PHARMACOMETRIC BENCHMARKING: QUANTITATIVE METHODS TO ASSESS THE PREDICTIVE PERFORMANCE OF POPULATION PHARMACOKINETIC MODELING PROGRAMS

by

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ABSTRACT

Throughout much of history, safe and effective drug doses have been discovered through trial-and-error and validated via anecdote. Such approaches are limited in their ability to define how a drug's safety and effectiveness are influenced by the addition of other co-administered medications and the presence of other acute and/or chronic diseases. Consideration of all these pharmacological and pathophysiological factors is impractical given the complexity of the many interactions that may occur.

To further advance clinical pharmacology, it has become necessary to leverage the increasing speed and storage capacity of computers. Developments in mathematics, statistics, and computer science have revolutionized the field of clinical pharmacology by making computers far more than glorified calculators. Today, sophisticated algorithms can be used to interrogate and learn from pharmacological datasets and make informed predictions about the safety and effectiveness of drug dosing regimens. The goal of these population pharmacokinetic analyses is to yield accurate predictions of clinically-relevant pharmacokinetic parameters and improve our understanding of the biological processes that mediate drug disposition.

In this dissertation, we present the results of three pharmacokinetic studies that demonstrate the clinical utility of population pharmacokinetic modelling, along the way challenging conventional dosing strategies for vancomycin in preterm neonates and zolpidem among severely burned children. Additionally, we developed a simulation-based

parameter estimation algorithms. This work lays the foundation for a transparent dialogue regarding the relative strengths and weaknesses of individual algorithms, which heretofore has not been possible. We conclude with a discussion of the additional unanswered questions that may now be investigated using the benchmarking framework developed here.

The results of the studies described in this dissertation underscore the importance of enhancing the clinical adoption of population pharmacokinetic models. However, these models must be rigorously evaluated to ensure that they are unbiased and precise. In simulations, three of the most commonly used pharmacokinetic parameter estimation algorithms differentiated themselves when they were applied in different clinical scenarios. This finding highlights an intriguing practical fact that algorithm selection should be guided by the clinical question at hand.

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LIST OF ABBREVIATIONS

AIC	Akaike information criterion
APGAR	Newborn scale based on appearance, pulse, grimace, activity, and respiration
AUC	Area under the concentration versus time curve
AUC_{24}	24 hour area under the concentration versus time curve
BMI	Body mass index
BSV	Between subject variability
CDC	United States Centers for Disease Control and Prevention
CF	Cystic fibrosis
CFU	Colony forming units
CI	Confidence interval
CL	Clearance
CL/F	Apparent clearance
C_{max}	Maximum concentration
C_{min}	Minimum concentration
Cr	Serum creatinine
CRRT	Continuous renal replacement therapy
CV	Coefficient of variation
df	Degrees of freedom
EM	Expectation maximization
EMA	European Medicines Agency
FDA	United States Food and Drug Administration
GFR	Glomerular filtration rate
IPRED	Individual predicted concentration
IQR	Interquartile range
K_a	Absorption rate
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NICU	Neonatal intensive care unit
NONMEM	Nonlinear mixed effects models
NPAG	Nonparametric adaptive grid
NPDE	Normalized prediction distribution error
OFV	Objective function value
PCH	Primary Children's Hospital
PMA	Postmenstrual age
PRED	Predicted concentration

Q	Intercompartmental clearance
Q / F	Apparent intercompartmental clearance
RSE	Relative standard error
RUV	Residual unexplained variability
SAEM	Stochastic approximation expectation maximization
TBSAB	Cumulative percentage of the total burned body surface area
V	Volume of distribution
V_c / F	Apparent central volume of distribution
V_d	Volume of distribution
V_d / F	Apparent volume of distribution
V_p / F	Apparent peripheral volume of distribution
V1	Central volume of distribution
V2	Peripheral volume of distribution
WT	Body weight
θ	Population pharmacokinetic parameter typical value
ε	Residual variability
η	Between subject variability
σ	Residual variability standard deviation
ω	Between subject variability standard deviation
ω^2	Between subject variability variance
Ω	Variance-covariance matrices for η
Σ	Variance-covariance matrices for ε

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CHAPTER 1

POPULATION PHARMACOKINETIC MODELING

The Relationship between Clinical Trials and Population Pharmacokinetics

Confirmatory clinical trials aim to reject the null hypothesis that the treatment regimen under investigation has no effect.[1] By design, these trials study a limited number of doses in relatively homogeneous patient populations.[2] Unfortunately, these trials often only answer the very first question of interest to clinicians – is my patient likely to benefit from this therapy? If the answer is yes, then several other practical questions must also be asked, including:

1) What is an appropriate initial dose for my patient?
2) How soon will beneficial (and potentially harmful) effects start?
3) How long will the beneficial (and potentially harmful) effects last?
4) Will tolerance develop?
5) What is the likelihood that the initial dose will need to be changed?
6) What metrics should be used to determine if the dosing regimen needs to be changed?
7) At what point should the dosing regimen be changed, and is a large or a small change appropriate?

Sheiner raised these questions several years ago to illustrate the difference between confirmatory clinical trials and population pharmacokinetic studies.[2] Population pharmacokinetic analyses are well suited to the types of questions described above as they allow us to quantify and understand the variability in drug responses among a population of patients, which then makes it possible to develop personalized dosing regimens after establishing how an individual patient differs from the population at large.[3]

Individual *vs.* Population Pharmacokinetics

Traditionally, pharmacokinetic studies have involved intensive serial blood sampling performed in a limited number of healthy, male, adult volunteers.[4-6] These studies allow the investigator to estimate the variability in plasma drug concentrations between individuals following the administration of a certain dose. In contrast, the population pharmacokinetic approach allows the investigator to characterize the pharmacokinetics of the drug of interest using fewer blood samples by treating all of the individuals in the study as a random sample from a larger population. From these data, it is then possible to estimate measures of central tendency for the pharmacokinetic parameters of the entire population, while simultaneously estimating within and between subject variability and quantifying the amount of residual, unexplained variability.[7] This improves the population mean and variance estimates and improves accuracy when selecting an initial dosing regimen or adjusting a dosing regimen in response to therapeutic drug monitoring data.

History of Population Pharmacokinetics

Historically, pharmacokinetic analyses were conducted using a two-stage procedure in which each individual's pharmacokinetic parameters were calculated using nonlinear regression methods. The parameters calculated for each individual were then averaged together to yield summary descriptive statistics for the population, including estimates of the mean pharmacokinetic parameter values and their variances. Similarly, other factors that influence the drug's concentration-time profile were identified using classical statistical approaches (e.g., linear regression or covariance analysis). Although this approach has been shown to yield unbiased pharmacokinetic parameter estimates for the population mean, the variance and covariance are often overestimated.[8-11]

To better meet the needs of individual patients, Sheiner, Rosenberg, and Melmon developed a 'conceptual scheme and associated statistical methodology designed to provide the basis for a clinically useful computer program to suggest optimal dosing regimens for a number of drugs' in 1972.[12] This conceptual scheme and statistical methodology was purpose built to perform well in clinical scenarios with sparse amounts of data, where the traditional two-stage procedure failed. The principal factor that differentiates this approach is that it considers the cohort of patients being treated (the 'population') as the unit of analysis, rather than the individual. Consequently, estimation of the pharmacokinetic parameters can be performed despite the use of sparse, unbalanced, or fragmented data, as compared with the two-stage procedure that required rigid, intensive sampling designs akin to those observed in prospective randomized controlled trials. Additionally, the nonlinear mixed effects modeling approach outlined by Sheiner, Rosenberg, and Melmon models the mean pharmacokinetic parameter values for the

population (derived from fixed effects terms) as well as the variability within the population (derived from random effects terms). For the remainder of this dissertation, this approach shall be interchangeably referred to as 'population pharmacokinetic modeling' and 'nonlinear mixed effects modeling'.

Building from their population pharmacokinetic conceptual framework, Sheiner and Beal developed the first version of NONMEM (nonlinear mixed effects modeling) in 1980, which employed a first-order parameter estimation algorithm.[13, 14] Independently, in 1986, Mallet et al. developed the first nonparametric pharmacokinetic parameter estimation algorithm.[15] In 1990, Lindstrom and Bates developed the first-order conditional estimation algorithm for nonlinear mixed effects models with repeated measures data.[16] Shortly thereafter, Schumitzky developed a nonparametric expectation maximization algorithm in 1991.[17] More recently, stochastic approximation expectation maximization algorithms were developed and simulations with interaction terms were conducted, which demonstrated their superiority over traditional first-order conditional estimation methods.[18-20] Additional details regarding the statistical methodologies employed in each of these iterative advancements in pharmacokinetic parameter estimation algorithms are described below.

General Mathematical Formulation

Population pharmacokinetic models involve the fitting of nonlinear mixed effects models to drug concentration data collected from multiple patients with the purpose of simultaneously estimating: (1) the pharmacokinetic parameters for the typical individual in the population; (2) the variability within the population; and (3) the unexplained

variability that may result from measurement error or a poorly specified model.[13] The general mathematical formulation for a nonlinear mixed effects model is:

$$y_{ij} = f\left(t_{ij}, g(\theta, \eta_i, x_i, z_i)\right) + h(t_{ij}, g(\theta, \eta_i, x_i, z_i), \varepsilon_{ij}) \quad (1.1)$$

where $f()$ is the function used to describe the structure of the model and $h()$ is the function used to describe the residual error model. t_{ij} represents the drug concentration measured for individual i at time j. $g()$ is a vector function that defines the ith individual's pharmacokinetic parameters given the vector of typical value parameters θ, the ith individual's random effects η_i, the ith individual's vector of study design variables x_i (e.g., the dosing regimen), and z_i, the ith individual's covariate vector (e.g., body weight, postmenstrual age, creatinine clearance, etc.).

It is unlikely that the pharmacokinetic parameters of the ith individual will perfectly match the typical pharmacokinetic parameter values for the population (θ); therefore, the individual pharmacokinetic parameters are said to deviate from θ by a vector of random effects terms of the same length as the number of pharmacokinetic parameters being estimated for the ith individual (η_i), where $\eta_i \sim N(0, \Omega)$. Here Ω is a covariance matrix that reflects the correlations between the individual pharmacokinetic parameters. The diagonal components of Ω reflect the between subject variability for each pharmacokinetic parameter.

The residual error model (ε_{ij}) describes the difference between the individual predicted concentration and the measured drug concentration, which is assumed to follow a normal distribution of the form $\varepsilon_{ij} \sim N(0, \Sigma)$. ε_{ij} is a vector of residual error terms and Σ is the covariance matrix that reflects the correlations between the ε_{ij} terms.

Methodological Approaches Employed in Population Pharmacokinetic Analyses

To derive estimates of the population pharmacokinetic parameters maximum likelihood estimation methods are used. The maximum likelihood method specifies the probability density function for individual i that optimizes the pharmacokinetic parameter estimates needed to maximize the likelihood of observing the vector of measured drug concentrations given the patient's dosing record and covariate vectors. The joint probability distribution for y_i and η_i can be expressed as:

$$p(y_i, \eta_i | \psi) = L_i(\psi | y_i, \eta_i) = p(y_i | \psi, \eta_i) * p(\eta_i | \psi) \quad (1.2)$$

in which L_i is the ith individual's likelihood given y_i and η_i. ψ is the vector of the typical parameter values (θ) and the variance-covariance matrices (Ω and Σ). $p(y_i | \psi, \eta_i)$ is the conditional probability density of the measured drug concentrations (y_i) given ψ and η_i. Lastly, $p(\eta_i | \psi)$ is the conditional probability density of η_i given ψ; however, since η_i cannot be measured experimentally the marginal distribution of the measured drug concentrations (y_i) is reformulated to yield the following likelihood function:

$$L(y | \psi) = \int p(y_i | \eta_i, \psi) \cdot p(\eta_i, \psi)\, d\eta_i \quad (1.3)$$

where $p(y_i | \eta_i, \psi)$ is the conditional probability of the measured drug concentrations (y_i) given the vector of random effects (η_i) and ψ. $p(\eta_i, \psi)$ denotes the joint population parameter density of the individual random effects.

For nonlinear functions, the likelihood cannot be maximized with a closed form solution. Therefore, several specialized software programs have been developed to approximate the maximum likelihood estimation. The most commonly used is the nonlinear mixed effects modeling program NONMEM (ICON Development Solutions, Ellicott Bay, MD, United States), which approximates the integrand and yields a closed

form expression for $L(y|\psi)$ that is computationally tractable.[21] Initially, NONMEM utilized a first-order algorithm, which is known to result in biased parameter estimates with high between subject variability.[22] More recently, NONMEM has adopted a first-order conditional estimation algorithm in which the individual random effects estimates from the current iteration of the model are conditionally estimated from the random effects estimates obtained from the previous iteration of the linearized model.[23]

An alternative pharmacokinetic parameter estimation algorithm is employed in the software program Monolix (Lixoft, Orsay, France), which uses expectation maximization (EM) methods that integrate the posterior density by performing Monte Carlo sampling over all possible individual parameters during the expectation step, which is then followed by a single iteration maximization step that moves the pharmacokinetic parameter value closer toward the maximum likelihood.[24] Delyon et al. demonstrated that the EM algorithm converges under very general conditions and Kuhn and Lavielle further established that the coupling of the EM algorithm with the Markov Chain Monte Carlo procedure rapidly converses toward the maximum likelihood estimate.[25, 26]

Pmetrics is another commonly used population pharmacokinetic modeling program that makes no assumptions regarding the distribution of the density function.[17] Pmetrics uses a nonparametric adaptive grid algorithm that performs no formal numerical optimization; rather it quasi-Monte Carlo methods to generate a grid of Faure points that can be rapidly tested to assess whether they improve the likelihood beyond the grid consisting of points derived from the model's initial estimates.[27] More specifically, this deterministic set of Faure points is used to approximate the integration featured in Equation 1.3.[27] This process is iteratively repeated to yield a final, discrete nonparametric

distribution of pharmacokinetic parameter estimates.[27] This algorithm has the advantage of appropriately identifying sub-populations with different pharmacokinetic profiles (e.g., varying hepatic formation clearances of CYP3A-metabolized drugs due to genetic polymorphisms that affect the level of CYP3A expression).[28]

As noted above, several specialized population pharmacokinetic modeling programs have emerged over the last 30 years that employ different pharmacokinetic parameter estimation algorithms, which have the potential to lead to dramatically different results.[21, 24] For this reason, benchmarks are needed to experimentally assess the strengths and weaknesses of current population pharmacokinetic modeling programs. The accuracy and precision of these estimates may impact clinical decisions and lead to alterations in medical management, such that the selection of a pharmacokinetic parameter estimation method with lower bias and higher precision is desirable.

Applications of Population Pharmacokinetics

The drug development process involves several iterative stages in which compounds are evaluated to confirm their safety and efficacy prior to regulatory approval, marketing, and widespread use.[29] Population pharmacokinetic modeling is used to increase our understanding of the quantitative relationships between drug dosing regimens, patient characteristics, and drug pharmacokinetics. Today, the use of population pharmacokinetic modeling is actively encouraged by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA).[29, 30] Despite the widespread acceptance of population pharmacokinetic methods in the drug approval process, relatively few population pharmacokinetics studies have been conducted among

children. Rectifying this scarcity of pediatric-specific population pharmacokinetic data has the potential to: (1) result in optimized dosing regimens that improve therapeutic effectiveness across the pediatric age spectrum from neonates to adolescents; (2) reduce the incidence of adverse drug reactions; and (3) generate substantial cost savings to the healthcare system.[31]

Dosing Optimization

Deriving the 'optimal' individualized dose that is neither ineffective nor toxic is the ultimate goal of many physicians, pharmacologists, regulatory agencies, and pharmaceutical companies.[32] Achieving this goal is challenging for many drugs due to pharmacokinetic variability within and between patients. For drugs with narrow therapeutic windows (a small margin separates sub-therapeutic from toxic concentrations), it is necessary to conduct population pharmacokinetic studies to determine whether predictable factors (covariates) can be identified that influence the extent and peak of drug exposure.[33] If substantial variability remains after such investigations and a target concentration range has been established, then it may be prudent to measure drug concentrations in each patient (a practice known as therapeutic drug monitoring).[34, 35] Drug concentration measurements obtained from therapeutic drug monitoring can then be used to refine the model's pharmacokinetic parameter predictions for that patient in a Bayesian manner.[36]

Statement of Objectives

The objective of this dissertation is to demonstrate the clinical utility of population pharmacokinetic models and to assess the predictive performance of several population pharmacokinetic modeling programs that are commonly used in evaluating drug concentration time profiles and the response to therapy.

The specific aims are as follows:

1) Define the population pharmacokinetics of two drugs belonging to different drug classes in a selection of rarely-studied pediatric patient populations. These analyses include an evaluation of the population pharmacokinetics of:
   - Vancomycin among children with invasive methicillin-resistant *Staphylococcus aureus* (MRSA) infections; and
   - Zolpidem among children with severe burn injuries.
2) Assess the performance of several commonly used population pharmacokinetic software programs in establishing precise and unbiased pharmacokinetic parameter estimates with varying: amounts of error / noise, sample sizes, and numbers of samples from each patient.

The foundation for the first specific aim is outlined in Chapter 2. Chapters 3 and 4 discuss two clinical applications of vancomycin population pharmacokinetic models among children with cystic fibrosis and neonates with invasive bacterial infections, respectively. Chapter 5 describes a population pharmacokinetic study involving the sedative agent zolpidem, which was administered in an effort to restore normal sleep

architecture among a cohort of severely burned children. Chapter 6 describes a simulation-based approach to benchmarking population pharmacokinetic software programs and is under preparation for submission. Publications that have stemmed directly from this work include:

- Stockmann C, Roberts JK, Yu T, et al. Vancomycin pharmacokinetic models: informing the clinical management of drug-resistant bacterial infections. *Expert Review of Anti-infective Therapy* **2014**; 12(11): 1371-88.
- Stockmann C, Sherwin CM, Zobell JT, et al. Population pharmacokinetics of intermittent vancomycin in children with cystic fibrosis. *Pharmacotherapy* **2013**; 33(12): 1288-96.
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During the course of this dissertation, several fruitful collaborations have also led to publications that are not discussed within this dissertation, which are featured in Appendix A.

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CHAPTER 2

VANCOMYCIN PHARMACOKINETIC MODELS: INFORMING THE CLINICAL MANAGEMENT OF DRUG-RESISTANT BACTERIAL INFECTIONS

Abstract

This review aims to critically evaluate the pharmacokinetic literature describing the use of vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Guidelines recommend that trough concentrations be used to guide vancomycin dosing for the treatment of MRSA infections; however, numerous *in vitro*, animal model, and clinical studies have demonstrated that the therapeutic effectiveness of vancomycin is best described by the area under the concentration versus time curve (AUC) divided by the minimum inhibitory concentration (MIC) of the infecting organism (AUC/MIC). Among patients with lower respiratory tract infections, an AUC/MIC≥400 was associated with a superior clinical and bacteriological response. Similarly, patients with MRSA bacteremia who achieved an Etest AUC/MIC≥320 within 48 hours were 50% less likely to experience treatment failure. For other patient populations and different clinical syndromes (e.g.,

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children, the elderly, patients with osteomyelitis, etc.) pharmacokinetic/pharmacodynamic studies and prospective clinical trials are needed to establish appropriate therapeutic targets.

Background

Vancomycin was first approved for use by the United States Food and Drug Administration (FDA) in 1958.[1] Despite more than 50 years of experience with this antibiotic, uncertainty remains regarding the most appropriate vancomycin dosing strategy.[2] This is primarily attributable to its variable pharmacokinetic profile, the emergence of vancomycin resistance, and its toxic effects.[3] Currently, intravenous vancomycin is reserved nearly exclusively for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, which until the late 1970s and early 1980s were extremely rare and confined to a few large hospitals.[4] More recently, other factors, such as the controversial 'MIC creep' phenomenon and a heightened awareness of the potential for sub-therapeutic dosing have further complicated vancomycin dosing. At the level of an individual patient, high between subject variability complicates efforts to develop simplified or standardized vancomycin dosing regimens.[5] Despite these challenges, pharmacokinetic and pharmacodynamic modeling techniques may be used to inform vancomycin dosing, even for adult populations, for which large amounts of data exist.[2]

Over the last 10 years, vancomycin dosing regimens have shifted toward larger, more frequent doses.[6] This has likely occurred in response to *in vitro* studies, which demonstrated that low vancomycin concentrations exert a selective pressure that drives the emergence of more resistant *S. aureus* isolates.[7] To prevent such occurrences,

professional society guidelines have increased vancomycin exposure targets in an effort to more rapidly achieve and maintain therapeutic concentrations.[8] Consequently, many patients who receive vancomycin today are being maintained at concentrations that are closer to levels associated with nephrotoxicity than ever before, which makes therapeutic drug monitoring imperative.[9] Simultaneously, there has been an increase in the incidence of hospital- and community-associated MRSA.[10] In 2003, nearly 60% of *S. aureus* isolates obtained from patients in the intensive care unit were methicillin-resistant.[11, 12] More recently, the US Centers for Disease Control and Prevention (CDC) estimated that the incidence of MRSA infections declined 31% from 2007 to 2012.[13] Although the reasons for this decline are unclear, the continued widespread use of vancomycin in the context of fewer invasive MRSA infections poses substantial risks and must be evaluated in light of the potential for promoting vancomycin resistance.

The safe and effective administration of vancomycin at the level of the individual patient, especially in light of current practice patterns, may require more sophisticated techniques than merely dosing by total body weight and estimated renal function.[14] Population pharmacokinetic models, which leverage data from a population of patients to derive an optimal population-specific dosing strategy, are one such example.[15] When applied to direct patient care, the purpose of population pharmacokinetic modeling is to provide quantitative and semi-quantitative guidelines for dose optimization. Unlike traditional pharmacokinetic evaluations, the population pharmacokinetic approach is unique in that it: (1) derives pharmacokinetic parameter estimates that are representative of the population being treated; (2) recognizes sources of variability (e.g., between subject, intra-subject, and inter-occasion variability); (3) identifies factors that influence the

pharmacokinetic behavior of the drug; and (4) quantitatively expresses the magnitude of the unexplained variability within the patient population being treated.[15] For patients requiring antibiotic therapy for the treatment of drug-resistant bacterial infections it is critical to quickly establish an effective and safe dosing regimen. Consequently, the purpose of this review is to critically evaluate the vancomycin pharmacokinetic literature with respect to its use in the treatment of MRSA infections. A secondary objective is to identify special patient populations who may require alternative vancomycin dosing regimens as a consequence of their demographic factors, physiologic status, or co-morbid conditions. The patient populations investigated in this review include: neonates and infants, children and adolescents, the elderly, obese patients, cancer patients, patients requiring continuous renal replacement therapy, patients with cystic fibrosis, and the critically ill. Emphasis will be placed on the integration of vancomycin population pharmacokinetic models into clinical care and the role that vancomycin therapeutic drug monitoring plays in preserving the utility of this antibiotic, as few suitable alternatives exist.

Pharmacokinetic Profile

The chemical structure of vancomycin is presented in *Figure 2.1*.

Absorption

Vancomycin is not well absorbed through the gastrointestinal tract and therefore achieves high colonic concentrations, which have been reported to range from 500-1000 mcg/mL following oral doses of 500 mg every 6 hours.[16] Due to this unique absorption

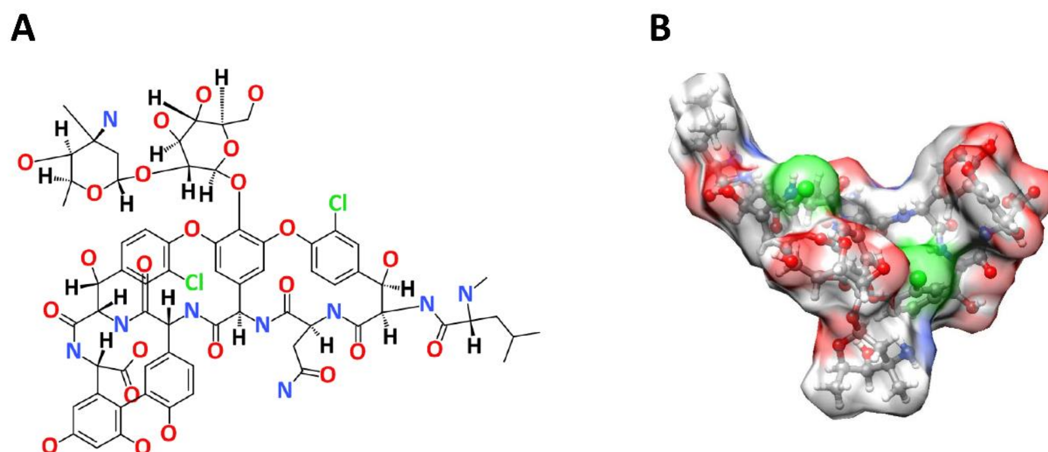

Figure 2.1. Chemical (A) and molecular (B) structures of the glycopeptide antibiotic vancomycin.

profile, vancomycin is recommended for the treatment of *Clostridium difficile* infections.[17] However, it must be noted that patients with bowel inflammation can have increased absorption following oral vancomycin administration.[18] Moreover, patients with severe renal disease and inflammatory bowel disease have the potential to reach toxic serum concentrations.[18, 19] Other modes of administration, such as intraperitoneal, intraventricular, and intrathecal dosing, have been rarely reported. Bunk et al. compared a single 10 mg/kg dose of vancomycin administered intravenously to a 10 mg/kg dose given intraperitoneally and noted that 65% of the intraperitoneal dose was absorbed, which yielded a peak plasma concentration of 6.3 mcg/mL.[20] They suggested that therapeutic plasma concentrations in excess of 10 mcg/mL could be reached with an intraperitoneal dose of 30 mg/kg followed by 1-5 mg/kg in each peritoneal exchange for patients in chronic renal failure. Intraventricular and intrathecal dosing have been described in several case reports, all of which reported high cerebrospinal fluid (CSF) concentrations without evidence of high serum concentrations.[21, 22] For the treatment of MRSA infections, vancomycin is commonly administered intravenously due to its poor oral bioavailability and extreme pain associated with intramuscular administration.[23] Therefore, the remainder of this review will focus on the intravenous use of vancomycin, which is delivered as a slow intravenous infusion with a standard infusion time of approximately 1 hour for a 1 g dose to avoid red man syndrome.[24-26]

Distribution

Vancomycin time-versus-concentration profiles have been reported as mono-, bi-, or triphasic, though the majority of the literature suggests a biphasic process after intravenous administration.[4, 27-30] The α-distribution phase ranges from 0.5-1 hr and

the β-elimination half-life is between 6-12 hrs in adults with normal renal function, which demonstrates its high between subject variability.[4, 28-31] After a single administration of the recommended dose of 15 mg/kg, peak serum concentrations at 2 hrs after infusion reach approximately 25 mcg/mL.[32, 33] Vancomycin is highly hydrophilic, with a volume of distribution at steady state comparable to that of total body water.[34, 35] The volume of distribution has been reported to range from 0.39-2.04 L/kg at steady state and is influenced by age, gender, and body weight.[4, 28-31, 36] The volume of the central compartment is approximately 10% of the volume of distribution, which is similar to the total volume of blood.[31] Protein binding in the serum is moderate, with most reports ranging from 50-55%.[37, 38] Due to its large volume of distribution, vancomycin readily crosses into ascitic, pericardial, synovial, and pleural fluids.[39] In addition, concentrations in abscess fluid are similar to those in serum.[40] Very low concentrations of vancomycin cross the blood brain barrier (0-0.18 CSF to serum ratios), unless the meninges are inflamed, which can then result in CSF to serum ratios of 0.36-0.48.[41, 42] Concentrations in lung tissue range from 5-50% of serum concentrations and have an overall blood to epithelial lining fluid ratio of 6:1 in critically ill patients.[43-46] Vancomycin concentrations in the bone are approximately 10% of serum concentrations, though this increases to 20-30% in infected bone.[47] An exploratory analysis of the pharmacokinetics and tissue penetration of vancomycin administered via continuous infusion as prophylaxis for vascular surgery found that vancomycin concentrations in the arterial wall were approximately 50% of those found in the serum; however, penetration into fat was lower at 22%.[48]

Metabolism

Early pharmacokinetic studies indicated that vancomycin is not efficiently metabolized.[32, 49] However, more recent studies have suggested that hepatic clearance may occur to a small degree, although these reports failed to find evidence of a prolonged vancomycin half-life among patients with impaired hepatic function.[29, 30] In addition, it is estimated that non-renal clearance may account for between 5-30% of total clearance.[24, 50]

Elimination

Up to 90% of the vancomycin dose is excreted unchanged within 24 hrs.[25] Renal excretion occurs primarily through glomerular filtration.[31] Nielsen et al. reported a vancomycin clearance to creatinine clearance ratio of 0.53 ± 0.11.[51] Similarly, Krogstad et al. reported a mean vancomycin clearance to creatinine clearance ratio of 0.68 ± 0.07.[31] These discrepancies may be suggestive of renal tubular reabsorption; however, no definitive reports have documented renal tubular reabsorption in humans and this discrepancy may be explained by moderate serum protein binding.[39] In adult population pharmacokinetic models, vancomycin clearance has been found to be highly correlated with creatinine clearance, weight, and age.[52-58] If creatinine clearance is not measured directly, the Cockroft-Gault equation may be used, which includes body weight, sex, age, and serum creatinine concentrations, to estimate creatinine clearance.[59]

Considerations for Pharmacokinetic Modeling

Many vancomycin population pharmacokinetic models have been published for adults in the last 20 years. In this section of the review, we will focus on studies that evaluated adults with serious drug-resistant bacterial infections. In coming sections, studies evaluating patients with renal impairment and other pathophysiologic process and co-morbidities will be discussed at length.

Seven seminal adult vancomycin pharmacokinetic modeling studies are presented in *Table 2.1*. The age ranges across these studies varied from 17-95 years.[52-54, 56-58] With regard to the development of the vancomycin structural model, several used one-compartment models and several others used two-compartment models. Vancomycin is well-known to feature a biphasic distribution and elimination phase, which is revealing as studies that involved intensive sampling often fit a two-compartment model; whereas sparse sampling schemes often could only fit a one-compartment model. Between subject variability was modeled using an exponential,[53, 55, 57, 58] a combined,[52] a proportional,[54] and an additive error model.[56] The between subject variability ranged from 19.8-38.5% and 18.2-36.4% for clearance and volume of distribution, respectively. Residual unexplained variability was modeled as an additive,[53, 56] combined,[52, 54, 55] and an exponential error model.[57, 58] Residual variability for the exponential and additive models ranged from 12.7-24.9% and 1.6-18.5%, respectively. The estimates for clearance ranged from 0.031-0.086 L/hr/kg in adults.[52-58] Estimates reported for the volume of distribution from the central compartment were quite variable, ranging from 0.39-2.04 L/kg.[57, 60] The majority of the population pharmacokinetic studies reported an effect of creatinine clearance or the rate of glomerular filtration on clearance.[2, 52] In

Table 2.1. A comparison of adult vancomycin population pharmacokinetic studies.

Study (Year)			**Ref.**
Revilla *et al.* (2010)	*Patient population*	Intensive care unit patients	53
	Number of patients studied	191	
	Age, years (median [range], mean ± standard deviation)	61.1 ± 16.3 [18-85]	
	Number of compartments	One	
	Final model (central compartment)	$CL = \theta_1 \times CL_{CR} + Age^{\theta_2}$ $V = \theta_3 \times \theta_4^A$, where A = 0 if SCr ≤1 mg/dL and A = 1 if SCr >1 mg/dL	
	Clearance	0.67 mL/min/kg	
	Volume of distribution	0.82 L/kg	
	Between subject variability model	Exponential	
	Residual variability model	Additive	
	Validation	External	
Thomson *et al.* (2009)	*Patient population*	Adults who received vancomycin	82
	Number of patients studied	398	
	Age, years (median [range], mean ± standard deviation)	66 [16-97]	
	Number of compartments	Two	
	Final model	$CL = \theta_1 \times CL_{CR}$ $V_1 = \theta_3$ x Total body weight $Q = \theta_4$ $V_2 = \theta_5$ x Total body weight	
	Clearance	CL = 2.99 L/hr Q = 2.28 L/hr	
	Volume of distribution	V_1 = 0.675 L/kg V_2 = 0.732 L/kg	
	Between subject variability model	Exponential	
	Residual variability model	Combined	
	Validation	External	

Table 2.1. Continued.

Staatz *et al.* (2006)	*Patient population*	Cardiothoracic surgery wound infections	55
	Number of patients studied	102	
	Age, years (median [range], mean ± standard deviation)	66 [17-81]	
	Number of compartments	One	
	Final model (central compartment)	$CL = \theta_1 \times (1 + \theta_2 \times (CL_{CR} - CL_{CR,\ median}))$ $V = \theta_3$	
	Clearance	2.97 L/hr	
	Volume of distribution	1.24 L/kg	
	Between subject variability model	Exponential	
	Residual variability model	Combined	
	Validation	External	
Tanaka *et al.* (2010)	*Patient population*	MRSA infections	56
	Number of patients studied	164	
	Age, years (median [range], mean ± standard deviation)	74 [17-94]	
	Number of compartments	One	
	Final model (central compartment)	$CL = \theta_1 \times GFR$ $V = \theta_2$	
	Clearance	0.88 L/hr	
	Volume of distribution	0.86 L/kg	
	Between subject variability model	Additive	
	Residual variability model	Additive	
	Validation	Internal	
Llopis-Salvia and Jimenez-Torres (2006)	*Patient population*	Intensive care unit patients	52

Table 2.1. Continued.

	Number of patients studied	50	
	Age, years (median [range], mean ± standard deviation)	60 [18-81]	
	Number of compartments	Two	
	Final model (central compartment)	$CL = \theta_1 + \theta_2 \times CL_{CR}$ $V = \theta_3 \times WT$	
	Clearance	0.03 L/hr	
	Volume of distribution	0.41 L/kg	
	Between subject variability model	Combined	
	Residual variability model	Combined	
	Validation	Internal	
Sanchez *et al.* (2010)	*Patient population*	Adults who received vancomycin	54
	Number of patients studied	141	
	Age, years (median [range], mean ± standard deviation)	55 ± 14.6	
	Number of compartments	Two	
	Final model (central compartment)	$CL = \theta_1 + \theta_2 \times CL_{CR}$ $V = \theta_3 \times WT$	
	Clearance	0.16 L/hr	
	Volume of distribution	0.28 L	
	Between subject variability model	Proportional	
	Residual variability model	Combined	
	Validation	External	
Yamamoto *et al.* (2009)	*Patient population*	Gram-positive infections	57
	Number of patients studied	100	
	Age, years (median [range], mean ± standard deviation)	65.4 ± 15.1 [25.8-99.7]	

Table 2.1. Continued.

	Number of compartments	Two	
	Final model (central compartment)	CL = θ_1 V = θ_4 x WT	
	Clearance	3.83 L/hr	
	Volume of distribution	0.48 L	
	Between subject variability model	Exponential	
	Residual variability model	Exponential	
	Validation	Internal	
Yasuhara *et al.* (1998)	*Patient population*	MRSA infections	58
	Number of patients studied	190	
	Age, years (median [range], mean ± standard deviation)	64.3 ± 13.8 [19.3-89.6]	
	Number of compartments	Two	
	Final model (central compartment)	CL = θ_1 V = θ_3	
	Clearance	3.51 L/hr	
	Volume of distribution	60.7 L (steady state)	
	Between subject variability model	Exponential	
	Residual variability model	Exponential	
	Validation	None	

addition, weight and age were the most common covariates that affected the volume of distribution. No categorical covariates had a significant effect on clearance or the volume of distribution. Model evaluations were evenly distributed between internal (bootstrap or visual predictive check)[52, 56, 57] and external validation procedures.[53-55]

Pharmacodynamic Profile

The vast majority of vancomycin pharmacodynamic studies have been conducted *in vitro*. However, in the last decade a few *in vivo* pharmacodynamic studies have been conducted, many of which will be discussed at length in this section of the review.

Exposure-Response Profiles

When evaluating the exposure-response profile of an antibiotic it is necessary to consider the magnitude of the drug exposure and its potency against a specific bacterial pathogen.[61] In developing exposure-response profiles, both of these may be quantitatively expressed as a ratio of the drug exposure (e.g., maximum concentration or the area under the concentration time curve [AUC]) and its potency (expressed as the minimum inhibitory concentration [MIC]).[61] As a consequence of the wide range of MICs among different pathogenic microorganisms, these pharmacokinetic / pharmacodynamic ratios have a broader range when compared to those that include a single measure of drug exposure or potency alone.[61]

Pharmacokinetic / Pharmacodynamic Indices

In vivo antibacterial activity may be predicted from two factors: (1) the antibiotic concentration at the effect site and (2) the duration of time that the pathogen is exposed to the antibiotic.[62] Consequently, numerous *in vitro* and animal studies have been conducted to evaluate which pharmacokinetic / pharmacodynamic index best predicts vancomycin antibacterial activity.[63-65] These studies demonstrated that the relationship between vancomycin concentrations and bacterial killing is best described by the AUC divided by the MIC of the infecting pathogen (AUC / MIC) (*Figure 2.2*).[64, 66] In murine infection models, the vancomycin AUC / MIC was the best predictor of bacterial killing against MSSA, MRSA, and vancomycin intermediate *S. aureus* (VISA).[67, 68]

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing results range from quantitative (e.g., actual MIC values) to qualitative (e.g., susceptible, intermediate, resistant).[62] For the latter, it is critical to determine whether the MIC breakpoints are chosen to detect drug resistance or to predict the antibacterial activity of a drug for a patient receiving a typical dose of the antibiotic.[69] These are two fundamentally different questions that are often not clarified when breakpoint MICs are selected. For the purpose of conducting pharmacokinetic / pharmacodynamic analyses, it is preferable to use actual MIC values and establish MIC breakpoints (if needed) based upon the intended goals of the analysis.

In seeking to predict whether a given vancomycin regimen is likely to be effective for an individual patient there are several factors that must be considered, including the variability in vancomycin pharmacokinetics and the range of MICs encountered in clinical

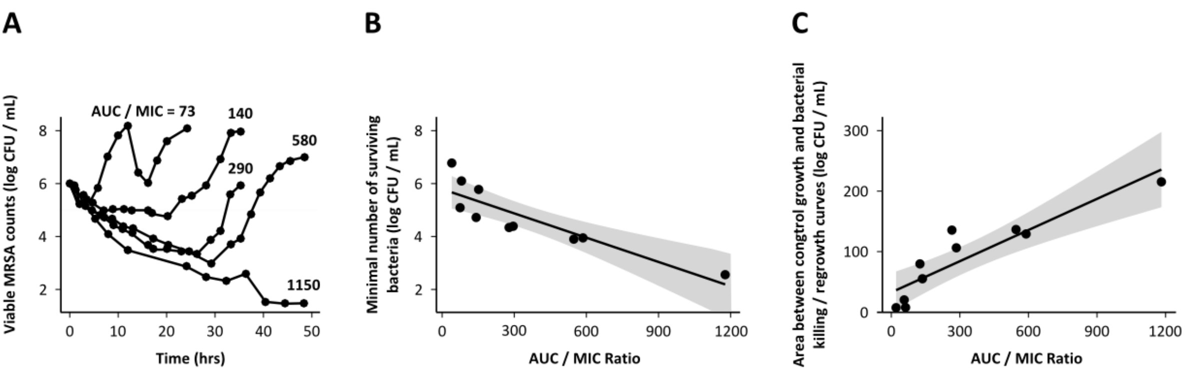

Figure 2.2. Vancomycin concentration versus bacterial activity relationship. (A) MRSA killing kinetics as a function of time and the ratio of the vancomycin area under the concentration versus time curve to MIC (AUC/MIC). (B) Minimal number of surviving bacteria (log CFU/mL) as a function of the vancomycin AUC/MIC ratio. (C) Relationship between the area between the control growth curve and the killing / regrowth curve as a function of the vancomycin AUC/MIC ratio. Data adapted from Cui et al.[71]

practice. Reliable predictions are challenging to develop as MIC-based vancomycin susceptibility tests are based primarily on the *in vitro* determination of the inhibition of growth for standardized low inocula (10^5-10^6), exponential-phase staphylococci.[3] As such, the MIC does not take into account the effects of vancomycin on higher inocula, such as those seen in the setting of critical illness.[70-73] Additionally, the effects of biofilm formation and stationary-phase growth are not taken into account with current vancomycin susceptibility testing methods.[73] Consequently, others have suggested that alternative pharmacokinetic / pharmacodynamic indices, such as the time above a multiple of the MIC, the time above the stationary-phase maximum bactericidal concentration, or the time above an inoculum-corrected MIC may be more accurate predictors of vancomycin efficacy in clinical practice.[3] However, these targets have not been evaluated in clinical trials yet and the AUC / MIC ratio remains the most widely accepted vancomycin pharmacokinetic / pharmacodynamic index.

Considerations for Pharmacodynamic Modeling

To optimize antibacterial activity and improve patient safety, vancomycin therapeutic drug monitoring is recommended for patients with invasive MRSA infections by the Infectious Diseases Society of America.[74] This practice involves the collection of serum samples that are assayed to determine the concentration of vancomycin at specified time-points.[75] These data may then be used in combination with MIC data to derive optimal patient-specific vancomycin dosing regimens.[75] Although this approach represents a major advance in the field of personalized medicine several limitations hinder its widespread adoption, including: the requirement for collecting multiple blood samples,

rapid determination of the MIC, and the need for sophisticated modeling software to integrate the pharmacokinetic / pharmacodynamic data and provide individualized dosing recommendations.[62]

Population pharmacokinetic / pharmacodynamic modeling leverages historical data describing the variability in vancomycin pharmacokinetics and the range of bacterial MICs encountered in clinical practice to derive probability density functions for the likelihood of achieving specific pharmacokinetic / pharmacodynamic targets (*Figure 2.3*).[76] Monte Carlo simulations are commonly used for this purpose.[77] In the case of invasive MRSA infections, a local hospital may establish its range of MICs using data from previous years and develop a probability density function that describes the likelihood that an MRSA isolate will have an MIC <1, 1-2, or ≥2 mcg/mL. Additionally, historical data may be used to establish the likelihood that a patient treated with a typical dose of vancomycin will have an AUC <200, 200-400, or >400 mcg*hr/mL. These distributions may then be used as inputs to develop a large number of computer simulations exploring possible AUC / MIC ratios. For each simulation, a single random AUC value is chosen along with a random MIC value, in accordance with their respective probabilities. As the simulation proceeds, large numbers of AUC and MIC pairs are developed allowing one to summarize the resulting AUC / MIC ratios as a function of their probability distribution. It is then possible to set a desired target (e.g., AUC / MIC >400) and calculate the probability of achieving that target.

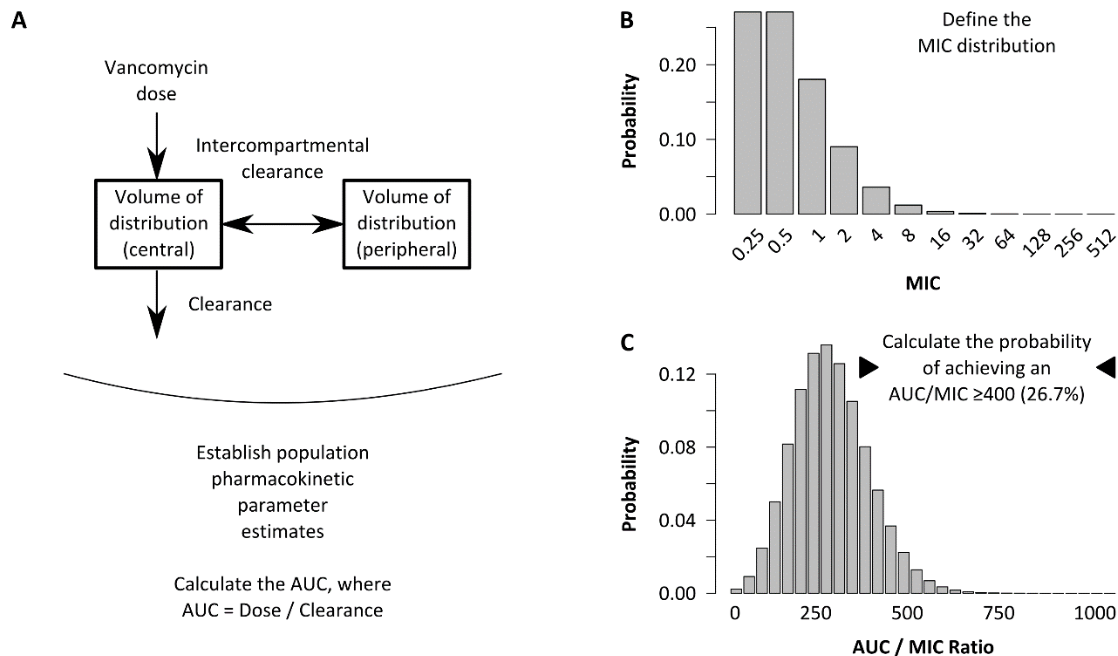

Figure 2.3. Probability distribution for a hypothetical population of patients receiving vancomycin. In this example, (A) a compartmental model is used to describe vancomycin pharmacokinetics, which allows the AUC to be determined by dividing the dose by the estimated clearance rate. (B) The distribution of methicillin-resistant *Staphylococcus aureus* MIC values is used to define the range of MICs observed in the hypothetical population of patients. (C) The AUC / MIC ratio may be determined using Monte Carlo simulation, which in this example yields a 26.7% probability of achieve an AUC / MIC ratio ≥400.

Dosing Optimization

AUC/MIC Targets

Vancomycin dosing guidelines recommend that pharmacokinetic / pharmacodynamic targets be used to guide the clinical management of MRSA infections.[75] These guidelines rely heavily upon a study by Moise-Broder et al. that evaluated 108 patients hospitalized with *S. aureus* lower respiratory tract infections who required vancomycin treatment.[78] In this study, the authors found that the clinical and bacteriological response was superior among patients with an AUC / MIC ≥400. Moreover, an AUC / MIC ≥400 was associated with a decreased time to bacterial eradication and a decrease in the time to improved pneumonia scores. In contrast, no relationship was defined between the time above the MIC and the clinical response to therapy. It should be noted that MICs were determined using the broth microdilution method in this study, which has been recently shown to result in higher AUC / MIC targets when compared to the Etest method.[2, 78] Additionally, the vancomycin AUC was not calculated from measured vancomycin concentrations but was instead predicted from renal function (creatinine clearance).

Following the publication of consensus recommendations from several leading professional societies that endorsed a vancomycin AUC / MIC ≥400 target for all serious MRSA infections, Holmes et al. evaluated this target among 182 patients with *S. aureus* bacteremia (77% MRSA).[79] The authors found that a broth microdilution AUC / MIC ≥400 was not associated with lower 30-day all-cause or attributable mortality from *S. aureus* bacteremia. However, using classification and regression tree methods, it was found that an AUC / MIC >373 within the first 96 hours of therapy was associated with a

reduction in mortality, following adjustment for potential confounders. This effect persisted in subgroup analyses limited comparing cases of MRSA versus MSSA and low versus high MICs (defined as an Etest MIC value >1.5 mcg/mL).

Several recent studies have suggested that the AUC / MIC ≥400 target may not be universally applicable for all MRSA-associated clinical syndromes.[80, 81] Brown et al. found that an AUC / MIC <211 was associated with increased mortality among patients with MRSA bacteremia and infective endocarditis.[80] Additionally, Gawronski et al. investigated the association between AUC / MIC ratios and the time to microbiological clearance in patients with MRSA bacteremia and osteomyelitis.[81] The authors used the classification and regression tree method to determine that an AUC / MIC >293 yielded the greatest difference in the time to microbiological clearance. For patients with an AUC / MIC >293 the mean time to clearance was two days shorter (4 vs. 6 days of bacteremia). Additional prospective clinical trials are warranted to confirm these findings.

Trough Concentration Targets

Historically, vancomycin trough concentrations have been used as a marker of vancomycin exposure.[74] Current clinical practice guidelines recommend that a minimum trough concentration of 10 mcg/mL is recommended to achieve antibacterial activity and to avoid promoting bacterial resistance.[74] For invasive MRSA infections, a therapeutic trough concentration target of 15-20 mcg/mL is recommended, which was predictive of an AUC / MIC >400 among adults with *S. aureus* lower respiratory tract infections.[78] However, Gawronski et al. found that trough concentrations did not correlate with AUC / MIC ratios among patients with MRSA bacteremia and osteomyelitis.[81] Consequently,

the authors recommended that total drug exposure be estimated by measuring the AUC / MIC for each patient to ensure optimal dosing.

Empiric and Definitive Dosing Regimens

Vancomycin is frequently prescribed empirically for the treatment of presumed MRSA infections. Conventional dosages (1 g every 12 hrs or 15-20 mg/kg actual body weight every 8-12 hrs) are recommended for adult patients with normal renal function.[75] However, to reliably achieve a vancomycin trough concentration of 10-15 mcg/mL or an AUC / MIC > 400, Thomson et al. demonstrated that alternative dosing strategies may be needed.[82] The authors developed a population pharmacokinetic model using data from 398 patients with a median age of 66 years (range 16-97) who received vancomycin from 1991-2004 and subsequently evaluated its performance using data from 100 patients (median age 71 years [range 22-91]) treated with vancomycin from 2004-2007. Using conventional dosing guidelines 19% of patients achieved a trough within 10-15 mcg/mL. The authors then used the population pharmacokinetic parameter estimates derived from their model to predict vancomycin trough concentrations in a simulated dataset of 110 patients with varying weights (40-120 kg) and creatinine clearance (15-125 mL/min). Revised dosing recommendations were then generated by examining the likelihood of achieving a target trough concentration of 10-15 mcg/mL at doses with fixed increments of 250 mg administered at intervals of 12, 24, and 48 hours. This process was repeated until the authors established a series of dosing recommendations that resulted in 55% of patients in the simulated dataset achieving the target trough concentration of 10-15

mcg/mL. Using these dosing recommendations, 87% of the simulated patients were predicted to achieve an AUC / MIC >400.

For MRSA isolates with an MIC equal to 1 mcg/mL, definitive vancomycin doses should be maintained at a higher level (60 mg/kg/day) to achieve trough concentrations of 15-20 mcg/mL.[8] Despite the use of larger doses, however, Patel and colleagues used Monte Carlo simulation techniques and found that the probability of achieving an AUC / MIC >400 was only 57% with an aggressive dosing regimen of 2 g every 12 hrs when the MIC was equal to 2 mcg/mL; in contrast to approximately 100% target attainment when MICs were ≤1 mcg/mL.[14] A similar trend was noticed by Gawronski et al., in which only 9% of patients were able to reach an AUC / MIC >400 with MICs >1 mcg/mL.[81] Based on these findings, alternative antibiotics (e.g., linezolid) should be considered for MRSA isolates with MICs >1 mcg/mL.

Continuous Infusion

Continuous infusion of vancomycin features several practical advantages, including lower costs, decreased pharmacokinetic variability, and increased ease of monitoring when compared to intermittent infusion.[83, 84] A recent meta-analysis evaluated one randomized controlled trial and five observational studies and found that continuous infusion was associated with a lower risk of nephrotoxicity and no difference in mortality.[85] This finding is in agreement with a study by Hutschala et al., which found that critically ill patients undergoing cardiac surgery had a lower incidence of acute renal failure requiring venovenous hemofiltration after vancomycin was administered via continuous infusion as compared to intermittent infusion.[86] Roberts et al. evaluated the

population pharmacokinetics of vancomycin administered via continuous infusion and found that high loading (35 mg/kg) and maintenance (35 mg/kg/day) doses were needed to rapidly achieve therapeutic vancomycin concentrations of 20 mcg/mL among critically ill patients.[87] Additional studies are needed to determine whether larger doses of vancomycin administered via continuous infusion affect mortality or the time to clinical and microbiological response. However, a report by Ingram et al. suggests that there may be an upper limit for steady-state vancomycin concentrations (≥28 mcg/mL) beyond which toxicity was frequently noted.[88]

Special Populations

Neonates and Infants

Establishing appropriate vancomycin dosing regimens for neonates and infants is challenging due to physiological and developmental factors that contribute to high pharmacokinetic variability.[89] For example, neonates have a high proportion of water by weight and rapidly changing renal function in the post-natal period, all of which have the potential to alter vancomycin pharmacokinetics.[90] These factors change most rapidly during the first week of life.[90]

Although many studies have investigated vancomycin pharmacokinetics in neonates and infants, an optimal dosing regimen has not yet been evaluated clinically.[34, 90] Variation in vancomycin clearance among pre-term (gestational age <37 weeks) and term (≥37 weeks gestation) neonates has been shown to be influenced by weight, post-menstrual age, and renal function.[91-94] This is reflected in the wide range of vancomycin half-lives (2-12 hours) reported among neonates.[90, 95] Population pharmacokinetic

studies have been reported to improve the likelihood of achieving target vancomycin concentrations among neonates.[96] Although two-compartment models with a long distribution phase best reflect vancomycin pharmacokinetics, one-compartment models perform reasonably well in providing individual pharmacokinetic parameter estimates that may be used for the purpose of developing individualized neonatal dosing regimens.[89, 97] An added advantage of this approach is the ability to determine vancomycin pharmacokinetic parameters while obtaining relatively few blood samples.[89] Post-hoc dose adjustments are frequently required to achieve target concentrations due to unexplained pharmacokinetic variability, which continues to make therapeutic drug monitoring essential despite advances in neonatal-specific population pharmacokinetic models.[90, 96]

Few studies have been able to link neonatal vancomycin pharmacokinetic / pharmacodynamic targets with positive clinical outcomes.[98] Currently, neonatal dosing is based on trough concentration targets derived from studies involving adults receiving treatment for MRSA infections.[98] Translation of these targets to neonatal medicine is challenging due to the fact that MRSA infections are relatively rare.[97, 99] In this patient population, coagulase-negative *S. aureus* is the most commonly identified infectious organism, which may require alternative vancomycin dosing regimens and the use of different pharmacokinetic / pharmacodynamic targets.[97, 99]

Recently, Zhao et al. evaluated vancomycin continuous infusion regimens for neonates and found that the regimens used varied widely, presumably owing to a paucity of pharmacokinetic data.[100] To address this need, the authors evaluated 116 neonates who received vancomycin via continuous infusion and found that 41% had therapeutic

vancomycin concentrations (15-25 mcg/mL). Moreover, the distribution of observed concentrations varied widely (range 5.1-61.5 mcg/mL). Using a one-compartment population pharmacokinetic model, the authors developed an optimized dosing regimen incorporating birth weight, current weight, postnatal age, and serum creatinine. In a prospective evaluation of this optimized regimen, 71% of the 58 neonates evaluated achieved the target range of 15-25 mcg/mL. However, the proportion that achieved an AUC / MIC > 400 is unknown.

Children and Adolescents

Similar to adults, the emergence of MRSA has led to a significant increase in the use of vancomycin among children.[101] Therapeutic target trough concentrations have rapidly evolved over the last decade.[8] Although treatment failure is rare, sub-therapeutic vancomycin trough concentrations (<5 mcg/mL) have been reported to be associated with a heightened risk for treatment failure.[102-104] Collectively, reports of rare treatment failures, inconsistent target attainment rates, and the absence of a clear causal relationship between vancomycin concentrations and toxicity in pediatric patients has led to upward revisions in pediatric dosing recommendations from several leading professional societies.[105-107]

After two years of age, without allometric scaling, weight-related vancomycin clearance declines with increasing age and serum creatinine concentration.[108] Dosing regimens that account for the influence of age, serum creatinine, and the susceptibility of the target organism (e.g., MIC) have been reported to improve target attainment rates among children.[108] This is particularly true for children <12 years of age, for which

higher doses are typically required, and also for critically ill children who require admission to the pediatric intensive care unit.[107, 109]

Pediatric therapeutic drug monitoring is common and is primarily based on trough concentration targets.[8] Current guideline recommendations suggest targeting steady state troughs >10 mcg/mL, with 15-20 mcg/mL recommended for the treatment of invasive MRSA infections.[8] These targets reflect a change in the primary motivation for vancomycin therapeutic drug monitoring, which is now less focused on detecting toxicity and more focused on ensuring that vancomycin concentrations are likely to be therapeutic.[110] It must be noted, however, that these targets are extrapolated from adult studies and may not directly correlate with pediatric outcomes.[110] Consequently, several institutions are in the process of re-evaluating their pediatric vancomycin therapeutic drug monitoring practices and are shifting away from the use of troughs exclusively in an effort to establish AUC-based dosing regimens, which have the potential to better describe vancomycin exposure over the entire dosing interval.[111] Frymoyer et al. determined that between 75-90% of children with vancomycin trough concentrations of approximately 7-10 mcg/mL achieve an AUC / MIC >400 when the MIC is ≤1 mcg/mL.[101, 109] However, no studies have evaluated the rate of treatment failure among children with MRSA infections with trough concentrations of 7-10 mcg/mL and MICs ≤1 mcg/mL, which has led to wide variations in pediatric therapeutic drug monitoring and vancomycin dosing practices.[112, 113]

The Elderly

The clinical pharmacokinetics of many antimicrobials, including vancomycin, are altered among the elderly.[114] This occurs principally due to a decrease in renal clearance that occurs with increasing age and is characterized by a prolonged half-life and increased AUC.[114] These changes may be amplified among patients with severe infections who are prescribed nephrotoxic agents.

In a study by Cutler et al., the pharmacokinetics of vancomycin were investigated in six healthy elderly men (61-77 years of age) and six healthy, young men (20-26 years of age).[115] It was reported that these individuals had an increased volume of distribution, increased tissue binding (calculated indirectly), an increased half-life, and significantly reduced vancomycin clearance.[115] However, the coefficients of variation for the derived pharmacokinetic parameters were relatively low (14-16%), suggesting that this relatively homogeneous population may not accurately reflect the true variability in vancomycin pharmacokinetics among elderly patients who are receiving vancomycin for the treatment of invasive MRSA infections. Guay et al. evaluated 148 elderly patients (≥60 years of age) who received vancomycin for the treatment of suspected or documented gram-positive or mixed infections.[116] The authors observed a significant increase in the volume of distribution, half-life, and a decreased rate of vancomycin clearance when compared to younger adults. In their population pharmacokinetic model, it was determined that advanced age was a strong predictor of vancomycin clearance, half-life, and volume of distribution. Due to these effects, it was established that elderly patients with normal renal function (serum creatinine values ≤1.5 mg/dL) require smaller daily doses as compared

with younger patients (18-59 years) to maintain similar target peak and trough concentrations (18.2 ± 5.8 vs. 25.2 ± 7.8 mg/kg/day).

Recently, Mizokami et al. studied 94 elderly patients (75-99 years) with hospital-acquired MRSA pneumonia and compared their clinical outcomes using trough- and AUC-based vancomycin therapeutic drug monitoring methods.[117] The authors found that trough concentrations were not predictive of 28-day mortality, whereas an AUC <250 or >450 mcg*hr/mL was strongly associated with an increased risk of death (odds ratio 23.2, 95% confidence interval 6.8-78.7). However, the authors did not report the method used to identify these thresholds (e.g., classification and regression tree analysis). Additionally, this retrospective study had a relatively small number of survivors with an AUC / MIC >450 (n = 11) and those who did not survive had more severe infections, which makes it difficult to determine whether a target AUC / MIC of 250-450 mcg*hr/mL improves treatment outcomes for elderly patients with hospital-acquired MRSA pneumonia.

Obese Patients

A limited number of studies have investigated vancomycin pharmacokinetics among morbidly obese individuals. Bauer et al. evaluated morbidly obese and non-obese individuals and found that vancomycin clearance rates were similar (1.2 mL/min/kg vs. 1.1 mL/min/kg, respectively).[118] The authors recommended that the total daily dose of 30 mg/kg total bodyweight be divided every 6 or 8 hrs.[118] Similarly, Blouin et al. found no difference with respect to vancomycin clearance when clearance was expressed per kg of total bodyweight.[28] Additionally, they recommended that the total daily dose should be divided every 4 or 6 hrs to prevent high peak concentrations, which are associated with a

heightened risk for nephrotoxicity.[28] In accordance with these results, Vance-Bryan et al. reported that total bodyweight had a significant influence upon vancomycin clearance and volume of distribution in a study cohort in which 47% of the patients were obese.[119] As a consequence of the lack of difference in clearance per kg bodyweight, all reports have concluded that vancomycin should be dosed on total bodyweight with total daily doses varying from 20-30 mg/kg.

Review articles evaluating vancomycin dosing in obese individuals have primarily summarized the above mentioned clinical studies, all of which emphasize the need for dosing regimens to be based upon total bodyweight and for dosing three or more times per day.[120-123] As vancomycin target concentrations are now higher than those targeted at the time that these studies were published it seems reasonable that obese individuals should now receive a total daily dose of at least 37.5 mg/kg divided three times per day to prevent potentially toxic high peak concentrations. When individual doses exceed 1 g (e.g., 1.5 and 2 g), the infusion period should be extended to 1.5-2 hrs.[75] If vancomycin is administered by continuous infusion, a dose of 30 mg/kg/day is expected to result in exposures similar to those achieved with three intermittent infusion doses of 12.5 mg/kg. To rapidly reach steady state, a loading dose of 25 mg/kg may be considered.[75] Therapeutic drug monitoring is recommended for all morbidly obese individuals, even when there is no evidence of renal insufficiency. Prospective clinical studies are needed to evaluate these dosing proposals for morbidly obese individuals in light of the increased target vancomycin concentrations that are currently aimed for.

Patients with Cancer

Patients with cancer frequently receive empiric vancomycin for episodes of febrile neutropenia and courses of definitive therapy for the treatment of infections caused by gram-positive organisms.[124] As patterns of vancomycin use have adapted to relatively recent recommendations that target higher pharmacokinetic / pharmacodynamic targets, patients with cancer are at a disadvantage owing to their more rapid clearance when compared to adults, children, and elderly patients without cancer.[8, 125-131] In a vancomycin pharmacokinetic / pharmacodynamic study conducted among adults with hematologic malignancies, it was found that only patients with normal renal function who received a standard 2 g/day dose achieved therapeutic vancomycin concentrations.[132] Among children with cancer, Krivoy et al. reported an increase in vancomycin clearance and lower trough concentrations when compared to children without cancer.[130] On the basis of these findings, high vancomycin doses are recommended for patients with cancer.[124]

Large vancomycin doses are required to achieve therapeutic concentrations for patients with cancer; however, patients with cancer are at an elevated risk for nephrotoxicity and warrant close monitoring.[133] Recognition of the potential for additive nephrotoxicity due to the use of vancomycin and the co-prescribing of other nephrotoxic agents (e.g., chemotherapy, cyclosporine, aminoglycosides, etc.) often leads to the use of low vancomycin doses (e.g., 1 g every 12 hrs for adults), which increases the risk for treatment failure and the emergence of resistance.[133] Moreover, patients with cancer are more likely to have less susceptible *S. aureus* isolates.[134] Rolston et al. tested the *in vitro* activity of vancomycin against 392 gram-positive isolates from patients with cancer and

found that 100% of the MRSA isolates and 98% of the MSSA isolates had MICs ≥1 mcg/mL, which has been strongly associated with treatment failure.[134]

There are major limitations associated with extrapolations from small, retrospective analyses often based solely on vancomycin trough concentrations. Consequently, there is a need for prospective, disease-specific population pharmacokinetic / pharmacodynamic studies to determine whether patients with cancer have an altered vancomycin pharmacokinetic profile. Currently, therapeutic drug monitoring is essential to individualize vancomycin therapy, thereby balancing therapeutic efficacy with the potential for developing toxicity among patients with cancer.

Continuous Renal Replacement Therapy

Multiple studies have examined the effect of continuous renal replacement therapy (CRRT) on vancomycin pharmacokinetics in critically ill patients.[87, 135-138] Many of these arrived at conflicting results regarding the influence of CRRT on vancomycin clearance and volume of distribution. DelDot et al. evaluated 10 critically ill patients receiving continuous venovenous hemodiafiltration (CVVHDF) and found that the mean total body clearance of vancomycin was 2.5 ± 0.7 L/hr, whilst that cleared by CVVHDF was 1.8 ± 0.4 L/hr (76% of total body clearance).[135] Another vancomycin pharmacokinetics study conducted among patients undergoing continuous venovenous hemofiltration (CVVH) found that CVVH represented approximately 50% of total vancomycin clearance.[139] Additionally, varying volume of distribution estimates have been reported.[87, 136] Theoretically, it is expected that the pathophysiology of acute kidney injury would result in impaired water and solute excretion, which would result in a

larger extracellular fluid compartment and lead to an increase in the volume of distribution.[140] In one of the largest studies to date this was not found to occur; however, the authors acknowledge that they were unable to account for fluid maintenance in the intensive care unit, which likely represents a critical covariate needed to accurately model vancomycin pharmacokinetics for this patient population.[136]

In a recent study, Covajes et al. evaluated 85 patients requiring CRRT and determined that higher vancomycin doses were needed for patients with the highest CRRT intensity (>40 mL/kg/hr).[141] The authors targeted a steady state concentration of 20-30 mcg/mL and reported that the two significant factors that influenced vancomycin target attainment rates within the first three days of therapy were the daily dosage amount and the intensity of CRRT. On day one of therapy, 51% of patients had adequate steady state vancomycin concentrations, 20% had supra-therapeutic concentrations, and 29% had sub-therapeutic concentrations. The majority of patients with adequate steady state vancomycin concentrations received a daily dose of 16-35 mg/kg. Due to the rapid evolution of acute kidney injury and the potential for rapid deterioration, therapeutic drug monitoring should be performed as early as 6 hours after administration of the first vancomycin dose, with maintenance dosing established after additional samples have been drawn.[142] This is especially critical as up to 49% of patients undergoing CRRT have been reported to have sub-therapeutic vancomycin concentrations.[143] Moreover, as the duration of CRRT increases non-renal clearance decreases, eventually approaching vancomycin clearance rates observed in patients with chronic renal failure.[50] Due to high variability in non-renal clearance over time, individualized vancomycin dosing regimens are essential for patients with acute renal failure.[50]

Patients with Cystic Fibrosis

The prevalence of pulmonary MRSA infections has increased among patients with cystic fibrosis.[144, 145] In 2008, the Cystic Fibrosis Foundation reported that 50% of patients with cystic fibrosis were infected with *S. aureus* and 23% were infected with MRSA. Among patients with cystic fibrosis, MRSA is primarily isolated from children and young adults.[147]

Pleasants et al. evaluated vancomycin pharmacokinetics among 10 adults with acute pulmonary exacerbations of cystic fibrosis.[148] The volume of distribution, total body clearance, and terminal elimination rate were similar among patients with cystic fibrosis when compared to previous pharmacokinetic parameter estimates derived from studies with healthy adult volunteers.[148, 149] However, Stockmann et al. found that vancomycin clearance was slower among children with cystic fibrosis (0.08 L/hr/kg) when compared to children without cystic fibrosis (0.10-0.16 L/hr/kg).[150-153] It is unclear whether lower vancomycin doses are needed to accommodate the decreased clearance among children with cystic fibrosis; however, vancomycin may be dosed similarly for adults with and without cystic fibrosis. As for all patients, therapeutic drug monitoring is highly recommended to prevent sub-therapeutic dosing and toxicity.[8]

Critically Ill Patients

Critically ill patients are an inherently heterogeneous population with varying extents of organ dysfunction, often requiring mechanical support for failing organ systems, and the administration of many life-saving medications.[52] All of these factors may modify the pharmacokinetic profile of vancomycin.

Llopis-Salvia et al. conducted a population pharmacokinetic study among 50 critically ill adults who required vancomycin for the treatment of suspected or proven gram-positive infections.[52] The authors used a two-compartment model and determined that the total body clearance for a 60 kg patient was 60 mL/min, with approximately 28% occurring via non-renal mechanisms. The volume of distribution in the central and peripheral compartments were estimated as 0.41 and 1.32 L/kg, respectively, and were linearly related to total body weight. In contrast, Rodvold et al. reported a volume of distribution in the central compartment of 0.21-0.24 L/kg in a population of patients with renal dysfunction.[154] The increased volume of distribution among critically ill patients may be explained, at least in part, by the physiologic changes in body compartments that occur as a consequence of fluid overload and/or from the accumulation of fluid in the third space due to tissue edema.[155]

A Bayesian pharmacokinetic approach has been proposed to feature excellent predictive performance in evaluating vancomycin dosing regimens for critically ill patients.[52, 156] Ito et al. developed a two compartment vancomycin infusion algorithm that resulted in a mean bias of 7.7 ± 7.6 mcg/mL and a mean precision of 8.9 ± 6.2 mcg/mL for estimating vancomycin trough concentrations.[156] More recently, Llopis-Salvia et al. established a Bayesian model that yielded improved predictive performance with regard to both the trough bias (-0.2 mcg/mL) and precision (3.9 mcg/mL).[52]

Current guidelines recommend that vancomycin be administered via intermittent infusion; however, some investigators prefer continuous infusion, particularly for critically ill patients.[74, 157, 158] For septic patients with a large volume of distribution continuous infusion may be preferable.[52, 159, 160] Saugel et al. retrospectively evaluated 164 adults

admitted to their medical intensive care unit and reported that a vancomycin continuous infusion regimen with a median daily dose of 960 (95% confidence interval 526-1723) mg resulted in a median vancomycin concentration of 19.8 (9.8-29.4) mcg/mL.[161] Using a target of 15-25 mcg/mL, the authors found that serum vancomycin concentrations were frequently sub-therapeutic on day one (44%), day two (29%), and day three (23%). These findings suggest that therapeutic drug monitoring is essential to ensure attainment of therapeutic vancomycin concentrations. Moreover, it may be speculated that higher doses may be necessary for critically ill patients.

Expert Commentary and Five-Year View

More than 50 years after the discovery of vancomycin, dosing regimens continue to evolve. Historically, pharmacokinetic / pharmacodynamic targets have been based on the use of trough concentrations, which are crude surrogates that are unable to capture the overall shape and extent of drug exposure over the entire dosing interval.[102] Within the last five years there has been a movement toward AUC-based therapeutic drug monitoring, which is a more accurate representation of vancomycin exposure.[75] Over the next five years, we expect that pharmacokinetic and pharmacodynamic models will continue to be vital tools used to establish targets for the treatment of invasive MRSA-associated bacteremia, osteomyelitis, skin and soft tissue infections, and meningitis. Moreover, we expect that studies will evaluate the AUC/MIC >400 target that is currently recommended for adults with MRSA-associated lower respiratory tract infections in other patient populations, which may feature altered vancomycin pharmacokinetic profiles.[74]

In addition to their utility in establishing therapeutic targets, these pharmacokinetic / pharmacodynamic models may be used to evaluate the efficacy and safety of alternative vancomycin administration methods. Currently, fewer than 50% of neonates reach therapeutic vancomycin concentrations with guideline-recommended intermittent dosing regimens.[97, 162] The reasons for this are likely to be multifactorial and may include: uncertainties regarding an appropriate therapeutic concentration; developmental considerations related to the acquisition of renal function; differences in the etiological agents of neonatal sepsis; unknown vancomycin concentrations at the site of action; and an unclear mechanism for vancomycin-induced nephrotoxicity, all of which contribute to the need for prospective, clinical trials guided by population pharmacokinetic / pharmacodynamic modeling.[98]

Continuous infusion of vancomycin is increasingly being used for adults with invasive MRSA infections; however, many studies report differing vancomycin target concentrations.[85] Further research is needed to establish a link between clinical outcomes and steady state vancomycin concentrations achieved with continuous infusion regimens. With such data, it would be possible to develop pharmacokinetic / pharmacodynamic models to identify optimal dosing regimens that would be likely to achieve positive clinical outcomes by maximizing the likelihood of attaining desired vancomycin target concentrations. In the event that this occurs within the next five years, it will be critical to evaluate whether such dosing regimens are appropriate for other patient populations, for which limited data currently exist.

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CHAPTER 3

POPULATION PHARMACOKINETICS OF INTERMITTENT VANCOMYCIN IN CHILDREN WITH CYSTIC FIBROSIS

Abstract

Background: Vancomycin is the drug-of-choice for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in children with cystic fibrosis. However, no studies have characterized the pharmacokinetic profile of vancomycin among pediatric cystic fibrosis patients.

Objective: To evaluate the pharmacokinetics of intermittent vancomycin administration in children with cystic fibrosis and identify covariates that significantly influence vancomycin efficacy and safety.

Methods: Therapeutic drug monitoring data were obtained from two cystic fibrosis care centers that identified children <18 years who received vancomycin treatment for an acute pulmonary exacerbation from 2005-2010. Trough and peak serum concentrations were determined before and after the third or fourth dose. Nonlinear mixed effects models

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were developed to evaluate the population pharmacokinetics of vancomycin.

Results: Among the 67 children (mean age 12.1$\pm$5.3 years), the mean vancomycin dose was 17.4$\pm$4.4 mg/kg. The mean trough concentration (C_{min}) was 10.3$\pm$3.8 mg/L. The mean daily area under the serum concentration time curve (AUC_{24}) was 282.5$\pm$816.9 mg*hr/L. A one-compartment model with first-order elimination best described the data. Weight significantly influenced vancomycin clearance ($P<0.001$). In the final model, clearance was estimated as 5.57 L/hr/70 kg, and the volume of distribution was 44.1 L/70 kg. The between subject variabilities for clearance and volume of distribution were 27% and 40%, respectively.

Conclusions: Using a one-compartment model to evaluate the pharmacokinetic properties of vancomycin in children with cystic fibrosis, clearance increased with body weight. Pharmacodynamic studies are needed to establish an optimal vancomycin dosing regimen for the treatment of pediatric exacerbations of cystic fibrosis.

Background

Vancomycin is commonly used to treat severe methicillin-resistant *Staphylococcus aureus* (MRSA) infections and has been widely studied in adults.[2] The prevalence of MRSA lower respiratory tract infections in patients with cystic fibrosis (CF) is increasing.[3-5] From 1996 to 2006, the proportion of patients with CF who had one or more positive culture(s) for MRSA increased from 2% to 19%. According to the 2008 Cystic Fibrosis Foundation (CFF) Patient Registry, more than 50% of patients with CF are infected with *S. aureus*, and 23% are infected with MRSA.[6,7] Among patients with CF, MRSA is primarily isolated from children and young adults.[8]

The importance of proper vancomycin dosing has been highlighted in a consensus national guideline endorsed by several leading professional societies.[2] Guideline recommendations were supported by data from adults without CF. Pharmacokinetic data to guide the optimal dosing of vancomycin in patients with CF are limited across all age groups. A small pharmacokinetic analysis of vancomycin in 10 adults with CF was published in 1996.[9] This study found that the disposition and pharmacokinetics of vancomycin were similar in adults with CF and healthy adult volunteers. However, there is a critical shortage of pharmacometric data for children and young adults with CF, who are most frequently infected with MRSA.

Although MRSA possesses virulence factors that can damage host tissue, the clinical consequences of MRSA infection in children with CF are poorly understood.[10, 11] In a large observational study, Ren et al. reported that patients who were culture-positive for MRSA had greater airway obstruction compared with patients with methicillin-sensitive *S. aureus* (MSSA).[12] A follow-up study found that despite greater airway obstruction, aggressive treatment with antibiotics, and more frequent hospitalizations, the rate of lung function decline was not significantly different for patients with MRSA or MSSA.[13] However, Dasenbrook and colleagues retrospectively examined 19,833 patients with CF and found evidence of an increased risk of death among patients with MRSA infection versus patients with MSSA infection.[14]

The objective of this study was to characterize the pharmacokinetic parameters of vancomycin in a population of children recruited from two CF care centers. Potential covariates were assessed for their effect upon vancomycin pharmacokinetic parameters.

Methods

Setting and Study Population

This retrospective study consisted of 67 pediatric patients who received vancomycin for treatment of a CF pulmonary exacerbation. Children who received treatment at Intermountain Primary Children's Medical Center, Salt Lake City, Utah and Cardinal Glennon Children's Medical Center, St. Louis, Missouri from January 1, 2005 through December 31, 2010 were eligible for inclusion. Patient demographics, including age, sex, weight, height, and serum creatinine were recorded through a combination of electronic and manual abstraction from the medical record. This study was reviewed, approved, and granted a waiver of informed consent by Institutional Review Boards at both study sites.

Drug Administration and Sample Collection

Intermountain Primary Children's Medical Center and Cardinal Glennon Children's Medical Center routinely evaluate for evidence of MRSA infection among children with CF undergoing treatment for an acute pulmonary exacerbation. Vancomycin is used as the first-line therapy for MRSA at both study sites.[15] All patients received a 60-minute infusion of vancomycin using a syringe pump at doses of 15-20 mg/kg administered 2, 3, or 4 times daily. The mean dose was 16.5 $\pm$ 4.2 mg/kg for patients treated at Intermountain Primary Children's Medical Center and 17.9 $\pm$ 4.5 mg/kg for patients treated at Cardinal Glennon Children's Medical Center. Dosing adjustments were made for peak concentrations less than 20 mg/L, peak concentrations greater than 40 mg/L, and trough concentrations greater than 20 mg/L.

Blood samples were collected from all patients for therapeutic drug monitoring as part of routine medical care. Samples were drawn within 30 minutes before the dose (trough concentration) and 30 minutes after the end of the intravenous infusion (peak concentration). Treatment duration was typically 10 to 14 days, based on clinical status and pulmonary function testing results.

Vancomycin Assay

Serum drug concentrations were measured using a fluorescence polarization immunoassay (Abbott AxSYM, Abbott Park, IL).[16] Assay validation was performed for clinical purposes. The lower and upper limits of quantification were 2.0 mg/L and 100.0 mg/L, respectively. At Cardinal Glennon Children's Medical Center, the intra-day relative standard deviation (a measure of precision) ranged from 3.5-4.3% and the inter-day relative standard deviation ranged from 0.6-0.9%. The relative error (a measure of accuracy) ranged from 2.9-4.3%. At Primary Children's Medical Center, the intra-day and inter-day relative standard deviations ranged from 4.7-7.1%.

Pharmacokinetic Analysis

Vancomycin pharmacokinetic parameters were evaluated using NONMEM 7.2 (non-linear mixed effects modeling; ICON Development Solutions, Ellicott City, MD). Data from the two centers were initially assessed separately. Results were found to be comparable, and the data were pooled for all further analyses. Pooled data were fitted with one- and two-compartment first-order conditional estimation with interaction models. The one-compartment model estimated vancomycin clearance (CL) and the volume of

distribution (V_D).

Structural models were selected for further assessment using the Akaike information criterion (AIC) and the Schwarz Bayesian criterion (SBC).[17] Diagnostic plots were used to visually inspect the model's fit, including observed vs. population predicted vancomycin concentrations and observed vs. individual predicted vancomycin concentrations. Residuals and conditional weighted residuals (CWRES) were also plotted vs. time or population predicted vancomycin concentrations. Models were further compared by assessing the precision of the parameter estimates, measures of variability, and the objective function value (OFV). A reduction in the OFV of more than 5.99 (-2 log likelihood difference) was considered to be statistically significant with two degrees of freedom and a $P<0.05$.[18]

Model variability and random effects were classified as one of two types of error: 1) between-subject variability (BSV) and 2) residual unexplained variability (RUV). BSV is the variability inherent between different patients and was assumed to be log-normally distributed according to an exponential equation of the form:

$$P_i = \theta_{pop} * \exp(\eta_i^\theta); \qquad \eta_i^\theta \text{ i.i.d.} \sim \mathrm{N}(0, \omega_\theta^2) \tag{3.1}$$

where P_i is the value of the pharmacokinetic parameter for the ith individual, θ_{pop} is the population mean for P, and η_i^θ represents the between subject random effect for the ith individual on θ, each of which are independent and identically distributed with a mean of zero and a variance of ω^2.[19]

The RUV was the second source of variability and reflects the difference between the model prediction for the individual and the measured observation. This includes the error in the assay, errors in drug dose, errors in the time of measurement, etc.[20] During

model development, RUV was evaluated using additive, proportional, and combined error models. A combined residual error model resulted in the greatest improvement in the OFV. The equation for the combined error model was:

$$Y_{ij} = Y_{mij}\left(1 + \varepsilon_{ij}\right) + \varepsilon_{ij} \quad (3.2)$$

where Y_{ij} is the observed concentration for the ith individual at time j, Y_{mij} is the model prediction, and ε_{ij} is a normally-distributed random error with a mean of zero and a variance of σ^2.

The area under the plasma concentration vs. time curve over a day (AUC_{24}) for vancomycin was calculated for each patient using the following equation:

$$AUC_{24} = \frac{Daily\ vancomycin\ dose}{Vancomycin\ clearance} \quad (3.3)$$

Covariate Analysis

Potential covariates were initially identified through generalized additive modeling. Further testing was performed by evaluating potential covariates using stepwise forward addition and then stepwise backward elimination procedures. A reduction in the OFV of >5.99 ($P<0.05$) was required to retain covariates in the forward addition step. In the backward elimination step, covariates were retained if they resulted in a reduction in the OFV of >9.21 ($P<0.01$).

Age, weight, height, sex, and serum creatinine were included in the covariate analysis. To adjust for differences in body size and metabolic rate, allometric scaling was applied to standardize body weight between the parameter estimates determined for this pediatric population and values reported for a typical 70 kg adult by fixing the exponents in the allometric model to 0.75 for clearance (equation 3) and to 1 for the V_D.[21]

$$CL_i = \left(CL_{pop} * \left({}^{BW}/_{70}\right)^{\theta}\right) * \exp(\eta_{CL}) \tag{3.4}$$

where CL_i is the individual clearance in the ith individual, CL_{pop} is the estimate of the population clearance, η_{CL} is the random between subject variability, θ is the shift parameter describing the systematic dependence of clearance on individual body weight, and BW is the body weight of the ith individual.

Model Evaluation

Models were evaluated and selected based on the goodness of fit and unstable models were excluded from the model building process. The stability of the models was assessed by changing the number of significant digits and the initial parameter estimates for CL and V_D. Models were also compared using the Akaike information criterion (AIC) and Schwarz information criterion (SIC) to discriminate between non-hierarchical models as part of the model selection criteria.[17] Nonparametric bootstrapping techniques were utilized to evaluate the stability of the final pharmacokinetic model and to quantify the uncertainty in parameter estimates.[22] PDx-Pop was used to derive 1000 bootstrap runs by randomly sampling with replacement from the original dataset. Standard errors were computed for both the estimated population parameters and random effect error models. Model stability was further assessed by generating visual predictive checks, in which the 90% confidence interval from the measured vancomycin concentrations were compared to the results obtained from 100 simulated vancomycin datasets.

Results

Patients and Pharmacokinetics

The median age of the study population was 13.9 years (interquartile range: 8-17), and a majority of patients were female (60%). The mean body weight of the patients was 40.6 $\pm$ 19.5 kg. Additional demographic characteristics are summarized in *Table 3.1*. From these 67 patients, there were 227 unique hospitalizations. Cardinal Glennon contributed 337 vancomycin concentration measurements (mean 7.9 $\pm$ 9.6 concentrations per patient), and Intermountain Primary Children's Medical Center contributed 149 (mean 6.3 $\pm$ 6.8 concentrations per patient). The mean peak (C_{max}) and trough (C_{min}) vancomycin concentrations were 25.4 $\pm$ 11.0 mg/L and 10.1 $\pm$ 3.8 mg/L, respectively. The mean AUC_{24} was 282.5 $\pm$ 816.9 mg*hr/L.

Population Pharmacokinetic Models

Several structural models were explored to determine the model that best fit the vancomycin concentration data. One-compartment and two-compartment structural models with first-order elimination were assessed with additive, proportional, and combined error models. Structural models also incorporated the rate and duration of the IV infusion for each subject.

A one-compartment model was used to describe the serum concentrations of vancomycin in this patient population. The base model was a one-compartment model with first-order elimination, which was selected as the initial base model on the basis of the OFV, AIC, and SBC. Base model parameter estimates for CL were similar for the Cardinal Glennon and Intermountain Primary Children's models, 2.38 and 3.24 L/hr, respectively.

Table 3.1. Demographic characteristics among children with cystic fibrosis who received vancomycin for the treatment of an acute pulmonary exacerbation.

Characteristic	Cardinal Glennon Number (%) (*n*=43)	Primary Childrens Number (%) (*n*=24)	Combined Number (%) (*n*=67)
Age, yrs			
Median	14	12.5	13.9
Interquartile range	9 – 17	7.1 – 15.5	8 – 17
Sex			
Male	19 (44)	8 (33)	27 (40)
Female	24 (56)	16 (67)	40 (60)
Weight, kg			
Median	43.2	36.6	41.2
Interquartile range	27.1 – 59.9	23.1 – 46.8	25.5 – 56.8
Height, cm			
Median	154	140	150
Interquartile range	108 – 163	124 – 159	118.5 – 159.5

Similarly, the base model estimates for V_D were 46.6 and 18.6 L for the Cardinal Glennon and Intermountain Primary Children's data, respectively. Due to the similarity of these estimates, data from the two centers were combined for a pooled analysis. The base model estimated CL as 2.70 L/hr and V_D as 44.8 L for the pooled analysis.

A covariate analysis was undertaken in which each covariate was added to the model. Following univariate analyses, weight (P<0.001) and serum creatinine (P<0.05) were identified as having a significant influence on vancomycin pharmacokinetics. However, in multivariate analyses accounting for weight, serum creatinine had no significant influence. The inclusion of allometric scaling significantly improved the OFV (P<0.001). This model determined that vancomycin clearance increased with increasing weight.

The final covariate model was chosen as it produced the most significant minimization of the OFV (Δ 37.9), reduced the BSV, and decreased the RUV. The parameter estimates derived from the final covariate model are shown in *Table 3.2*. Also presented are several metrics used to assess the stability and robustness of the final model including standard errors, coefficients of variation, 95% confidence intervals, and bootstrapped estimates (n=1000). The 95% confidence interval surrounding the bootstrapped point estimate for the BSV in V_D includes 0.

Model Evaluation

Diagnostic plots were generated for assessing model fit between observed vancomycin concentrations versus population predicted and individual predicted values (*Figure 3.1*). Plots of the conditional weighted residuals (based on the first-order

Table 3.2. Vancomycin pharmacokinetic parameter estimates and bootstrap estimates from the final one-compartment covariate model across the entire study population.

Parameters	**Parameter Estimates**	**% RSE**	**% CV**	**95% CI**	**Boostrap Mean (*n*=1000)**	**95% CI**
Pharmacokinetic parameters						
Clearance (CL), L/hr	5.57	4.24	--	5.11-6.03	5.52	4.91-6.06
Volume of distribution (V_D), L	44.1	6.53	--	38.5-49.7	44.2	35.4-56.2
Between subject variability (BSV)						
BSV (ω) – clearance (CL)	0.07	35.5	26.9	0.02-0.12	0.08	0.02-0.18
BSV (ω) – volume of distribution (V_D)	0.16	47.5	39.9	0.01-0.31	0.16	0.00-0.37
Residual unexplained variability (RUV)						
RUV (σ) – proportional error (%)	0.08	18.2	0.29	0.05-0.11	0.08	0.04-0.13
RUV (σ) – additive error (mg/L)	12.3	30.8	351	4.87-19.7	12.2	0.00-20.6

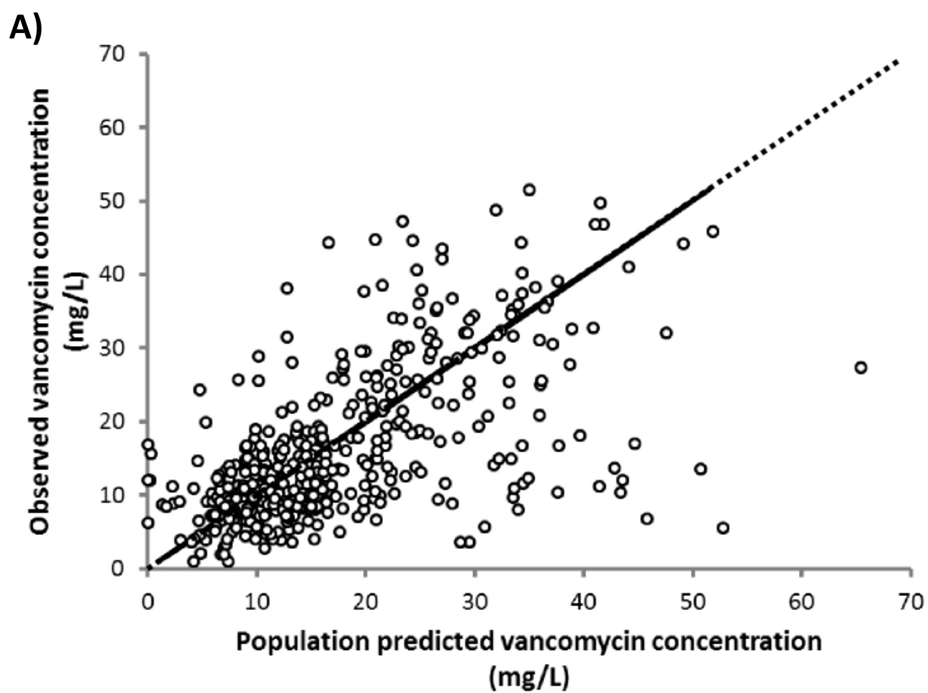
conditional estimation method) versus the population predicted vancomycin concentrations were also examined (*Figure 3.1*). In aggregate, visual inspection revealed that the final covariate model fit the data more tightly than the initial base model, indicating superior performance.

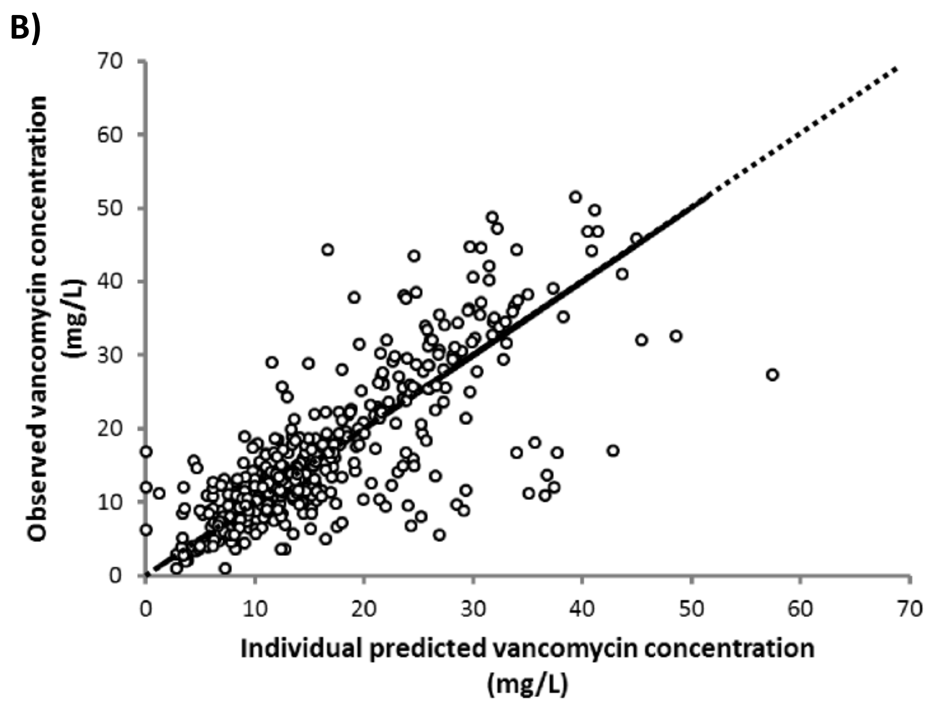
Bootstrapping techniques were also used to assess the robustness of the final covariate model. Mean estimates from the 1000 bootstrap runs were similar to the population estimates derived from the final covariate model. Bootstraps were successfully generated 88% of the time for the pooled data from both Cardinal Glennon and Intermountain Primary Children's Medical Center. Simulations from the final covariate model were derived from the observed vancomycin data in an effort to reveal evidence of model misspecification, which is not easily detected by other methods.[23] Visual predictive checks (VPC) present a graphical comparison of the observed vancomycin data and simulated data and are shown in *Figure 3.2*, with the median simulated value compared to the 5th, 10th, 90th, and 95th quantiles. Of the 48,600 simulated observations 93% fell within the 90% confidence interval of the observed vancomycin concentrations, demonstrating reasonable model stability and agreement.

Discussion

Monitoring of vancomycin concentrations is common to prevent sub-therapeutic dosing and toxicity.[24] Despite extensive study among other patient populations,[25-27] vancomycin population pharmacokinetics have not been described for children with CF. In this study, vancomycin CL and V_D were estimated using a one-compartment model with data derived from children with CF from two centers. Vancomycin CL increased with

Figure 3.1. Diagnostic plots of the final model. (A) Observed versus population-predicted vancomycin concentrations; (B) observed versus individual-predicted vancomycin concentrations; (C) conditional weighted residuals versus population-predicted vancomycin concentrations; and (D) conditional weighted residuals versus the time after dose. (Solid black lines indicate regression lines and dashed black lines represent lines of identity.)


A)
Observed vancomycin concentration (mg/L)
Population predicted vancomycin concentration (mg/L)
0
10
20
30
40
50
60
70


B)
Observed vancomycin concentration (mg/L)
Individual predicted vancomycin concentration (mg/L)
0
10
20
30
40
50
60
70

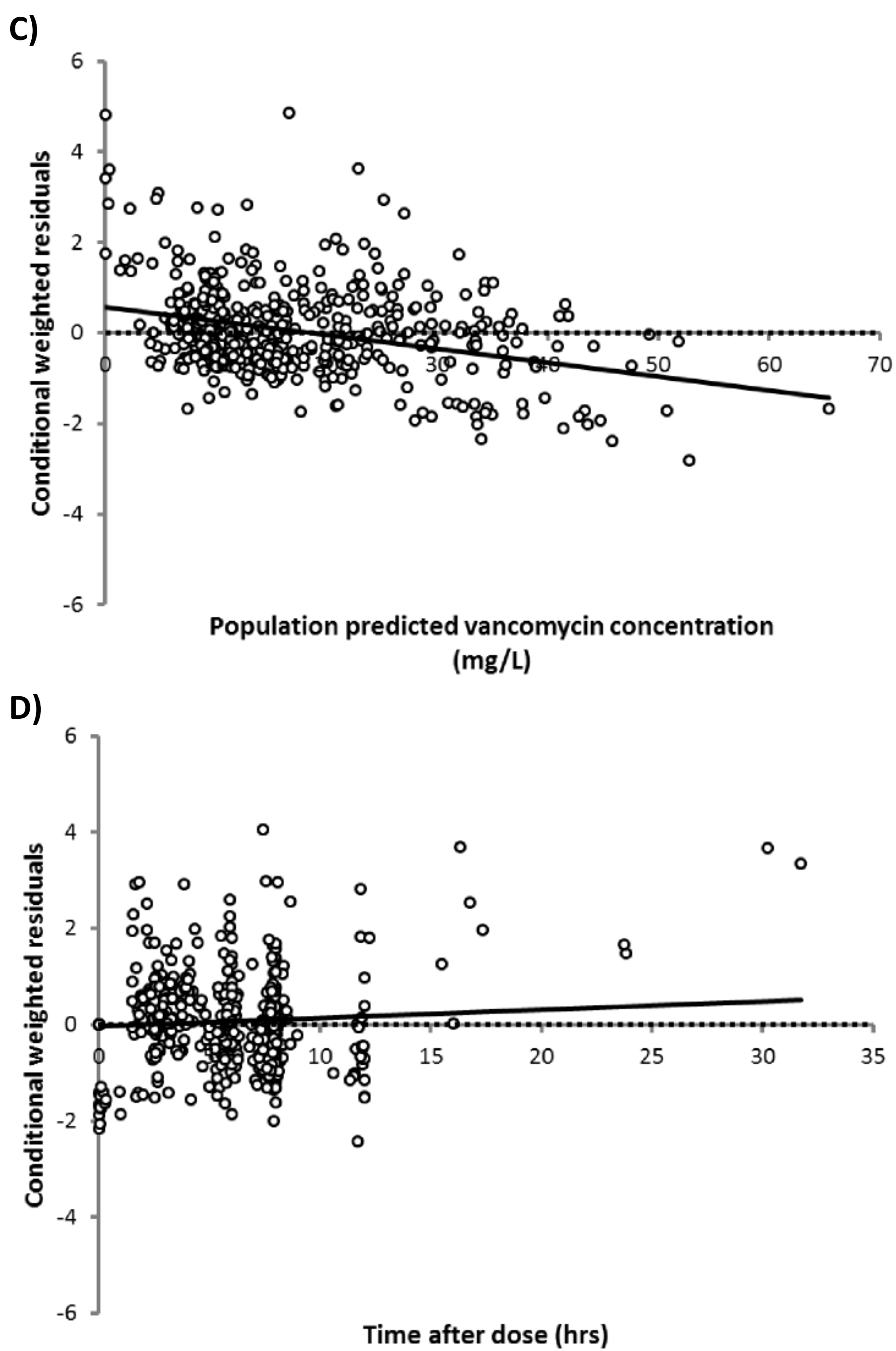


Figure 3.1. Continued.

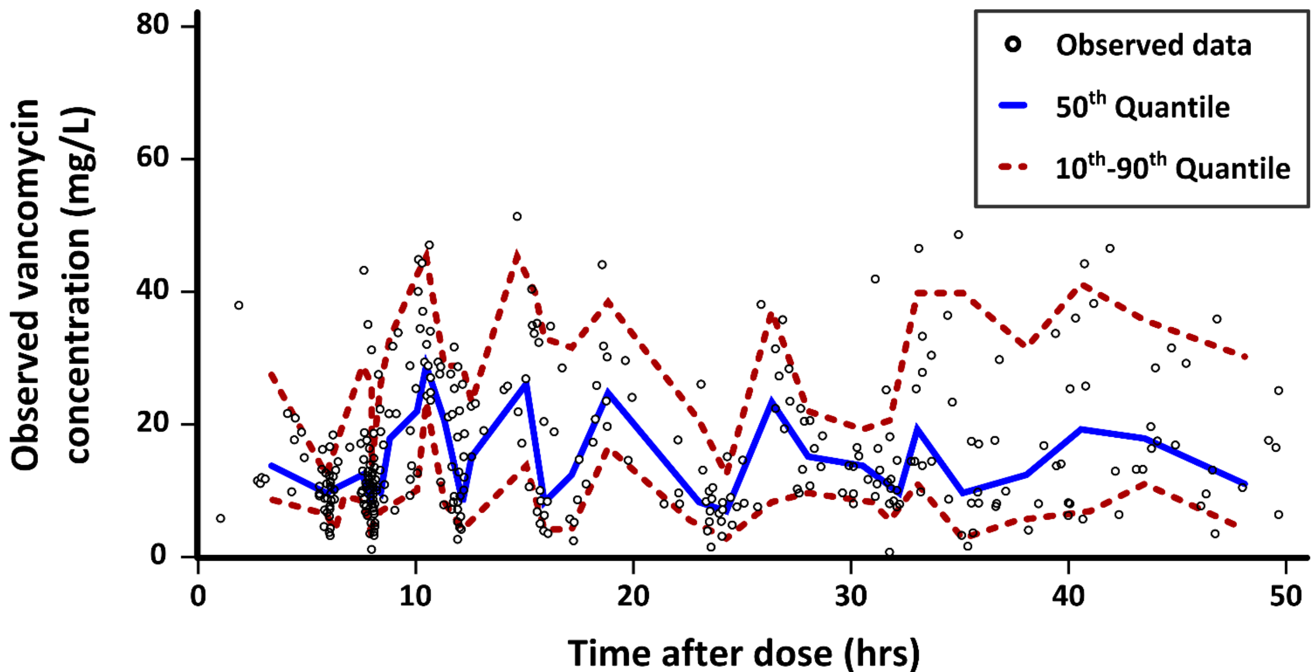


Figure 3.2. Visual predictive check for the final covariate model. Observed vancomycin concentrations are compared with the 10th, 50th, and 90th percentiles for 100 simulated datasets. Comparison of median (solid blue line) and the $10\text{-}90^{th}$ percentile interval (dashed red lines).

increasing weight and is an important covariate that merits consideration in establishing initial dosing regimens for children with acute pulmonary exacerbations of CF.

The pathophysiology of CF affects the pharmacokinetics of many antibacterials prescribed for the treatment of acute pulmonary exacerbations.[28, 29] To evaluate whether vancomycin dosage requirements differ between patients with CF and those without, Pleasants et al. evaluated the pharmacokinetics of vancomycin among 10 adults with CF.[9] The authors reported pharmacokinetic parameter estimates that were similar to earlier studies among healthy adult volunteers. In an earlier study that evaluated vancomycin pharmacokinetics among adults with burn injuries, CL was estimated as 5.3 L/hr/70 kg.[30] This compares favorably with our CL estimate of 5.57 L/hr/70 kg. Among 56 adults with varying levels of renal impairment, the mean V_D at steady-state was 0.72 L/kg.[31] The mean V_D among our cohort of pediatric patients with CF was 0.63 L/kg. These findings suggest that vancomycin pharmacokinetics in children are not substantially different from values that have been reported in adult populations with normal to minor renal impairment. In contrast, vancomycin CL among children without CF has been reported to range from 0.10 to 0.16 L/hr/kg.[32-34] It is possible that the relatively young ages of the children included in these studies (mean ages of 3.9, 5.6, and 7.6 years, respectively) or the pathophysiologic changes that result from CF may account for the difference between the CL estimate of 0.08 L/hr/kg reported here among a population of slightly older children (mean age of 12.1 years). Despite this, the mean V_D in the present population was 0.63 L/kg, which is similar to estimates obtained in several earlier studies conducted among different populations of children without CF.[32-35] In aggregate, these data support the notion that vancomycin CL among older children with CF may more

closely resemble adult CL estimates, while the V_D of vancomycin is similar to values reported among healthy children.

In the present study, current body weight was an important covariate that influenced vancomycin clearance among children with CF. Previous studies in both children and adults without CF have also identified current weight as an important determinant of vancomycin pharmacokinetics.[36, 37]

Controversy exists as to whether a one-compartment or a two-compartment model is more appropriate for characterizing vancomycin pharmacokinetics.[38] Albrecht et al. reported that the half-life of vancomycin ranged from 0.2 to 0.8 hours.[39] This led the authors to conclude that a one-compartment model using two serum concentrations is acceptable for pharmacokinetic modeling studies.[39] In evaluating a Bayesian approach, Pryka et al. assessed the relative predictive utility of one- and two-compartment models and determined that a two-compartment model was more precise and less biased.[40] However, Rosell et al. proposed that it is difficult to ethically-justify the number of serum concentrations needed to rigorously evaluate a two-compartment model.[41] In clinical practice, post-distributive vancomycin concentrations are frequently obtained, which allow the use of one-compartment model equations to describe the pharmacokinetics of a two-compartment drug.[42] This study utilized data that were collected during routine therapeutic drug monitoring and therefore featured a limited number of vancomycin concentrations for each patient. With the limited data available, we found that a one-compartment model tended to under-predict low vancomycin concentrations and over-predict high concentrations among children with CF. It is likely that more frequent sampling may have supported the use of a two-compartment model.

Interpretation of these findings is subject to several limitations. Data were collected during routine therapeutic drug monitoring, and a limited number of vancomycin concentrations were measured for each patient. Additionally, this study was not designed to correlate vancomycin pharmacokinetics with clinical efficacy, although trough concentrations of <10 mg/L have been associated with treatment failure, which may be due to poor tissue penetration and selection of vancomycin-heteroresistant *S. aureus*.[43] This emphasizes the importance of achieving appropriate vancomycin serum concentrations for each patient, which requires individualized dosing and knowledge of important covariates that influence vancomycin pharmacokinetics.

The pathophysiology of CF has been reported to alter aminoglycoside pharmacokinetics, making it difficult to establish dosing regimens that optimize antibacterial efficacy and safety.[44] As the prevalence of MRSA has increased over the last 30 years, vancomycin use has also increased.[45, 46] Despite this, relatively little is known about the pharmacokinetics of vancomycin in children with CF. In this study, vancomycin pharmacokinetics were adequately described with a one-compartment first-order elimination model. Vancomycin CL was lower than has been reported among studies of younger children without CF. Clearance was also significantly influenced by current body weight. The V_D was similar among children with CF, healthy children, and heterogeneous adult populations. Future pharmacodynamic studies are needed to establish markers of efficacy and safety, which may be used to develop an optimal vancomycin dosing regimen for the treatment of acute pulmonary exacerbations of CF among children.

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CHAPTER 4

PREDICTIVE PERFORMANCE OF A VANCOMYCIN POPULATION PHARMACOKINETIC MODEL IN NEONATES

Abstract

Introduction: The pharmacokinetics of vancomycin are highly variable among neonates, which makes dosing challenging in this population. However, adequate drug exposure is critical, especially when treating methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Utilization of population pharmacokinetic models and Bayesian methods offers the potential for developing individualized therapeutic approaches. To meet this need, a neonatal vancomycin population pharmacokinetic model was recently published. The current study sought to externally evaluate the predictive performance and generalizability of this model.

Methods: A retrospective chart review of neonates who received vancomycin and had ≥1 peak and ≥1 trough concentrations at five Intermountain Healthcare neonatal intensive care units from 2006-2013 was performed and served as the external validation

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cohort. The published population pharmacokinetic model was implemented in NONMEM 7.2 with the structural and variance parameter values set equal to the estimates reported previously. The model was then used to predict the first peak and trough concentration for each neonate in the validation cohort and the model prediction error and absolute prediction error were calculated. Normalized prediction distribution errors (NPDE) were also evaluated.

Results: A total of 243 neonates were studied with a median postmenstrual age of 33 (interquartile range [IQR]: 28-39) weeks and a median weight of 1.6 (IQR: 1.0-2.9) kg. The model predicted the observed vancomycin concentrations with reasonable precision. For all vancomycin concentrations, the median prediction error was -0.8 (95% CI: -1.4 to -0.4) mg/L and the median absolute prediction error was 3.0 (95% CI: 2.7 to 3.5) mg/L. No trends in NPDE across weight, postmenstrual age, serum creatinine or time after dose were observed.

Conclusions: An evaluation of a recently published neonatal vancomycin population pharmacokinetic model in a large external dataset supported the predictive performance and generalizability of the model. This model may be useful in evaluating neonatal vancomycin dosing regimens and estimating the extent of drug exposure.

Introduction

Optimizing vancomycin dosing to rapidly achieve adequate drug exposure is imperative in treating neonatal sepsis, particularly when treating invasive methicillin-resistant *Staphylococcus aureus* (MRSA) infections.[1] However, this has been challenging in neonates as the pharmacokinetics of vancomycin are highly variable among

neonates due to developmental and pathophysiological changes.[2, 3] Recent studies have shown that standard neonatal vancomycin dosing strategies, such as those outlined in NeoFax®, do not reliably achieve trough concentrations >10 mg/L.[4, 5] In addition, the ratio of the 24-hour area under the concentration-time curve (AUC_{24}) to the minimum inhibitory concentration (MIC) – the best predictor of successful outcomes when treating invasive MRSA infections – is not routinely utilized to assess the appropriateness of vancomycin dosing in neonates, presumably due to practical limitations associated with calculating the AUC_{24}.

Innovative vancomycin dosing strategies are therefore needed in neonates that 1) incorporate known patient-specific determinants of vancomycin pharmacokinetics such as size, maturation, and renal function in the dose selection and 2) allow for assessment of AUC_{24} based on the dosing history and vancomycin concentration(s) measured as part of routine therapeutic drug monitoring.[3, 6, 7] To develop such an individualized therapeutic approach in neonates, utilization of population pharmacokinetic models and Bayesian methods will be essential.[8-11] We recently developed a neonatal vancomycin population pharmacokinetic model that capitalized on patient data readily available in the electronic medical record: weight (an indicator of size), postmenstrual age (an indicator of maturation), and serum creatinine (an indicator of renal function).[7] The model has the potential to improve our ability to define vancomycin dosing regimens that reliably achieve recommended exposure targets; however, it is critical to first evaluate whether this model and its findings are generalizable to neonates outside of the original population used to develop the model. The objective of the current study was to conduct an external evaluation

of this published pharmacokinetic model and to enhance our understanding of the relationship between vancomycin trough concentration and AUC_{24} in neonates.

Methods

Validation Cohort

Approval to conduct this study was granted by the University of Utah and Primary Children's Hospital (PCH) Institutional Review Boards. PCH is a freestanding children's hospital with a level IV neonatal intensive care unit that is staffed by University of Utah neonatologists. PCH is owned and operated by Intermountain Healthcare, which is a large, not-for-profit, vertically-integrated healthcare delivery system that serves Utah, Idaho, Wyoming, Nevada, and Montana. In addition to PCH, four other level II-III neonatal intensive care units operated by Intermountain Healthcare were included in this study.

A retrospective chart review was conducted for all neonates who had vancomycin therapeutic drug monitoring performed from 2006-2013 at five Intermountain Healthcare neonatal intensive care units. Neonates were included if they were <54 weeks postmenstrual age and had ≥2 doses of vancomycin, ≥1 peak concentration, ≥1 trough concentration, and ≥1 serum creatinine level. Vancomycin concentrations were quantified using a particle-enhanced turbidimetric inhibition immunoassay on an Abbott Architect cSystem platform (Abbott Laboratories, Abbott Park, Illinois). Vancomycin concentrations were defined based on their temporal relationship to dosing records. Trough concentrations were defined as concentrations obtained within three hours of the next vancomycin dose and peak concentrations were defined as concentrations obtained within three hours of the preceding dose. Serum creatinine levels collected within ±48 hours of vancomycin dosing

and concentration records were carried forward and backward and were used in the analyses. To account for the known difference in measured serum creatinine concentrations between the Jaffe method (used in the original model derivation cohort) and the enzymatic method (used in the current external validation cohort), a previously described linear conversion factor was applied to all of the enzymatic serum creatinine concentrations included in this external validation ($enzymatic\ concentration = 1.050 * Jaffe\ method\ concentration - 0.122$).[12] Exclusion criteria included a diagnosis of congenital kidney disease, major congenital heart disease (other than ventricular septal defect, atrial septal defect, or patent ductus arteriosus), or extracorporeal membrane oxygenation (ECMO) during the vancomycin course.

Model Evaluation

The published neonatal vancomycin population pharmacokinetic model was implemented in the non-linear mixed effects modeling software NONMEM 7.2 (ICON Development Solutions, Ellicott City, MD) as previously described.[7] Briefly, a one compartment model with first-order elimination was used to describe vancomycin pharmacokinetics. Clearance (CL) was predicted by weight (an indicator of size), postmenstrual age (PMA; an indicator of maturation) and serum creatinine (Cr; an indicator of renal function) according to the following equation:

$$CL\ (L/h) = 0.345 \cdot \left(\frac{Weight}{2.9\ kg}\right)^{0.75} \cdot \frac{1}{1 + \left(\frac{PMA_{weeks}}{34.8}\right)^{-4.53}} \cdot \left(\frac{1}{Cr_{mg/dL}}\right)^{0.267} \qquad (4.1)$$

Volume of distribution (V) was predicted by weight:

$$V\,(L) = 1.75 \cdot \left(\frac{Weight}{2.9\,kg}\right) \quad (4.2)$$

After accounting for known predictors, the remaining variation between neonates was described by an exponential error model for both CL (% coefficient of variation [% CV] 21.6%) and V (% CV 10.9%). Residual variability (a measure of the difference between the model predicted concentration for a neonate and the observed concentration in that neonate) was captured using a combined proportional (% CV 20.5%) and additive error model (standard deviation [SD] ± 1.3 mg/L).

For each neonate in the external validation cohort, vancomycin concentrations were then predicted by using the parameters of the population pharmacokinetic model and simulating the actual dosing regimen given to the neonate (using the NONMEM MAXEVAL=0 POSTHOC command). Only concentrations at times for which a neonate had therapeutic drug monitoring performed were simulated. Model-predicted vancomycin concentrations (PRED from the NONMEM output) were then compared with the corresponding observed vancomycin concentrations. As described by Sheiner and Beal,[13] the bias and precision of the model were assessed by calculating the median prediction error and median absolute prediction error for the first trough and peak concentration according to the following formulas:

Prediction error (bias): $$\left(\frac{Conc_{pred} - Conc_{obs}}{Conc_{obs}}\right) \quad (4.3)$$

Absolute prediction error (precision): $$\left(\frac{|Conc_{pred} - Conc_{obs}|}{Conc_{obs}}\right) \quad (4.4)$$

where $Conc_{pred}$ refers to the model-predicted vancomycin concentration and $Conc_{obs}$ refers to the observed vancomycin concentration. Model predicted vancomycin concentrations calculated using each patient's individual Bayesian estimate of CL and V (i.e. the IPRED

from the NONMEM output which incorporates the patient's drug concentrations in addition to the fixed covariate effects in the model predictions) were also evaluated using the same approach.

The predictive performance of the model was further evaluated using simulation-based diagnostic methods. Normalized prediction distribution errors (NPDE) were calculated by simulating 1000 datasets and comparing the predicted concentrations to the observed concentrations using the NPDE command in NONMEM.[14, 15] The NPDE should follow a normal distribution with a theoretical mean of 0 and variance equal to 1.[14]

Trough Concentration and AUC_{24} Relationship

Following model evaluation, the relationship between trough concentration and AUC_{24} was examined. Bayesian estimates of CL for each neonate from the population pharmacokinetic model were used to calculate AUC_{24} at the time that vancomycin trough concentrations were collected.[8] AUC_{24} was calculated as the daily dose ÷ CL. For a given trough concentration, the proportion of neonates with that trough concentration who achieved an AUC_{24} ≥400 was calculated. An AUC_{24} ≥400 mg*hr/L would predict an AUC_{24}/MIC ≥400 for an MIC of ≤1 mg/L. AUC_{24} calculations, descriptive statistics, and graphical analyses were performed in R 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

External Validation Cohort

Overall, 243 neonates had vancomycin dose and concentration data available and served as the external validation cohort. The median dose was 15.5 mg/kg (interquartile range [IQR]: 13.9-19.3) and the median dosing interval was 11.5 hrs (IQR: 8.0-12.5 hrs). Demographic and clinical characteristics of the neonates in the external validation cohort are shown in *Table 4.1*. For comparison, demographic and clinical characteristics of the neonates in the cohort used to develop the original published pharmacokinetic model are also shown. Overall, the validation cohort was of lower weight and age and had higher serum creatinine concentrations.

In the external validation cohort, a total of 734 vancomycin concentrations were available for analysis. Each neonate contributed a mean of 3.0 (± 1.8) vancomycin concentrations. The time of vancomycin concentration collection relative to the previous dose is shown in *Table 4.2*. All neonates had at least one concentration measured within three hours of the end of the vancomycin infusion. No concentrations were below the lower limit of quantitation.

Model Evaluation

The vancomycin pharmacokinetic model adequately described the observed vancomycin concentrations in the external cohort of neonates (*Figure 4.1A*). Model predicted vancomycin concentrations (PRED) were slightly lower than the observed concentrations (median prediction error -0.8 [95% CI: -1.4 to -0.4] mg/L). The precision of the model was reasonable with a median absolute prediction error of 3.1 (95% CI: 2.7

Table 4.1. Demographic and clinical characteristics of neonates who received vancomycin and had therapeutic drug monitoring performed.

Characteristic	***Model Development Cohort (n=249)*** [a]		***External Validation Cohort (n=243)*** [b]	
	Median / No.	***Range***	***Median / No.***	***Range***
Female, *n* (%)	121 (49%)	--	103 (42%)	--
Gestational age, weeks	34	23 – 42	30	22 – 41
Birthweight, kg	2.0	0.4 – 4.4	1.3	0.5 – 5.1
Weight, kg	2.9	0.5 – 6.3	1.6	0.4 – 6.8
Postnatal age, days	19	0 – 173	12	0 – 196
Postmenstrual age, weeks	39	24 – 53	33	23 – 54
APGAR at 5 minutes	8	1 – 10	8	1 – 10
Serum creatinine, mg/dL [d]	0.4	0.1 – 2.7	0.6	0.3 – 1.5

[a] Patient characteristics of the 249 neonates used to develop the neonatal vancomycin population pharmacokinetic model described by Frymoyer et al.[7]

[b] Patient characteristics of the 243 neonates used in the current external validation.

[c] The serum creatinine concentration in the model derivation cohort was measured using the Jaffe method. The serum creatinine concentration in the external validation cohort was measured using the enzymatic method and was converted to a Jaffe-standardized equivalent using a linear equation described by Srivastava et al.[12] Converted values are presented in the table above.

Table 4.2. Timing of 734 neonatal vancomycin concentrations relative to the end of the most recent 1 hour infusion.

Time since the end of the most recent infusion	***N (%)***
0 – 1 hr	122 (17%)
1 – 2 hrs	192 (26%)
2 – 4 hrs	27 (4%)
4 – 6 hrs	63 (9%)
6 – 8 hrs	66 (9%)
8 – 12 hrs	152 (21%)
12 – 24 hrs	107 (15%)
>24 hrs	5 (1%)

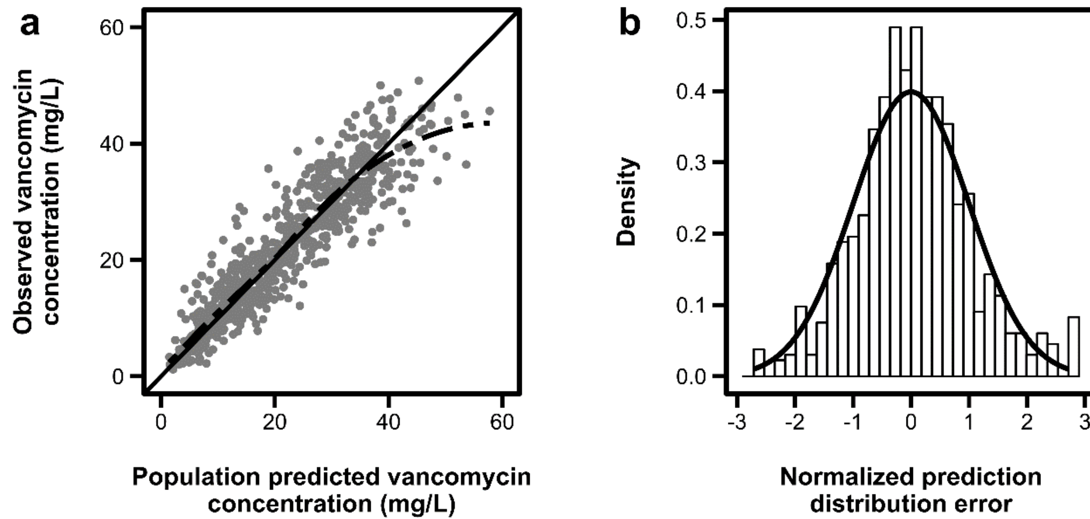

Figure 4.1. An external evaluation of the predictive performance of a previously published neonatal vancomycin population pharmacokinetic model. (A) Diagnostic plot depicting the model fit for observed versus population-predicted vancomycin concentrations. The dashed black line represents the locally weighted scatterplot smoothed fit of the data. (B) Kernel density plot of the normalized prediction distribution errors with a histogram depicting a normal, Gaussian distribution overlaid for comparative purposes.

to 3.2 mg/L). The predictive performance of the model for peak and trough concentrations is featured in *Table 4.3*. When incorporating patient concentrations to obtain Bayesian estimates of PK parameters for each neonate, the precision of the model predicted vancomycin concentrations (IPRED) improved (*Table 4.4*). For example, the median absolute prediction error of IPRED was 1.7 (95% CI: 1.5 to 1.8) mg/L.

Simulation based diagnostics of the vancomycin pharmacokinetic model demonstrated a mean NPDE of 0.05 and a variance of 0.96, indicating no bias and an ability of the model to reasonably capture the underlying variability in the external validation cohort. Additionally, there were no trends in NPDE across weight, postmenstrual age, serum creatinine, or time after dose (*Figure 4.2*).

Trough Concentration and AUC_{24} Relationship

A linear relationship between increased AUC_{24} and higher trough concentrations was observed in the external validation cohort ($r^2 = 0.60$; *Figure 4.3A*). AUC_{24} was highly variable at a given trough concentration (i.e., a 2 to 3 fold range of AUC_{24} was achieved at a given trough concentration), and therefore, AUC_{24} could not be precisely predicted for an individual neonate based on a trough concentration alone. However, a trough concentration of 11 mg/L predicted the achievement of an $AUC_{24} \geq 400$ in 93% of neonates (*Figure 4.3B*). The median (range) AUC_{24} at this trough concentration was 542 (308 to 649) mg*hr/L.

Table 4.3. Predictive performance of the neonatal population pharmacokinetic model in the external validation cohort.

	All concentrations	***First peak***	***First trough***
Prediction error			
Median	-0.8	-2.0	-0.1
95% confidence interval	-1.4 to -0.4	-2.9 to -1.4	-0.5 to 0.2
Percent prediction error			
Median	-4.5%	-7.5%	-1.5%
95% confidence interval	-7.2% to -2.2%	-9.4% to -4.9%	-4.5% to 2.7%
Absolute prediction error			
Median	3.0	3.9	2.1
95% confidence interval	2.7 to 3.5	3.4 to 4.1	1.7 to 2.7
Absolute percent prediction error			
Median	15.2%	12.6%	20.1%
95% confidence interval	14.1% to 17.3%	10.9% to 14.4%	16.8% to 24.0%

Table 4.4. Predictive performance of the neonatal population pharmacokinetic model in the external validation cohort after incorporating patient drug concentrations in predictions (e.g., IPRED method).

	All concentrations	***First peak***	***First trough***
Prediction error			
Median	-0.7	-1.7	-0.2
95% confidence interval	-0.9 to -0.5	-2.2 to -1.4	-0.4 to 0.1
Percent prediction error			
Median	-3.8%	-5.8%	-1.7%
95% confidence interval	-4.9% to -3.2%	-7.5% to -4.6%	-3.4% to 0.6%
Absolute prediction error			
Median	1.7	2.7	0.9
95% confidence interval	1.5 to 1.8	2.1 to 3.1	0.7 to 1.1
Absolute percent prediction error			
Median	8.8%	8.4%	9.1%
95% confidence interval	8.1% to 9.7%	7.3% to 9.6%	7.3% to 10.8%

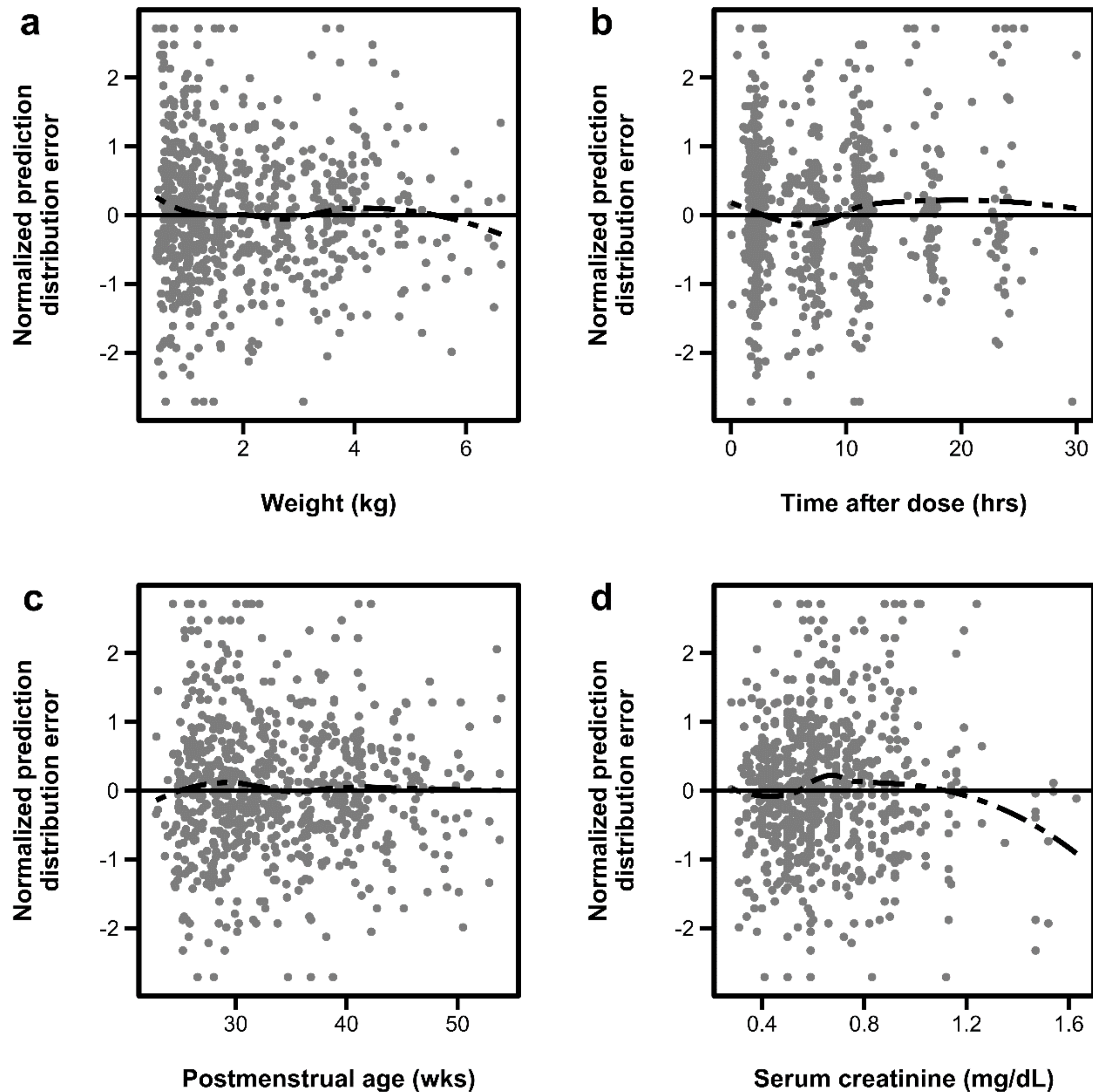

Figure 4.2. Assessment of the predictive performance of the neonatal vancomycin population pharmacokinetic model. (A) Normalized prediction distribution errors versus weight, measured in kilograms. (B) Normalized prediction distribution errors versus the time elapsed since the last vancomycin dose, measured in hours. (C) Normalized prediction distribution errors versus postmenstrual age, measured in weeks. (D) Normalized prediction distribution errors versus serum creatinine concentrations, measured in milligrams per deciliter. The dashed black lines represent locally weighted scatterplot smoother fits of the data.

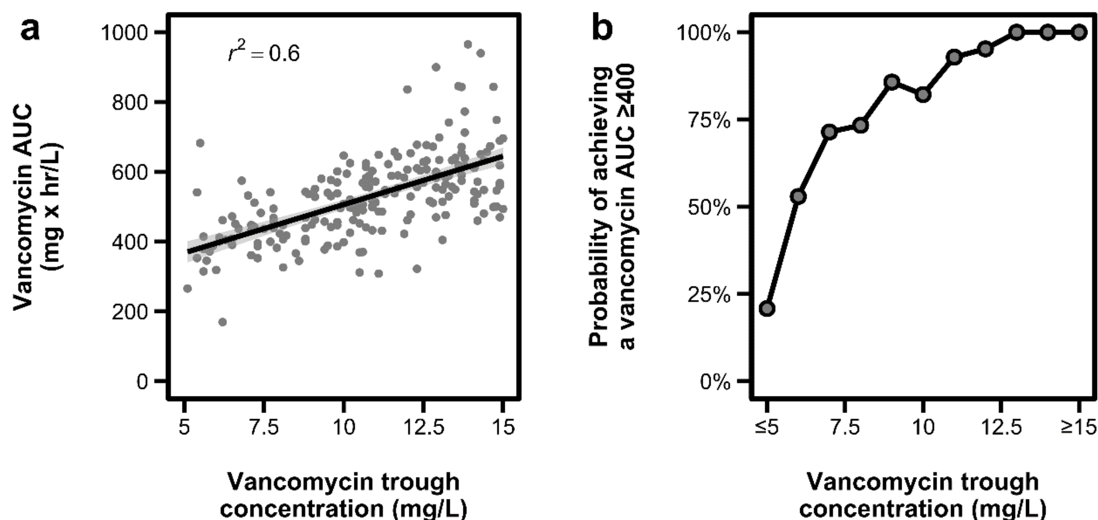

Figure 4.3. The association between vancomycin trough concentrations and the extent of drug exposure, as measured by the 24-hour area under the curve (AUC_{24}). (A) Higher vancomycin trough concentrations were associated with higher AUC_{24} values, although substantial variability was noted. (B) The probability of achieving a pharmacokinetic / pharmacodynamic target associated with clinical and microbiological success for invasive methicillin-resistant *Staphylococcus aureus* infections (an AUC_{24} ≥400) increased with higher vancomycin trough concentrations. All neonates with a trough ≥12 mg/L had an AUC_{24} ≥400, although many neonates achieved the AUC_{24} target with lower trough concentrations.

Discussion

External validation of a population pharmacokinetic model is described by the United States Food and Drug Administration (FDA) as "the most stringent method for testing a developed model".[16] Yet, external validation is performed in <10% of published pharmacokinetic models and concerns about the clinical utility of the model often remain.[17] The external validation performed in the current study strengthens a previously published neonatal vancomycin population pharmacokinetic model. Namely, we found the pharmacokinetic model to be unbiased across the largest cohort of neonates used in a validation study to date. The precision of the model when utilizing only a neonate's postmenstrual age, weight, and serum creatinine was 12.6% for peak concentrations and 20.1% for trough concentrations. When a neonate's drug concentrations are incorporated into the model (such as would occur after therapeutic drug monitoring in the NICU), the precision further improved to 8.4% and 9.1% for peak and trough concentrations, respectively. This level of precision suggests that the model may be useful in evaluating vancomycin dosing regimens and estimating the extent of drug exposure in the clinical setting.

A recent clinical study by Ringenberg et al. highlights the current challenges with vancomycin dosing in neonates. In a multicenter retrospective evaluation, vancomycin dosing guidelines from Neofax resulted in only 25% of the neonates studied achieving a target trough concentration of 10-20 mg/L with empiric dosing.[4] Moreover, the authors reported that 20% of the neonates included in their study had a trough concentration <5 mg/L (Theresa Ringenberg personal communication, April 15, 2015). Even after therapeutic drug monitoring and dose-adjustment, only 45% of neonates achieved the goal

trough concentration of 10-20 mg/L at any point during their course of therapy. This study clearly reveals the significant clinical challenge associated with reliably achieving therapeutic and safe vancomycin concentrations in this highly variable patient population.[4] More innovative vancomycin dosing strategies and approaches are needed in neonates that can help providers personalize empiric dose selection, interpret therapeutic drug monitoring data, and adjust dosing so that exposure targets are achieved.

Population pharmacokinetic models are a powerful tool that can aid clinicians and help inform dosing decisions.[18, 19] By incorporating patient-specific characteristics, dosing information, drug concentrations, and consideration of the variability between patients, population pharmacokinetic models offer the opportunity to provide a more personalized approach to therapeutic decision making. This is especially valuable in a highly variable population, such as neonates, receiving a narrow therapeutic window drug such as vancomycin.

In adults, Bayesian approaches utilizing population pharmacokinetic models have already been shown to have the potential to help support vancomycin dosing decisions.[19, 20] Advancement of similar approaches in neonates is needed. The development and external validation of a neonatal vancomycin population pharmacokinetic model lays the foundation for this future work. For example, our group is currently developing a model-based approach to individualize the empiric dose in neonates that incorporates the predictors of weight, postmenstrual age, and serum creatinine. Using a simulation framework, the vancomycin dose for a given neonate that is most likely to achieve an AUC_{24} >400 while still maintaining a trough concentration <20 mg/L is calculated. A user-friendly, web-based application is currently being developed to facilitate the adoption of

this model in our neonatal intensive care units, including integration into the electronic health record. In addition, the ability to estimate AUC_{24} and assist providers with dose adjustment within the clinical workflow would be of high value.

Until more robust clinical dosing support tools are developed, clinicians will continue to rely on trough concentration monitoring to help guide vancomycin dosing in neonates. Our findings reinforce the large variability observed in vancomycin trough concentrations among neonates and the inability of a trough concentration alone to reliably predict an individual neonate's AUC_{24}. Targeting an $AUC_{24}/MIC \geq 400$ is recommended by the Infectious Disease Society of America when treating invasive MRSA infections, and a trough concentration of 15-20 mg/L is suggested in adults to achieve this target.[1, 21] The current study provides further support that in neonates a vancomycin trough concentration of 15-20 mg/L is unnecessary to achieve an $AUC_{24}/MIC \geq 400$ with an MIC ≤ 1 mg/L and that lower trough concentrations are likely adequate based on AUC_{24} considerations.[7] Accordingly, a trough concentration of approximately 10 mg/L is likely a reasonable first-line target that will provide adequate exposure for invasive MRSA while also appropriately covering for coagulase negative staphylococcal infections. Further dose adjustment and individualization of the therapeutic approach should be guided by the specific pathogen identified, susceptibility testing, clinical status, etc. For example, for MRSA infections with MICs ≥ 2 mg/L, an alternative to vancomycin may be necessary since an $AUC_{24}/MIC \geq 400$ will not be achieved in neonates even at trough concentrations of 15-20 mg/L.[7] Lastly, the extent to which the target $AUC_{24}/MIC \geq 400$ is generalizable to neonates is unclear and requires further study.

Conclusions

In summary, an evaluation of a recently published neonatal vancomycin population pharmacokinetic model in a large external dataset supported the predictive performance and generalizability of the model. The model may be useful in evaluating vancomycin dosing regimens and estimating the extent of drug exposure in neonates.

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CHAPTER 5

PRELIMINARY ASSESSMENT OF ZOLPIDEM PHARMACOKINETICS IN PEDIATRIC BURN PATIENTS

Abstract

Purpose: Severely burned patients frequently experience sleep fragmentation and insomnia. This study evaluated the population pharmacokinetics of the sleep-enhancing agent zolpidem among burned children.

Methods: Zolpidem was administered according to the following age-based dosing schedule: 2-4 years, 2.5 mg/dose; 5-10 years, 5.0 mg/dose; and >10 years, 10 mg/dose. Serum samples were collected pre-dose, 1, 2, 4, 5, 6, and 8 hours post-dose. The population pharmacokinetic analysis modelled zolpidem concentrations using non-linear mixed effects models.

Results: Eleven patients with a mean ($\pm$SD) age of 8.3$\pm$4.0 years and a mean total burn surface area of 56$\pm$22% were recruited. Seventy-three zolpidem concentrations were

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measured with a mean C_{max} of 291±140 ng/mL. A two-compartment model with first-order absorption best described the data. Zolpidem clearance was estimated at 0.03 L/hr/kg (relative standard error, 55%) and increased with body weight ($P<0.05$). The central compartment volume of distribution was estimated at 0.05 L/kg (relative standard error, 25%), which was inversely related to the proportion of the body surface with third degree burns ($P<0.001$).

Conclusions: A population pharmacokinetic model has been developed that reliably characterized the pharmacokinetic parameters of zolpidem when used as a sleep-enhancing agent among pediatric burn patients. Further studies are needed to link this pharmacokinetic model with pharmacodynamic data, which may include an assessment of the effects of higher zolpidem doses and/or more frequent administration upon sleep architecture.

Introduction

Zolpidem tartrate is an imidazopyridine sedative and hypnotic agent that is rapidly absorbed, metabolized, and eliminated.[1] In studies among adult subjects, zolpidem has been shown to decrease the number of night-time awakenings, decrease the time required to fall asleep, increase total sleep time, and improve sleep quality among insomniacs.[2] Zolpidem has been used for the management of sleep disorders for more than 20 years and has been reported to be safe and effective for a variety of sleep-related complaints.[3, 4]

Burn injuries are marked by a dramatic catabolic phase that is characterized by an increase in energy expenditure, protein catabolism, and cachexia.[5, 6] Alterations in sleep patterns have been shown to be independent risk factors for poor pain tolerance in adults

with severe burn injuries.[7] Among burn patients, restorative sleep may be impaired due to physiological, psychological, environmental, and treatment-related stimuli that interfere with normal sleep patterns.[8-10] These detrimental effects suggest that sleep-enhancing agents, such as zolpidem, may be useful for improving burn survival and recovery.[11]

In a recent study, children with severe burn injuries were randomized to receive zolpidem or haloperidol and had continuous polysomnographic recordings obtained to evaluate the effects of these agents on sleep architecture.[12] Forty patients were enrolled in this blinded crossover study, in which each patient alternately received zolpidem one week and haloperidol the next. Zolpidem was found to have a small, but significant, effect in improving the proportion of stage 3 and rapid eye movement sleep (0.8 vs. 0.6 hours), but did not affect the total duration of sleep. In contrast, haloperidol increased total sleep time (5.3 vs. 4.3 hours) and increased stage 2 sleep (3.3 vs. 2.4 hours). The authors concluded that sleep was marginally improved with both drugs and there were no significant differences between the two therapeutic agents. It was noted, however, that the relatively short half-life of zolpidem (mean 2.5 hours) may have attenuated its beneficial effects upon sleep architecture. To date, no studies have examined zolpidem pharmacokinetics in burn patients; however, drug metabolism is generally thought to be elevated as a consequence of the pathophysiology of burn injuries, including: altered protein binding, bioavailability, and tissue blood flow; heightened renal clearance; and a higher volume of distribution.[13, 14] As a consequence of these pharmacokinetic changes and the relatively short half-life of zolpidem, it is possible that this agent may fail to prevent sleep fragmentation as it wears off, thereby decreasing the amount of restorative sleep among burn patients.

The primary aim of this study was to develop a population pharmacokinetic model to explore the pharmacokinetics of zolpidem among children with severe burn injuries.

Materials and Methods

Subjects and Study Design

This study was conducted as an open-label inpatient pharmacokinetic study that involved pediatric burn patients who consented to receive zolpidem tartrate for use as a sleep-enhancing agent over four consecutive days. Acutely burned children were screened at the time of their admission to the burn unit for enrollment into this prospective pharmacokinetic study. Inclusion criteria included a total burn surface area (TBSA) greater than 20%, age between 3 and 18 years, and admission within 5 days of the burn injury. Children were excluded from enrollment if there were pre-existing neurological, sleep or psychiatric disorders; a history of brain injury; endocrine disease; questionable 72 hour survival; severe obesity (body mass index >97th percentile); or if administration of other sleep-inducing agents was planned within 24 hours. Demographic data were collected for all study participants.

This study was reviewed and approved by the University of Cincinnati Institutional Review Board. Parental permission and informed assent (when appropriate) were obtained prior to the performance of any study-related procedures.

Drug Administration

Historically, zolpidem dosing was based upon the age of the child. In this study, we aimed to examine the pharmacokinetics of zolpidem when dosed according to routine

clinical practice. Dose amounts for each child are presented in *Table 5.1*. Two pilot subjects received a single 5 mg night-time dose of zolpidem at 2200 hours. Nine subsequent patients received a second dose at 0200 hours in an attempt to maintain sleep throughout the night. Zolpidem was administered as a crushed tablet that was dissolved in 5 mL of water and was given via a nasoenteric Frederick-Miller feeding tube, followed by a 5 mL flush.

Sample Collection

Zolpidem concentration-time data were prospectively collected for each enrolled subject. On the fourth and final day of zolpidem therapy, blood samples were collected over an 8-hour period from an indwelling catheter. Samples were drawn in non-heparinized tubes immediately prior to the first dose and at 1, 2, 4, 5, 6, and 8 hours post-dose. The total amount of blood drawn at each sampling interval did not exceed 3.0 mL. Whole blood samples were centrifuged at 1500 *g* (approximately 3000 rev/min) for 10 min at 4°C. Centrifuged samples were then stored at -80°C prior to pharmacokinetic analysis.

Analytical Assay

Zolpidem serum concentrations were analyzed using a validated high-performance liquid chromatography (HPLC) assay tethered to a fluorescence detector.[15] The assay was linear over the range from 25-1000 ng/mL. Intra- and inter-day coefficients of variation were less than 8.2%.

Table 5.1. Demographic and clinical characteristics of severely burned children who received zolpidem as a sleep-enhancing agent.

Patient number	Sex	Age, yrs	Weight, kg	BMI, kg/m^2	TBSAB, %	Third-degree burn, %	Days after burn	No. of doses	Dose amount, mg
1	Male	7	34	18	85	83	44	1	5
2	Male	4	20	19	43	43	14	1	5
3	Male	4	28	23	51	51	16	2	2.5
4	Male	4	17	16	83	82	8	2	2.5
5	Male	8	25	16	78	74	14	2	5
6	Female	12	45	16	50	25	16	2	10
7	Male	8	23	15	28	23	11	2	5
8	Female	8	35	18	35	29	15	2	5
9	Male	7	22	15	31	19	11	2	10
10	Female	17	60	21	83	22	19	2	20
11	Male	12	45	19	54	46	30	2	8
Mean ± SD		8.3 ± 4.0	32.1 ± 13.2	17.8 ± 2.5	56.4 ± 22.0	45.2 ± 24.5	18.0 ± 10.3	--	7.1 ± 5.0

Pharmacokinetic Analysis

Zolpidem pharmacokinetic parameters were estimated using Monolix 4.2 (Lixoft, Orsay, France), interfaced through Matlab R2012b (The Mathworks Inc., Natick, MA, United States). Monolix employs a stochastic approximation expectation maximization parameters without approximating the statistical model.[16] The consistency and minimum variance of the estimates have been optimized in the Monolix SAEM implementation.[17] Furthermore, Markov Chain Monte Carlo (MCMC) simulations were performed in Monolix using a simulated annealing procedure to accelerate the algorithm's convergence toward a solution. Descriptive statistics were used to characterize the study population with Stata 11.2 (StataCorp, College Station, TX, United States) and R version 2.15.1 (Cran.R-project.org).

One- and two-compartment structural models were fitted to the naïve pooled data with and without lags. All compartmental models were parameterized to give estimates of zolpidem clearance (CL/F) and the apparent volume of distribution (V_d/F). Models were evaluated and selected based on the goodness of fit. Unstable models were excluded from the model building process, as well as models that produced non-physiological results (e.g., negative clearance). Model stability was assessed by changing the initial estimates for CL/F and V_d/F.

Selection of structural models was facilitated using the Akaike information criterion (AIC) and the Bayesian information criterion (BIC).[18] During model development several diagnostic plots were used to visually assess the model's fit, including observed versus population predicted zolpidem concentrations and observed versus individual predicted zolpidem concentrations. Plots of the residuals and conditional

weighted residuals (CWRES) versus time or population predicted zolpidem concentrations were also visually inspected. Models were also compared by examining the precision of parameter estimates, measures of variability, and the objective function value (OFV). Model fit was based on minimization of the OFV. A reduction of more than 3.84 (-2 log likelihood difference) was considered statistically significant with $P<0.05$ and one degree of freedom.

A mixed effects model was built to incorporate intra- and inter-individual variability and residual unexplained variability (RUV). Inter-individual variability was assumed to be log-normally distributed and was assessed using an exponential equation of the form:

$$CL_i/F = \theta_{pop} * \exp(\eta_i) \tag{5.1}$$

where CL_i/F is the clearance value parameter for the ith individual, θ_{pop} is the population mean for zolpidem CL, and η represents the inter-individual random effect with a mean of zero and a variance of ω^2.

During model development, RUV was evaluated using a combined additive and constant coefficient of variation error model. This followed the form of:

$$\mathrm{Y} = \mathrm{IPRED} * \left(1 + \varepsilon_{\mathrm{prop}}\right) + \varepsilon_{\mathrm{add}} \tag{5.2}$$

where Y is the observed zolpidem concentration, IPRED is the individual predicted concentration, and $\varepsilon_{\mathrm{prop}}$ and $\varepsilon_{\mathrm{add}}$ are the RUV terms for the additive and proportion error models.

Base Model Development

The base model was developed using an empirical approach that focused on assessing several structural models. An evaluation of multiple absorption models was performed to identify the model that best described zolpidem absorption in the dataset. Lag time and absorption rate constants (K_a) were also assessed to determine if they improved estimations of the zolpidem absorption process.

Covariate Analysis

Several demographic and clinical characteristics were investigated for their influence upon zolpidem pharmacokinetics. Patient age, gender, race, ethnicity, type and extent of burn injury, elapsed time since the burn injury, current body weight, height, and body mass index (BMI), C-reactive protein (CRP), and serum creatinine were evaluated. An exploratory analysis was performed to identify relationships between zolpidem pharmacokinetic parameters and the above characteristics. The empirical Bayesian estimates from the individual parameters obtained from the base model were plotted against the covariate values and were compared by visual inspection.

To correct for differences in body size and metabolic rate, allometric scaling was applied to zolpidem CL/F and V_d/F, which were standardized to a body weight of 70 kg.[19] After the initial analysis, final covariates were selected for inclusion within the model following a stepwise inclusion approach. Covariates were added within the model until there was no further decrease in the OFV. A backward stepwise approach was used to remove covariates from the model.

Model Evaluation

Model performance was assessed numerically and graphically, in which observed drug concentrations were visually inspected for their correlation with predicted concentrations. As described by Goobie et al., conditional weighted residual plots were constructed and assessed using the empirical -2 to +2 region criterion, in the absence of any serial correlation or heteroscedasticity.[20] Standard errors were assessed for both the estimated population parameters and random effects error models. Goodness-of-fit plots and visual predictive checks were used to evaluate model fit. The final covariate model was also assessed by generating numeric and visual predictive checks. Further, the final model was also assessed with plots of the normalized prediction distribution error (NPDE) as a function of time and population predicted zolpidem concentrations.[21]

Results

Subjects and Pharmacokinetics

Data were collected from 11 children with acute burn injuries, 8 of whom were male and 3 were female. The mean age of the subjects included in this study was 8.3 $\pm$ 4.0 years. Additional demographic characteristics of the study cohort are featured in *Table 5.1*. The extent of the mean TBSA burn was 56% $\pm$ 22%. All 11 children had thermal burn injuries and 2 (18%) patients also suffered an inhalation injury.

Two pilot subjects received a single dose of zolpidem just prior to sleep onset (10:00 p.m.). Nine subjects also received a second dose of zolpidem 4 hours after the first dose (2:00 a.m.). There were 73 zolpidem serum concentrations measured with a median of 7 (range 5-7) per patient.

Initial exploratory analysis of the data revealed characteristic concentration-time profiles for all subjects. *Figure 5.1A* displays the raw zolpidem concentration data at each sample time for the two pilot patients who received a single dose of zolpidem. *Figure 5.1B* presents the raw zolpidem concentration data for the subsequent nine patients who received two doses. For these patients, the mean peak serum concentration was 291 $\pm$ 140 ng/mL.

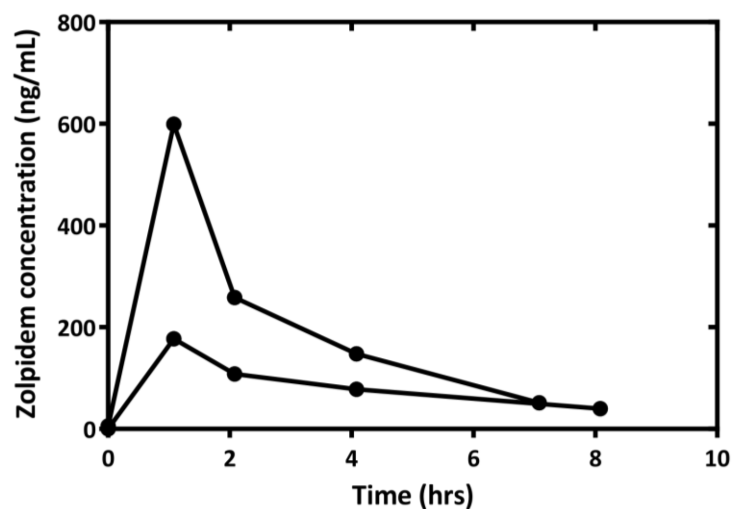
Population Pharmacokinetic Models

The population pharmacokinetic analysis included all 73 measured zolpidem concentrations from the 11 study participants. A two-compartment model with first order absorption was identified as the base model that best described the data, as assessed by the OFV, AIC, and BIC. This model was utilized for subsequent covariate model development.

Covariate Models

Inclusion of body weight in the initial covariate analysis revealed that allometrically-scaled body weight exerted a substantial influence upon zolpidem CL/F. Additionally, the volume of distribution in the central compartment (V_c/F) was inversely associated with the proportion of the body surface with third degree burns. None of the other covariates (e.g., age, gender, race, ethnicity, type of burn injury, total body burn surface area, elapsed time since the burn injury, CRP, and serum creatinine) influenced zolpidem pharmacokinetic parameter estimates.

The final covariate model was selected as it produced the most significant reduction of the OFV (Δ 13.5), reduced the inter-individual variability, and decreased the RUV. Additionally, visual inspection of the diagnostic plots was used to confirm the selection of



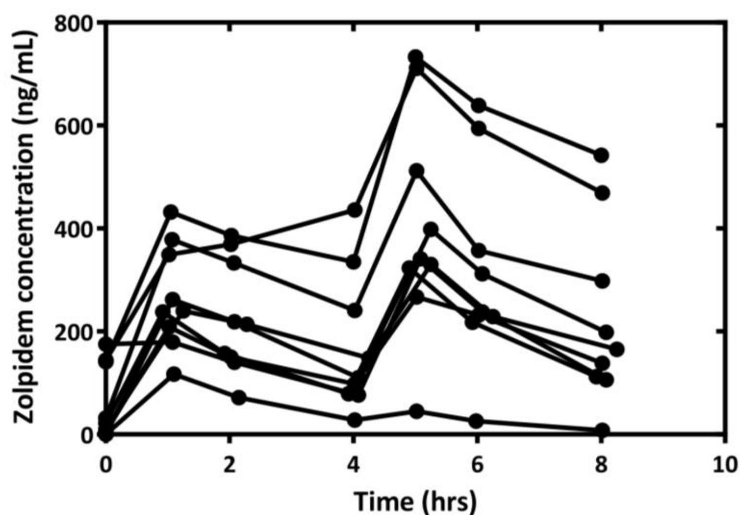


Figure 5.1. Zolpidem concentration versus time curves. (A) Two pilot subjects received a single dose of zolpidem and (B) nine subjects received an additional dose of zolpidem four hours after receiving their first dose.

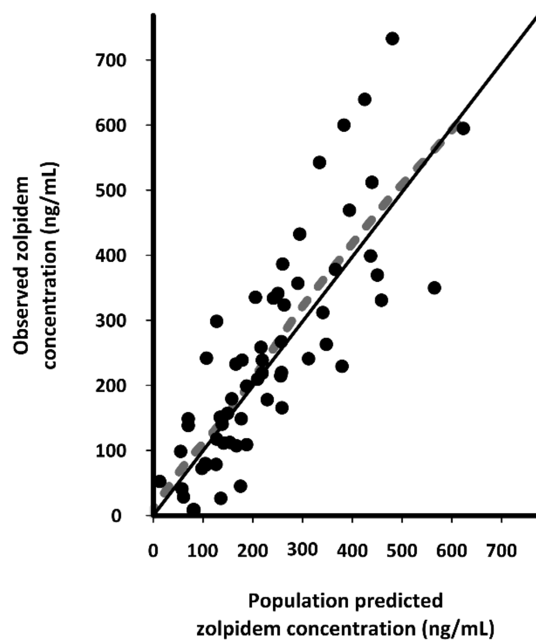
the final model (*Figure 5.2*). The parameter estimates derived from the final covariate model are featured in *Table 5.2*.

Model Evaluation

Diagnostic plots of the population and individually predicted zolpidem concentrations were compared against the observed concentrations for all patients (*Figure 5.2*). Conditional weighted residuals and NPDE metrics revealed a random distribution around 0, with nearly all values within the -2 to +2 range (*Figure 5.3*). Simulations from the observed zolpidem concentration data are presented in *Figure 5.4* using a visual predictive check, with the median value compared to the 10th and 90th percentiles. Approximately 94% of the simulated observations fell within the 90% prediction interval, demonstrating reasonable agreement between the observed and simulated zolpidem concentration data.

Discussion

This is the first study to evaluate the pharmacokinetics of zolpidem when used as a sleep-enhancing agent among children with severe burn injuries. Zolpidem pharmacokinetics are strongly influenced by both body weight and the extent of third degree burn injuries. Due to the relatively short half-life of zolpidem, a single dose failed to result in sleep that persisted throughout the night for two pilot subjects. The remaining nine participants received a second dose administered 4 hours later, which improved the duration of sleep throughout the latter half of the night.



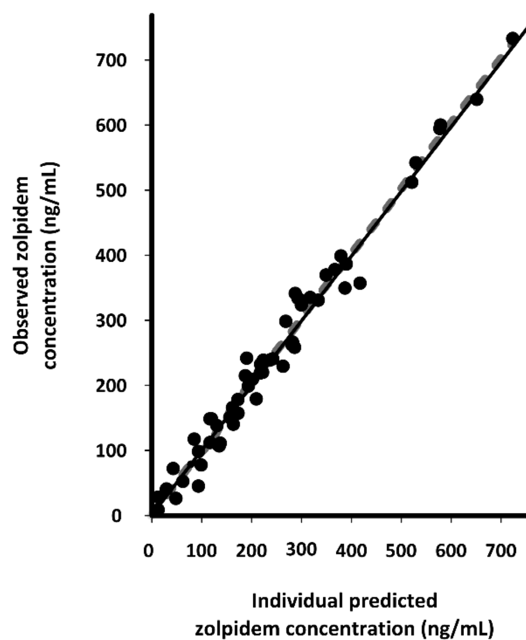
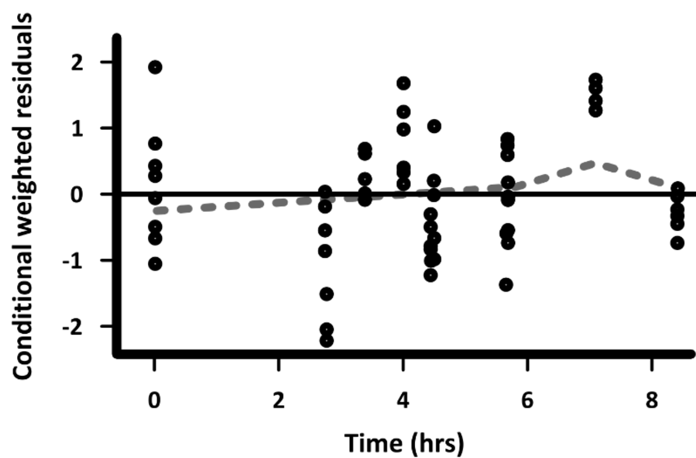


Figure 5.2. Zolpidem final covariate model observed versus (A) population-predicted concentrations and (B) individual-predicted concentrations. The line of identity is shown as a solid black line. The dashed gray line represents the spline of the data.

Table 5.2. Zolpidem population pharmacokinetic parameter estimates from the final two-compartment covariate model.

Parameters			Mean Parameter Estimate	Standard Error	% RSE
Θ – Pharmacokinetic Parameters					
K_a	-	Absorption rate (hr^{-1})	0.18 (fixed)	--	--
CL/F	-	Clearance (L/hr/kg)	0.03	0.015	55
V_c/F	-	Volume of distribution in the central compartment (L/kg)	0.05	0.012	25
Q/F	-	Intercompartmental clearance (L/hr/kg)	0.04	0.020	47
V_p/F	-	Volume of distribution in the peripheral compartment (L/kg)	0.69	2.9	421
ω – Between-Subject Variability					
CL/F	-	Clearance	0.25	0.26	103
V_c/F	-	Volume of distribution in the central compartment	0.37	0.09	26
Q/F	-	Intercompartmental clearance	0.96	0.28	30
V_p/F	-	Volume of distribution in the peripheral compartment	4.3	3.2	75
ε – Residual Unexplained Variability			28	3.1	11



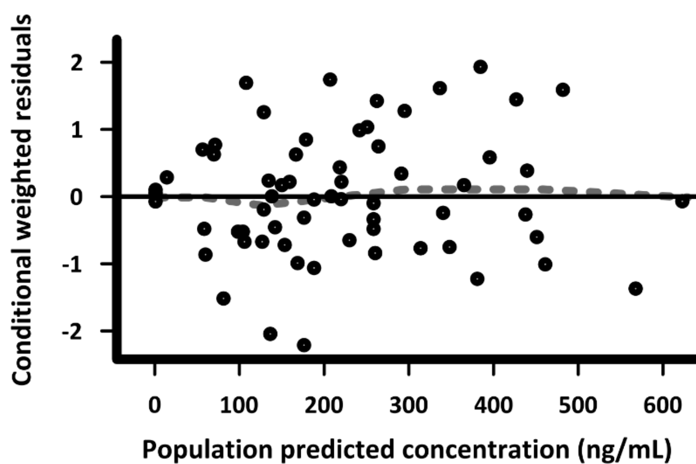
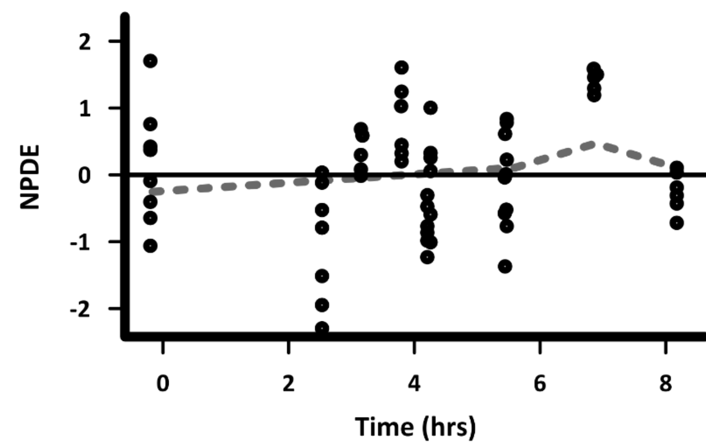


Figure 5.3. Zolpidem final covariate model (A) conditional weighted residuals versus time and population-predicted concentrations and (B) normalized prediction distribution errors as a function of time and population-predicted concentrations.

B) Normalised prediction distribution errors (NPDE)



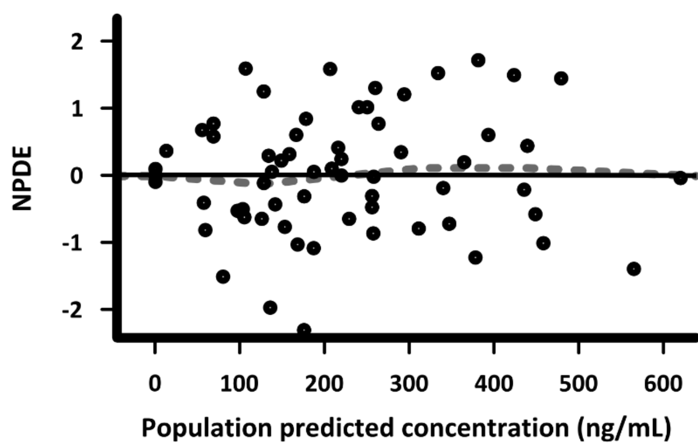


Figure 5.3. Continued.

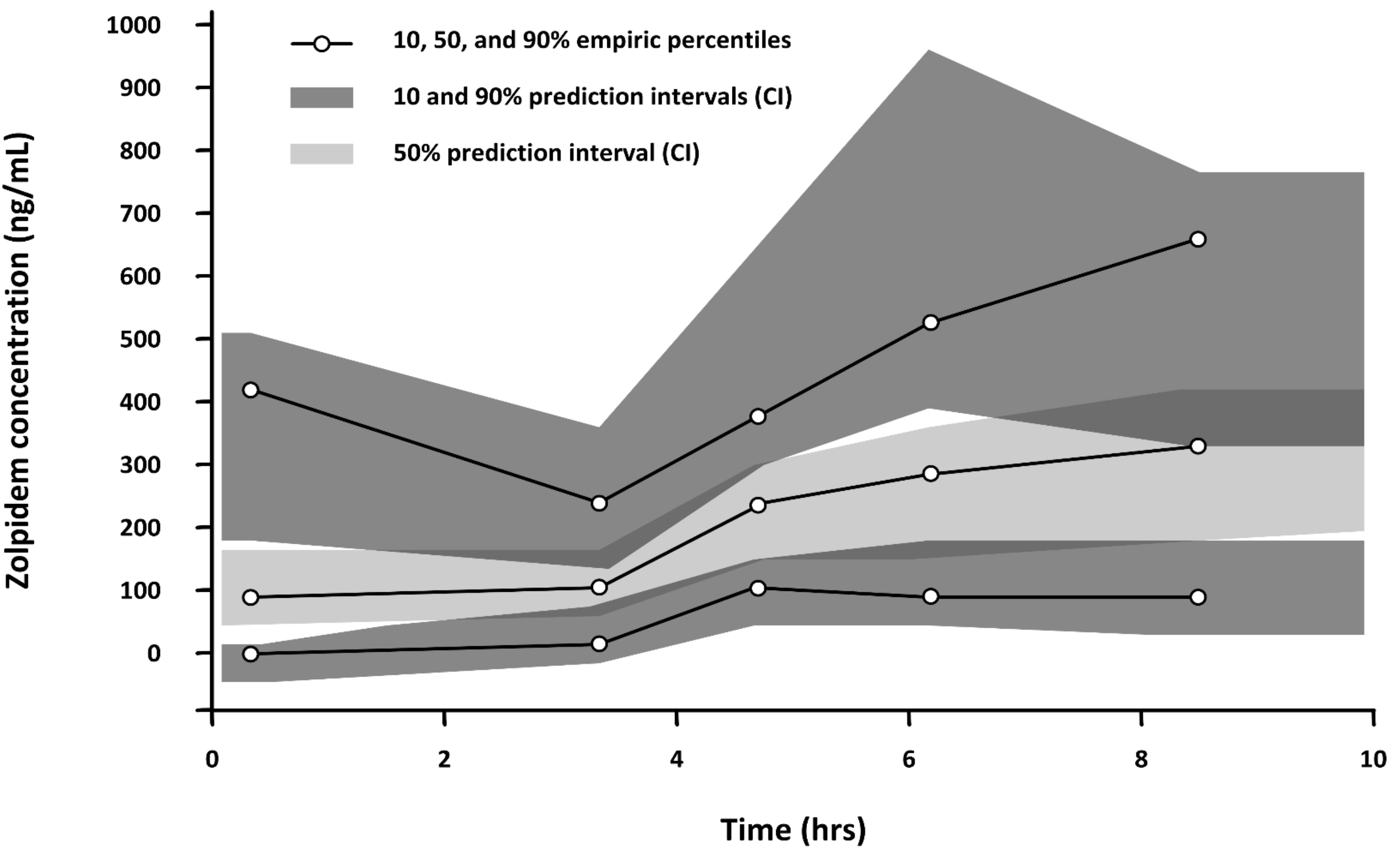


Figure 5.4. Visual predictive check for observed zolpidem concentrations using the final covariate pharmacokinetic model.

Few studies have established therapeutic dosage regimens for sleep-enhancing agents among children.[22] The only other pediatric zolpidem pharmacokinetic study was conducted by Blumer et al., which utilised a single dose escalation design to evaluate three zolpidem dosage regimens (0.125, 0.25, and 0.5 mg/kg) among otherwise healthy children with insomnia.[23] The authors found that zolpidem doses of $\geq$0.25 mg/kg (maximum of 10 mg/day) were safe, well tolerated, and potentially efficacious. However, the appropriateness of this dosing regimen for children with severe burn injuries is unknown. Burn injuries evolve over time and can profoundly impact the pharmacokinetics and pharmacodynamics of many drugs.[24] A few days after the burn injury, patients enter a hypermetabolic state with high blood flow to the liver and kidneys, increased α_1-acid-glycoprotein concentrations, and loss of the drug due to exudate leakage – all of which contribute to altered protein binding, drug distribution, and clearance.[25] Clinically, many hypermetabolic burn patients eliminate drugs rapidly, necessitating higher doses and/or shorter dosing intervals to maintain therapeutic serum concentrations. In a previous study, we evaluated the effects of a single zolpidem dose of 0.5 mg/kg upon sleep duration and architecture among burned children.[12] The authors found that this dose was ineffective in restoring normal sleep architecture and speculated that sleep fragmentation may have occurred later in the night as a consequence of sub-therapeutic serum concentrations. Further pharmacodynamic assessments are needed to assess whether a second dose administered 4 hours after the first improves sleep quality in this critically-ill pediatric population.

In the previously mentioned study conducted by Blumer et al., the pharmacokinetics of zolpidem were evaluated among otherwise healthy children suffering

from insomnia.[23] Age was found to significantly influence the zolpidem area under the concentration time curve, half-life, and mean residence time. In this study, body weight was significantly associated with zolpidem clearance, which likely reflects the cachexia associated with severe burn injuries. Additionally, the extent of third degree burn injuries was inversely associated with the volume of distribution in the central compartment. As a consequence, the children enrolled in this study achieved higher serum zolpidem concentrations than have been previously reported among healthy children.[23] This may be at least partially attributable to pathophysiologic changes in the volume of distribution among burn patients, which strongly affect hydrophilic drugs such as zolpidem tartrate.[26] Despite the relatively high zolpidem concentrations measured in this study, it was noted by clinical investigators that with two doses each night sleep quantity and quality were still suboptimal. Future studies are warranted to define the relationship between the sleep-enhancing effects of zolpidem (pharmacodynamics) and measured serum concentrations. Defining this relationship will be critical to ensure that a therapeutic range is developed for children with severe burn injuries.

Recently, the U.S. Food and Drug Administration (FDA) approved new labelling changes, which advocate a lowering of the recommended initial dose of immediate- and extended-release zolpidem formulations.[27, 28] These changes were made to decrease the risk of next-day driving impairment among adults; however, the appropriateness of this recommendation for pediatric patients is not known. In this study, two pilot subjects who received a single 5 mg dose of zolpidem failed to sustain sleep throughout the night. The next nine subjects received a second dose of zolpidem during the latter half of the night, which was found to improve sleep duration. According to recent FDA guidelines,[27] the

recommended initial dose of the immediate-release formulation of zolpidem is now 5 mg for women and 5 or 10 mg for men. The gender-specific difference in dosing recommendations is a function of adult pharmacokinetic studies conducted among healthy volunteers, which reported that women clear zolpidem at a slower rate than men.[29] Sex was not identified as a significant covariate in this pediatric burns study; however, the limited sample size may have obscured our ability to detect a subtle variation in zolpidem clearance between male and female participants.

Interpretation of these findings warrants the consideration of several limitations. First, the association between zolpidem pharmacokinetics and pharmacodynamic endpoints, including changes in sleep architecture, remains unknown for pediatric burn patients. Further research is needed to link zolpidem dosing with polysomnographic evidence of clinical efficacy. Second, children in this study were enrolled at a mean of 18 days post-burn. As such, the findings described herein may not generalize to children in the hypermetabolic stage immediately following their burn injury nor after significant wound healing and convalescence has occurred. Third, this study featured a limited number of serum samples from eleven children with severe burn injuries. This relatively small sample size limits our ability to precisely define the covariates that influence the disposition of zolpidem tartrate; however, this is the first study to date that has sought to systematically evaluate the pharmacokinetics of this drug in this unique patient population. Lastly, inadequate pain control in the burn population has been associated with poor sleep quality.[8] In this study, it was not possible to correlate pain control with sleep disturbances, although this may be an important component of future pharmacodynamic studies.

Conclusions

The current study characterized the pharmacokinetic parameters of zolpidem when used as a sleep-enhancing agent among children with severe burn injuries. Assessments from the clinical team treating the first two pilot subjects suggested that a single 5 mg dose of zolpidem was insufficient to achieve sustained sleep throughout the night. Nine subsequent patients received a second dose 4 hours after the first, which increased the duration of sleep through the latter half of the night. These findings suggest that future clinical trials should investigate higher doses and/or more frequent dosing for children with acute burn injuries. Recent FDA recommendations to decrease adult zolpidem doses are not likely to be appropriate for severely burned children. Further studies are needed to define the target zolpidem concentration required to improve sleep architecture among burned children. Additionally, pharmacodynamic studies and simulations may be helpful in developing appropriate dosing regimens for this vulnerable population.

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CHAPTER 6

SIMULATION-BASED PHARMACOMETRIC BENCHMARKING OF MONOLIX, NONMEM, AND PMETRICS

Introduction

Pharmacometric models can be used to quantify the variability in drug concentrations over time by fitting mathematical equations to clinical data. Population pharmacokinetic analyses involve the fitting of nonlinear mixed effects models to dosing and concentration data from multiple subjects at multiple points in time.[1-4] Mixed effects modeling involves the simultaneous quantification of the effects of random variability (between subject and residual variability) and fixed effects (such as weight, age, or renal function) on plasma drug concentrations.[5] These models are useful in then defining influential sources and correlates of variability in drug concentrations among patients, which can be used to derive personalized dosing regimens.[6]

Multiple software platforms have emerged over the last 30 years for conducting population pharmacokinetic analyses, many of which feature different parameter estimation methods that have the potential to lead to different results when the same structural model is fit to the same dataset.[2, 3] For this reason, benchmarks are needed to

experimentally assess the strengths and weaknesses of current pharmacometric modeling programs and their estimation methods. The accuracy and precision of these pharmacokinetic parameter estimates may impact clinical decisions and lead to alterations in medical management, such that the selection of a pharmacokinetic parameter estimation method with lower bias and higher precision is desirable. Additionally, it is unknown whether the selection of the optimal modeling program and parameter estimation method differs based on the design of the clinical trial. The objective of this study was to assess the predictive performance of three of the most commonly used population pharmacokinetic modeling programs and their parameter estimation algorithms when applied to simulated data under a variety of clinical trial designs with varying amounts of error, varying sample sizes, and varying numbers of concentrations obtained from each subject.

Methods

Experimental Design

This study was designed to assess the predictive performance of three commonly used population pharmacokinetic modeling programs when their respective pharmacokinetic parameter estimation algorithms were applied to simulated datasets. Construction of the simulated one compartment datasets was performed using a structural model of the form:

$$\text{One-compartment model: } \frac{dA_1}{dt} = -k_{el} * A_1 \qquad (6.1)$$

Initially, a dataset was simulated with 160 neonatal subjects, each of whom received a single 140 mg/kg dose of the drug and had a total of 10 plasma concentrations measured at 5, 15, 30, 45, 60, 90, 120, 180, 360, and 540 minutes post-dose.

To assess the impact of varying the amount of error on the predictive performance of each software program, new datasets were constructed in which each concentration was randomly permuted by 20%, 40%, 60%, and 80%. Additionally, to assess the influence of the study design parameters on the predictive performance of the software programs, the number of subjects (*n* = 10, 20, 40, 80, and 160) and the number of concentrations obtained from each subject (*n* = 2, 4, 6, 8, and 10) was varied. These permutations of the original simulated dataset resulted in the generation of 100 datasets due to the creation of unique datasets with each combination of the error, sample size, and number of concentration terms (4 error terms * 5 sample size terms * 5 concentration number terms = 100 unique datasets). A survey of the literature was performed to confirm the sample size, number of concentrations per subject, and error terms included in the simulations covered a sufficient range of values that may be reasonably expected to be feasible when designing a prospective clinical trial.[6-8] These datasets are included in Appendix C.

The 100 simulated datasets were generated by MGS and CMTS and were labeled with coded identifiers, such that the investigator performing the analysis (CS) was blinded to the true values used to initialize the simulation and to the amount of error included in each dataset.

Software Programs Tested

Three population pharmacokinetic modeling programs were employed in this benchmarking study. Each of these software programs allows the user the opportunity to employ different estimation algorithms, which are discussed separately in the following paragraphs:

1) ***Monolix*** (Lixoft, Orsay, France): Monolix uses expectation maximization (EM)

methods to integrate the posterior density by performing Monte Carlo sampling over all possible individual parameters during the expectation step, followed by a single iteration maximization step that moves the fixed-effect parameter values closer toward the maximum likelihood.[9, 10] Monte Carlo-based methods have the advantage of not using a linearized approximation to the integral and are theoretically less biased.[9] EM algorithms are inherently stochastic and are therefore less likely to be forced into a local minimum but they may yield less precise results.[11]

2) ***NONMEM*** (nonlinear mixed effects modelling; ICON Development Solutions, Ellicott City, MD, USA): NONMEM uses the Broyden-Fletcher-Goldfarb-Shanno (BFGS) quasi-Newton algorithm to maximize the approximated likelihood.[12] With the first-order conditional estimation with interaction (FOCEI) method, the likelihood is linearized with respect to the random effects using a first-order Taylor series expansion.[2] The integration step is performed by assuming that the posterior density can be approximated by a multivariate normal density with respect to the individual parameters.[2]

3) ***Pmetrics*** (Laboratory of Applied Pharmacokinetics and Bioinformatics, University of Southern California, Los Angeles, CA, USA): Pmetrics uses a nonparametric adaptive grid (NPAG) algorithm to estimate the unknown probability distribution (F) of the pharmacokinetic model parameter values using a set of discrete distributions with the same number of support points as the number of subjects included in the study.[13] To define F, a large grid of potential support points (G^0) is laid out on the surface of F. To determine probabilities of the support points and

the corresponding likelihood, a primal-dual interior point method is used.[14, 15] The vast majority of the support points have exceedingly low probabilities (<10^{-12}), which are then deleted from the grid yielding a smaller grid (G^1). New support points are then added around each of the remaining support points, which leads to a new expanded grid (G^2) with an improved likelihood. This process is iteratively repeated thereby giving rise to G^k grids with larger likelihoods. When the difference between the previous grid and the current grid is extremely small, the model is said to have converged.

Model Implementation

The simulated datasets featured a strong influence of weight (kg) on the pharmacokinetic parameters of clearance and volume of distribution. As an additional sensitivity analysis, the parameter estimates obtained from the three population pharmacokinetic modeling programs were compared using incorrectly-specified one-compartment models that did not account for the influence of weight on clearance or the volume of distribution. Correctly-specified models that incorporate the influence of weight on clearance and the volume of distribution were also tested to assess the sensitivity of these software programs to model misspecification. The influence of weight on clearance and the volume of distribution was modeled with an estimated power function of the form:

$$\theta_i = \theta_{pop} * WT_i^{\theta_{cov}} * e^{\eta_i} \quad (6.2)$$

where θ_i is the individual model-predicted pharmacokinetic parameter (e.g., clearance or volume of distribution) for individual i with a weight of WT_i (kg), θ_{pop} is the population mean pharmacokinetic parameter θ, θ_{cov} is the effect of the weight covariate, and η_i is the

between-subject random effect on the pharmacokinetic parameter θ with a mean of 0 and a variance of Ω. The initial estimates for the fixed effects in each model script were uniformly set equal to 1.

A proportional error model was used to describe the residual variability, which took the form:

$$Y_{ij} = \hat{Y}_{ij} + (\hat{Y}_{ij} * \varepsilon_{ij}) \tag{6.3}$$

where Y_{ij} is the measured drug concentration for the ith individual at time j, $\hat{Y}_{ij}$ is the model-predicted drug concentration, and ε_{ij} is a normally-distributed random error term with a mean of 0 and a variance of Σ.

Statistical Analysis

The predictive performance of Monolix, NONMEM, and Pmetrics' respective pharmacokinetic parameter estimation algorithms was compared with the true clearance and volume of distribution values used to develop the simulated one-compartment pharmacokinetic datasets. As described by Sheiner and Beal,[16] the performance of each algorithm was assessed by computing its relative bias and precision according to the following equations:

Percent prediction error (bias): $$100 * \left(\frac{\theta_{est} - \theta_{true}}{\theta_{true}}\right) \tag{6.4}$$

Absolute percent prediction error (precision): $$100 * \left(\frac{|\theta_{est} - \theta_{true}|}{\theta_{true}}\right) \tag{6.5}$$

The bias and precision were calculated for both clearance and the volume of distribution estimates derived from each of the population pharmacokinetic modeling programs. Analyses were performed globally with the results of all of the datasets pooled together, which were then followed by sub-analyses that were stratified by the error, sample

size, and number of concentrations per subject terms. Descriptive statistics were calculated and the bias and precision were compared for each of the population pharmacokinetic modeling programs using the nonparametric Wilcoxon-Mann-Whitney test. All statistical analyses were performed in R 3.1.3 (R Foundation for Statistical Computing, Vienna, Austria) with a two-tailed alpha of 0.05.

Results

Across the 100 datasets, there were a total of 37,200 simulated concentrations. The simulated concentration-time profiles for these simulated neonatal subjects are featured in *Figure 6.1*. The median postmenstrual age of the simulated study population was 38.0 (interquartile range [IQR]: 37.1-39.0) weeks and the median current body weight was 2.17 (IQR: 1.23-3.42) kg.

Correctly-Specified Models with Covariates

The median parameter estimates and the IQR of the parameter estimates for all estimated parameters for the correctly-specified models that incorporated the influence of current body weight on clearance and the volume of distribution are featured in *Figures 6.2A* and *6.2B*, respectively, for each of the population pharmacokinetic modeling programs. In this figure, the values of the parameters were normalized by dividing the model-estimated parameter value by the known, true value. The solid gray horizontal line corresponds to the true value of each pharmacokinetic parameter. The x-axis features each of the three population pharmacokinetic modeling programs that were tested. The heavy solid black horizontal line within each box plot denotes the median normalized value of

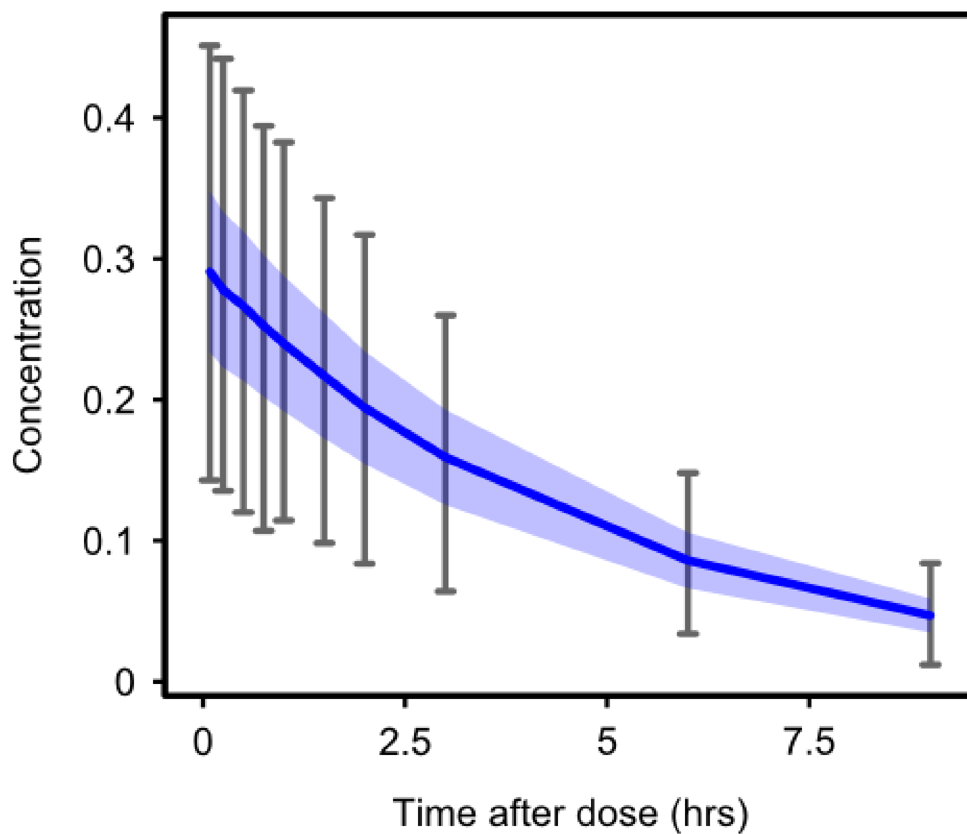

Figure 6.1. Simulated drug concentration vs. time profiles. A total of 37,200 concentrations were simulated across 100 datasets with varying sample sizes, varying numbers of concentrations obtained from each subject, and varying error terms. The solid blue line depicts the loess spline of the data and the blue shaded region depicts the 95% confidence interval for the loess spline. The vertical gray error bars represent 95% confidence intervals for the simulated drug concentrations at each time point.

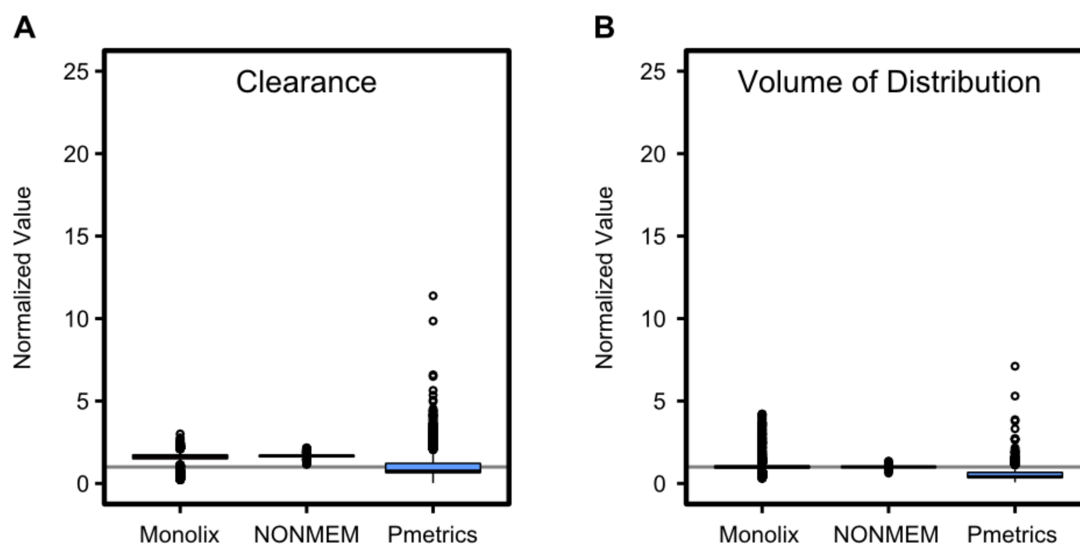

Figure 6.2. Clearance (A) and volume of distribution (B) parameter estimates using correctly-specified covariate models. For each parameter, the estimated value was normalized by the true value. The gray horizontal line depicts the true normalized values (equal to 1). The box plots depict the 25th, 50th, and 75th percentiles. The vertical lines extending from each boxplot extend to 1.5 times the interquartile range, with values beyond this point denoted by unfilled circles.

the pharmacokinetic parameters and the lighter weight solid black lines forming the box denote the 25th and 75th percentiles. The whiskers extending from each box plot extend to 1.5 times the IQR. Observations beyond the whiskers are presented as unfilled circles. Generally, clearance estimates were biased slightly higher than their true values with both Monolix and NONMEM, whereas the volume of distribution estimates were very close to their true values with both of these programs. In contrast, clearance estimates were generally unbiased with Pmetrics, whereas volume of distribution estimates were biased slightly lower than their true values. Notably, the range of normalized values was considerably larger for Pmetrics than Monolix, which was in turn slightly larger than that observed with NONMEM.

The bias of the population pharmacokinetic modeling programs was tested with a 20% error applied to the pharmacokinetic parameters with varying sample sizes and numbers of concentrations from each subject. For all three programs, bias decreased with larger numbers of concentrations from each subject ($P<0.001$) and larger sample sizes ($P=0.005$). However, the pharmacokinetic parameter estimates were biased for all three population pharmacokinetic modeling programs. As seen in *Table 6.1*, pharmacokinetic parameter estimates obtained from both Monolix and NONMEM were positively biased, whereas the estimates obtained from Pmetrics were negatively biased. Monolix was less biased than NONMEM, which was less biased than Pmetrics ($P<0.001$ for all comparisons).

The precision of the population pharmacokinetic parameters was tested with 20% error (*Figure 6.3A* and *Table 6.1*). In simulations with larger numbers of concentrations from each subject, the percent absolute prediction error decreased with Monolix,

Table 6.1. Bias and precision of the estimated population pharmacokinetic parameter values obtained using Monolix, NONMEM, and Pmetrics for the correctly-specified models with covariates.

	Monolix	NONMEM	Pmetrics
Simulated with 20% error			
Median percent prediction error (IQR)[a]	8.9% (-1.1% to 64.4%)	19.1% (0.0% to 67.5%)	-27.8% (-44.0% to 1.4%)
Median percent absolute prediction error (IQR)[b]	40.6% (3.8% to 65.5%)	21.5% (0.5% to 67.5%)	37.3% (22.7% to 60.7%)
Simulated with 40% error			
Median percent prediction error (IQR)[a]	13.2% (-1.7% to 62.8%)	22.7% (0.1% to 67.8%)	-33.1% (-59.7% to -8.0%)
Median percent absolute prediction error (IQR)[b]	35.8% (5.7% to 64.2%)	22.8% (0.9% to 67.8%)	40.5% (26.7% to 63.3%)
Simulated with 60% error			
Median percent prediction error (IQR)[a]	16.9% (-2.4% to 61.4%)	26.1% (0.5% to 66.9%)	-32.9% (-60.4% to -11.4%)
Median percent absolute prediction error (IQR)[b]	37.1% (7.1% to 63.5%)	26.1% (0.9% to 66.9%)	41.8% (26.7% to 62.8%)
Simulated with 80% error			
Median percent prediction error (IQR)[a]	18.2% (-3.6% to 60.8%)	20.9% (-0.5% to 66.2%)	-43.2% (-62.8% to -28.2%)
Median percent absolute prediction error (IQR)[b]	37.8% (8.2% to 62.5%)	26.6% (1.9% to 66.2%)	49.6% (30.9% to 63.6%)

[a] Percent prediction error is a measure of bias.
[b] Percent absolute prediction error is a measure of precision.

NONMEM, and Pmetrics (*P*<0.001). In contrast, increasing sample sizes had a negligible effect on percent absolute prediction errors (*P*=0.3). Overall, with 20% error and varying sample sizes and numbers of concentrations from each subject, percent absolute prediction errors were lowest for NONMEM, followed by Pmetrics, and then Monolix (*P*<0.001 for all comparisons).

When the modeling programs were tested with datasets that featured 80% error, bias increased and precision decreased (*Figure 6.3B* and *Table 6.1*). Pharmacokinetic parameter estimates were positively biased for both Monolix and NONMEM, whilst Pmetrics pharmacokinetic parameter estimates were negatively biased (*P*<0.001 for all comparisons). Increasing the number of concentrations from each subject decreased percent absolute prediction errors (*P*<0.001). Additionally, increasing the number of subjects slightly decreased percent absolute prediction errors (*P*=0.05). With 80% error, percent absolute prediction errors were lowest for NONMEM, followed by Monolix, and then Pmetrics (*P*<0.001 for all comparisons).

Incorrectly-Specified Models without Covariates

In *Figures 6.4A* and *6.4B* the normalized values of the pharmacokinetic parameter estimates are presented for incorrectly-specified models that did not incorporate the influence of weight on clearance or the volume of distribution. Monolix, NONMEM, and Pmetrics clearance estimates were positively biased, whereas the volume of distribution estimates were unbiased. Notably however, the range of normalized values for Pmetrics was considerably larger than that observed for Monolix and NONMEM, which may be interpreted as evidence of model misspecification.

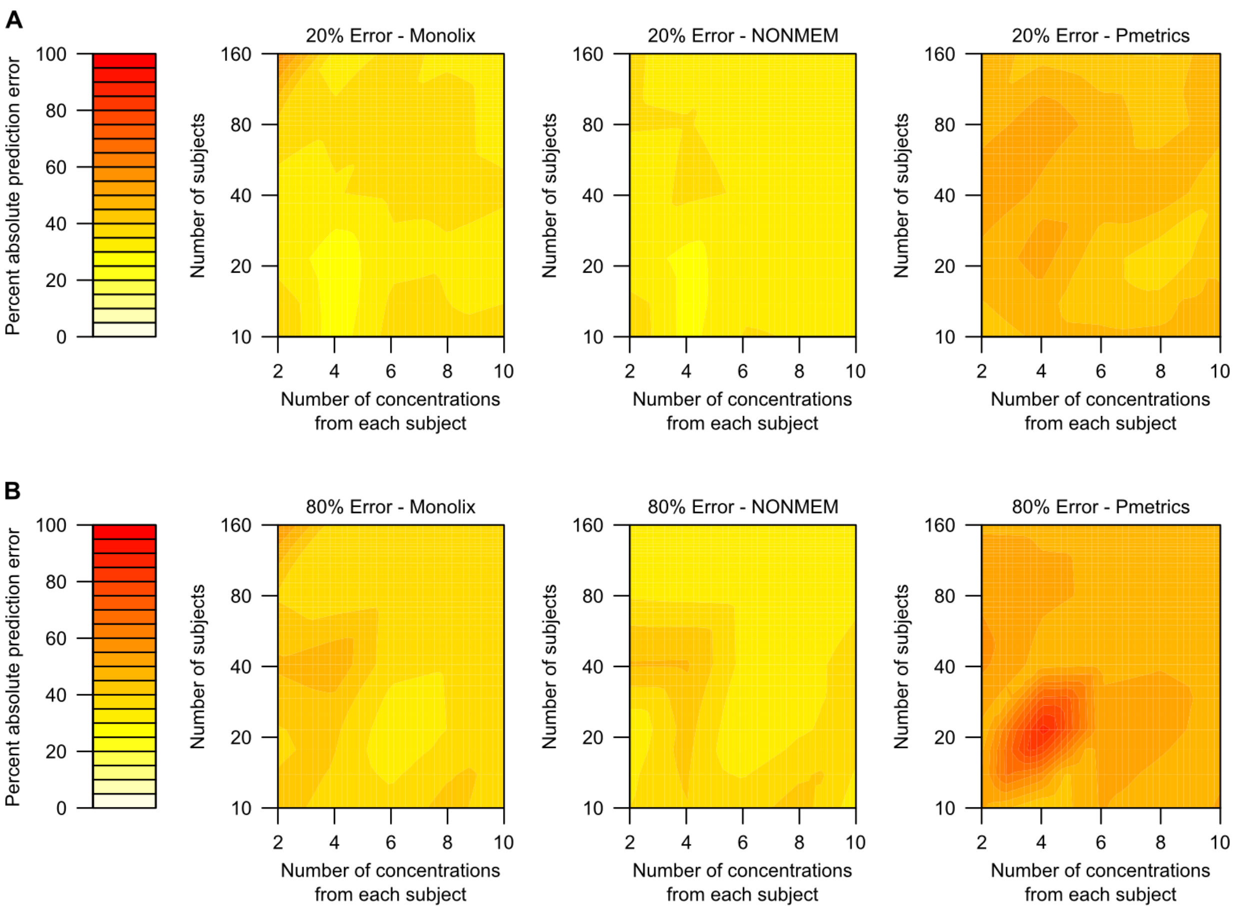

Figure 6.3. Precision of the population pharmacokinetic parameter estimates as a function of sample size and the number of concentrations obtained from each subject with 20% error (A) and 80% error (B), using correctly-specified covariate models.

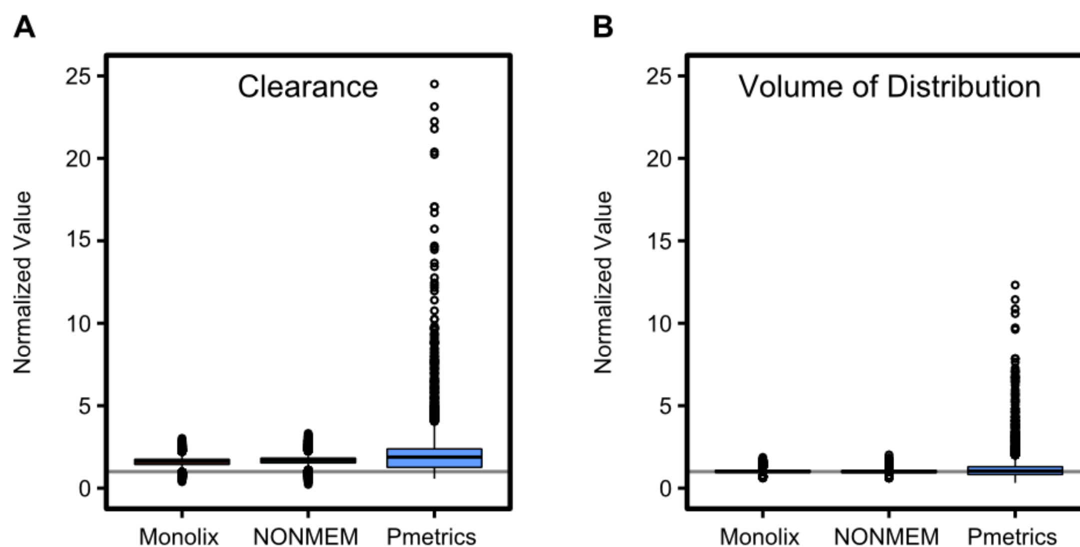

Figure 6.4. Clearance (A) and volume of distribution (B) parameter estimates using incorrectly-specified models without covariates. For each parameter, the estimated value was normalized by the true value. The gray horizontal line depicts the true normalized values (equal to 1). The box plots depict the 25th, 50th, and 75th percentiles. The vertical lines extending from each boxplot extend to 1.5 times the interquartile range, with values beyond this point denoted by unfilled circles.

When the incorrectly-specified models were applied to simulated datasets with 20% error, the results of all three modeling programs were significantly biased (*P*<0.001 for all). Neither increasing the number of concentrations per subject (*P*=0.2) nor increasing the sample size (*P*=0.3) reduced the bias. As seen in *Table 6.2*, the magnitude of the bias was comparable among all three modeling programs (*P*>0.1 for all comparisons). Similarly, increasing the number of concentrations per subject (*P*=0.7) and increasing the sample size (*P*=0.2) did not improve the precision of the parameter estimates. The precision of the pharmacokinetic parameter estimates was slightly higher for NONMEM than for Monolix (*P*=0.02) (*Figure 6.5A*); however, the precision of the pharmacokinetic parameter estimates obtained with Pmetrics was substantially higher than that observed with NONMEM and Monolix (*P*<0.001 for both).

Similar to the findings observed with 20% error, when the incorrectly-specified models were applied to datasets with 80% error, all three modeling programs yielded significantly biased pharmacokinetic parameter estimates (*P*<0.001 for all). Larger sample sizes (*P*<0.001) and increased numbers of samples obtained from each subject (*P*=0.01) were associated with increased bias. Pmetrics was substantially more biased than both NONMEM and Monolix (*P*<0.001 for both); however, NONMEM was slightly more biased than Monolix (*P*=0.002). The precision of the pharmacokinetic parameter estimates was not affected by changes in the number of samples obtained from each subject (*P*=0.7). In contrast, precision decreased with larger sample sizes (*P*<0.001). With the incorrectly-specified models, the median percent absolute prediction error was highest for Pmetrics, followed by NONMEM, and then Monolix (*Figure 6.5B*).

Table 6.2. Bias and precision of the estimated population pharmacokinetic parameter values obtained using Monolix, NONMEM, and Pmetrics for the incorrectly-specified models without covariates.

	Monolix	NONMEM	Pmetrics
Simulated with 20% error			
Median percent prediction error (IQR) [a]	16.4% (0.6% to 31.7%)	17.2% (0.1% to 67.3%)	29.9% (-5.8% to 90.7%)
Median percent absolute prediction error (IQR) [b]	16.5% (3.9% to 64.1%)	17.3% (3.8% to 67.3%)	32.7% (13.8% to 90.7%)
Simulated with 40% error			
Median percent prediction error (IQR) [a]	16.9% (0.0% to 62.1%)	19.7% (-0.4% to 67.4%)	24.8% (-6.3% to 90.4%)
Median percent absolute prediction error (IQR) [b]	19.6% (5.9% to 62.1%)	23.0% (5.8% to 67.4%)	34.5% (14.8% to 90.4%)
Simulated with 60% error			
Median percent prediction error (IQR) [a]	18.6% (-0.8% to 58.0%)	21.2% (-1.0% to 66.5%)	31.2% (-2.9% to 101.5%)
Median percent absolute prediction error (IQR) [b]	21.9% (7.5% to 58.0%)	25.1% (7.7% to 66.6%)	38.3% (16.3% to 101.5%)
Simulated with 80% error			
Median percent prediction error (IQR) [a]	18.3% (-1.9% to 55.4%)	20.0% (-2.3% to 66.2%)	38.3% (-0.2% to 114.9%)
Median percent absolute prediction error (IQR) [b]	22.4% (8.5% to 55.4%)	26.7% (8.9% to 66.2%)	42.6% (17.5% to 114.9%)

[a] Percent prediction error is a measure of bias.
[b] Percent absolute prediction error is a measure of precision.

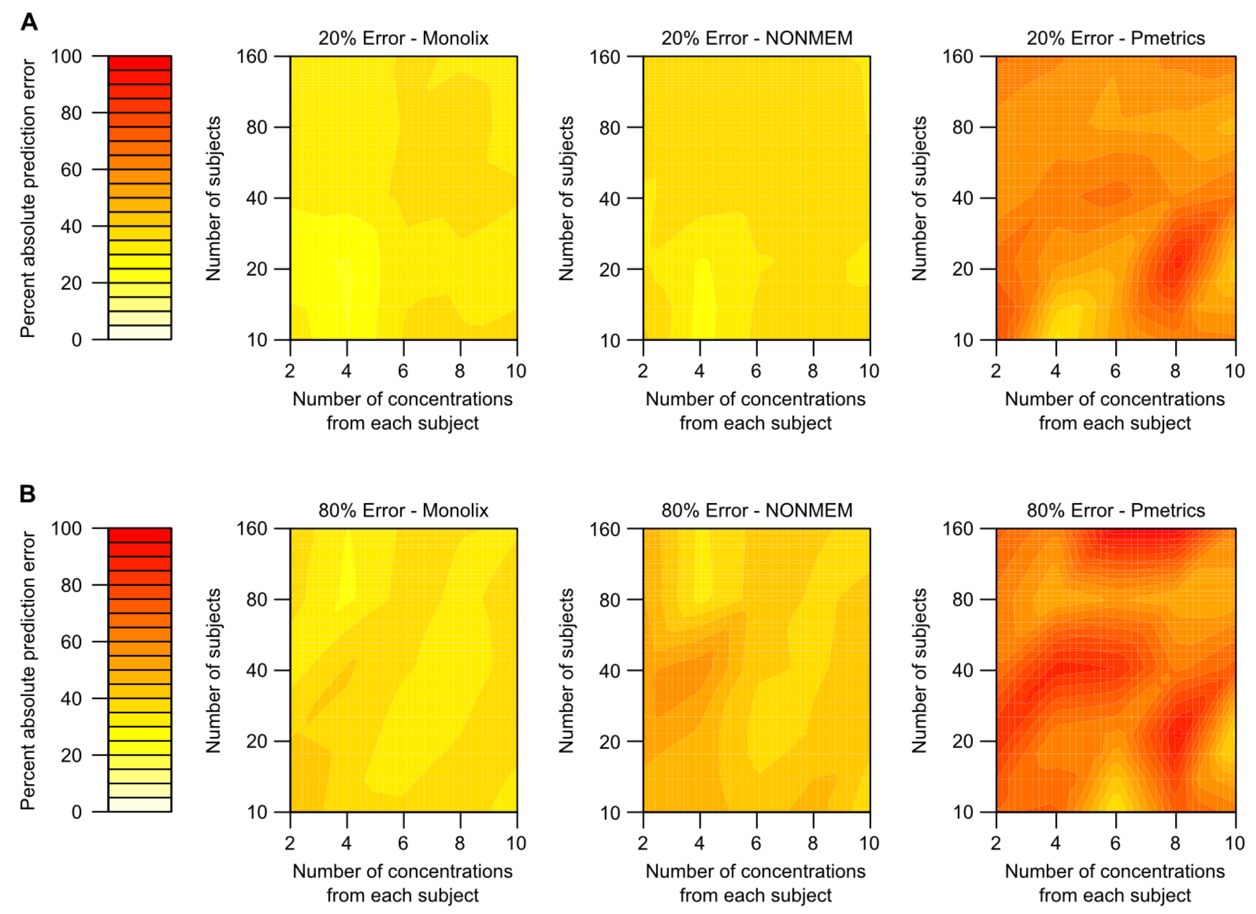

Figure 6.5. Precision of the population pharmacokinetic parameter estimates as a function of sample size and the number of concentrations obtained from each subject with 20% error (A) and 80% error (B), using incorrectly-specified models without covariates.

Discussion

Across a wide range of simulated error terms, sample sizes, and varying numbers of concentrations obtained from each subject, with correctly-specified covariate models, the three population pharmacokinetic modeling programs tested were modestly biased (median percent prediction error of 32%) and imprecise (median percent absolute prediction error of 37%). Several consistent themes emerged for all three population pharmacokinetic modeling programs, including a decrease in bias with larger numbers of concentrations obtained from each subject and a more modest decrease in bias with larger sample sizes for correctly-specified models. Additionally, increasing the number of concentrations obtained from each subject, but not larger sample sizes, improved the precision of the pharmacokinetic parameter estimates. Despite these consistent findings across all three population pharmacokinetic modeling programs, relative strengths and weaknesses were also identified with each of the modeling programs parameter estimation algorithms tested.

As expected, the stochastic approximation EM algorithm implemented in Monolix yielded less biased parameter estimates than those obtained with the FOCEI method in NONMEM and the NPAG method in Pmetrics, with the correctly-specified covariate model. Also as expected, Monolix pharmacokinetic parameter estimates were less precise than those obtained with the FOCEI method implemented in NONMEM. Monolix performed equally as well as NONMEM but considerably worse than Pmetrics at identifying model misspecification, as evidenced by relatively low percent prediction and percent absolute prediction errors with the incorrectly-specified model. Nevertheless, Monolix is freely available to students and academics and features a user-friendly graphical

interface.

The FOCEI algorithm implemented in NONMEM was more precise than the EM algorithm implemented in Monolix and the NPAG algorithm implemented in Pmetrics. Additionally, NONMEM's FOCEI algorithm was less biased than Pmetrics' NPAG algorithm when applied to correctly-specified models. Similar to Monolix, NONMEM struggled to identify misspecified models; however, unlike Monolix, NONMEM requires an annual license and does not come packaged with a graphical interface.

The NPAG algorithm implemented in Pmetrics yielded more biased and imprecise pharmacokinetic parameter estimates than both Monolix and NONMEM when applied to incorrectly-specified models that did not incorporate the influence of weight on clearance or the volume of distribution. However, when the NPAG algorithm was applied to correctly-specified models, the pharmacokinetic parameter estimates were more biased than those obtained with Monolix and NONMEM. With correctly-specified models, similar precision was achieved with Pmetrics and Monolix; however, Pmetrics' NPAG algorithm yielded less precise parameter estimates when compared with NONMEM's FOCEI algorithm. As with Monolix, Pmetrics is available at no cost to students and academics. Although Pmetrics is not bundled with a standalone graphical interface, it is interfaced through the open source statistical software program R.

This study is limited by its use of simulated drug concentration time profiles, which cannot possibly capture the breadth of biological processes involved in human pharmacokinetic studies. Nevertheless, this simulation-based design was adopted so that it would be possible to compare the pharmacokinetic modeling programs' results with known, true values, which are unknowable in clinical pharmacokinetic studies.

Additionally, the metrics used to benchmark these population pharmacokinetic modeling programs included the percent prediction error and the percent absolute prediction error; however, alternative metrics exist. Nevertheless, the percent prediction error and the percent absolute prediction error were used as they provide a cogent way to simultaneously compare the results of different pharmacokinetic parameter estimation algorithms employed in different modeling programs across a wide range of study designs. A further limitation of this study is that the length of time required to run the models was not captured; however, no modeling program took longer than 1 hr to run a single model.

In pharmacokinetic simulations with study designs mimicking those observed in previously published clinical trials, the bias and precision of three of the most commonly used population pharmacokinetic modeling programs was approximately 30-35%. This suggests that additional efforts are needed to develop less biased and more precise pharmacokinetic parameter estimation algorithms. However, these simulations revealed relative strengths and weaknesses of Monolix, NONMEM, and Pmetrics, which should be considered when attempting to identify the most appropriate estimation algorithm and modeling program for each project. Across all three population pharmacokinetic modeling programs, bias was found to decrease substantially with the collection of additional concentrations from each subject, and to a lesser degree with increasing sample sizes. In contrast, precision was found to improve only with a greater number of concentrations collected from each subject.

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CHAPTER 7

CONCLUSIONS

By and large, clinical pharmacology – and population pharmacokinetics in particular – remains a field that is ripe for investigation as many questions are still unanswered despite decades of experience using well-established methods and modeling programs. In this dissertation, we revisited the clinical utility of population pharmacokinetic modeling, along the way challenging conventional dosing strategies for vancomycin in preterm neonates and zolpidem among severely burned children, in an effort to shed light on the importance of conducting clinical pharmacokinetics studies.

Ideally, population pharmacokinetic studies should be designed to reflect clinical practice and involve participants who are similar to those for whom the study's results are intended to be applied to. Unfortunately, there is a dearth of well-designed pediatric population pharmacokinetic studies, which has hampered the development of safe and effective dosing regimens for many medications that are used in children's hospitals around the world.[1] Although multiple factors likely underlie the shortage of pediatric population pharmacokinetic studies, the perception that pediatric studies are costly, time consuming, and logistically challenging is likely one of the leading factors.[2] To dispel this fallacy, we leveraged vancomycin therapeutic drug monitoring data from two vulnerable pediatric

populations to provide evidence supporting the safety and effectiveness of current dosing regimens for children with cystic fibrosis and validated new dosing regimens that more reliably achieve therapeutic targets for preterm neonates with invasive bacterial infections.[3, 4] Additionally, we identified evidence of sub-therapeutic dosing among severely burned children treated with the sleep-enhancing agent zolpidem in a prospective clinical trial.[5]

It is of equal – if not more – importance to also assess the validity of population pharmacokinetic modeling programs against a true gold standard, thereby ensuring that the recommendations that are developed from population pharmacokinetic studies are unbiased and accurate. Toward this effort, we developed a simulation-based framework for benchmarking the bias and accuracy of population pharmacokinetic parameter estimates and confirmed the average bias and precision of three of the most commonly used population pharmacokinetic modeling programs to be approximately 30-35%. This finding suggests that although these models may perform adequately for drugs with wide therapeutic windows, additional research is needed to improve the predictive performance of the pharmacokinetic parameter estimation algorithms employed in these programs, particularly when they will be applied to characterize the pharmacokinetic properties of drugs with narrow therapeutic windows.

The main contribution of our simulation-based benchmarking paradigm was identification of the relative strengths and weaknesses of the pharmacokinetic parameter estimation algorithms implemented in the three modeling programs we studied. These differences in the performance characteristics of the estimation algorithms ought to be considered when designing population pharmacokinetic studies. For first-in-human studies

or trials involving patient populations in which the investigational drug has not been studied before, gaining insight into the factors that influence the drug's pharmacokinetics may be more important than predictive accuracy. In cases such as these, one might consider using the NPAG algorithm implemented in Pmetrics, as it performed best in identifying ill-specified covariate models. Conversely, for clinical use (e.g., therapeutic drug monitoring), the correct covariate-model structure has likely already been defined previously and the goal is instead to accurately predict a patient's pharmacokinetic parameters, for which Monolix's EM algorithm or NONMEM's FOCEI algorithm may be preferred.

This preliminary work sets the stage for many additional directions of future investigation, including those of a practical and a theoretical nature. We are currently recruiting participants as part of a clinical trial that will compare target rates of treatment failure and nephrotoxicity among neonates who are dosed according to the vancomycin model described in Chapter 4 as compared with historical controls at the University of Utah and Stanford University. Additionally, we submitted a grant requesting funding to perform a pharmacokinetic-guided dosing study of zolpidem among children with severe burn injuries who are cared for in the Shriners' network of children's hospitals. From a more theoretical standpoint, we believe that the simulation-based framework for benchmarking population pharmacokinetic parameter estimation algorithms described in this dissertation can be applied to many additional interesting questions, which include (but are not limited to):

1) Do the same patterns identified in Chapter 6 hold true with more complex pharmacokinetic models (e.g., transit absorption compartments, enterohepatic

recirculation, etc.)?

2) Which algorithms perform best for pharmacodynamic analyses? What are the influential factors in pharmacodynamic analyses that affect bias and precision and to what extent can these be controlled for with optimal clinical trial design?
3) Which algorithms perform best for mixture models? Under what circumstances is bias minimized and precision maximized?
4) How much of an effect do date and time errors in dosing and concentration records have on pharmacokinetic parameter estimates? Does the effect vary with different estimation algorithms?

Overall, this dissertation lays the foundation for a permanent re-assessment of the role of population pharmacokinetic analyses in drug development and clinical practice. The clinical utility demonstrated in the three real-world case studies presented in Chapters 3, 4, and 5 should foster the development of new pharmacokinetic parameter estimation algorithms that can further improve the predictive accuracy and interpretability of population pharmacokinetic analyses. Additionally, the novel simulation-based benchmarking framework that we developed here can easily be extended to provide a true gold standard to guide the development of new algorithms. With clinical impact driving the development of these new algorithms, it should be understood that these models cannot be thought of as 'black boxes', but rather they must be thought of as rational tools that can provide insights into the underlying biology and pharmacology hidden within the data, whilst simultaneously yielding accurate pharmacokinetic predictions that may be used to guide drug dosing.

As we look to the future and consider the situations in which population pharmacokinetic models may be most useful, it is worth noting that the factors influencing pharmacokinetic variability differ between drugs, disease states, and the presence / absence of other co-prescribed medications. Many drugs feature wide therapeutic windows for which a single well-designed population pharmacokinetic study may be sufficient to establish an effective and safe range of exposures. For drugs such as these, population pharmacokinetic modeling can facilitate the development of dosing regimens designed to reliably achieve the target exposure range for the vast majority of patients.

The situation is more complicated for medications that are used in the hospital setting as these often feature narrow therapeutic windows and may be administered to critically-ill patients. In situations such as these, a one size fits all approach to dosing is unlikely to be maximally efficacious and safe. Instead, collection of one or two samples to measure drug concentrations from an individual patient may be performed to develop a personalized pharmacokinetic model. Moreover, these additional concentrations can be leveraged to better inform the dosing recommendations for the population at large via Bayesian updating of the population pharmacokinetic model.

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APPENDIX A

VANCOMYCIN POPULATION PHARMACOKINETIC ANALYSIS CODE

```
$PROBLEM   Neonatal vancomycin population pharmacokinetic model

$INPUT     C ID TIME AMT DV MDV RATE WT SCR JSCR PMA PNA TYPE
           DOSE

  ;   ID          =          Subject identifier
  ;   TIME        =          Time (measured in hours)
  ;   AMT         =          Dose amount (mg)
  ;   DV          =          Drug concentration (mg/L)
  ;   MDV         =          Missing dependent variable
  ;   RATE        =          Infusion rate (mg/hr)
  ;   WT          =          Weight (kg)
  ;   SCR         =          Serum creatinine concentration (mg/dL)
  ;   JSCR        =          Linear conversion of enzymatic SCr to
                             the Jaffe method
  ;   PMA         =          Postmenstrual age (weeks)
  ;   PNA         =          Postnatal age (days)
  ;   TYPE        =          Drug concentration type (0 = trough,
                             1 = peak)
  ;   DOSE        =          Daily dose (mg/day)

$DATA NEONATAL_VANCO_DATA.CSV     IGNORE=C
$SUB       ADVAN1      TRANS2

$PK
  TH1        =          THETA(1)
  TVCL       =          TH1 * (WT/2.9)**0.75 *
                        (1/(1+(PMA/THETA(3))**(-(THETA(4))))) *
                        (1/JSCR)**(THETA(5))
  CL         =          TVCL * EXP(ETA(1))
  TH2        =          THETA(2)
  TVV        =          TH2 * (WT/2.9)**1.0
  V          =          TVV * EXP(ETA(2))
  TM50       =          THETA(3)
  HILL       =          THETA(4)
  CR         =          THETA(5)
  S1         =          V
  AUC        =          DOSE / CL

IF(AMT.GT.0) THEN
  TDOS       =          TIME
  TAD        =          0
ENDIF

IF(AMT.EQ.0) THEN
  TAD        =          TIME - TDOS
```

```
ENDIF

$THETA
  0.345     FIXED      ;     Population clearance
  1.75      FIXED      ;     Population volume of distribution
  34.8      FIXED      ;     TM50
  4.53      FIXED      ;     Hill coefficient
  0.267     FIXED      ;     Creatinine effect

$OMEGA BLOCK(2) FIXED
  0.0465
  0.00734  0.0119

$SIGMA
  0.0421    FIXED      ;     Proportional error
  1.168     FIXED      ;     Additive error

$ERROR
  A1        =     A(1)
  Y         =     F + F * ERR(1) + ERR(2)
  IPRED     =     F

$ESTIMATION METHOD = 1 INTERACTION MAXEVAL = 0 POSTHOC
$TABLE ID TIME DV MDV CL V TH1 TH2 TM50 HILL CR WT PMA JSCR AUC
TYPE IPRED NOPRINT ONEHEADER FILE = neonatal_vanco.fit
```

APPENDIX B

ZOLPIDEM POPULATION PHARMACOKINETIC ANALYSIS CODE

```
DESCRIPTION:
  ambien_final.mlxtran

DATA:
  path = "%MLXPROJECT%/",
  file = "zolpidem_data.csv",
  headers = {ID, TIME, Y, MDV, AMT, COV, COV},
  columnDelimiter = ","

VARIABLES:
  THIR [use = cov],
  WT,
  t_WT = log(WT) [use = cov,  centeredBy = mean]

INDIVIDUAL:
  Cl = {distribution = logNormal, covariate = t_WT, iiv = yes},
  Q = {distribution = logNormal, iiv = yes},
  V1 = {distribution = logNormal, covariate = THIR, iiv = yes},
  V2 = {distribution = logNormal, iiv = yes},
  ka = {distribution = logNormal, iiv = yes}

STRUCTURAL_MODEL:
  file = "oral1_2cpt_kaClV1QV2",
  path = "%MLXPATH%/libraries/PKLibrary",
  output = {Cc}

OBSERVATIONS:
  y1 = {type = continuous, prediction = Cc, error = combined1}

TASKS:
  ; settings
  globalSettings = {
    withVariance = no,
    settingsGraphics = "%MLXPROJECT%/ambien_final_graphics.xmlx",
    settingsAlgorithms =
    "%MLXPROJECT%/ambien_final_algorithms.xmlx",
    resultFolder = "%MLXPROJECT%/ambien_final"},
  ; workflow
  estimatePopulationParameters(
    initialValues = {
      pop_Cl = 1,
      beta_{Cl,t_WT} = 0.75 [method = FIXED],
      pop_Q = 1,
      pop_V1 = 1,
      beta_{V1,THIR} = 0,
      pop_V2 = 1,
```

```
        pop_ka = 0.18 [method = FIXED],
        a_y1 = 1,
        b_y1 = 0.3,
        omega_Cl = 1,
        omega_Q = 1,
        omega_V1 = 1,
        omega_V2 = 1,
        omega_ka = 1
    }),
estimateFisherInformationMatrix(method = {linearization}),
estimateIndividualParameters(method = {conditionalMode}),
estimateLogLikelihood(method = {linearization}),
displayGraphics(),
```

APPENDIX C

RELATIONSHIP BETWEEN ZOLPIDEM CONCENTRATIONS AND SLEEP PARAMETERS IN PEDIATRIC BURN PATIENTS

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Relationship Between Zolpidem Concentrations and Sleep Parameters in Pediatric Burn Patients

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Zolpidem is a short-acting non-benzodiazepine hypnotic that is used to improve sleep architecture in patients with burn injuries. This study evaluated the relationship between zolpidem administration and sleep parameters in a cohort of children with severe burn injuries. Standard age-based zolpidem dosing practices were employed. Polysomnography data were recorded at 30-second intervals throughout the night. Serum concentrations of zolpidem were measured at 0, 1, 2, 4, 5, 6, and 8 hours after administration of the first dose. The relationship between zolpidem concentrations and sleep parameters was evaluated using Markov mixed-effects pharmacodynamic models. Ten children received two doses of zolpidem at 22:00 and 02:00 hours. The median total amount of sleep was 361.0 (interquartile range [IQR]: 299.0–418.5) minutes; approximately 65% of the normal reference value for an 8-hour period. Slow-wave and rapid eye movement (REM) sleep were also dramatically reduced (18–37% of normal). With two doses of zolpidem, stage 2 sleep was 99% of normal levels. Higher peak zolpidem concentrations were associated with increased stage 2 sleep (r^2 = .54; P = .04). Despite this, a median of 120.0 (IQR: 99.5–143.5) transitions between nocturnal sleep stages were recorded, with a median of 55.5 (IQR: 36–75) night-time awakenings per patient. In pediatric burn patients, higher zolpidem serum concentrations were associated with restoration of stage 2 sleep to normal levels. Nonetheless, slow-wave and REM sleep were profoundly depressed with frequent transitions between sleep stages, suggesting that alternative hypnotic agents may be required to restore normal sleep architecture in severely burned children. (J Burn Care Res 2015;36:137–144)

Severe burn injuries are accompanied by a pronounced catabolic phase that is characterized by an increase in energy expenditure, protein catabolism, and cachexia.[1,2] Sleep disturbances have been reported to affect approximately 50% of patients hospitalized with burn injuries and alterations in normal sleep architecture have been associated with impaired ventilation, poor wound healing, immunosuppression, heightened perception of pain, and increased mortality.[3–12] These detrimental effects suggest that medications that enhance sleep may reduce morbidity and mortality associated with severe burn injuries.[13] However, the use of sedative and hypnotic agents in children remains understudied.[14]

Zolpidem tartrate is a short-acting, non-benzodiazepine sedative, and hypnotic agent, which has been used for more than two decades to improve sleep duration and quality among insomniacs.[15–18] In 2008, we randomized 40 children with severe burn injuries to receive zolpidem or haloperidol with continuous polysomnographic recording to evaluate the effects of these agents on sleep architecture.[19] Zolpidem was found to have a small but significant effect in improving the proportion of stage 3/4 and rapid

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eye movement (REM) sleep (0.8 vs 0.6 hours) but did not improve the total duration of sleep. More recently, we evaluated the pharmacokinetics of zolpidem in a cohort of severely burned children and reported sub-therapeutic effects following a single dose at the onset of night-time and recommended that a second dose be administered 4 hours after the first.[20] The objective of the current study was to characterize the effects of this second dose of zolpidem on multiple measures of sleep efficacy for the same cohort of severely burned children.

METHODS

Subjects and Study Design

Children 3 to 18 years of age with severe burn injuries were recruited to participate in this open-label pharmacokinetic and pharmacodynamic study if they were willing to receive zolpidem for use as a sleep-enhancing agent. To be eligible for inclusion in the study, the total burn surface area must have been ≥20% and they must have been admitted to Shriners Hospital for Children within 5 days of the burn injury. Exclusion criteria included: pre-existing neurological, sleep, or psychiatric disorders; brain injury; endocrine disease; a body mass index >97th percentile; questionable 72-hour survival; or planned administration of other sleep-inducing agents within the next 24 hours. Clinical and demographic data were collected for all study subjects.

The University of Cincinnati Institutional Review Board reviewed and approved this study. Parental permission and assent (when applicable) were obtained before conducting any study-related procedures.

Drug Administration

A standard age-based zolpidem dosing regimen was employed, in which children 2 to 4 years of age received 2.5 mg, children 5 to 10 years received 5 mg, and children >10 years received 10 mg of zolpidem. Zolpidem tablets were crushed, dissolved in 5 ml of water, and administered via a nasoduodenal feeding tube, followed by a 5 ml flush, in conjunction with continuous delivery of small amounts of liquid enteral nutrition substrate.

Sample Collection

Serial blood samples were obtained at 0, 1, 2, 4, 5, 6, and 8 hours after administration of the first dose of zolpidem. The total volume of blood collected during each sampling interval did not exceed 3.0 ml. Whole-blood samples were centrifuged at 1500*g* (~3000 rpm) for 10 minutes at 4°C. Centrifuged samples were then stored at −80°C.

Analytical Assay

Serum zolpidem concentrations were established using a validated high-performance liquid chromatography assay with a fluorescence detector.[21] The assay was linear over the range from 25 to 1000 ng/ml. Intra- and interday coefficients of variation were <8.2%.

Sleep Measurement

Each study participant went to sleep at their habitual bedtime and underwent polysomnography evaluation for 8 hours. The polysomnography recording began at the time that the subject received their first dose of zolpidem. Polysomnography recordings were conducted using standard methods, which included

Table 1. Demographic and clinical characteristics of 10 severely burned children who received zolpidem

Participant Characteristics	
Gender	
Male	7 (70%)
Female	3 (30%)
Age (yrs)	
Median	8.0
Interquartile range	7.0–12.0
Range	4.0–17.0
Weight (kg)	
Median	30.75
Interquartile range	23.0–45.0
Range	17.0–59.8
Height (cm)	
Median	131.5
Interquartile range	119.0–153.0
Range	102.0–170.0
Body mass index (kg/m^2)	
Median	17.0
Interquartile range	16.3–19.2
Range	14.9–23.1
TBSA* (%)	
Median	52.5
Interquartile range	35.3–82.5
Range	28.0–84.9
Third-degree burns (%)	
Median	37.6
Interquartile range	23.0–74.3
Range	19.0–83.2
Days after burn	
Median	15.5
Interquartile range	11.0–19.0
Range	8.0–44.0

*TBSA reflects the cumulative percentage of the burned area for each major section of the body.

evaluation of frontal, central, parietal and occipital electroencephalograms, submental electromyogram, electrooculogram, anterior tibialis electromyogram, and electrocardiogram.[22]

Polysomnography data were digitally recorded and scored for each 30-second interval (epoch). Sleep stages were determined according to previously published criteria, in which the subject was determined to be either awake, in stage 1, stage 2, stage 3, stage 4, or REM sleep for each epoch.[23] Stage 3 and stage 4 were collectively aggregated into a single stage called "slow-wave sleep."[24]

Thirteen sleep efficacy measurements proposed by Kjellsson et al[24] were evaluated to quantify sleep quantity and quality in this study. A list of these measurements is featured in Table 2.

Dataset Preparation

The dataset was prepared for analysis with sleep stage as the dependent variable. Coded patient identifiers and a date/time stamp were recorded for each 30 second interval throughout the night. Also included in the dataset were measurements of the sleep stage for the previous observation (30 seconds before) and for the subsequent observation (30 seconds later). Additionally, the dataset included measurements of the relative bedtime (defined as the time elapsed since going to bed divided by the total time spent asleep) and a flag to identify transitions between sleep stages. Lastly, we included individually-predicted zolpidem pharmacokinetic parameters for each participant using data from a previously published analysis.[25]

The objective of this study was to model the probability of transitioning from one sleep stage to another and to determine the effect of zolpidem serum concentrations on the likelihood of entering and exiting each sleep stage. There were five distinct sleep stages that a participant could potentially inhabit over the course of the night: wake, stage 1, stage 2, slow-wave, and REM. On the basis of prior literature describing the effects of zolpidem on different sleep stages and to reduce the computational time required to model each of the 21 possible transitions, we focused our analysis on the probability of transitioning from stage 1 sleep to: wakefulness, stage 2 sleep, slow-wave sleep, and REM sleep.[26]

Table 2. Measures of sleep efficacy among 10 severely burned children who received zolpidem

Sleep Efficacy Measurements	Definition	Observed Data Median (Interquartile Range)
Latency to persistent sleep (min)	Elapsed time since the start of the polysomnography recording to sleep lasting at least 5 minutes	19.7 (9.0–33.5)
Total sleep time (min)	Time from sleep onset to final awakening minus the time spent awake	361.0 (299.0–418.5)
Sleep efficiency (%)	The proportion of sleep, defined as total sleep time divided by the amount of time in which polysomnography data were recorded	62.3 (58.7–65.6)
Number of awakenings	Number of arousals to wakefulness during the night	55.5 (36.0–75.0)
Number of body movements	Number of interruptions in polysomnography recording because of body movements during the night	99.5 (60.0–139.0)
Stage transitions	Number of transitions from one stage of sleep to another during the night (excluding transitions from periods of movement)	212.0 (146.0–279.0)
Sleep onset latency (min)	Elapsed time from the start of the polysomnography recording until stage 1 sleep is recorded	6.0 (3.7–17.0)
REM sleep latency (min)	Elapsed time from the start of the polysomnography recording until REM sleep is recorded	121.0 (61.7–283.0)
Total time in stage 1 (min)	Total time spent in stage 1 during the night	40.5 (25–53)
Total time in stage 2 (min)	Total time spent in stage 2 during the night	216.3 (177.5–230.0)
Total time in slow-wave sleep (min)	Total time spent in stage 3 or stage 4 (slow-wave sleep) during the night	16.3 (8.0–93.0)
Total time in REM sleep (min)	Total time spent in REM sleep during the night	13.3 (0.5–44.5)
Total time in non-REM sleep (min)	Total time spent in stage 1, stage 2, stage 3, or stage 4 sleep during the night	321.0 (248.0–369.5)

REM, rapid eye movement.
Sleep efficacy measurements were adapted from Kjellsson et al.[24]

Pharmacodynamic Analysis

Traditional mathematical models assume independence between measurements; however, stochastic Markov models make it possible to model processes in which a future state depends entirely on the current state (eg, tomorrow's weather is likely to depend on today's weather). For this reason, Markov models are ideally suited to the analysis of longitudinal polysomnography data.

In this study, we developed Markov models that allow estimation of the probability of observing a transition between sleep stages. For example, it is possible to estimate the probability of measuring a specific sleep stage (eg, stage 2 sleep) as a function of the fact that the subject was in a different stage in the preceding 30 second period (eg, stage 1). This probability is represented as *P* (Stage 2|Stage 1).

Using techniques developed by Karlsson et al[27] and Kjellsson et al,[24] we developed Markov mixed-effects models to analyze the relationship between zolpidem concentrations and sleep quantity and quality. Sleep transitions were modelled separately as a function of zolpidem exposure, which was based on the individual pharmacokinetic parameters estimated previously.[25] To calculate the average probability that stage 2 sleep will occur directly after stage 1 sleep [*P* (Stage 2|Stage 1)], the complete dataset may be reduced to only those observations of stage 1 sleep that are immediately preceded by stage 2 sleep. This transition probability can then be estimated independently of all other sleep stage transitions, which do not have to be included in the *P* (Stage 2|Stage 1) dataset.

To simultaneously estimate the transition probability between any two sleep stages for all 10 participants, we developed a mixed-effects model that was implemented in NONMEM 7.2 (nonlinear mixed effects modelling; ICON Development Solutions, Ellicott City, MD).

RESULTS

Participant Characteristics

Data were collected from 10 children with acute burn injuries, seven of whom were male and three were female (Table 1). The median age of the subjects included in this study was 8.0 (interquartile range [IQR]: 7.0–12.0) years. Additional demographic characteristics of the study cohort are featured in Table 1. The extent of the median TBSA burn was 52.5% (IQR: 35.3–82.5%). All 10 children had thermal burn injuries and 2 (20%) patients also suffered an inhalation injury.

Sleep Patterns and Efficacy Parameters

The median amount of time from the onset of sleep to final awakening, minus the time spent awake, was 361.0 (IQR: 299.0–418.5) minutes. A minimum of 218.5 minutes of total sleep time was recorded in one patient and a maximum of 427.0 minutes was recorded in another patient. The distribution

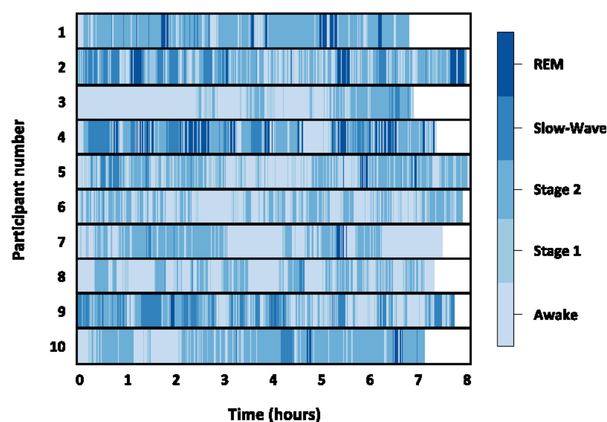

Figure 1. Distribution of sleep stages for 10 severely burned children who received zolpidem. Each participant had polysomnographic measurements obtained every 30 seconds for the duration of the night. The total duration of sleep was approximately 65% of normal age- and gender-matched reference values. This was primarily because of markedly lower amounts of slow-wave and rapid eye movement (REM) sleep than would be expected for healthy children. Two doses of zolpidem effectively restored the amount of stage 2 sleep to normal levels. The sleep patterns observed in these children exhibit signs of sleep fragmentation, including more than 50 nocturnal awakenings, which has been previously reported among children with severe burn injuries.[20]

of sleep stages is presented graphically for each of the study participants in Figure 1. Overall, 40.8% of the night was spent in stage 2, 8.2% in stage 1, 7.8% in slow-wave, and 4.9% in REM sleep. In this population, 29.1% of the night was spent awake and a further 9.2% of the time the patient was moving and a reliable polysomnographic measurement could not be established.

Table 3. Distribution of the number of sleep stage transitions among 10 severely burned children who received the sleep-enhancing agent zolpidem

		Sleep Stage in the Next 30 Seconds				
Hourly Interval	**Current Sleep Stage**	**Awake**	**Stage 1**	**Stage 2**	**Slow-Wave**	**REM**
0	Awake		32	3		
	Stage 1	6		27		
	Stage 2	14	3		33	2
	Slow-wave	2		29		
	REM			2		
1	Awake		32	2		
	Stage 1	5		25	1	1
	Stage 2	13	2		22	7
	Slow-wave	1		18		
	REM	3		4		
2	Awake		23	5		1
	Stage 1	5		27		
	Stage 2	9	7		32	9
	Slow-wave	2	1	28		
	REM	4	1	4		
3	Awake		44	5	1	
	Stage 1	16		28		
	Stage 2	19	3		16	6
	Slow-wave	4		11		
	REM	1		4		
4	Awake		46	6		
	Stage 1	13		26		
	Stage 2	12	6		10	9
	Slow-wave	1	1	9		3
	REM	3		3	3	
5	Awake		42	3		
	Stage 1	6		31		3
	Stage 2	16	6		9	7
	Slow-wave			5		
	REM	2	1	4		
6	Awake		39	3		
	Stage 1	7		34		3
	Stage 2	18	7		12	10
	Slow-wave	2		12		
	REM	3	1	4	1	
7	Awake		36	8		
	Stage 1	18		28		
	Stage 2	16	7		7	12
	Slow-wave			4		
	REM	2	1	4		
8	Awake		10	2		
	Stage 1	1		11		
	Stage 2	12	3		10	4
	Slow-wave	1		8		
	REM	2		3		

REM, rapid eye movement.

Additional sleep efficacy measures are featured in Table 2, including the relative sleep efficiency, the number of body movements recorded, the number of transitions between sleep stages, and the number of minutes spent in each sleep stage. Characteristic of this study population,[22] the median number of night-time awakenings was 55.5 (IQR: 36–75).

Sleep Stage Transitions

Transitions between sleep stages occurred often in this population, with a median of 212.0 (IQR: 60.0–139.0) transitions recorded per patient. The distribution of the number of transitions, divided into hourly intervals, is presented in Table 3. The most common transition was to stage 2 sleep (34.2%). Transitions into slow-wave and REM sleep were infrequently recorded (12.5 and 6.1%, respectively).

Consistent with earlier studies involving zolpidem use among insomniacs,[26] the probability of transitioning from stage 1 into stage 2 sleep increased following administration of the second dose of zolpidem. This is depicted graphically in Figure 2, in which the probability of transitioning from stage 1 sleep is modelled as a function of the night-time (scaled from 0 to 1 to represent the proportion of the night). Critically, administration of the second dose of zolpidem half-way through the night (denoted by the black arrows at 0.5) also decreased the probability of waking from stage 1 sleep. However, zolpidem did not increase the probability of transitioning to slow-wave or REM sleep.

Associations Between Zolpidem Concentrations and Sleep Parameters

Zolpidem serum concentrations were significantly higher 1 hour after the administration of the second dose as compared to 1 hour after the first dose (mean = 407.7 ± 72 vs 269 ± 34 ng/ml; $P < .01$). The area under the concentration time curve was also significantly higher in the second half of the night as compared to the first (mean = 1193 ± 248 vs 809 ± 138 ng × hour/ml; $P < .01$). Compared to previously published polysomnography data for children with severe burn injuries, the administration of zolpidem was not associated with statistically significant changes in the total amount of time spent asleep or the amount of time spent in REM sleep ($P > .05$ for both). However, the amount of time spent in stage 2 sleep was highly correlated with zolpidem exposure, with higher peak concentrations associated with increased stage 2 sleep ($r^2 = .54$; $P = .04$). Additionally, administration of a second dose of zolpidem was associated with an increase in the total amount of sleep when compared with burned children who received only a single dose (two dose mean = 344.8 ± 79 vs one dose mean = 288 ± 18 minutes; $P < .01$).

DISCUSSION

In this study, the total amount of sleep recorded among children with burn injuries who received two doses of zolpidem was approximately 65% of the normal reference value for age-matched healthy children.[28] This aligns well with our earlier study in which 40 children with severe burn injuries were randomized to receive a single dose of zolpidem and had a mean recorded duration of sleep that was 49% of normal.[19,28] The current study employed a two dose regimen, which was associated with a slightly longer total sleep time.

Slow-wave and REM sleep were similarly disrupted, with values ranging from 18 to 37% of

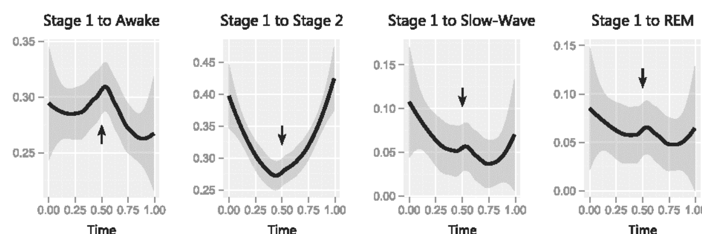

Figure 2. The probability of transiting between sleep stages changes following zolpidem administration. The *x*-axis represents the total duration of the night-time and is scaled from 0 (initial sleeplessness) to 1 (final awakening), as a proportion of the 8-hour study period. Solid black lines depict the predicted transition probabilities within the next 30 seconds for severely burned children who received zolpidem (shaded grey areas represent the 95% confidence intervals). Black arrows indicate the timing of the second administration of zolpidem. Administration of the second dose of zolpidem was associated with an increased likelihood of transitioning from stage 1 to stage 2 sleep. The second dose of zolpidem was also associated with a decrease in the likelihood of waking from stage 1 sleep. In contrast, the second dose of zolpidem was associated with a reduced likelihood of transitioning from stage 1 sleep to slow-wave and rapid eye movement (REM) sleep.

normal age and gender matched reference values.[28] In contrast, the amount of time spent in stage 2 sleep was restored to 99% of normal.[28] In studies conducted among adults with insomnia, zolpidem has been widely reported to improve the duration of stage 2 sleep.[26,29,30] Unlike benzodiazepine hypnotics, these adult studies demonstrated that zolpidem had no effect on slow-wave sleep or REM sleep. Normal pediatric reference ranges suggest that healthy children spend 50% of their total sleep time in slow-wave or REM sleep.[28] However, in this study, slow-wave and REM sleep accounted for 20% of the total sleep time, demonstrating the profound alterations to normal sleep architecture that are observed among children with severe burn injuries.

Further exacerbating normal sleep patterns, the children in this study experienced more than double the number of night-time awakenings reported among control subjects.[19] This suggests that two doses of zolpidem are insufficient to sustain sleep throughout the night for children with severe burn injuries and that alternative hypnotic agents may be required to achieve long-lasting improvements in sleep quality and duration. However, until more effective agents are discovered, a second dose of zolpidem halfway through the night appears to improve the duration of stage 2 sleep and achieves a slightly longer duration of sleep than has been reported following administration of a single dose.

In 2013, the United States Food and Drug Administration recommended that the initial dose of immediate- and extended-release formulations of zolpidem be lowered to reduce the risk of driving impairment the following morning.[31,32] Although the updated product labelling did not provide any recommendations on the dosage of zolpidem to be administered to children, our data suggest that lowering the dose of zolpidem is not likely to be appropriate for children with severe burn injuries.

Interpretation of these findings should be considered in light of several limitations. First, only 10 subjects were evaluated in this study. Nevertheless, this is the first study to conduct an integrated assessment of the pharmacokinetics and pharmacodynamics of zolpidem in pediatric burn patients. Although approximately 1000 polysomnographic measurements were obtained for each participant over the course of the night (typically an 8 to 10-hour period), these results cannot be interpreted to reflect the total amount of time slept over a 24-hour period. Second, because of computational constraints, our Markov model analyses were limited to evaluating transitions from stage 1 sleep. Additionally, the children enrolled in this study underwent polysomnographic evaluation at a median of 18 days after the burn injury. Therefore, the findings described here may not be generalizable to children during the shock phase (lasting 3–5 days postinjury), peak hypermetabolic stage (approximately 5–12 days postburn), nor after substantive wound healing and convalescence has occurred. It should also be noted that zolpidem was administered in conjunction with small amounts of liquid enteral nutrition, which has been reported to decrease zolpidem exposure.[33] Lastly, limited demographic and clinical data were collected after informed consent was obtained and we did not have access to information regarding other medical and environmental factors that may have influenced our patients' sleep patterns.

This study reinforces the fact that alterations in normal sleep patterns are common among children with severe burn injuries. Use of the sleep-enhancing agent zolpidem resulted in a modest improvement in sleep efficacy parameters, including a notable increase in stage 2 sleep. However, these children had profoundly fragmented sleep patterns, with more than 50 awakenings per night. Additionally, large aberrations in slow-wave and REM sleep that are characteristic of severely burned patients were not ameliorated despite the administration of two doses of zolpidem. This finding suggests that zolpidem improves cycling to non-slow-wave sleep and that alternative hypnotic agents are needed to restore deep sleep architecture in children with severe burn injuries.

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APPENDIX D

POPULATION PHARMACOKINETIC

BENCHMARKING DATASETS

A representative sample of the population pharmacokinetic datasets is included in the table below. To obtain access to all of the datasets used in Chapter 6 please contact Chris.Stockmann@hsc.utah.edu.

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
20	a2	1	3.749	39.714	524.8			0.314					0.157		
20	a2	2	3.339	39.429	467.5			0.254					0.159		
20	a2	3	3.184	39.143	445.7			0.249					0.151		
20	a2	4	1.915	37.714	268.1			0.232					0.167		
20	a2	5	1.488	37.286	208.3			0.288					0.174		
20	a2	6	3.84	39	537.6			0.304					0.174		
20	a2	7	0.39	36.429	54.6			0.238					0.1		
20	a2	8	3.683	38.857	515.6			0.305					0.161		
20	a2	9	4.417	39.571	618.3			0.302					0.163		
20	a2	10	2.171	38.143	303.9			0.265					0.181		
80	e1	1	1.956	38	273.9	0.269	0.152	0.164	0.252	0.155	0.235	0.18	0.129	0.117	0.037
80	e1	2	3.966	39.429	555.2	0.281	0.421	0.395	0.384	0.343	0.169	0.253	0.217	0.108	0.034

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT X MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	3	4.269	39.429	597.6	0.406	0.261	0.278	0.274	0.269	0.222	0.22	0.259	0.085	0.031
80	e1	4	2.407	38	336.9	0.204	0.213	0.168	0.231	0.235	0.185	0.211	0.102	0.108	0.062
80	e1	5	2.452	38.286	343.3	0.337	0.297	0.189	0.28	0.18	0.269	0.244	0.242	0.093	0.066
80	e1	6	4.001	39.571	560.2	0.358	0.214	0.163	0.247	0.277	0.281	0.17	0.208	0.065	0.062
80	e1	7	2.442	38.143	341.8	0.359	0.378	0.317	0.26	0.206	0.234	0.109	0.099	0.056	0.065
80	e1	8	3.82	39	534.8	0.296	0.345	0.242	0.186	0.358	0.166	0.241	0.128	0.118	0.068
80	e1	9	4.066	39.714	569.2	0.371	0.234	0.293	0.174	0.285	0.15	0.257	0.16	0.073	0.052
80	e1	10	1.22	37.143	170.9	0.241	0.144	0.166	0.283	0.229	0.27	0.227	0.162	0.078	0.031
80	e1	11	0.496	36.571	69.4	0.195	0.219	0.282	0.157	0.276	0.237	0.161	0.122	0.064	0.02
80	e1	12	4	39.143	560	0.391	0.429	0.234	0.232	0.348	0.252	0.222	0.212	0.148	0.052
80	e1	13	4.49	40	628.5	0.302	0.216	0.325	0.33	0.222	0.191	0.221	0.152	0.048	0.058
80	e1	14	2.165	37.714	303	0.357	0.21	0.255	0.294	0.132	0.151	0.109	0.096	0.076	0.058
80	e1	15	4.043	39.286	566	0.323	0.366	0.205	0.286	0.3	0.335	0.157	0.176	0.055	0.07
80	e1	16	1.106	37.143	154.8	0.19	0.327	0.284	0.192	0.293	0.146	0.177	0.129	0.049	0.044
80	e1	17	1.632	37.429	228.4	0.347	0.274	0.226	0.226	0.15	0.153	0.127	0.096	0.107	0.065

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	18	2.267	38	317.4	0.266	0.266	0.23	0.179	0.252	0.163	0.219	0.227	0.088	0.042
80	e1	19	0.36	36.429	50.5	0.193	0.204	0.194	0.229	0.128	0.115	0.182	0.155	0.037	0.022
80	e1	20	1.953	38	273.5	0.221	0.273	0.34	0.268	0.283	0.28	0.227	0.117	0.102	0.055
80	e1	21	3.378	38.714	473	0.228	0.37	0.181	0.268	0.152	0.168	0.117	0.21	0.127	0.068
80	e1	22	3.094	39	433.1	0.315	0.272	0.372	0.186	0.285	0.285	0.296	0.172	0.063	0.032
80	e1	23	4.235	39.857	592.9	0.217	0.253	0.239	0.209	0.225	0.178	0.168	0.127	0.078	0.033
80	e1	24	0.704	36.714	98.6	0.276	0.316	0.263	0.18	0.165	0.146	0.206	0.18	0.07	0.033
80	e1	25	5.044	40	706.2	0.288	0.172	0.27	0.231	0.376	0.306	0.317	0.222	0.081	0.05
80	e1	26	4.232	39.286	592.5	0.378	0.353	0.154	0.257	0.22	0.182	0.242	0.219	0.117	0.039
80	e1	27	0.756	36.714	105.9	0.151	0.251	0.2	0.306	0.205	0.147	0.212	0.124	0.043	0.036
80	e1	28	1.956	38	273.8	0.241	0.201	0.27	0.313	0.181	0.165	0.114	0.23	0.052	0.067
80	e1	29	4.393	40	615	0.339	0.366	0.304	0.297	0.171	0.221	0.279	0.158	0.057	0.056
80	e1	30	2.886	38.857	404.1	0.321	0.374	0.323	0.252	0.265	0.178	0.232	0.199	0.063	0.065
80	e1	31	0.859	36.857	120.2	0.352	0.275	0.204	0.311	0.199	0.192	0.139	0.181	0.085	0.053
80	e1	32	4.41	40	617.4	0.178	0.193	0.332	0.283	0.275	0.331	0.294	0.214	0.06	0.047

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	33	2.268	38.143	317.5	0.368	0.315	0.244	0.238	0.274	0.304	0.244	0.159	0.118	0.058
80	e1	34	2.25	37.857	315	0.362	0.302	0.199	0.147	0.174	0.134	0.145	0.169	0.113	0.025
80	e1	35	3.966	39.571	555.2	0.294	0.228	0.343	0.293	0.153	0.17	0.162	0.165	0.117	0.068
80	e1	36	2.671	38.286	374	0.185	0.325	0.237	0.285	0.183	0.19	0.137	0.136	0.084	0.071
80	e1	37	4.421	39.857	618.9	0.224	0.364	0.322	0.196	0.2	0.283	0.184	0.197	0.124	0.029
80	e1	38	3.418	39.143	478.5	0.442	0.172	0.173	0.218	0.148	0.235	0.177	0.188	0.069	0.046
80	e1	39	3.127	38.571	437.8	0.386	0.224	0.261	0.214	0.295	0.242	0.186	0.212	0.137	0.08
80	e1	40	2.126	38.143	297.6	0.175	0.157	0.266	0.199	0.221	0.307	0.253	0.165	0.057	0.061
80	e1	41	1.947	37.857	272.6	0.334	0.286	0.314	0.231	0.27	0.294	0.206	0.161	0.069	0.041
80	e1	42	2.275	38.143	318.5	0.409	0.249	0.287	0.198	0.146	0.247	0.212	0.152	0.099	0.047
80	e1	43	4.071	39.571	570	0.327	0.23	0.198	0.252	0.142	0.158	0.134	0.184	0.088	0.065
80	e1	44	0.521	36.571	72.9	0.332	0.138	0.234	0.301	0.265	0.177	0.167	0.11	0.084	0.027
80	e1	45	0.654	36.714	91.6	0.329	0.249	0.334	0.266	0.137	0.229	0.23	0.167	0.083	0.03
80	e1	46	0.568	36.571	79.5	0.292	0.169	0.163	0.273	0.19	0.225	0.158	0.163	0.095	0.042
80	e1	47	1.592	37.571	222.9	0.182	0.156	0.263	0.246	0.126	0.209	0.239	0.18	0.077	0.054

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	48	1.981	38	277.4	0.194	0.163	0.329	0.185	0.182	0.196	0.228	0.111	0.088	0.042
80	e1	49	4.013	40	561.9	0.253	0.344	0.229	0.324	0.186	0.265	0.274	0.181	0.124	0.06
80	e1	50	2.585	38.571	361.8	0.38	0.337	0.395	0.244	0.225	0.171	0.237	0.115	0.103	0.04
80	e1	51	4.327	39.571	605.8	0.224	0.236	0.254	0.311	0.164	0.174	0.242	0.24	0.077	0.031
80	e1	52	0.433	36.429	60.6	0.319	0.18	0.322	0.205	0.267	0.232	0.156	0.073	0.079	0.027
80	e1	53	4.331	39.857	606.4	0.325	0.356	0.417	0.197	0.308	0.308	0.314	0.116	0.134	0.053
80	e1	54	1.558	37.571	218.2	0.316	0.202	0.263	0.258	0.15	0.178	0.269	0.167	0.077	0.067
80	e1	55	3.119	38.857	436.6	0.291	0.26	0.361	0.383	0.312	0.323	0.14	0.158	0.063	0.05
80	e1	56	2.173	37.714	304.2	0.344	0.234	0.159	0.298	0.332	0.22	0.163	0.111	0.113	0.032
80	e1	57	1.94	37.571	271.6	0.231	0.175	0.159	0.319	0.257	0.223	0.169	0.107	0.06	0.035
80	e1	58	1.105	37	154.8	0.387	0.293	0.208	0.23	0.297	0.16	0.19	0.131	0.079	0.036
80	e1	59	3.48	38.714	487.3	0.304	0.295	0.267	0.27	0.334	0.316	0.269	0.25	0.062	0.065
80	e1	60	4.073	39.714	570.2	0.39	0.189	0.198	0.38	0.264	0.244	0.154	0.18	0.103	0.045
80	e1	61	2.811	38.429	393.5	0.33	0.21	0.365	0.227	0.359	0.223	0.288	0.234	0.075	0.036
80	e1	62	1.562	37.286	218.7	0.357	0.282	0.154	0.358	0.286	0.294	0.157	0.152	0.084	0.039

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	63	0.505	36.571	70.7	0.294	0.306	0.197	0.209	0.224	0.163	0.219	0.089	0.045	0.041
80	e1	64	2.883	38.286	403.7	0.281	0.365	0.22	0.253	0.145	0.249	0.274	0.157	0.092	0.059
80	e1	65	4.622	40	647.1	0.362	0.375	0.237	0.206	0.239	0.193	0.192	0.172	0.094	0.056
80	e1	66	0.874	36.857	122.4	0.368	0.275	0.166	0.191	0.15	0.141	0.159	0.127	0.08	0.04
80	e1	67	0.467	36.429	65.3	0.282	0.264	0.304	0.296	0.237	0.199	0.093	0.159	0.085	0.035
80	e1	68	2.076	38	290.7	0.344	0.226	0.195	0.205	0.221	0.287	0.133	0.183	0.119	0.051
80	e1	69	2.994	38.571	419.2	0.235	0.227	0.301	0.312	0.201	0.175	0.195	0.196	0.119	0.042
80	e1	70	2.476	38.143	346.7	0.159	0.331	0.182	0.23	0.221	0.274	0.285	0.236	0.118	0.05
80	e1	71	2.16	38.143	302.4	0.287	0.268	0.237	0.211	0.293	0.146	0.118	0.209	0.064	0.033
80	e1	72	0.816	36.714	114.2	0.175	0.138	0.34	0.14	0.286	0.197	0.219	0.169	0.091	0.027
80	e1	73	2.597	38.714	363.6	0.317	0.366	0.329	0.162	0.263	0.3	0.28	0.22	0.075	0.067
80	e1	74	1.157	37.143	162	0.337	0.209	0.345	0.323	0.176	0.278	0.148	0.092	0.071	0.05
80	e1	75	2.141	38.143	299.8	0.285	0.223	0.358	0.287	0.303	0.315	0.233	0.171	0.091	0.063
80	e1	76	2.99	38.429	418.6	0.418	0.315	0.334	0.183	0.353	0.263	0.212	0.139	0.096	0.033
80	e1	77	0.552	36.571	77.3	0.26	0.307	0.164	0.276	0.182	0.164	0.224	0.185	0.078	0.026

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	78	3.276	39	458.7	0.2	0.17	0.312	0.249	0.242	0.255	0.222	0.12	0.089	0.063
80	e1	79	1.599	37.571	223.8	0.246	0.21	0.306	0.189	0.225	0.205	0.262	0.138	0.113	0.043
80	e1	80	1.219	37	170.6	0.347	0.191	0.295	0.293	0.271	0.176	0.149	0.206	0.084	0.029
80	e1	81	0.593	36.571	83	0.346	0.335	0.248	0.277	0.146	0.187	0.182	0.116	0.082	0.043
80	e1	82	1.425	37.429	199.5	0.172	0.352	0.195	0.275	0.302	0.213	0.193	0.165	0.049	0.038
80	e1	83	1.196	37.143	167.4	0.16	0.15	0.335	0.232	0.229	0.154	0.115	0.136	0.084	0.024
80	e1	84	3.862	40	540.7	0.298	0.179	0.385	0.379	0.274	0.242	0.142	0.195	0.126	0.063
80	e1	85	3.679	39.143	515.1	0.328	0.166	0.387	0.341	0.318	0.297	0.292	0.113	0.133	0.046
80	e1	86	1.017	36.857	142.4	0.289	0.349	0.138	0.223	0.285	0.202	0.145	0.126	0.06	0.047
80	e1	87	1.92	37.714	268.8	0.197	0.385	0.334	0.241	0.187	0.246	0.271	0.196	0.126	0.021
80	e1	88	4.508	39.571	631.2	0.283	0.248	0.31	0.286	0.229	0.3	0.236	0.133	0.071	0.062
80	e1	89	1.94	37.571	271.6	0.162	0.354	0.31	0.318	0.29	0.231	0.281	0.132	0.067	0.055
80	e1	90	0.591	36.571	82.8	0.315	0.21	0.213	0.196	0.239	0.123	0.113	0.124	0.07	0.035
80	e1	91	3.904	39.286	546.6	0.191	0.248	0.27	0.262	0.288	0.322	0.167	0.134	0.137	0.058
80	e1	92	1.227	37	171.8	0.346	0.276	0.317	0.344	0.184	0.158	0.257	0.148	0.098	0.03

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	93	2.412	38.429	337.7	0.178	0.273	0.232	0.318	0.224	0.127	0.213	0.114	0.078	0.06
80	e1	94	3.508	39.286	491.2	0.38	0.253	0.288	0.3	0.174	0.157	0.224	0.19	0.133	0.032
80	e1	95	1.254	37.143	175.5	0.212	0.285	0.165	0.139	0.205	0.211	0.185	0.177	0.051	0.04
80	e1	96	2.395	38	335.3	0.23	0.228	0.331	0.218	0.325	0.322	0.288	0.117	0.066	0.054
80	e1	97	4.47	40	625.8	0.236	0.415	0.166	0.206	0.175	0.305	0.257	0.237	0.058	0.056
80	e1	98	2.855	38.286	399.6	0.402	0.205	0.198	0.317	0.341	0.254	0.12	0.113	0.102	0.059
80	e1	99	2.583	38.286	361.7	0.37	0.335	0.373	0.164	0.163	0.263	0.129	0.194	0.07	0.065
80	e1	100	3.064	38.857	428.9	0.175	0.228	0.197	0.317	0.267	0.285	0.174	0.202	0.103	0.056
80	e1	101	1.28	37.143	179.3	0.367	0.298	0.152	0.19	0.259	0.285	0.214	0.122	0.107	0.039
80	e1	102	3.884	39.429	543.8	0.185	0.339	0.231	0.203	0.199	0.249	0.188	0.109	0.116	0.06
80	e1	103	3.475	39.429	486.5	0.361	0.419	0.233	0.263	0.31	0.265	0.164	0.206	0.111	0.048
80	e1	104	1.239	37.143	173.5	0.193	0.237	0.183	0.317	0.238	0.203	0.257	0.119	0.112	0.043
80	e1	105	0.401	36.429	56.1	0.261	0.205	0.162	0.141	0.121	0.224	0.221	0.134	0.076	0.031
80	e1	106	1.561	37.571	218.6	0.376	0.313	0.19	0.33	0.284	0.242	0.227	0.214	0.059	0.057
80	e1	107	1.342	37.286	187.9	0.215	0.38	0.149	0.223	0.177	0.19	0.236	0.162	0.115	0.047

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	108	0.926	36.857	129.6	0.203	0.25	0.341	0.278	0.176	0.269	0.239	0.097	0.102	0.034
80	e1	109	3.786	39.429	530.1	0.369	0.339	0.299	0.174	0.218	0.132	0.253	0.209	0.083	0.026
80	e1	110	1.895	37.857	265.2	0.375	0.371	0.249	0.306	0.298	0.164	0.195	0.226	0.063	0.02
80	e1	111	0.913	37	127.8	0.172	0.273	0.349	0.175	0.232	0.184	0.106	0.16	0.038	0.046
80	e1	112	1.908	37.714	267.1	0.382	0.371	0.377	0.235	0.174	0.126	0.202	0.21	0.059	0.041
80	e1	113	1.897	38	265.6	0.314	0.385	0.199	0.243	0.318	0.199	0.219	0.147	0.118	0.031
80	e1	114	2.595	38.286	363.3	0.41	0.192	0.346	0.201	0.324	0.129	0.148	0.17	0.106	0.051
80	e1	115	0.376	36.429	52.7	0.21	0.328	0.146	0.107	0.148	0.208	0.13	0.154	0.077	0.019
80	e1	116	1.42	37.286	198.9	0.193	0.373	0.359	0.131	0.239	0.14	0.157	0.2	0.07	0.051
80	e1	117	3.318	38.571	464.6	0.378	0.263	0.304	0.357	0.26	0.227	0.234	0.191	0.103	0.063
80	e1	118	2.748	38.429	384.7	0.399	0.359	0.37	0.204	0.148	0.207	0.227	0.234	0.053	0.064
80	e1	119	4.112	39.286	575.7	0.287	0.344	0.28	0.351	0.203	0.19	0.201	0.199	0.115	0.061
80	e1	120	0.365	36.429	51.1	0.143	0.314	0.142	0.196	0.143	0.146	0.183	0.092	0.036	0.022
80	e1	121	1.15	37	161	0.367	0.239	0.295	0.25	0.184	0.245	0.206	0.182	0.088	0.05
80	e1	122	1.227	37	171.8	0.299	0.197	0.313	0.309	0.182	0.147	0.158	0.142	0.073	0.04

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	123	2.975	38.571	416.4	0.362	0.298	0.395	0.361	0.163	0.301	0.258	0.227	0.069	0.047
80	e1	124	0.51	36.571	71.4	0.178	0.221	0.295	0.13	0.125	0.119	0.09	0.118	0.09	0.045
80	e1	125	1.582	37.429	221.5	0.156	0.382	0.276	0.285	0.135	0.29	0.122	0.128	0.109	0.034
80	e1	126	4.428	40	619.9	0.349	0.17	0.22	0.355	0.212	0.193	0.123	0.17	0.1	0.04
80	e1	127	1.849	37.857	258.8	0.174	0.321	0.184	0.247	0.298	0.149	0.25	0.166	0.106	0.05
80	e1	128	2.092	38.143	292.9	0.242	0.225	0.199	0.159	0.324	0.307	0.17	0.092	0.076	0.053
80	e1	129	2.181	38.286	305.3	0.411	0.274	0.321	0.283	0.153	0.216	0.145	0.212	0.089	0.035
80	e1	130	3.987	39.571	558.2	0.25	0.435	0.258	0.194	0.201	0.19	0.172	0.167	0.082	0.045
80	e1	131	3.414	38.857	478	0.198	0.215	0.291	0.205	0.172	0.262	0.269	0.201	0.117	0.054
80	e1	132	1.544	37.571	216.2	0.282	0.166	0.243	0.205	0.189	0.184	0.13	0.195	0.062	0.041
80	e1	133	3.499	38.714	489.9	0.352	0.305	0.238	0.171	0.316	0.161	0.131	0.216	0.09	0.051
80	e1	134	2.597	38.714	363.6	0.399	0.218	0.31	0.285	0.288	0.188	0.16	0.165	0.072	0.049
80	e1	135	0.384	36.429	53.8	0.311	0.212	0.284	0.168	0.132	0.242	0.141	0.167	0.044	0.035
80	e1	136	3.788	39.286	530.3	0.451	0.204	0.202	0.36	0.215	0.127	0.208	0.224	0.133	0.062
80	e1	137	1.148	37	160.7	0.357	0.214	0.207	0.165	0.21	0.123	0.186	0.126	0.057	0.033

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	**15**	**30**	**45**	**60**	**90**	**120**	**180**	**360**	**540**
80	e1	138	3.042	39.143	425.9	0.263	0.233	0.148	0.326	0.242	0.264	0.218	0.222	0.108	0.043
80	e1	139	4.328	39.571	605.9	0.22	0.319	0.316	0.217	0.22	0.163	0.127	0.106	0.147	0.056
80	e1	140	4.542	39.857	635.8	0.312	0.228	0.21	0.152	0.366	0.342	0.3	0.139	0.081	0.054
80	e1	141	2.146	37.714	300.4	0.353	0.289	0.149	0.354	0.192	0.314	0.145	0.17	0.071	0.066
80	e1	142	3.21	39.143	449.4	0.213	0.204	0.29	0.265	0.198	0.202	0.139	0.158	0.124	0.044
80	e1	143	3.94	39.429	551.6	0.307	0.257	0.309	0.257	0.302	0.246	0.142	0.2	0.112	0.063
80	e1	144	2.97	38.714	415.8	0.172	0.254	0.22	0.318	0.304	0.209	0.126	0.122	0.096	0.059
80	e1	145	1.749	37.571	244.9	0.264	0.222	0.172	0.227	0.226	0.212	0.16	0.169	0.108	0.047
80	e1	146	1.981	38	277.3	0.284	0.229	0.263	0.267	0.218	0.293	0.122	0.108	0.123	0.061
80	e1	147	1.383	37.286	193.6	0.308	0.26	0.326	0.311	0.216	0.139	0.258	0.186	0.104	0.058
80	e1	148	0.828	36.714	115.9	0.348	0.164	0.287	0.148	0.219	0.224	0.201	0.102	0.076	0.049
80	e1	149	0.614	36.571	85.9	0.217	0.315	0.231	0.178	0.168	0.25	0.149	0.075	0.072	0.03
80	e1	150	2.483	38.429	347.6	0.335	0.386	0.172	0.179	0.255	0.138	0.243	0.195	0.055	0.05
80	e1	151	0.798	36.714	111.8	0.279	0.262	0.159	0.289	0.277	0.177	0.127	0.107	0.053	0.029
80	e1	152	2.051	37.714	287.2	0.217	0.296	0.212	0.356	0.295	0.208	0.115	0.175	0.115	0.032

ERROR	NUM	ID	WT_KG	AGE_WKS	DOSE	DRUG CONCENTRATIONS AT *X* MINUTES POST DOSE									
						5	15	30	45	60	90	120	180	360	540
80	e1	153	0.84	36.714	117.6	0.143	0.275	0.274	0.271	0.146	0.158	0.248	0.145	0.082	0.038
80	e1	154	2.753	38.429	385.5	0.421	0.357	0.294	0.213	0.278	0.17	0.237	0.242	0.048	0.077
80	e1	155	0.814	36.714	114	0.162	0.155	0.289	0.272	0.269	0.272	0.246	0.166	0.074	0.033
80	e1	156	0.747	36.714	104.6	0.229	0.189	0.318	0.211	0.187	0.182	0.175	0.074	0.073	0.049
80	e1	157	1.835	37.571	256.9	0.202	0.376	0.294	0.16	0.226	0.286	0.206	0.16	0.077	0.031
80	e1	158	0.974	36.857	136.3	0.297	0.352	0.234	0.273	0.177	0.148	0.242	0.181	0.044	0.036
80	e1	159	0.437	36.429	61.2	0.202	0.247	0.299	0.294	0.181	0.127	0.192	0.077	0.077	0.019
80	e1	160	2.915	38.429	408.1	0.281	0.296	0.366	0.324	0.177	0.205	0.223	0.24	0.08	0.079