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Authors

Parsley, Ruth A

Mutlow, Adrian G

Hansted, Jacqueline

et al.

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Application of different pharmacokinetic models to describe and predict pharmacokinetics of voriconazole in magellanic penguins following oral administration

Ruth A. Parsley[1] | Adrian G. Mutlow[2] | Jacqueline Hansted[2] | Femke J. Taverne[3] | Lisa A. Tell[1] | Ronette Gehring[3]

[1]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California

[2]San Francisco Zoo, San Francisco, California

[3]Veterinary Pharmacotherapeutics and Pharmacy Group, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

Correspondence
Ronette Gehring, Veterinary Pharmacotherapeutics and Pharmacy Group, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands.
Email: r.gehring@uu.nl
and
Lisa Tell, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA.
Email: latell@ucdavis.edu

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Abstract

Aspergillosis is a condition causing serious morbidity and mortality in captive penguins and other bird species. It can be treated with antifungal drugs, such as voriconazole. However, the pharmacokinetics of voriconazole are variable between different animal and bird species. Therefore, the pharmacokinetics of voriconazole were investigated in this study in Magellanic penguins. Pharmacokinetic models were constructed and applied to predict the pharmacokinetics of voriconazole during long-term treatment in Magellanic penguins, since the voriconazole treatment duration in chronic aspergillosis cases can last up to several months. Plasma voriconazole concentration–time data from adult Magellanic penguins (*Spheniscus magellanicus; n* = 15) following a single oral (PO) dose of either 2.5 mg/kg or 5 mg/kg in a herring in three separate study periods 7–12 months apart were collected. Mean plasma voriconazole concentrations were above the targeted MIC for *Aspergillus fumigatus* for 2 hr following a single 2.5 mg/kg voriconazole dose while the plasma concentrations exceeded the MIC for least 24 hr following a 5 mg/kg dose. Nonlinear mixed-effects modeling was used to fit two pharmacokinetic models, one with first-order and another with saturable elimination, to the single-dose data. Fits were good for both, as long as dose was included as a covariate for the first-order model so that clearance was lower and the half-life longer for animals receiving the 5 mg/kg dose. Although the single-dose data suggested saturated elimination at higher concentrations, the model with saturable elimination did not predict plasma voriconazole concentrations well for a clinical aspergillosis case receiving long-term treatment, possibly because of induction of metabolizing enzymes with chronic exposure. Pharmacokinetic models should accurately predict plasma drug concentrations for different dosage regimens in order to be applicable in the field. Future studies should focus on determining clearance at steady-state to be able to refine the pharmacokinetic models presented here and improve model performance for long-term oral voriconazole administration in Magellanic penguins.

KEYWORDS

aspergillosis, modeling, penguins, pharmacokinetics, voriconazole

1 | INTRODUCTION

Aspergillosis is a major cause of morbidity in captive penguins and is the most important cause of mortality in indoor exhibited penguins (Cranfield, 2003). Scarce data are available on clinical *Aspergillus* isolates in penguins, but Talbot and co-workers isolated *Aspergillus fumigatus* from a captive Little penguin (Talbot, Thompson, Vogelnest, & Barrs, 2017). This is in concordance with the most common *Aspergillus* spp. (*Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*) isolated from other bird species (Akan, Haziroğlu, Ilhan, Sareyyüpoğlu, & Tunca, 2002; Friend, Franson, & Ciganovich, 1999; Martin et al., 2007; Nardoni, Ceccherelli, Rossi, & Mancianti, 2006; Talbot et al., 2017). *Aspergillus* spp. are often found in indoor facilities such as zoologic collections, and rehabilitation and medical facilities. Healthy, well-adapted penguins can be exposed to low levels of spores without developing disease, but disease frequently occurs in stressed or debilitated birds. Stressors include substandard air quality, poor ventilation, overcrowding, excessive environmental heat or cold and social incompatibility (Filho et al., 2015; McMillian & Petrak, 1989; Phalen, 2000; Redig, 2000; Verstappen & Dorrestein, 2005; Wallace, 2015). *Aspergillus* spp. are transmitted by air, and in birds, they mainly infect the pulmonary parenchyma and air sac membranes (Seyedmousavi et al., 2015). In affected birds, chronic aspergillosis most commonly exhibits as voice change, anorexia, biliverdinuria, lethargy or depression, dyspnea, and emaciation. Occasionally, ataxia or paralysis can occur if the central nervous system is affected (Dahlhausen, 2006). Chronic cases of aspergillosis in captive birds have a persistent clinical course that can last weeks or months (Neumann, 2016). In contrast, acute cases, which are caused by high levels of fungal spores and/or severe immunosuppression, progress very quickly and penguins are often dead within 24–48 hr (Kearns & Loudis, 2003).

Voriconazole[a] is a triazole antifungal drug effective against a broad spectrum of fungi, including *Aspergillus* species (Antonissen & Martel, 2018). Voriconazole acts by inhibiting fungal cytochrome P-450-mediated 14 alpha-lanosterol demethylation, thus interrupting an important stage in fungal ergosterol biosynthesis (Antonissen & Martel, 2018). *In* vitro, voriconazole demonstrates high fungicidal activity against *Aspergillus* spp. (Sabino et al., 2016). Efficacy and exposure are positively correlated, with an AUC/MIC ratio of >25 established as the target for acceptable clinical outcomes in mice (Andes, Marchillo, Stamstad, & Conklin, 2003). Clinical breakpoints for voriconazole in *Aspergillus* spp. isolates from birds are not established; however, aspergillosis in both humans and birds is usually caused by *Aspergillus fumigatus* (Seyedmousavi et al., 2015). Instead of a clinical breakpoint, the epidemiological cutoff (ECOFF) value may be used for veterinary species. The ECOFF for voriconazole for *Aspergillus fumigatus* has been established as a MIC of 1.0 mg/L (EUCAST, 2018).

The pharmacokinetics of voriconazole have been reasonably well-studied in different bird species including red-tailed hawks (Gentry et al., 2014; Parsley, Tell, & Gehring, 2017), several species of parrots (Flammer et al., 2008; Sanchez-Migallon Guzman et al., 2010), chickens (Burhenne, Haefeli, Hess, & Scope, 2008), and Japanese quail (Souza, Redig, & Cox, 2017; Tell et al., 2010). To date, only one study assessed voriconazole pharmacokinetics in penguins (Hyatt, Wiederhold, Hope, & Stott, 2017). Hyatt and co-workers studied the pharmacokinetics of orally administered voriconazole in African penguins (*Spheniscus demersus)* as a single-dose and a once-daily repeated dosage regimen. The most striking findings were the fast absorption of voriconazole after oral administration in penguins (T_{max} 0.4 hr) compared to voriconazole absorption in other bird species studied (only studies with fasted animals considered) and the prolonged elimination half-life in penguins receiving the daily dosage regimen ($T_{½}$ 10.9 hr) compared to elimination half-lives in other bird species ($T_{½}$ 1.0–1.7 hr).

The objective of this study was to evaluate the pharmacokinetics of voriconazole in Magellanic penguins (*Spheniscus magellanicus*) and develop a pharmacokinetic model to predict concentration–time profiles following different dosage regimens. This was achieved by measuring voriconazole in plasma samples collected after a single dose and analyzing the time–concentration data using different pharmacokinetic models. To test the predictive value of these models, plasma samples were also collected from a single penguin to which voriconazole was administered on an ongoing basis to control clinical aspergillosis. The in vivo measured voriconazole concentrations in plasma over time in this clinical long-term voriconazole administration case were compared to the voriconazole plasma concentration–time profile predicted by the pharmacokinetic model to assess model performance.

2 | MATERIALS AND METHODS

2.1 | Animals

2.1.1 | Study 1–3

Fifteen adult captive Magellanic penguins (*Spheniscus magellanicus*, seven females and eight males, ages 2–11 years) at the San Francisco Zoo, with body weights ranging from 3.70 kg to 6.69 kg (mean 4.69 kg ± 0.71 kg) were used in this study. The sex of the birds was determined by DNA testing. All birds were considered healthy based on physical examination and evaluation of medical history. Birds were moved from their normal exhibit 48 hr prior to initiation of the study and housed in temporary holding pens. They had access to water at all times other than the first 2 hr of the study period. Birds were fed according to their normal schedule on the day prior to the study initiating. On the day of the study, the morning feeding was replaced with a single medicated fish, and normal feeding was resumed at 7.5 hr following drug administration.

2.1.2 | Clinical case

An adult (8.5 years, bodyweight on presentation 3.33 kg) male penguin presented with a recurrent cough and weight loss over 6 weeks.

[a]Vfend™, Pfizer Pharmaceuticals, New York, NY.

Aspergillus fumigatus was isolated from a bronchial wash and oral treatment with voriconazole was initiated. He was held in a smaller pen with his mate during treatment periods when he was ill and not eating consistently. During these times, he had access to water and a normal feeding schedule.

All procedures relating to this study were performed in strict accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

2.2 | Drug formulations

For the three study parts, oral voriconazole powder[a] was reconstituted according to manufacturer's instructions, by adding 11.5 ml of deionized ultrafiltered water to 11.295 g powder to obtain a suspension with a final voriconazole concentration of 40 mg/ml. Due to limitations regarding drug administration options in captive animals, the voriconazole suspension was injected into the coelomic cavity of a herring and fed to the penguin. A 25-gauge needle was used for injecting the fish with voriconazole suspension to minimize drug loss from the injection site. For the clinical case, the formulation of voriconazole was compounded from five voriconazole tablets (50 mg) that were crushed using a mortar and pestle and suspended in 10 ml of sterile water with a targeted concentration of 25 mg/ml. New suspension was prepared every 2 weeks.

2.3 | Treatment and sampling protocols

2.3.1 | Study 1

Each penguin received a single oral dose of voriconazole (dosage = 2.5 mg voriconazole per kg body weight) that was injected into a herring. Blood samples (1.5–3 ml per sampling time) were collected from jugular veins. Samples were collected before voriconazole administration (blank), and 0.5, 1, 2, 5, 8, 12, and 24 hr postadministration. Five penguins were sampled at each time point, and each individual was only bled at two of the seven time points in order to minimize the handling of the animals. The data were then pooled for each time point. The blood tubes were centrifuged at 1,500 *g* for 10 min. The plasma was decanted, labeled, and frozen at −80°C until the assays were performed.

2.3.2 | Study 2

Each penguin received a single oral dose of voriconazole (dosage = 5 mg voriconazole per kg body weight) in a herring, and samples were collected before voriconazole administration (blank), and 0.5, 1, 2, 5, 8, 12, and 24 hr postadministration as described above. The blood was processed as described above, and the data were pooled for each time point.

2.3.3 | Study 3

Each penguin received a single oral dose of voriconazole (dosage = 5 mg voriconazole per kg body weight) in a herring, and samples were collected before voriconazole administration (blank), and 0.5, 1, 2, 5, 8, 12, 24, 32, 48, 56, 72 and 96 hr postadministration as described above. Five penguins were sampled at each time point, and each individual was only bled at four of the time points in order to minimize the handling of the animals. The blood was processed as described above, and the data were pooled for each time point.

2.3.4 | Clinical case

This single penguin with clinical aspergillosis received a treatment consisting of repeated oral doses of voriconazole (starting dose of approximately 8 mg/kg, decreased to 5 mg/kg and adjusted for weight gain over time). The dose was administered once daily in a fish. Blood samples were collected over a period of 43 days to monitor plasma drug concentrations. Details of the dosage regimen and sampling schedule are given in Table 1.

2.4 | Sample analysis: ultraperformance liquid chromatography

Voriconazole plasma concentrations were measured using an ultra performance liquid chromatography (UPLC) method coupled with ultraviolet (UV)-detection. Plasma samples (250 µl) were prepared on C18 solid-phase extraction cartridges (Hypersep[b] C18, 100 mg, 1 ml, Thermo Scientific, Bellefonte, PA, USA) based on the protocol described in Pennick, Clark, Sutton, and Rinaldi (2003). Methanol extracts were dried under nitrogen at 40°C, reconstituted in 250 µl mobile phase, and centrifuged at 14,000 *g* for 5 min at 20°C prior to analysis on the UPLC system (Acquity UPLC™)[c]. Due to limited sample volume, all samples were analyzed without duplication. The isocratic mobile phase was 0.02% trifluoroacetic acid in acetonitrile:water (37:63 vol/vol), the flow rate was 0.25 ml/min, and the injection volume was 5 µl. Voriconazole was separated by a BEH C18 column, 1.7 µm, 2.1 × 50 mm (Waters Corporation[d]) and detected by UV absorption at 263 nm. Calibration standards of voriconazole prepared in mobile phase ranged from 0.02 to 2.0 µg/ml while quality control plasma samples from nontreated penguins were spiked with 0.2, and 1.0 µg/ml voriconazole. Average interassay variability, as measured by relative standard deviation, was 14.9%. Intra-assay variability was 9.2%, based on selected assay dates when quality control samples were analyzed in duplicate. Average recovery was 85.4%. Limit of detection and limit of quantification, calculated by measuring the baseline at the expected retention time in the 0 QCs and adding 3 and 10 times the standard deviation of the measurements respectively, were 0.07 and 0.18 µg/mL. Samples were analyzed from 2 to 8 months after collection. Freezer stability was not evaluated.

[b]Bond-Elut CN-E, 50 mg, 1 ml, Varian Inc, Palo Alto, CA

[c]Acquity UPLC™ with TUV detector, Waters Corporation, Taunton, MA

[d]Waters Corporation, Taunton, MA

TABLE 1 Dosage regimen and sampling schedule for a single clinical case to which voriconazole was repeatedly administered orally

Time (hr)	Concentration (μg/ml)	Dose (mg)	Weight (kg)
0	0	25	3.6
2	0.57		
19		17.5	3.55
24	5.66		
43		17.5	
67	4.13	17.5	3.65
69	5.31		
91		17.5	
115		17.5	
139	2.70	17.5	
141	4.72		3.85
163		20	
187	1.95	20	
189	4.16		
195	4.51		
211	2.04	20	
235		20	
259	1.34	20	
261	2.30		
283		20	4
307		20	
331		20	
355		20	4.15
379	1.59	20	
381	3.70		
387	3.47		
403	2.21	20	4.2
427		20	
451		20	4.15
475		20	
499		20	4.15
523		20	
547	1.25	20	4.2
571		20	
595		20	
619		20	
643	3.52	20	
667	4.61		
691	1.93		
715	0.15	20	
739		20	4.15
763		20	
787	3.45	20	
811		20	
835		20	
859		20	
883		20	4.05
907		20	
931		20	
955		20	
979		20	
1004	1.24		
1027	0.61	20	4.05

(Continues)

TABLE 1 (Continued)

2.5 | Pharmacokinetic analysis

2.5.1 | Noncompartmental analysis

To determine initial estimates for the pharmacokinetic parameters, and to test for nonlinearity, a noncompartmental analysis was performed on the single-dose data using the commercially available software program Phoenix 64 WinNonlin® (Certara[e] USA, Princeton, NJ). The sparse sampling option was selected, with the two dose levels (2.5 and 5 mg/kg) analyzed separately. This option within the program first calculates a mean concentration–time curve by taking the mean concentration value for each unique time point. The usual noncompartmental parameters are then calculated from this mean profile (Gabrielsson & Weiner, 2012).

2.5.2 | Compartmental analysis

A compartmental pharmacokinetic model using nonlinear mixed-effects regression as implemented in the commercially available software program Phoenix NLME® (Certara[e] USA, Princeton, NJ) was fit to the plasma drug concentration–time data. The fits of both one- and two-compartment structural models with and without saturable clearance (Michaelis–Menten) were explored. Additive, multiplicative and power (Poisson) models were explored for the residual error. Bodyweight was explored as a covariate for total body clearance and apparent volume of distribution. Since the bioavailability of the oral administration in fish is unknown, the total body clearance and apparent volume of distribution are both in relation to bioavailability. All references to clearance and volume of distribution in this study are to those values in relation to the bioavailability. Model performance was compared based on the value of Akaike's Information Criterion (AIC), which is a method used to compare model performance that describes the balance of the goodness of fit of the model compared to the data loss resulting from applying a more complex model. A model with a lower value of the AIC compared to another model therefore has a better balance between model fit as compared

[e]Phoenix 64®, Certara, Princeton, NJ

to information loss and is therefore considered the better model to describe the collected data (Akaike, 1974). Since there is no official cutoff value to establish whether a certain increase or decrease in AIC is significant, it is important take biological/physiological processes in the study population into consideration when choosing the most appropriate pharmacokinetic model to describe and predict pharmacokinetics. Therefore, also visual inspection of plots of the observed vs. predicted concentration data and visual inspection of the plot of residuals vs. time (an error model that resulted in a consistent spread of residuals around an average of zero was chosen) were used to assess model performance.

2.6 | First-order elimination model

A one-compartment open model with first-order absorption and elimination and a Poisson (power = 0.75) distribution for the residual error was fit assuming linear kinetics to describe the rate of change of voriconazole concentrations in the plasma over time for both the low and high voriconazole dose (Equation 1).

$$\frac{dC}{dt} = k_a \times A_a - CL \times C \tag{1}$$

$$C = \frac{A}{Vd} \tag{2}$$

where C is the voriconazole concentration in the plasma, A is the amount of drug in the body, Vd is the apparent volume of distribution (in relation to bioavailability), k_a is the absorption rate constant, A_a is the amount of drug at the site of administration, CL is the total body clearance (in relation to bioavailability), and t is time.

2.7 | Population-based first-order elimination model

The model was refined since interindividual variability was high and was incorporated into the model using Equations (3) and (4), with dose level included in the final model as a categorical covariate to explain the difference in CL between the groups receiving low and high voriconazole doses (Equation 5).

$$Vd_i = tvVd \times e^{\eta Vd_i} \tag{3}$$

$$k_{a_i} = tvk_a \times e^{\eta k_{a_i}} \tag{4}$$

$$CL_i = tv\,CL \times \left(1 + dCL_{dose} \times doselevel\right) \times e^{\eta CL_i} \tag{5}$$

where i denotes the value of the parameter for the individual, tv denotes the typical value of the parameter for the population, η denotes the parameter that adjusts the typical value for the population to each individual, and dCL_{dose} adjusts the typical value for the population to account for doselevel (low = 1 and high = 0).

2.8 | Michaelis–menten elimination model

A model that incorporated the Michaelis–Menten equation to account for saturable clearance was built by incorporating Equation 6.

$$\frac{dC}{dt} = k_a \times A_a - \frac{V_{max} \times C}{k_m + C} \tag{6}$$

where V_{max} denotes maximal clearance rate, and k_m denotes the drug concentration at which clearance is 50% of maximum.

2.9 | Population-based michaelis–menten elimination model

Interindividual variability was incorporated into the model using Equations (7) through 10 with bodyweight as a covariate.

$$k_{a_i} = tv\,k_a \times e^{\eta k_{a_i}} \tag{7}$$

$$V_{max_i} = tvV_{max} \times e^{\eta V_{max_i}} \tag{8}$$

$$k_{m_i} = tv\,k_m \times e^{\eta k_{m_i}} \tag{9}$$

$$Vd_i = tv\,Vd \times \frac{\text{Body weight}}{\text{Average body weight}} \times dV\,dweight \times e^{\eta Vd_i} \tag{10}$$

where i denotes the value of the parameter for the individual, tv denotes the typical value of the parameter for the population, η denotes the parameter that adjusts the typical value for the population to each individual, and dVdweight denotes the parameter that adjusts the value of the volume of distribution for bodyweight.

2.10 | Simulation and model validation

The final two models (the first-order elimination kinetic model and the Michaelis–Menten elimination kinetic model) were used to predict plasma drug concentrations for the dosage regimen that was administered to the penguin with clinical aspergillosis (see Clinical case under *Treatment and Sampling Protocols*). A Monte Carlo simulation with 100 iterations was used to construct a 95% confidence interval for predicted values to which the observed concentrations in the collected samples were compared.

3 | RESULTS

The measured plasma voriconazole concentration vs. time data are given in Figure 1, showing a steeper slope for the terminal elimination

phase in the profiles with the lower concentrations (corresponding to the animals that were given the 2.5 mg/kg dose) compared to the profiles with the higher concentrations (corresponding to the animals that were given the 5 mg/kg dose). Plasma concentrations were above the targeted MIC of 1.0 μg/ml for susceptible *Aspergillus fumigatus* (EUCAST, 2018) only at the 2 hr sample time after the 2.5 mg/kg dose, whereas concentrations remained above this level for up to 56 hr after the administration of the 5 mg/kg dose. The noncompartmental pharmacokinetic parameters calculated for the two different doses using naïve pooling of the sparse data are summarized in Table 2. The parameter values for the initial (no covariates) and final (dose level a covariate of CL) versions of the first-order and Michaelis–Menton elimination models are given in Tables 3 and 4.

3.1 | Comparison of pharmacokinetic models

The pharmacokinetic model that incorporated the Michaelis–Menten equation to account for saturable clearance fit the data well, as did the model with first-order elimination once a covariate was added for dose level, although the AIC value for the Michaelis–Menten elimination model was higher compared to the final model with first-order elimination (153.8 vs. 147.2, respectively). Comparison of the plots of the individual predicted vs. observed concentrations suggested that the model with Michaelis–Menten elimination kinetics predicted the observed data slightly better than the first-order elimination model, although some of the higher concentrations were underpredicted by the model (Figure 2a,b). In addition, the residual error decreased from 0.36 to 0.30 for the Michaelis–Menten elimination model compared to the first-order elimination model. The calculated values of model parameters for the Michaelis–Menten-based model (without and with bodyweight as a covariate for volume of distribution) are summarized in Table 4.

3.2 | Simulations and model validation

The pharmacokinetic model with Michaelis–Menten elimination kinetics predicted drug accumulation and plasma voriconazole concentrations that continued to rise over time. Figure 3a shows the model predictions (within a 95% confidence interval) for the pharmacokinetic model with Michaelis–Menten elimination kinetics against the plasma voriconazole concentrations measured in vivo. It is clear that the predictions were not in line with the plasma voriconazole concentrations that were measured in the samples collected during long-term voriconazole treatment. Instead, the data were better predicted by the model that assumed first-order elimination without dose level as a covariate, with the majority of observed concentrations falling within the predicted 95% confidence interval (Figure 3b).

4 | DISCUSSION

The pharmacokinetics of voriconazole have been described in various veterinary species, but this is the first pharmacokinetic study performed in Magellanic penguins. Also, this study compared two pharmacokinetic compartmental models to describe the pharmacokinetics of voriconazole in Magellanic penguins and assessed their performance in predicting voriconazole plasma drug concentrations in a case of long-term oral voriconazole administration to a Magellanic penguin with clinical aspergillosis. The performance of both pharmacokinetic models was overall considered good following a single dose. However, the model describing elimination as saturable using the Michaelis–Menten equation overpredicted plasma voriconazole concentrations following long-term administration (from about 125 hr after the first dose), despite the fact that results of the single-dose studies suggested that elimination was saturated at the higher dose. In contrast, the model that assumed first-order elimination underpredicted some of the initial concentrations, but overall the concentrations measured during long-term administration fell within the 95% confidence interval of the predicted values. These findings are, however, in line with pharmacokinetic studies of voriconazole in other bird species. Nonlinear, saturated elimination kinetics (Michaelis–Menten kinetics) with disproportionate increases in C_{max} and AUC for higher single doses of voriconazole have been reported in other bird species, including African Grey parrots and Hispaniolan Amazon parrots (Flammer et al., 2008; Sanchez-Migallon Guzman et al., 2010;). Lower-than-expected concentrations following repeated and/or long-term administration, as was the case with the penguin with aspergillosis in this study, have also been observed in African Grey parrots, Hispaniolan Amazon parrots, and pigeons (Beernaert et al., 2009; Flammer et al., 2008; Sanchez-Migallon Guzman et al., 2010).

A possible explanation for these observations is that metabolizing enzymes in these birds became induced after long-term administration, increasing the body's capacity to eliminate the drug and therefore converting an initially saturable process to a first-order one. It is also possible that CYP enzymes of another subfamily were able to take over part of the metabolism of voriconazole; however, both these hypotheses have never been studied in penguins, or in

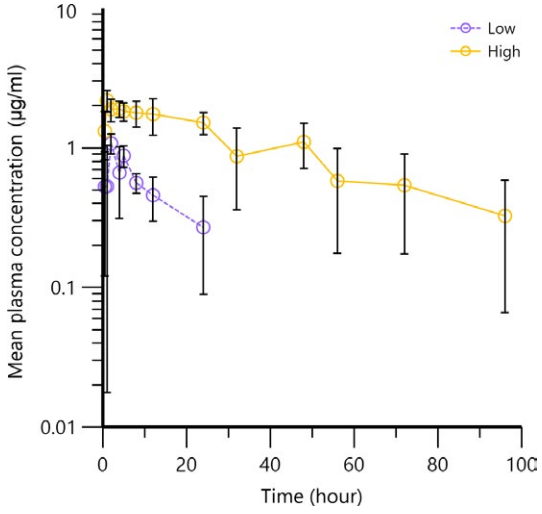

FIGURE 1 Plasma voriconazole concentration–time data (mean and standard deviation) collected from 15 Magellanic penguins administered two doses (low = 2.5 mg/kg and high = 5 mg/kg) over the course of three separate studies [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Comparison of noncompartmental pharmacokinetic parameters for voriconazole administered orally to Magellanic penguins at two different doses (2.5 and 5 mg/kg)

Pharmacokinetic parameter	Units	2.5 mg/kg	5 mg/kg
λ_Z	1/hr	0.10	0.02
$T_{½\lambda_Z}$	hr	7.26	33.71
T_{max}	hr	2.02	1.03
C_{max}	μg/ml	1.08	2.59
$C_{max/Dose}$	$kg*\mu g*ml^{-1}*mg^{-1}$	4.32	5.18
AUC_{last}	hr*μg/ml	10.43	92.43
$AUC_{0-\infty}$	hr*μg/ml	14.55	108.29
$AUC_{0-\infty/Dose}$	$hr*kg*\mu g*ml^{-1}*mg^{-1}$	58.20	216.58
% $AUC_{extrapolated}$	%	28.30	14.64
V_z/F	ml/kg	179.89	224.58
CL/F	$ml*hr^{-1}kg^{-1}$	17.18	4.62
$MRT_{0-\infty}$	hr	15.16	50.33

TABLE 3 Parameter value estimates for the one-compartment pharmacokinetic model with first-order absorption and elimination with or without dose level as a covariate for clearance

	Initial model		Final model		
Parameter	tv	IIV	tv	IIV	CV%
tvk_a (1/hr)	2.49	2.8×10^{-6}	2.19	2.8×10^{-6}	17.51
tvV[a] (ml)	11863.40	5.6×10^{-6}	11054.90	5.6×10^{-6}	4.86
tvCL[a] (ml/hr)	256.93	0.15	230.00	0.11	10.15
dCL_{dose}	NA	NA	2.64	NA	26.76
Residual error	0.39	NA	0.36	NA	7.36
AIC	169.1	NA	147.2	NA	NA

Notes. CL, total body clearance; CV%, Co-efficient of variation (measure of accuracy with which tv is predicted); dCL_{dose}, fixed effect accounting for difference in clearance between low and high dose groups; IIV, inter-individual variability (variance of η's); k_a, absorption rate constant; NA, Not applicable; tv, typical value of the parameter for the population; *V*, apparent volume of distribution

[a]*V* and CL values are confounded by unknown bioavailability of voriconazole following oral administration.

TABLE 4 Parameter values for the one-compartment model with first-order absorption and nonlinear elimination with or without bodyweight as a covariate for volume of distribution

	Initial model		Final model		
Parameter	tv	IIV	tv	IIV	CV%
tvk_a (1/hr)	2.92	4.0×10^{-5}	2.68	3.20×10^{-5}	16.32
tvV[a] (ml)	12825.30	0.077	12325.70	0.0087	4.00
tvK_m (μg/ml)	0.16	4.01×10^{-5}	0.27	3.23×10^{-5}	45.39
tvV_{max} (μg/hr)	279.52	0.057	384.32	0.25	16.36
dVd_{weight}	NA	NA	1.25	NA	18.04
Residual error	0.30	NA	0.30	NA	8.28
AIC	158.14	NA	139.19	NA	NA

Notes. CL, total body clearance; CV%, Coefficient of variation (measure of accuracy with which tv is predicted); dVdweight, fixed effect to explain how interindividual variability in volume of distribution is related to body weight; IIV, interindividual variability (variance of η's); k_a, absorption rate constant; NA, Not applicable; tv, typical value of the parameter for the population; *V*, apparent volume of distribution

[a]*V* and CL values are confounded by unknown bioavailability of voriconazole following oral administration.

other bird species. It is known that voriconazole is primarily metabolized by CYP2C19 (of which polymorphisms are known to exist in humans) and to a lesser extent by CYP2C9 and CYP3A4 in humans (Pfizer, Vfend package insert). For Magellanic penguins, it is unknown whether such polymorphisms or other variations in CYP enzyme profiles exist; however, there is evidence for differences in CYP expression between other bird and penguin species. A study by Almeida and co-workers (Almeida et al., 2016) on the genome of a large variety of bird species studying the presence of metabolic enzymes of the CYP2 family on a DNA level revealed a striking difference in the profile between Adelie penguins and Emperor penguins, even though both species live in the same environmental conditions and have a comparable diet. Adelie penguins appeared to have a broad spectrum of CYP2 enzymes, but lacking sufficient CYP2C enzymes, while the Emperor penguin only had three out of twelve CYP2 isoforms tested in the study. Since such differences are found between those two species of penguins, it cannot be ruled out that specific CYP enzymes are also lacking or expressed to a different extent in Magellanic penguins.

Metabolic differences may not only exist between bird/penguin species but also between individuals of the same species. These differences may result in drug toxicity in individuals due to large differences in achieved voriconazole plasma concentrations. Twenty-four cases of suspected voriconazole toxicity in six species of penguin have been reported by Hyatt and co-workers (Hyatt et al., 2015). Clinical signs of toxicity included anorexia, abnormal

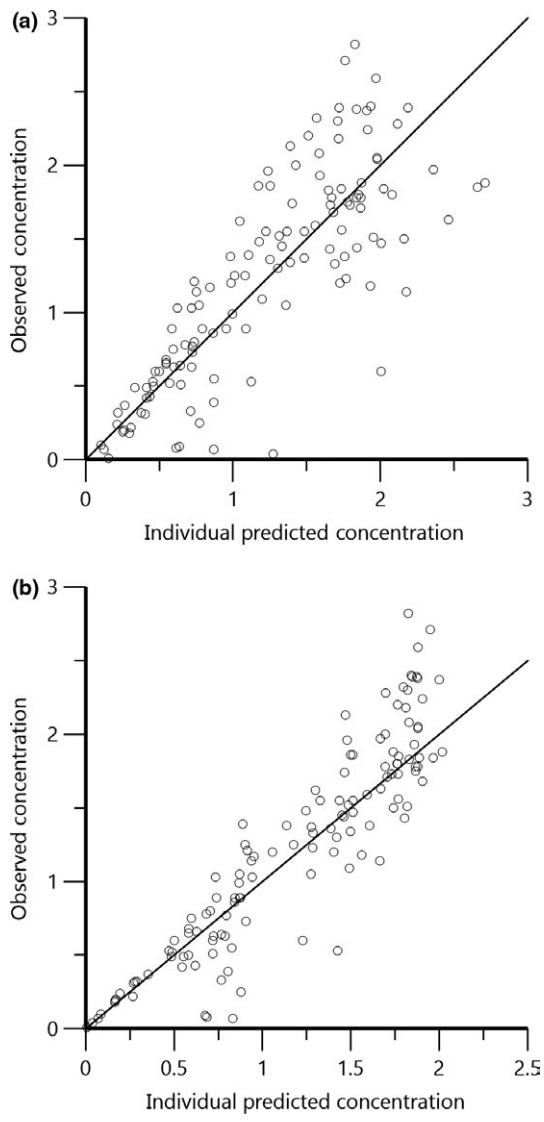

FIGURE 2 Plots of the predicted vs. observed plasma voriconazole concentrations for the model assuming first-order elimination with dose level incorporated as a covariate for CL (a) and the nonlinear incorporating saturable clearance with bodyweight as a covariate explaining interindividual variability in the volume of distribution (b)

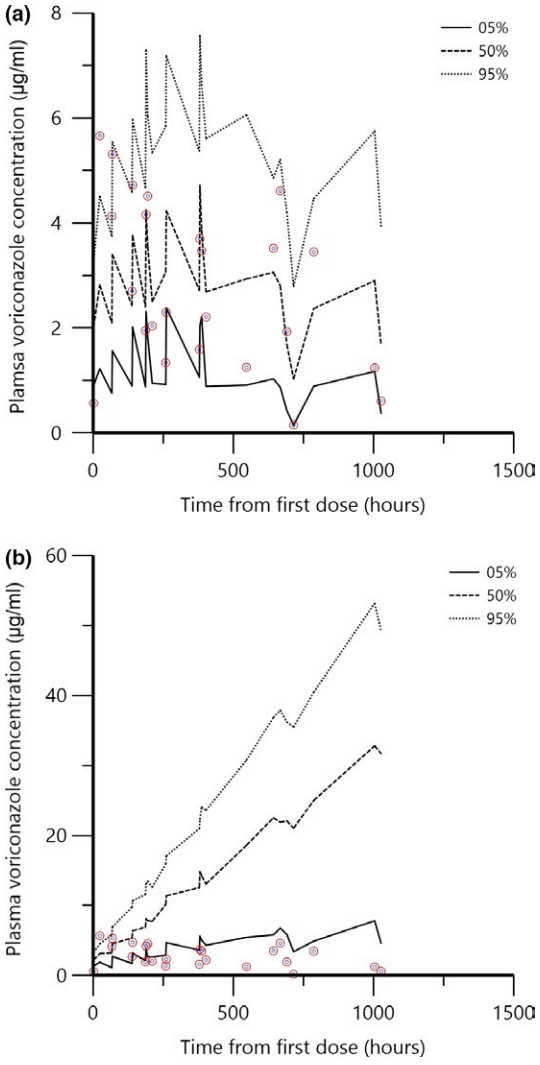

FIGURE 3 (a) Comparison of plasma voriconazole concentrations predicted by the pharmacokinetic model that assumes first-order elimination with observed concentrations following long-term administration in a clinical patient. (b) Comparison of plasma voriconazole concentrations predicted by the nonlinear pharmacokinetic model with saturable elimination with observed concentrations following long-term administration in a clinical patient [Colour figure can be viewed at wileyonlinelibrary.com]

behavior (lethargy, changes in mentation, depression, isolating self, staring off into the distance), or neurologic signs (weakness, ataxia, paresis, apparent changes in vision, seizure-like activity, and seizures) in penguins and elevated liver enzymes and bile acids in other birds (Hyatt et al., 2015). In the Hyatt et al. (2015), signs of toxicity in penguins occurred within 4 to 99 days of start of treatment in animals dosed at least once daily and after 448 and 511 days of treatment in penguins treated every 72 hr and doses in excess of 5 mg/kg were used in all cases. Plasma voriconazole in the affected penguins ranged from >10 to 64.17 μg/ml. In contrast, for our study no adverse reactions were observed in the penguins during the single-dose pharmacokinetic studies. A dose of 5 mg/kg once daily was associated with anorexia after 24 days of voriconazole administration to the Magellanic penguin with clinical aspergillosis described as the clinical case in this study. However, this penguin resumed normal appetite and activity within 36 hr of discontinuation of the voriconazole. Subsequent treatment with a treatment cycle of 5 days on treatment followed by a 2-day off period for several years subsequently resulted in no further episodes of apparent voriconazole toxicity. These observations suggest toxicity is likely to develop due to voriconazole accumulation after repeated administration as a result of a diminished elimination capacity in certain individual birds. However, since the initial single-dose study, voriconazole has been used routinely by San Francisco Zoo for both prophylaxis and treatment of clinical aspergillosis. At the time of writing, 67 individual Magellanic penguins have been treated using 5 mg/kg once daily orally in fish in cycles of 5 days on and 2 days off with courses of varying lengths but including two birds that were treated for more than 3 years. After initiation of voriconazole therapy, none of these penguins showed any toxicity-associated neurological disease or liver biochemistry abnormalities and nonspecific signs such as anorexia, lethargy resolved with response to therapy. Efficacy was considered to be very high, as assessed by resolution of clinical signs and heterophilia, temporal size reduction in suspected granulomatous lesions on computed tomography scans, and substantially increased survival times for clinical cases compared to treatment with itraconazole. This dose also corresponds with the dose suggested by Hyatt and co-workers for African penguins, another *Spheniscus* species (Hyatt et al., 2017).

With regard to assessing both voriconazole effective doses and risk of possible toxic effects, the formulation used for drug administration in penguins has to be considered. Although the manufacturer instructs to avoid administration of voriconazole with high-fat food due to a significant reduction of C_{max} and AUC (34% and 24% for tablets and 58% and 37% reduction for the oral suspension, respectively), voriconazole is usually administered to penguins in fish to minimize stress due to handling of the animals. Also, crushed tablets in water are often used instead of the suspension for oral administration due to economic reasons. The manufacturer states that bioequivalence was established between voriconazole tablets and the 40 mg/ml of suspension for oral administration when the same dose of voriconazole in mg was administered orally to humans (Pfizer, Vfend package insert). However, this bioequivalence seems no longer present when the drug is administered together with food (since there is a different effect on C_{max} and AUC for the tablets compared to the oral solution). With regard to the fish feeding, Hyatt and co-workers (Hyatt et al., 2017) tested both oral gavage and administration of voriconazole in fish to African penguins. They describe a mean lag phase of 1.53 hr in the pharmacokinetic concentration–time profile in penguins dosed with voriconazole in fish, compared to penguins dosed by oral gavage. Furthermore, in the same study the plasma concentrations measured on day 2 of the multiple dose study where already much higher (3.36 μg/ml 2 hr postdose) than the mean plasma C_{max} (1.89 μg/ml at 0.4 hr postdose) measured in the single-dose study in the penguins receiving oral gavage. Therefore, the influence of the fish was apparently not significantly high to strongly reduce the amount absorbed after oral administration, although part of this may have been obscured by the long elimination half-life of voriconazole (10.9 hr) measured in these penguins.

In conclusion, the pharmacokinetic models presented in this study accurately predict saturation elimination processes after a single dose of voriconazole and predict the switch in elimination of voriconazole to a first-order process during long-term treatment. Given the possible high interindividual variability in pharmacokinetics and the lack of information regarding metabolic pathways in Magellanic penguins, dose and dosing intervals of voriconazole may need to be adjusted for individual penguins based on monitoring plasma voriconazole concentrations during long-term treatment to ensure that safe and effective concentrations are maintained.

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CONFLICTS OF INTEREST

The authors have no conflict of interests to declare.

AUTHOR CONTRIBUTIONS

The authors contributed to this study as follows: LT contributed to conceptualization, project administration, supervision, and

resources; RP, AM, JH, and LT involved in investigation; LT and RG contributed to methodology; RG involved in data analysis and interpretation and pharmacokinetic modeling; FT contributed to pharmacokinetic analysis; and LT, AM, and JH involved in funding acquisition. All authors have read and approved the final manuscript.

ORCID

Femke J. Taverne http://orcid.org/0000-0001-5873-0565
Lisa A. Tell http://orcid.org/0000-0003-1823-7420
Ronette Gehring http://orcid.org/0000-0002-1329-201X

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