with other antifungal drugs.

Aspergillosis of the CNS is the most frequent and devastating manifestation of dissemination, with mortality rate nearing 100%. Until recently, the standard drug for the treatment of this disease was amphotericin B, but the response is poor (Denning and Stevens, 1990; Collette *et al*, 1989). Caspofungin has also shown poor penetration into the CSF and brain tissue (Hajdu *et al*, 1997). Voriconazole has become the drug of choice for invasive aspergillosis. In a large randomized study involving 392 patients diagnosed with invasive aspergillosis, complete or partial response was achieved in 53% of the voriconazole group, versus 32% in amphotericin B group (Herbrecht *et al*, 2002b). Furthermore, patient survival was 71% in the voriconazole group and 58% in the amphotericin B group. Voriconazole has been clearly shown to be more effective than amphotericin B as initial therapy for patients with invasive aspergillosis in this study (Walsh *et al*, 2002a). In a cohort study of cerebral aspergillosis, of the patients receiving voriconazole, 35% had a complete or partial response with a 31% survival rate (Schwartz at al, 2005). Voriconazole has also been used successfully for the treatment of cerebral abscess due to rare fungal infections caused by *Cladophialophora bantiana*, *Chrysosporium* species, *Fusarium solani*, *Pseudallescheria boydii* and meningitis caused by *Scedosporium apiospermum* (Lyons *et al*, 2005; Steininger *et al*, 2005; Vincent *et al*, 2003; Neskey *et al*, 2000; Poza *et al*, 2000; Danaher *et al*, 2004).

Historically, miconazole had the best *in vitro* activity against *S. apiospermum*, but voriconazole shows much better CNS penetration and has fewer side effects than miconazole. Therefore, voriconazole is the drug of choice for the treatment of *S. apiospermum* brain abscesses (Buzina *et al*, 2006). CNS infections of *Scedosporium apiospermum* have been successfully treated with voriconazole in several reported

clinical cases (Mursch *et al*, 2006; Chakraborty *et al*, 2005; Danaher and Walter, 2004). *S. apiospermum* displays *in vitro* resistance to fluconazole, amphotericin B, and 5-flucytosine, and this fungal species also exhibits variable susceptibility to miconazole, caspofungin, and posaconazole. However, voriconazole has been used with success for the resolution of disseminated infections caused by *Scedosporium apiospermum, Fusarium*, and other angioinvasive fungi, even though the patient was refractory with prior therapy of other antifungals and undergoing immunosuppressive treatment (Husain *et al*, 2005; Klopfenstein *et al*, 2003; Perfect *et al*, 2003; Munoz *et al*, 2000; Girmenia *et al*, 1998). Overall, the success rate of voriconazole has been reported to be 30% for the treatment of scedosporiosis (Perfect *et al*, 2003).

Among patients with pulmonary manifestation of invasive fungal infections, long term therapies are usually required, therefore, the oral formulation of voriconazole is the more attractive option compared to amphotericin B, itraconazole, or caspofungin that has either only intravenous formulation or oral formulation with variable absorption. For outpatients who are unable to take itraconazole, voriconazole is the only viable alternative (Jain and Denning, 2006). Voriconazole has also been used successfully for the treatment of pulmonary aspergillosis in children with cystic fibrosis (Hillard *et al*, 2005).

In cases of pulmonary aspergillosis from lung transplant patients, there have been many reports of resolution of the disease after long term (6-9 months) treatment with voriconazole (Zoumot *et al*, 2006; McCallum *et al*, 2005; Eibl *et al*, 2004; Garbino *et al*, 2003; Mattei *et al*, 2002; van't Hek *et al*, 1998). Various immunologic, anatomic, and surgical factors contribute to the risk for fungal infection in solid organ transplant

recipients. Immunological risk factors include the use of immunosuppressive drug therapy and immunomodulatory conditions. Anatomical predispositions are tissue ischemia and damage. Also, the transplanted organ is often infected, causing secondary fungal disease of the biliary duct (liver transplant), urinary tract (kidney transplant), respiratory tract (lung transplant), and chest (heart transplant) in the recipient (Golan, 2005). In a reported case of pulmonary aspergillosis after heart transplantation, the infection was resolved after voriconazole administration in a patient refractory to amphotericin B treatment (Wieland *et al*, 2005). And a patient refractory to itraconazole treatment after *Pseudoallescheria boydii* infection of the lungs had resolution of the disease with voriconazole (Perlroth and Miller, 2004).

Voriconazole has also been used successfully for the treatment of osteomyelitis of *Scedosporium* (O'Doherty *et al*, 2005; Stratov *et al*, 2003; Studahl *et al*, 2003). In a reported case of *Scedosporium apiospermum* involving the bone, voriconazole was initiated after the patient failed to respond to other antifungal drugs. The patient tolerated voriconazole well for 18 months without any serious adverse reaction, and surgical amputation was avoided (Porte *et al*, 2006). Voriconazole also has been used for the resolution of osteomyelitis caused by *Myceliophthora thermophila* and *Aspergillus* species (Destino *et al*, 2006; Sohail *et al*, 2004). Patients with bone aspergillosis had a global response rate of 55% to voriconazole (Mouas *et al*, 2005).

For the treatment of fungal endophthalmitis, the most important therapeutic principle is early diagnosis and correct identification of the fungus, because early treatment is more likely to yield a better visual outcome and avoidance of enucleation (Jones, 1978). The treatment is usually complicated by the difficulty of the drug reaching

the avascular vitreous tissue. Another important criterion of effectiveness of an antifungal therapy would be the prevention of the loss of retinal function. The electroretinogram (ERG) has been used successfully as a non-invasive measure of retinal function, thus permitting serial measurements in the same animal subjects, as opposed to enucleation or tissue biopsy (Harrison *et al*, 2005). Previous studies of intravitreal injection of amphotericin B have suggested retinal toxicity and the concentration of the drug after intravenous administration barely reached the MIC against *Candida parapsilosis*, a common cause of endophthalmitis (Souri and Green, 1974; Axelrod *et al*, 1973;). In addition, because of numerous adverse effects related to systemic administration of amphotericin B, intravenous administration of amphotericin B is not recommended for routine use for treating fungal endophthalmitis (O'Day *et al*, 1986). Fluconazole has been found to be able to achieve adequate levels in vitreous humor of the rabbits, but fluconazole lacked a broad spectrum of coverage against many of the most commonly encountered organism found to cause fungal endophthalmitis (Breit *et al*, 2005; O'Day *et al*, 1990). Alternatively, intravitreal voriconazole concentration of up to 25 μg/ml caused no histologic or electroretinographic abnormalities to the rat retina, and intravitreal injections of voriconazole have been used successfully in several case reports of fungal endophthalmitis, of which resolution of infection was achieved, and visual acuity was ameliorated or maintained (Gao *et al*, 2004; Sen *et al*, 2006; Scott *et al*, 2004). Patients with endophthalmitis involving *Candida* species were resolved with intravenous and oral voriconazole, and the oral formulation of voriconazole was used successfully for the resolution of *Aspergillus* endophthalmitis (Varma *et al*, 2005; Breit *et al*, 2005; Aliyeva *et al*, 2004).

Similar to fungal endophthalmitis, fungal scleritis can be caused by hematogenous dissemination or direct inoculation by surgery or external trauma (Rodriguez-Ares *et al*, 1995; Jager *et al*, 1994). The use of systemic antifungal therapy may be necessary to salvage the eye and avoid enucleation (Howell *et al*, 2005). Treatment of infectious scleritis is complicated by poor vascularity of the sclera (Reynolds and Alfonso, 1991). Echinocandins do not penetrate the blood-brain barrier, thus they are not recommended for therapies involving the optic nerve or retina. However, oral voriconazole was used successfully to treat scleritis and keratitis caused by *Fusarium* and *Scedosporium* species in case reports (Kim *et al*, 2003; Nulens *et al*, 2003). However the level was not sufficient for the treatment of *Fusarium* species. Nevertheless, the combination of intravenous and topical administrations of voriconazole led to concentrations recommended for the treatment of *Fusarium* (Klont *et al*, 2005). *Aspergillus* involvement of the eye is usually a sign of disseminated disease, but a combination of caspofungin and voriconazole was used successfully for the treatment of *Aspergillus* scleritis (Aliyeva *et al*, 2004; Howell *et al*, 2005).

Regarding cutaneous fungal infections in immunosuppressed patients, certain superficial dermatophyte infections have little potential for dissemination, but opportunistic fungal skin infections can manifest into disseminating and invasive fungal diseases. Voriconazole was effectively used for the treatment of a rare form of disseminated cutaneous aspergillosis that was unresponsive to therapies with amphotericin B and itraconazole (La Nasa *et al*, 2004). Voriconazole is the only agent available that is considered to have any efficacy against fusariosis, and it has been used successfully for the treatment of dermatological manifestation of *Fusarium dimerum*

infection in a stem cell transplant patient and in a patient taking prednisone for autoimmune disease (Mays *et al*, 2006; Bigley *et al*, 2004; Guimera-Martin-Neda *et al*, 2004).

Voriconazole has shown success in the treatment of disseminated fusariosis with a 46% response rate (Perfect *et al*, 2003). Another type of emerging infection is cryptococcosis. Treatment of choice for invasive *Cryptococcus* infections had been amphotericin B, with or without flucytosine and fluconazole (Saag *et al*, 2000). Voriconazole has good activity against this species, and it has been proven to be effective in the treatment of cerebral cryptococcosis (Pfaller *et al*, 1999; Sabbatani *et al*, 2004).

Empirical and prophylactic use of voriconazole in patients with febrile neutropenia and suspected of fungal infection were also evaluated. The risk of invasive aspergillosis is strongly related to the duration and degree of neutropenia. In most cases, small foci of invasive fungal disease may be clinically unapparent during the initial cycle of neutropenia, but manifest during a subsequent cycle (Segal and Walsh, 2006). The expense and toxicity of the medication must be justified, taking into account the patient's risk for invasive fungal infection, to ensure that resources are used appropriately and unnecessary toxicity is avoided (Morris and Villman, 2006). Furthermore, when voriconazole was used as prophylaxis in hematopoietic stem cell transplant (HSCT) patients, an increase in *Zygomycetes* infection emerged (Marty *et al*, 2004; Siwek *et al*, 2004; Imhof *et al*, 2004). It is imperative that the new antifungals be used responsibly so that their efficacy will be maintained. Nonetheless, voriconazole has been shown to be more effective than amphotericin B as empirical therapy in patients with neutropenia and persistent fever (Walsh *et al*, 2002a).

2.12.8 Interindividual Variations

Voriconazole displays minimal intra-invidivual but high inter-individual pharmacokinetic variations. In patients administered oral voriconazole, there was low correlation (r=0.26; P=0.099) between the dose per kg given and the plasma voriconazole level observed (Trifilio *et al*, 2005).

The variation could be explained partly by pharmacogenomic factors. Pharmacogenomics is the study of the impact of genetic variants on the determination of pharmacokinetics of drugs. The cytochrome P450 enzyme CYP2C19 demonstrates genetic polymorphism, with 15-20% of Asians estimated to be poor metabolizers, and 3-5% in Caucasians and Blacks (Shimada *et al*, 1994). In humans, it has been found that the terminal elimination half life of voriconazole in CYP2C19 extensive metabolizers is 8 hours, whereas it is 15 hours in poor metabolizers. Poor metabolizers receive 4-fold higher voriconazole exposure than extensive metabolizers (Theuretzbacher *et al*, 2006). However, currently no dosage adjustments have been recommended regarding this difference (Theuretzbacher *et al*, 2006).

Other causes of interindividual variability include age and sex. Voriconazole is approved for children >2 years, but there is little information regarding metabolism of voriconazole in children. In children under 12 years of age, clinical studies revealed that voriconazole follows linear pharmacokinetics in pediatric patients over the dosage range of 3 and 4 mg/kg every 12 hours, and the clearance is more rapid, requiring higher doses to achieve AUCs similar to those of adults. In a dose escalation study involving pediatric patients, the patients were allowed to serve as their own control for variables such as CYP2C19 genotype and body weight, and pediatric dose of 11 mg/kg twice a day is

recommended, compared to the recommended 4 mg/kg maintenance dose in adults (Walsh *et al*, 2002b). Body weight has been shown to account for the kinetic interindividual variability in children. The authors surmised that children have a higher elimination capacity than adults on a body weight basis, and this difference between pediatric and adult patients was the most important factor accounting for this difference in clearance of voriconazole (Walsh *et al*, 2004b). In a case study involving a 4-year-old boy who developed osteomyelitis from *Myceliophthora thermophila* after direct inoculation from trauma, the patient was unresponsive to liposomal amphotericin B treatment. Voriconazole was given as salvage therapy, initiated with doses recommended for adults (loading dose of 6 mg/kg every 12 hours for 24 hours, followed by 4 mg/kg every 12 hours as maintenance). However, periodic sampling of blood indicated a higher dosage (13.4 mg/kg every 12 hours) was needed to achieve the same plasma peak levels as in adults (Destino *et al*, 2006). A case series study involving pediatric patients revealed that children have a higher rate of elimination of voriconazole per unit of body weight compared to adults,

In multiple oral dosing to healthy volunteers, the mean C_{max} and AUC values were 85% and 113%, respectively, with higher drug exposure observed in young females than for young males. However, no significant differences were observed between healthy elderly men and women. In clinical trials, elderly patients have 80-90% higher plasma concentration than those found in younger patients, after both intravenous and oral administrations. Currently, no dose adjustment is recommended for sex and age differences (Pfizer, 2006).

2.12.9 Adverse Effects

Voriconazole is generally well tolerated. The most commonly reported adverse event is visual disturbances, which typically occur around 30 minutes after dosing and is spontaneously resolved within 1 hour. Visual disturbances include altered color discrimination, blurred vision, the appearance of bright spots and wavy lines, and photophobia. The visual effects are associated with electroretinogram tracings, which revert to normal with the cessation of treatment, and no permanent damage to the retina has been noted (Naithani and Kumar, 2005).

Hepatotoxicity is observed in approximately 13% of patients receiving voriconazole, with an increase in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase. Therefore, routine monitoring of hepatic enzyme levels during therapy is recommended. However, researchers determined that there was no correlation between voriconazole plasma level and ALT, creatinine, and bilirubin levels, but there was a significant correlation between voriconazole, AST (aspartate aminotransferase), and alkaline phosphatase levels (Trifilio *et al*, 2005). As there is no statistically significant relationship between plasma voriconazole concentrations and ALT serum level abnormalities, and only weak association between plasma voriconazole concentrations and AST, ALP, and bilirubin serum level abnormalities, the researchers asserted that there is little value in measuring the plasma voriconazole concentration in the absence of an abnormal liver function test (Tan *et al*, 2006; Lutsar *et al*, 2003b). In patients with mild to moderate hepatic insufficiency, single and multiple oral dosing caused a 3.2-fold increase in AUC compared with subjects with normal hepatic function, and in patients with cirrhosis,

voriconazole clearance was about half of those observed in subjects with normal hepatic function. Therefore, patients with cirrhosis are recommended to have a regimen of the standard loading dose, but half of the maintenance dose (Peng and Lien, 2005).

Because voriconazole is predominantly cleared by biotranformation of the liver, the drug itself has no impact on renal clearance (Pfizer, 2006). However, as voriconazole has limited aqueous solubility, the intravenous form is solubilized in the solvent vehicle sulfobutylether-β-cyclodextrin (SBECD), at 16 mg per mg of voriconazole, as a novel delivery system (Walsh *et al*, 2000). In healthy subjects, SBECD is rapidly eliminated with a $t_{1/2}$ of 1.6 hours, and the clearance is linearly related to creatinine clearance. In animal experiments, the minimal lethal dose was 20,000 mg/kg, and the target organs for toxic effects in rodents were kidney and liver with obstruction of renal tubules and single cell necrosis in the liver. In patients with renal impairment, oral voriconazole is recommended because SBECD can accumulate in patients with creatinine clearance <50 mL/min, and the clearance and AUC of SBECD are increased 4-fold and 50%, respectively, compared to subjects with normal renal function (Hoffman and Rathbun, 2002). However, in critically ill patients, sometimes it is not possible to administer the drug orally, due to extensive gastric reflux, gastrointestinal bleeding, or mucositis, and intravenous administration might be necessary. In a case series study involving patients with renal failure who received repeated intravenous dosing of voriconazole, the concentration of SBECD in plasma were determined to be within toxic range, fortunately there was no evidence of toxic effect related to the SBECD concentration measured. Nonetheless, the authors recommended intravenous voriconazole therapy to be switched to oral administration as soon as possible in patients with renal impairment, as SBECD is

nephrotoxic through its reuptake from glomerular filtrate by the kidney tubules and should be avoided (von Mach *et al*, 2006). No dose adjustment is needed for patients with renal impairment if the oral dose is administered.

Compare to amphotericin B, voriconazole has less nephrotoxicity and less infusion-related toxicity (Walsh *et al*, 2002a). In a large randomized study involving 392 patients diagnosed with invasive aspergillosis, the researchers found that overall voriconazole was better tolerated than amphotericin B, with significant less adverse events (Herbrecht, 2002c). There has been an overall decreased use of amphotericin B in Germany in the past decade, particularly due to its decreased use in the hematology-oncology wards and association with increased use of voriconazole (de With *et al*, 2005).

Skin rashes occur in about 6% of patients, especially after long term treatment but are usually mild. Other reported side effects include headache, nausea and vomiting, diarrhea, abdominal pain, and visual hallucinations (Walsh *et al*, 2004a; Lazarus *et al*, 2002; Purkins *et al*, 2002). Photosensitivity reactions in patients receiving voriconazole have been reported, thus patients are advised to avoid direct sunlight during therapy (Swift and Denning, 1998). Voriconazole is contraindicated in patients with known hypersensitivity to voriconazole or to any of the excipients (Naithani and Kumar, 2005).

2.12.10 Interactions

Single and multiple oral administration of voriconazole with food reduced the bioavailability by 22% and delayed the absorption by 1.1 hours, compared with dose administered in fasted state. Furthermore, high fat meals reduce mean C_{max} and AUC values by 34% and 24%, respectively. Therefore, it is recommended that oral dosing of voriconazole be administered either 1 hour before or after meals (Purkins *et al*, 2003c).

Because voriconazole is both a substrate and inhibitor of CYP2C19, CYP2C9, and CYP3A4, it has the potential for drug interaction with any chemical that has the ability to alter the activities of these enzymes also. Voriconazole serum concentrations are significantly reduced by rifampicin, phenytoin, carbamazepine, and long-acting barbiturates. For example, coadministration with rifampicin reduces the AUC of voriconazole by 93% and by 70% with phenytoin (Gothard and Rogers, 2004; Purkins *et al*, 2003d). Therefore, coadministration with these drugs is contraindicated. In addition, plasma concentrations of rifabutin and phenytoin are increased when given concomitantly with voriconazole, thus frequent monitoring of phenytoin and rifabutin levels are recommended (Johnson and Kauffman, 2003). Other contraindicated drugs include immunosuppressants such as sirolimus, tacrolimus, and cyclosporine, due to the observed increase in plasma concentration of the immunosuppressive agent. For example, after standard dosing of voriconazole, liver transplant patients experienced a 10-fold increase in tacrolimus trough levels, and coadministration with cyclosporine led to a 1.6 to 1.7-fold increase in AUC in liver and bone marrow transplant patients (Venkataramanan *et al*, 2002; Romero *et al*, 2002; Groll *et al*, 2004). Coadministration with ergot alkaloids is also contraindicated due to the possible increase in plasma concentration of the ergo alkaloids, which would lead to ergotism. Similarly, coadministration with quinidine and pimozide can cause potential QT interval prolongation and possibility of torsade de pointes. When voriconazole is given with warfarin, monitoring of prothrombin time is recommended, as coadministration potentiates warfarin-induced prothrombin time prolongation (Pfizer, 2006).

Coadministration with St. John's Wort leads to a short term but clinically irrelevant increase, followed by a prolonged reduction in voriconazole exposure (Rengelshausen *et al*, 2005).

2.12.11 Pharmacoeconomics

In several pharmacoeconomic evaluations based on analytic model of using voriconazole versus amphotericin B as the initial therapy for the treatment of invasive aspergillosis, the researchers determined that the initial therapy of voriconazole is both cost-saving and results in better clinical outcomes compared to initial therapy of amphotericin B. The main difference in cost was derived from decreased renal toxicity from amphotericin B and reduced number of days of hospital stay. This makes voriconazole the dominant cost-effective option for initial therapy of invasive aspergillosis, despite very low drug acquisition cost of conventional amphotericin B (Jansen *et al*, 2006; Wenzel *et al*, 2005; Garbino *et al*, 2006).

The risk of unnecessary over-treatment of toxicity and acquisition cost must be weighed against the high fatality rate in patients not given antifungal treatment unless their infection has been proved unequivocally (Maschmeyer and Ruhnke, 2004).

2.12.12 Use in Veterinary Medicine

To date, there have been no published reports regarding the efficacy of voriconazole in veterinary medicine. This could be mainly due to the lack of pharmacokinetic studies of this drug in veterinary animals, thus there are no dosage regimen recommendations available to clinicians. Because of possibilities of variable absorption, autoinduction of metabolism, and saturable elimination that have been

observed in humans and laboratory animals, it is impossible to extrapolate pharmacokinetic parameters across species using allometric scaling.

Only recently was one study published on the pharmacokinetics of voriconazole after administration to healthy horses (Davis *et al*, 2006). After single IV dosing to six horses, the mean half-life value of voriconazole was 13.11 hours, the mean volume of distribution at steady state value was 1.35 L/kg, and the mean clearance value was 0.11 L/kg/hr. The systemic bioavailability of voriconazole in horses was 135.75% with a single administration of the powder formulation of the drug, and the peak plasma concentration was observed at 2.92 hours postdose. No clear evidence of nonlinear pharmacokinetics was observed in horses.

Because the pharmacokinetics of voriconazole is known to alter during chronic dosing, multiple dosing studies in horses would be desirable. Furthermore, due to possible time- and dose-dependent characteristics of voriconazole pharmacokinetics in certain species and the inability to predict kinetic parameters across species, it is important to investigate the disposition of voriconazole after single and chronic dosing in specific species of interest with healthy subjects, before administrating the drug to diseased animals.

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3. PHARMACOKINETICS OF VORICONAZOLE AFTER SINGLE DOSE INTRAVENOUS AND ORAL ADMINISTRATION TO ALPACAS

Abstract

There is a paucity of drug disposition studies in alpacas, and pharmacokinetic information would be helpful in determining appropriate antifungal treatment regimens in alpacas in the absence of approved agents for this species. Voriconazole is a new antifungal drug that has shown effectiveness in treating serious fungal infections and has the potential for being used in large animal veterinary medicine. The objective of this study was to determine the plasma concentrations and pharmacokinetic parameters of voriconazole after single-dose intravenous (IV) and oral administration to alpacas. Four alpacas were treated with single 4 mg/kg intravenous and oral administrations of voriconazole. Plasma voriconazole concentrations were measured by a high-performance liquid chromatography method. The mean terminal half-life ($t_{1/2\beta}$) following IV and oral administration was 8.011 ± 2.879 and 8.748 ± 4.307 hours, respectively; mean maximum plasma concentrations (C_{max}) were 5.930 ± 1.132 and 1.700 ± 2.707 mg/L, respectively; and area under the curve from time zero extrapolated to infinity ($AUC_{0\text{-}inf}$) was 38.50 ± 11.11 and 9.484 ± 6.983 mg-hr/L, respectively. The mean apparent systemic oral availability was low with a value of 22.74 ± 9.48 %. The drug plasma concentrations remained above 0.1 μg/mL for at least 24 hours after single IV dosing. The IV administration of 4 mg/kg/day voriconazole may be a safe and appropriate option for

antifungal treatment of alpacas. Due to the low oral extent of voriconazole absorption in alpacas, doses of 20.4 to 33.9 mg/kg/day may be needed.

Introduction

Originating in South America, alpacas (*Lama pacos*) are considered to be one of the oldest domesticated animals in the world. In the past decade they have rapidly gained popularity as fiber producers, pets, and packing animals in the United States (Fowler, 1998). Although overall this species is quite healthy, as with many species, animals having chronic diseases and receiving prolonged antibiotic or immunosuppressive therapies are prone to developing fungal infections. Various invasive and disseminated forms of cryptococcosis, aspergillosis, and coccidioidomycosis have been reported to occur in alpacas (Severo *et al*, 1989; Thedford and Johnson, 1989; Goodchild *et al*, 1996; Coulton *et al*, 1997; Muntz, 1999). There is an absence of pharmacokinetic, drug disposition, and bioavailability studies in alpacas (Chakwenya *et al*, 2002; Drew *et al*, 2002; Hunter *et al*, 2004; Gandolf *et al*, 2005; Lakritz *et al*, 2006). Because there are no drugs approved by the Food and Drug Administration (FDA) for use in alpacas, usually the dosages given to alpacas are based on study results from other ruminants. However, alpacas are not taxonomically classified as ruminants, even though they are functional ruminants (von Engelhardt *et al*, 1984; Fowler, 1998). Therefore, the practice of extrapolating antifungal dosages across species can be invalid, especially if the drug has dose-dependent pharmacokinetic characteristics.

One drug of such characteristic is voriconazole. Voriconazole is a new antifungal that has demonstrated activity against some of the pathogenic fungi *in vitro*. Against *Candida* species, on a milligram-to-milligram basis, voriconazole is 60 to 100-fold more

potent than fluconazole, and among *Candida* species the overall minimum inhibitory concentration to inhibit the growth of 90% of the organisms (MIC_{90}) is 0.25 μg/mL, with 99% of the tested isolates inhibited by concentrations ≤1 μg/mL (Pfaller *et al*, 2006). Voriconazole exhibits fungicidal activity against *Candida* species and is fungistatic against *Aspergillus* species. Voriconazole also has very low minimum inhibitory concentration (MIC) values against the most commonly found *Aspergillus* species (Theuretzbacher *et al*, 2006). In an *in vitro* comparative study, voriconazole had higher activity than amphotericin B and itraconazole against various *Aspergillus* species (Guinea *et al*, 2006).

Clinically, voriconazole has been shown to be more effective than amphotericin B as initial therapy for patients with invasive aspergillosis. Complete or partial response was achieved in 53% of the voriconazole group, versus 32% in the amphotericin B group (Walsh *et al*, 2002). Furthermore, compared to amphotericin B, voriconazole is better tolerated than amphotericin B, with significantly less reported adverse events (Herbrecht *et al*, 2002).

Voriconazole is available commercially as a lyophilized powder for solution for intravenous (IV) infusion, as a film-coated tablet for oral administration, and as a powder for oral suspension. The pharmacokinetics of voriconazole have been well characterized in humans. The standard dosing of voriconazole to adult humans is IV 6 mg/kg loading dose every 12 hours for 24 hours, then 4 mg/kg every 12 hours as the maintenance dose. For oral administration, voriconazole is given at the rate of 100 mg to patients under 40 kg and 200 mg to those over 40 kg, twice a day (Pfizer, 2006).

There are published studies of voriconazole pharmacokinetics in other animal species. In a preclinical study following a single IV dose in several species, voriconazole pharmacokinetics was characterized by non-linear elimination (Roffey *et al*, 2003). After oral administration in this study, voriconazole was absorbed with a time for the observed peak plasma concentration (T_{max}) between 1 and 8 hours, and high extents of absorption in all species investigated: mouse, 81%; male rat, 159%; female rat, 88%; guinea pig, 75%; rabbit, 87%; and dog, 138%. In addition, autoinduction of voriconazole metabolism has been observed in some species, but not in rabbit, guinea pig, or human (Purkins *et al*, 2002; Roffey *et al*, 2003). There are many factors, such as hepatic metabolism, that could cause differences in pharmacokinetic parameters among species and thus lead to a need to possibly change the therapeutic doses and interval of a compound based on the species of the animal (Short, 1994). Therefore, it may be difficult to accurately predict the pharmacokinetics of voriconazole across species and thus therapeutic multiple dosing regimens.

One recent study was published regarding pharmacokinetics of voriconazole in the veterinary medicine setting and the safety of the drug after single 1 mg/kg IV and 4 mg/kg of powder via oral dosing in equine. The results show that voriconazole was well tolerated by healthy adult horses and no adverse effects were detected (Davis *et al*, 2006). Furthermore, drug loss during oral administration was minimal, with apparent systemic bioavailability of 135.75 ± 18.41% and peak plasma concentration of 2.43 ± 0.4 μg/mL at 2.92 ± 1.20 hours postdose. Since the intravenous and oral dose varied so widely, estimates of the extent of oral absorption could not be accurately estimated, and an extent of absorption value greater than 100% could suggest nonlinear drug elimination, but no

clear evidence of nonlinear elimination was observed in the plasma concentration versus time curves in horses (Davis *et al*, 2006). The possibility of saturable metabolism and nonlinearity in horses should be further investigated. Although initial pharmacokinetic studies of voriconazole have been performed in rats, mice, rabbits, guinea pigs, and dogs by Roffey *et al* (2003), to our knowledge, the safety and pharmacokinetics of voriconazole had not been investigated for the use in veterinary medicine in any other species.

The objectives of this study were to investigate the pharmacokinetics of voriconazole when administered to alpacas as a single dose by the IV and oral routes and to generate information that can be used to develop dosing regimens that will result in plasma concentrations in alpacas that are within the therapeutic range. This information should be helpful in determining appropriate antifungal treatment regimens in alpacas in the absence of approved agents for this species.

Materials and Methods

Animals

Four clinically normal adult alpacas were used in this study. The alpacas weighed between 25 and 63 kg (mean, 50 kg) and were part of The Auburn University Food Animal Research Program. Results of complete blood count (CBC) and serum biochemical analyses performed the day before the study were within reference values for our laboratory. All alpacas were vaccinated and dewormed at least 4 weeks prior to the pharmacokinetic study. Alpacas were maintained on pasture and acclimated to the barn and housed in one large stall twelve hours prior to performance of the study. Water and grass hay were available *ad libitum*. General physical examination was performed once a

day throughout the study. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of Auburn University.

Drug administration

Voriconazole was administered intravenously and then orally to each alpaca, separated by a minimum of one week of washout period. For the collection of blood samples, a 16-gauge 2-inch catheter (Surflo®, Terumo Medical Corp., Somerset, NJ) was aseptically inserted into the right jugular vein and attached to a 32-inch extension with male rotating luer lock and stopcock (Arrow International Inc., Reading, PA); the extension and stopcock were secured to the alpaca by use of elastic tape (Elastikon®, Johnson & Johnson, New Brunswick, NJ). For IV drug administration (4 mg/kg), an additional catheter was placed in the left jugular vein. Lidocaine 2% (Hospira Inc., Lake Forest, IL) was used as a local anesthetic to alleviate any pain. The IV treatment consisted of voriconazole injectable (Vfend®, Pfizer Inc., New York, NY), 2 mg/ml mixed in sterile saline solution (0.9% sodium chloride, Abbott Laboratories, Abbott Park, IL), and injected into the jugular vein, using a 73-inch primary IV set (Hospira Inc., Lake Forest, IL). The IV set was vented with a flashback bulb, via the catheter in the left jugular vein (infusion time 3-9 minutes; mean, 5.5 minutes) and this was immediately followed by 6 mL of saline solution containing 10 units/mL heparin (American Pharmaceutical Partners Inc., Schaumburg, IL). For the oral treatment, each dose of voriconazole powder (4 mg/kg; Maithri Laboratories Pvt. Ltd., Andhra Pradesh, India) was mixed with 15 ml of corn syrup (T.J. Blackburn Syrup Works Inc. Jefferson, TX) and administered via a 60-ml syringe (Becton Dickinson, Franklin Lakes, NJ).

Blood collection

Blood samples (6 mL) were taken with 10-mL syringes (Becton Dickison, Franklin Lakes, NJ) via the catheter in the right jugular vein immediately before drug administration (time 0), and after IV administration at 0.083, 0.167, 0.25, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24 and 48 hours, and after oral administration at 0.083, 0.167, 0.25, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, and 33 hours. Blood samples were immediately placed in lithium heparin-coated tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ) and kept on ice. The catheter inserted in the jugular vein was flushed with heparinized saline solution (6 mL) between subsequent collection of blood samples. At the time of each sample collection, 6 mL of saline solution was injected to flush the heparinized saline solution out of the extension system, 6 mL of blood was withdrawn to remove the saline solution from the extension system, and another 6 mL of blood was collected as sample. Samples were centrifuged at 1,163 × g for 10 minutes (Clay Adams Brand Compact II Centrifuge, Becton Dickinson, Franklin Lakes, NJ) within 30 minutes of collection, and plasma was removed with disposable polyethylene transfer pipettes (Denville Scientific Inc., Metuchen, NJ) and stored in cryogenic storage vials (Fisher Scientific Inc., Waltham, MA) at -80ºC until assay.

Drug analysis

The plasma samples were assayed using a modified version of a previously published liquid-liquid extraction method followed by reverse-phase high-performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detection (Roffey *et al*, 2003). The HPLC system (Dionex, Sunnyvale, CA) consisted of a pump (GP-40), autosampler (AS3500), UV/visible light absorption detector (AD-20), column (Thermo BetaBasic-18,

4.6 mm × 15 cm, 5 µm, Bellefonte, PA), silica guard column (4 mm × 1.25 cm), and computer for data collection and analysis (Gateway 2000, P4D-66, Irvine, CA)

Voriconazole and internal standard (ketoconazole) were eluted with a mobile phase consisting of 35% 0.1 M *N,N,N',N'*-tetramethylethylenediamine (Fisher Scientific Inc., Waltham, MA), pH 7.4, and 65% methanol (Fisher Scientific Inc., Waltham, MA) at a flow rate of 1.0 ml/min at room temperature. The mobile phase was degassed by vacuum filtration with 0.45 µm nylon membrane filters (Whatman plc, Middlesex, UK). The injection volume was 100 µL. Voriconazole and internal standard were detected at a wavelength of 254 nm, and the retention times were 3.7 and 13.5 minutes, respectively.

To produce calibration curves, stock solutions of 1 mg/mL voriconazole were prepared by dissolving voriconazole powder in mobile phase. The stock solution was kept refrigerated in a borosilicate glass tube (Fisher Scientific Inc., Waltham, MA), tightly sealed with flexible film (Parafilm®, Alcon Inc., Menasha, WI). Spiking solutions for the calibration curve were prepared by diluting the stock solution with mobile phase to yield solutions with voriconazole solutions ranging from 1 to 100 µg/mL. By adding 50 µL of each of the spiking solutions to 500 µL of alpaca plasma or filtered and distilled (Barnstead Fistreem II, Barnstead International, Dubuque, IA) water, calibration standards with voriconazole concentrations ranging from 0.1 to 10 µg/mL were prepared. Plasma obtained from an alpaca that was not treated with voriconazole was used as a blank control. A new calibration curve was prepared daily before samples were analyzed.

To prepare samples and calibration standards for HPLC, 500 µL of each sample or standard, followed by 15 µL of internal standard, were pipetted into a disposable

borosilicate glass tube and vortexed for 10 seconds. The internal standard solution (1mg/mL) was prepared by dissolving ketoconazole powder (PCCA, Houston, TX) in mobile phase. Voriconazole and internal standard were extracted with 4 mL ethyl acetate (Fisher Scientific Inc., Waltham, MA), followed by 1mL of 0.2 M sodium borate (J.T. Baker Chemical, Phillipsburg, NJ) at pH 9. The mixture was vortexed for 30 seconds and centrifuged at 4,500 × g for 10 minutes (IEC Clinical Centrifuge, International Equipment Company, Needham Heights, MA). The organic phase was transferred to a separate glass tube and evaporated with nitrogen gas in a 50°C sand bath. The dried residue was reconstituted with 200 µL of the mobile phase and transferred to an HPLC vial and insert (250 µL glass flat bottom, Alltech Associates Inc., Deerfield, IL) for analysis.

Chromatograms were integrated by use of computer software (Dionex PeakNet® 4.11, Sunnyvale, CA). Calibration curves of peak area ratios (voriconazole/internal standard) versus concentration were calculated by the use of linear-regression analysis (R^2=0.999). The lower limit of detection for voriconazole was 0.1 µg/mL, and the lower limit of quantification was 0.5 µg/mL. The mean intra-day variation was 1.96% and the mean inter-day variation was 5.01%. Recovery was 100%.

Pharmacokinetic analysis

Pharmacokinetic parameters for each alpaca were determined for IV and oral drug administration. Non-compartmental and compartmental analyses were performed on the data obtained from IV dosing, and non-compartmental analysis was performed on the data obtained from oral dosing, using the computer program WinNonlin® 02.1A (Pharsight, Cary, NC) and Excel® MicroSoft Office 2003. Pharmacokinetic parameters

measured included observed peak plasma concentration (C_{max}), time of observed peak concentrations (T_{max}), area under the curve (AUC), half-life of the elimination phase ($t_{1/2\beta}$), clearance (Cl), mean residence time (MRT), and volume of distribution at steady state (Vd_{ss}) (Tables 3.2 and 3.3). The elimination rate constant, K_{el}, was estimated from the log-linear terminal decline in the natural log (LN) of the plasma concentration versus time with a weighting of 1/[concentration]. The AUC was measured by the use of the log-trapezoidal method. The area under the plasma concentration-time curve from time zero extrapolated to infinity ($AUC_{0\text{-}inf}$) was calculated by determining the AUC up to the last sample point ($AUC_{0\text{-}LT}$) and then adding the terminal portion estimated from the terminal rate constant. The C_{max} and T_{max} were observed directly from the data. The $t_{1/2\beta}$ was the LN of 2 (0.693) divided by the terminal rate constant (K_{el}).

Mean residence times were accessed from dividing the area under the moment curve (AUMC) by the AUC. The mean residence time of absorption (MRT_{abs}) is equal to the difference of $MRT_{oral} - MRT_{IV}$, where MRT_{oral} is the mean residence time following oral administration, and MRT_{IV} is the mean residence time following intravenous administration. The extent of oral absorption, F, was calculated by use of the following equation:

$$F = ([AUC_{oral} \times Dose_{IV}]/[AUC_{IV} \times Dose_{oral}])$$

where AUC_{IV} and $Dose_{IV}$ are the AUC and dose, respectively, for the IV route of administration, and AUC_{oral} and $Dose_{oral}$ are the AUC and dose, respectively, for the oral route of administration.

Plasma concentrations versus time following IV administration were evaluated to determine the best-fit pharmacokinetic model for compartmental analysis (Figure 3.2). A

two-compartmental model was chosen for analysis based on the sum of squares and residual plots. The model used was characterized by the equation:

$$C = (A\,[1 - e^{-\alpha T}]e^{-\alpha t})/[\alpha\, T] + (B\,[1 - e^{-\beta T}]e^{-\beta t})/[\beta\, T]$$

where C is the plasma concentration of voriconazole at time post dose; t is time after drug administration, T is the infusion period; A and α are the coefficient and slope, respectively, of the distribution phase; B and β are the coefficient and slope, respectively, of the elimination phase; and e is the base of natural logarithm. The coefficients are defined in terms of the microrate constants as:

$$A = (\text{Dose}\,[\alpha - k_{21}])/(V_c\,[\alpha - \beta])$$

$$B = (\text{Dose}\,[k_{21} - \beta])/(V_c\,[\alpha - \beta])$$

The hybrid rate constant can also be described in terms of the microrate constants of the model as:

$$\alpha = ([k_{12} + k_{21} + k_{10}] + \sqrt{[k_{12} + k_{21} + k_{10}]^2 - 4k_{21}k_{10}})/2$$

$$\beta = ([k_{21} + k_{12} + k_{10}] - \sqrt{[k_{12} + k_{21} + k_{10}]^2 - 4k_{21}k_{10}})/2$$

The above and below equations used for the calculation of pharmacokinetic parameters were based on methods described by Gibaldi and Perrier (1982). The value for the volume of distribution of the central compartment (V_c) was calculated by use of the following equation:

$$V_c = \text{Dose}/(C_0)$$

where C_0 is equal to the sum of A plus B. Cl was calculated as:

$$Cl = V_c \times k_{10}$$

where k_{10} is defined as $(\alpha\beta)/k_{21}$, and k_{21} is equal to $(A\beta + B\alpha)/C_0$. The value for the volume of distribution based on the AUC (Vd_{area}) is the quotient of Cl/β. Vd_{ss} was calculated by use of the following equation:

$$Vd_{ss} = V_c + V_p$$

where V_p is the volume of distribution of the peripheral compartment and is equal to $(k_{12}V_c)/k_{21}$. The value for the extrapolated volume of distribution (Vd_{ext}) was calculated by the use of the following equation:

$$Vd_{ext} = Dose/B$$

The AUC based on the compartmental model is equal to $(A/\alpha) + (B/\beta)$, and the MRT was calculated as follows:

$$MRT = AUMC/AUC$$

where AUMC is the area under the moment curve and is equal to $(A/\alpha^2) + (B/\beta^2)$. The value of distribution clearance (Cl_{12}) is the product of V_cK_{12}.

The oral absorption kinetics of voriconazole was examined in greater detail from plots of the data expressed as the percent remaining to be absorbed (Ravis *et al*, 1987). The equations used for the calculation of the percent remaining to be absorbed were based on methods described by Wagner (1983).

Statistical analysis

Statistical comparison between noncompartmental and two-compartmental analyses of pharmacokinetic parameters was carried out applying the two-way analysis of variance (ANOVA) test using Microsoft® Excel 2002. The level of significance was $P \leq 0.05$.

Results

No adverse effects were observed in any of the alpacas after administration of either IV or oral voriconazole. After 4 mg/kg of single IV dosing in alpacas, the mean ± standard deviation (SD) of elimination half-life ($t_{1/2\beta}$) was 8.011 ± 2.879 hours, and the clearance (Cl) was 0.1093 ± 0.0251 L/hr/kg using noncompartmental analysis. The area under the plasma concentration-time curve from time zero extrapolated to infinity (AUC_{0-inf}) values ranged from 30.86 to 55.02 µg-hr/mL, with a mean ± SD value of 38.50 ± 11.11 µg-hr/mL. The AUC from time zero to 24 hours accounted for at least 90% of the AUC to infinity. The drug plasma concentrations remained above 0.1 µg/mL for at least 24 hours postdose (Figure 3.2).

When two-compartmental modeling was applied for the analysis of the plasma concentrations versus time data following IV administration of voriconazole, the calculated mean distribution half-life ($t_{1/2\alpha}$) was 0.1698 ± 0.0260 hours, and the mean $t_{1/2\beta}$ was 7.833 ± 0.770 hours (Table 3.3). The mean ± SD values of Cl and Vd_{area} using two-compartmental analysis were 0.1118 ± 0.0145 L/hr/kg and 1.256 ± 0.134 L/kg, respectively. Other relevant pharmacokinetic parameters obtained by modeling after IV administration of voriconazole were summarized (Table 3.3).

Following a 4 mg/kg single oral administration, the extent of absorption based on AUC values after oral and IV dosing was 22.74 ± 9.48% in the four alpacas. The mean ± SD value of AUC_{0-24} was 7.812 ± 6.548 µg-hr/mL, the AUC_{0-inf} was 9.484 ± 6.983 µg-hr/mL, and the dose-normalized AUC_{0-inf} value (AUC_{0-inf}/Dose) was 2.371 ± 1.746 kg-hr/L. The AUC up to the last sample time of 33 hours accounted for approximately 90% of the total AUC to infinity. The calculated mean residence time of absorption (MRT_{abs})

was variable with an average value of 3.610 ± 6.407 hours. The total body clearance divided by the extent of absorption (Cl/F) obtained after 4 mg/kg oral administration of voriconazole powder to four alpacas ranged from 0.2019 to 0.8420 L/hr/kg. Larger inter-subject variations were observed in the oral route pharmacokinetic parameters, with coefficients of variation as high as 159.23% among C_{max} values.

The absorption kinetics after oral administration were further examined by percent remaining to be absorbed methods. Final graphs of the amount remaining to be absorbed as a function of time were illustrated (Figures 3.9 and 3.10). The plots of the percent remaining to be absorbed showed multi-phasic characteristics.

Discussion

The current population of alpacas in the United States is approximately 25,000 and the number continues to rise (Smith, 1998). Therefore it is of increasing importance to have the pharmacokinetic data and dosing regimen recommendation of significant drugs available to veterinarians. This study is the first to our knowledge that investigates the pharmacokinetics of voriconazole in a non-monogastric species. Due to the low availability of alpacas for research purposes, only four animals were utilized.

After IV administration of voriconazole to four alpacas, the mean clearance value (0.1093 L/hr/kg) was lower than that observed in the mouse (0.240 L/hr/kg), male rat (0.538 L/hr/kg), female rat (0.123 L/hr/kg), rabbit (2.727 L/hr/kg), guinea pig (0.260 L/hr/kg), and human (0.498 L/hr/kg), but higher than that of the dog (0.093 L/hr/kg) (Roffey *et al*, 2003; Purkins *et al*, 2003a). However total body clearance in alpacas was similar to that observed in horses (0.11 L/hr/kg) (Davis *et al*, 2006). The mean dose-normalized $AUC_{0\text{-}inf}$ value (9.626 kg-hr/L) was similar to that of horses (9.23 kg-hr/L)

that received single dosage of 1 mg/kg as observed by Davis *et al* (2006). When comparing to previously investigated species that received 3 mg/kg intravenously, the alpaca's mean AUC/Dose value was much higher than that of the healthy adult humans (2.008 kg-hr/L) and rabbits (0.37 kg-hr/L), and it was slight lower than that found in the dogs (10.70 kg-hr/L). The mean AUC/Dose value of alpaca was also higher than that of mouse, rat, and guinea pig, but those species had received much higher dosages (10 to 30 mg/kg) compared to the other species mentioned before (Roffey *et al*, 2003). At these high doses of 10-30mg/kg, dose-dependent pharmacokinetics has been reported in certain species for voriconazole (Roffey *et al*, 2003). The mean $t_{1/2\beta}$ value following a single IV dose to alpacas was similar to that previously reported in the horse (8.89 ± 2.31 hours), but longer than that found in the human of 5.6 hours (Purkins *et al*, 2003a; Davis *et al*, 2006). Half-life values after single IV dosing in other species are not available.

Log-linear plot of voriconazole plasma concentration versus time after IV administration to the four alpacas (Figure 3.4) indicated multi-compartmental features, therefore, the data was also subjected to compartmental as well as noncompartmental analysis. The pharmacokinetic parameters obtained following noncompartmental analysis were in excellent agreement with those obtained by the two-compartmental modeling methods. The mean $t_{1/2\beta}$ value obtained using noncompartmental analysis after IV administration of voriconazole was 8.011 ± 2.879 hours. This value was comparable to the $t_{1/2\beta}$ calculated using the two-compartmental analysis (7.833 ± 0.770 hours). Furthermore, there was no significant difference observed in clearance, Vd_{ss}, AUC, and MRT_{IV} values obtained between the two different analysis methods. This seems to

indicate that the two-compartment model adequately describes the pharmacokinetics of voriconazole in alpacas.

The mean $t_{1/2\alpha}$ value obtained after two-compartmental analysis was 0.1698 ± 0.0260 hours, which indicates a short distribution phase, that was followed by a more prolonged elimination phase. The distribution phase appeared complete by 50-60 minutes. Further evidence for rapid distribution was found in the similarity in the average Vd_{ss} and Vd_{area} values (Duran *et al*, 1987). The slight convex fall in the log-linear plasma concentration versus time in the mean graph (Figure 3.2) and in the individual plots of two of the alpacas (Figure 3.4) may suggest some capacity limited elimination. Since voriconazole is eliminated predominantly by metabolism in all species investigated in the pre-clinical study, the authors of that study believed that the saturation of metabolic clearance is the cause of the nonlinearity (Roffey *et al*, 2003). No clear evidence of saturation of voriconazole metabolism was observed in the other two alpacas nor has it been reported in horses. It is possible that in the present study, the voriconazole concentrations did not reach levels that would display clear evidence of saturable metabolism in all four alpacas. Since only one dosage for each route was given, the nonlinearity of voriconazole in alpacas needs to be further studied.

Pharmacokinetic variations of voriconazole among species could be due to possible interspecies differences in the expression of cytochrome P450 enzymes that are responsible for voriconazole metabolism, since voriconazole is metabolized primarily in the liver. Little information regarding drug metabolism pathways for alpacas is available at present. However, because expression of hepatic cytochrome P450 isoenzymes is species specific and the various isoforms of P450 are substrate selective, differences in

amount and type of metabolites formed have been observed in other species in the preclinical study. For example, in humans, the primary metabolite is formed by N-oxidation of the fluoropyrimidine ring, whereas in rabbits, it is through hydroxylation (Roffey *et al*, 2003). Further evidence of species differences in the metabolism of voriconazole can be observed in the decrease in AUC values in the mouse, rat, and dog, but not in the rabbit, guinea pig, or human after multiple dosing, compared with single administration (Roffey *et al*, 2003; Purkins *et al*, 2003a)

The extent of hepatic cytochrome P450 isoenzyme activities can also differ within species. Voriconazole is extensively metabolized in all animal species investigated in the pre-clinical study and in humans (Roffey *et al*, 2003). Metabolism of voriconazole in humans is primarily by the hepatic enzymes CYP2C9, CYP2C19, and CYP3A4, and up to twice the $t_{1/2\beta}$ has been observed in those exhibiting the cytochrome P450 enzyme CYP2C19 polymorphism, compared to extensive metabolizers (Hyland *et al*, 2003; Theuretzbacher *et al*, 2006). Another cause of inter-individual variability is the age of the subjects. In children under 12 years of age, clinical studies revealed that voriconazole is more rapidly cleared compared to that of adults, but the authors surmised that children have a higher elimination capacity than adults on body weight basis, and this difference between pediatric and adult patients was the most important factor accounting for the difference in clearance of voriconazole (Walsh *et al*, 2004). In the present study, although the clearance and volume of distribution values were the highest in the alpaca with the heaviest body weight, there was no trend observed regarding body weight in the pharmacokinetic parameters in the other three alpacas, despite up to two-fold difference in body weights. In addition, elderly human patients have 80 to 90% higher plasma

concentrations than those in younger patients after IV administration (Pfizer, 2006). No age-related trend in pharmacokinetic values was observed in alpacas with an age range of 0.83 to 6.5 years.

Liver function is variable from animal to animal, even within a given species (Ayers et al, 1984). The pharmacokinetics of voriconazole is affected by liver impairment with lower doses recommended in patients with severe chronic cirrhosis (Tan *et al*, 2006). Analysis of the serum biochemistry in the alpacas showed that the serum levels that assess liver and kidney functions were all within normal ranges and there were no age- or size-dependent trends among the four alpacas. It is possible that hepatic enzyme polymorphism, age, and liver functions differences all could have contributed to the inter-subject variations in plasma concentrations found in alpacas after single IV dosing of voriconazole, as observed in humans.

The mean AUC values following oral administration showed that voriconazole pharmacokinetics in the rat and human are gender-dependent, with a lower systemic exposure in male animals, but this phenomenon was not observed in dogs (Roffey *et al*, 2003; Pfizer, 2006). There was no evidence of pharmacokinetic differences due to gender in alpacas. However, only one male subject was included in the present study and its voriconazole absorption or disposition did not appear different from female subjects.

The pharmacokinetics of voriconazole given by the oral route was also investigated in alpacas. Several drugs are commonly prescribed and administered orally to Camelids to alleviate the need for repetitive parenteral administration to sick animals and to improve owner compliance (Chakwenya *et al*, 2002). Consequently, the identification of safe and efficacious antifungals, which can be administered orally, is

highly desirable. In the present study, a powder formulation of the drug was mixed with corn syrup and administered orally. Following the oral dose, voriconazole appeared rapidly in the plasma with drug concentrations detected at 5 minutes postdose in all four alpacas. The mean half-life value (8.748 ± 4.307 hours) in alpacas after single oral dosing of voriconazole was similar to that after IV dosing. This half-life is slightly longer than that observed in humans (6.0 hours) but shorter than that in horses (13.11 hours) (Purkins *et al*, 2003b; Davis *et al*, 2006). The C_{max} values observed in the present study varied widely from 0.280 to 5.761 μg/mL, with one alpaca exhibiting considerably higher C_{max} concentration than observed in the other three subjects.

The mean T_{max} value after single oral administration in alpacas was 5.367 ± 3.364 hours, which is longer than that in mice (2 hours), female rats (1 hour), rabbits (1 hour), dogs (3 hours), humans (1 hour), and horses (2.92 hours), but shorter than that in male rats (6 hours) and guinea pigs (8 hours) (Roffey *et al*, 2003; Purkins *et al*, 2003b). Although voriconazole appeared rapidly in the plasma, the absorption rate seemed slow with a peak plasma concentration at nearly 5.4 hours. The extent of absorption, F, is much lower in alpacas (22.74 ± 9.48%) than in other species investigated so far (75 to 159%) (Roffey *et al*, 2003; Pfizer, 2006; Davis *et al*, 2006). This phenomenon has been observed in ruminants with other antimicrobials. Certain drugs that showed high systemic availability after enteral administration to monogastric animals are poorly absorbed in ruminants due to inactivation or dilution of the drug in the rumen (Abdennebi *et al*, 1994; Christensen *et al*, 1996). This could also explain the low extent of absorption observed in alpacas after oral dosing of voriconazole compared to prior studies in non-ruminants.

In addition, as in other species, large inter-individual variation in pharmacokinetic parameters was observed in alpacas after oral administration. Wide variability was evident in the range and the coefficient of variation of MRT_{abs}, Cl/F, and Vd_{area}/F values. Highly variable plasma concentrations and pharmacokinetic parameters can be expected when drugs have low extents of oral absorption as seen in this case of voriconazole in alpacas. The variations are especially evident in drugs that are highly cleared by the liver before entering the general circulation, due to differences in inter-subject hepatic enzyme activities. This phenomenon could also substantially decrease systemic availability of drugs that undergo extensive hepatic metabolism. Estimating the hepatic extraction ratio using the alpaca hematocrit level of 35.5%, hepatic blood flow rate of 55 ml/min/kg as obtained from the sheep, another ruminant of similar body weight, because the hepatic blood flow rate of alpacas is unavailable, and assuming nearly complete elimination by metabolism, the average first-pass loss would be only 5.14%, which is low (Reynafarje *et al*, 1968; Katz and Bergman, 1969). However, the first-pass effect has not been established with voriconazole in other species because of the high extent of absorption values obtained after oral administration.

There are several other possible reasons for the low extent of absorption and substantial inter-individual variations. In one study with a small ruminant species, the sheep, investigators found that crushed tablets of enrofloxacin, another antimicrobial, fed free choice with grain results in superior systemic drug absorption, compared with absorption of tablets crushed and force-fed with water as a drench (Bermingham and Papich, 2002). They theorized that dilution of the drug in the liquid fraction of the rumen may be a possible factor in the poorer absorption following drench administration. In the

present study, the powder formulation of voriconazole was mixed and force-fed with corn syrup, but no water was added. However, is it possible that the corn syrup contributed to the lower extent of absorption observed in alpacas, through voriconazole binding to the components of corn syrup and thus decreasing free drug for absorption. Although the same carrier substance has also been used in the voriconazole study with horses by Davis *et al* (2006) and by our research group with high extents of oral voriconazole absorption, horses are considered to be a modified monogastric species, whereas alpacas are classified as pseudo-ruminants (Davis *et al*, 2006).

Another factor that might have contributed to the inter-species and inter-subject differences in the extent of absorption values observed could be due to drug binding to food materials in the gastrointestinal tract. In this study, the animals were not fasted prior to drug administration to better simulate how voriconazole would be administered in practice, whereas in a study by Davis *et al* (2006) with horses, voriconazole was administered orally with 12 hours of fasting prior to dosing. Under field conditions, alpacas often have access to feed at all times (Chakwenya *et al*, 2002). Drugs binding to hay in horses and to ruminal contents in alpacas could subsequently influence systemic absorption and has been observed by investigators (Maitho *et al*, 1986; Konigsson *et al*, 2003). In humans, the systemic availability of voriconazole was reduced by 22% when taken with food, compared to fasting (Purkins *et al*, 2003c).

In the present study, IV and oral doses (4 mg/kg) and thus plasma concentrations were over a similar range, permitting a better estimate of bioavailability. Dosage difference of several fold between IV and oral dosage as given in previous investigations with the mouse (10 and 30 mg/kg, respectively), rat (10 and 30 mg/kg, respectively),

rabbit (3 and 10 mg/kg, respectively), dog (3 and 6 mg/kg, respectively), and horse (1 and 4 mg/kg, respectively) reported extent of absorption values ranging from 81 to 159% (Roffey *et al*, 2003, Davis *et al,* 2006). In one study where the same IV and oral doses (10 mg/kg) were given to guinea pigs, the extent of absorption was 75% and this value was the lowest among the five species investigated in that study (Roffey *et al*, 2003). When pharmacokinetic parameters are obtained from non-equal oral and IV doses, an inaccurate overestimation of the drug disposition may occur, especially for drugs such as voriconazole that may display nonlinear elimination characteristics.

To better understand the apparent complex oral absorption of voriconazole in alpacas, percent remaining to be absorbed calculations using the microrate constants obtained from the two-compartmental analysis were used. Evaluation of the percent remaining to be absorbed plots showed multi-phasic absorption process in all four alpacas (Figure 3.10). The percent remain to be absorbed plots showed multiple absorption phases or periods, suggesting complex absorption processes. As with phenobarbital, it appears that there was an initial rapidly absorbed period for voriconazole from the gastrointestinal tract of alpacas and then absorption proceeded at a slower rate. It is possible that this drug is rapidly absorbed in the stomach and more slowly absorbed in the intestine (Ravis *et al*, 1987). Observed multiple plasma concentration peaks can be an effect of delayed absorption of a portion of the dose due to slow release of drug from ruminal contents after initial drug binding to hay in the stomach (Konigsson *et al*, 2003). Recycling of drug that back partitions into the GI fluids could also explain secondary plasma concentration peaks. Secondary peaks after oral administration can also indicate enterohepatic circulation with reabsorption of the drug after hepatic metabolism (Gibson

& Skett, 1986). However, because of the low extent of absorption, it was difficult to analyze whether or not there was first order absorption from the percent remaining to be absorbed plots.

From the data determined in this study, it appears that administration of a single IV dose of 4 mg/kg to alpacas resulted in plasma levels of voriconazole that exceeded the MIC values against important fungal pathogens reported in humans. Furthermore, voriconazole concentrations in plasma remained above 0.1 μg/mL over the 24-hour period postdose. Because of the long half-life values of voriconazole in alpacas, once daily dosing could be considered . Assuming that MIC values of voriconazole against various fungi is the same or similar in alpacas as in humans, an average target concentration of 1.5 to 2.5 μg/mL, as reported in humans, could also be used to calculate recommended dosing regimen in alpacas. Based on the average Cl values and the equation:

$$\text{Dose} = \text{Cl} \times \text{desired concentration} \times \text{dosing interval}$$

An IV dosing regimen of 3.9 to 6.6 mg/kg of voriconazole every 24 hours to mature, healthy alpacas seems appropriate. Based on a half-life value of 8 hours and once daily dosing, no loading dose may be needed but could be considered with once daily dosing. Because pharmacokinetics of voriconazole is known to differ between single and chronic dosing in other species, additional studies will be needed to determine the safety and possible changes in pharmacokinetic parameters of voriconazole for use in the long term treatment of alpacas.

The oral dosage of 4 mg/kg used in the present study reached C_{max} values that are above the MIC values for some fungal species. However, due to the low oral extent of

absorption in alpacas, higher dosages might be needed depending on the susceptibility profile of the fungal strain involved. Oral maintenance dose can be calculated as equal to the product of desired concentration, Cl/F, and dosing interval. For oral dosing using similar administration procedures with the powder, a regimen of 20.4 to 33.9 mg/kg/day appears appropriate.

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TABLE 3.1 - Demographics of study subjects.

		Alpaca			
		A	B	C	D
Age	(years)	2	0.83	4	6.5
Sex		M	F	F	F
Weight	(kg)	62.7	25.5	59.4	50.5

M = male; F = female.

TABLE 3.2 - Noncompartmental pharmacokinetic parameters of voriconazole given intravenously (4 mg/kg) to alpacas.

		Alpaca						
		A	B	C	D	Mean	SD	CV %
Infusion Time	(hr)	0.15	0.05	0.10	0.07	NA	NA	NA
C_{max}	(µg/mL)	5.545	4.505	6.709	6.960	5.930	1.132	19.10
AUC_{0-24}	(µg-hr/mL)	28.58	33.04	41.09	32.69	33.85	5.23	15.46
AUC_{0-inf}	(µg-hr/mL)	30.86	34.09	55.02	34.05	38.50	11.11	28.86
AUC_{0-inf}/Dose	(kg-hr/L)	7.715	8.522	13.755	8.513	9.626	2.778	28.86
K_{el}	(hr^{-1})	0.09950	0.11840	0.05660	0.09950	0.09350	0.02616	27.98
$t_{1/2\beta}$	(hr)	6.966	5.855	12.257	6.966	8.011	2.879	35.94
MRT_{IV}	(hr)	10.17	9.76	17.74	8.56	11.56	4.18	36.16
Cl	(L/hr/kg)	0.1296	0.1173	0.0727	0.1175	0.1093	0.0251	22.93
Vd_{ss}	(L/kg)	1.318	1.145	1.290	1.006	1.190	0.144	12.11
Vd_{area}	(L/kg)	1.303	0.991	1.286	1.181	1.190	0.143	12.03

C_{max} = peak plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours; AUC_{0-LT} = area under the plasma concentration-time curve from time zero to the last observed time; AUC_{0-inf} = area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC_{0-inf}/Dose = area under the plasma concentration-time curve from time zero extrapolated to infinity/dose; K_{el} = elimination rate constant; $t_{1/2\beta}$ = elimination half-life; MRT_{IV} = mean residence time following intravenous administration; Cl = total body clearance; Vd_{ss} = volume of distribution at steady state; Vd_{area} = volume of distribution as calculated by the area under the plasma concentration-time curve method; NA = not applicable; SD = standard deviation; CV% = percent coefficient of variation.

TABLE 3.3 - Two-compartmental pharmacokinetic parameters of voriconazole given intravenously (4 mg/kg) to alpacas.

		Alpaca						
		A	B	C	D	Mean	SD	CV %
Dose	(mg/kg)	4	4	4	4	4	NA	NA
Infusion Time	(hr)	0.15	0.05	0.10	0.07	NA	NA	NA
C_{max}	(µg/mL)	5.545	4.505	6.709	6.960	5.930	1.132	19.10
$AUC_{0\text{-}inf}$	(µg-hr/mL)	32.31	33.95	44.03	34.88	36.29	5.27	14.51
$AUC_{0\text{-}inf}$/Dose	(kg-hr/L)	8.078	8.488	11.007	8.720	9.073	1.316	14.51
α	(hr^{-1})	3.359	4.688	4.466	4.080	4.148	0.583	14.05
$t_{1/2\alpha}$	(hr)	0.2063	0.1478	0.1552	0.1698	0.1698	0.0260	15.32
β	(hr^{-1})	0.08900	0.08727	0.07928	0.10096	0.08913	0.00895	10.04
$t_{1/2\beta}$	(hr)	7.787	7.941	8.741	6.864	7.833	0.770	9.82
MRT_{IV}	(hr)	10.83	11.22	12.29	9.54	10.97	1.14	10.37
Cl	(L/hr/kg)	0.1238	0.1178	0.0909	0.1147	0.1118	0.0145	12.93
Cl_{12}	(L/hr/kg)	1.070	1.517	1.184	1.043	1.204	0.218	18.10
V_c	(L/kg)	0.5906	0.6433	0.4693	0.4534	0.5391	0.0926	17.18
Vd_{area}	(L/kg)	1.391	1.350	1.146	1.136	1.256	0.134	10.65
Vd_{ss}	(L/kg)	1.341	1.323	1.117	1.094	1.218	0.131	10.79
Vd_{ext}	(L/kg)	1.444	1.379	1.177	1.181	1.295	0.137	10.58

C_{max} = peak plasma concentration; $AUC_{0\text{-}inf}$ = area under the plasma concentration-time curve; $AUC_{0\text{-}inf}$/Dose = area under the plasma concentration-time curve/dose; α = slope of the distribution phase; t1/2α = half-life of distribution; β = slope of the elimination phase; $t_{1/2\beta}$ = half-life of elimination; MRT_{IV} = mean residence time following intravenous administration; Cl = total body clearance; Cl_{12} = distribution clearance; V_c = volume of distribution of the central compartment; Vd_{area} = volume of distribution as calculated by the area under the plasma concentration-time curve method; Vd_{ss} = volume of distribution at steady state; Vd_{ext} = extrapolated volume of distribution; NA = not applicable; SD = standard deviation; CV% = percent coefficient of variation.

TABLE 3.4 - Noncompartmental pharmacokinetic parameters of voriconazole given orally (4 mg/kg) to alpacas.

		Alpaca						
		A	B	C	D	Mean	SD	CV %
C_{max}	(µg/mL)	0.370	0.391	5.761	0.280	1.700	2.707	159.23
T_{max}	(hr)	5.867	3.867	1.933	9.800	5.367	3.364	62.68
AUC_{0-24}	(µg-hr/mL)	4.743	4.071	17.621	4.814	7.812	6.548	83.82
AUC_{0-LT}	(µg-hr/mL)	5.065	4.372	18.857	6.752	8.762	6.804	77.66
AUC_{0-inf}	(µg-hr/mL)	5.786	4.751	19.810	7.588	9.484	6.983	73.63
AUC_{0-inf}/Dose	(kg-hr/L)	1.447	1.188	4.952	1.897	2.371	1.746	73.63
F	(%)	18.75	13.94	36.00	22.28	22.74	9.48	41.68
K_{el}	(hr^{-1})	0.0698	0.0634	0.0590	0.2908	0.1208	0.1135	93.96
$t_{1/2\beta}$	(hr)	9.927	10.941	11.739	2.384	8.748	4.307	49.23
MRT_{oral}	(hr)	14.57	13.30	13.17	19.63	15.17	3.04	20.05
MRT_{abs}	(hr)	4.403	3.540	-4.570	11.068	3.610	6.407	177.48
Cl/F	(L/hr/kg)	0.6913	0.8420	0.2019	0.5272	0.5656	0.2744	48.52
Vd_{area}/F	(L/kg)	9.900	13.291	3.420	1.813	7.106	5.406	76.07

C_{max} = peak plasma concentration; T_{max} = time of peak plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours; AUC_{0-LT} = area under the plasma concentration-time curve from time zero to last observed time; AUC_{0-inf} = area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC_{0-inf}/Dose = area under the plasma concentration-time curve from time zero extrapolated to infinity/dose; F = extent of absorption; K_{el} = elimination rate constant; $t_{1/2\beta}$ = elimination half-life; MRT_{oral} = mean residence time following oral administration; MRT_{abs} = mean residence time of absorption; Cl/F = total body clearance/extent of absorption; Vd_{area}/F = volume of distribution as calculated by the area under the plasma concentration-time curve method/extent of absorption; SD = standard deviation; CV% = percent coefficient of variation.

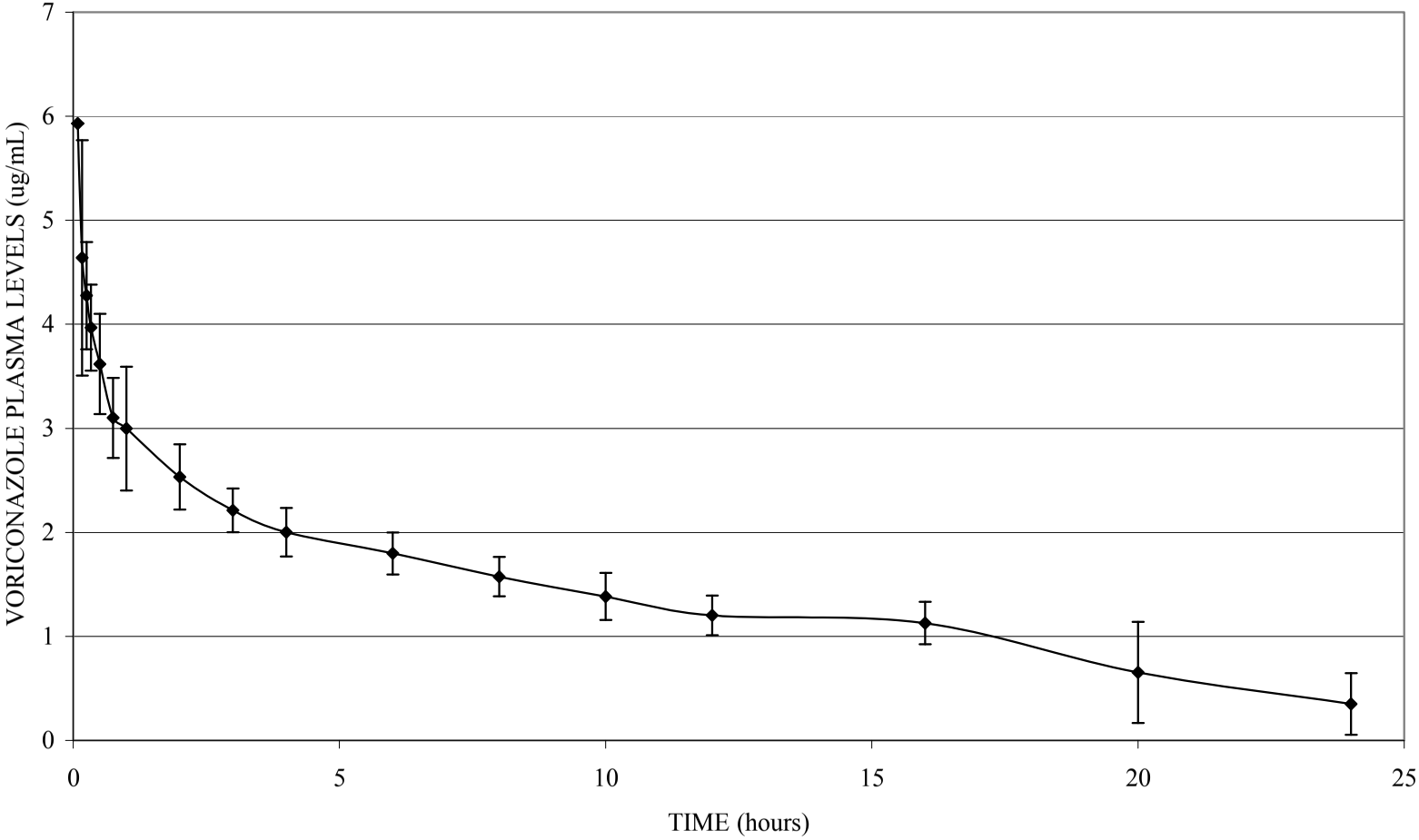

FIGURE 3.1. Mean plasma concentrations of voriconazole in alpacas after single intravenous administration at the dose of 4 mg/kg to four alpacas on linear coordinates (bars indicate standard deviations).

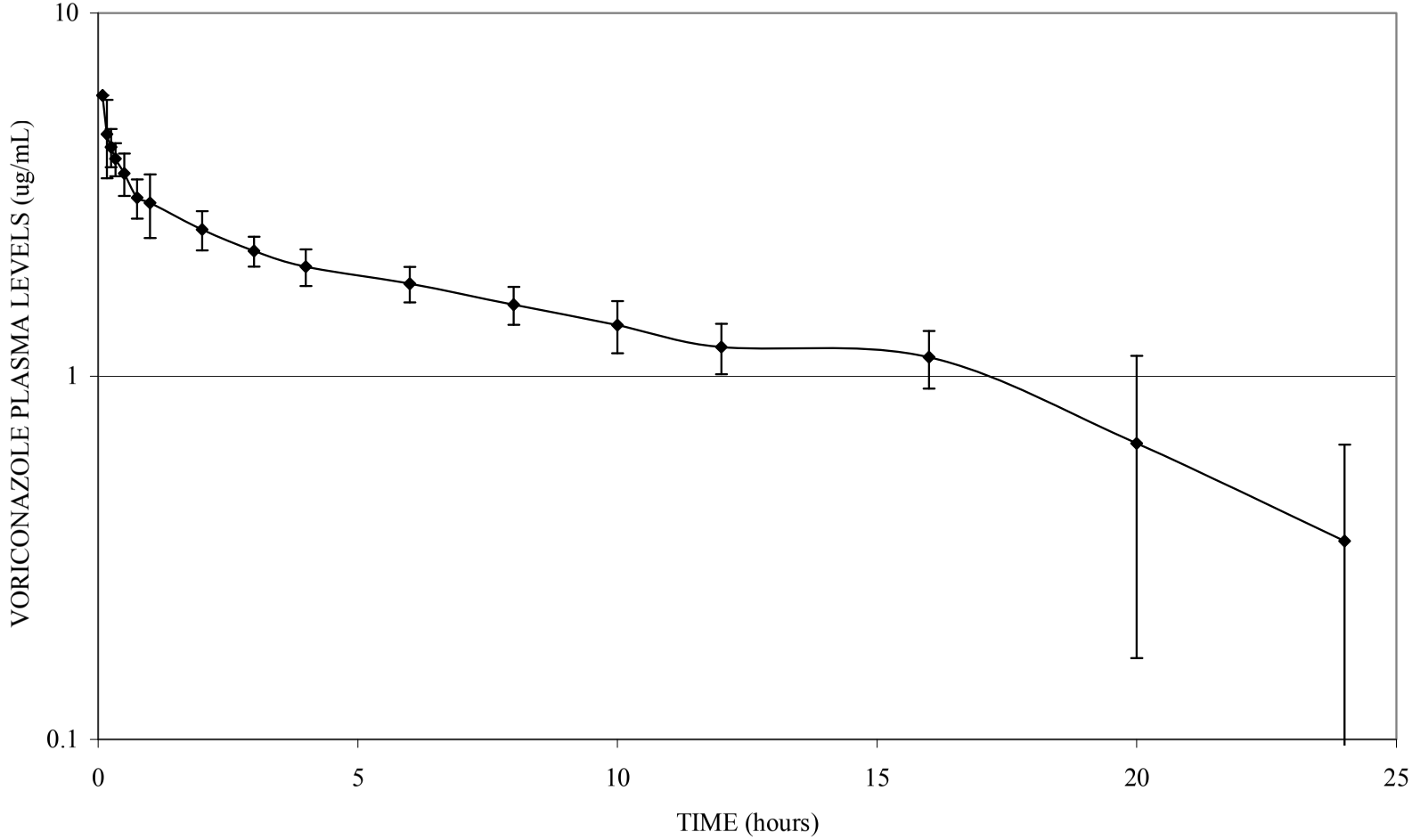

FIGURE 3.2. Mean plasma concentrations of voriconazole in alpacas after single intravenous administration at the dose of 4 mg/kg to four alpacas on log-linear coordinates (bars indicate standard deviations).

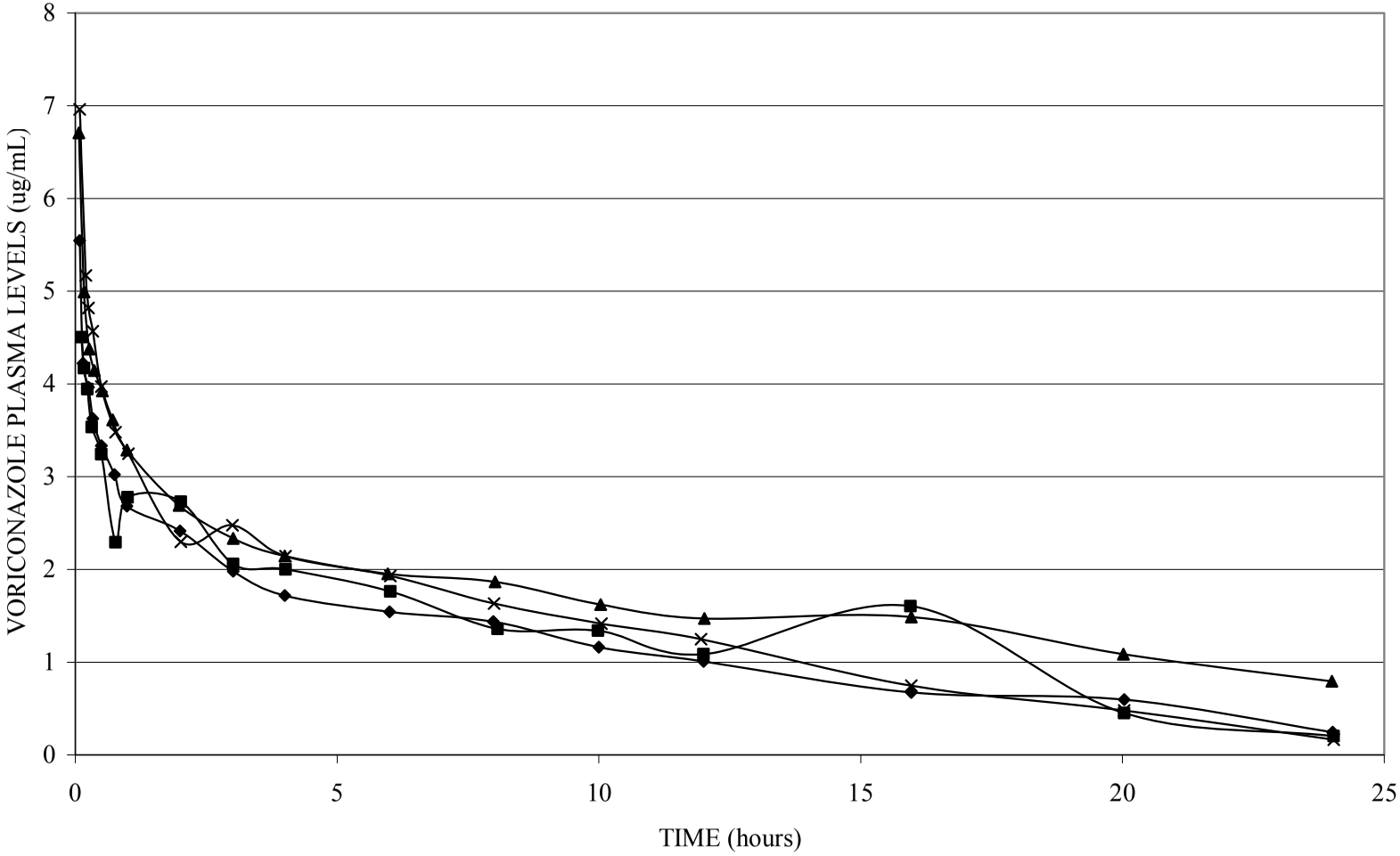

FIGURE 3.3. Plasma concentrations of voriconazole in alpacas after a single intravenous administration at a dose of 4 mg/kg to Alpaca A (♦), Alpaca B (■), Alpaca C (▲), and Alpaca D (×) on linear coordinates.

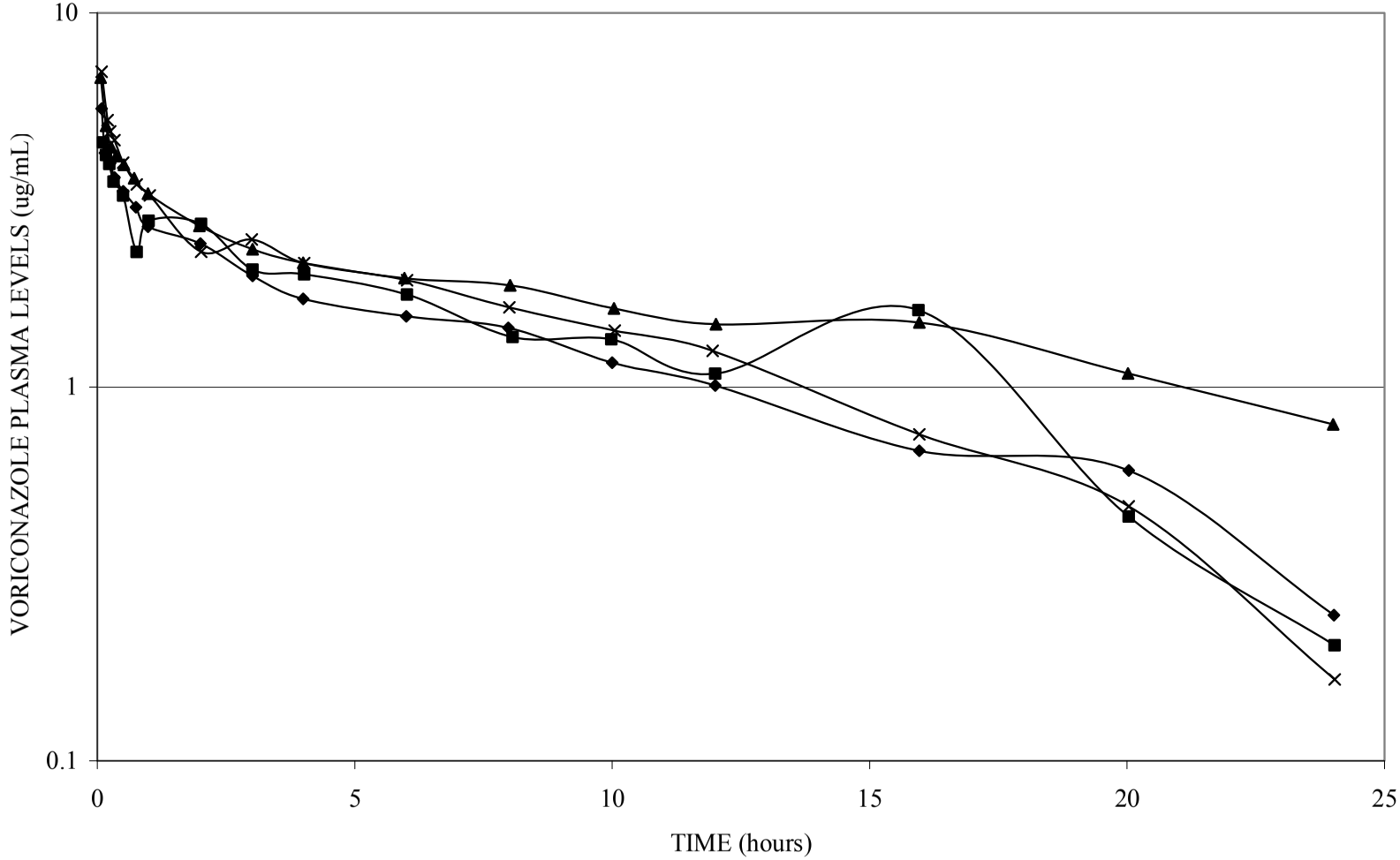

FIGURE 3.4. Plasma concentrations of voriconazole in alpacas after a single intravenous administration at a dose of 4 mg/kg to Alpaca A (♦), Alpaca B (■), Alpaca C (▲), and Alpaca D (×) on log-linear coordinates.

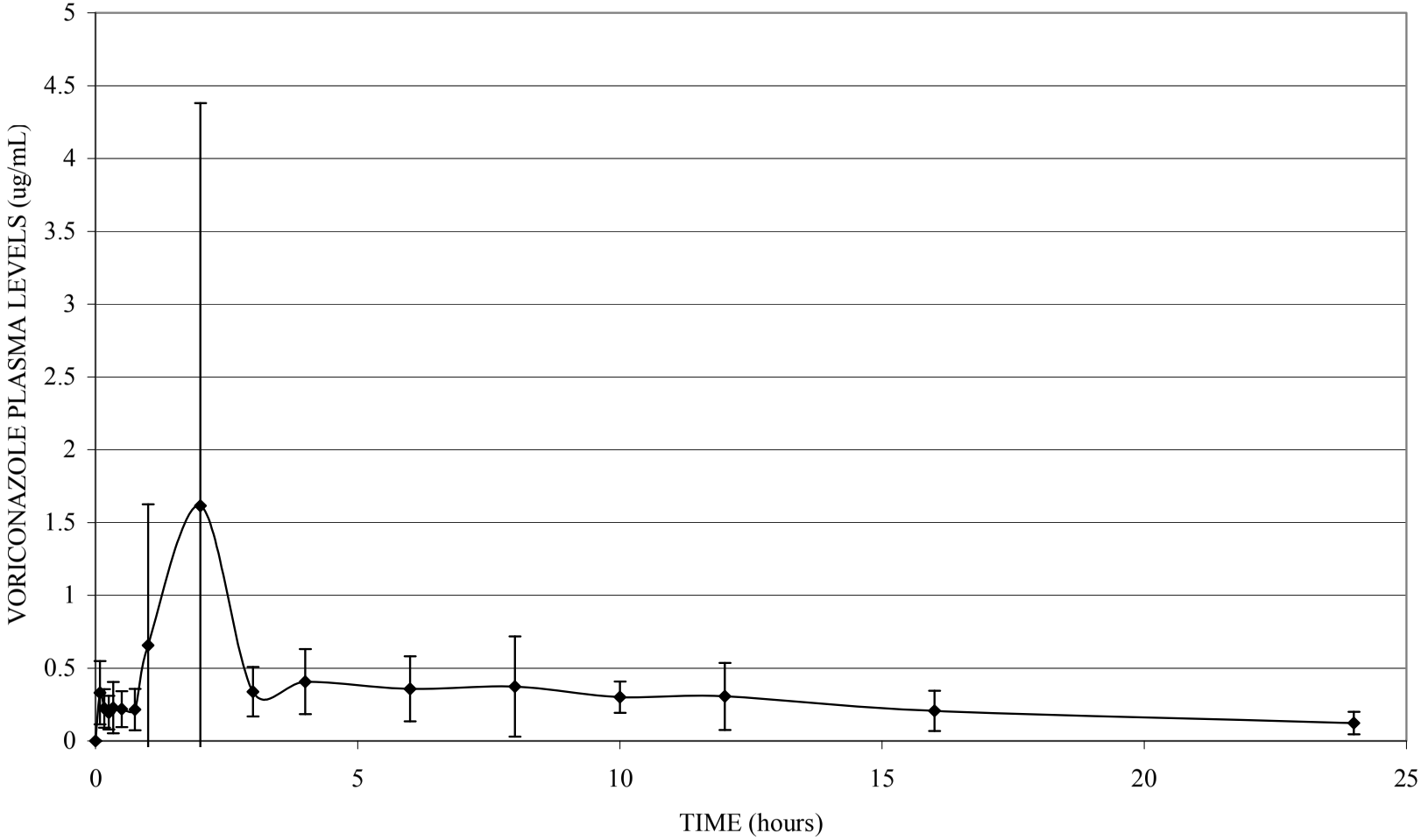

FIGURE 3.5. Mean plasma concentrations of voriconazole in alpacas after a single oral administration at the dose of 4 mg/kg with powder to four alpacas on linear coordinates (bars indicate standard deviations).

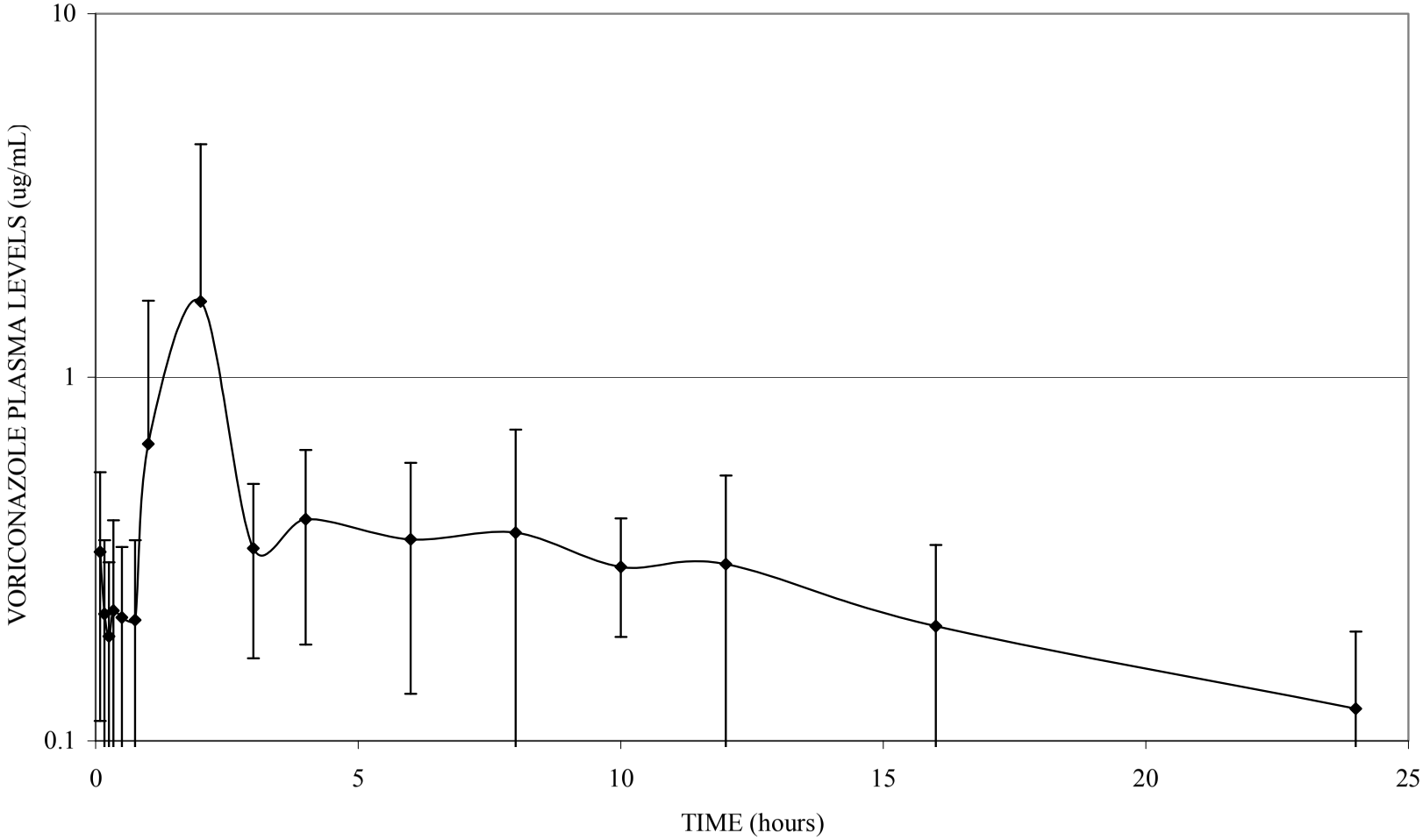

FIGURE 3.6. Mean plasma concentrations of voriconazole in alpacas after a single oral administration at the dose of 4 mg/kg with powder to four alpacas on log-linear coordinates (bars indicate standard deviations).

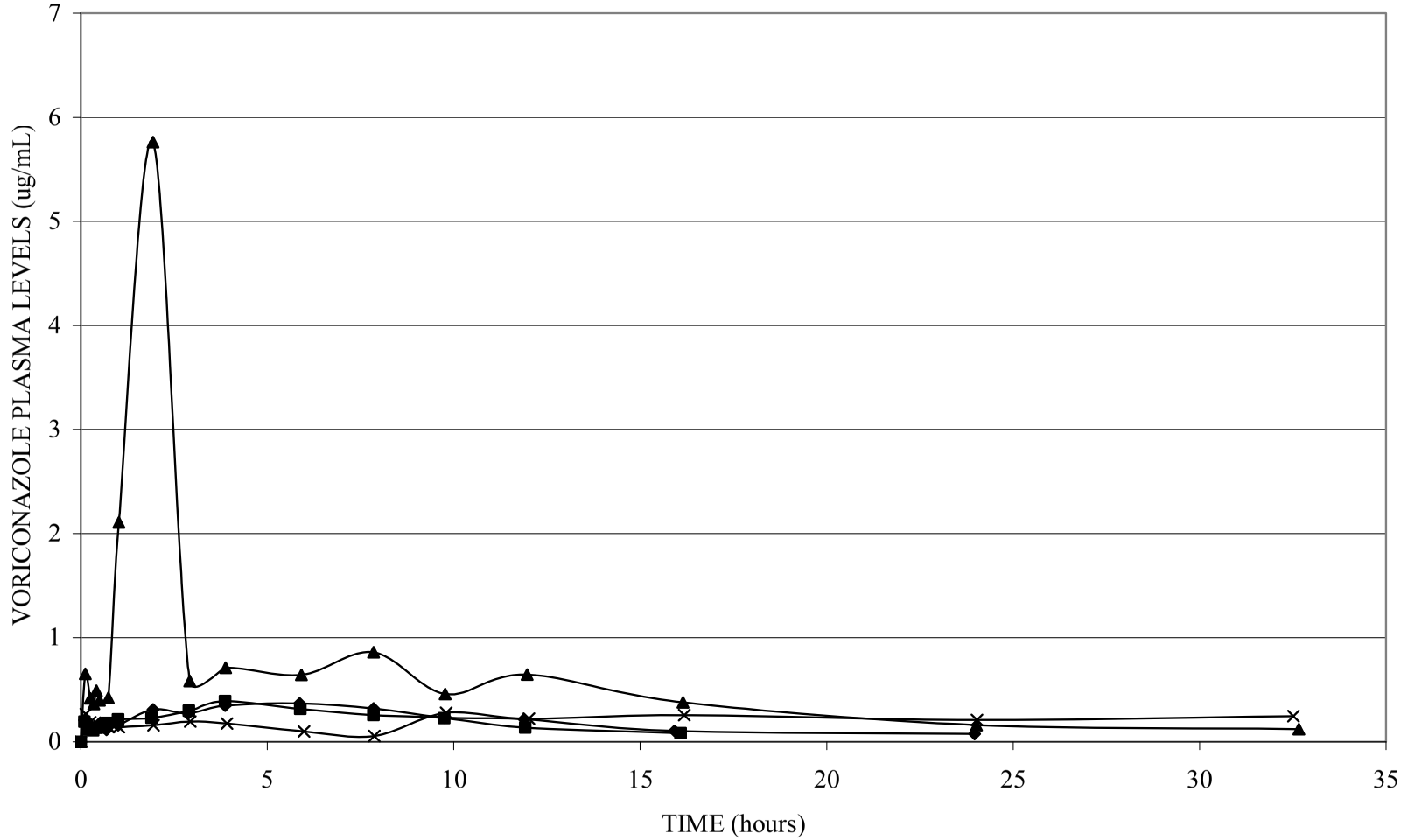

FIGURE 3.7. Plasma concentrations of voriconazole in alpacas after a single oral administration at a dose of 4 mg/kg with powder to Alpaca A (♦), Alpaca B (■), Alpaca C (▲), and Alpaca D (×) on linear coordinates.

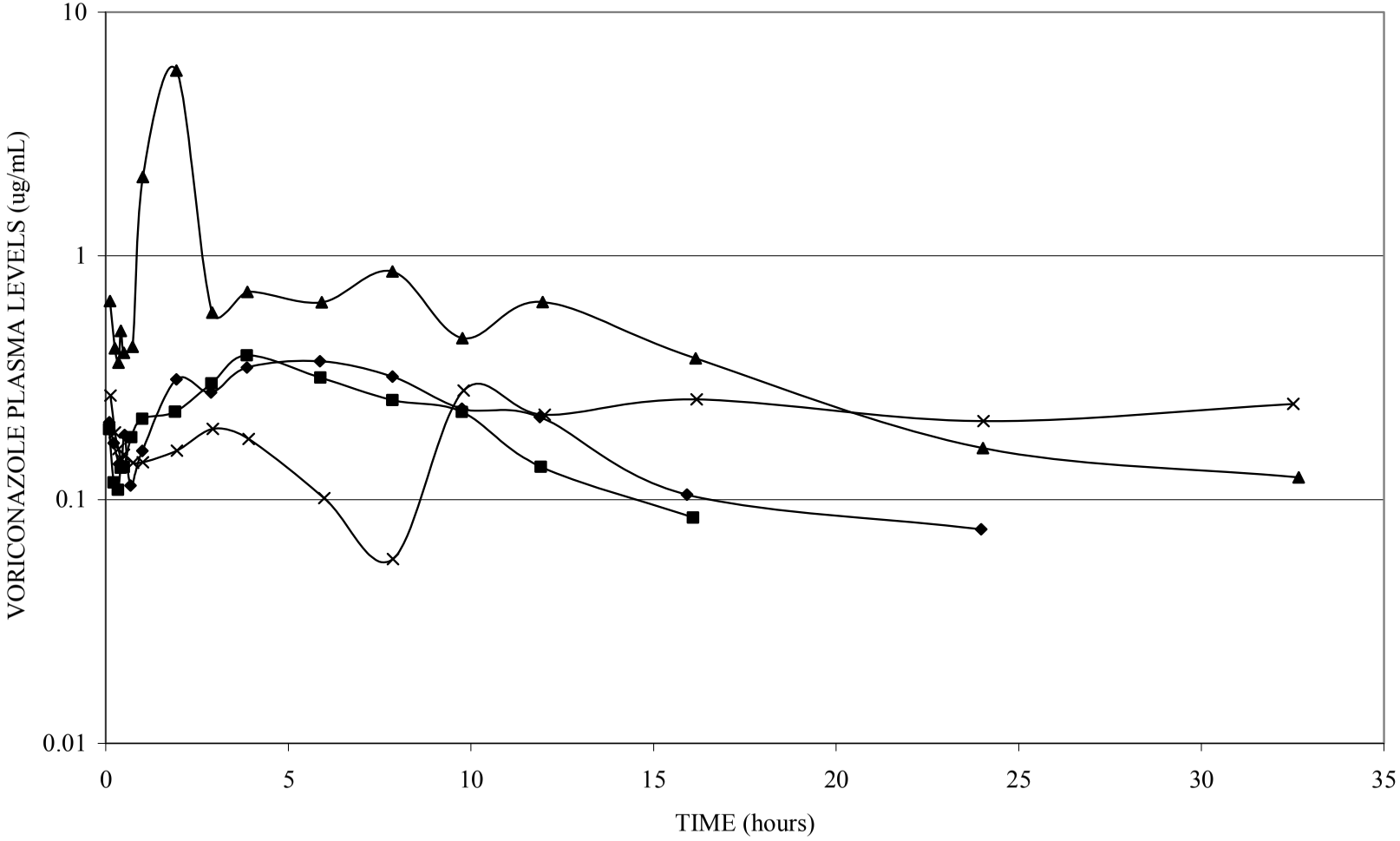

FIGURE 3.8. Plasma concentrations of voriconazole in alpacas after a single oral administration at a dose of 4 mg/kg with powder to Alpaca A (♦), Alpaca B (■), Alpaca C (▲), and Alpaca D (×) on log-linear coordinates.

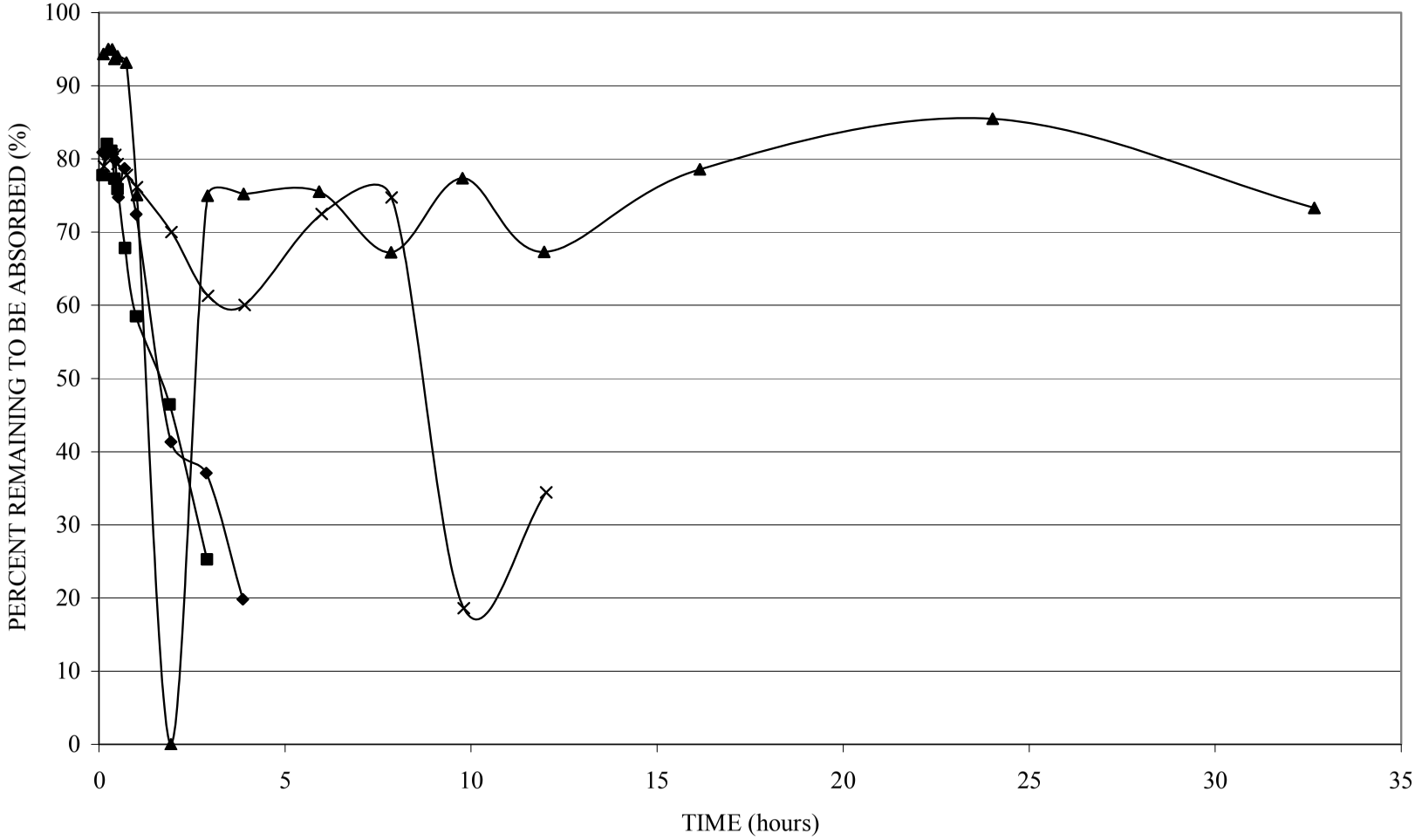

FIGURE 3.9. Percentage of available dose remaining to be absorbed following oral administration of voriconazole at a dose of 4 mg/kg with powder to Alpaca A (♦), Alpaca B (■), Alpaca C (▲), and Alpaca D (×) on linear coordinates.

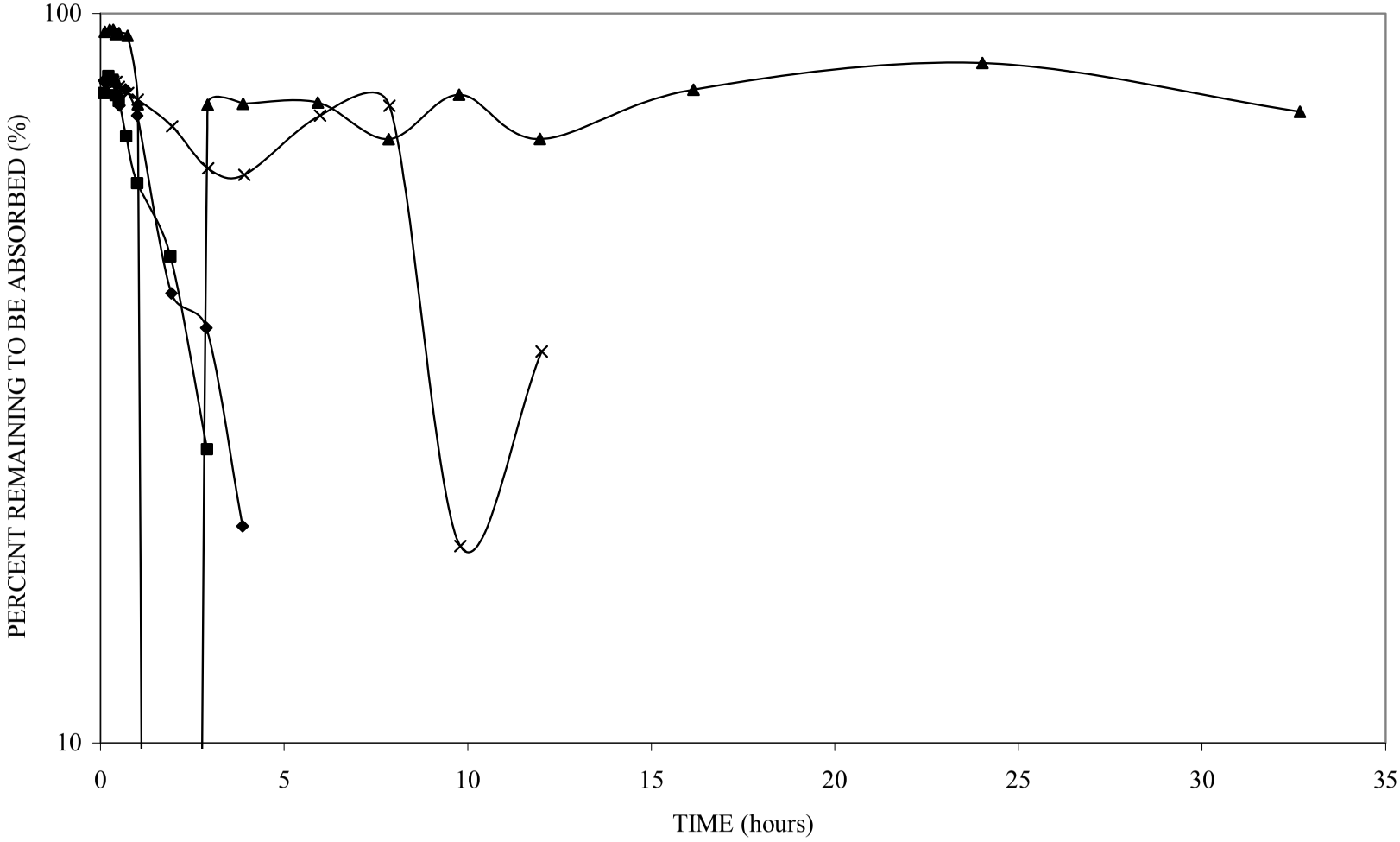

FIGURE 3.10. Percentage of available dose remaining to be absorbed following oral administration of voriconazole at a dose of 4 mg/kg with powder to Alpaca A (♦), Alpaca B (■), Alpaca C (▲), and Alpaca D (×) on log-linear coordinates.

4. PHARMACOKINETICS OF VORICONAZOLE AFTER SINGLE INTRAVENOUS AND ORAL (TABLET AND POWDER FORMULATIONS) ADMINISTRATION TO HORSES

Abstract

Voriconazole is a new antifungal drug that has shown effectiveness in treating serious fungal infections and has the potential for being used in large animal veterinary medicine. The objective of this study was to determine the plasma concentrations and pharmacokinetic parameters of voriconazole after single-dose intravenous (IV) and oral administration to horses. Two horses were used for the treatment of 2 mg/kg single dose intravenous and 3 mg/kg oral administration of crushed tablet formulation of voriconazole, and four horses were used for the treatment of 4 mg/kg single dose intravenous and oral administration of powder formulation of voriconazole. Plasma voriconazole concentrations were measured by the use of high-performance liquid chromatography. The mean elimination half-life ($t_{1/2\beta}$) following IV, oral crushed tablet, and oral powder administration were 12.22 ± 3.54, 9.482 ± 0.899, and 15.28 ± 3.50 hours, respectively; mean maximum plasma concentration (C_{max}) were 4.355 ± 1.850, 0.7515 ± 0.1195, and 1.6910 ± 0.3240 μg/mL, respectively; mean dose-adjusted area under plasma concentration-time curve from time zero extrapolated to infinity (AUC_{0-inf}/Dose) were 9.976 ± 2.069, 5.924 ± 1.251, and 10.059 ± 2.229 kg-hr/L, respectively. The mean apparent extent of absorption, F, for the crushed tablet and the powder

formulations were 57.88 ± 8.62% and 103.07 ± 9.15%, respectively. The IV and oral administration of a single dose of voriconazole yielded ratios of $AUC_{0\text{-}inf}$ to minimum inhibitory concentration (MIC) >25 for many fungal pathogens common to horses. Based on linear pharmacokinetic predictions, the administration of 2 to 4 mg/kg IV or 3 to 4 mg/kg oral voriconazole once daily may be appropriate for antifungal treatment of horses.

Introduction

There has been a steady increase in frequency and severity of fungal infections in human medicine in recent years, and the species involved in these infections are becoming more diverse (Kauffman, 2006). Although the exact incidence of fungal infections in veterinary medicine has not been established, it is believed that the trend has paralleled the increase seen in human medicine (Wiebe *et al*, 2005). Fungal infections in horses include localized infections of the dermis, eyes, central nervous system (CNS), guttural pouches, respiratory system, and reproductive tract, as well as disseminated systemic infections involving multiple organs and body cavities (Davidson, 1991; Steckel *et al*, 1982; Rawlinson and Jones, 1978; Sweeney, 1997; Swerczek and Donahue, 1990; Rochette *et al*, 2003). Some of the most commonly reported fungi species found in infected horses include *Aspergillus*, *Fusarium*, *Paecilomyces*, *Acremonium*, *Histoplasma*, *Rhinosporidium*, *Cryptococcus*, *Penicillium*, *Alternaria*, *Cladosporium*, and *Candida* (Blomme *et al*, 1998; Plumlee and Galey, 1994; Foley *et al*, 2002; Latch, 1985; Ajello, 1998; Wallin *et al*, 2001; Steckel *et al*, 1982; Samuelson *et al*, 1984; Brooks, 1999). One of the most frequent clinical manifestations of fungal infections in horses is keratitis, and

Aspergillus species are the most commonly isolated organisms from horse eyes (Brooks *et al*, 1998).

The effectiveness of drug therapy for the treatment of fungal infections is dependent on the susceptibility of fungal isolates to the drug, bioavailability of the antifungal agent, ability of the drug to penetrate the affected tissues to achieve sufficient concentrations, and potential toxicity and drug interactions (Moore *et al*, 1995; Thomas, 2003). In addition, a long course of therapy of several weeks to months is often required for the treatment of fungal infections.

Successful antifungal therapy for affected horses is limited to only a few drugs (Latimer *et al*, 2001). Amphotericin B was the first available drug for the treatment of systemic fungal infections (Brook, 1982). The disadvantages associated with the use of this drug include required intravenous (IV) administration and resultant phlebitis, poor perfusion into the cerebralspinal fluid (CSF), and renal toxicity with prolonged administration (Speller, 1979). Griseofulvin is the most commonly used systemic antifungal in horses (Rochette *et al*, 2003). However, its spectrum of activity is limited to *Trichophyton*, *Epidermophyton*, and *Microsporum* species (Vanden Bossche *et al*, 2003). Of the azoles, the most commonly used is miconazole. Miconazole is active against *Aspergillus* species, but poor levels are observed in the CSF (Hamor and Whelan, 1999; Vanden Bossche *et al*, 2003). Ketoconazole is moderately effective against *Aspergillus* species, but considerably less effective against *Fusarium* species, and it is not absorbed well from the digestive tract of horses (Prades, 1989; Brooks *et al*, 1998). Although fluconazole has excellent absorption and tissue distribution after oral administration in horses, it is intrinsically resistant to certain *Aspergillus* species (Brooks *et al*, 1998).

Another azole, itraconazole, has substantial *in vitro* activity against *Aspergillus* and many other filamentous fungi, but it was found to have variable absorption with the capsule formulation, relatively low extent of absorption (65%) with the improved solution formulation as well as poor penetration into the aqueous humor of horses and inconsistent plasma concentrations for the treatment of fusariosis (Wong-Beringer and Kriengkauykiat, 2003; Davis *et al*, 2005).

A few new antifungal drugs were recently approved for human use and could potentially provide effective treatments for horses (Vanden Bossche *et al*, 2003). Voriconazole was approved by the Food and Drug Administration (FDA) in 2002 for human use. Among azoles, voriconazole has the broadest antifungal spectrum (Kulemann *et al*, 2005). It is indicated for the treatment of invasive aspergillosis, candidaemia in non-neutropenic patients, disseminated infections caused by *Candida* species, and in patients with scedosporiosis and fusariosis who are refractory to or intolerant of other antifungal therapy (Pfizer, 2006).

The pharmacokinetics of voriconazole has been evaluated in several species. In a preclinical study following a single IV administration, voriconazole pharmacokinetics was characterized by non-linear elimination (Roffey *et al*, 2003). Using a one-compartment model analysis with elimination based on the Michaelis-Menten equation, the volume of distribution (Vd) was calculated to be 2.1 and 1.3 L/kg in male rat and in dog, respectively. After oral administration in this study, voriconazole was absorbed with a time at observed peak plasma concentration (T_{max}) between 1 and 8 hours, and high extents of absorption in all species investigated: mouse, 81%; male rat, 159%; female rat, 88%; guinea pig, 75%; rabbit, 87%; and dog, 138%. There was greater than

proportional increases in area under the plasma concentration-time curve (AUC) with increasing dose following single oral administration. The authors believed that since voriconazole is eliminated predominantly by metabolism in the species investigated, it is likely that the saturation of metabolic clearance is the cause of dose-dependency, and the nonlinearity of elimination following intravenous administration further supports this theory. They further stated that the apparent bioavailability values of >100% obtained by comparison of dose-normalized AUC values reflect the capacity-limited pharmacokinetic profile of voriconazole. The bioavailability of voriconazole in healthy volunteers has been determined to be 96% (Purkins *et al*, 2002). In addition, autoinduction of voriconazole metabolism is observed in some species, but not in human, rabbit, or guinea pig (Roffey *et al*, 2003). Therefore, it maybe difficult to accurately predict the pharmacokinetics of voriconazole across species and during multiple dosing regimens.

Equine pharmacokinetic data would be helpful to guide the treatment of horses with fungal infections (Drusano, 1988). Pharmacokinetic and therapeutic data of antifungal agents for horses are limited. Ocular fluid disposition after repeated topical dosing and pharmacokinetics after single intravenous and oral administrations of voriconazole have been characterized in horses. Following 0.2 mL topical administrations of 0.5, 1.0, and 3.0% concentrations of voriconazole solution every 4 hours for 7 doses, the mean voriconazole concentration in the aqueous humor resulting from application of the 0.5% solution was significantly less than those resulting from application of the 1.0% and 3.0% solutions, but there was no significant difference in aqueous humor concentrations resulting from application of the 1.0% versus 3.0% solutions (Clode *et al*, 2006). Voriconazole was detected at low concentrations in the

plasma of all horses after repeated topical ocular administration with all concentrations of voriconazole being less than the limit of quantification (0.005 μg/mL) 1 hour after the final dose. After a single orally administered dose, voriconazole was detected in the aqueous humor of all horses with a mean concentration of 0.86 ± 0.22 μg/mL, which is 38.8 ± 8.62% of the plasma concentration at the same time point of 2.5 hours post-dose (Clode *et al*, 2006).

In another study, a single dose of voriconazole 1 mg/kg IV infusion and 4 mg/kg of powder for oral administration were given to horses (Davis *et al*, 2006). The investigators did not observe clear evidence of nonlinear elimination after intravenous administration, and it was proposed that oral administration of voriconazole at a dosage of 4 mg/kg every 24 hours will attain plasma concentrations adequate for treatment of horses with fungal infection for which the fungi have a minimum inhibitory concentration (MIC) ≤ 1 μg/mL. However, the voriconazole intravenous dose administered was lower than in any other species that have been investigated so far, and the plasma concentrations obtained following intravenous administration were low for the susceptibility of some fungi, such as *Aspergillus* species, when compared to the reported MIC values. After single oral administration of voriconazole powder in corn syrup, the bioavailability was 135.75 ± 18.41%. The pharmacokinetics of crushed tablets, the usual form of oral delivery to equine patients, has not been characterized in horses.

The purpose of this study was to determine the pharmacokinetics of voriconazole in adult horses after a single intravenous and oral administration which displays therapeutic plasma concentrations. Pharmacokinetic parameters and information will then be applied to propose therapeutic dose regimens.

Materials and Methods

Animals

Six clinically normal adult mares were used in this study. The horses weighed between 404 and 514 kg (mean, 470 kg) and were part of The Auburn University Equine Research Program. Results of complete blood count (CBC) and serum biochemical analyses performed the day before the study were within reference values for our laboratory. All horses were vaccinated and dewormed at least 4 weeks prior to the pharmacokinetic study. Horses were maintained on pasture and acclimated to the barn and housed in individual box stalls twelve hours prior to performance of the study. Water and grass hay were available *ad libitum*. Horses were placed in stocks 30 minutes prior to dosing and returned to the stalls 2 hours after drug administration. General physical examination was performed once a day throughout the study. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of Auburn University.

Drug administration

Voriconazole was administered intravenously and then orally to each horse, separated by a minimum of a one-week washout period. For the collection of blood samples, a 16-gauge 2-inch catheter (Surflo®, Terumo Medical Corp., Somerset, NJ) was aseptically inserted into the right jugular vein and attached to a 32-inch extension with male rotating luer lock and stopcock (Arrow International Inc., Reading, PA); the extension and stopcock were secured to the horse by use of elastic tape (Elastikon®, Johnson and Johnson, New Brunswick, NJ). For intravenous (IV) drug administration, an additional catheter was placed in the left jugular vein. Lidocaine 2% (Hospira Inc., Lake Forest, IL) was used as a local anesthetic to alleviate any pain. The IV treatment

consisted of voriconazole injectable (Vfend®, Pfizer Inc., New York, NY), 2 mg/ml mixed in sterile saline solution (0.9% sodium chloride, Abbott Laboratories, Abbott Park, IL), and injected into the jugular vein, using a 73-inch primary IV set (Hospira Inc., Lake Forest, IL). The IV set was vented with a flashback bulb, via the catheter in the left jugular vein (infusion time 5-15 minutes; mean, 9.5 minutes) and this was immediately followed by 7 mL of saline solution containing 10 units/mL heparin (American Pharmaceutical Partners Inc., Schaumburg, IL). For the oral treatment, each dose of voriconazole powder (Maithri Laboratories Pvt. Ltd., Andhra Pradesh, India) or crushed tablets (Vfend®, Pfizer Inc., New York, NY) was mixed with 30 ml of corn syrup (T.J. Blackburn Syrup Works Inc. Jefferson, TX) and administered via a 60-ml syringe (Becton Dickinson, Franklin Lakes, NJ). The syringe was then rinsed with 10 mL of water to ensure adequate delivery of the drug. Horses A and B received 2 mg/kg of voriconazole via IV infusion and 3 mg/kg with crushed tablets orally. Horses C, D, E, and F received 4 mg/kg of intravenous voriconazole and 4 mg/kg from powder for oral administration.

Blood collection

Blood samples (7 mL) were taken with 10-mL syringes (Becton Dickison, Franklin Lakes, NJ) via the catheter in the right jugular vein immediately before drug administration (time 0), and after IV administration at 0.083, 0.167, 0.25, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 36 (Horses C, D, E, and F), and 48 hours, and after oral administration at 0.083, 0.167, 0.25, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hours. Blood samples were immediately placed in lithium heparin-coated tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ) and kept on ice. The catheter

inserted in the jugular vein was flushed with heparinized saline solution (7 mL) between subsequent collection of blood samples. At the time of each sample collection, 7 mL of saline solution was injected to flush the heparinized saline solution out of the extension system, 7 mL of blood was withdrawn to remove the saline solution from the extension system, and another 7 mL of blood was collected as sample. Samples were centrifuged at 1,163 × g for 10 minutes (Clay Adams Brand Compact II Centrifuge, Becton Dickinson, Franklin Lakes, NJ) within 30 minutes of collection, and plasma was removed with disposable polyethylene transfer pipets (Denville Scientific Inc., Metuchen, NJ) and stored in cryogenic storage vials (Fisher Scientific Inc., Waltham, MA) at -80ºC until assay.

Drug analysis

The plasma samples were assayed using a modified version of a previously published liquid-liquid extraction method followed by reverse-phase high-performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detection (Roffey et. al., 2003). The HPLC system (Dionex, Sunnyvale, CA) consisted of a pump (GP-40), autosampler (AS3500), UV/visible light absorption detector (AD-20), column (Thermo BetaBasic-18, 4.6 mm × 15 cm, 5 µm, Bellefonte, PA), silica guard column (4 mm × 1.25 cm), and computer for data collection and analysis (Gateway 2000, P4D-66, Irvine, CA)

Voriconazole and internal standard (ketoconazole) were eluted with a mobile phase consisting of 35% 0.1 M *N,N,N',N'*-tetramethylethylenediamine (Fisher Scientific Inc., Waltham, MA), pH 7.4, and 65% methanol (Fisher Scientific Inc., Waltham, MA) at a flow rate of 1.0 ml/min at room temperature. The mobile phase was degassed by vacuum filtration with 0.45 µm nylon membrane filters (Whatman plc, Middlesex, UK).

The injection volume was 100 µL. Voriconazole and internal standard were detected at a wavelength of 254 nm, and the retention times were 3.7 and 13.5 minutes, respectively.

To produce calibration curves, stock solutions of 1 mg/mL voriconazole were prepared by dissolving voriconazole powder in mobile phase. The stock solution was kept refrigerated in a borosilicate glass tube (Fisher Scientific Inc., Waltham, MA), tightly sealed with flexible film (Parafilm®, Alcon Inc., Menasha, WI). Spiking solutions for the calibration curve were prepared by diluting the stock solution with mobile phase to yield solutions with voriconazole ranging from 1 to 100 µg/mL. By adding 50 µL of each of the spiking solutions to 500 µL of horse plasma or filtered and distilled (Barnstead Fistreem II, Barnstead International, Dubuque, IA) water, calibration standards with voriconazole concentrations ranging from 0.1 to 10 µg/mL were prepared. Plasma obtained from a horse that was not treated with voriconazole was used as the blank control. A new calibration curve was prepared daily before samples were analyzed.

To prepare samples and calibration standards for HPLC, 500 µL of each sample or standard, followed by 15 µL of internal standard, were pipetted into a disposable borosilicate glass tube and vortexed for 10 seconds. The internal standard solution (1mg/mL) was prepared by dissolving ketoconazole powder (PCCA, Houston, TX) in mobile phase. Voriconazole and internal standard were extracted with 4 mL ethyl acetate (Fisher Scientific Inc., Waltham, MA), followed by 1 mL of 0.2 M sodium borate (J.T. Baker Chemical, Phillipsburg, NJ) at pH 9. The mixture was vortexed for 30 seconds and centrifuged at 4,500 × g for 10 minutes (IEC Clinical Centrifuge, International Equipment Company, Needham Heights, MA). The organic phase was transferred to a

separate glass tube and evaporated with nitrogen gas in a 50°C sand bath. The dried residue was reconstituted with 200 µL of the mobile phase and transferred to an HPLC vial and insert (250 µL glass flat bottom, Alltech Associates Inc., Deerfield, IL) for analysis.

Chromatograms were integrated by use of computer software (Dionex PeakNet® 4.11, Sunnyvale, CA). Calibration curves of peak area ratios (voriconazole/internal standard) versus concentration were calculated by the use of linear-regression analysis (R^2=0.999). The lower limit of detection for voriconazole was 0.1 µg/mL, and the lower limit of quantification was 0.5 µg/mL. The mean intra-day variation was 2.758% and the mean inter-day variation was 8.700%. Recovery was 100%.

Pharmacokinetic analysis

Pharmacokinetic parameters for each horse were determined for IV and oral drug administration. Non-compartmental and compartmental analyses were performed on the data obtained from IV dosing, and non-compartmental analysis was performed on the data obtained from oral dosing, using the computer program WinNonlin® 5.0.1 (Pharsight, Cary, NC). Pharmacokinetic parameters measured included observed peak plasma concentration (C_{max}), AUC, half-life of the elimination phase ($t_{1/2\beta}$), clearance (Cl), mean residence time (MRT), and volume of distribution at steady state (Vd_{ss}) (Tables 4.2 and 4.4). The elimination rate constant, K_{el}, was estimated from the log-linear terminal decline in the natural log (LN) of the plasma concentration versus time curve with a weighting of 1/[concentration]^2. The AUC was measured by the use of the log-trapezoidal method. The area under the plasma concentration-time curve from time zero extrapolated to infinity ($AUC_{0\text{-}inf}$) was calculated by determining the AUC up to the

last sample point ($AUC_{0\text{-}LT}$) and then adding the terminal portion estimated from the terminal rate constant. The C_{max} and T_{max} were observed directly from the data. The $t_{1/2\beta}$ was the LN of 2 (0.693) divided by the terminal rate constant.

Mean residence times were accessed from dividing the area under the moment curve by the AUC. The mean residence time of absorption (MRT_{abs}) is equal to the difference of $MRT_{oral} - MRT_{IV}$, where MRT_{oral} is the mean residence time following oral administration, and MRT_{IV} is the mean residence time following intravenous administration. The extent of oral absorption, F, was calculated by use of the following equation:

$$F = ([AUC_{oral} \times Dose_{IV}]/[AUC_{IV} \times Dose_{oral}])$$

where AUC_{IV} and $Dose_{IV}$ are the AUC and dose, respectively, for the IV route of administration, and AUC_{oral} and $Dose_{oral}$ are the AUC and dose, respectively, for the oral route of administration.

Plasma concentrations versus time following IV administration were evaluated based on sum of the squares and residual values to determine the best-fit pharmacokinetic model for compartmental analysis. A two-compartmental model was chosen for analysis(Table 4.3). The model used was characterized by the equation:

$$C = (A\,[1 - e^{-\alpha T}]e^{-\alpha t})/[\alpha\, T] + (B\,[1 - e^{-\beta T}]e^{-\beta t})/[\beta\, T]$$

where C is the plasma concentration of voriconazole at time t; t is time after drug administration, T is the infusion period; A and α are the coefficient and slope, respectively, of the distribution phase; B and β are the coefficient and slope, respectively, of the elimination phase; and e is the base of natural logarithm. The coefficients are defined in terms of the microrate constants as:

$$A = (\text{Dose}\ [\alpha - k_{21}])/(V_c\ [\alpha - \beta])$$

$$B = (\text{Dose}\ [k_{21} - \beta])/(V_c\ [\alpha - \beta])$$

The hybrid rate constant can also be described in terms of the microrate constants of the model as:

$$\alpha = ([k_{12} + k_{21} + k_{10}] + \sqrt{[k_{12} + k_{21} + k_{10}]^2 - 4k_{21}k_{10}})/2$$

$$\beta = ([k_{21} + k_{12} + k_{10}] - \sqrt{[k_{12} + k_{21} + k_{10}]^2 - 4k_{21}k_{10}})/2$$

The above and below equations used for the calculation of pharmacokinetic parameters were based on methods described by Gibaldi and Perrier (1982). The value for the volume of distribution of the central compartment (V_c) was calculated by use of the following equation:

$$V_c = \text{Dose}/(C_0)$$

where C_0 is equal to the sum of A plus B. Cl was calculated as:

$$Cl = V_c \times k_{10}$$

where k_{10} is defined as $(\alpha\beta)/k_{21}$, and k_{21} is equal to $(A\beta + B\alpha)/C_0$. The value for the volume of distribution based on the AUC (Vd_{area}) is the quotient of Cl/β. Vd_{ss} was calculated by use of the following equation:

$$Vd_{ss} = V_c + V_p$$

where V_p is the volume of distribution of the peripheral compartment and is equal to $(k_{12}V_c)/k_{21}$. The value for the extrapolated volume of distribution (Vd_{ext}) was calculated by the use of the following equation:

$$Vd_{ext} = \text{Dose}/B$$

The AUC based on the compartmental model is equal to $(A/\alpha) + (B/\beta)$, and the MRT was calculated as follows:

$$MRT = AUMC/AUC$$

where AUMC is the area under the moment curve and is equal to $(A/\alpha^2) + (B/\beta^2)$. The value of distribution clearance (Cl_{12}) is the product of V_cK_{12}.

The oral absorption kinetics of voriconazole was examined in greater detail from plots of the data expressed as the percent remaining to be absorbed (Ravis *et al*, 1987). The equations used for the calculation of the percent remaining to be absorbed were based on methods described by Loo and Riegelman (1968).

Results

No adverse effects were observed in any of the horses after administration of either oral or intravenous voriconazole. After 2 and 4 mg/kg IV administration, the mean ± standard deviation (SD) value for clearance of voriconazole, calculated using noncompartmental model anaylsis, was low (0.1041 ± 0.0224 L/hr/kg), and the mean Vd at steady state was high (1.744 ± 0.473 L/kg). The mean $AUC_{0\text{-}inf}$/dose value was 9.976 ± 2.069 kg-hr/L. The values of terminal half-life ranged from 7.72 to 16.68 hours, with the two lowest values obtained from the horses that received the 2 mg/kg intravenous dose, and a mean of 12.22 ± 3.54 hours.

Using two-compartmental analysis, the mean ± SD distribution half-life was 0.3600 ± 0.1487 hours, and the terminal (elimination) half-life was 11.86 ± 1.93 hours. The mean Vd of the central compartment (Vc) and of the peripheral compartments (Vp) were 0.7081 ± 0.2479 L/kg and 0.9814 ± 0.3657 L/kg, respectively. Other relevant pharmacokinetic variables obtained by modeling after IV administration of voriconazole are summarized (Table 4.3).

Drug loss during oral administration was minimal in the four horses that received the 4mg/kg voriconazole in powder form. The mean ± SD systemic bioavailability of

voriconazole powder was 103.07 ± 9.15%. The C_{max} was 1.6910 ± 0.3240 µg/mL at 8.38 ± 8.81 hours after administration. Mean terminal half-life was 15.284 ± 3.497 hours, and the mean $AUC_{0\text{-inf}}$ value was 40.24 ± 8.92 µg-hr/mL.

Plasma concentration over time following oral dosing of 3 mg/kg crushed tablets to the other two horses was plotted (Figures 4.5 and 4.6). The maximum observed plasma concentration was 0.7515 ± 0.1195 µg/mL at 10.96 ± 1.36 hours after drug administration. The mean $AUC_{0\text{-inf}}$ value was 17.77 ± 3.75 µg-hr/mL. After oral administration of the crushed tablets, mean ± SD bioavailability was 57.88 ± 8.63%, and the mean terminal half-life was 9.482 ± 0.899 hours. The mean Cl/F value was 0.1727 ± 0.0364 L/hr/kg.

The absorption kinetics after oral administration was further examined by percent remaining to be absorbed methods. Final graphs of the amount remaining to be absorbed as a function of time are illustrated (Figures 4.7 and 4.8). The plots of the percent remaining to be absorbed showed multi-phasic characteristics.

Discussion

The plasma profile observed following intravenous administration displayed multi-compartment features with log-linear elimination after 8 hours (Figure 4.2). Thereby, a 2-compartment pharmacokinetic model was used in describing voriconazole plasma concentration during and after the infusion (Table 4.3). Plasma concentrations decreased rapidly during the distributions phase with a $t_{1/2\alpha}$ value of 0.3600 ±0.1487 hours and it is followed by a slower elimination phase with a $t_{1/2\beta}$ of 11.86 ±1.93 hours. Pharmacokinetic parameters obtained using noncompartmental (Table 4.2) and 2-compartmental methods showed good agreement. The elimination half-life and volume

of distribution values appeared lower for the two horses that received 2 mg/kg IV dose than those of the horses that received the 4 mg/kg dose (Table 4.2). Total body clearance (Cl) seemed similar between 2 and 4 mg/kg IV dosed horse.

Further evidence for rapid distribution was found in the similarity in the average Vd_{ss} and Vd_{area} values (Duran *et al*, 1987). The apparent total volume of distribution (Vd_{ss} = 1.744 L/kg) is smaller than that observed in humans of 2.2 L/kg after single dosing of IV voriconazole (Purkins *et al*, 2003a). As expected for this model, $Vd_{ext} > Vd_{area} > Vd_{ss} > V_c$, with the extrapolation of the linear portion of the log concentration versus time plot to obtain Vd_{ext} and it being an overestimation of the apparent volume of distribution (Gibaldi and Perrier, 1982). Moderate inter-individual variability was observed in the volume of distribution at steady state values with coefficient of variation of 27.13%.

The average dose-adjusted $AUC_{0\text{-}inf}$ value (9.976 ± 2.069 kg-hr/L) in this study is in close agreement to that reported for dogs of 10.7 kg-hr/L after intravenous administration of 3 mg/kg but higher than those obtained from mouse, male rat, female rat, rabbit, guinea pig, and human, which were 4.17, 1.86, 8.16, 0.37, 3.85, and 2.008 kg-hr/L, respectively (Roffey *et al*, 2003; Purkins *et al*, 2003a). In the present study, the range of dose-adjusted $AUC_{0\text{-}inf}$ values of voriconazole after intravenous administration was 7.278 to 12.940 kg-hr/L, which is consistent with finding of an earlier report of 9.23 kg-hr/L in the horse (Davis *et al*, 2006). Dose-dependency characteristics of voriconazole have been described in other animal species and in humans (Purkins *et al*, 2002; Roffey *et al*, 2003). In this present study, the dose-adjusted $AUC_{0\text{-}inf}$ parameter after single IV administration does not show a trend between $AUC_{0\text{-}inf}$/Dose values and

the dose administered. Since clearance is the inverse of AUC/Dose, there appears to be no decrease in clearance with increased IV dose and thus no evidence for non-linear or saturable elimination. However, it is recognized that this represents only a 2-fold range in dose and a larger dosing range may show such trends.

Elimination half-life ($t_{1/2\beta}$) in humans after single IV administration was 5.6 hours (Purkins *et al*, 2003a). Voriconazole $t_{1/2\beta}$ after IV dosing in horses was observed to be 12.22 ± 3.54 hours. This $t_{1/2\beta}$ is longer that reported previously in horses (8.89 ± 2.31 hours) (Davis *et al*, 2006). The mean $t_{1/2\beta}$ after oral voriconazole powder (4 mg/kg) in the present study was 15.284 hours, which is longer than the findings in humans (6.0 hours) after 4 mg/kg once daily dosing and similar to an earlier report in horses (13.11 hours) (Purkins *et al*, 2003b; Davis *et al*, 2006). The apparent $t_{1/2\beta}$ is a function of clearance and volume of distribution. Over a wide dose range, $t_{1/2\beta}$ of voriconazole may vary due to potential saturable drug metabolic clearance. The $t_{1/2\beta}$ in horses given 4 mg/kg IV was 14.36±1.61 hours, which is shorter than the average of the two horses administered 2 mg/kg IV of 7.95 hours. The mean $t_{1/2\beta}$ for the two horses receiving 3 mg/kg of crushed tablets was 9.482 hours, which appears shorter than the voriconazole $t_{1/2\beta}$ of the horses given the 4 mg/kg of powder. However, different dosage forms were administered and the dose difference was modest, making it difficult to evaluate dose-dependent elimination.

Oral administration resulted in rapid absorption, with voriconazole detected in plasma at 5 minutes after dosing in all six horses (Figure 4.3). The mean maximum plasma concentrations of voriconazole occurred at 8.38 hours after oral administration of the powder formulation (Table 4.4). One investigation has examined voriconazole

disposition and elimination after a single oral dose in horses (Davis *et al*, 2006). They gave 4 mg/kg voriconazole powder in corn syrup and 1 mg/kg IV during fasting. In that study, the extent of absorption was 135.75 ± 18.41% and the C_{max} was 2.43 ± 0.4 μg/mL at 2.92 ± 1.2 hours after administration. In the present study, the absorption of voriconazole was essentially complete with the powder formulation, with an average extent of absorption of 103.07% (Table 4.4) as determined by comparing the $AUC_{0\text{-}inf}$ values after IV and oral dosing of the same dose (4 mg/kg). For the two horses which received the crushed tablets for a dose of 3 mg/kg and 2 mg/kg intravenously, the extent of voriconazole oral absorption appeared less with F values of 63.98 and 51.78%. Oral absorption of voriconazole in horses seems to be lower from tablets as opposed to powder dosing. The non-equal oral and IV doses in the previous study by Davis *et al* and potential drug nonlinear elimination may explain the inaccurate overestimate of voriconazole oral extent of absorption in horses. In the present study, IV and oral doses and thus plasma concentrations were similar, permitting a better estimate of bioavailability.

Comparison of the apparent clearance after oral administration (Cl/F) among species, the mean Cl/F of the four horses (0.1034±0.0227 L/hr/kg) after a single oral dosing of 4 mg/kg of powder was lower than that reported in mouse, male rat, female rat, rabbit, guinea pig, and human, which were observed to be 0.304, 0.333, 0.139, 3.125, 0.345, and 0.678 L/hr/kg, respectively, but higher than that of the dog (0.068 L/hr/kg). (Roffey *et al*, 2003). There was no evidence of dose- or formulation-dependency in values of clearance divided by extent of absorption (Cl/F). Although the two horses that received 3 mg/kg of crushed tablets had Cl/F values of 0.1469 and 0.1984 L/hr/kg, oral

absorption from the tablets is less, leading to larger overestimates of clearance. The longer half-life observed for oral administration in 3 of the 6 horses, compared with that for the IV formulation in the horses, might be attributable to a prolonged absorption phase. Interestingly, voriconazole extent of absorption appeared lower from tablets than powder but the rate of drug absorption from crushed tablets was faster. The average mean residence time of absorption, the time for 50% to be orally absorbed, is 9.005 hours for the powder formulation and 4.970 hours for the tablet formulation.

In the present study, there was a marked variability in the plasma voriconazole concentrations after oral dosing with multiple peak concentrations in several instances. The MRT_{abs} varied from 3.478 to 13.719 hours (mean 9.005 hours) in horses administered the powder. To better understand the apparent complex oral absorption of voriconazole in horses, percent remaining to be absorbed calculations and plots were performed. Evaluation of the percent remaining to be absorbed plots showed multi-phasic absorption process in all six horses (Figure 4.8). The percentage to absorbed plots showed multiple absorption phases or periods suggesting complex absorption processes. As with phenobarbital, it appears that it is initially rapidly absorbed from the gastrointestinal tract and later absorbed at a slower rate. Rapid tablet dissolution with absorption, followed by drug precipitation to another less soluble crystalline form may cause this effect. Also, it is possible that this drug is rapidly absorbed in the stomach and more slowly absorbed in the intestine (Ravis *et al*, 1987).

In this study, food was not withheld in order to better simulate how voriconazole would be administered in practice. The high variability in oral drug absorption could be attributed to free access to hay, except during the time when the horses were in the

stocks, in this study, compared to 12 hours of fasting in the previous report, prior to drug administration. The horse is a grazing animal, and in normal situations, its stomach is almost never empty, even after 24 hours of fasting (Andrews, 2001). Despite 12 hours of fasting, variable absorption after drug administration has been observed (Baggot, 1977). The horse, like the human, dog, and cat, is monogastric, but differs from other species in that microbial digestion of polysaccharides takes place in the specialized caecum and colon. It has been postulated that, while some of the dose may be absorbed in the small intestine, some may be adsorbed on to the feed and be subsequently released by fermentative digestion to be absorbed in the colon or caecum (Baggot, 1992). Double peaks in the plasma concentration versus time curves, as shown in this study (Figure 4.3), were also observed in ponies after oral administration of phenylbutazone when given free choice of hay, possibly due to drug binding to hay and subsequent release in the distal part of the rumen (Maitho *et al*, 1986). A secondary peak after oral administration can also indicate enterohepatic circulation and reabsorption of the drug after hepatic metabolism (Gibson and Skett, 1986).

The marked variability in the plasma voriconazole concentrations versus time profiles could also be related to the drug dosage form administered. However gastric pH does not play significant role in absorption of voriconazole in other species. The bioavailability of the crushed tablet formulation is assessed against the powder formulation, as both products are subject to first pass clearance. The mean relative oral availability of the crushed tablet formulation, based on two horses, is 56.12%. Bioavailability depends on drug stability, interactions with gastrointestinal (GI) components, drug permeation across the GI wall, gastric emptying, drug dissolution, and

intestinal and first-pass metabolism. Oral absorption of drugs may be influenced by drug release from the dosage form, stability of the drug in the stomach or rumen, and the degree of ionization and lipid solubility. Because dissolution is the rate-limiting step in the absorption of most orally administered lipophilic drugs, the powder formulation with greater surface area per dose ratio would be expected to dissolve faster and be absorbed better than would the crushed tablet formulation. Drug interactions with excipients in the tablet formulation can also cause a decrease in GI concentrations of active substance available for absorption. One of the excipients found in the commercially available voriconazole tablets is magnesium stearate, which is a commonly used lubricant in the manufacturing of tablets. The presence of magnesium stearate has been observed to cause slower release of active components from capsules and tablets in dissolution tests (Yu *et al*, 2002).

Incomplete systemic availability of orally administered drugs also may be due to metabolism in the intestinal mucosa or liver before reaching the systemic circulation. All orally administered drugs must pass through the liver before entering the general circulation, and those drugs which are highly cleared by the liver will have diminished systemic availability. This phenomenon is known as the first-pass effect. Due to the high activity of hepatic microsomal-associated metabolic pathways in horses, the first-pass effect could substantially decrease systemic availability of drugs, such as voriconazole, that undergo extensive hepatic metabolism. Extraction ratio (ER) represents the fraction of drug in the portal circulation which is cleared by the liver . Voriconazole is extensively metabolized in all species studied and the primary pathway is

N-oxidation followed by glucuronidation of the metabolite. (Hyland *et al*, 2003). The hepatic extraction ratio can be estimated by the equation:

$$ER = Cl/PF_H$$

where PF_H is the average hepatic plasma flow of a normal horse and is equal to:

$$PF_H = \text{hepatic blood flow } (1 - \text{hematocrit})$$

The average equine hepatic blood flow is equal to 23.8 mL/min/kg and hematocrit value is 37% for a normal horse (Dyke *et al*, 1998; Geor, 1995). The mean ± SD value for ER using clearance obtained after intravenous administration was 0.116± 0.025 in the present study. Therefore, voriconazole in horses would be considered a drug with a low extraction ratio (ER <0.4). In the case of drugs which are poorly extracted by the liver, it is expected that nearly all of the dose gets through the liver first-pass and does not affect the extent of absorption. Even doubling or halving the minor proportion extracted by the liver does not make any significant difference to apparent bioavailability (Birkett, 2002). The fact that voriconazole displayed nearly complete extent of absorption from the powder dosage form supports the absence of first-pass metabolism of this drug in horses.

Of the four horses that were administered the powder, the two Arabian horses displayed more rapid absorption with a shorter time of peak and smaller mean residence time for absorption, MRT_{abs} (Figure 4.5). Despite this evidence, to the authors' knowledge, there has been no report of breed-dependent pharmacokinetics or oral absorption reported in horses. In addition, the Arabian horses were the youngest subjects with ages of 9 and 11 years as compared to the other horses with ages of 16, 19, 20, and 23 years. Age is also a factor that may influence the disposition and absorption of some drugs and can be related to metabolic and renal elimination abilities and changes in the

volume of distribution caused by body weight increases and tissue and plasma protein binding. Voriconazole has been reported to have increased C_{max} in elderly humans (Pfizer, 2006). Based on IV results, the younger Arabian horses showed higher total body clearance than the other four horses with Cl values of 0.1232 and 0.1374 mL/hr/kg.

In humans, up to twice the $t_{1/2\beta}$ has been observed in those exhibiting the cytochrome P450 enzyme CYP2C19 polymorphism after both intravenous and oral administrations of voriconazole, compared to extensive metabolizers (Theuretzbacher *et al*, 2006). This gene has not been identified in horses, but generally, liver function is variable from animal to animal, even within a given species (Ayres *et al*, 1984). The pharmacokinetics of voriconazole is affected by liver function with lower doses recommended in patients with severe chronic cirrhosis (Tan *et al*, 2006). Evaluation of the serum liver enzymes levels in these 6 horses showed that they were within normal ranges and there was no age-, breed-, or size-dependent trends among the horses in the serum levels that assess liver and kidney functions. The AUC following oral administration showed that voriconazole pharmacokinetics in the rat and human are gender-dependent, with a lower systemic exposure in male animals (Pfizer, 2006). This phenomenon was not observed in dogs (Roffey *et al*, 2003). However, we were not able to assess the gender-dependency aspect of voriconazole pharmacokinetics in horses, as only female horses were used in the present study.

In ten clinical trials, the median values for the average and maximum voriconazole plasma concentrations in individual patients across these studies (N=1121) was 2.51 μg/mL and 3.79 μg/mL, respectively (Pfizer, 2006). Although the reported minimum inhibitory concentration (MIC) values from different laboratories and for

different fungal species vary, it is generally between 0.5 and 4 μg/mL for voriconazole. Assuming that MIC values of voriconazole against various fungi is the same or similar in horses as in humans, an average target concentration could be 1.5 - 2.5 μg/mL for horses. However, in addition to extraction ratio, the extent of plasma protein binding has to be considered as well, since it has been shown that only the protein unbound drug contributes to antimicrobial activities. There is a significant difference between the extent of plasma protein binding of voriconazole in horses (31.68%) compared to humans (58%), mouse (67%), rat (66%), rabbit (60%), guinea pig (45%), and dog (51%) (Craig and Welling, 1977; Pfizer, 2006; Roffey *et al*, 2003). Based on average clearance values and the equation:

$$\text{Dose} = \text{Cl} \times \text{desired concentration} \times \tau$$

where τ is the dosing interval, an IV dosing regimen of 3.7 – 6.2 mg/kg/day of voriconazole to mature, healthy horses seems appropriate. Based on an 8 to 9 hour $t_{1/2\beta}$ and once daily dosing, no loading dose should be needed. Oral maintenance dose can be calculated as equal to the product of desired concentration, Cl/F, and τ. For oral dosing with powder, similar regimens of 3.7 – 6.2 mg/kg/day appear appropriate. In the case of crushed tablets, higher oral daily doses of 6.2 – 10.4 mg/kg/day may need to be recommended.

An alternative, pharmacodynamic method of calculating dosing regimen for antimicrobials is with the use of AUC values. An $AUC_{0\text{-}24}$/MIC ratio at steady-state of 20 to 25 has been associated with efficacy of triazoles for the treatment of candidiasis (Andes *et al*, 2003; Pai *et al*, 2007). Using a MIC value of 0.25 μg/mL, the AUC/MIC obtained from all six horses and the two oral formulations yielded ratios of 71 for

3mg/kg/day with tablets and 131 for 4 mg/kg/day with powder. The same calculation using pharmacokinetic parameters obtained after IV administration resulted in AUC/MIC ratios of 86 for 2 mg/kg/day and 137 for 4 mg/kg/day. Therefore, the dose used in this study may be appropriate for the treatment of fungal infections in horses. Because pharmacokinetics of voriconazole is known to change during chronic dosing in other species, the recommended maintenance dosing regimen needs to be examined during repetitive dosing.

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TABLE 4.1 - Demographics of study subjects.

		Horse					
		A	B	C	D	E	F
Age	(years)	20	19	9	11	23	16
Sex		F	F	F	F	F	F
Breed		AQH	AQH	Arabian	Arabian	TWH	APH
Weight	(kg)	498.6	500.0	472.7	429.5	404.5	513.6

F = female; AQH = American Quarter Horse; TWH = Tennessee Walking Horse; APH = American Paint Horse.

TABLE 4.2 - Noncompartmental pharmacokinetic parameters of voriconazole given intravenously (2 or 4 mg/kg) to horses.

		Horse								
		A	B	C	D	E	F	Mean	SD	CV %
Dose	(mg/kg)	2	2	4	4	4	4	NA	NA	NA
Infusion Time	(hr)	0.25	0.22	0.08	0.13	0.13	0.13	NA	NA	NA
C_{max}	(µg/mL)	2.588	2.005	6.864	4.022	5.027	5.623	4.355	1.850	42.48
AUC_{0-24}	(µg-hr/mL)	17.58	17.15	23.94	21.30	34.92	29.53	24.07	7.00	29.10
AUC_{0-LT}	(µg-hr/mL)	20.93	19.63	29.97	27.20	47.40	38.89	30.67	10.74	35.02
AUC_{0-inf}	(µg-hr/mL)	21.28	19.47	32.47	29.11	51.76	44.59	33.11	12.82	38.72
AUC_{0-inf}/Dose	(kg-hr/L)	10.642	9.733	8.117	7.278	12.940	11.147	9.976	2.069	20.74
K_{el}	(hr^{-1})	0.08470	0.08980	0.05050	0.05340	0.04930	0.04160	0.06155	0.02035	33.06
$t_{1/2\beta}$	(hr)	8.18	7.72	13.72	12.97	14.05	16.68	12.22	3.54	28.95
MRT_{IV}	(hr)	12.54	12.45	17.28	16.95	20.19	22.16	16.93	3.94	23.25
Cl	(L/hr/kg)	0.0940	0.1027	0.1232	0.1374	0.0773	0.0897	0.1041	0.0224	21.50
Vd_{ss}	(L/kg)	1.179	1.279	2.129	2.329	1.561	1.988	1.744	0.473	27.13
Vd_{area}	(L/kg)	1.110	1.145	2.438	2.572	1.567	2.159	1.832	0.646	35.27

C_{max} = peak plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours; AUC_{0-LT} = area under the plasma concentration-time curve from time zero to the last observed time; AUC_{0-inf} = area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC_{0-inf}/Dose = area under the plasma concentration-time curve from time zero extrapolated to infinity/dose; K_{el} = elimination rate constant; $t_{1/2\beta}$ = elimination half-life; MRT_{IV} = mean residence time following intravenous administration; Cl = total body clearance; Vd_{ss} = volume of distribution at steady state; Vd_{area} = volume of distribution as calculated by the area under the plasma concentration-time curve method; NA = not applicable; SD = standard deviation; CV% = percent coefficient of variation.

TABLE 4.3 - Two-compartmental pharmacokinetic parameters of voriconazole given intravenously (2 or 4 mg/kg) to horses

		Horse								
		A	B	C	D	E	F	Mean	SD	CV %
Dose	(mg/kg)	2	2	4	4	4	4	NA	NA	NA
Infusion Time	(hr)	0.25	0.22	0.08	0.13	0.13	0.13	NA	NA	NA
C_{max}	(µg/mL)	2.588	2.005	6.864	4.022	5.027	5.623	4.355	1.850	42.48
$AUC_{0\text{-}inf}$	(µg-hr/mL)	21.02	21.93	30.45	27.60	51.65	42.58	32.54	12.17	37.39
$AUC_{0\text{-}inf}$/Dose	(kg-hr/L)	10.509	10.965	7.612	6.901	12.913	10.644	9.924	2.252	22.70
α	(hr^{-1})	2.074	1.110	2.863	1.598	2.996	2.349	2.165	0.728	33.64
$t_{1/2\alpha}$	(hr)	0.3342	0.6241	0.2421	0.4336	0.2313	0.2950	0.3600	0.1487	41.29
β	(hr^{-1})	0.07119	0.05903	0.06751	0.06233	0.04741	0.05056	0.05967	0.00933	15.63
$t_{1/2\beta}$	(hr)	9.73	11.74	10.27	11.12	14.62	13.71	11.86	1.93	16.28
MRT_{IV}	(hr)	13.46	16.58	13.73	15.11	20.49	18.77	16.36	2.82	17.25
Cl	(L/hr/kg)	0.0952	0.0912	0.1314	0.1449	0.0774	0.0940	0.1057	0.0263	24.88
Cl_{12}	(L/hr/kg)	0.6349	0.3256	0.9287	0.8054	1.0745	0.8462	0.7692	0.2610	33.94
V_c	(L/kg)	0.6060	1.0997	0.4750	0.9311	0.5842	0.5528	0.7081	0.2479	35.01
Vd_{area}	(L/kg)	1.337	1.545	1.946	2.325	1.634	1.858	1.774	0.348	19.59
Vd_{ss}	(L/kg)	1.281	1.512	1.804	2.189	1.587	1.764	1.690	0.309	18.29
Vd_{ext}	(L/kg)	1.396	1.581	2.103	2.475	1.682	1.960	1.866	0.393	21.06

C_{max} = peak plasma concentration; $AUC_{0\text{-}inf}$ = area under the plasma concentration-time curve from time zero extrapolated to infinity; $AUC_{0\text{-}inf}$/Dose = area under the plasma concentration-time curve from time zero extrapolated to infinity/dose; α = slope of the distribution phase; t1/2α = half-life of distribution phase; β = slope of the elimination phase; $t_{1/2\beta}$ = half-life of elimination phase; MRT_{IV} = mean residence time following intravenous administration; Cl = total body clearance; Cl_{12} = distribution clearance; V_c = volume of distribution of the central compartment; Vd_{area} = volume of distribution as calculated by the area under the plasma concentration-time curve method; Vd_{ss} = volume of distribution at steady state; Vd_{ext} = extrapolated volume of distribution; NA = not applicable; SD = standard deviation; CV% = percent coefficient of variation.

TABLE 4.4 - Noncompartmental pharmacokinetic parameters of voriconazole given orally (3 mg/kg crushed tablets or 4 mg/kg powder) to horses.

		Horse									
		A	B	C	D	E	F	$Mean_{A-B}$	$Mean_{C-F}$	SD_{C-F}	$CV\%_{C-F}$
Dose	(mg/kg)	3	3	4	4	4	4	3	4	NA	NA
Formulation		Tablet	Tablet	Powder	Powder	Powder	Powder	Tablet	Powder	NA	NA
C_{max}	(µg/mL)	0.8360	0.6670	1.9240	2.0110	1.3610	1.4690	0.7515	1.6910	0.3240	19.15
T_{max}	(hr)	11.92	10.00	1.00	0.50	16.00	16.02	10.96	8.38	8.81	105.20
AUC_{0-24}	(µg-hr/mL)	15.75	11.19	24.91	21.12	22.27	22.87	13.47	19.69	5.17	26.28
AUC_{0-inf}	(µg-hr/mL)	20.43	15.12	33.12	32.00	46.86	48.96	17.77	40.24	8.92	22.16
AUC_{0-inf}/Dose	(kg-hr/L)	6.808	5.040	8.279	8.001	11.716	12.241	5.924	10.059	2.229	22.16
F	(%)	63.98	51.78	102.00	109.94	90.54	109.82	57.88	103.07	9.15	8.87
K_{el}	(hr^{-1})	0.07840	0.06850	0.06900	0.04140	0.04000	0.04070	0.07345	0.04778	0.01416	29.64
$t_{1/2\beta}$	(hr)	8.847	10.118	10.051	16.732	17.337	17.016	9.482	15.284	3.497	22.88
MRT_{oral}	(hr)	16.83	18.10	17.21	22.21	32.22	32.49	17.47	26.03	7.58	29.13
MRT_{abs}	(hr)	4.287	5.652	3.478	7.098	11.726	13.719	4.970	9.005	4.612	51.21
Cl/F	(L/hr/kg)	0.1469	0.1984	0.1208	0.1250	0.0860	0.0817	0.1727	0.1034	0.0227	21.94
Vd_{area}/F	(L/kg)	1.875	2.896	1.752	3.017	2.151	2.005	2.385	2.231	0.549	24.62

C_{max} = peak plasma concentration; T_{max} = time of peak plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours; AUC_{0-inf} = area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC_{0-inf}/Dose = area under the plasma concentration-time curve from time zero extrapolated to infinity/dose; F = extent of absorption; K_{el} = elimination rate constant; $t_{1/2\beta}$ = elimination half-life; MRT_{oral} = mean residence time following oral administration; MRT_{abs} = mean residence time of absorption; Cl/F = total body clearance/extent of absorption; Vd_{area}/F = volume of distribution as calculated by the area under the plasma concentration-time curve method/extent of absorption; NA = not applicable; $Mean_{A-B}$ = average of values obtained for Horses A and B; $Mean_{C-F}$ = average of values obtained for Horses C, D, E, and F; SD_{C-F} = standard deviation of values obtained from Horses C, D, E, and F; $CV\%_{C-F}$ = percent coefficient of variation of values obtained from Horses C, D, E, and F.

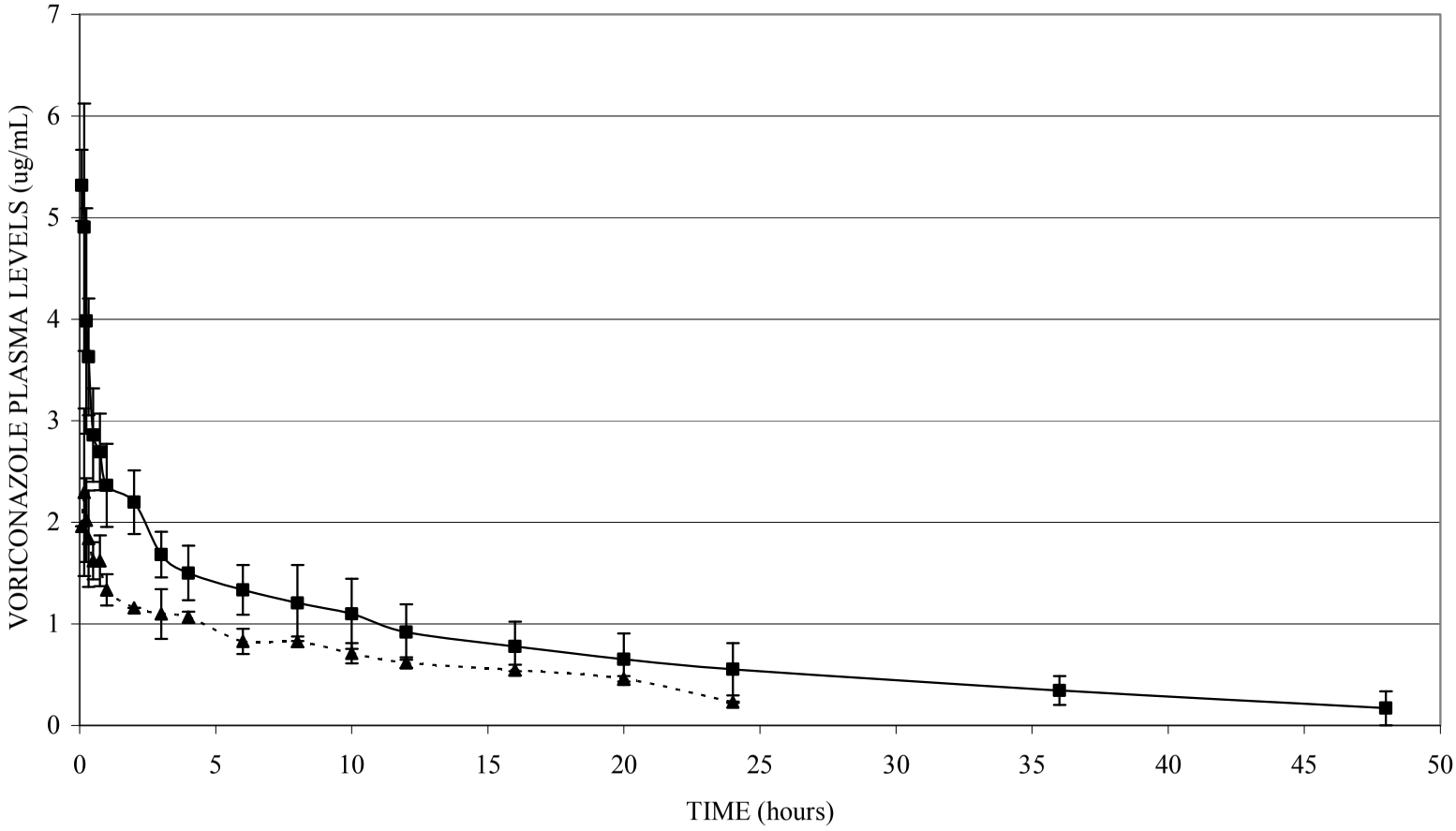

FIGURE 4.1. Mean plasma concentrations of voriconazole in horses after single intravenous administration at the dose of 2 mg/kg (--▲--) to two horses and 4 mg/kg (—■—) to four horses on linear coordinates (bars indicate standard deviations).

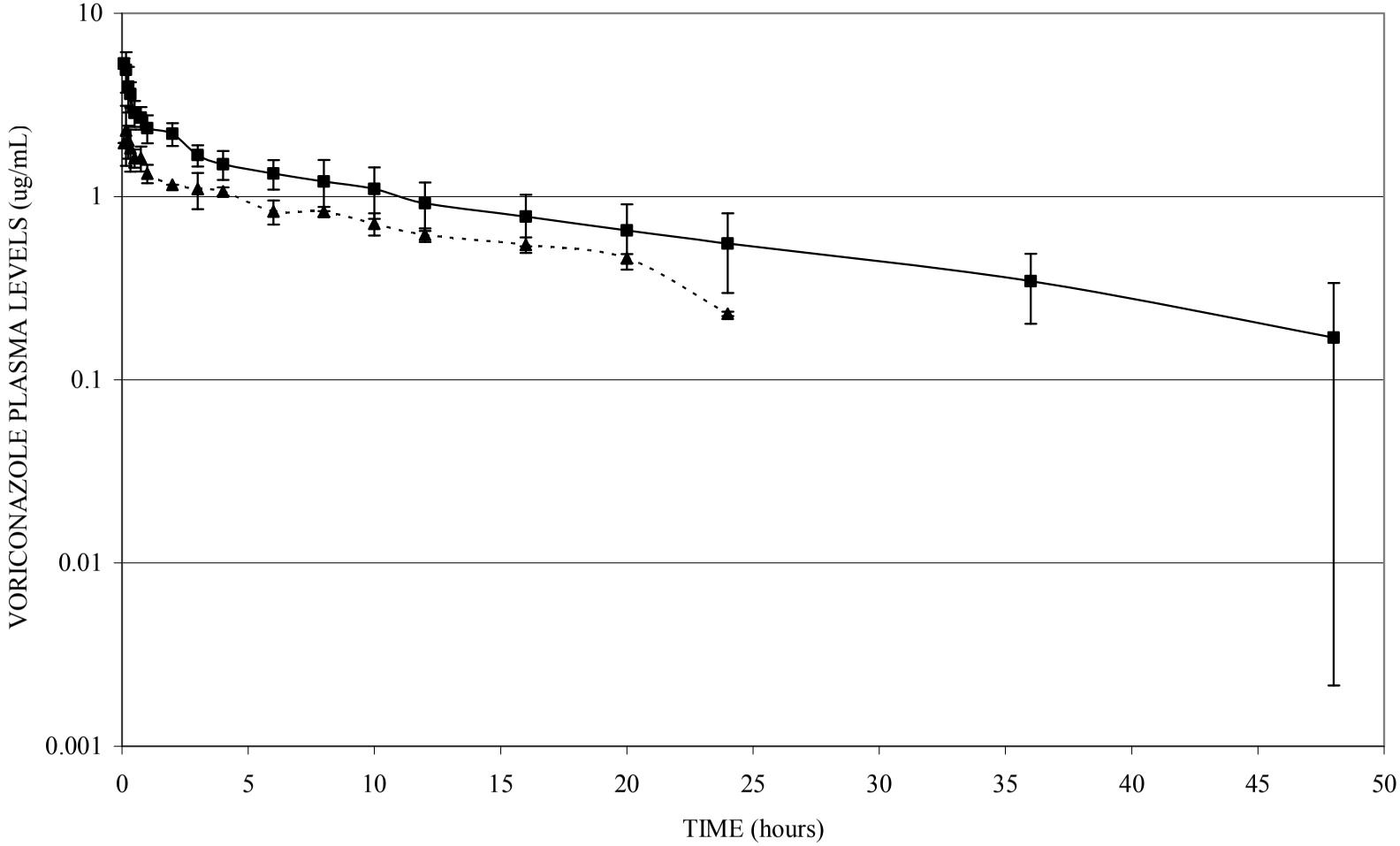

FIGURE 4.2. Mean plasma concentrations of voriconazole in horses after single intravenous administration at the dose of 2 mg/kg (--▲--) to two horses and 4 mg/kg (—■—) to four horses on log-linear coordinates (bars indicate standard deviations).

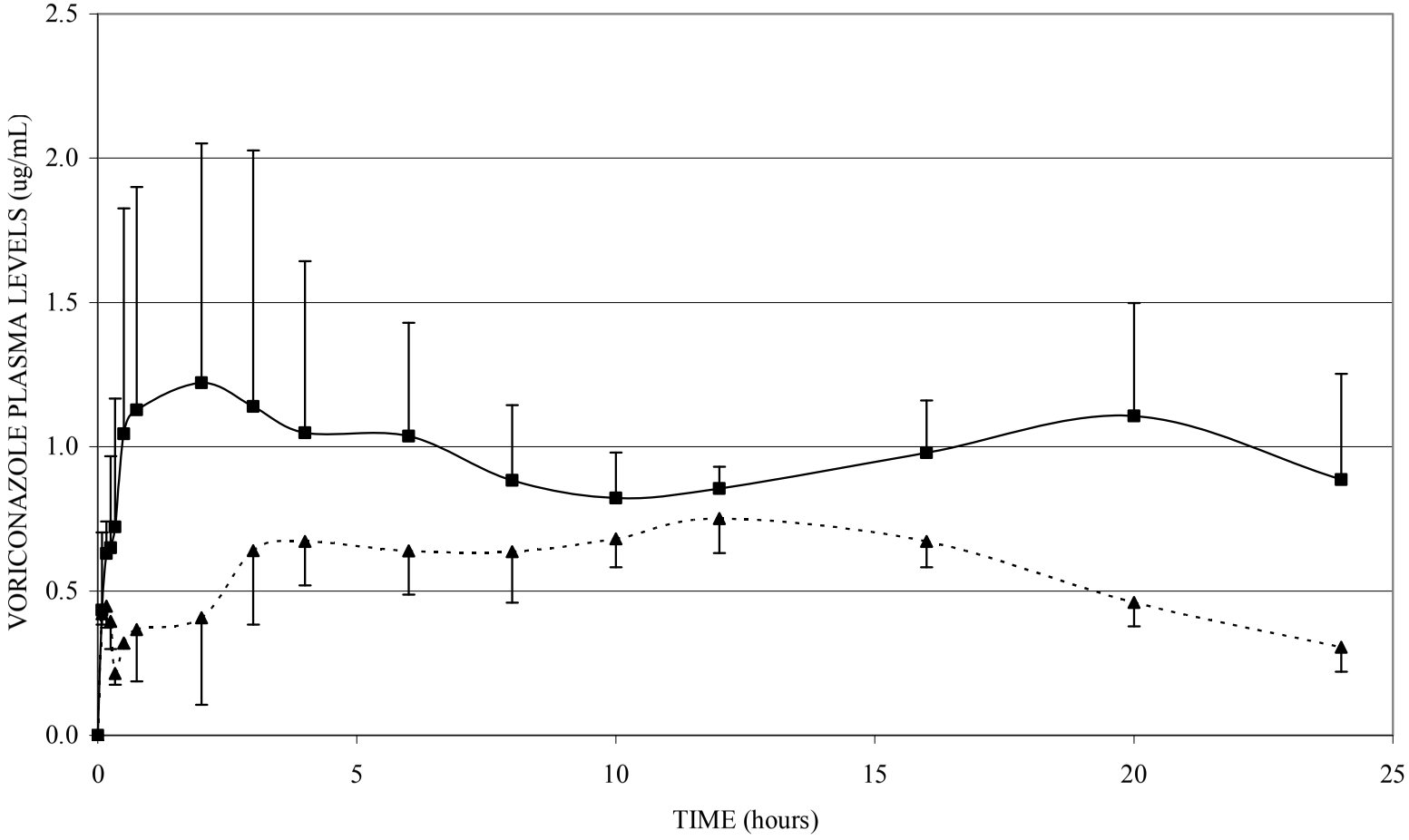

FIGURE 4.3. Mean plasma concentrations of voriconazole in horses after a single oral administration at the dose of 3 mg/kg with tablets (--▲--) to two horses and 4 mg/kg with powder (—■—) to four horses on linear coordinates (bars indicate standard deviations).

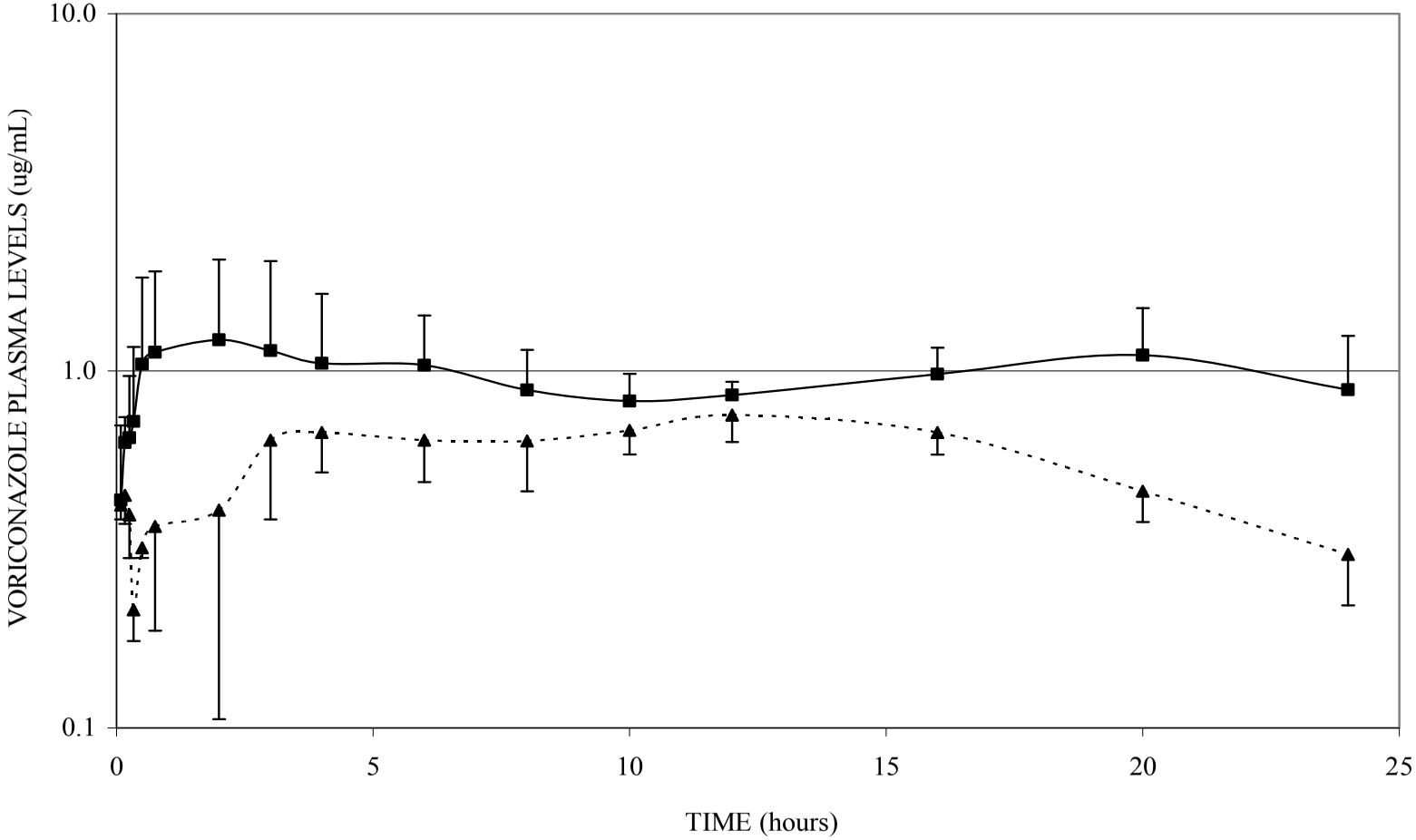

FIGURE 4.4. Mean plasma concentrations of voriconazole in horses after a single oral administration at the dose of 3 mg/kg with tablets (--▲--) to two horses and 4 mg/kg with powder (—■—) to four horses on log-linear coordinates (bars indicate standard deviations).

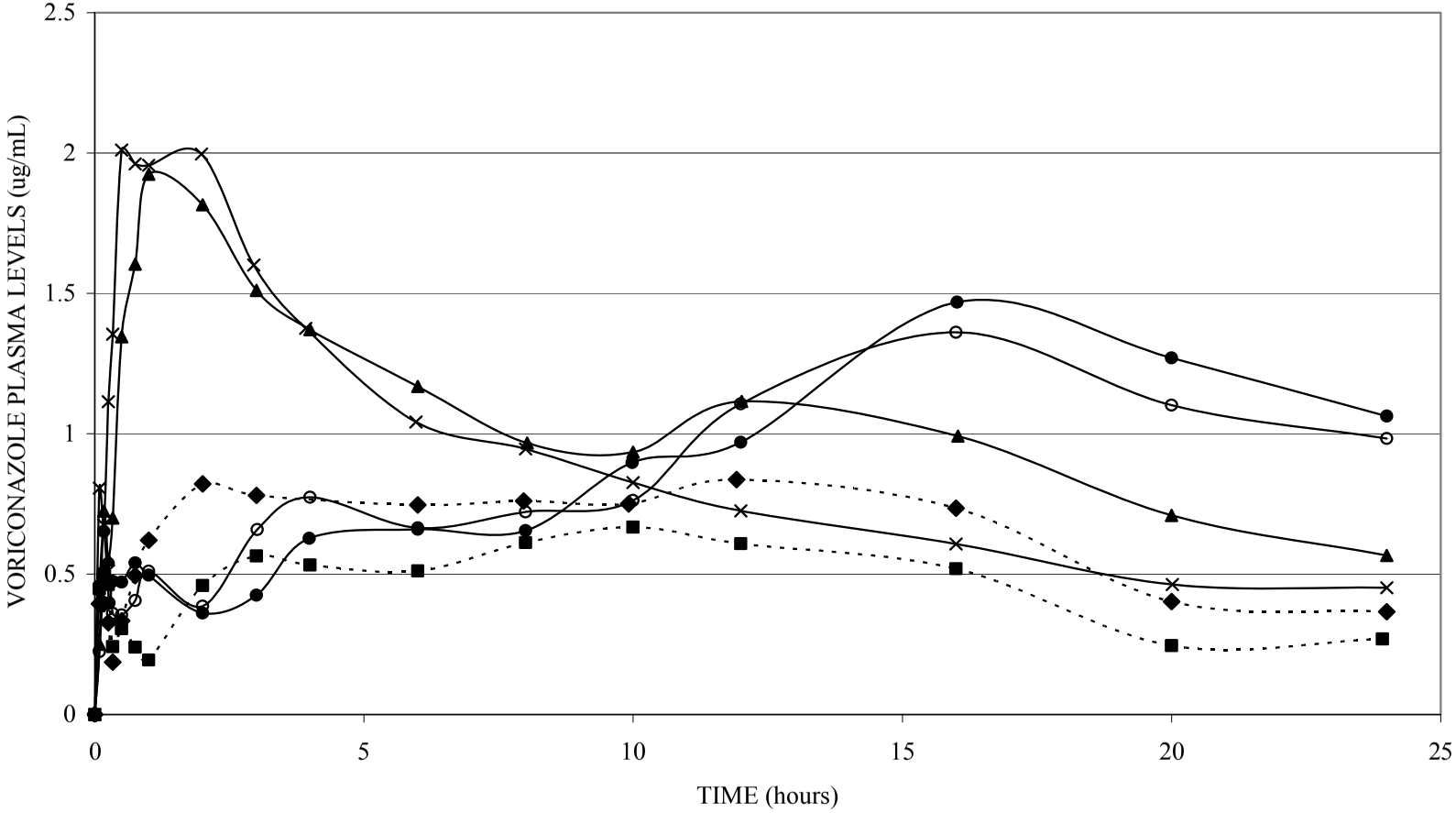

FIGURE 4.5. Plasma concentrations of voriconazole in horses after a single oral administration at a dose of 3 mg/kg with tablets to Horse A (--♦--) and Horse B (--■--), and 4 mg/kg with powder to Horse C (—▲—), Horse D (—×—), Horse E (—○—), and Horse F (—●—) on linear coordinates.

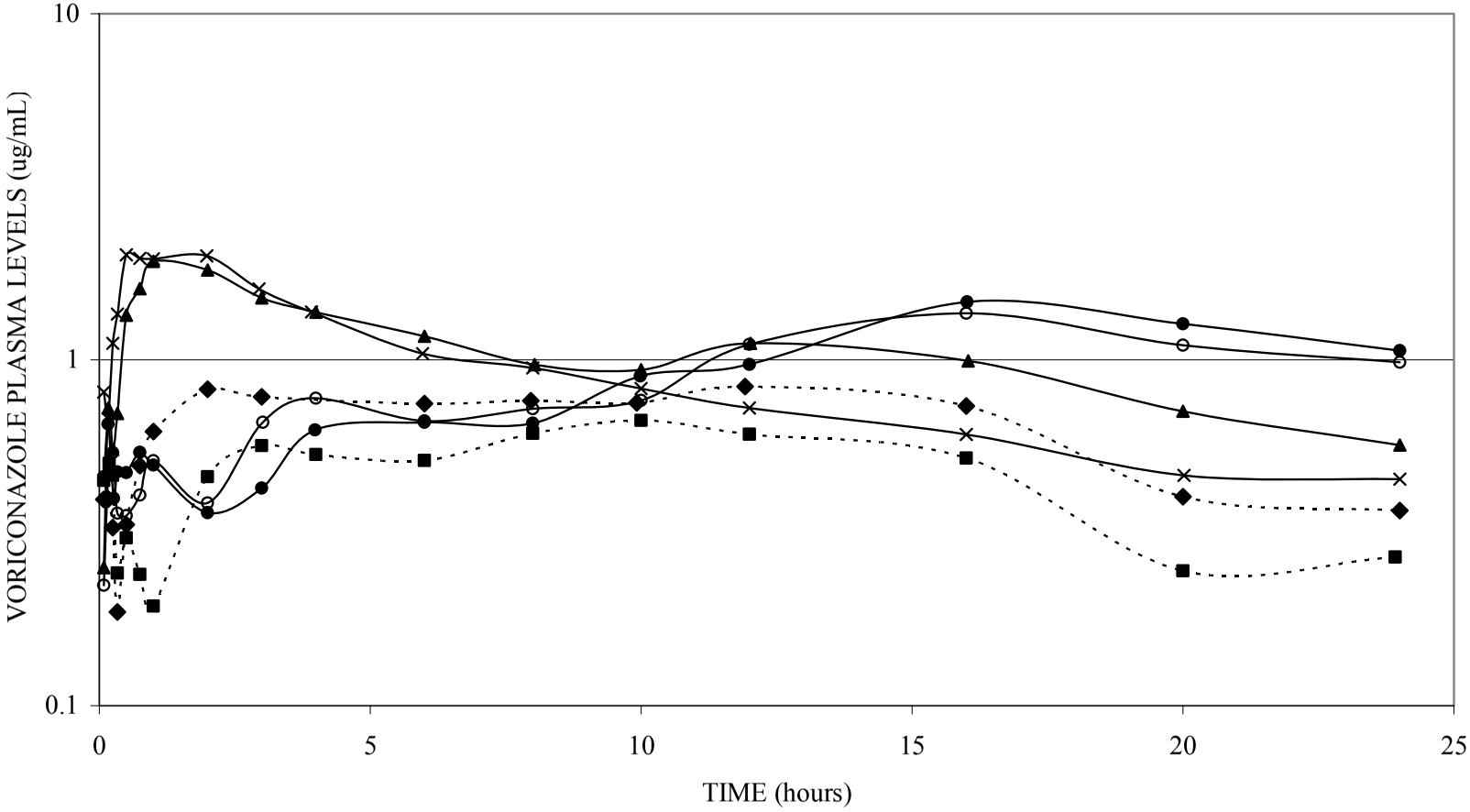

FIGURE 4.6. Plasma concentrations of voriconazole in horses after a single oral administration at a dose of 3 mg/kg with tablets to Horse A (--♦--) and Horse B (--■--), and 4 mg/kg with powder to Horse C (—▲—), Horse D (—×—), Horse E (—○—), and Horse F (—●—) on log-linear coordinates.

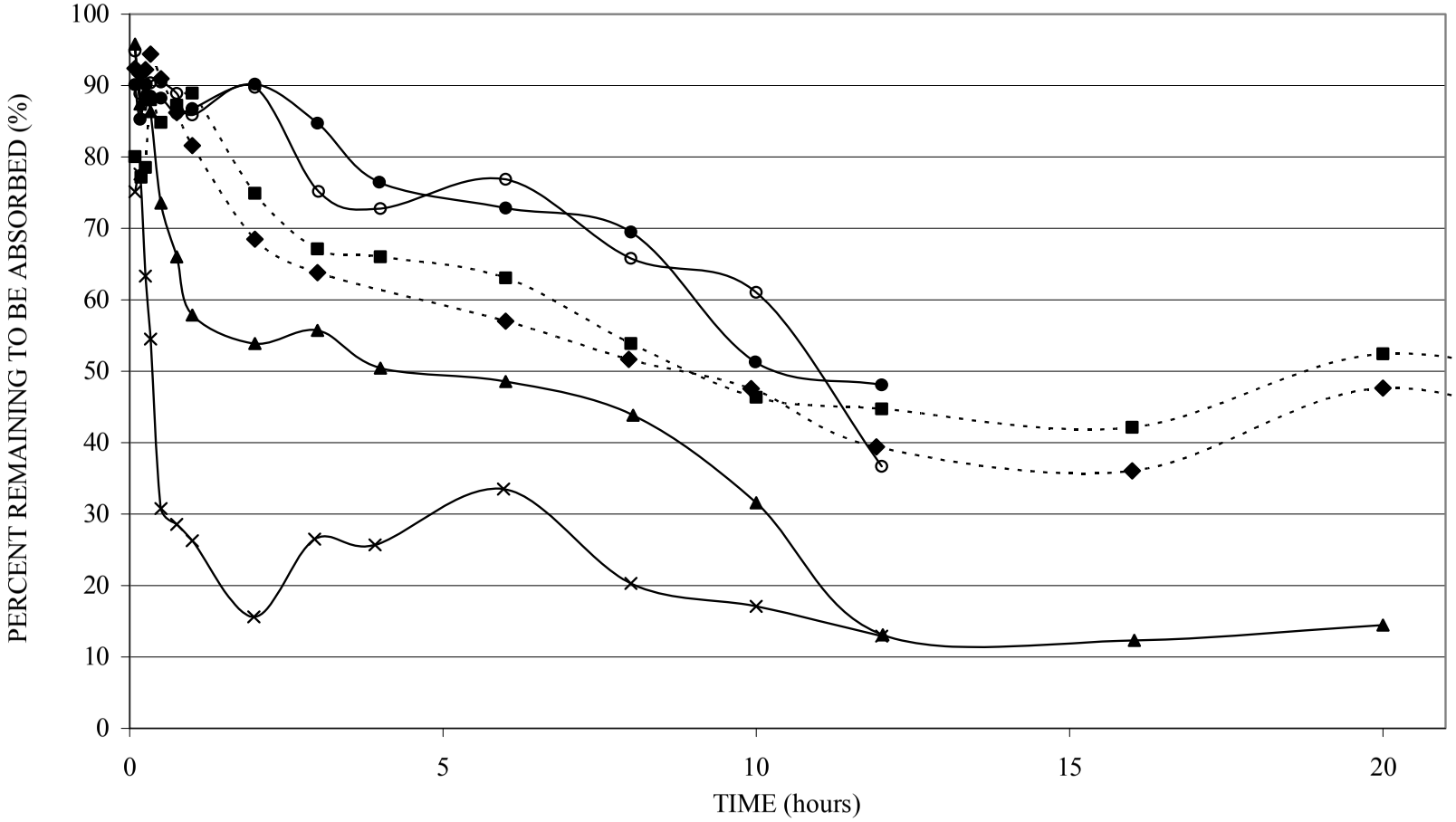

FIGURE 4.7. Percentage of available dose remaining to be absorbed following oral administration of voriconazole at a dose of 3 mg/kg with tablets to Horse A (--♦--) and Horse B (--■--), and 4 mg/kg with powder to Horse C (—▲—), Horse D (—×—), Horse E (—○—), and Horse F (—●—) on linear coordinates.

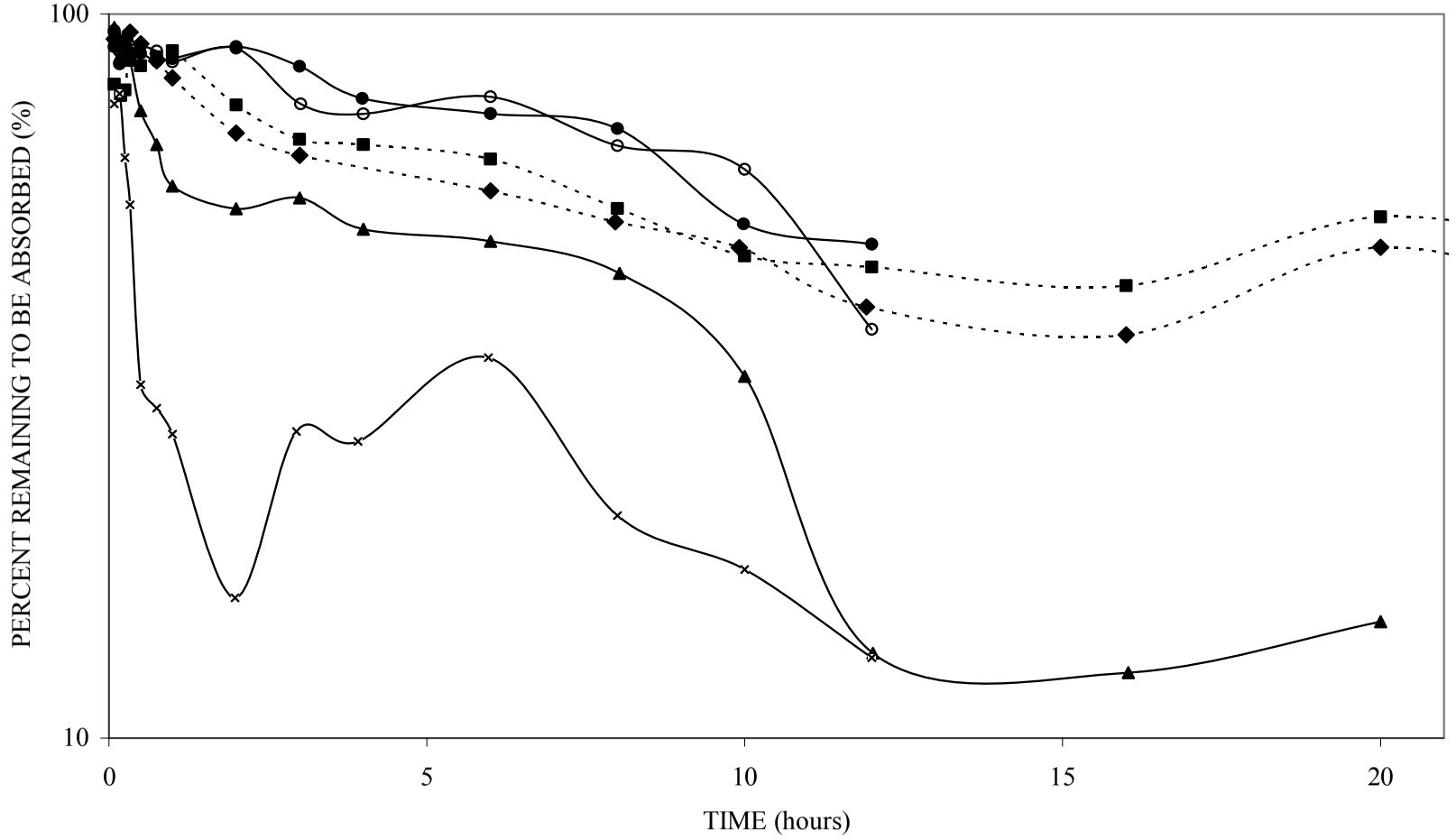

FIGURE 4.8. Percentage of available dose remaining to be absorbed following oral administration of voriconazole at a dose of 3 mg/kg with tablets to Horse A (--♦--) and Horse B (--■--), and 4 mg/kg with powder to Horse C (—▲—), Horse D (—×—), Horse E (—○—), and Horse F (—●—) on log-linear coordinates.

5. PHARMACOKINETICS OF VORICONAZOLE AFTER MULTIPLE ORAL (TABLET AND POWDER FORMULATIONS) ADMINISTRATIONS TO HORSES

Abstract

The objective of this study was to determine the pharmacokinetics of voriconazole after oral multiple administrations to horses. Two horses were used for the treatment of 3 mg/kg oral administration of a crushed tablet formulation of voriconazole, and four horses were used for the treatment of 4 mg/kg oral administration of a powder formulation of voriconazole every 24 hours. Plasma voriconazole concentrations were measured by the use of high-performance liquid chromatography. On day 14/15 of the treatment, the mean elimination half-life ($t_{1/2\beta}$) following oral 3 mg/kg/day crushed tablet and 4 mg/kg/day powder administration was 10.054 and 7.141 hours, respectively; mean maximum plasma concentration (C_{max}) was 1.977 and 1.892 μg/mL, respectively; mean dose-adjusted area under the curve from time zero to 24 hours (AUC_{0-24}/Dose) was 9.433 and 5.944 kg-hr/L, respectively. The mean accumulation factor calculated from the AUC values on day 14/15 compared to that of day 1 was 2.202 and 1.062, respectively, for the crushed tablet and the powder formulations. The oral administration of voriconazole yielded plasma concentrations above the minimum inhibitory concentration for many fungal pathogens common to horses during the 14- or 15-day treatment period. Results of this study suggest the oral administration of 3 mg/kg crushed tablets or 4 mg/kg

powder formulation of voriconazole once daily may be appropriate for the long-term treatment of certain fungal pathogens in horses.

Introduction

Voriconazole is a new, lipophilic, broad-spectrum antifungal drug that was developed from fluconazole, by a substitution of the fluoropyrimidine ring for one of the azole groups and an added α-methyl group. Voriconazole's mode of action is through its inhibition of cytochrome P450-dependent 14α-demethylase and ligation of the iron heme cofactor through a nitrogen atom. This inhibition leads to the depletion of ergosterol and accumulation of 14α-methyl sterol such as lanosterol, affecting the integrity and function of the fungal membrane. Voriconazole is about 250-fold more active against the fungal demethylase enzyme than against the mammalian P450-dependent steroid hormone biosynthesis (Ghannoum & Kuhn, 2002).

Voriconazole is indicated for the treatment of invasive fungal infections by *Aspergillus*, *Candida*, *Scedosporium*, and *Fusarium* species, and it has shown effectiveness for the treatment of various emerging fungal pathogens and for strains that are resistant to prior therapy with an older antifungal drug, such as amphotericin B and fluconazole (Pfizer, 2006). A typical feature of systemic fungal infection is that it needs long-term therapy of weeks to months. Generally the antifungal treatments for invasive infections are initiated by intravenous (IV) administration, but long-term IV therapy can cause effusion or phlebitis, due to the need to puncture veins frequently, and high risk of additional infection and deterioration of the venous reservoir related to daily use. In addition, although voriconazole itself has no impact on renal clearance, the solvent vehicle, sulfobutylether-β-cyclodextrin, in the commercially available IV formulation is

nephrotoxic and should be avoided in patients with renal impairment. Therefore, when the therapy lasts longer than 7-10 days, the need to replace IV with oral administration becomes a high priority. Furthermore, oral therapy should mean much lower costs than IV therapy, due to the savings in intravenous administration and decreased hospital stay or elimination of visiting an outpatient clinic. However, the switch should not mean a risk of reduced efficacy or an increase in adverse effects (Perea *et al*, 2004).

The pharmacokinetic characteristics of an oral formulation of voriconazole have been well established in humans (Purkins *et al*, 2003a). During a phase I clinical trial study, healthy, young, male volunteers were given 2 mg/kg twice-daily, 1.5 mg/kg three-times daily, 4 mg/kg once-daily, 2 mg/kg three-times daily, or 3 mg/kg twice-daily of voriconazole orally. The mean time at the observed peak plasma concentration (T_{max}) ranged from 0.9 to 1.7 hours after single and multiple dosing, indicating that voriconazole was absorbed rapidly after oral administration, and the plasma concentrations showed that there was a biphasic decline in plasma levels once T_{max} had been attained. The mean observed peak plasma concentration (C_{max}) was 0.485. 0.364, 1.570, 0.646, and 1.194 μg/mL on day 1, and 1.007, 1.106, 2.066, 2.184, and 2.356 μg/mL on day 12 after administration of 2 mg/kg twice-daily, 1.5 mg/kg three-times daily, 4 mg/kg once daily, 2 mg/kg three-times daily, and 3 mg/kg twice-daily, respectively. The investigators reported that steady state was attained between day 5 and 7, as there was no significant inter-day difference in C_{max} or area under the plasma concentration-time curve from time zero to the end of the dosing interval ($AUC_{0-\tau}$) after these days. When the data between treatment groups were compared, it was shown that the ratio of $AUC_{0-\tau}$ /Dose values of day 12 to that of day 1 ranged from 2.12 to 4.37, indicating that multiple dosing resulted

in significant accumulation of voriconazole than was predicted from the single-dose data across all dosage groups. Furthermore, a super-proportional increase in $AUC_{0-\tau}$ with respect to the increase in dose was observed. Mean $t_{1/2\beta}$ values were greater after multiple dosing than after the single dose, and the observed mean accumulation ratios ranged from 1.32 to 3.48 for C_{max} and from 2.24 to 6.30 for $AUC_{0-\tau}$.

Voriconazole exhibits dose- and time-dependent nonlinear pharmacokinetics in other animal species as well. In a pre-clinical study, pharmacokinetics of voriconazole after single and multiple IV and oral administrations was examined in male mouse, male and female rat, female rabbit, female guinea pig, and male and female dog (Roffey *et al*, 2003). Comparison of area under the plasma concentration-time curve (AUC) values obtained following oral and IV administration showed that apparent oral bioavailability is high in all animal species, with 81% in mouse, 159% in male rat, 88% in female rat, 87% in rabbit, 75% in guinea pig, and 138% in dog. Multiple dosing of IV and oral voriconazole resulted in an appreciable decrease in AUC compared with single administration in the mouse, rat and dog, and in guinea pig after IV dosing, but a slight increase in AUC values was observed in the rabbit after repeated IV and oral dosing and in the guinea pig after oral administration for five days. Furthermore, a greater than proportional increase in AUC values with increasing dose was evident after both single and multiple oral administrations in the rat and dog, and there was an increase in hepatic cytochrome P450 content in liver from both rats and dogs treated with multiple doses of voriconazole. The volume of distribution (Vd) values following multiple IV dosing were 2.1 and 1.3 L/kg in the male rat and in the dog, respectively. After multiple oral administration, the clearance divided by extent of absorption (Cl/F), as calculated by

dividing the reported AUC value by the dose, is 0.850, 0.929, 0.523, 2.273, 0.310, and 0.115 L/hr/kg in the mouse, male rat, female rat, rabbit, guinea pig, and dog, respectively. Since voriconazole is extensively metabolized in all the species investigated, the authors theorized that the saturation of metabolic clearance is the cause of the nonlinearity, and because of these characteristics, it is not possible to extrapolate the data for accurate prediction of pharmacokinetics in other species.

One study investigating the pharmacokinetics of voriconazole after a single IV dose of 1 mg/kg and 4 mg/kg oral administrations in horses has recently been published (Davis *et al*, 2006). It showed a high oral bioavailability of 135.75%, a long half-life of 13.11 hours after oral dosing, and no adverse effects after administration of a single dose, but a biphasic elimination profile after IV dosing was observed. The authors recommended an oral dose of 4 mg/kg once per day would be adequate for the treatment of most invasive fungal infections. The high bioavailability of the oral formulation of voriconazole in all of the species investigated so far offers the distinct advantage of this drug over most of the older antifungals, because the patients can be easily switched from the IV to the oral form when appropriate. This makes voriconazole more attractive for long-term use.

Another study in horses examined the extent of drug disposition into aqueous humor after repeated topical and single oral administrations of voriconazole (Clode *et al*, 2006). Although topical antifungals are often used for the treatment of eye infections in horses and voriconazole was detected in the aqueous humor after topical administration every 4 hours for 7 doses, infection in the posterior segment of the eye requires systemic therapy, as most topical medications do not penetrate to that area. Furthermore, topical

antifungal drops are often initially applied every hour for the first 4 to 6 hours, then decreased to every 2 to 3 hours for the remaining 24 to 48 hours, then to every 4 hours for the next 24 to 48 hours. Treatment should continue at this frequency if there is no improvement (Hamor and Whelan, 1999). Because of the pain associated with most ocular conditions as well as the strong equine orbicularis oculi muscle, regular topical treatment in most animals becomes difficult to nearly impossible as well as potentially dangerous. In cases in which there is decreased corneal integrity such as deep corneal ulcers or deep corneal lacerations, putting pressure on the globe in an attempt to open the eyelids can result in corneal perforation and permanent loss of vision (Hamor and Whelan, 1999). The investigators reported that after single oral dosing, voriconazole concentration detected in the aqueous humor at 2.5 hours after dosing was 38.8% of that in the plasma at the same time point. However, because of the non-linearity characteristics and the potential for accumulation, multiple-dosing studies were recommended. To our knowledge, the safety and possibility of changes in disposition over time after repeated dosing of voriconazole has not been investigated for use in veterinary medicine in any species.

The objectives of this study were to investigate the plasma concentrations of voriconazole after chronic dosing (7/8 and 14/15 days) by the oral route to horses and to generate data that can be used to develop dosing regimens that will result in plasma concentrations in horses that are within the therapeutic range.

Materials and Methods

Animals

Six clinically normal adult mares were used in this study. The horses weighed between 404 and 514 kg (mean, 470 kg) and were part of The Auburn University Equine Research Program. Results of complete blood count (CBC) and serum biochemical analyses performed on the day before (day -1) and on days 6 (Horse A) or 7 (Horses B, C, D, E, and F) and 13 (Horse A) or 14 (Horses B, C, D, E, and F) of the study were within reference values for our laboratory. All horses were vaccinated and dewormed at least 4 weeks prior to the pharmacokinetic study. Horses were maintained on pasture and acclimated to the barn and housed in individual box stalls twelve hours prior to the performance of the study. Water and grass hay were available *ad libitum*. Horses were placed in stocks 30 minutes prior to dosing and returned to the stalls 2 hours after drug administration on days 1, 3, 7 (Horse A) or 8 (Horses B, C, D, E, and F), and 14 (Horse A) or 15 (Horses B, C, D, E, and F). General physical examination was performed once a day throughout the study. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of Auburn University.

Drug administration

Voriconazole was administered orally to each horse every 24 hours. For the collection of blood samples, 16-gauge 2-inch catheter (Surflo®, Terumo Medical Corp., Somerset, NJ) was aseptically inserted into the right jugular vein and attached to a 32-inch extension with male rotating luer lock and stopcock (Arrow International Inc., Reading, PA); the extension and stopcock were secured to the horse by use of elastic tape (Elastikon®, Johnson & Johnson, New Brunswick, NJ). Lidocaine 2% (Hospira Inc.,

Lake Forest, IL) was used as a local anesthetic to alleviate any pain. For the oral treatment, each dose of voriconazole powder (Maithri Laboratories Pvt. Ltd., Andhra Pradesh, India) or crushed tablets (Vfend®, Pfizer Inc., New York, NY) was mixed with 30 ml of corn syrup (T.J. Blackburn Syrup Works Inc. Jefferson, TX) and administered via a 60-ml syringe (Becton Dickinson, Franklin Lakes, NJ). The syringe was then rinsed with 10 mL of water to ensure adequate delivery of the drug. Horses A and B received 3 mg/kg of voriconazole with crushed tablets orally. Horses C, D, E, and F received 4 mg/kg of powder for oral administration.

Blood collection

Blood samples (7 mL) were taken with 10-mL syringes (Becton Dickison, Franklin Lakes, NJ) via the catheter in the right jugular vein immediately before drug administration (time 0), and after oral administration at 0.083, 0.167, 0.25, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 36 (Horses C, D, E, and F) and 48 hours on days 1, 3, 7 (Horse A) or 8 (Horses B, C, D, E, and F), and 14 (Horse A) or 15 (Horse B, C, D, E, and F). Blood samples were immediately placed in lithium heparin-coated tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ) and kept on ice. Blood samples were also collected at 1.8 hours postdose for the measurement of serum urea and creatinine levels. The catheter inserted in the jugular vein was flushed with heparinized saline solution (7 mL) between subsequent collections of blood samples. At the time of each sample collection, 7 mL of saline solution was injected to flush the heparinized saline solution out of the extension system, 7 mL of blood was withdrawn to remove the saline solution from the extension system, and another 7 mL of blood was collected as sample. Samples were kept on ice and centrifuged at 1,163 × g for 10 minutes (Clay

Adams Brand Compact II Centrifuge, Becton Dickinson, Franklin Lakes, NJ) within 30 minutes of collection, and plasma was removed with disposable polyethylene transfer pipettes (Denville Scientific Inc., Metuchen, NJ) and stored in cryogenic storage vials (Fisher Scientific Inc., Waltham, MA) at -80ºC until assay.

Drug analysis

The plasma samples were assayed using a modified version of a previously published liquid-liquid extraction method followed by reverse-phase high-performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detection (Roffey et. al., 2003). The HPLC system (Dionex, Sunnyvale, CA) consisted of a pump (GP-40), autosampler (AS3500), UV/visible light absorption detector (AD-20), column (Thermo BetaBasic-18, 4.6 mm × 15 cm, 5 µm, Bellefonte, PA), silica guard column (4 mm × 1.25 cm), and computer for data collection and analysis (Gateway 2000, P4D-66, Irvine, CA)

Voriconazole and internal standard (ketoconazole) were eluted with a mobile phase consisting of 35% 0.1 M *N,N,N',N'*-tetramethylethylenediamine (Fisher Scientific Inc., Waltham, MA), pH 7.4, and 65% methanol (Fisher Scientific Inc., Waltham, MA) at a flow rate of 1.0 ml/min at room temperature. The mobile phase was degassed by filtration with 0.45 µm nylon membrane filters (Whatman plc, Middlesex, UK). The injection volume was 100 µL. Voriconazole and internal standard were detected at a wavelength of 254 nm, and the retention times were 3.7 and 13.5 minutes, respectively.

To produce calibration curves, stock solutions of 1 mg/mL voriconazole were prepared by dissolving voriconazole powder in mobile phase. The stock solution was kept refrigerated in a borosilicate glass tube (Fisher Scientific Inc., Waltham, MA), tightly sealed with flexible film (Parafilm®, Alcon Inc., Menasha, WI). Spiking solutions

for the calibration curve were prepared by diluting the stock solution with mobile phase to yield solutions with voriconazole solutions ranging from 1 to 100 µg/mL. By adding 50 µL of each the spiking solution to 500 µL of horse plasma or filtered and distilled (Barnstead Fistreem II, Barnstead International, Dubuque, IA) water, calibration standards with voriconazole concentrations ranging from 0.1 to 10 µg/mL were prepared. Plasma obtained from a horse that was not treated with voriconazole was used as blank control. A new calibration curve was prepared daily before samples were analyzed.

To prepare samples and calibration standards for HPLC, 500 µL of each sample or standard, followed by 15 µL of internal standard, were pipetted into a disposable borosilicate glass tube and vortexed for 10 seconds. The internal standard solution (1 mg/mL) was prepared by dissolving ketoconazole powder (PCCA, Houston, TX) in mobile phase. Voriconazole and internal standard were extracted with 4 mL ethyl acetate (Fisher Scientific Inc., Waltham, MA), followed by 1 mL of 0.2 M sodium borate (J.T. Baker Chemical, Phillipsburg, NJ) at pH 9. The mixture was vortexed for 30 seconds and centrifuged at 4,500 × g for 10 minutes (IEC Clinical Centrifuge, International Equipment Company, Needham Heights, MA). The organic phase was transferred to a separate glass tube and evaporated with nitrogen gas in a 50°C sand bath. The dried residue was reconstituted with 200 µL of the mobile phase and transferred to an HPLC vial and insert (250 µL glass flat bottom, Alltech Associates Inc., Deerfield, IL) for analysis.

Chromatograms were integrated by use of computer software (Dionex PeakNet® 4.11, Sunnyvale, CA). Calibration curves of peak area ratios (voriconazole/internal standard) versus concentration were calculated by the use of linear-regression analysis

(r^2=0.999). The lower limit of detection for voriconazole was 0.1 µg/mL, and the lower limit of quantification was 0.5 µg/mL. The mean intra-day variation was 2.758% and the mean inter-day variation was 8.700%. Recovery was 100%.

Pharmacokinetic analysis

Pharmacokinetic parameters for each horse were determined for oral drug administration. Non-compartmental analysis was performed on the data obtained from oral dosing, using the computer program WinNonlin® 5.0.1 (Pharsight, Cary, NC). Pharmacokinetic parameters measured included the C_{max}, AUC, $t_{1/2\beta}$, Cl, mean residence time (MRT), and volume of distribution as calculated by the area under the plasma concentration-time curve method/extent of absorption (Vd_{area}/F). The elimination rate constant, K_{el}, was estimated from the log-linear terminal decline in the natural logarithm (LN) of the plasma concentration versus time with a weighting of 1/[concentration]. The AUC was measured by the use of the log-trapezoidal method. The area under the plasma concentration-time curve from time zero extrapolated to infinity ($AUC_{0\text{-}inf}$) was calculated by determining the AUC up to the last sample point ($AUC_{0\text{-}LT}$) and then adding the terminal portion estimated from the terminal rate constant. The C_{max} and T_{max} were observed directly from the data. The above and below equations used for the calculation of pharmacokinetic parameters were based on methods described by Gibaldi and Perrier (1982). The $t_{1/2\beta}$ was the LN of 2 (0.693) divided by the terminal rate constant, K_{el}.

Mean residence times were accessed from dividing the area under the moment curve (AUMC) by the AUC. The mean residence time of absorption (MRT_{abs}) is equal to

the difference of $MRT_{oral} - MRT_{IV}$, where MRT_{oral} is the mean residence time following oral administration and was calculated by the use of the following equation:

$$MRT_{oral} = (AUMC_{0-\tau} + \tau\,[AUC_{0-inf} - AUC_{0-\tau}]) / AUC_{0-\tau}$$

where τ is the dosing interval, which equals to 24 hours, and $AUMC_{0-\tau}$ is the AUMC from time zero to the dosing interval. MRT_{IV} is the mean residence time following single intravenous administration obtained from another study by our group, with each horse serving as its own control. The extent of oral absorption, F, was calculated by use of the following equation:

$$F = ([AUC_{oral} \times Dose_{IV}]/[AUC_{IV} \times Dose_{oral}]) \times 100$$

where AUC_{IV} and $Dose_{IV}$ are the AUC and dose, respectively, for the IV route of administration, obtained from a previous single intravenous administration study by our group, with each horse serving as its own control, and AUC_{oral} and $Dose_{oral}$ are the AUC and dose, respectively, for the oral route of administration. The AUC_{oral} used for the calculation of F was AUC_{0-inf} on day 1 and AUC_{0-24} on the other days. The accumulation ratio, R, on days 3, 7 or 8, and 14 or 15 is the quotient of AUC_{0-24} on the day 3, 7, 8, 14, or 15 divide by the AUC_{0-24} obtained from day 1.

The observed Cl/F on day 1 is equal $Dose/AUC_{0-inf}$, whereas the Cl/F on the other days is equal to the $Dose/AUC_{0-24}$ obtained on that day. Vd_{area}/F was calculated according to the formula:

$$Vd_{area}/F = (Cl/F) / K_{el}$$

Average steady state plasma voriconazole concentration (C_{ss}) on days 3, 7 or 8, and 14 or 15 was calculated from AUC_{0-24} divided by τ, and the trough plasma concentration (C_{min}) was the average of the concentrations observed at 0 and 24 hours on the respective days.

Statistical comparisons

Effects of dose/dosage form and dosing day on the pharmacokinetic estimates were examined by general linear model (GLM) procedures with SAS 9.1 (SAS Institute Inc, Cary, NC). Parameters and values were evaluated with natural logarithm transformation and significance level of $P<0.05$.

Results

Horse E developed noticeable urticarial skin reaction on day 2 and was given 15 mL of tripelennamine HCl (Re-Covr®, Fort Dodge Animal Health, Fort Dodge, IA) at 20 mg/mL intramuscularly. Upon further examination, the horse was deemed fit to continue with the study, and was provided with a daily injection of tripelennamine at two hours prior to voriconazole dosing. Of the five remaining horses, no adverse reaction was observed during the daily physical examination. Results of CBC and serum chemical analyses performed on days 7 (Horse A), 8 (Horses B, C, D, E, and F), 14 (Horse A), and 15 (Horses B, C, D, E, and F) were within reference values for all six horses.

The pharmacokinetic values obtained after a single dose (Table 5.2) show that the mean extent of absorption (F) was essentially complete for the powder formulation. The mean F after dosing with crushed tablets was significantly ($P=0.004$) lower at 57.88%. There was no significant difference ($P=0.087$) in the mean $t_{1/2\beta}$ values between the two formulations on day 1, and their ranges overlap (8.847 to 10.118 hours for the crushed tablets, 10.051 to 17.337 hours for the powder). Both formulations were rapidly absorbed, with drug concentrations detected in the plasma at 5 minutes after dosing in all six horses. No significant difference ($P=0.438$) between the mean T_{max} values following oral administration of crushed tablets and powder formulations was observed, despite the

extreme variation (105.16%) from the data obtained after dosing of the powder formulation.

Oral administration for a 3-day period results in similar mean C_{max} values between the two formulations (Table 5.3). There were also no significant differences in the mean F and AUC_{0-24}/Dose values between the two formulations. The apparent extent of absorption values of both oral formulations are near completion at around 100%. The MRT_{oral} ranged from 20.35 to 35.20 hours following administration of the crushed tablet, and 18.64 to 28.81 hours after oral powder dosing. The mean accumulation factor values calculated from the AUC obtained on day 3 and compared to that of day 1 was 2.338 and 1.718 for the crushed tablet and powder formulations, respectively. A multiple intravenous dosing experiment was not performed, but it was assumed that pharmacokinetic parameters after single intravenous administration was similar to those after multiple dosing intravenously, and the MRT obtained from the same six horses following single IV dose administration (MRT_{IV}, ranged from 12.45 to 22.16 hours) was used for the calculation of MRT_{abs} values. The mean MRT_{abs} for the crushed tablets and powder formulations were 15.282 and 4.343 hours, respectively.

It was assumed from the $t_{1/2\beta}$ on day 1 that voriconazole had reached steady state by day 3 or 4. Therefore, the pharmacokinetic values obtained from Horse A on day 7 and from Horse B on day 8, both horses dosed with 3 mg/kg every 24 hours with crushed tablet, were grouped together and the mean values were obtained. After 7 or 8 days of once daily voriconazole administration orally, the mean C_{max} and AUC_{0-24} was higher in the horses that received 3 mg/kg of voriconazole in the crushed tablet form, compared to those that received 4 mg/kg once daily of the powder. The mean AUC_{0-24}/Dose values

were 12.930 and 6.009 kg-hr/L for the crushed tablet and the powder formulations, respectively. The Cl/F values, as calculated by the inverse of the AUC_{0-24}/Dose, was found to be significantly different (P=0.008) between the two formulations on day 7/8. The mean Cl/F value obtained after administration of crushed tablets on day 7/8 was also found to be significantly different (P=0.027) from that of day 1, but not when compared with day 3. In addition, the mean Cl/F values obtained following repeated power dosing showed significant difference when compared day 8 to day 1 and also to 3 (P=0.004). The mean $t_{1/2\beta}$ value of the crushed tablet formulation on day 7/8 (18.26 hours) was not significantly different (P=0.088) when compared to that of day 1 (9.482 hours) and with that of day 3 (19.83 hours). Similarly, as with the tablet formulation, there was no significant difference observed in the mean $t_{1/2\beta}$ values of the powder formulation between days 1 and 3 and between days 1 and 8.

The pharmacokinetic parameters obtained after 15 days of voriconazole powder dosing showed high inter-individual variation (13.66 to 174.85%). The mean Cl/F and Vd_{area}/F were 0.1832 L/hr/kg and 1.776 L/kg, respectively, for the four horses that received the powder formulation daily for 15 days, and 0.1072 L/hr/kg and 1.554 L/kg, respectively, for the two horses that received the crushed tablet formulation. The mean Cl/F value on day 15 obtained following crushed tablet administration of voriconazole was not significantly different to the three previously sampled days, but mean Cl/F value from the horses that received the powder formulation showed significant differences (P=0.004) between day 15 and that of the three earlier sampled periods. When comparing the AUC values on day 15 to that of day 1, the mean accumulation factor was 2.202 for the crushed tablet formulation on day 14/15, and 1.062 for the powder

formulation on day 15. The plasma concentration versus time plots (Figures 5.2 and 5.4) show that amount of voriconazole in plasma remained above 0.1 μg/mL over the 15-day dosing period for both formulations. Using the pharmacokinetic values obtained after a single-dose administration, the predicted plasma concentration at steady state (C_{pred}) was calculated by the equation:

$$C_{pred} = (F \times Dose) / (Cl \times \tau)$$

The C_{pred} for day 14/15 was 0.5446 and 0.3262 μg/mL, respectively, for Horses A and B that received the crushed tablets, and the observed average steady state plasma voriconazole concentration (C_{ss}) on day 15 was 1.054 and 1.304 μg/mL, respectively. However, the mean ± SD of mean C_{pred} based on the pharmacokinetic values obtained on day 1 for the horses that received the powder formulation was 1.7202 ± 0.3807 μg/mL, but the observed C_{ss} on day 15 was 0.9906 ± 0.3033 μg/mL. Thus for crushed tablets, day 15 voriconazole plasma concentrations were greater than expected from a single dose while for subjects receiving the powder, voriconazole plasma concentrations on day 15 were less than predicted by a single dose.

Discussion

Due to possibilities of variable absorption, autoinduction of metabolism, and saturable elimination that have been observed in certain animal species but not in others, it would be difficult to extrapolate pharmacokinetic parameters using allometric scaling to accurately predict voriconazole disposition across species. This is especially true during multiple dosing, when pharmacokinetics of this drug is known to alter compared to single dosing. In addition, pharmacokinetic studies of oral formulations of voriconazole to different veterinary species may be further complicated by the

administration of the drug to animals with different digestive anatomies and physiologies. Only recently was one study published on the pharmacokinetics of voriconazole after single administration to healthy horses (Davis *et al*, 2006).

In the present study, the disposition and absorption kinetics of voriconazole during multiple dosing was determined in six healthy horses. The results indicated that significant differences in half-life and Cl/F values existed between single and repeated dosing, especially in horses that received the powder formulation repeatedly. The mean $t_{1/2\beta}$ (15.284 ± 3.497 hours), $AUC_{0\text{-inf}}$ (40.24 ± 8.92 µg-hr/mL), and Cl/F (0.1032 ± 0.0228 L/hr/kg) determined on day 1 following 4 mg/kg of powder administered orally were similar to those reported previously (13.11 hours, 50.81µg-hr/mL, 0.079 L/hr/kg, respectively) after single oral dosing in mature horses (Davis *et al*, 2006). However, the mean T_{max} value (8.380 ± 8.813 hours) is longer than the observed value of 2.92 ± 1.20 hours in fasting horses found by Davis *et al* (2006). In the present study, food was not withheld in order to better simulate how voriconazole would be administered in practice.

Based on the half-life values obtained on day 1, it was predicted that voriconazole would reach steady state on day 3 or 4 in horses. Changes in $t_{1/2\beta}$, Cl/F, and F were examined between days and dosage forms. Considerable variations in plasma concentrations and pharmacokinetic parameters were observed among the horses that received the two different doses and dosage forms and among the four different sampled periods. When the two dose groups were combined for analysis, no particular trend in the mean Cl/F values was noted from day 1 to day 15. There was also no day-dependent trend observed in the mean $t_{1/2\beta}$ values when the four sampled days were compared, except that the mean $t_{1/2\beta}$ value on day 15 was significantly different (P=0.022) from the

other three sampled days. However, when day effects were examined within each dosing group, additional significant differences in pharmacokinetic parameters were observed over the four sampled periods. The directions of these trends were different (Figure 5.8), depending on whether the horses received 3 mg/kg of voriconazole as tablets or 4 mg/kg as powder.

For the two horses that received the crushed tablet formulation, an increasing trend was observed in the mean AUC_{0-24}/Dose values over 15 days of dosing (Figure 5.7). Their mean Cl/F value, as calculated by the amount of the administered dose and AUC_{0-24} values obtained, on day 7/8 (0.07942 L/hr/kg) was 54% lower than and significantly different from the mean value obtained after a single dose, and that of day 14/15 was 38% less than day 1 but was not significantly different. In contrast, with the four horses that received the 4 mg/kg voriconazole powder, the mean Cl/F values on days 8 and 15 were approximately 60-80% greater than those observed on days 1 and 3.

We do not have an immediate explanation for the conflicting results in the mean Cl/F values of voriconazole between horses that receive the powder and those that received the crushed tablet formulation. Both formulations were mixed with 30 mL of syrup and all six horses were place in the stocks and returned to the stalls at approximately the same time periods. Voriconazole reportedly alters the pharmacokinetics of a large number of drugs. The majority of these interactions arise because voriconazole is both a substrate and inhibitor of the cytochrome P450 enzymes (Leveque *et al*, 2006). Because of this property, clearance of voriconazole may be increased when coadministered with another drug that is also extensively metabolized by the cytochrome P450 pathway. However, none of the drugs used for anesthesia or for the

treatment of allergic reaction in this study were reported to affect the absorption of voriconazole from the gastrointestinal tract or interfere with hepatic metabolic function. The increase in the mean Cl/F values over 15 days of treatment from the horses that received the powder formulation could be attributed to acceleration of biotransformation, or autoinduction. A similar increasing trend in Cl/F values was observed in the mouse, rat, and dog after multiple oral dosing (Roffey *et al*, 2003). There was an increase in hepatic cytochrome P450 content in liver from both rats and dogs treated with multiple doses of voriconazole, and the enzyme induction appeared to be dose-related (Roffey *et al*, 2003). These effects are completely reversible, and cytochrome P450 concentrations in rats that underwent a 1-month recovery period returned to levels that were similar to those of control animals.

On the other hand, the decreased mean Cl/F values of voriconazole on days 7/8 and 14/15, compared to day 1, in the horses that received 3 mg/kg of crushed tablets, as observed in the rabbit, guinea pig, and human after repeated oral dosing, could also be attributed to inhibition of metabolism of the parent drug by one or more of its metabolites (Roffey *et al*, 2003; Purkins *et al*, 2003a). The exact mechanisms involved in the elimination of voriconazole in the horse have not been determined, but in all species investigated so far, voriconazole is eliminated predominantly by metabolism. In human, mouse, rat, guinea pig, and dog, the major elimination involves N-oxidation followed by glucuronidation of the metabolite, and in the rabbit, the major metabolite was attributed to hydroxylation of the fluoropyrimidine ring of the parent compound (Hyland *et al*, 2003; Roffey *et al*, 2003). Hepatic metabolism is thought to be a saturable and rate-limiting process in the elimination of voriconazole in humans and other animal species,

and a convex plasma profile that is indicative of capacity-limited elimination was observed in all animal species in the pre-clinical study. In horses, no clear evidence of saturable metabolism from the post-distribution phase of the plasma concentration versus time curve has been observed.

It is interesting that the trends in the mean Cl/F values of dosing appear to be dosage form- or dose-dependent. Because Cl/F is a function of both clearance and extent of absorption, changes in either pharmacokinetic parameter could produce the increasing or decreasing trends in Cl/F values. Half-life is also a function of clearance. In horses administered 3 mg/kg of crushed voriconazole tablets, $t_{1/2\beta}$ values were not significantly different over the 14/15 days of dosing. With horses given 4 mg/kg powder, the mean $t_{1/2\beta}$ value on day 15 was significantly shorter than the three previously sampled days. The decrease in the mean $t_{1/2\beta}$ value obtained on day 15, compared to that of day 1, 3, and 8, further supports that there was no evidence of saturable metabolism observed in horses. However, the $t_{1/2\beta}$ values calculated for the first three sampled periods were estimated from plasma concentration-time points up to the dosing period of 24 hours, whereas those obtained on day 15 were determined using plasma concentration-time points up to 48 hours postdose and thus might be a more accurate representation of voriconazole $t_{1/2\beta}$ values.

An increase or decrease in the mean Cl/F values without significant changes in $t_{1/2\beta}$ or k_{el} values could suggest that Cl/F differences noted between days may be related to alterations in F values during the 15-day dosing period. The extent of absorption, F, was calculated for each dosing period, with the assumption that other pharmacokinetic parameters such as clearance, volume of distribution, and $t_{1/2\beta}$ values remained constant

throughout the 15 days. For horses that received 3 mg/kg of crushed voriconazole tablets, the mean F value on day 7/8 was 121% greater than day 1. The opposite trend was noted for horses that received 4 mg/kg of voriconazole powder per day, with the mean F value being approximately 40% less on days 8 and 15, compared to days 1 and 3. The mean F values was significantly different between the dosage forms on day 1, but was not significantly different on days 3 through 15. Since $t_{1/2\beta}$ appeared unchanged and $t_{1/2\beta}$ reflects changes in Cl and Vd, it is most likely that the changes in Cl/F and thus changes in C_{ss} values over the 15 days of dosing were related to varying and changing F and not Cl, and this would reflect alterations in the voriconazole elimination or metabolism capabilities.

Variable absorption appears to explain the large variability and trends in plasma concentrations and pharmacokinetic parameters over the 15 days. Despite the small number of subjects used and only 15 days of treatment were sampled, it seems that the extent of absorption, F, improved with continuing dosing of the crushed tablets, but decreased with ongoing dosing of the powder. Although the extent of absorption values appear to change and are different depending on the day of sampling and dosage form, the absorption rates were too variable to detect any day or dosage form effects. Even though the mean T_{max} values were also highly variable and ranged from 0.8750 to 12.158 hours, there was no significant difference observed between days or between formulations. Because T_{max} values can serve as an indicator of change in absorption rates in bioequivalence studies, it is assumed that there was probably no significant difference in the rate of absorption between the two oral voriconazole formulations in horses

(Basson *et al*, 1996). Furthermore, the mean values of T_{max} and MRT_{abs} did not seem to be affected by the type of dosing material or the length of the dosing period.

The large inter-subject variability in plasma concentrations after oral voriconazole dosing could be attributed to free access to hay, possibly due to drug binding to hay and its subsequent release back into the gastrointestinal fluid for absorption into the systemic compartment (Maitho *et al*, 1986). In addition, the marked difference in the plasma voriconazole concentrations between the two formulations may be a result of variable absorption from the gastrointestinal tract, due to possible differences in dissolution rates from variable drug particle sizes and the presence of various inactive ingredients that can cause slower release of active components from the tablet formulation (Yu *et al*, 2002). Furthermore, the corn syrup, a carrier substance commonly used for administration of oral drugs in equine medicine, might have also contributed to the high inter-individual variation, due to the possibility of the syrup hindering the absorption by interaction with voriconazole or with excipients in the tablet formulation. In healthy human volunteers, the multiple administration of oral voriconazole with food and high fat meals reduced bioavailability compared with fasting (Purkins *et al*, 2003b). Another source of inter-subject variation in pharmacokinetic parameters could be intrinsic differences in the metabolizing rate by hepatic enzymes.

The high mean Vd_{area}/F values of voriconazole (2.385 and 2.228 L/kg, for crushed tablets and powder, respectively, on day 1, and 1.554 and 1.776 L/kg, respectively, on day 14/15) after oral administration indicate that the drug is widely distributed in the extravascular tissues.

One horse in our study developed urticarial skin reactions. The reported adverse events of voriconazole in humans are uncommon and include visual disturbances, hepatotoxicity, headache, nausea and vomiting, diarrhea, and abdominal pain. Skin rashes occur in about 6% of patients (Lazarus *et al*, 2002; Purkins *et al*, 2002). In horses, as in humans, inter-individual variation in Cl/F values and possible hepatic autoinduction associated with continued administration indicate the need to monitor liver enzyme values and possibly voriconazole plasma concentration during long term administration. On the basis of theses observed increases in Cl/F after oral dosing with powder in horses, it would appear that dosage adjustment may be required during the first 14 days of treatment. For this reason, further studies in which doses are adjusted weekly or monthly on the basis of plasma concentration and liver enzyme values observed over several months are needed to confirm the dose recommended in the present study. The total protein, albumin, sorbitol dehydrogenase (SDH), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), total bilirubin, creatinine, bile acids levels and red blood cell (RBC) counts are within normal ranges as observed on days -1, 6/7, and 13/14, and there was no age-, breed-, or size-dependent trends among the horses in the serum levels that assess liver or kidney functions.

In conclusion, the results from this study with long-term voriconazole dosing showed that the voriconazole concentration in the plasma is highly variable over two weeks of dosing, and the variability was probably related to changes in extent of absorption and is dependent upon dosage form and administration. Future studies may require larger number of subjects for each dose and dosage form. The findings from this study may indicate oral dosing regimens that are useful for extended treatment. Because

of the long mean $t_{1/2\beta}$ value of 7.141 to 10.054 hours in horses on day 14/15, doses can be administered orally once daily. A potentially therapeutic voriconazole plasma concentration of > 0.1 μg/mL was maintained in all six horses throughout the treatment period. Voriconazole levels achieved and maintained in plasma exceed the minimum inhibitory concentration (MIC) values of susceptible opportunistic fungi commonly found in sites of deep and invasive infections in horses. As voriconazole is fungistatic against *Candida* and *Cryptococcus* species, it is important that blood concentrations above MIC values are achieved as long as possible. However, the MIC ranges are wide, and the infections caused by some strains would be unlikely to respond to voriconazole doses of 3 mg/kg/day of crushed tablets or 4 mg/kg/day of powder, and thus a higher dosage might be needed.

Based on the *in vitro* susceptibility pattern of the common fungal pathogens that affect horses, the amount of protein binding, and an average target plasma concentration of 1.5 to 2.5 μg/mL, a maintenance dose can be calculated from the mean Cl/F value obtained on day 14/15 by the following equation:

$$\text{Maintenance dose of voriconazole} = C_{desired} \times Cl/F \times \tau.$$

where $C_{desired}$ is the average target plasma concentration. For an oral dosing regimen with crushed tablets, 3.9 to 6.4 mg/kg/day appear appropriate. In the case of powder, higher oral daily doses of 6.6 to 11.0 mg/kg/day may need to be recommended. Because of observed changes and high inter-subject variations in pharmacokinetic parameters in horses, changes in the dosage regimen during long term administration might be needed, and due to possible hepatotoxicity, monitoring of liver enzyme levels is recommended.

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TABLE 5.1 - Demographics of study subjects.

		Horse					
		A	B	C	D	E	F
Age	(years)	20	19	9	11	23	16
Sex		F	F	F	F	F	F
Breed		AQH	AQH	Arabian	Arabian	TWH	APH
Weight	(kg)	498.6	500.0	472.7	429.5	404.5	513.6

F = Female; AQH = American Quarter Horse; TWH = Tennessee Walking Horse; APH = American Paint Horse.

TABLE 5.2 - Noncompartmental pharmacokinetic parameters of voriconazole given orally (3 mg/kg crushed tablets or 4 mg/kg powder) to horses on day 1.

		Horse									
		A	B	C	D	E	F	$Mean_{A-B}$	$Mean_{C-F}$	SD_{C-F}	CV $\%_{C-F}$
Dose	(mg/kg)	3	3	4	4	4	4	3	4	NA	NA
Formulation		Tablet	Tablet	Powder	Powder	Powder	Powder	Tablet	Powder	NA	NA
C_{max}	(µg/mL)	0.8360	0.6670	1.9240	2.0110	1.3610	1.4690	0.7515	1.6913	0.3240	19.16
T_{max}	(hr)	11.917	10.000	1.000	0.500	16.000	16.020	10.959	8.380	8.813	105.16
AUC_{0-24}	(µg-hr/mL)	15.75	11.19	24.91	21.12	22.27	22.87	13.47	22.79	1.59	6.96
AUC_{0-inf}	(µg-hr/mL)	20.43	15.12	33.12	32.00	46.86	48.96	17.77	40.24	8.92	22.16
AUC_{0-24}/Dose	(kg-hr/L)	5.250	3.730	6.228	5.280	5.568	5.718	4.490	5.698	0.397	6.96
AUC_{0-inf}/Dose	(kg-hr/L)	6.808	5.040	8.280	8.000	11.715	12.240	5.924	10.059	2.229	22.16
F	(%)	63.99	51.77	102.00	109.93	90.53	109.80	57.88	103.07	9.14	8.87
K_{el}	(hr^{-1})	0.07840	0.06850	0.06900	0.04140	0.04000	0.04070	0.07345	0.04778	0.01416	29.64
$t_{1/2\beta}$	(hr)	8.847	10.118	10.051	16.732	17.337	17.016	9.482	15.284	3.497	22.88
MRT_{oral}	(hr)	18.04	19.36	18.10	21.21	40.18	41.55	18.70	30.26	12.32	40.73
MRT_{abs}	(hr)	5.497	6.907	0.822	4.259	19.986	19.395	6.202	11.115	10.003	90.00
Cl/F	(L/hr/kg)	0.1469	0.1984	0.1208	0.1250	0.0854	0.0817	0.1726	0.1032	0.0228	22.13
Vd_{area}/F	(L/kg)	1.873	2.897	1.750	3.019	2.134	2.007	2.385	2.228	0.551	24.75

C_{max} = peak plasma concentration; T_{max} = time of peak plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours; AUC_{0-inf} = area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC_{0-24}/Dose = area under the plasma concentration-time curve from time zero to 24 hours/dose; AUC_{0-inf}/Dose = area under the plasma concentration-time curve from time zero extrapolated to infinity/dose; F = extent of absorption; K_{el} = elimination rate constant; $t_{1/2\beta}$ = elimination half-life; MRT_{oral} = mean residence time following oral administration; MRT_{abs} = mean residence time of absorption; Cl/F = total body clearance/extent of absorption; Vd_{ss}/F = volume of distribution at steady state/extent of absorption; Vd_{area}/F = volume of distribution as calculated by the area under the plasma concentration-time curve method/extent of absorption; NA = not applicable; $Mean_{A-B}$ = average of values obtained for Horses A and B; $Mean_{C-F}$ = average of values obtained for Horses C, D, E, and F; SD_{C-F} = standard deviation of values obtained from Horses C, D, E, and F; CV%C-F = percent coefficient of variation of values obtained from Horses C, D, E, and F.

TABLE 5.3 - Noncompartmental pharmacokinetic parameters of voriconazole given orally (3 mg/kg crushed tablets or 4 mg/kg powder) to horses on day 3.

		Horse									
		A	B	C	D	E	F	$Mean_{A-B}$	$Mean_{C-F}$	SD_{C-F}	$CV\ \%_{C-F}$
Dose	(mg/kg)	3	3	4	4	4	4	3	4	NA	NA
Formulation		Tablet	Tablet	Powder	Powder	Powder	Powder	Tablet	Powder	NA	NA
C_{max}	(µg/mL)	1.959	2.570	2.110	2.722	2.365	2.839	2.265	2.509	0.334	13.30
T_{max}	(hr)	1.0000	0.7500	2.0000	1.0000	10.0000	3.9830	0.8750	4.2458	4.0315	94.95
AUC_{0-24}	(µg-hr/mL)	29.76	31.18	31.65	32.46	39.38	52.47	30.47	38.99	9.63	24.71
AUC_{0-24}/Dose	(kg-hr/L)	9.920	10.393	7.913	8.115	9.845	13.118	10.157	9.748	2.408	24.71
F	(%)	93.2	106.8	97.5	111.5	76.1	117.7	100.0	100.7	18.5	18.33
R		1.890	2.786	1.271	1.537	1.768	2.294	2.338	1.718	0.435	25.33
K_{el}	(hr^{-1})	0.02720	0.04880	0.06010	0.04480	0.04780	0.04340	0.03800	0.04903	0.00761	15.52
$t_{1/2\beta}$	(hr)	25.45	14.22	11.53	15.46	14.49	15.98	19.83	14.37	1.99	13.86
MRT_{oral}	(hr)	35.20	20.35	18.64	20.85	28.81	25.66	27.78	23.49	4.60	19.59
MRT_{abs}	(hr)	22.660	7.905	1.357	3.896	8.616	3.502	15.282	4.343	3.060	70.46
Cl/F	(L/hr/kg)	0.10081	0.09622	0.12638	0.12323	0.10157	0.07623	0.09851	0.10685	0.02320	21.71
Vd_{area}/F	(L/kg)	3.706	1.972	2.103	2.751	2.125	1.757	2.839	2.184	0.414	18.95

C_{max} = peak plasma concentration; T_{max} = time of peak plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours; AUC_{0-24}/Dose = area under the plasma concentration-time curve from time zero to 24 hours/dose; F = extent of absorption; R = accumulation factor; K_{el} = elimination rate constant; $t_{1/2\beta}$ = elimination half-life; MRT_{oral} = mean residence time following oral administration; MRT_{abs} = mean residence time of absorption; Cl/F = total body clearance/extent of absorption; Vd_{ss}/F = volume of distribution at steady state/extent of absorption; Vd_{area}/F = volume of distribution as calculated by the area under the plasma concentration-time curve method/extent of absorption; NA = not applicable; $Mean_{A-B}$ = average of values obtained for Horses A and B; $Mean_{C-F}$ = average of values obtained for Horses C, D, E, and F; SD_{C-F} = standard deviation of values obtained from Horses C, D, E, and F; $CV\%_{C-F}$ = percent coefficient of variation of values obtained from Horses C, D, E, and F.

TABLE 5.4 - Noncompartmental pharmacokinetic parameters of voriconazole given orally (3 mg/kg crushed tablets or 4 mg/kg powder) on day 7 (Horse A) or 8 (Horses B, C, D, E, and F).

		Horse									
		A	B	C	D	E	F	$Mean_{A-B}$	$Mean_{C-F}$	SD_{C-F}	$CV\ \%_{C-F}$
Dose	(mg/kg)	3	3	4	4	4	4	3	4	NA	NA
Formulation		Tablet	Tablet	Powder	Powder	Powder	Powder	Tablet	Powder	NA	NA
C_{max}	(µg/mL)	2.032	3.536	1.437	2.508	1.799	1.559	2.784	1.826	0.479	26.24
T_{max}	(hr)	2.000	0.733	2.933	1.000	0.500	0.250	1.367	1.171	1.216	103.82
AUC_{0-24}	(µg-hr/mL)	32.51	45.07	19.58	28.45	23.20	24.91	38.79	24.04	3.69	15.34
AUC_{0-24}/Dose	(kg-hr/L)	10.837	15.023	4.895	7.113	5.800	6.228	12.930	6.009	0.922	15.34
F	(%)	101.85	154.32	60.30	97.73	44.82	55.86	128.09	64.68	22.98	35.52
R		2.064	4.028	0.786	1.347	1.042	1.089	3.046	1.066	0.230	21.56
K_{el}	(hr^{-1})	0.04550	0.03260	0.03810	0.03760	0.07700	0.08580	0.03905	0.05963	0.02540	42.60
$t_{1/2\beta}$	(hr)	15.24	21.29	18.21	18.45	9.01	8.08	18.26	13.44	5.66	42.15
MRT_{oral}	(hr)	22.88	29.14	43.82	25.60	16.70	17.24	26.01	25.84	12.66	48.98
MRT_{abs}	(hr)	10.344	16.691	26.537	8.647	-3.486	-4.917	13.517	6.695	14.560	217.47
Cl/F	(L/hr/kg)	0.09228	0.06656	0.20429	0.14060	0.17241	0.16058	0.07942	0.16947	0.02667	15.74
Vd_{area}/F	(L/kg)	2.028	2.042	5.362	3.739	2.239	1.872	2.035	3.303	1.593	48.22

C_{max} = peak plasma concentration; T_{max} = time of peak plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours; AUC_{0-24}/Dose = area under the plasma concentration-time curve from time zero to 24 hours/dose; F = extent of absorption; R = accumulation factor; K_{el} = elimination rate constant; $t_{1/2\beta}$ = elimination half-life; MRT_{oral} = mean residence time following oral administration; MRT_{abs} = mean residence time of absorption; Cl/F = total body clearance/extent of absorption; Vd_{ss}/F = volume of distribution at steady state/extent of absorption; Vd_{area}/F = volume of distribution as calculated by the area under the plasma concentration-time curve method/extent of absorption; NA = not applicable; $Mean_{A-B}$ = average of values obtained for Horses A and B; $Mean_{C-F}$ = average of values obtained for Horses C, D, E, and F; SD_{C-F} = standard deviation of values obtained from Horses C, D, E, and F; $CV\%_{C-F}$ = percent coefficient of variation of values obtained from Horses C, D, E, and F.

TABLE 5.5 - Noncompartmental pharmacokinetic parameters of voriconazole given orally (3 mg/kg crushed tablets or 4 mg/kg powder) on day 14 (Horse A) or 15 (Horses B, C, D, E, and F).

		Horse									
		A	B	C	D	E	F	$Mean_{A-B}$	$Mean_{C-F}$	SD_{C-F}	$CV\ \%_{C-F}$
Dose	(mg/kg)	3	3	4	4	4	4	3	4	NA	NA
Formulation		Tablet	Tablet	Powder	Powder	Powder	Powder	Tablet	Powder	NA	NA
C_{max}	(µg/mL)	1.328	2.625	0.992	3.262	1.758	1.557	1.977	1.892	0.969	51.21
T_{max}	(hr)	24.033	0.283	24.000	0.750	1.000	0.750	12.158	6.625	11.584	174.85
AUC_{0-24}	(µg-hr/mL)	25.30	31.30	14.42	31.36	22.18	27.14	28.30	23.78	7.28	30.61
AUC_{0-LT}	(µg-hr/mL)	44.22	39.74	24.01	37.50	28.20	39.06	41.98	32.19	7.26	22.56
AUC_{0-24}/Dose	(kg-hr/L)	8.433	10.433	3.605	7.840	5.545	6.785	9.433	5.944	1.820	30.61
F	(%)	79.26	107.17	44.41	107.73	42.85	60.87	93.22	63.96	30.29	47.36
R		1.606	2.797	0.579	1.485	0.996	1.187	2.202	1.062	0.380	35.75
K_{el}	(hr^{-1})	0.06950	0.06840	0.13140	0.07290	0.10540	0.09620	0.06895	0.10148	0.02419	23.84
$t_{1/2\beta}$	(hr)	9.972	10.136	5.276	9.509	6.573	7.204	10.054	7.141	1.771	24.80
MRT_{oral}	(hr)	21.07	14.72	19.47	13.02	14.58	18.40	17.89	16.37	3.06	18.72
MRT_{abs}	(hr)	8.527	2.266	2.190	-3.927	-5.613	-3.756	5.397	-2.777	3.416	-123.02
Cl/F	(L/hr/kg)	0.1186	0.0958	0.2774	0.1276	0.1803	0.1474	0.1072	0.1832	0.0665	36.30
Vd_{area}/F	(L/kg)	1.706	1.401	2.111	1.750	1.711	1.532	1.554	1.776	0.243	13.66

C_{max} = peak plasma concentration; T_{max} = time of peak plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours; AUC_{0-LT} = area under the plasma concentration-time curve from time zero to last observed time; AUC_{0-24}/Dose = area under the plasma concentration-time curve from time zero to 24 hours/dose; F = extent of absorption; R = accumulation factor; K_{el} = elimination rate constant; $t_{1/2\beta}$ = elimination half-life; MRT_{oral} = mean residence time following oral administration; MRT_{abs} = mean residence time of absorption; Cl/F = total body clearance/extent of absorption; Vd_{ss}/F = volume of distribution at steady state/extent of absorption; Vd_{area}/F = volume of distribution as calculated by the area under the plasma concentration-time curve method/extent of absorption; NA = not applicable; $Mean_{A-B}$ = average of values obtained for Horses A and B; $Mean_{C-F}$ = average of values obtained for Horses C, D, E, and F; SD_{C-F} = standard deviation of values obtained from Horses C, D, E, and F; $CV\%_{C-F}$ = percent coefficient of variation o

TABLE 5.6. Comparative pharmacokinetic values between day 1 and days 3, 7 (Horse A) or 8 (Horses B, C, D, E, and F), and 14 (Horse A) or 15 (Horses B, C, D, E, and F) of repeated treatment (3 mg/kg crushed tablets or 4 mg/kg powder) once every 24 hours.

		Horse								
		A	B	C	D	E	F	$Mean_{A-B}$	$Mean_{C-F}$	SD_{C-F}
Dose	(mg/kg)	3	3	4	4	4	4	3	4	NA
Formulation		Tablet	Tablet	Powder	Powder	Powder	Powder	Tablet	Powder	NA
Day 1										
C_{max}	(µg/mL)	0.8360	0.6670	1.9240	2.0110	1.3610	1.4690	0.7515^{cde}	1.6913^{c}	0.3240
Cl/F	(L/hr/kg)	0.1469	0.1984	0.1208	0.1250	0.0854	0.0817	0.1726^{d}	0.1032^{de}	0.0228
C_{pred}	(µg/mL)	0.5446	0.3262	1.4076	1.4657	1.7677	2.2399	0.4354	1.7202	0.3807
T_{max}	(hr)	11.917	10.000	1.000	0.500	16.000	16.020	10.959	8.380	8.813
F	(%)	63.99	51.77	102.00	109.90	90.53	109.80	57.88^{d}	103.07^{ade}	9.13
$t1/2_{\beta}$	(hr)	8.847	10.120	10.050	16.730	17.340	17.020	9.484	15.284^{e}	3.499
Day 3										
C_{max}	(µg/mL)	1.959	2.570	2.110	2.722	2.365	2.839	2.265^{b}	2.509^{b}	0.334
C_{min}	(µg/mL)	0.7797	0.7153	0.7686	0.6944	1.2429	1.3746	0.7475	1.0201^{e}	0.3389
Cl/F	(L/hr/kg)	0.10081	0.09622	0.12638	0.12323	0.10157	0.07623	0.09851	0.10685^{de}	0.02320
C_{ss}	(µg/mL)	1.240	1.299	1.319	1.353	1.641	2.186	1.270	1.625	0.401
T_{max}	(hr)	1.0000	0.7500	2.0000	1.0000	10.0000	3.9830	0.8750	4.2458	4.0315
F	(%)	93.2	106.8	97.5	111.5	76.1	117.7	100.0	100.7^{de}	18.5
$t1/2_{\beta}$	(hr)	25.45	14.22	11.53	15.46	14.49	15.98	19.83	14.37^{e}	1.99
Day 7/8										
C_{max}	(µg/mL)	2.032	3.536	1.437	2.508	1.799	1.559	2.784^{b}	1.826	0.479
C_{min}	(µg/mL)	0.7902	1.1777	0.7887	0.6863	0.6961	0.8395	0.9839	0.7526	0.0740
Cl/F	(L/hr/kg)	0.09228	0.06656	0.20429	0.14060	0.17241	0.16058	0.07942^{b}	0.16947^{abc}	0.02667
C_{ss}	(µg/mL)	1.355	1.878	0.816	1.185	0.967	1.038	1.616	1.001	0.154
T_{max}	(hr)	2.000	0.733	2.933	1.000	0.500	0.250	1.367	1.171	1.216
F	(%)	101.80	154.30	60.30	97.70	44.82	55.86	128.09^{b}	64.68^{bc}	23.00
$t1/2_{\beta}$	(hr)	15.24	21.29	18.21	18.45	9.01	8.08	18.27	13.44^{e}	5.66
Day 14/15										
C_{max}	(µg/mL)	1.328	2.625	0.992	3.262	1.758	1.557	1.977^{b}	1.892	0.969
C_{min}	(µg/mL)	1.0146	1.5674	0.6863	0.4590	0.5635	0.8821	1.2910	0.6477^{c}	0.1818
Cl/F	(L/hr/kg)	0.1186	0.0958	0.2774	0.1276	0.1803	0.1474	0.1072	0.1832^{bc}	0.0665
C_{ss}	(µg/mL)	1.0542	1.3042	0.6008	1.3067	0.9242	1.1308	1.1792	0.9906	0.3033
T_{max}	(hr)	24.033	0.283	24.000	0.750	1.000	0.750	12.158	6.625	11.584
F	(%)	79.30	107.20	44.41	107.70	42.85	60.87	93.20	63.96^{bc}	30.29
$t1/2_{\beta}$	(hr)	9.972	10.140	5.276	9.509	6.573	7.204	10.046	7.141^{bcd}	1.771

C_{max} = observed peak plasma concentration; C_{min} = observed trough plasma concentration; Cl/F = total body clearance/extent of absorption; C_{pred} = predicted plasma concentration at steady state; C_{ss} = observed plasma concentration at steady state; T_{max} = time of observed peak plasma concentration; NA = not applicable; a = significantly different from crushed tablet formulation; b = significantly different from Day 1; c = significantly different from Day 3; d = significantly different from Day 7/8; e = significantly different from Day 15.

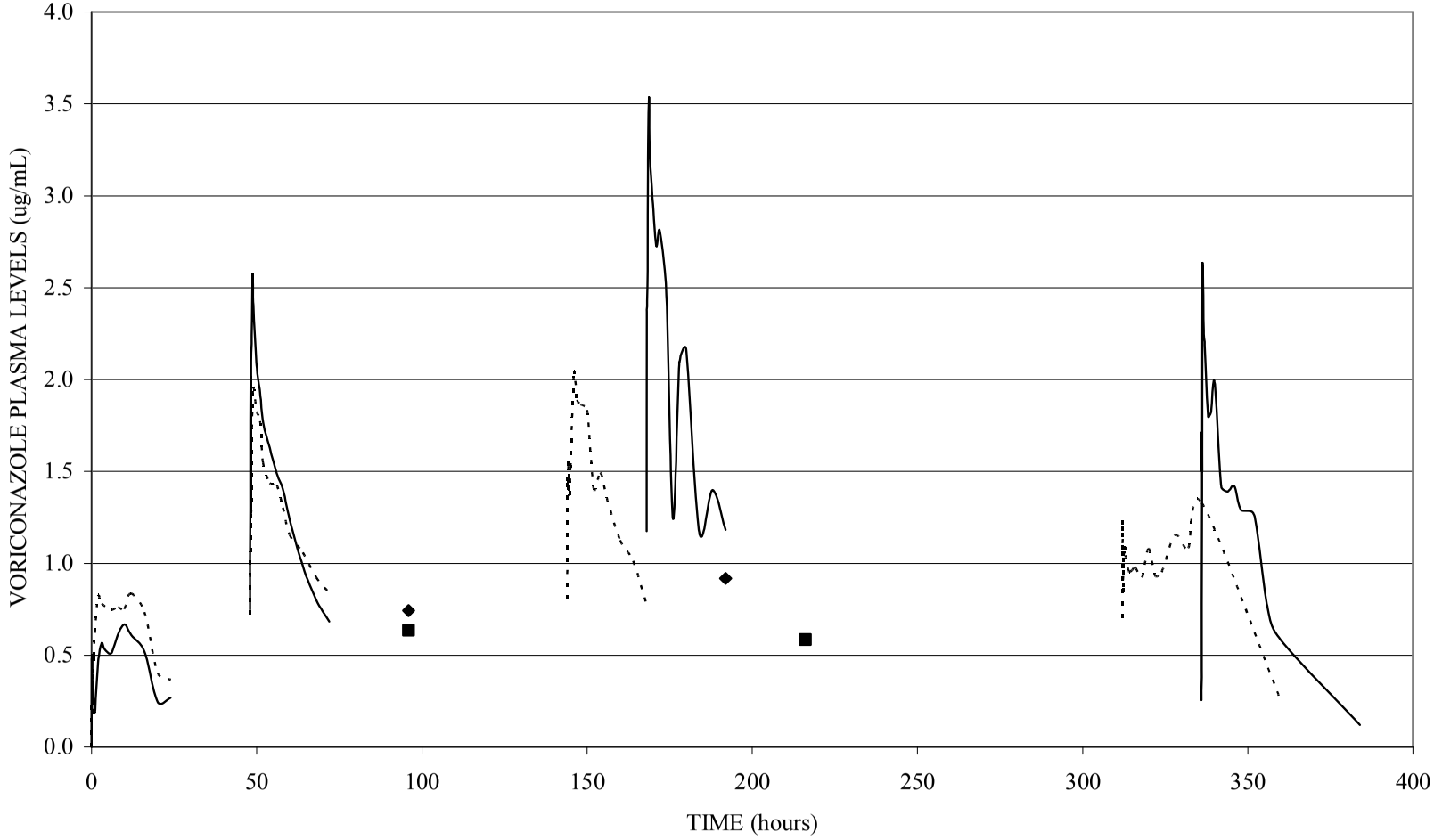

FIGURE 5.1. Plasma concentrations of voriconazole on days 1, 3, 7 (Horse A) or 8 (Horse B), and 14 (Horse A) or 15 (Horse B) after repeated oral administration at a dose of 3 mg/kg every 24 hours with crushed tablets to Horse A (----♦----) and Horse B (——■——) on linear coordinates.

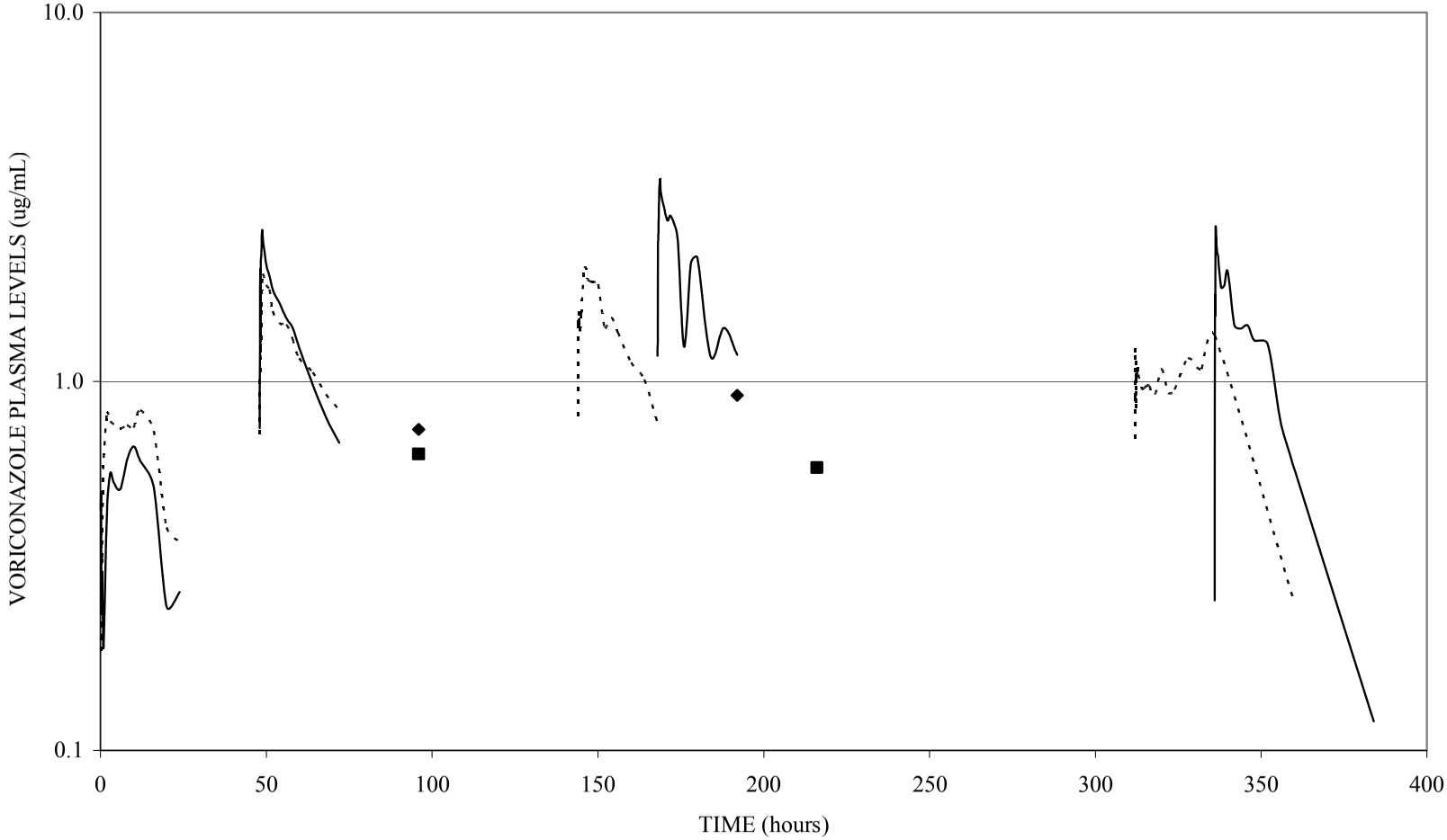

FIGURE 5.2. Plasma concentrations of voriconazole on days 1, 3, 7 (Horse A) or 8 (Horse B), and 14 (Horse A) or 15 (Horse B) after repeated oral administration at a dose of 3 mg/kg every 24 hours with crushed tablets to Horse A (----♦----) and Horse B (——■——) on log-linear coordinates.

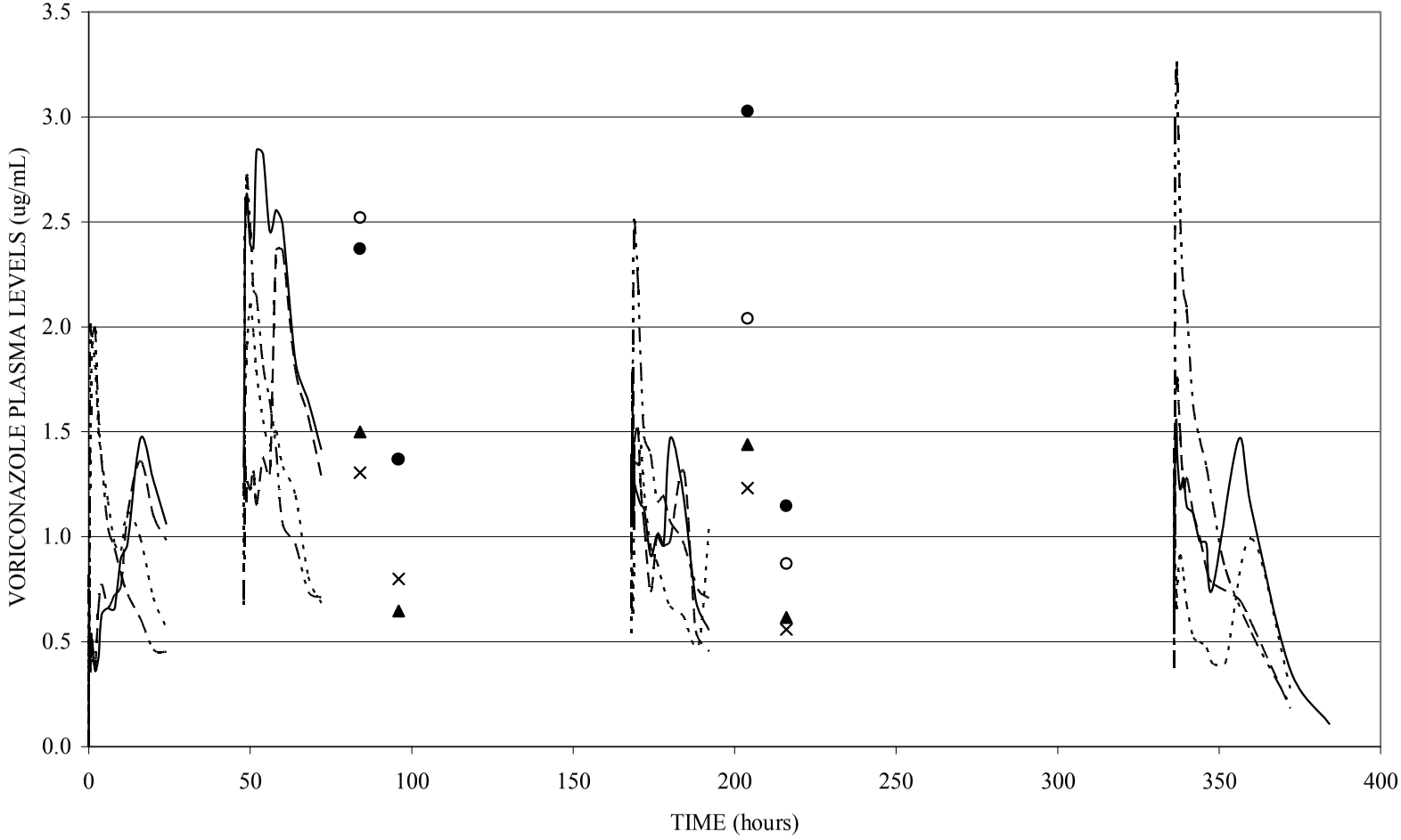

FIGURE 5.3. Plasma concentrations of voriconazole on days 1, 3, 8, and 15 in horses after repeated oral administration at a dose of 4 mg/kg every 24 hours with powder to Horse C (----▲----), Horse D (— -- × — --), Horse E (— — ○ — —), and Horse F (——●——) on linear coordinates.

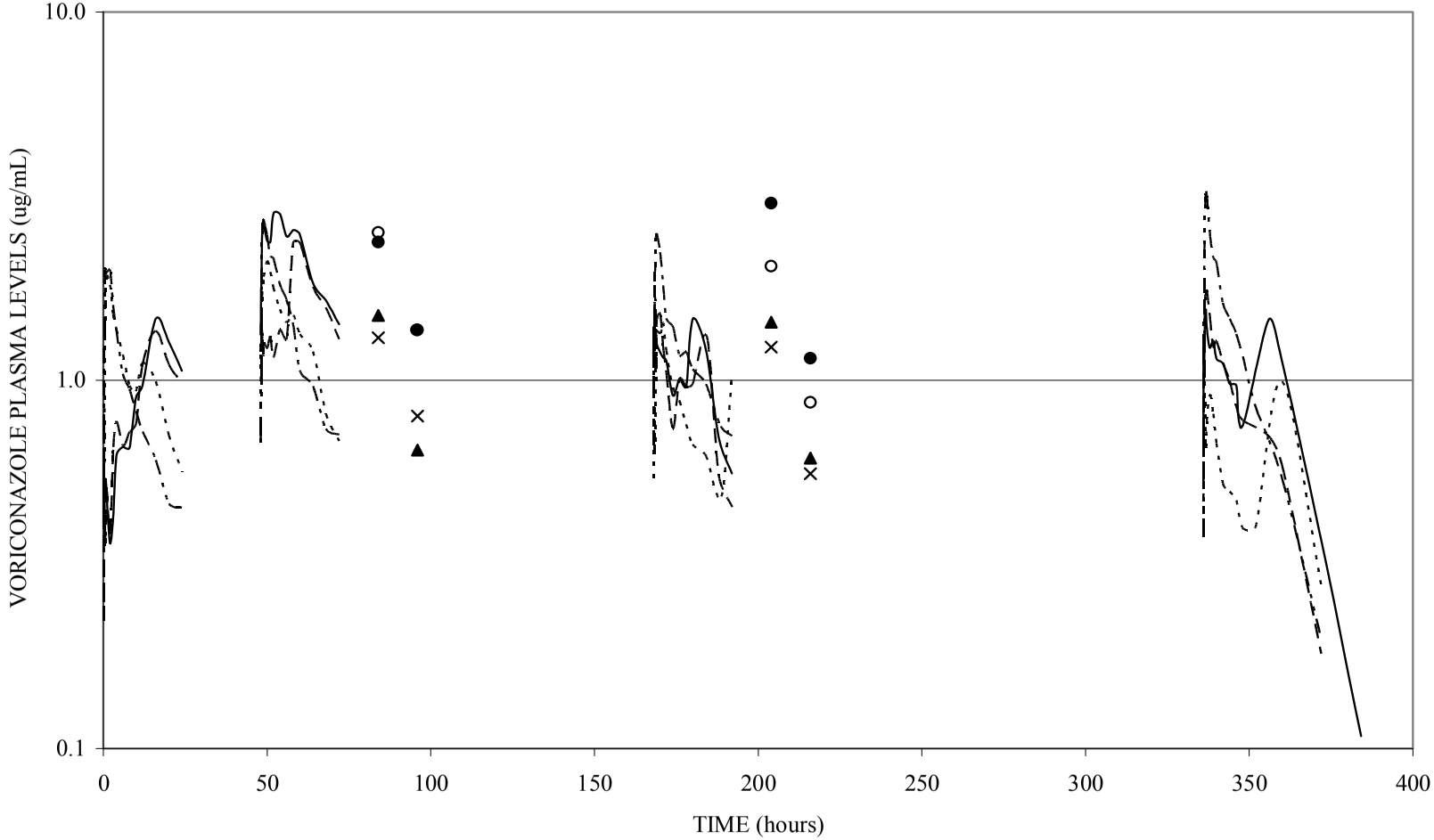

FIGURE 5.4. Plasma concentrations of voriconazole on days 1, 3, 8, and 15 in horses after repeated oral administration at a dose of 4 mg/kg every 24 hours with powder to Horse C (----▲----), Horse D (— -- × — --), Horse E (— — ○ — —), and Horse F (——●——) on log-linear coordinates.

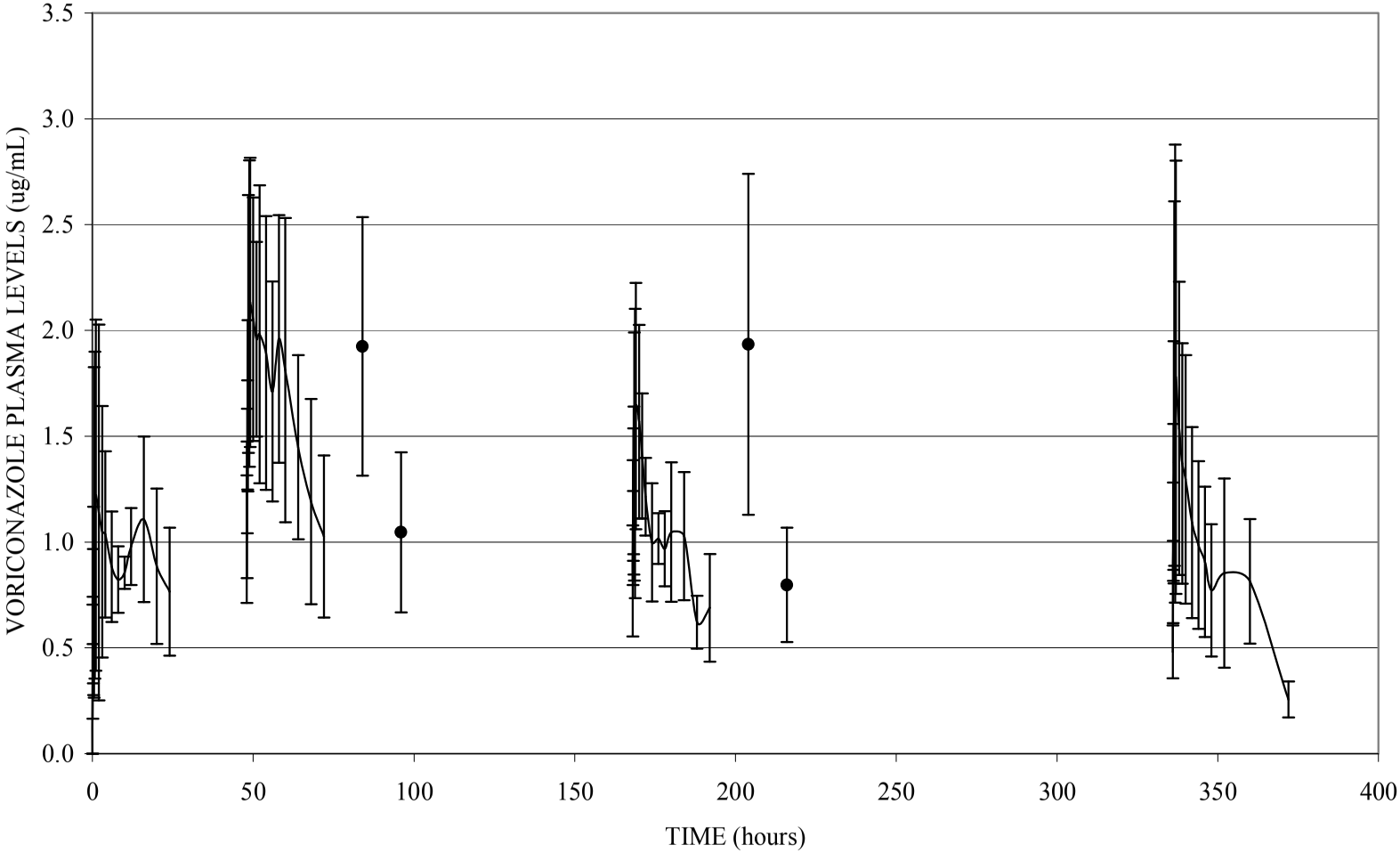

FIGURE 5.5. Mean plasma concentrations of voriconazole on days 1, 3, 8, and 15 in horses after repeated oral administration at a dose of 4 mg/kg with powder to four horses every 24 hours on linear coordinates (bars indicate standard deviations).

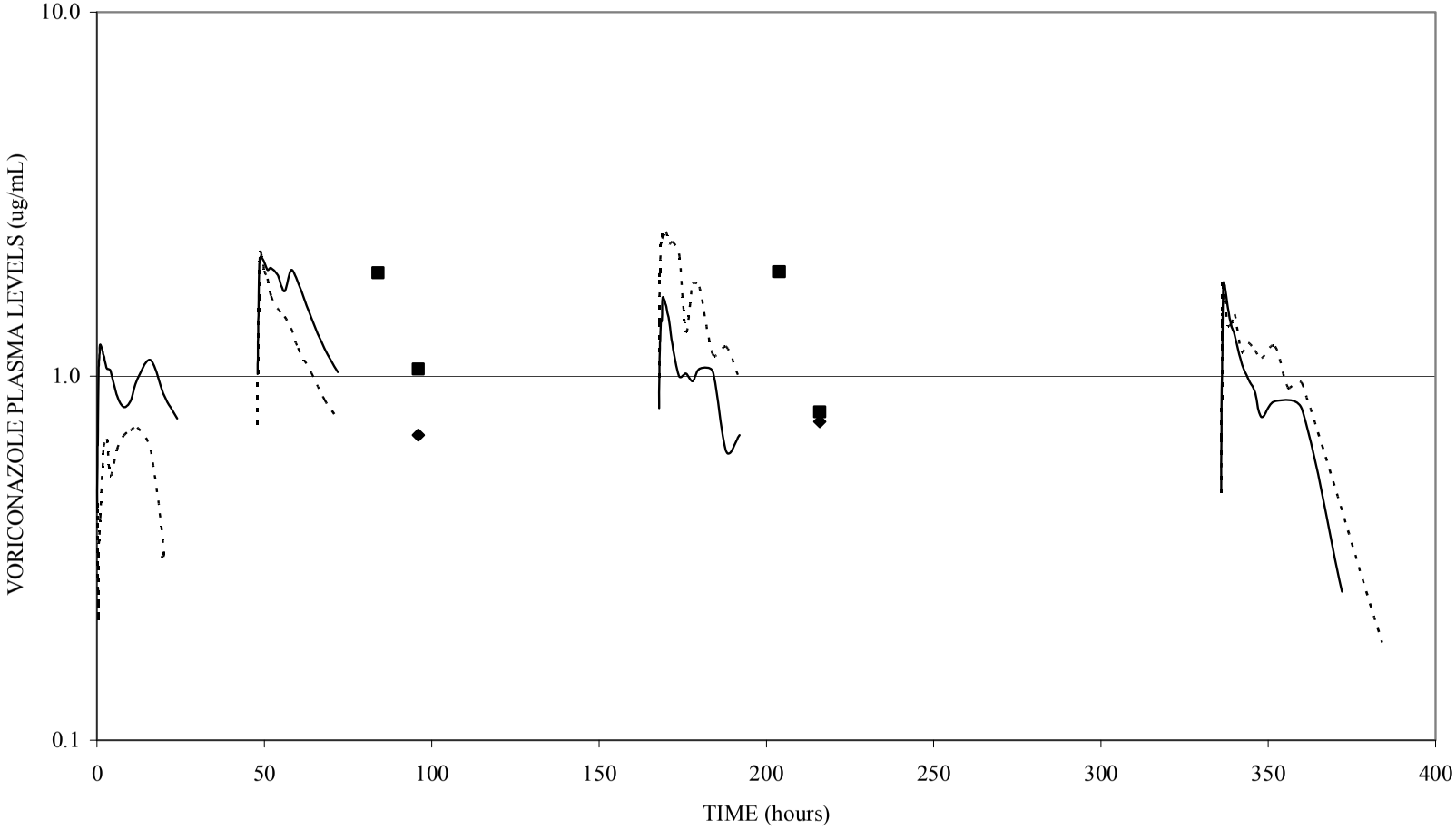

FIGURE 5.6. Mean plasma concentrations of voriconazole on days 1, 3, 8, and 15 in horses after repeated oral administration at a dose of 3 mg/kg with crushed tablets (---♦---) to two horses and 4 mg/kg with powder (—■—) to four horses every 24 hours on log-linear coordinates.

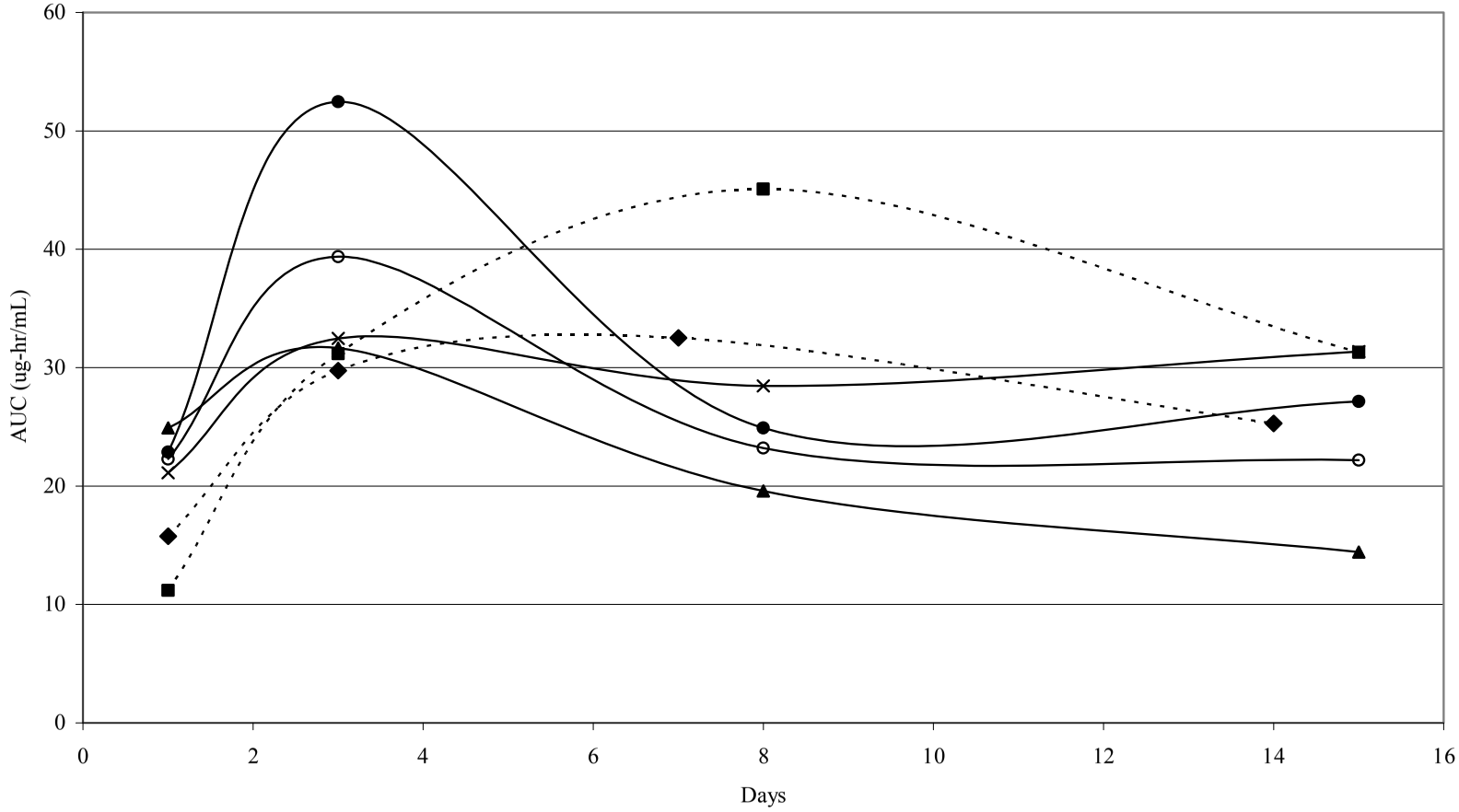

FIGURE 5.7. Area under the plasma concentration-time curve (AUC) values of voriconazole on days 1, 3, 7 (Horse A) or 8 (Horses B, C, D, E, and F), and 14 (Horse A) or 15 (Horses B, C, D, E, and F) in horses after repeated oral administration at a dose of 3 mg/kg with crushed tablets to Horse A (--♦--) and Horse B (--■--) and 4 mg/kg with powder to Horse C (—▲—), Horse D (—×—), Horse E (—○—), and Horse F (—●—) every 24 hours.

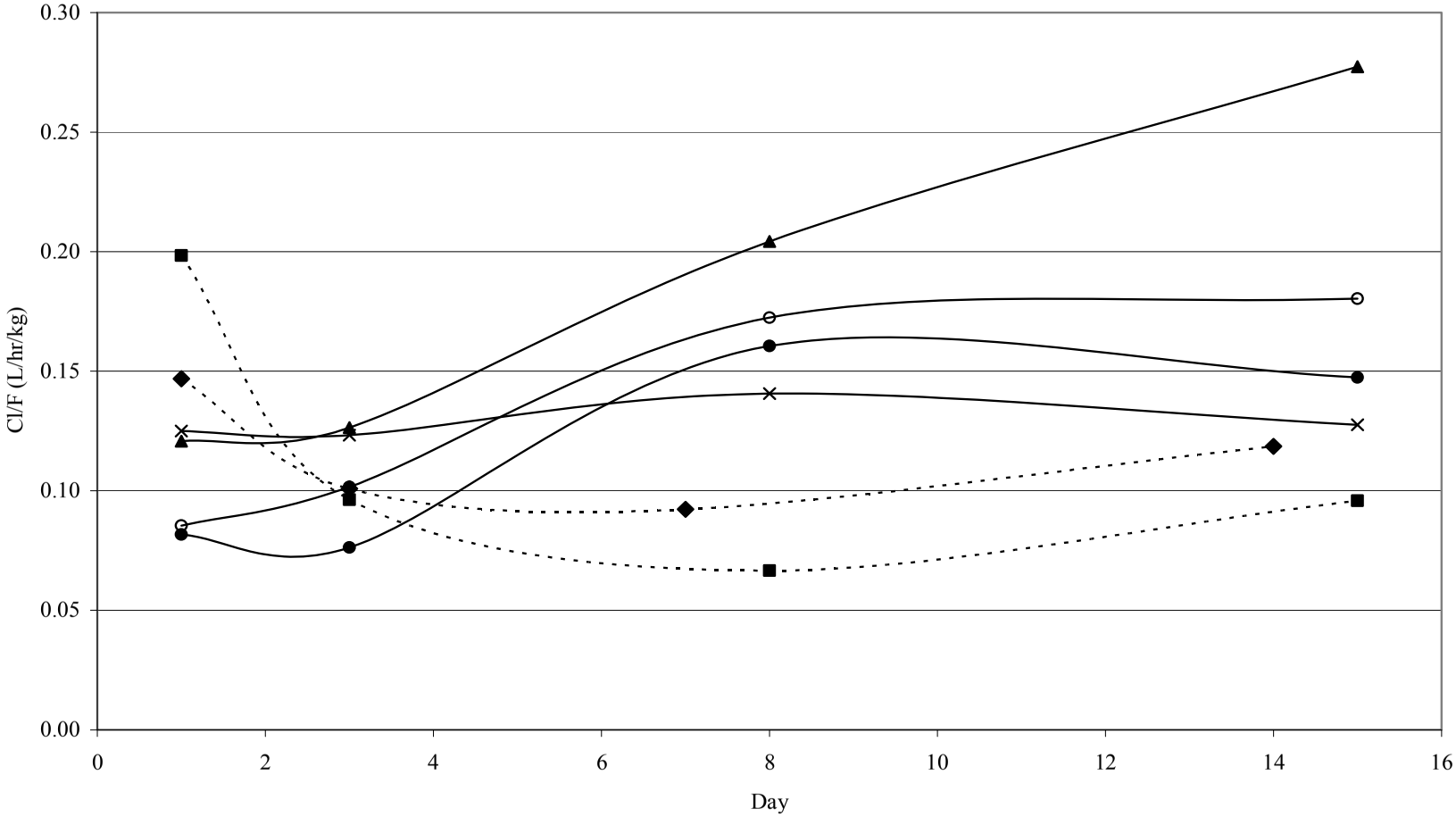

FIGURE 5.8. Total body clearance over extent of absorption (Cl/F) values of voriconazole on days 1, 3, 7 (Horse A) or 8 (Horses B, C, D, E, and F), and 14 (Horse A) or 15 (Horses B, C, D, E, and F) in horses after repeated oral administration at a dose of 3 mg/kg with crushed tablets to Horse A (--♦--) and Horse B (--■--) and 4 mg/kg with powder to Horse C (—▲—), Horse D (—×—), Horse E (—○—), and Horse F (—●—) every 24 hours.

6. SUMMARY AND CONCLUSIONS

Invasive and systemic fungal infections in veterinary medicine are uncommon, but some can be life threatening and are often difficult to eradicate. Despite the considerable progress in the pharmaceutical management of invasive mycosis, the majority of antifungal agents are approved for human use only, and successful antifungal therapy for affected animals is limited to only a few drugs (Latimer *et al*, 2001). Usually the dosages given to large animals are based on study results extrapolated from humans and laboratory animals. However, the pharmacokinetic profiles of many drugs can not be extrapolated across species without adjusting for some individual species characteristics (Riviere, 1999). For example, herbivorous species generally metabolize lipid-soluble drugs more rapidly than do carnivorous species (Baggot, 1992).

A relatively new and lipophilic antifungal agent, voriconazole, is known to have unpredictable pharmacokinetic profiles across species due to variable absorption, autoinduction of metabolism, and saturable elimination that have been observed in certain animal species but not in others. Furthermore, the pharmacokinetics of voriconazole is known to change from single to multiple dosing. To date, there have been no published reports regarding the efficacy of voriconazole in veterinary medicine, and only recently was one study published on the pharmacokinetics of voriconazole after single dose administration to healthy horses (Davis *et al*, 2006). The present study examined the

pharmacokinetics of voriconazole after single dosing to alpacas and of tablet and powder formulations of voriconazole after single and multiple dosing to horses.

Healthy adult animals were enrolled into the study. All samples were analyzed by high-performance liquid chromatography using liquid-liquid extraction and an internal standard. The sensitivity and variability of the assay were assessed.

Alpacas, a South American Camelid and pseudoruminant species, are considered to be one of the oldest domesticated animals in the world, and in the past decade, they have rapidly gained popularity in the United States. To our knowledge, the safety and pharmacokinetics of voriconazole had not been investigated in a non-monogastric species. After single intravenous (IV) administration of 4 mg/kg voriconazole to four alpacas, the pharmacokinetic parameters obtained following two-compartmental modeling analysis were in excellent agreement with those obtained by the noncompartmental methods. The mean distribution half-life ($t_{1/2\alpha}$) value obtained after two-compartmental analysis was 0.1698 ± 0.0260 hours, which indicates a short distribution phase, that was followed by a more prolonged elimination phase (elimination half-life value, $t_{1/2\beta}$ = 7.833 ± 0.770 hours). The slight convex fall in the log-linear plasma concentration versus time in the mean graph and in the individual plots of two of the alpacas may suggest some capacity limited elimination. It is possible that in the present study, the voriconazole concentrations did not reach levels that would display clear evidence of saturable metabolism in all four alpacas. Since only one dosage for each route was given, the nonlinearity of voriconazole in alpacas needs to be further studied.

Following the oral dose, voriconazole appeared rapidly in the plasma with drug concentrations detected at 5 minutes postdose in all four alpacas. The mean $t_{1/2\beta}$ (8.748 ± 4.307 hours) in alpacas after single oral dosing of voriconazole was similar to that after IV dosing. The extent of absorption based on AUC values after oral and IV dosing of 4 mg/kg of voriconazole in alpacas was 22.74 ± 9.48%, which is much lower in alpacas than in other species investigated so far (75 to 159%) (Roffey *et al*, 2003; Pfizer, 2006; Davis *et al*, 2006). This phenomenon has been observed in ruminants with other antimicrobials, and it has been theorized that this phenomenon is due to inactivation or dilution of the drug in the rumen. Although voriconazole appeared rapidly in the plasma after oral dosing, the absorption rate seemed slow with a peak plasma concentration at nearly 5.4 hours. There was high inter-individual variation in voriconazole plasma concentrations and pharmacokinetic parameters, but no age- or gender-dependent trends were observed. Evaluation of the percent remaining to be absorbed plots showed a multi-phasic absorption process in all four alpacas, possibly due to an initial rapid absorption period of voriconazole from the upper gastrointestinal tract and then absorption proceeded at a slower rate in the lower gastrointestinal tract.

After 4 mg/kg IV administration, voriconazole concentrations in plasma remained above 0.1 μg/mL over the 24-hour period postdose, and an IV dosing regimen of 3.9 to 6.6 mg/kg of voriconazole every 24 hours to mature, healthy alpacas seems appropriate. Because pharmacokinetics of voriconazole is known to differ between single and chronic dosing in other species, additional studies will be needed to determine the safety and possible changes in pharmacokinetic parameters of voriconazole for use in the long term treatment of alpacas. Due to the variable and low extent of absorption of the oral

formulation of voriconazole in alpacas, higher dosages of 20.4 to 33.9 mg/kg/day might be needed depending on the susceptibility profile of the fungal strain involved.

One of the most frequent clinical manifestations of fungal infections in large animals is equine keratitis. The pharmacokinetics of single administration of voriconazole has been characterized in horses previously. However, in that study, only the powder formulation was used for oral administration, not the more commercially available tablets, and the IV dosage of 1 mg/kg was much lower than the amount given to any other species before (Davis *et al*, 2006). In the present study, after 2 (n=2) and 4 (n=4) mg/kg IV administration, terminal half-life values ranged from 7.72 to 16.68 hours, with the two lowest numbers obtained from horses that received the 2 mg/kg intravenous dose. The overall mean half-life was 12.22 ± 3.54 hours. Using two-compartmental analysis, the mean ± SD distribution half-life was 0.3600 ± 0.1487 hours, and the elimination half-life was 11.86 ± 1.93 hours. No evidence of non-linear or saturable elimination was observed in horses.

Like in alpacas, voriconazole following oral dosing in horses was rapidly absorbed, with the drug detected in plasma at 5 minutes after dosing in all six horses. The completeness of voriconazole absorption was high in the four horses that received the 4mg/kg voriconazole in powder form. The mean ± SD systemic extent of absorption from voriconazole powder was 103.07 ± 9.15%, and the mean terminal half-life was 15.284 ± 3.497 hours. In the present study, IV and oral doses and thus plasma concentrations were similar, permitting a better estimate of voriconazole bioavailability in horses. Pharmacokinetics of oral administration of 3 mg/kg crushed tablets to the other two horses showed that the mean ± SD bioavailability was only 57.88 ± 8.63%, and

the mean terminal half-life was 9.482 ± 0.328 hours. Oral absorption of voriconazole after single administration in horses appeared lower from tablets as opposed to powder dosing. Either the difference in surface area per dose ratio of the drug molecules or drug interactions with excipients in the tablet formulation could have caused the disparity in extents of absorption between powder and crushed tablet formulations.

The percent remaining to be absorbed plots showed multiple absorption phases or periods suggesting complex absorption processes. It is possible that voriconazole is rapidly absorbed in the stomach and more slowly absorbed in the intestine in horses. The high variability in oral drug absorption could be attributed to drug binding to hay in the stomach or colonic reabsorption, because the horses were not fasted prior to drug dosing.

Because a long course of therapy of several weeks to months is often required for the treatment of fungal infections, pharmacokinetics of voriconazole in horses after multiple oral dosing were also investigated. To our knowledge, the safety and possibility of changes in drug disposition and absorption over time after repeated dosing of voriconazole had not been investigated previously in veterinary species. The six horses used in the single dose studies were continued on doses of either 3 mg/kg of crushed tablets or 4 mg/kg powder every 24 hours for 14 or 15 days. One of the horses developed noticeable urticarial skin reaction on day 2 after receiving voriconazole, but the results from complete blood count and serum chemical analyses performed during the study were within reference values for all six horses.

The pharmacokinetic analysis indicated that significant differences in half-life and clearance divided by extent of absorption (Cl/F) values existed between single and repeated dosing periods, especially in horses that received the powder formulation. The

trends in the mean Cl/F values of dosing appear to be dosage form- or dose-dependent. For the two horses that received the 3 mg/kg crushed tablet formulation, the mean Cl/F value on day 7/8 was 54% and significantly lower than the mean value obtained after a single dose and Cl/F on day 14/15 was 38% less than the single dose but not significantly different. In contrast, for the four horses that received the 4 mg/kg voriconazole powder, the mean Cl/F values on days 8 and 15 were approximately 60-80% greater ($P<0.05$) than that observed on a single dose or on day 3 of multiple dosing.

No clear evidence of saturable elimination or autoinduction was observed. However, an increase or decrease in the mean Cl/F values without significant changes in $t_{1/2\beta}$ or k_{el} values could suggest that Cl/F differences noted between days may be related to alterations in extent of absorption during the 15-day dosing period. This assumes that other pharmacokinetic parameters such as clearance, volume of distribution, and $t_{1/2\beta}$ values remained constant throughout the 15 days. Since $t_{1/2\beta}$ appeared unchanged, it is most likely that the changes in Cl/F over the 15 days of dosing were related to varying and changing F and not Cl. Changes in Cl would reflect alterations in the voriconazole elimination or metabolism capabilities during multiple dose administration.

Variable absorption appears to explain the large variability in plasma concentrations and pharmacokinetic parameters over the 15 days. Although the extent of absorption appeared to change and were different depending on the day of sampling and dosage form, the absorption rates were too variable to detect any day or dosage form effects. The large inter-subject variability in plasma concentrations after oral voriconazole dosing could be further attributed to the corn syrup hindering the absorption by an interaction with voriconazole or with excipients in the tablet formulation.

However, the contributions of changes in intrinsic metabolizing rates among the horses during multiple dosing towards the variability in plasma concentrations can not be completely excluded.

Potentially therapeutic voriconazole plasma concentrations of > 0.1 μg/mL were maintained in all six horses throughout the treatment period. Voriconazole levels achieved and maintained in plasma exceed the minimum inhibitory concentration (MIC) values of susceptible opportunistic fungi commonly found in sites of deep and invasive infections in horses. For an oral dosing regimen with crushed tablets, 3.9 to 6.4 mg/kg/day appears appropriate in horses. In the case of powder, higher oral daily doses of 6.6 to 11.0 mg/kg/day may need to be recommended. This appears to be true since the extent of absorption increases during multiple dosing for the tablets and decreases during multiple dosing for the powder. These studies also emphasis the importance of the need for multiple dosing studies for drugs which will be administered in this fashion.

The extent of absorption is substantially lower in alpacas compared to horses, humans, mice, rats, rabbits, guinea pigs, and dogs, and high inter-individual variations are observed across all species (Roffey *et al*, 2003, Purkins *et al*, 2003a; Purkins *et al*, 2003b; Davis *et al*, 2006). A drug such as voriconazole with a low extraction ratio may show inter-individual and inter-species variations in drug disposition because clearance is a function of the plasma protein binding and the intrinsic metabolic capability of the individual and species, which is largely dependent upon isoenzyme expression and other genetic factors (Riviere, 1999). In addition, there is no correlation between the concentration of hepatic microsomal cytochrome P450 and activity of the enzymes that catalyze biotransformation reactions (Dalvi *et al*, 1987). However, comparative studies

have shown that in general, the drug metabolizing capacity of the liver is greater and systemic availability of orally administered drugs is lower in herbivorous species than in humans and dogs (Baggot, 1992).

Another confounding factor that may contribute to inter-species variations in clearance values is limited metabolic capacity for specific pathways in certain species, such as glucuronidation in cats (Riviere, 1999). If the therapeutic dose in some of the species or the species of interest produces concentrations that saturate the metabolizing mechanisms, nonlinear elimination would result and this has been observed with voriconazole. However, it is difficult to predict in which species this phenomenon will occur. Furthermore, for extensively metabolized drugs, the contribution of the various metabolic pathways differs and could cause variable elimination rates across species (Baggot, 1992). However, saturation of metabolism generally does not pose a problem for most pharmacological doses (Riviere, 1997).

Despite possible species differences in cytochrome P450 isoenzyme makeup and nonlinear pharmacokinetics with drugs that are extensively metabolized, allometric scaling based on body weights and pharmacokinetic parameters from previously investigated species can be used to obtain interspecies extrapolation of voriconazole pharmacokinetics in order to predict drug elimination and distribution parameters in species that have not yet been studied. Equations in which a parameter is related to a mathematical function of a metric such as body weight is termed an allometric relationship.

It was discovered that over 30 anatomical, physiological, and biochemical parameters can be correlated with body weight across species (Adolph, 1949). According to Adolph, the following equation:

$$Y = a\ W^b$$

where Y is the parameter under study, a is an allometric coefficient (intercept) that is constant for a drug, W is the species average body weight, and b is the allometric exponent, can be used to describe allometric relationships. When double-logarithmic plots are used, the exponential equation becomes a linear function after logarithmic transformation, so that estimates of the intercept, a, and the slope, b, can be computed by linear regression according to the following equation:

$$\text{Log } Y = \text{Log } a + b\ (\text{Log } W)$$

A review of published articles suggests that for most physiological processes, the allometric exponent, b, is between 0.67 and 1.0, and as most pharmacokinetic parameters are dependent upon physiological functions, it is possible to use scaling across species based upon the general allometric relationship (Riviere, 1997). Pharmacokinetic parameters extrapolated from data in species of which the pharmacokinetic studies have already been conducted, using allometry, could be used to calculate the predicted effective dose of a drug in another species.

The effectiveness of antimicrobials is basically dependent on the concentration of drug present in the extracellular fluid and there are generally no pharmacodynamic differences between animal species toward the same target bacterial or fungal species (Riviere, 1997). Therefore, for these drugs, factors such as unique physiology or species-specific biochemical receptors should contribute only minimally when adjusting for

species differences in pharmacokinetic parameters, such as clearance, when deriving the optimal dose for each animal species of interest (Riviere, 1997).

Allometric techniques are more effective when there is a wide range of body weights because of the logarithmic transformation involved (Riviere, 1999). Allometric plot obtained for voriconazole was based on eight species, and the mean body weights ranged from 0.03 kg in mice to 526 kg in horses (Roffey *et al*, 2003; Purkins *et al*, 2003a; Davis *et al*, 2006). Logarithmic values of mean body weights and clearance were calculated and plotted (Figure 6.1). The allometric equation that best characterizes this relationship is:

$$\text{Log (Clearance)} = 0.900 \text{ Log (Body Weight)} - 0.552$$

There is a clear double-logarithmic relationship between clearance and body weight, and the allometric data for voriconazole total body clearance values in these species suggest size dependence. The coefficient of determination (r^2) equals 0.903. Some variability in the allometric relation could be due to using data from numerous laboratories (different assay methods, sample times, doses, etc.) and small numbers of subjects in the surveyed animal studies. Allometric scaling with voriconazole using other pharmacokinetic parameters, such as volume of distribution, was not investigated because of the lack of the data available in some species.

In summary, these pharmacokinetic studies of voriconazole showed variable oral absorption, with respect to rate and extent, after oral administration in both horses and alpacas. Following intravenous administration, voriconazole pharmacokinetics appeared more consistent among subjects with evidence of a short distribution half-life and large volume of distribution. The voriconazole mean elimination half-life value was longer in

horses than in alpacas, but the mean total body clearance values for these two herbivorous species after single IV dosing were similar. However, the extent of absorption is substantially lower in alpacas compared to other species previously studied, and high inter-individual variations in absorption were noted. Because of observed changes and high inter-subject variations in pharmacokinetic parameters in horses after oral dosing, adjustments in the dosage regimen during long term administration might be needed. These adjustments may be needed due to variable oral absorption and possible hepatotoxicity, as recommended for other species.

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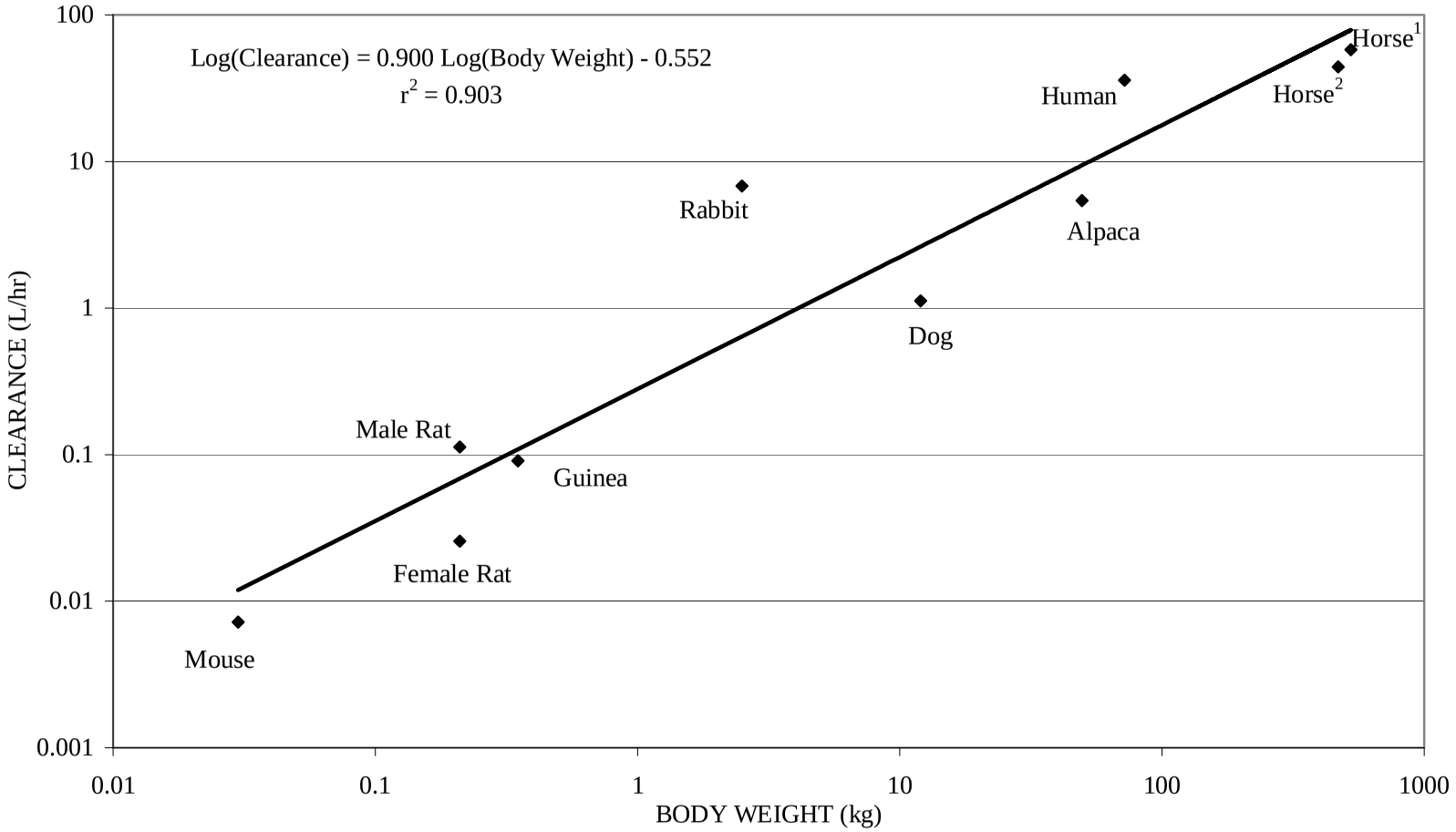

FIGURE 6.1. Allometric scaling for voriconazole in various species. Mouse, male rat, female rat, guinea pig, rabbit, and dog from Roffey *et al* , 2003; Human data from Purkins *et al* , 2003; Horse[1] data from Davis *et al* , 2006; Horse[2] and alpaca data from Chapters 3 and 4 of this dissertation.