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EXAMPLES OF POPULATION PHARMACOKINETIC MODELING IN DRUG DEVELOPMENT USING NONMEM

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ABSTRACT

The population approach to pharmacokinetic analysis, and its application to the identification of patient characteristics that affect a drug's pharmacokinetic parameters, is achieving greater prominence in the drug development process. Specifically, population analyses are a way to gather information that might be difficult to capture in some subpopulations. In the fall of 1997, the Food and Drug Administration proposed new legislation, commonly known as the "Pediatric Rule". This new legislation required pharmaceutical companies to collect pediatric data for drugs with indications applicable to children before the compound would be approved. Other than conducting traditional pharmacokinetic clinical trials, another way to collect this information would be to perform a population pharmacokinetic analysis. Two different examples of this approach are presented. The first study was conducted on traditional pharmacokinetic data (intense sampling) pooled from four pediatric trials. The second study is an example of the ability of the population approach to take advantage of sparse data obtained as a secondary objective of a clinical study.

A population pharmacokinetic analysis was conducted for azithromycin on data from pediatric patients enrolled in four separate clinical trials. A two compartment model with parallel zero- and first-order absorption was found to best fit the data. Potential covariates were assessed for oral clearance (CL/F), oral volume of distribution in the peripheral compartment (V2/F), intercompartmental oral clearance (Q/F), and the first-

order absorption rate constant (ka). Weight was found to be a significant covariate for both CL/F and V2/F. No covariates were found to be significant for Q/F or ka.

A population pharmacokinetic analysis was conducted for prednisolone on data from thoracic organ transplant patients. A one compartment model with a fixed first order rate of absorption was found to best fit the data. Potential covariates were assessed for oral clearance (CL/F) and oral volume of distribution (V/F). Sex and concomitant ciprofloxacin use were found to be significant covariates for CL/F. No covariates were found to be significant for V/F. Data was also available on plasma concentrations of prednisolone's metabolite, prednisone. It was not possible to derive a robust and clinically meaningful model that incorporated the metabolite data.

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PREFACE

This document was prepared in the format of the manuscript plan in accordance to section 11-10 of the Graduate School Manual at the University of Rhode Island. The dissertation is divided into three sections.

Section I contains a general introduction to the objectives of the research. Section II consists of the main body of this dissertation. This section is composed of three manuscripts written in the format required for each scientific journal to which they are, or will be submitted. A statement of overall conclusions for the entire dissertation is also included in this section. Section III contains one appendix that includes additional information and experimental details useful to the understanding of the work in Section II. A bibliography follows Section III in which all sources used as references in this document are cited.

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INTRODUCTION

In February 1999 the Food and Drug Administration (FDA) issued a final guidance governing the development, conduct, and analysis of population pharmacokinetic clinical trials (1). The guidance states that population pharmacokinetic analyses are ideal to investigate variability and alternative dosing regimens when there is prior knowledge that certain factors may affect drug behavior. Traditionally, alternative dosing regimens have required a large clinical trial or many smaller clinical trials in sub-populations. Instead of running additional clinical trials, the FDA guidance has suggested that an alternate method of analysis, population pharmacokinetic analysis, may provide the same information.

The need to modify the usual dose of a drug in certain populations is determined by comparing the pharmacokinetics of the sub-group to the population as a whole. There may be so many different sub-populations that it is often unrealistic to run a separate clinical trial for each group. Many times, dosages are adjusted empirically across sub-populations; using either clinician experience or assuming dose proportionality with either body weight or age. These empirical approaches increase the tendency for serious adverse events or sub-therapeutic concentration levels (2;3). In order to address the inadequate dosing information, many researchers have been focusing on new approaches to pharmacokinetic analyses and model building.

Originally, pharmacokinetic modeling concentrated on the individual. For example, in a traditional standard two stage analysis (STS), a clinical trial is comprised of a small number of subjects from whom a large (12-20) number of serial blood samples are collected over a dosing interval. Trials are restricted to representative subjects from a particular population to limit variability between subjects. An analysis of this type of data is done in two stages. For the first stage, plasma concentration time data are modeled using nonlinear regression to produce estimates of the pharmacokinetic parameters. For the second stage, the individual pharmacokinetic parameters are combined and descriptive summary statistics are computed (e.g. group mean and group variance). Analysis of the dependencies between the parameter and any covariates use a classical statistical approach (stepwise linear regression, cluster analysis, etc.) (1;4;5). This type of analysis moves from an individual (unit of analysis) out to the population, and as a result, the parameter estimates are unbiased and the random effects are overestimated (1). There are several logistical issues associated with this approach, primarily revolving around the need to perform extensive blood sampling and homogeneity of the population (1;6). These reasons have led to an alternative approach known as nonlinear mixed effects modeling.

A second approach, nonlinear mixed effects modeling, is a way to directly study the population's pharmacokinetics. Nonlinear mixed effects modeling is less stringent than a STS analysis; it allows for the use of sparse data (2 or more samples not necessarily from the same dosing interval per patient) from a large number of representative patients in the population (1;4;5;7). The population method pools all data collected and calculates

population pharmacokinetic parameters (e.g. volume of distribution). Additionally the focus of the analysis is on the source and correlation of variability in drug concentrations among individuals in the population. Thus, population pharmacokinetics focuses on the target population (unit of analysis) and moves out to the individual. Population analyses also provide quantitative estimates of both the interindividual and intraindividual variabilities of the population (4;5). Interindividual variability may be accounted for by adding specific patient characteristics into the population model. Patient characteristics that cause changes in the dose-concentration relationship can be identified and assessed and then appropriate dosing modifications can be determined (1).

Nonlinear mixed effects modeling will be performed using a software package called NONlinear Mixed Effect Model (NONMEM) version 5 level 1.1. NONMEM is suitable to analyze these types of data and has been extensively utilized by others (8;9). Both fixed and random effects are modeled using NONMEM. Fixed effects (e.g. time or dose) structure the actual pharmacokinetic parameters (structural portion of model). Random effects are comprised of random interindividual variability (unexplainable error produced by each individual's variability not accounted by the fixed effects) and intraindividual variability (explainable error accounting for the difference between actual and predicted concentration values) in the pharmacokinetic parameters (statistical portion of model). NONMEM provides estimates of both inter- and intraindividual (i.e. residual random error) variabilities in the pharmacokinetic parameters (4;7;9).

HYPOTHESIS TESTED

To date, there are no published population pharmacokinetic models for azithromycin in the pediatric population and prednisolone in organ transplant patients. For azithromycin, small clinical trials have been conducted in pediatric patients to determine alternative dosing regimens. The drug's label includes results from some of these trials and only has information on adjusting dose by weight (kg). For prednisolone, it appears that a standard dose produces a large variability in prednisolone concentrations. There is little information on the cause of this variability and on what adjustments should be made to doses in certain sub-populations. For the use of prednisolone in organ transplant patients, it is critical that an optimum prednisolone concentration be achieved. It has been shown that a patient with a higher prednisolone clearance is more likely to suffer an allograft loss, while a patient with high prednisolone concentration levels (i.e. low prednisolone clearance) is more likely to suffer from adverse events (2;10).

The hypothesis to be tested in this investigation is that the population pharmacokinetic modeling approach can be used to evaluate and describe the concentration time data collected in the azithromycin and prednisolone clinical trials. Using this approach, precise estimates of the pharmacokinetic parameters and their variability will be quantifiable and significant covariates will be identified.

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OBJECTIVES

The specific aims of this dissertation are as follows:

Azithromycin example:

1) To develop a population pharmacokinetic model for pediatric patients taking azithromycin. This model will include the following pharmacokinetic parameters: clearance (CL/F), volume of distribution (VD/F), interindividual variability, and intraindividual variability.
2) To identify individual characteristics such as demographic information, disease status, and concomitant medications which effect values of pharmacokinetic parameters.

Prednisolone example:

3) To develop a population pharmacokinetic model for prednisolone including oral clearance (CL/F) and oral volume of distribution (V/F) and to assess the interindividual variability in thoracic organ transplant patients
4) To investigate various individual characteristics such as demographic information, disease status, and concomitant medications as potential covariates to reduce interindividual variability
5) To develop a population pharmacokinetic model for the evaluation of the optimal prednisolone dosing based on individual characteristics.

MANUSCRIPT I

Summary of Recent Proposed Regulation for Assessment of Safety and Effectiveness of Drugs and Biological Products in the Pediatric Population Published in Clinical Research and Regulatory Affairs 15(2):79-90. 1998

ABSTRACT

In the fall of 1997, the United States Food and Drug Administration (FDA) proposed to add onto the existing 1994 regulations dealing with the "pediatric use" subsection of prescription drug labels. These new regulations were titled Docket No. 97N-0165 "Regulations Requiring Manufacturers to Assess the Safety and Effectiveness of New Drugs and Biological Products in Pediatric Patients"(1). These new rules will require pharmaceutical companies to collect data for those drugs whose indications may be applicable to usage in children before the compound will be approved (or soon thereafter). In some cases, manufacturers will also have to provide this information (within a length of time determined by both the FDA and the manufacturer) for drugs already marketed. It is proposed that, by including safety and effectiveness information on the label, the pediatric population will be less likely to have serious adverse events or subtherapeutic treatments. This article will cover in detail the 1997 proposed regulations and what it will mean for industry.

INTRODUCTION

The United States Food and Drug Administration (FDA) claims that pediatric labeling often is incomplete, inadequate, and even nonexistent for many prescription drugs. In most cases, the label contains no information on safe and effective doses for children. To address this issue, FDA passed regulations in December of 1994 which made it easier for manufacturers to include pediatric information in the "pediatric use" subsection of prescription drug labels. The 1994 regulations amended an earlier set of regulations from 1979 requiring full clinical trials in the pediatric population as a basis for labeling for use in this population. The 1994 legislation built on to the 1979 regulations with three points: 1) data could be extrapolated from adult studies, if the course of the disease and the drug effects were similar in both the pediatric and adult populations, 2) companies had to reexamine data to determine whether pediatric labeling of their marketed products needed to be modified, and 3) FDA was given authority to request specific pediatric use information. The purpose of these regulations was to make it easier for manufacturers to include pediatric information on the labeling of their products. Although the Pharmaceutical Research and Manufacturers of America (PhRMA) believes that industry has been in compliance with the regulations (2), the FDA believes many companies are still not providing adequate information (Proposed Rule Section II (1)). Consequently, in the fall of 1997, FDA proposed new regulations to address what it perceives as the pharmaceutical industry's poor compliance to the 1994 regulations.

Post 1994 Regulations to Present

FDA states clinical studies in the pediatric population have been conducted for only a small fraction of drugs currently on the market. The labeling of many of these drugs contain limited, if any, information on either the use of the drug in the pediatric patient or on specific dosing requirements for the different pediatric age groups. The FDA conducted a survey and found that although there was adequate pediatric labeling for vaccines and antibiotics, the labeling for many drugs used to treat common childhood illnesses and other more serious conditions, contained little information for pediatrics. From data collected by IMS America, Ltd. regarding prescription drug usage, FDA compiled the 10 most prescribed drugs in pediatric patients, on an outpatient basis (Table 1). For these 10 drugs, FDA claims the label either lacked information for the subpopulation for which the drug was being prescribed, or the information was inadequate (Proposed Rule, Section I (1)). PhRMA responded to these claims by noting that the data was obtained in 1994 and therefore out-dated. After 1994, the manufacturers claim that either they have provided additional pediatric information within the label or that there is no need for additional labeling information- particularly for Ampicillin and Auralgan (2). The Center for Drug Evaluation and Research (CDER) identified the top ten drugs prescribed (on both an inpatient and outpatient basis) in the pediatric population and asked the companies that market these drugs to voice their concerns over the proposed changes in regulations (Proposed Rule, Section II (1)). FDA claims that physicians have to either guess on an appropriate dosage (causing a potential for subtherapeutic levels or adverse events due to toxicity) or prescribe only

those drugs with which they have had experience prescribing in the pediatric population (causing a potential for a less effective form of therapy) (Proposed Rule, Section I (1)).

An informal study by the American Academy of Pediatrics in 1990 found that only 20% of the new molecular entities (NME's) approved between 1984-1990 had pediatric information (not all of the NME's had potential use in the pediatric population) and that 56% of the NME's approved in 1991 that had potential use in the pediatric population had some pediatric labeling at the time of approval. In 1996 (2 years after the passing of the 1994 regulations regarding pediatric use labeling) only 37% of the NME's that had potential use in the pediatric population had some pediatric labeling at the time of approval. The pediatric labeling that was present on the NME's in 1991 and 1996 may not have been adequate for all groups within the pediatric population (Proposed Rule Section III (1)). PhRMA states that 20 of the approved drugs in 1996 would have potential use in the pediatric population. Of these 20, 19 have been studied or will be studied in pediatric patients, showing an improvement in industry's response to the 1994 regulations (2).

Description of the Proposed Rule

The proposed rule would be intended for new chemical entities and new biological drug products. A new chemical entity is defined as "a drug that contains no previously approved active moiety." There are three main points to this proposed rule: 1) before approval, a new chemical entity must have safety and efficacy information on relevant pediatric age groups for the claimed indication, 2) drugs already marketed will need to

provide more pediatric information if the label is lacking in relevant information, and 3) FDA can call for meetings to discuss the need for pediatric studies early in the development process and postmarketing. FDA has broken down the pediatric population into 4 subgroups: 1) neonates- birth to one month of age, 2) infant- one month to two years of age, 3) children - two years to twelve years of age, and 4) adolescent - twelve years to sixteen years of age. A safety and efficacy assessment would be required for pediatric patients, in all age groups, for the claimed indication. A manufacturer would not be responsible for providing information for any off-label indications. Companies would not need to provide new information for any supplements filed for new indications (Proposed Rule Section V.A (1)).

Pediatric formulations would be required in the studies to ensure bioavailability and the consistency of the dosing. By using a pediatric formulation in a study, data will be more meaningful and an accurate analysis can be made for safety and effectiveness in the pediatric population. If a manufacturer were unable to produce an appropriate pediatric formulation for a given age group, then a waiver would be granted. FDA was seeking comments on using cost of generating a formulation to be grounds for a waiver (Proposed Rule Section V.E (1)).

Waivers

Pediatric studies would not be necessary if FDA granted a full or partial waiver (Proposed Rule Section V.B.4 (1)). Pediatric assessments are not necessary if 1) the product will not be a meaningful therapeutic benefit over already existing treatments and

if it will not be widely used in the pediatric population, 2) if studies would be impossible or impractical to carry out, and 3) if the compound would pose undue risk to the pediatric patients. A full waiver would be granted if one or more of the conditions above applied to the entire pediatric population. A partial waiver would be granted if there was a need to avoid studies in a specific age group within the pediatric population. FDA was seeking comments regarding whether there should be other situations that might merit a waiver - e.g. costs.

One of the questions that FDA faced was how to quantify "meaningful therapeutic advances". FDA addressed this issue by deciding that it would be meaningful if a substantial number of patients were to use this new compound. The proposal discusses two different methods for determining a substantial number of patients. The first method would be to assess the number of times the drug would be used annually within the pediatric population. If it is estimated that 100,000 or more prescriptions may be written for patients within the pediatric population, then the drug would qualify as being given to a substantial number in all age groups. A partial waiver would be granted if fewer than 15,000 prescriptions were to be written for a specific age group. The second method would assess the number of patients affected by the disease or condition that the drug is designed to treat. If 100,000 pediatric patients were affected, then the compound would be used in a substantial number of pediatric patients. A partial waiver would be granted if fewer than 15,000 patients comprised a particular age group (Proposed Rule Section V.B.4 (1)). PhRMA argues over the true representation of the diseased population by using prescription numbers as a basis for calculating a drug as being used in a substantial

number of patients. For many diseases (e.g. asthma) multiple prescriptions are refilled several times in a given year for a single patient. PhRMA believes that there would be potential for gross exaggerations of diseased children for certain diseases. PhRMA recommends deciding a meaningful therapeutic advance by unmet medical needs and not by arbitrarily decided numbers which may not be a true measurement of the diseased population (2).

There may be instances where the deferral of submissions of pediatric studies may be necessary (e.g. if the New Drug Application (NDA) submission or approval is ready for adults before pediatric testing is complete). It may be inappropriate to begin pediatric testing before the safety and efficacy data in adults has been collected. The deferred submission would need to be provided not more than 2 years after the date of the initial approval. Applicants would need to provide pediatric information in their Annual Progress Reports (APRs) to show compliance (Proposed Rule Section V.B.3 (1)).

Legal Ramifications for Inadequate Pediatric Labeling Information

In the proposed rule, FDA states "Denying or withdrawing approval of an otherwise safe and effective drug or biological product is not a satisfactory remedy, because removal of a product from the marketplace could deprive other patients of the benefits of a useful medical product." Therefore, FDA is looking into injunctive actions against companies that fail to provide the necessary pediatric information. Violation of an injunctive action could result in the manufacturer being fined (Proposed Rule Section V.G (1)).

What legal authority would FDA have over enforcing this proposal? The proposal cites provisions that apply to FDA's authority over enforcing this proposal. For example, FDA has authority to classify a drug as misbranded if the label is false or misleading, dangerous to health when prescribed, recommended, or suggested in its labeling, or fails to provide adequate directions for each intended use. There are other legal reasons cited in Section VI of the regulations. Still, industry questions whether FDA has any legal authority over forcing manufacturers to provide this data (2).

Analysis of Impact

An assessment of the impact of the proposed regulation is difficult to ascertain. The FDA has estimated the number of additional studies and the cost that would have accrued had these regulations been in place over the period 1991-1995. The drugs approved over this period were categorized according to their potential use in the pediatric population. The drugs were divided into 3 categories: 1) therapeutically important, 2) other approvals, and 3) all other approvals. The "therapeutically important" drug category was composed of those drugs that would have a potential use in the pediatric population. The "other approval" category comprised drugs that would have a potential to be used extensively in the pediatric population. The final category, "all other approvals", consisted of drugs that would not be used for a pediatric patient. This data was tabulated in Table 2. Of the 142 drugs approved, 60 (42%) were estimated to have pediatric use and 82 (58%) did not. To estimate the additional studies that would be required to provide adequate data, FDA assumed that much of the data could be extrapolated from previous studies. Therefore, the 60 drugs that required

pediatric information were further sub-divided into 3 groups according to the amount of additional work required. The first of these 3 groups consisted of 30 drugs that would have required the least amount of new data; 23 drugs that already had some pediatric labeling information and 7 drugs that already had ongoing pediatric studies at the time of approval. Of these 30 drugs, FDA estimated that 15 would need limited additional data in the form of a study with approximately 50 patients. Of the remaining 30, 23 of the drugs would have needed additional studies with approximately 100 patients, and 7 drugs would have required extensive safety and effectiveness involving 300 patients. Therefore, if these regulations had been in effect, the manufacturers would have needed additional studies for 45 of the 60 drugs. This would have involved 5,150 patients (15·50+23·100+7·300) or clinical trials of 9 drugs involving 1,030 patients per year. In addition, FDA is also authorized to request additional data for already marketed compounds. FDA estimated two additional already marketed drugs per year into their assessment. FDA further estimated that these additional 2 drugs require an additional 400 patients. Thus, totaling NMEs and already marketed drugs, there are 11 drugs and 1,430 patients per year. FDA has also noted that not all compounds will be approved. To account for the additional pediatric studies that will occur for drugs that will not ultimately be approved, FDA has further padded the numbers by increasing the estimate by 30%, or 14 drugs and 1,850 pediatric patients per year (Proposed Rule Section X.B (1)).

Costs of studies vary proportionately with the complexity of the clinical trial. FDA hired a private consulting firm to estimate the costs of Phase IV trials. The firm estimates that

for a fever or vaccine trial, the cost would range from $300-$500 per patient, for renal disease the cost would be $3600 per patient, and for epilepsy the cost would be $5,000 per patient. Many researchers estimate $1,500-$3,400 per patient as an average cost. To include all costs incurred during a study, FDA has estimated the cost to be $5,000-$9,000 per patient. Based on this estimate, the annual cost to conduct the additional studies for the 1,850 patients in any given year would have cost the industry $9.25 million-$16.65 million per year. This estimate does not include any additional expenditure for the manufacturing of the pediatric formulation. FDA estimates that the cost of the additional formulations will not cost more than $1 million per year for each drug (estimating that a total of 4 drugs per year will need additional formulations bringing the total to $4 million for additional formulations). There will also be additional paperwork due to the increased regulations and FDA estimates these costs at $220,000 per year. The total estimation comes in at $13.5 million - $20.9 million per year (Proposed Rule Section X.C.(1)). Delays in the submittal of a NDA might result in a further potential impact for the manufacturer due to extended drug development timelines. These estimates do not include additional staff that will be needed by FDA to process the supplements to already existing NDAs, increases in the number of studies included in future NDAs, and the additional meetings being held during the development process to ensure adequate pediatric trials (2).

Benefits of Regulations

These regulations address providing adequate dosing information in the label for pediatric age groups. This information will be used to avoid adverse drug reactions and

undertreatment in this population. Additionally, the information should increase the availability of newer medications to the pediatric population. FDA compiled a list of the top 25 NME's responsible for the highest number of adverse events in pediatric patients. Eight (8) of these NME's had no pediatric labeling information (1,273 adverse events) and 5 lacked label information for children under 12 (434 adverse events). Out of these 13 NME's, 11 would have been required to submit further pediatric labeling information under these proposed regulations (Proposed Rule Section X.E.(1)).

DISCUSSION

What will these proposals mean for industry? Obviously, there will be more initial cost for manufacturers in that will need to run more clinical studies and create new pediatric formulations. What about compounds that are off patent or are unpatentable drugs? What sort of incentive is there for companies to spend additional resources on drugs that they no longer have patent protection? Some of the smaller manufacturers will simply not be able to fund these additional requirements. It is argued that the FDA estimates of the cost of additional studies and the creation of new formulations are far too low. PhRMA believes that there could be potential delays in drug development time and NDA approvals. Additionally, the issue of legal consent will be hotly disputed. Will parental consent be considered enough? Industry is concerned that children recruited in these studies will possibly be injured and subsequently sue for unlawful consent (2).

PhRMA has suggested that FDA follow European countries, Canada, and Japan when looking to create additional regulations regarding pediatric clinical trials. In Europe,

pediatric studies begin after the completion of Phase III trials and their age groups are different from those assigned by FDA. Canada tests drugs in children after safety and efficacy has been determined in the adult population. Japan excludes children from Phase I and II trials and conducts trial in neonates and infants only after older children have been studied (2).

Clearly, there is much debate over whether these regulations should be passed, by FDA and industry, as well as health care providers and parents. There is a need for further discussion on this matter both from the viewpoint of the child and also the realistic requirements that can be placed on the pharmaceutical industry. It has been recommended that a committee be formed, comprised of these individuals, to address these many difficult questions. Until then, there are too many unresolved issues to proceed with further implementation of the current proposed regulations as they stand.

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Table 1 Listing of Top 10 Out-patient Prescribed Drugs in Pediatric Patients

DRUG	INDICATION	# OF PRESCRIPTIONS	PhRMA Comments
1. Albuterol	asthma	1,626,000 under 12	Inhalation solution 2 strengths for ages 2+, aerosol for children age 4+.
2. Phenergan	allergic reactions	663,000 under 2	Label includes relevant information in various subsections.
3. Ampicillin	infections	639,000 under 12	No label information, however physicians have dosing knowledge.
4. Auralgan	ear pain	600,000 under 16	Grandfather clause - no NDA on file (marketed for over 40 years).
5. Lotrisone	topical infections	325,000 under 12	Statement in label not to use in diaper dermatitis due to harmful concentrations in infants and young children.
6. Prozac	depression and OCD	349,000 under 16 (3,000 under 1)	Studies nearing completion.
7. Intal	asthma	109,000 under 2 aerosol 399,000 under 5	Solution in age 2+ and metered dose inhaler for age 5+.
8. Zoloft	depression	248,000 under 16	Pediatrics use 10/97.
9. Ritalin	attention deficit disorders, narcolepsy	226,000 under 6	Evaluation for under 6 years of age in process.
10. Alupent	asthma	184,000 under 6	Revised label 2/97 for dose age 6 and over and one dose for 12 and older.
Total		over 5 million /year	

Abbreviations: OCD=obsessive compulsive disorder, PhRMA= Pharmaceutical Research and Manufacturers of America, NDA=new drug application

Table 2
Pediatric Labeling of NME's and Biological Products Approved 1991-1995
with Potential Pediatric Use

	#NMEs	% of All Approved	% Some Pediatric Labeling	% No Pediatric Information
Some Potential Pediatric Use	47	33	34 % (16/47)	66 % (31/47)
Wide Pediatric Use	13	9	54 % (7/13)	46 % (6/13)
Total	60	42	38 % (23/60)	62% (37/60)

#Of the 142 NME and Biological Products approved in this period, 60 were deemed to have potential use in the pediatric population.

Abbreviations: NME=new molecular entity

MANUSCRIPT II

Population Pharmacokinetics of Azithromycin in the Pediatric Population

ABSTRACT

A population pharmacokinetic analysis was conducted for azithromycin on data from pediatric patients enrolled in four separate clinical trials. The data, which consisted of 526 serum concentrations from 58 patients administered one to five daily oral doses of azithromycin ranging from 5 mg/kg to 12 mg/kg per day, was analyzed in NONMEM. A two compartment model with parallel zero-order and first-order absorption was found to best fit the data. Potential covariates were assessed for oral clearance (CL/F), oral volume of distribution in the peripheral compartment (V2/F), intercompartmental oral clearance (Q/F), and the first-order absorption rate constant (ka). Models were initially developed using the first order (FO) method and subsequently refined using the first order conditional estimation (FOCE) method. Weight was found to be a significant covariate for both CL/F and V2/F. Neither age, gender, the presence of anemia, cancer, pneumonia, nausea, colitis, nor the concomitant usage of albuterol, amikacin, captopril, ceftazidime, ceftriaxone, digoxin, diphenhydramine, dopamine, fentanyl, furosemide, midazolam, morphine, nystatin, ranitidine, sulfamethotrexate, ticarcillin, nor vancomycin appeared significant for any pharmacokinetic parameter.

INTRODUCTION

Azithromycin is an azalide antibiotic (a subset of macrolide antibiotics), is active against a wide spectrum of microorganisms, and has a low side effect profile (1-4). Azithromycin is indicated for pediatric usage for the treatment of acute otitis media, community-acquired pneumonia, and pharyngitis/tonsillitis (5). Following administration, azithromycin undergoes extensive and rapid distribution in tissue. Thereafter, distribution from tissue is the rate limiting process for elimination of azithromycin, thus leading to a long terminal half-life (around 55-70 hours for both adult and pediatric populations) (1;2;4-6). Because of these properties, azithromycin is administered once daily and for a shorter duration than other macrolide antibiotics (1;2;7-10). Appendix I provides a more extensive overview of the pharmacokinetics of azithromycin.

Clinical trials have been conducted in pediatric patients to determine the pharmacodynamic and pharmacokinetic characteristics in this group compared to the adult population (9-11). These studies, and other safety clinical trials, showed that once daily dosing was well tolerated and efficacious in pediatric patients (7-12). Results from two of these studies have been used for the development of dosing guidelines in pediatrics (5;10;11). Current recommended dosages for pediatric patients are determined by indication. For the indications of otitis media and community-acquired pneumonia, pediatric recommendations are for 10 mg/kg on day 1 and 5 mg/kg doses on days 2-5. A higher dose of 12 mg/kg for days 1-5 is recommended for children with pharyngitis/tonsillitis (5). In contrast, a 500 mg single dose given on day 1, followed by

250 mg single doses on days 2-5 is recommended for adults with these indications (5;13).

After a single oral dose of 500 mg azithromycin in an adult population, the following pharmacokinetic parameters have been reported: peak serum concentration (Cmax) of around 0.4 mg/L, time to peak concentration (Tmax) of 2.5 hours, area under the concentration time curve from 0-24 hours (AUC_{0-24}) of 2.36-2.60 μg·hr/mL, steady state volume of distribution of 23-31 L/kg, clearance of 9 mL/min/kg (0.54 L/hr/kg), and bioavailability of 37% (no standard deviations were provided with these results) (1;2;4;5;13-15). When azithromycin was administered to children 0-5 years of age (10 mg/kg day 1; 5 mg/kg days 2-5), Cmax was 0.224 +/- 0.120 μg/ml, Tmax was 1.8 +/- 0.4 hours, and AUC_{0-24} was 1.842 +/- 0.651 μg·hr/mL. In a different study, when azithromycin was administered at the same dose to children 6-15 years of age (10 mg/kg day 1; 5 mg/kg days 2-5), Cmax was 0.383 +/- 0.142 μg/mL, Tmax was 2.4 +/- 1.1 hours, and AUC_{0-24} was 3.109 +/- 1.033 μg·hr/mL (5;10;11). Comparing these two studies, children 0-5 years old versus children 6-15 years old have significantly lower Cmax and AUC values while their oral clearance is significantly higher (9;11). Thus, it is proposed that age may prove to be an important covariate for oral clearance. The coefficient of variation for oral clearance (CL/F=4.83 L/hr/kg) in a different pediatric study was reported to be 74%, but the cause of the variability was not identified (9). Otitis media and pharyngitis are very common infections, especially in the younger pediatric population (8;12) and an understanding of the contribution of age or other factors that may explain interpatient variability in clearance may prove beneficial. The

lack of information on variability may be addressed by utilizing nonlinear mixed effects modeling (i.e. population pharmacokinetic models) for azithromycin in the pediatric population.

Nonlinear mixed effects modeling permits the use of sparse data (2 or more plasma concentration samples not necessarily from the same dosing interval per patient) from a large number of representative patients in the population (16-18). The population method pools all data collected and calculates population pharmacokinetic parameters (e.g. CL/F). Additionally the focus of the analysis is on the source and correlation of variability in pharmacokinetic parameters among individuals in the population (19). Thus, population pharmacokinetics focuses on the study population (unit of analysis) initially and moves out to the individual. Population analyses also provide quantitative estimates of both the interindividual and intraindividual (i.e. residual) variabilities of the population (17;18). Interindividual variability may be accounted for by adding specific patient characteristics (e.g. demographic information, concomitant medication usage, etc.) into the population model. Patient characteristics that cause changes in the dose-concentration relationship can be identified, assessed, and then appropriate dosing modifications can be determined to enhance efficacy or to reduce the chance of adverse events (16;17).

The purpose of this investigation was to evaluate whether a population pharmacokinetic modeling approach could be used to develop a model for data combined from four

pediatric trials and to determine if any patient characteristics could be identified that might provide useful information when selecting a dose of azithromycin in children.

METHODS AND MATERIALS

Patients. Plasma concentration-time data were obtained from pediatric patients enrolled in four Phase I clinical studies (see Table 1). These four clinical trials were conducted to evaluate safety, efficacy, and pharmacokinetics after oral administration of azithromycin in pediatric patients. Results for three of the four studies (Protocols 054, 136, and 172) have been reported elsewhere (9-11). The fourth study (Protocol 043) was terminated early due to difficulties with patient enrollment. Protocol 043, 054, 136, and 172 were conducted during 1993, 1993, 1991, and 1992-1993 respectively. The appropriate institutional review boards approved all protocols. The patient's parent or a legal guardian gave written informed consent prior to inclusion in the study.

This retrospective combined data analysis was conducted on all pediatric patients with measurable azithromycin concentration-time data collected in the four Phase I clinical trials. A random selection of 20% of the patients from the combined dataset was reserved to assess the predictive performance of the model, i.e. internal validation of the final model. The data from the remaining 80% of the patients was used for the model development. Two patients in Protocol 054 (1 male 2 year old weighting 13kg and concomitantly medicated with captopril, furosemide, and morphine; 1 male 1 year old weighting 9kg with colitis and concomitantly medicated with captopril, digoxin, diphenhydramine, dopamine, fentanyl, furosemide, morphine, and nystatin) were

excluded from the combined data analysis because they had no measurable azithromycin concentration levels at any time point. Thus, there were a total of 58 pediatric patients used for the combined data analysis: 46 were included in the model development dataset and 12 were included in the validation dataset. Characteristics of the pediatric patients included in the dataset are presented in Table 2.

Azithromycin administration. Azithromycin (oral suspension) was used for dosing for each clinical trial as instructed in each of the four protocols. In Protocol 043, a single daily dose of azithromycin (12 mg/kg) was administered on days 1-5. In Protocol 054, a single daily dose of azithromycin (12 mg/kg) was administered on day 1. Pediatric patients that were enrolled in the multiple dose portion of the clinical trial also received single daily doses of azithromycin (12 mg/kg) administered on days 2-5 (9). In Protocols 136 and 172, a single daily dose of azithromycin (10 mg/kg) was administered on day 1 and single daily doses of 5 mg/kg on days 2-5 (10;11). For Protocols 043, 054 and 136, azithromycin powder was reconstituted to 100 ml volume to yield 40 mg/ml azithromycin concentration (9;10). For Protocol 172, azithromycin was provided in a 20 mg/ml suspension (11). Azithromycin was dosed in the morning either one hour before or two hours after the morning meal (9-11). In Protocols 043, 136 and 172, patients fasted overnight before receiving their final dose on day 5 (10;11)

Blood Collection and Sample Analysis. For Protocol 043, serum samples were collected at 0 (just prior to dosing), 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 144 hours post dose on day 5. In Protocol 054, serum samples were collected at 0 (just prior

to dosing), 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours post dose on day 1. For patients enrolled in the multiple dosing portion of the trial, additional samples were collected at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 hours post dose on day 5 (9). For Protocols 136 and 172, serum samples were collected at 0 (just prior to dosing), 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 hours post dose on day 5 (10;11). Concentrations of azithromycin in the serum samples, for all of the protocols, were determined by a high-performance liquid chromatography-mass spectrometry method as published elsewhere (9-11;20).

There were a total of 526 concentration values used in the model development dataset and 148 concentration values in the validation dataset. No steady state assumptions were made with any concentration values.

Data Preparation and Pharmacokinetic Analysis. Demographic, plasma collection time, medical history, concomitant medication, concentration, dosing history, physical examination, and adverse event data relevant to the pharmacokinetic analysis were extracted from raw data sources and merged using SAS v6.12 on a VAX/VMS mainframe (Digital Equipment Corporation, Maynard, MA.). Twenty percent (20%) of patients from each protocol were then randomly removed from the model building dataset to form the validation dataset. The remaining 80% of the data was used for the model development dataset. The pharmacokinetic analysis was performed using NONlinear Mixed Effect Model (NONMEM) version 5 level 1.1 double precision on a Pentium III computer with a Visual Fortran 5.0 compiler (21-25).

Data Analysis Strategy.

An approach proposed by Mandema, *et.al.* (1992) was used for the data analysis: 1) a base model was developed for the population, 2) the estimates found during step 1 were used to explore potential covariates with the base model, and 3) a mixed effects model was developed to describe the relationship between the covariates and pharmacokinetic parameters (26). In this analysis, forward addition of covariates was used to generate the full model, while a backwards elimination approach from the full model was used to determine the final model.

Pharmacokinetic and statistical models were evaluated to determine the model that best described the model development dataset (n=46 patients). To discriminate between models, the following criteria were used: 1) a decrease in the objective function value (which is proportional to minus twice the log-likelihood of the data) of 3.84 (χ^2 distribution, df=1, $p< 0.05$) or greater following the addition of a single parameter was deemed statistically significant; 2) diagnostic plots (e.g. predicted concentration versus observed concentration data, predicted concentrations overlaying all concentration data versus time, weighted residuals versus predicted concentration values), 3) minimization of variances: reduction of interindividual variances and residual variability, and 4) the Akaike Information Criterion (AIC) (16;21;26;27).

Pharmacokinetic Model. To compare adult and pediatric models and estimates, a population model was initially developed using data from a traditional pharmacokinetic study conducted in healthy normal adult male subjects (age=27-54 years, weight=63-90

kg) (28). The subjects had no evidence of a history of disease, were taking no concomitant medications, and were enrolled in a study to evaluate azithromycin pharmacokinetics after single oral and intravenous doses. An intensive blood sampling regimen was used. A total of twelve subjects started the study but only ten completed both arms of the study. Two subjects dropped out after the first arm (one subject in each cohort), leaving eleven subjects that completed each arm. The eleven subjects from the oral azithromycin administration cohort contributed 120 concentration records to the modeling dataset; blood samples were collected at 0 (just prior to dosing), 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours post dose on day 1. Several pharmacokinetic models were evaluated to fit the adult data: one-compartment and two-compartment models with zero-order, first-order, and a combination zero- and first-order absorption. A two-compartment model with a combination parallel zero-order and first-order absorption best described the data. The two-compartment model with both absorption terms was parameterized as oral clearance (CL/F), oral volume of distribution in the central compartment (V1/F), oral volume of distribution in the peripheral compartment (V2/F), intercompartmental oral clearance (Q/F), the first-order absorption rate constant (ka), and the zero-order rate constant (R) (NONMEM subroutines ADVAN4 TRANS4).

As with the adult dataset, several pharmacokinetic models were used to evaluate the pediatric data: one, two, and three compartment models with zero-, first-, and combination parallel zero-and first-order absorption terms (14;29;30). For these models, the final parameter estimates from the adult model were used as the initial estimates for

the modeling of the pediatric data. The two compartment model with parallel zero- and first-order absorption rates best fit the pediatric data and was used as the base model.

Statistical Model. An additive, proportional, and exponential-error model were evaluated for interindividual variability of the pharmacokinetic parameters (23;31). For example:

Additive model: $\Theta_j = TV\Theta + \eta_{j,\Theta}$

Proportional model: $\Theta_j = TV\Theta \cdot (1 + \eta_{j,\Theta})$

Exponential model: $\Theta_j = TV\Theta \cdot EXP(\eta_{j,\Theta})$

where $\eta_{j,\Theta}$ is a random variable distributed with a zero mean and variance of ω^2_Θ and $TV\Theta$ is the population mean value for Θ.

Residual variability was modeled using a proportional-error model and an additive and proportional error model (23;31):

Proportional model: $C_{ij} = C_{pred,ij} \cdot (1+\varepsilon_{ij})$

Additive and Proportional model: $C_{ij} = C_{pred,ij} \cdot (1+\varepsilon_{1ij}) + \varepsilon_{2ij}$

where C_{ij} is the observed serum concentration value for the jth individual at time=i, $C_{pred,ij}$ is the model predicted serum concentration for the jth individual at time=i, and ε_{1ij} and ε_{2ij} are randomly distributed variables with a zero mean and variance of σ^2.

Analysis of Covariates. Once the base pharmacokinetic model was obtained, the posthoc Bayesian estimation (first order (FO) method) was implemented to obtain the individual parameter estimates to evaluate potential influences of covariates. An exponential error model for interindividual variability and a proportional error model for the residual variability were initially assumed for the covariate analysis. For each pharmacokinetic parameter, the potential influence of covariates on the individual pharmacokinetic parameter estimates was evaluated. This evaluation was performed using stepwise linear regression in S-Plus version 4.5. For each pharmacokinetic parameter, covariates were added and removed from the model in an iterative process based on a covariate's calculated residual sum of squares and the AIC. The covariate with the largest reduction in the AIC was then added or dropped from the model. The stepwise iterations stopped when no additional step decreased the AIC (32). Diagnostic plots were also used to screen for the potential influence of covariates on the pharmacokinetic parameters (i.e. covariate versus individual pharmacokinetic parameter estimate). Age and weight were treated as continuous variables. Gender was treated as an indicator variable (0=female, 1=male). The presence of asthma, anemia, cancer, pneumonia, nausea, colitis, albuterol, amikacin, captopril, ceftazidime, ceftriaxone, digoxin, diphenhydramine, dopamine, fentanyl, furosemide, midazolam, morphine, nystatin, ranitidine, sulfamethotrexate, ticarcillin, or vancomycin at any point in the study was represented as an indicator variable (0=no, 1=yes). Any concomitant medication, adverse event, or disease status in fewer than four of the patients was not tested.

Covariates that were found to be statistically significant from the initial screening in S-Plus were then evaluated in the base model using NONMEM. Each covariate was added one at a time into the base model. Covariates were deemed as statistically significant in NONMEM as outlined above; i.e. a change of >3.84 in the objective function value, diagnostic plots, reductions in variability, and the AIC (16;21;26;27). A large number of covariates were found to be statistically significant in both S-Plus and NONMEM. Because of the number of significant variables, the model development was done in a forward stepwise manner, in a manner similar to that published by Lee et. al.(33).

To generate the model in a forward stepwise manner, the change in the objective function value was used as the initial criteria for a covariate's inclusion into the model. The list of covariates that generated a change in the objective function value of greater than 3.84 for any pharmacokinetic parameter was sorted in descending order. The 95% confidence interval was calculated for the covariate parameter estimate that generated the largest change in the objective function value. If the 95% confidence interval did not include the null value, this parameter was then added to the base model. If the 95% confidence interval did include the null value, the parameter was not added and the covariate that generated the next largest change in the objective function value was then evaluated. Once the initial covariate was identified, the other covariates in the list (whose change in objective function value were greater than or equal to 3.84) were added individually. Any covariate that did not generate a further change in the objective function value of 3.84 or greater in this second run was discarded from the model building process. Again, the covariates were sorted by magnitude of the change in the

objective function value. The top covariate whose 95% confidence interval did not include the null value then became the second covariate to be added to the model. This process continued until there were no more covariates whose addition into the model would generate an objective function value change of greater than 3.84 and whose parameter estimate value would not include the null value, thus the full model was created. A backward elimination procedure was then performed on the full model. Each covariate was removed one at a time from the full model. If the objective function value increased by a more conservative value of 7.88 (χ^2 distribution, df=1, $p< 0.005$), the parameter was included in the final model.

The next step in the analysis was the validation of the model. Model validation was performed by fixing all parameter estimates (both fixed and random effects) to their final model value. The model was then run using the validation dataset (20% of the total data). The posthoc Bayesian estimation (POSTHOC option on $ESTIMATION) was invoked and the residuals calculated in $ERROR were saved in the $TABLE command. The data output from NONMEM was then exported to Microsoft Excel (version 98). Bias (mean prediction error) and precision (root mean square error) of the predicted concentration values were calculated to describe the predictive performance of the model (33-35):

$$precision = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(pei)^2}$$

$$bias = \frac{1}{N}\sum_{i=1}^{N} pe_i$$

where pe_i= the difference between the *i*th measured and predicted azithromycin concentration value at a given time and N=the number of pairs of predicted and observed azithromycin concentrations. Ideally, a value of zero is desired for both precision and bias; the smaller the magnitude of the residual, the lower the magnitude of the value of precision and bias (35). The 95% confidence intervals of precision and bias were also calculated by using the following equation (33-35):

$$X_{av} \pm t_{0.975,\,N-1} \bullet SE(X_{av})$$

As another form of validation, the NONMEM analysis using the final model was conducted on 100% of the data. The estimates of the pharmacokinetic and statistical parameters were compared to those obtained with the development of the final model dataset (80% of the data) (16;36;37).

Finally, the analysis was performed using the first order conditional estimation (FOCE) method using 100% of the data (17;22;38). Backwards elimination was performed on the final model obtained with the FO method to determine covariate significance. The FOCE estimates of the pharmacokinetic parameters, estimates of the statistical parameters, and diagnostic plots were compared with the final FO model using 100% of

the pediatric data. Bias and precision of the predicted concentration values were calculated to describe the predictive performance of the FOCE model.

RESULTS

Adult Pharmacokinetics:

The adult dataset was best described by using a two-compartment model with a zero-order rate of absorption. For the adult model, interindividual variability was described with an exponential error term on CL/F. The base model equations, parameter estimates, percent relative standard error (%RSE), and 95% confidence intervals for the adult dataset are given in Table 3 (mean weight=73.6 kg). A previous study modeled a similar dataset using a two-compartment model with zero-order absorption (14). The previous study found that a zero-order absorption rate was a superior fit for the data when compared to a first-order absorption rate model. The pharmacokinetic parameter values reported from the previous study were similar in values for oral clearance and oral volume of distribution to this analysis.

Pediatric Pharmacokinetics (FO Method) for Base Model – 80% data:

In contrast with the adult data, a two-compartment model with zero- and first- order absorption rate constants best fit the pediatric model development dataset. The parameter estimates calculated from the adult model were used as the initial estimates for the pediatric model development dataset. During model development, interindividual error terms on CL/F, V2/F, ka, and Q/F significantly improved the model, i.e. a decrease in the objective function of 3.84 (χ^2 distribution, df=1, $p< 0.05$). Interindividual error

terms on V1/F and R did not significantly improve the fit of the model and were excluded from further model development. Interindividual variability was best described by an exponential error model. Residual variability was best described by using a proportional error model. The base model equations, parameter estimates, percent relative standard error (%RSE), and 95% confidence intervals for the pediatric dataset are given in Table 4 (mean weight=26.5kg). When standardized by the mean weight, the parameter values generally compare well for the pediatric and adult populations (see Table 5), although the estimate for V2/F in the pediatric population was approximately double that of the adult population. The observed serum concentration versus predicted serum concentration values, residual versus predicted serum concentration values, and weighted residual versus predicted serum concentration values are plotted in Figures 1-3 respectively. In Figure 1, for larger concentration values, there is a larger spread seen in the data. There is a trend seen in the residual versus predicted serum concentration plot; the larger the value of concentration, the more negative the residual (Figure 2). This trend does not appear to be corrected by weighting the residual; smaller values of predicted serum concentrations now have larger variability (Figure 3).

Individual covariate testing (FO Method) for Full/Final Model – 80% data:

A summary of the forward stepwise model development for inclusion of covariates for pharmacokinetic parameters is provided in Table 6. The full model consisted of albuterol and weight as covariates for CL/F, ceftriaxone and weight as covariates for V2/F, dopamine as a covariate for Q/F, and morphine as a covariate for ka (Table 7).

Backwards elimination was then performed to generate the final model. Each covariate was removed individually from the model. A covariate was retained in the final model if there was a significant decrease in the goodness of fit (i.e., objective function value decreased by 7.88 (χ^2 distribution, df=1, $p< 0.005$). Following the backwards elimination procedure, only albuterol and weight were identified as significant covariates for CL/F and only ceftriaxone and weight for V2/F. Additionally, the parameter $\omega^2_{V2/F}$ was removed from the model since its 95% confidence interval included the null value. The final model equations, parameter estimates, percent relative standard error (%RSE), and 95% confidence intervals are given in Table 8. The diagnostic plots of CL/F versus weight, CL/F versus albuterol, V2/F versus weight, and V2/F versus ceftriaxone for the base model parameter estimates are shown in Figures 4-7 respectively. For the figures of CL/F and V2/F versus weight (Figures 4 and 6), the regression line has a positive slope; indicating as weight increases, so does the value of the pharmacokinetic parameter. For the albuterol and ceftriaxone plots (Figures 5 and 7), the box plots show the spread of the pharmacokinetic parameter values. The confidence intervals for the use of the concomitant medication overlap each other on both figures, indicating that there may not be any difference between the two groups. There are lower values for %RSE seen with Θ values (10.6-24.3%) than the variability parameters, ω^2 and σ^2 (20.0-44.1%). The %RSE for V1/F, Q/F, $\omega^2_{CL/F}$, ω^2_{ka}, and σ^2 are lower for the final model as compared to the base model (12.7 vs. 14.2, 10.6 vs. 12.9, 39.5 vs. 43.2, 36.3 vs. 42.0, and 20.0 vs. 24.9 respectively). The %RSE for ka, R, and $\omega^2_{Q/F}$ are higher for the final model as compared to the base model (15.5 vs. 14.5, 14.6 vs. 11.1, and 44.1 vs. 39.6

respectively). Parameter estimate ranges are as follows: CL/F=7-363 L/h, V2/F=900-4387 L, Q/F=14-246 L/h, and ka=0.2-19.1 L/h.

Validation dataset (FO Method) – 20% data:

The predicted performance of the validation dataset is shown in Table 9. There are both bias and imprecision in the model between the observed and predicted azithromycin concentrations as shown with the 95% confidence intervals not including the null value. The observed serum concentration versus predicted serum concentration values and weighted residual versus predicted serum concentration values plots are shown in Figures 8 and 9 respectively. In Figure 8, it appears that the model still had difficulty estimating the larger concentration values. In Figure 9, larger weighted residuals are seen for a few smaller predicted concentration values.

Final Model (FO Method) – 100% data:

The final model equations, pharmacokinetic and statistical parameter estimates, percent relative standard error (%RSE), and 95% confidence intervals generated using 100% of the data are shown in Table 10. The diagnostic plots of observed versus predicted serum concentration values, observed versus individual predicted serum concentration values, residuals versus predicted concentration values, and weighted residuals versus predicted serum concentration values are depicted in Figures 10-13. Figures 10, 11 and 12 show that the model fails to adequately predict large concentration values. Figure 13 shows that the weighted residuals are not biased, that is, there is a scatter of weighted residual values over the entire predicted concentration range. When 100% of the pediatric data

was used, weight and albuterol were retained as covariates for CL/F and weight and ceftriaxone for V2/F.

Final Model (FOCE Method) – 100% data:

The final model equations, pharmacokinetic and statistical parameter estimates, percent relative standard error (%RSE), and 95% confidence intervals generated using the FOCE method on 100% of the data are shown in Table 11. When the final model from the FO method was evaluated using the FOCE method, albuterol and ceftriaxone were no longer statistically significant covariates. Interindividual variability was best described by an exponential error model on CL/F and ka. Residual variability was best described by using a proportional error model. The diagnostic plots of observed versus predicted serum concentration values, observed versus individual predicted serum concentration values, residuals versus individual predicted serum concentration values, and weighted residuals versus individual predicted serum concentration values are depicted in Figures 14-17. Figures 14 and 15 show that the FOCE model better describes the dataset. There was a more uniform distribution of data points spread over the line of identity. Figure 16 shows that the residual plots do not differ much between the two models. Figure 17 shows that the weighted residuals are biased for smaller predicted concentration values. When 100% of the pediatric data was used with the FOCE method, weight remained as a covariate for CL/F and V2/F in the final model.

Validation dataset (FOCE Method) – 20% data:

The predicted performance of the validation dataset is shown in Table 12, where it can be observed that bias and imprecision were present between the observed and predicted azithromycin concentrations: the 95% confidence intervals did not include the null value. The values for precision and bias are similar to the values obtained using the FO method. The observed serum concentration versus predicted serum concentration values and weighted residual versus predicted serum concentration values plots are shown in Figures 18-19 respectively. In Figure 18, the model still had difficulty estimating the higher concentration values. In Figure 19, larger weighted residuals are seen for a few smaller predicted concentration values. Figure 20 shows the overall fit of the model by comparing predicted and observed concentration values versus time. At the later time points, the model consistently overpredicts the concentration values.

Model parameters versus published results:

A further validation of the population analysis can be found by comparing the individual study pharmacokinetic results with a prior published analysis. For the 054 study, oral clearance and V1/F were reported. There were 23 evaluable patients in the analysis resulting in values of CL/F=4.83 +/- 3.59 L/h/kg (CV=74%) and V1/F=38.1 L/kg (range of 9.6-184.6 L/kg – standard deviation and CV were not provided) (9). The FOCE analysis on 100% of the pediatric dataset gave the following estimates of parameters: CL/F(%CV) (L/h) = 18.2(31.3) + 2.25(14.3)·weight(kg). Using the mean weight for the population (26.5 kg), the value for CL/F was 2.93 L/h/kg. For the 100% pediatric dataset FOCE analysis, the value for V1/F was 5.1 (L/kg). While the value for CL/F

compares favorably between the two analyses, the value for V1/F in our analysis appears substantially smaller than the prior analysis.

DISCUSSION

The study demonstrated that a population pharmacokinetic modeling approach could be used to model azithromycin concentration-time data from four pediatric clinical trials. Additionally, the study demonstrated that it was possible to identify covariates to explain variability in the pharmacokinetic parameters. When the FOCE method was used, weight was found to be a significant covariate for CL/F and V2/F.

Weight was an anticipated covariate for both CL/F and V2/F and supports the current dosing recommendations for azithromycin based on weight (5). Age has been proposed as a potential significant covariate since a higher oral clearance has been seen in children 0-5 years of age as compared to children that are 6-15 years of age (9). However, the population analysis found that while age created a large difference in the objective function value when added individually to the base model, the 95% confidence interval of age's parameter estimate included the null value; age was not considered statistically significant. Additionally, weight and age were highly correlated covariates. Therefore age was not evaluated in further model development.

When the FO method was used in the analysis, two of the covariates identified as significant subsequently become insignificant when the FOCE method was used. Albuterol was identified as a statistically significant covariate for CL/F and ceftriaxone

for V2/F. Albuterol was given to 22% (13 out of 58) of the entire population, while ceftriaxone was given to 14% (8 out of 58). No plausible explanation for these effects could be found in the literature. However, it is interesting to note that many of the patients taking albuterol and ceftriaxone were also taking many concomitant medications. Additionally, all but one of the patients taking these medications were from the 054 study. The patients in study 054 were different from the children in the other three studies in that they received multiple concomitant medications and had more acute and chronic illnesses (all children were hospitalized); 12 of the 26 patients were cancer patients. Children in studies 043, 136, 172, and the non cancer patients in 054 were enrolled in their respective protocols for otitis media or pharyngitis (9-11). Consequently, all concomitant medications were taken by approximately half of the patients in study 054 (12 out of 58 patients, or 21%). Thus, this analysis may not have had sufficient power to fully evaluate the interacting potential of many of these drugs. When the FOCE method was used, both albuterol and ceftriaxone ceased to be significant in the model.

A previous analysis found a large interpatient variability in the parameter estimates for study 054 (9). In this study analysis, there were statistical differences ($p<0.0001$) in oral clearance in children <=5 years old (CL/F=4.27 L/hr/kg) compared to the group of children 6 years of age (2.27 L/hr/kg) and greater. In contrast the present analysis did not find age to be a statistically significant covariate for oral clearance.

Model misspecification may have led to the poor fit of the model for large concentration values. A two compartment model was found to best fit the data. However, more recent studies indicate that azithromycin follows three compartmental pharmacokinetics (29;30). This could possibly explain the under-prediction of concentrations at later times. In the previous analysis of study 054, a zero-order input was used to model drug absorption (9). In the present study, the data was best fit using both zero order input and simultaneous first order input. While the diagnostic plots and also the change in the objective function value signaled a better fitting model when both inputs were used, the plots still showed that there was bias and imprecision when calculating the predicted concentration values with this model. Additionally, the bias and imprecision in the model was seen with the results of the predictive performance using the validation dataset. In Figure 20, the predicted concentration values were consistently larger than the observed values. This finding would be indicative of a three compartment model being a potentially better fit. Further work could be done evaluating a three compartment model with various error models using the FOCE method.

Our models were generated using both FO and FOCE estimation methods in NONMEM. The FO estimation method obtains values for η values after the population parameter estimates have been obtained. Therefore these estimates of η are computed with the assumption that the variance model is that of the mean individual. The FOCE method does not use this assumption. The values of η are computed simultaneously with the population estimates (17;22). While using the FOCE method, some of the imprecision in the model was addressed, as seen in the improvement in the observed azithromycin

serum concentration versus predicted azithromycin serum concentration plots. However, the weighted residual plots showed an increase in bias for low concentration values.

In conclusion, this study has shown that population pharmacokinetic models can be used to model azithromycin serum concentration time data obtained after oral dosing in the pediatric population. A two-compartment model with a parallel NONMEM calculated zero-order and first order absorption rate constant was used to describe the concentration versus time data for the pediatric data pooled across four clinical studies. When using the FO method, weight and albuterol for CL/F and weight and ceftriaxone for V2/F were found to significantly decrease their respective parameter estimates. When using the FOCE method, weight for both CL/F and V2/F was found to significantly increase the value for their respective parameter estimates. The model using the FOCE method best describes the dataset, though there was still bias in the final model.

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Table 1. Features of Pediatric Studies Included in the Population Pharmacokinetic Analysis of Azithromycin

Protocol Number	Design	Azithromycin Oral Dosage	Total Number of Patients* Gender and Age
043**	Open label, noncomparative, non-randomized trial in patients with Group A beta-hemolytic streptococcal (GABHS) pharyngitis	Single dose 12 mg/kg for days 1-5	n=5 3 male; 2 female 2-12 years of age
054	Open label, noncomparative, non-randomized trial to characterize the disposition and tolerance of azithromycin after single and multiple oral doses in patients with and without cancer	Single dose 12 mg/kg on day 1; single dose 12 mg/kg on days 2-5	n=26 15 male; 11 female 0-15 years of age
136	Open label, noncomparative, non-randomized trial in patients with Group A beta-hemolytic streptococcal (GABHS) pharyngitis	Single dose 10 mg/kg on day 1; single dose 5 mg/kg on days 2-5	n=14 5 male; 9 female 6-15 years of age
172	Open label, noncomparative, non-randomized trial in patients with acute otitis media	Single dose 10 mg/kg on day 1; single dose 5 mg/kg on days 2-5	n=13 7 male;6 female 1-5 years of age

* Number of patients in study with measurable concentrations of azithromycin

** Protocol 043 was discontinued early due to poor study enrollment

Table 2. Characteristics of Pediatric Patients Evaluated in the Population Pharmacokinetic Analysis of Azithromycin

Characteristic	Model development dataset	Validation dataset
Total number of patients	46	12
Male	26	7
Female	20	5
Age (years)	5.85 (range=0-15)	4.83 (range=1-12)
Weight (kg)	26.5 (range=4-80)	25.2 (range=7-83)
Albuterol*	10	3
Amikacin*	5	1
Anemia*	6	1
Asthma	4	2
Cancer*	9	3
Captopril*	3	1
Ceftazidime*	4	2
Ceftriaxone*	6	2
Colitis	14	6
Digoxin*	4	1
Diphenhydramine*	4	1
Dopamine*	3	1
Fentanyl*	2	2
Furosemide*	5	1
Midazolam*	4	1
Morphine*	4	0
Nausea	9	1
Nystatin*	4	1
Pneumonia*	5	3
Ranitidine*	7	2
Sulfamethotrexate*	4	2
Ticarcillin*	5	0
Vancomycin*	10	4

* All of the children with these characteristics are from Protocol 054.

Table 3. Base Model Equations, Parameter Estimates, Percent Relative Standard Errors (%RSE), and 95% Confidence Intervals (Adult Dataset)

Parameters	Estimated Values	%RSE	95% Confidence Intervals
CL/F (L/h)=Θ_1	138	12.0	106,170
V1/F (L)= Θ_2	849	17.2	563,1140
Q/F (L/h)= Θ_3	240	16.6	162,318
V2/F (L)= Θ_4	2990	14.0	2170,3810
R (mg/h)= Θ_5	215	9.86	173,257
$\omega^2_{CL/F}$	0.229	41.6	0.042,0.416
σ^2	0.291	17.5	0.191,0.391

Abbreviations: CL/F = oral clearance, V1/F = oral volume of distribution in the central compartment, Q/F = intercompartmental oral clearance, V2/F = oral volume of distribution in the peripheral compartment, R= zero-order absorption rate constant, $\omega^2_{CL/F}$ =interpatient variability of CL/F, σ^2 =variance of residual error.

Table 4. Base Model Equations, Parameter Estimates, Percent Relative Standard Errors (%RSE), and 95% Confidence Intervals (Pediatric Model Development Dataset (80% of Data)) – FO Method

Parameters	Estimated Values	%RSE	95% Confidence Intervals
CL/F (L/h)=Θ_1	55.3	13.7	40.5,70.1
V1/F (L)= Θ_2	258	14.2	186,330
Q/F (L/h)= Θ_3	120	12.9	90,150
V2/F (L)= Θ_4	1900	12.3	1443,2357
ka (h^{-1})= Θ_5	1.86	14.5	1.33,2.39
R (mg/h)= Θ_6	89.6	11.1	70,109
$\omega^2_{CL/F}$	0.600	43.2	0.092,1.108
$\omega^2_{Q/F}$	0.245	39.6	0.055,0.435
$\omega^2_{V2/F}$	0.353	39.7	0.079,0.627
ω^2_{ka}	2.450	42.0	0.431,4.469
σ^2	0.215	24.9	0.109,0.321

Abbreviations: FO=first order, CL/F = oral clearance, V1/F = oral volume of distribution in the central compartment, Q/F = intercompartmental oral clearance, V2/F = oral volume of distribution in the peripheral compartment, ka= first-order absorption rate constant, R= zero-order absorption rate constant, $\omega^2_{CL/F}$ =interpatient variability of CL/F, $\omega^2_{Q/F}$ = interpatient variability of Q/F, $\omega^2_{V2/F}$ = interpatient variability of V2/F, ω^2_{ka} = interpatient variability of ka, σ^2 =variance of residual error.

Table 5. Comparison of Parameter Estimates for Adult and Pediatric Model

Development Dataset (80% of Data) on a Mean per kg Weight Basis

Parameters	Adult Parameter Estimate	Pediatric Model Development Parameter Estimate
CL/F (L/h/kg)=Θ_1	1.9	2.1
V1/F (L/kg)= Θ_2	11.5	9.7
Q/F (L/h/kg)= Θ_3	3.3	4.5
V2/F (L/kg)= Θ_4	40.6	71.7
ka (h^{-1})= Θ_5	--	1.86
R (mg/h/kg)= Θ_6	2.9	3.4

Abbreviations: CL/F = oral clearance, V1/F = oral volume of distribution in the central compartment, Q/F = intercompartmental oral clearance, V2/F = oral volume of distribution in the peripheral compartment, ka= first-order absorption rate constant, R= zero-order absorption rate constant

Table 6. Summary of Covariate Analysis (Model Development Dataset (80% of Data))

Covariate Analysis	Objective Function Value	Change in Objective Function Value
Step 1 Base Model	-2836.404	
Step 2 Add single covariate onto Base Model		
Age on CL/F *	-2994.688	158.284
Weight on CL/F	-2982.568	146.164
Albuterol on CL/F	-2950.983	114.579
Weight on V2/F	-2893.253	56.849
Age on V2/F	-2889.726	53.322
Nausea on V2/F	-2885.832	49.428
Diphenhydramine on V2/F	-2877.958	41.554
Morphine on ka	-2876.861	40.457
Midazolam on CL/F	-2873.100	36.696
Nausea on CL/F	-2866.496	30.092
Vancomycin on V2/F	-2866.369	29.965
Ticarcillin on CL/F	-2865.848	29.444
Anemia on CL/F	-2863.263	26.859
Colitis on V2/F	-2862.736	26.332
Furosemide on CL/F	-2861.176	24.772
Sulfamethotrexate on CL/F	-2858.981	22.577
Pneumonia on V2/F	-2858.892	22.488
Ceftriaxone on CL/F	-2858.552	22.148
Cancer on V2/F	-2856.941	20.537
Ceftriaxone on V2/F	-2856.429	20.025
Amikacin on CL/F	-2856.103	19.699
Dopamine on CL/F	-2855.336	18.932
Height on V2/F	-2854.314	17.910
Fentanyl on V2/F	-2852.693	16.289
Ticarcillin on V2/F	-2851.556	15.152
Ceftazidime on V2/F	-2849.378	12.974
Amikacin on V2/F	-2849.152	12.748
Digoxin on CL/F	-2848.791	12.387
Albuterol on V2/F	-2846.670	10.266
Pneumonia on CL/F	-2845.137	8.733
Dopamine on Q/F	-2842.936	6.532
Weight on Q/F	-2842.341	5.937
Furosemide on V2/F	-2842.154	5.750
Sulfamethotrexate on V2/F	-2841.608	5.204
Vancomycin on CL/F	-2841.493	5.089
Age on ka	-2840.979	4.575
Vancomycin on ka	-2840.791	4.387

Covariate Analysis	Objective Function Value	Change in Objective Function Value
Dopamine on V2/F	-2840.533	4.129
Ticarcillin on ka	-2840.486	4.082
Albuterol on ka	-2840.394	3.990
Weight on ka	-2840.354	3.950
Anemia on ka	-2840.133	3.729
Step 3 Add single covariate onto base model with weight on CL/F		
Albuterol on CL/F	-3029.745	47.177
Weight on V2/F	-3017.325	34.757
Age on V2/F	-3013.095	30.527
Colitis on V2/F	-3007.163	24.595
Ticarcillin on CL/F	-3003.158	20.590
Morphine on ka	-3002.987	20.419
Ceftriaxone on V2/F	-3000.477	17.909
Age on CL/F	-3000.030	17.462
Sulfamethotrexate on CL/F	-2997.110	14.542
Anemia on CL/F	-2996.912	14.344
Ceftriaxone on CL/F	-2995.645	13.077
Albuterol on ka	-2995.220	12.652
Weight on Q/F	-2994.211	11.643
Dopamine on Q/F	-2993.971	11.403
Furosemide on V2/F	-2992.992	10.424
Ticarcillin on ka	-2992.288	9.720
Ticarcillin on V2/F	-2990.290	7.722
Vancomycin on V2/F	-2989.823	7.255
Fentanyl on V2/F	-2989.478	6.910
Furosemide on CL/F	-2989.346	6.778
Midazolam on CL/F	-2988.727	6.159
Age on ka	-2988.611	6.043
Weight on ka	-2988.255	5.687
Amikacin on V2/F	-2987.120	4.552
Dopamine on CL/F	-2986.693	4.125
Vancomycin on ka	-2986.552	3.984
Amikacin on CL/F	-2985.769	3.201
Sulfamethotrexate on V2/F	-2985.578	3.010
Dopamine on V2/F	-2985.553	2.985
Diphenhydramine on V2/F	-2985.530	2.962
Cancer on V2/F	-2985.397	2.829
Vancomycin on CL/F	-2985.362	2.794
Height on V2/F	-2985.131	2.563
Albuterol on V2/F	-2985.095	2.527

Covariate Analysis	Objective Function Value	Change in Objective Function Value
Ceftazidime on V2/F	-2984.932	2.364
Nausea on V2/F	-2984.047	1.479
Digoxin on CL/F	-2983.903	1.335
Pneumonia on CL/F	-2982.725	0.157
Nausea on CL/F	-2982.570	0.002
Pneumonia on V2/F	-2982.377	-0.191
Step 4 Add single covariate onto base model with weight on CL/F, and albuterol on CL/F		
Weight on V2/F *	-3070.877	41.132
Age on V2/F *	-3062.283	32.538
Age on CL/F *	-3052.624	22.879
Ceftriaxone on V2/F	-3049.137	19.392
Colitis on V2/F	-3047.466	17.721
Furosemide on V2/F	-3044.101	14.356
Ticarcillin on CL/F	-3043.946	14.201
Anemia on CL/F	-3040.798	11.053
Morphine on ka	-3040.763	11.018
Sulfamethotrexate on CL/F	-3038.156	8.411
Dopamine on Q/F	-3035.479	5.734
Ticarcillin on ka	-3035.289	5.544
Ticarcillin on V2/F	-3034.070	4.325
Weight on ka	-3033.796	4.051
Amikacin on V2/F	-3033.720	3.975
Ceftriaxone on CL/F	-3032.002	2.257
Midazolam on CL/F	-3030.397	0.652
Age on ka	-3030.153	0.408
Dopamine on CL/F	-3029.803	0.058
Furosemide on CL/F	-3029.751	0.006
Albuterol on ka	-3027.386	-2.359
Vancomycin on ka	-3025.119	-4.626
Weight on Q/F	-3023.072	-6.673
Step 5 Add single covariate onto base model with weight on CL/F, albuterol on CL/F, and ceftriaxone on V2/F		
Weight on V2/F	-3082.405	33.268
Age on V2/F	-3078.131	28.994
Age on CL/F	-3074.059	24.922
Morphine on ka	-3062.637	13.500
Furosemide on V2/F	-3061.865	12.728
Ticarcillin on CL/F	-3057.697	8.560
Dopamine on Q/F	-3057.359	8.222

Covariate Analysis	Objective Function Value	Change in Objective Function Value
Colitis on V2/F	-3056.345	7.208
Ticarcillin on ka	-3054.317	5.180
Weight on ka	-3054.127	4.990
Ticarcillin on V2/F	-3053.223	4.086
Amikacin on V2/F	-3052.872	3.735
Sulfamethotrexate on CL/F	-3050.762	1.625
Anemia on CL/F	-3050.621	1.484
Step 6 Add single covariate onto base model with weight on CL/F, albuterol on CL/F, ceftriaxone on V2/F, and weight on V2/F		
Age on CL/F*	-3096.981	14.576
Dopamine on Q/F	-3089.298	6.893
Morphine on ka	-3088.954	6.549
Weight on ka	-3087.621	5.216
Ticarcillin on ka	-3087.414	5.009
Ticarcillin on CL/F	-3086.086	3.681
Furosemide on V2/F	-3085.382	2.977
Ticarcillin on V2/F	-3084.643	2.238
Age on V2/F	-3083.425	1.020
Colitis on V2/F	-3082.406	0.001
Step 7 Add single covariate onto base model with weight on CL/F, albuterol on CL/F, ceftriaxone on V2/F, weight on V2/F, and dopamine on Q/F		
Age on CL/F *	-3102.415	13.117
Morphine on ka	-3094.727	5.429
Ticarcillin on ka	-3094.110	4.812
Weight on ka	-3092.860	3.562
Step 8 Add single covariate onto base model with weight on CL/F, albuterol on CL/F, ceftriaxone on V2/F, weight on V2/F, dopamine on Q/F, and morphine on ka		
Ticarcillin on ka	-3095.264	0.537

* 95% confidence interval of parameter estimate includes the null value.

Abbreviations: CL/F = oral clearance, Q/F = intercompartmental oral clearance, V2/F = oral volume of distribution in the peripheral compartment, ka= first-order absorption rate constant

Table 7. Backward Elimination Results from Full Model (Model Development Dataset (80% of Data))- FO Method

Parameter	Value of parameter	Change in objective function value	p Value
CL/F			
Weight	$\Theta_7=0$	89.5	< 0.005
Albuterol	$\Theta_8=0$	57.0	< 0.005
V2/F			
Ceftriaxone	$\Theta_9=0$	9.5	< 0.005
Weight	$\Theta_{10}=0$	33.3	< 0.005
Q/F			
Dopamine	$\Theta_{11}=0$	6.9	NS
ka			
Morphine	$\Theta_{12}=0$	5.4	NS

Abbreviations: FO=first order, CL/F = oral clearance, V2/F = oral volume of distribution in the peripheral compartment, Q/F = intercompartmental oral clearance, ka= first-order absorption rate constant, NS=not significant.

Table 8. Final Model Equations, Parameter Estimates, Percent Relative Standard Errors (%RSE), and 95% Confidence Intervals (Model Development Dataset (80% of Data)) – FO Method

Parameters	Estimated Value	%RSE	95% Confidence Intervals
CL/F (L/h)=Θ_1 + Θ_7·weight+ Θ_8·albuterol			
Θ_1	43.9	14.6	31.4,56.4
Θ_7	1.22	24.3	0.64,1.80
Θ_8	–20.1	20.4	-28.1,-12.1
V1/F (L)= Θ_2	258	12.7	194,322
Q/F (L/h)= Θ_3	110	10.6	87,133
V2/F (L)= Θ_4+ Θ_9·cefr+ Θ_{10}·weight			
Θ_4	1270	14.8	902,1638
Θ_9	-588	18.9	-806,-370
Θ_{10}	32.4	20.4	19.5,45.3
ka (h^{-1})= Θ_5	1.81	15.5	1.26,2.36
R (mg/h)= Θ_6	105	14.6	75,135
$\omega^2_{CL/F}$	0.769	39.5	0.173,1.365
$\omega^2_{Q/F}$	0.286	44.1	0.039,0.533
$\omega^2_{V2/F}$	---	---	---
ω^2_{ka}	2.26	36.3	0.65,3.87
σ^2	0.23	20.0	0.14,0.32

Abbreviations: FO=first order, CL/F = oral clearance, V1/F = oral volume of distribution in the central compartment, Q/F = intercompartmental oral clearance, V2/F = oral volume of distribution in the peripheral compartment, ka= first-order absorption rate constant, R= zero-order absorption rate constant, $\omega^2_{CL/F}$ =interpatient variability of CL/F, $\omega^2_{Q/F}$ = interpatient variability of Q/F, $\omega^2_{V2/F}$ = interpatient variability of V2/F, ω^2_{ka} = interpatient variability of ka, σ^2 =variance of residual error, cefr=ceftriaxone use.

Table 9. Predictive Performance of Azithromycin Population Pharmacokinetic Model for Validation Dataset (20% of Data) – FO Method

Parameter	Value (ug/mL)	s.d. (ug/mL)	95% Confidence Interval (ug/mL)
Precision	0.1026	0.1644	(0.0781,0.1223)
Bias	0.0167	0.1016	(0.0001,0.0334)

Abbreviations: FO=first order, s.d.= standard deviation

Table 10. Final Model Equations, Parameter Estimates, Percent Relative Standard Errors (%RSE), and 95% Confidence Intervals with 100% of the Pediatric Data using FO Method

Parameters	Estimated Value	%RSE	95% Confidence Intervals
CL/F (L/h)=Θ_1 + Θ_7·weight+ Θ_8·albuterol			
Θ_1	43.5	15.9	29.9,57.1
Θ_7	1.47	20.0	0.89,2.05
Θ_8	–20.7	25.1	-30.9,-10.5
V1/F (L)= Θ_2	307	14.8	218,396
Q/F (L/h)= Θ_3	114	10.8	90,138
V2/F (L)= Θ_4+ Θ_9·cefr+ Θ_{10}·weight			
Θ_4	1230	13.3	911,1549
Θ_9	-597	19.3	-822,-372
Θ_{10}	38	26.0	26,50
ka (h^{-1})= Θ_5	1.94	14.9	1.37,2.51
R (mg/h)= Θ_6	106	12.4	80,132
$\omega^2_{CL/F}$	0.887	38.7	0.215,1.559
$\omega^2_{Q/F}$	0.293	39.6	0.066,0.520
$\omega^2_{V2/F}$	---	---	---
ω^2_{ka}	2.26	38.9	0.54,3.98
σ^2	0.31	25.4	0.16,0.46

Abbreviations: FO=first order, CL/F = oral clearance, V1/F = oral volume of distribution in the central compartment, Q/F = intercompartmental oral clearance, V2/F = oral volume of distribution in the peripheral compartment, ka= first-order absorption rate constant, R= zero-order absorption rate constant, $\omega^2_{CL/F}$ =interpatient variability of CL/F, $\omega^2_{Q/F}$ = interpatient variability of Q/F, $\omega^2_{V2/F}$ = interpatient variability of V2/F, ω^2_{ka} = interpatient variability of ka, σ^2 =variance of residual error, cefr=ceftriaxone use.

Table 11. Final Model Equations, Parameter Estimates, Percent Relative Standard Errors (%RSE), and 95% Confidence Intervals with 100% of the Pediatric Data Using FOCE Method

Parameters	Estimated Value	%RSE	95% Confidence Intervals
CL/F (L/h)=$\Theta_1 + \Theta_7 \cdot$weight			
Θ_1	18.2	31.3	7.1,29.4
Θ_7	2.25	14.3	1.62,2.88
V1/F (L)= Θ_2	134	21.3	78,190
Q/F (L/h)= Θ_3	103	11.1	81,125
V2/F (L)= $\Theta_4 + \Theta_8 \cdot$weight			
Θ_4	1180	14.2	851,1510
Θ_8	26.9	15.4	18.8,35.0
ka (h^{-1})= Θ_5	0.656	17.2	0.435,0.877
R (mg/h)= Θ_6	187	28.3	83,291
$\omega^2_{CL/F}$	0.428	20.0	0.260,0.596
ω^2_{ka}	0.761	40.2	0.160,1.360
σ^2	0.287	22.1	0.163,0.411

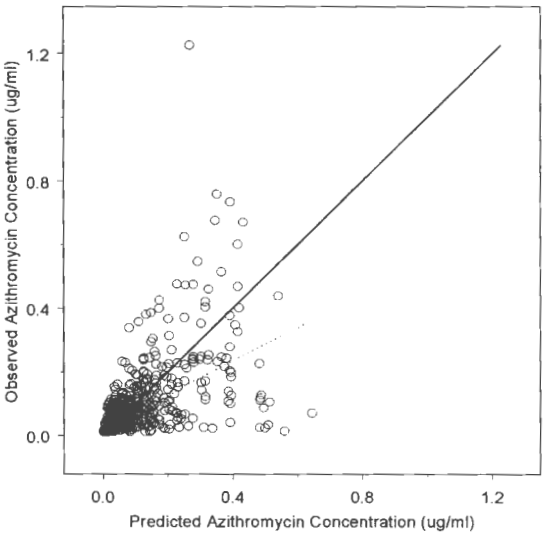
Abbreviations: FOCE=first order conditional estimation, CL/F = oral clearance, V1/F = oral volume of distribution in the central compartment, Q/F = intercompartmental oral clearance, V2/F = oral volume of distribution in the peripheral compartment, ka= first-order absorption rate constant, R= zero-order absorption rate constant, $\omega^2_{CL/F}$ =interpatient variability of CL/F, ω^2_{ka} = interpatient variability of ka, σ^2 =variance of residual error.

Table 12. Predictive Performance of Azithromycin Population Pharmacokinetic Model for Validation Dataset (20% of Data) – FOCE Method

Parameter	Value (ug/mL)	s.d. (ug/mL)	95% Confidence Interval (ug/mL)
Precision	0.1048	0.1699	(0.0791,0.1253)
Bias	0.0236	0.1025	(0.0068,0.0404)

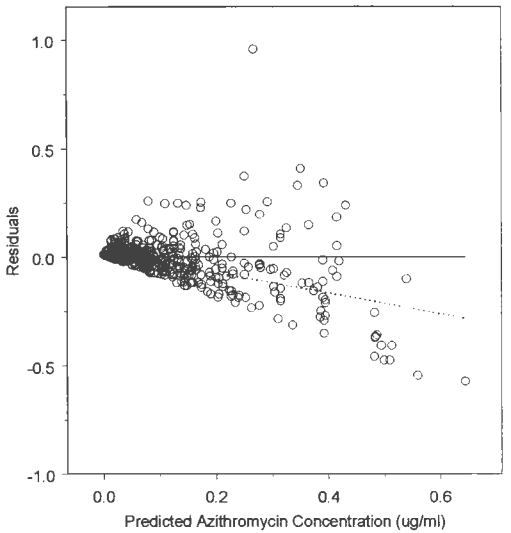
Abbreviations: FOCE=first order conditional estimation, s.d.= standard deviation

Figure 1. Observed Azithromycin Serum Concentration versus Predicted Azithromycin Serum Concentration Values for the Base Model (80% of data) – FO Method



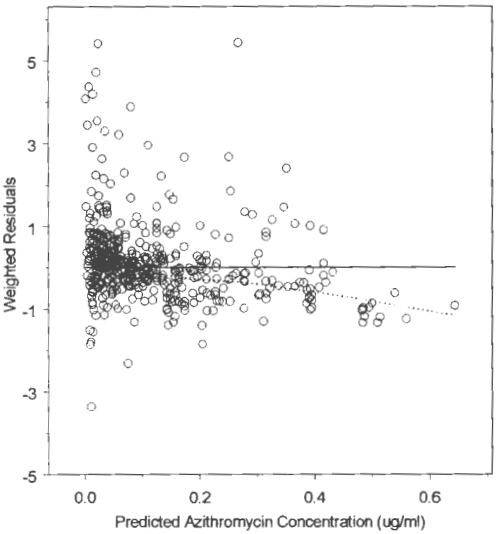
Abbreviations: FO=first order

Figure 2. Residuals versus Predicted Azithromycin Serum Concentration Values for the Base Model (80% of data) – FO Method



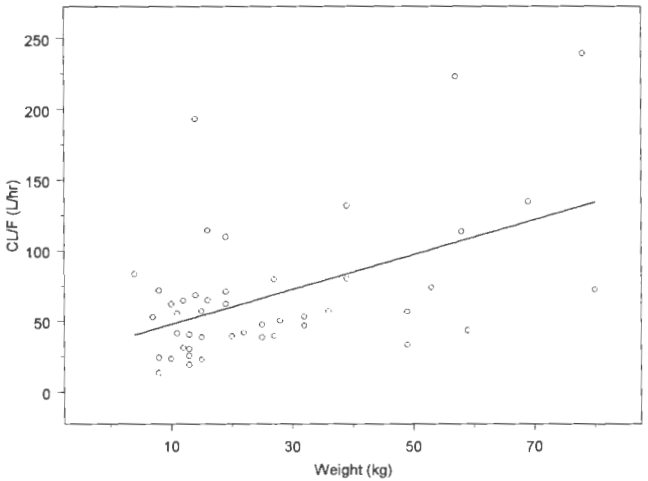
Abbreviations: FO=first order

Figure 3. Weighted Residuals versus Predicted Azithromycin Serum Concentration Values for the Base Model (80% of data) – FO Method



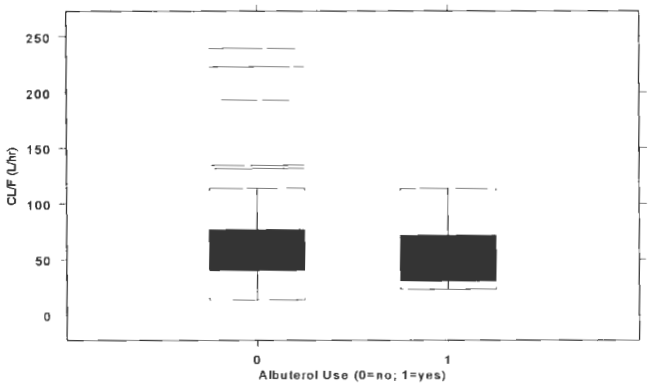
Abbreviations: FO=first order

Figure 4. CL/F versus Weight for Base Model parameter estimates (80% of data) – FO Method



Abbreviations: CL/F = clearance, FO=first order

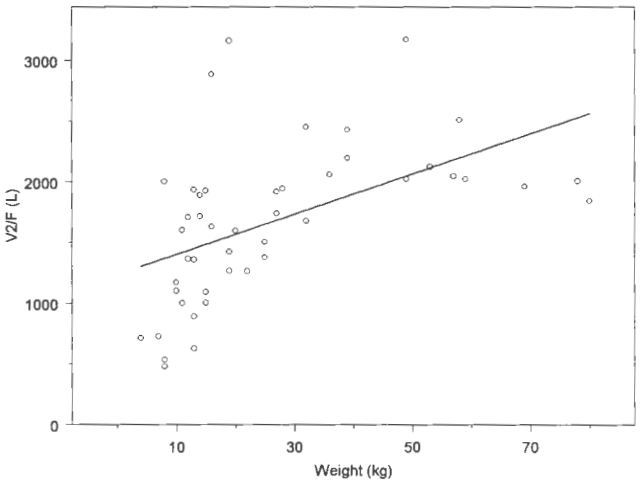
Figure 5. CL/F versus Albuterol for Base Model Parameter Estimates (80% of data) – FO Method



no albuterol use: n=36; albuterol use: n=10

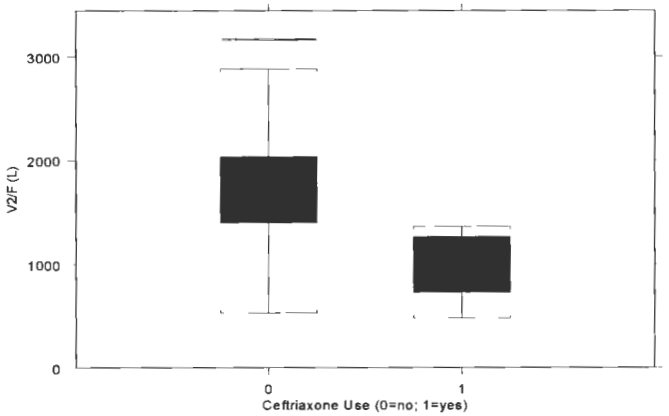
Abbreviations: CL/F = clearance, FO=first order

Figure 6. V2/F versus Weight for Base Model Parameter Estimates (80% of data) – FO Method



Abbreviations: V2/F = oral volume of distribution in the peripheral compartment, FO=first order

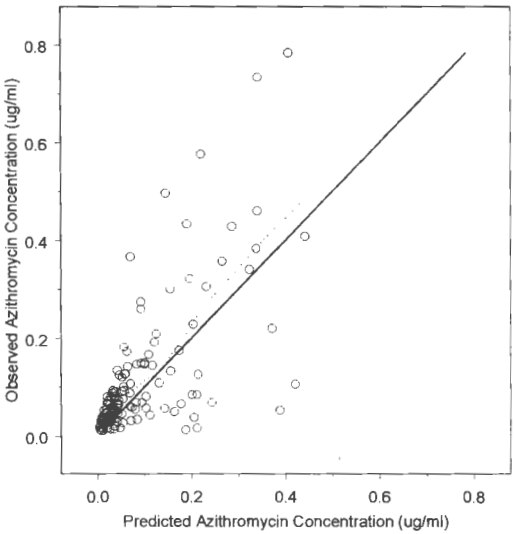
Figure 7. V2/F versus Ceftriaxone for Base Model Parameter Estimates (80% of data) – FO Method



no ceftriaxone use: n=40; ceftriaxone use: n=6

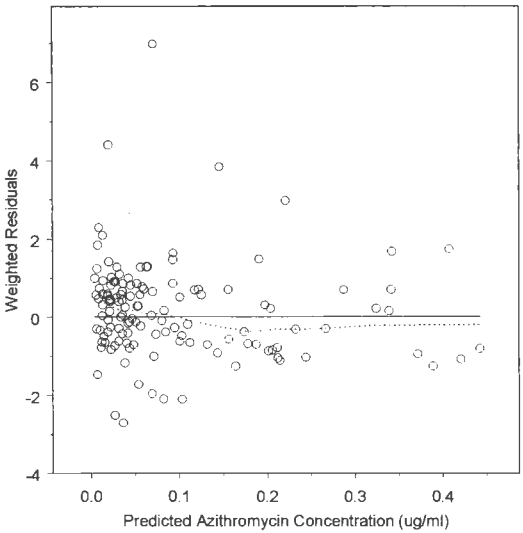
Abbreviations: V2/F = oral volume of distribution in the peripheral compartment, FO=first order

Figure 8. Observed Azithromycin Serum Concentration versus Predicted Azithromycin Serum Concentration Values for the Validation Dataset (using Final Estimates from Final Model with 80% data) – FO Method



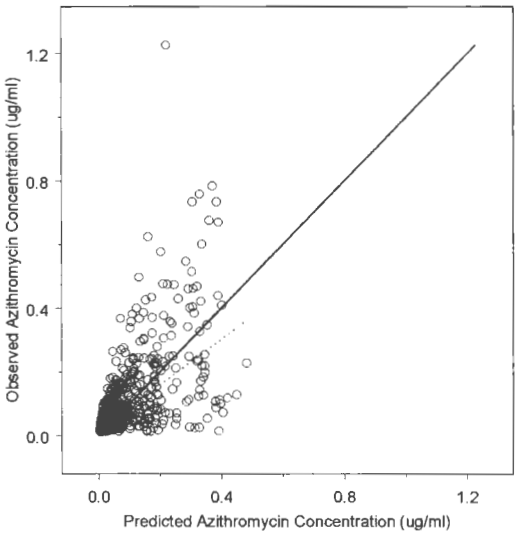
Abbreviations: FO=first order

Figure 9. Weighted Residuals versus Predicted Azithromycin Serum Concentration Values for the Validation Dataset (using Final Estimates from Final Model with 80% data) – FO Method



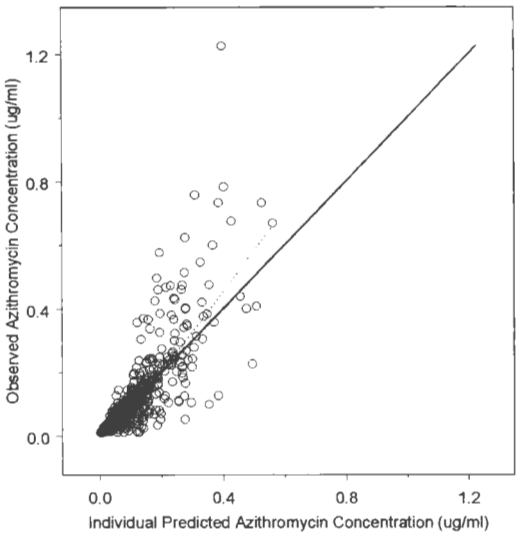
Abbreviations: FO=first order

Figure 10. Observed Azithromycin Serum Concentration versus Predicted Serum Azithromycin Concentration Values for 100% of the Pediatric Data Using FO Method



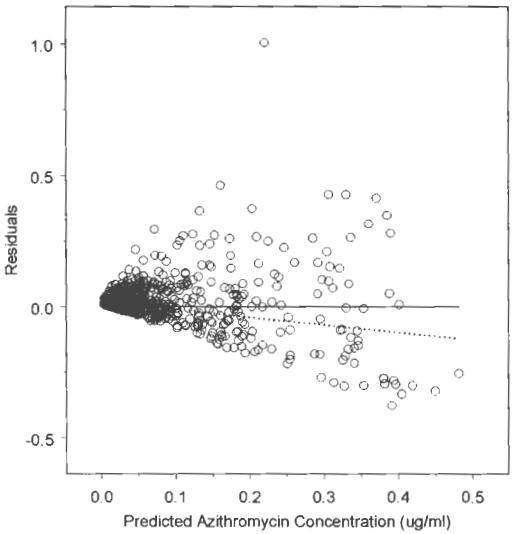
Abbreviations: FO=first order

Figure 11. Observed Azithromycin Serum Concentration versus Individual Predicted Serum Azithromycin Concentration Values for 100% of the Pediatric Data Using FO Method



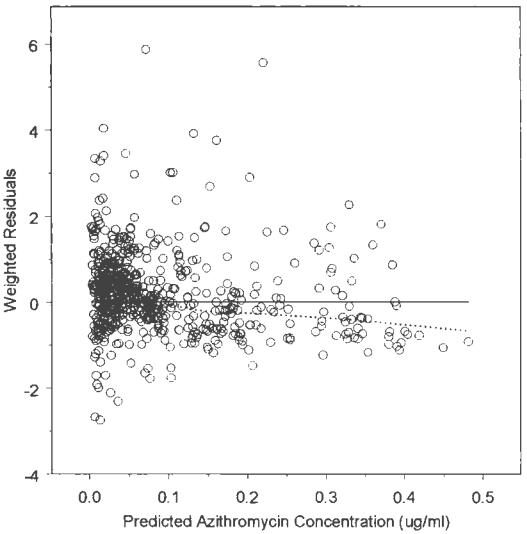
Abbreviations: FO=first order

Figure 12. Residuals versus Predicted Azithromycin Serum Concentration Values for 100% of the Pediatric Data Using FO Method



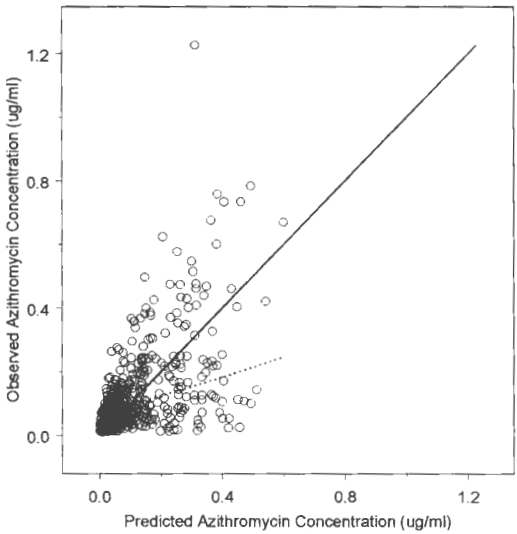
Abbreviations: FO=first order

Figure 13. Weighted Residuals versus Predicted Azithromycin Serum Concentration Values for 100% of the Pediatric Data Using FO Method



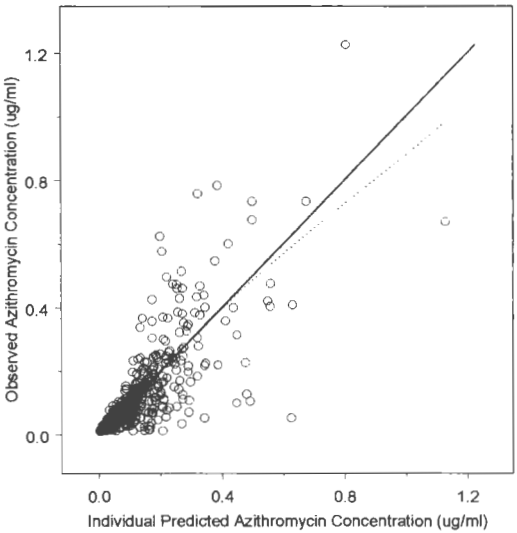
Abbreviations: FO=first order

Figure 14. Observed Azithromycin Serum Concentration versus Predicted Serum Azithromycin Concentration Values for 100% of the Pediatric Data Using FOCE Method



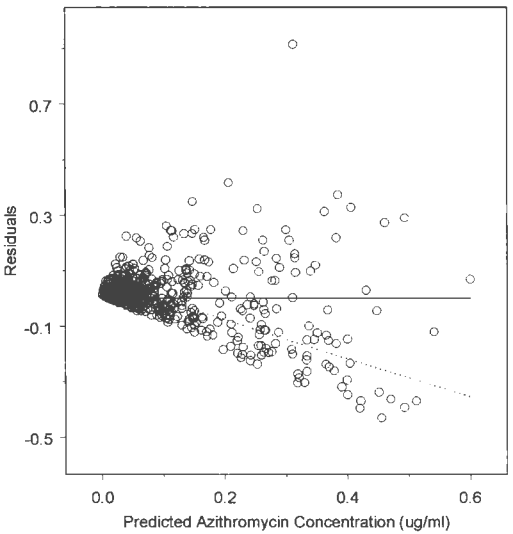
Abbreviations: FOCE=first order conditional estimation

Figure 15. Observed Azithromycin Serum Concentration versus Individual Predicted Serum Azithromycin Concentration Values for 100% of the Pediatric Data Using FOCE Method



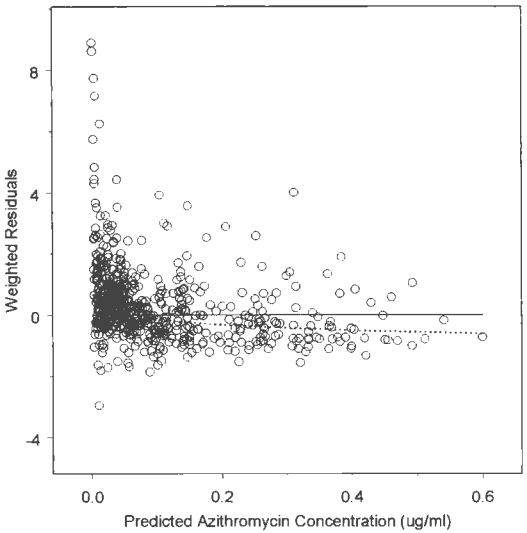
Abbreviations: FOCE=first order conditional estimation

Figure 16. Residuals versus Predicted Azithromycin Serum Concentration Values for 100% of the Pediatric Data Using FOCE Method



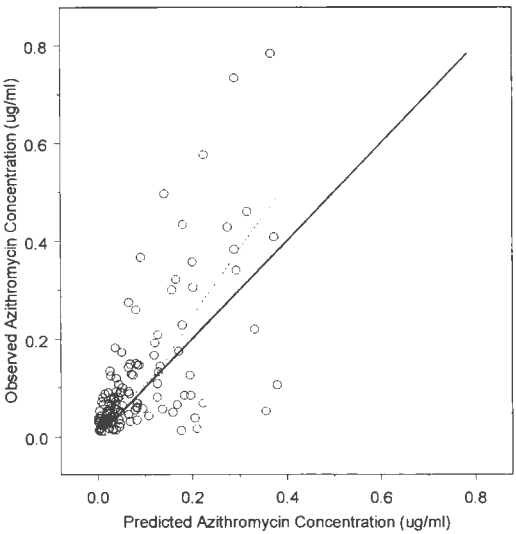
Abbreviations: FOCE=first order conditional estimation

Figure 17. Weighted Residuals versus Predicted Azithromycin Serum Concentration Values for 100% of the Pediatric Data Using FOCE Method



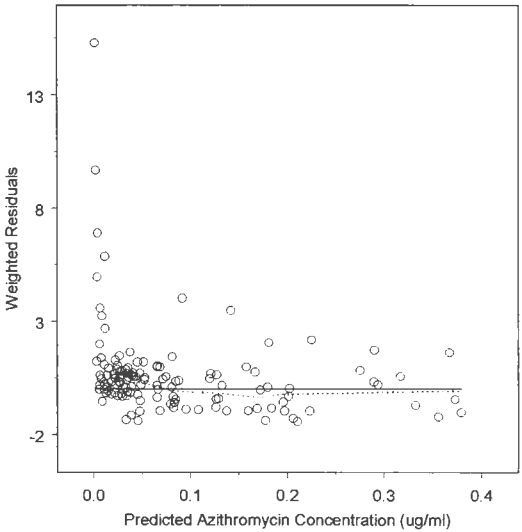
Abbreviations: FOCE=first order conditional estimation

Figure 18. Observed Azithromycin Serum Concentration versus Predicted Azithromycin Serum Concentration Values for the Validation Dataset (using Final Estimates from Final Model with 80% data) – FOCE Method



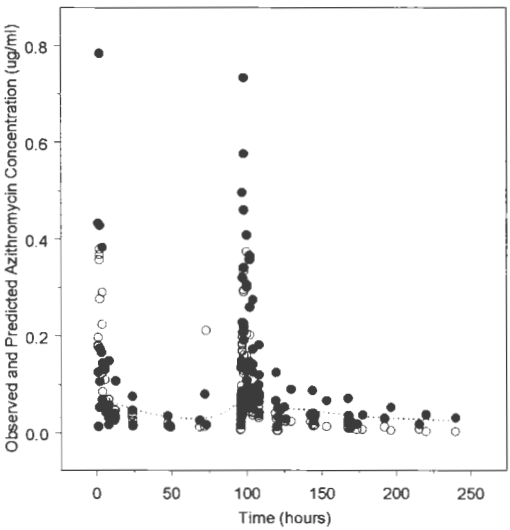
Abbreviations: FOCE=first order conditional estimation

Figure 19. Weighted Residuals versus Predicted Azithromycin Serum Concentration Values for the Validation Dataset (using Final Estimates from Final Model with 80% data) – FOCE Method



Abbreviations: FOCE=first order conditional estimation

Figure 20. Observed and Predicted Azithromycin Serum Concentration Values versus Time for the Validation Dataset (using Final Estimates from Final Model with 80% data) – FOCE Method



Open Circle: Observed Serum Concentration
Closed Circle: Predicted Serum Concentration
Abbreviations: FOCE=first order conditional estimation

MANUSCRIPT III

Population Pharmacokinetics of Prednisolone in Heart and Lung Transplant Patients

ABSTRACT

A population pharmacokinetic analysis was conducted for prednisolone on data from thoracic organ transplant patients. The data consisted of 496 plasma prednisolone concentrations and 496 plasma prednisone concentrations from 41 patients administered total daily oral doses of prednisolone ranging from 5-80 mg per day. A population pharmacokinetic analysis was conducted in NONMEM on estimated unbound prednisolone concentrations. A one compartment model with first order rate of absorption was found to best fit the data. Potential covariates were assessed for oral clearance (CL/F) and oral volume of distribution (V/F). Sex and concurrent ciprofloxacin use were found to be significant covariates for CL/F. No covariates were found to be significant for V/F. Neither age, weight, type of transplant, presence of cystic fibrosis, or concurrent use of acyclovir, amphotericin, cefotaxime, ceftazidime, flucloxacillin, ganciclovir, imipenem, itraconazole, lyposomal amphotericin, nor septrin appeared to be significant for any pharmacokinetic parameter. The unbound prednisolone and total prednisone concentration data were simultaneously modeled using the final parameter estimate of the apparent volume of distribution from the analysis of prednisolone alone and a literature value for the apparent volume of distribution of

prednisone was used. It was not possible to obtain meaningful models for the simultaneous modeling of the prednisolone/prednisone data.

INTRODUCTION

Prednisolone, a synthetic corticosteroid, is an important immunosuppressant used in organ transplant patients to suppress allograft rejection. Transplant patients are often treated chronically with empirically determined prednisolone doses (1;2). Because of the lack of individual dosing regimens, the tendency for increased serious adverse events or sub-therapeutic concentrations is great. The future of immunosuppression therapy will be geared towards individualizing a patient's dose (3).

General steroid information

Corticosteroids are prescribed for their immunosuppressive and anti-inflammatory effects. These effects are produced by the binding of the steroid to cytosolic receptors in many different tissues. These activated receptors then go on to the cell nucleus and increase the transcription of certain genes that regulate the synthesis of specific proteins, second messengers, or enzymes (2). Some effects are seen immediately (e.g. changes in cortisol plasma concentrations) and appear directly related to the pharmacokinetics of the steroid; other effects (e.g. eosinophil counts), have a slow onset (6-8 hours) and slow dissipation of the response (24-36 hours) back to baseline (2;4).

Because steroids are nonselective immunosuppressants (i.e. they affect many gene-mediated responses simultaneously), these drugs may predispose patients to a greater risk of infections and other side effects (5). These adverse events may include: cushingoid features, hemorrhage, psychoses, myopathy, osteoporosis, cataracts, hyperlipidemia, growth retardation in children, and hypertension (2;6-8). Often the

frequency of side effects are increased in patients undergoing chronic therapy, patients with low serum albumin concentrations, and patients receiving certain concomitant medications (e.g. oral contraceptives) that affect the protein binding and metabolism of prednisolone; all factors that increase a patient's steroid exposure. Patients with low serum albumin concentrations may have greater steroid exposure due to altered protein binding and/or a reduced hepatic function (2;9). A study of 240 medical inpatients receiving prednisone showed a correlation between the frequency of side effects, the mean daily prednisone dose, and the serum-albumin levels. Side effects were more common with those patients that received higher prednisone doses and in patients with low serum-albumin concentrations (4;9).

Prednisolone Pharmacokinetics

For immunosuppression of organ transplants, prednisolone is administered either orally or intravenously. When prednisolone is administered orally, it is administered as prednisolone or as its prodrug prednisone, which is metabolized to active prednisolone (2;10;11).

Absorption

Oral prednisolone has a bioavailability (F) of 60-100% (2;11-14). The lower bioavailability has been seen with higher steroid doses. Patients who exhibit sub-therapeutic responses with prednisolone often experience poor absorption of drug (4).

Prednisolone has a prompt rate of absorption with a peak concentration (Cmax) occurring between one and two hours post dose (Tmax) (1;2;4;15-17).

Distribution

The reversible binding of drug to proteins follows the law of mass action:

$$[D]+[P]\underset{k_2}{\overset{k_1}{\rightleftarrows}}[DP]$$

where D= the molar concentration of unbound drug, P= the unoccupied protein, DP= the drug protein complex, k1= the forward rate constant, and k2= the reverse rate constant. The ratio of k1/k2 is known as the equilibrium association constant or affinity constant (Ka) (18;19). Ka provides information as to the affinity between the drug and its binding site on the protein; drugs that are strongly protein bound have large values of Ka (19). The inverse of Ka (i.e. 1/Ka) is known as the equilibrium dissociation constant (Kd).

A drug's extent and ability to bind to proteins will affect its pharmacokinetic parameters, specifically clearance and volume of distribution (18;19). In the typical therapeutic concentration range for most drugs, the fraction unbound remains constant; only a small fraction of the binding sites on proteins are occupied. For a given concentration of protein, the fraction unbound is constant. Consequently, the pharmacokinetic parameters of most drugs are independent of dose (19). However, for some drugs, protein binding varies with concentration level and thus these drugs exhibit concentration dependent pharmacokinetics.

Plasma protein binding of prednisolone appears to be dose related, resulting in nonlinear pharmacokinetics (2;4;10;16;17;20-24). The nonlinearity is attributed to prednisolone binding to two different proteins; transcortin (i.e. corticosteroid binding globulin) which exhibits a low capacity and high affinity for prednisolone and albumin which exhibits a high capacity and low affinity for prednisolone (2;4;9;20;23-27). Prednisolone protein binding can be expressed:

$$D_b = \frac{N_t P_t K_t D_f}{(1 + K_t D_f)} + \frac{N_a P_a K_a D_f}{(1 + K_a D_f)}$$

where D_b is the concentration of prednisolone bound to both transcortin and albumin sites, N_t is the number of binding sites for transcortin, K_t is the affinity constant for transcortin, P_t is the molar concentration of transcortin protein, N_a is the number of binding sites for albumin, K_a is the affinity constant for albumin, P_a is the molar concentration of albumin, and D_f is the unbound concentration of prednisolone (16;23;24;28;29). Assuming $K_a D_f << 1$, there is one prednisolone binding site per molecule of albumin, and prednisolone only binds to transcortin and albumin (16;23;24;28;29), then bound concentration of prednisolone can be reduced to:

$$D_b = \frac{N_t P_t K_t D_f}{(1 + K_t D_f)} + P_a K_a D_f$$

Nonlinear prednisolone protein binding occurs because of limited concentrations of transcortin in plasma (24). At low concentrations of prednisolone, binding to transcortin is maximal at 90-95%, but at large concentrations, saturation occurs thus producing only 60% transcortin binding (2;4;10;24;30). Therefore at low doses, the increased fraction bound of prednisolone to transcortin makes less prednisolone available to distribute to

receptor sites (2). The binding capacity (N_tP_t) and Ka of prednisolone to transcortin have been reported to be (5.45-8.00)x10^{-7} M and (1.40-3.39)x10^{7} L/M respectively (2;9;18;28;31). The normal concentration of transcortin in plasma is approximately 0.7 μM and falls in proportion with serum-albumin levels (9). It is thought that only unbound prednisolone is biologically active (4;10;22). Cortisol also complicates the binding of prednisolone since it competes with prednisolone for binding sites to transcortin (31). There appears to be a circadian cycle affecting the binding capacity of transcortin to prednisolone; binding is least at 8a.m. when cortisol levels are high and greatest at midnight when cortisol levels are low (2;32).

The other protein that prednisolone extensively binds to is albumin. The binding capacity (N_aP_a) and Ka of prednisolone to albumin have been reported to be (6.23-7.00)x10^{-4} M and (1.40-3.00) x 10^{3} L/M, respectively (2;9;24;28;31). The normal concentration of albumin in plasma is 35-55 g/L (2;19;31). Albumin concentration decreases with age and is lower in cystic fibrosis patients (19).

At oral prednisolone doses of 15mg and 50mg, the protein binding of prednisolone has been reported at 87% and 74% respectively (19). The dose dependency of prednisolone pharmacokinetics has been primarily attributed to nonlinear protein binding (4). The apparent steady state volume of distribution based on total and unbound prednisolone concentrations were reported to be 35-114 L and 323-530 L respectively for doses ranging from 1.25 mg eight times daily to 100 mg once daily; larger doses produced larger values total prednisolone volume of distribution, while the free prednisolone

volume of distribution was not dose dependent (17). The apparent volume of distribution for total prednisone was reported to be 0.97 L/kg (33).

Metabolism

Prednisolone is extensively metabolized by both the liver and kidney (2;10;22;34). Prednisolone and prednisone undergo biotransformation to a variety of oxidation products (4;24;35). The four most important metabolites for prednisolone reported are: prednisone, 20-β-hydroxyprednisolone, 6-β-hydroxyprednisolone, and 20-α-hydroxyprednisolone. Another minor metabolite that has been reported is 20-β-hydroxyprednisone (11).

As discussed earlier, the bioavailability of prednisolone is high, thus there is limited presystemic metabolism of prednisolone (36). Prednisolone displays restrictive clearance; clearance is sensitive to fraction unbound in the plasma and the activity of the drug metabolizing enzymes (2;19;34).

Prednisolone undergoes reversible metabolism (interconversion) to prednisone. The enzyme 11-β-hydroxydehydrogenase is responsible for the interconversion process (10;13;20). Prednisone is also reconverted back to prednisolone. The interconversion of prednisolone and prednisone has been reported to be a nonlinear process. This nonlinearity can be seen in the area under the concentration versus time curve (AUC) for prednisolone and prednisone. The ratio of AUC prednisolone/AUC prednisone increases with increasing doses of prednisolone (2;11;20;24). If the interconversion were linear,

the ratio of the AUCs would remain constant with increasing doses; as the concentration of one of the steroids increases, the other steroid would increase in a proportional manner. Prednisolone dominates the interconversion process and the prednisolone concentrations can be as much as 10 times the prednisone concentrations (2;4;11;21;24). The dose-dependent interconversion of prednisone and prednisolone complicates an assessment of the pharmacokinetic parameters of prednisolone (2;22;24;29). Conventional linear pharmacokinetic parameter calculation methods that assume no interconversion underestimate clearance and overestimate volume of distribution (37). The absence of an intravenous formulation of prednisone for humans makes the exact assessment of the interconversion process difficult (10).

Excretion

The unchanged prednisolone and metabolites recovered in the urine from a dose of intravenous prednisolone are reported to be approximately 42% (11). Prednisolone, prednisone, and 6-β-hydroxyprednisolone recovered in the urine are reported to be approximately 20%, 2%, and 6-10% of the dose respectively (2;4;11;13;38). Three other metabolites, 20-β-hydroxyprednisolone, 20-α-hydroxyprednisolone, 20-β-hydroxyprednisone have reported values recovered in urine around 7%, 5%, and 0.6% respectively (11).

Garg *et.al.* preformed an extensive analysis on the interconversion of prednisolone and prednisone (11). They performed a two-way crossover study between two treatments: oral prednisone tablets and intravenous prednisolone sodium phosphate. They found the

irreversible elimination clearances of prednisone and prednisolone to be 53.9 ml/min and 196 ml/min respectively. The clearance for the conversion of prednisolone to prednisone was reported as 836 ml/min. The clearance for the conversion of prednisone to prednisolone was reported as 8822 ml/min; 10 times higher then the clearance of prednisolone to prednisone. Since the clearance of prednisone not reconverted to prednisolone was relatively small and the clearance of prednisone reconverted to prednisolone was relatively high, this implied that most prednisone was converted to prednisolone. The recycled fraction (RF), the probability of a molecule being converted to its metabolite and back at least once, was reported to be 0.76 for prednisolone. A large RF indicates a greater role of interconversion between a drug and its metabolite. A RF of 0.76 suggests that a large interconversion process occurs between prednisolone and prednisone (11).

As reported by Jusko *et.al.*, in a study with six normal male subjects dosed 5mg, 20mg, and 50mg of oral prednisone, the oral clearance of total prednisolone was around 8, 12, and 16 L/h respectively (4). The increase in oral clearance was statistically significant. Rohatagi *et.al.* found similar results for oral clearance of total and unbound prednisolone to be 6-19 L/h and 64-128 L/h respectively across oral prednisolone doses of 1.25 mg eight times daily-100 mg once daily (17). These investigators found dose dependent increases in clearance based on total but not unbound concentrations. The mean oral clearance of prednisone was reported to be 0.216 L/h/kg (39). It has been reported that prednisone does not exhibit nonlinear protein binding (10).

Nonlinearity of Prednisolone Pharmacokinetics

Total prednisolone concentrations exhibit both dose-dependent clearance and dose dependent steady state volume of distribution (2;4;10;16;17;24;37). Saturation of prednisolone binding to plasma transcortin, a saturation of the interconversion processes, saturation of elimination pathways, concentration dependent clearance of unbound prednisolone concentrations, and tissue-binding sites may all be responsible for the dose dependency (10;37). Apparent clearance and steady state volume of distribution increase two fold between 5-40mg of prednisolone (2;16;20;24;40). Because of the nonlinearity seen with total prednisolone concentrations, it has been recommended that unbound prednisolone concentrations be measured (2;24). When using unbound prednisolone concentrations, both clearance and steady-state volume of distribution become more constant with dose (17;23). However, some investigators report that some nonlinearity still exists. The remaining nonlinearity has been proposed to be caused by nonlinear renal clearance, dose dependency of the interconversion process, and differences in the degree of nonlinearity in the disposition of prednisolone and prednisone (4;10;11;20;24;35;40).

The half-life of unbound prednisolone appears to remain constant (range= 2.3-3.5 hours; mean=2.9 hours) over different doses. This is probably because the volume of distribution and clearance are equally affected by nonlinear effects (2;4;11;17).

Prednisolone population characteristics

Table 1 provides a comparison of some population pharmacokinetic parameters.

Age

One study reported that total prednisolone clearance was not different between children and adults (41), while another study found a 49% higher clearance (on a per kg basis) in children younger than 12 years of age than children over 12 years (42). No information was found regarding volume of distribution in children. Compared to young adults, elderly patients have a higher frequency of adverse events, lower unbound prednisolone clearance (Table 1) and smaller unbound prednisolone steady-state volume of distribution (14). These differences have been attributed to a decrease in both renal and nonrenal clearances. The clearance of 6-β-hydroxyprednisolone decreases linearly with the nonrenal clearance of unbound prednisolone, thus indicating the activity of liver enzymes responsible for prednisolone metabolism diminishes in the elderly (24).

Gender

While gender has been found to alter the pharmacokinetics of prednisolone, there have been reported differences in the effect. In two studies, both unbound and total prednisolone clearances in adults were reported as being 20% greater in females than males (Table 1) (40;43). No statistically significant differences in gender were found in volume of distribution for unbound and total prednisolone (40). In another study, Magee *et.al.* reported that unbound prednisolone clearance normalized to total body weight was approximately 20% higher in white males and 40% higher in black males as

compared to females (44). The unbound prednisolone apparent volume of distribution normalized to total body weight was approximately 30% higher in white males and 40% higher in black males than females (44).

Concomitant Medications

Inducers

It has been reported that the metabolism of prednisolone increased when administered concomitantly with anticonvulsants or rifampicin (2;4;10;22). Phenytoin increased both the total clearance (48%) and nonrenal clearance (77% females; 65% males) of unbound and total prednisolone (Table 1) (2;4;43;45). The increase in total clearance was due to the increase in nonrenal clearance. The urinary excretion of 6-β-hydroxyprednisolone was greater post phenytoin dosing (43). Phenytoin does not affect prednisolone's volume of distribution, protein binding, or renal clearance (24;43).

Inhibitors

The metabolism of prednisolone was inhibited when administered concomitantly with oral contraceptives. Oral contraceptives cause: 1) a decrease in unbound prednisolone clearance and steady-state volume of distribution, 2) an increase in serum transcortin concentrations, 3) an increase in half-life, and 4) lower affinity constants for both prednisolone-albumin and prednisolone-transcortin complexes (2;4;15;22;27;29;30;38;46). Three studies compared the effects of oral contraceptive use with different doses of prednisolone. Total prednisolone clearance, unbound prednisolone clearance, and total prednisolone volume of distribution were all lower in

oral contraceptive users than the control cohort (Table 1). The lower values for total body clearance were attributed to a reduction in nonrenal clearance and increased cortisol binding to transcortin (27;29;30). The reduction in nonrenal clearance has been attributed to a reduction in the activity of hepatic 6β-hydroxylase (27). Plasma cortisol concentrations have been reported as being twice as high in oral contraceptive users (30;46). At lower doses of prednisolone; cortisol displaces prednisolone from transcortin binding sites but not albumin binding sites (40;46). Oral contraceptive users have decreased unbound prednisolone clearances at low doses of prednisolone as compared to high doses (30;40).

Inhibition of prednisolone metabolism has also been seen with concomitant administration of other medications. Concomitant administration of diltiazem resulted in a reduction of the total clearance of prednisolone (Table 1), while naproxen and indomethacin reduced the clearance of unbound prednisolone by 35% and 40% respectively (2;47). Two studies found minor or no changes in AUC and half-life of prednisolone when given concomitantly with itraconazole (36;48). Both analyses concluded that CYP3A4 was a subsidiary pathway for prednisolone metabolism. Zurcher *et.al.* found that ketoconazole, a potent inhibitor of CYP3A4, decreased the total body clearance and volume of distribution of both unbound and total prednisolone. The AUC of unbound prednisolone increased by 50% with concurrent ketoconazole use. It was proposed that ketoconazole decreases renal clearance by impaired tubular secretion and nonrenal clearance by inhibited 6β-hydroxylase activity. Since the unbound prednisolone volume of distribution decreased while transcortin and albumin levels

remained constant, they concluded that altered protein binding was not the reason for the reduction in volume of distribution; the mechanism that reduced the volume of distribution was not known (13). Contrary to Zurcher's findings, Yamashita *et.al.* found no significant inhibition with concomitant ketoconazole use (49). In summary, the role of the CYP450 enzyme system with prednisolone pharmacokinetics remains unknown.

Cystic Fibrosis (CF)

Prednisolone clearance and steady-state volume of distribution were approximately 50% higher in adolescent CF patients compared to a control cohort of age matched adolescent asthmatic patients (50). It is believed that enhanced biotransformation is the underlying reason for the differences seen in clearance; more frequent steroid doses may be necessary in the treatment of CF patients (50).

Additional differences in pharmacokinetic parameters were seen comparing CF patients with normal subjects. Dove *et.al.* found that the total prednisolone nonrenal clearance and unbound fraction of prednisolone were larger in CF patients. Albumin and total protein serum concentrations for CF are low; therefore increased volume of distribution could be related to decreased protein binding (50).

Menopause

After a 25mg intravenous and 30mg oral dose of prednisolone in premenopausal and postmenopausal women, Harris *et.al.* found that total and unbound prednisolone clearances were smaller and half-lives were larger in postmenopausal women (Table 1).

There were no observed significant differences in volume of distribution, protein binding, or bioavailability of prednisolone between these groups of women. They proposed that a change in the activity of at least one enzyme system involved in the metabolism of prednisolone occurs in postmenopausal women (34).

Prednisolone specifics in organ transplantation patients

Rejection levels have been shown to be similar between high and low prednisolone clearance groups. While rejection levels were similar, an increased frequency of rejection and corresponding allograft loss was found in high prednisolone clearance patients; the number of rejection episodes has been shown to be an important risk factor for allograft failure (1;2;5;10;51;52). Combination drug therapy (e.g. cyclosporine) is typically used for adequate immunosuppression and to minimize adverse events (38;52;53). Bergrem *et.al.* evaluated cushingoid versus non-cushingoid transplant patients taking 10mg oral prednisolone and found that cushingoid patients had lower total and unbound prednisolone clearances (Table 1). The cushingoid patients had a poorer transplant function than the non-cushingoid patients, as determined by creatinine clearance (15). In a renal transplant study conducted by Ost *et.al.*, the total prednisolone clearance in cushingoid patients did not differ from non-cushingoid patients (1).

Specific Aims of this Research

Ultimately, the goal of immunosuppression is to taper the dose of prednisolone and eventually switch a patient to either the lowest efficacious dose or an alternate-day therapy regimen while not compromising a patient's therapeutic response (2;10). Steroid

dosage is tapered as rapidly as possible after transplantation, although without an objective guide to safe steroid withdrawal, this can hasten recurrent rejection (5). The overall patient survival rate is linearly correlated with frequency of rejection episodes; i.e. the more episodes, the less likely a patient is to survive (5;51;52). Ideally, a reduction in the maintenance dose is warranted if the disease symptoms are under control or if transplanted organ function is suitable (2). It is desirable to develop an individualized dosing regimen for prednisolone based on measurable parameters (23).

An understanding of the time course of concentration values would be helpful to optimize immunosuppressive therapy. To date, a population pharmacokinetic study of prednisolone has not been performed in humans. The intent of this research is to develop a population pharmacokinetic model of prednisolone, which can be used to optimize the dosing regimen of prednisolone in organ transplant patients.

Specific aims of this research are:

1) To develop a population pharmacokinetic model for prednisolone including oral clearance (CL/F) and oral volume of distribution (V/F) and to assess the interindividual variability in thoracic organ transplant patients
2) To investigate various individual characteristics such as demographic information, disease status, and concomitant medications as potential covariates to reduce interindividual variability
3) To develop population pharmacokinetic models for the evaluation of the optimal prednisolone dosing based on individual characteristics.

METHODS AND MATERIALS

Patients. A randomized, open-label clinical trial was conducted in 50 thoracic organ transplant patients to compare the pharmacokinetics and pharmacodynamics of two cyclosporine formulations (Neoral, the microemulsion formulation (n=28) versus Sandimmune, the original formulation (n=22)). The appropriate institutional review boards approved the protocol. The patients, prior to inclusion in the trial, gave written informed consent. The trial was conducted over the first postoperative year after organ transplant, with supplementary visits occurring approximately at the end of weeks 1, 2, 3, and 4 and at the end of months 3 (week=12), 6 (week=26), 9 (week=38), and 12 (week=52). Results from this trial have been previously published by A. Trull *et.al.* (51).

Of the 50 patients, 41 patients had serum samples that were assayed for prednisolone, prednisone, and cortisol on at least one of the supplementary visits. These 41 patients had the following types of transplants: heart and lung (n=19), double lung (n=7), or single lung (n=15) transplant. This retrospective data analysis was conducted on all of the 41 patients with measurable prednisolone concentrations. Characteristics of the patients are presented in Table 2.

Prednisolone Administration.

The dosing of prednisolone was individualized for each patient. Initially, a patient was dosed with a large dose (maximum dose of 40mg) of prednisolone every 12 hours (maximum daily dose of 80mg). Each day, the total daily dose of prednisolone was

reduced by 5mg (or in some cases 2.5mg) until the lowest possible maintenance dose was achieved for the patient. The larger doses of prednisolone (e.g. 25mg) were supplied by Hoechst Marion Roussel (West Malling, Kent), while the smaller doses (e.g. 5mg) were supplied by either APS Ltd (Eastbourne, East Sussex) or CP Pharmaceuticals Ltd (Wrexham, Clwyd). The dosing interval was increased to 24 hours once a daily dose of 15-20mg was achieved. Figure 1 shows the distribution of doses by plotting the percent of concentration samples versus the dose given.

A patient continued on the lowest daily maintenance dose unless they started to reject their transplant. Rejection episodes were treated with high intravenous doses (500-1000 mg/day) of methylprednisolone over a period of three consecutive days. If a dose of methylprednisolone was given the day prior, or on the day of the sample collection, then the sample collection was not used in this analysis (64 records).

Table 3 provides a partial account of the dosing history for patient #4. Patient #4 received a total daily dose of 50mg on day 1. The total daily dose was reduced by 5mg each day thereafter until day 8. The patient began to have symptoms of organ rejection and on day 11 they were dosed with intravenous methylprednisolone. The methylprednisolone treatments continued until day 14. On day 14, the patient once again started on a high oral prednisolone dose, and continued the 5mg step down in dose until day 22. The same dosing pattern was followed for the rejection episode that occurred on day 24.

Blood Collection and Sample Analysis.

Plasma samples were collected from patients approximately at the end of weeks 1, 2, 3, and 4 and at the end of months 3 (week=12), 6 (week=26), 9 (week=38), and 12 (week=56). The primary objective for the study was to collect cyclosporine concentrations at these visits for each patient. If enough sample remained, then an additional assay was conducted for total prednisolone, total prednisone, and total cortisol. Measurements of total prednisolone, total prednisone and total cortisol were made using a fully validated high-performance liquid chromatography (HPLC) technique as described in a previous publication (54).

Of the concentration records that were obtained, if the values of prednisolone, prednisone, and cortisol were all equal to 0, then that record was removed. Thus there remained a total of 496 prednisolone and 496 prednisone concentrations (n=992 concentration values). The lower limit of detection (LLD) and lower limit of quantification (LLQ) for both prednisone and prednisolone were 2.1 μg/L (signal-to-noise ratio no less than 3) and 7 μg/L (signal-to-noise ratio no less than 10) respectively (54). For prednisolone, there were 60 (12.7%) and 45 (9.1%) concentration values below the LLQ and LLD respectively. For prednisone, there were 147 (29.6%) and 74 (14.9%) concentration values below the LLQ and LLD respectively. Because we were able to address the residual error associated with low concentration values in our model, we used all concentration values regardless of whether they were below the LLQ or LLD (55;56).

Data Preparation and Pharmacokinetic Analysis. Demographic, plasma collection time, primary diagnosis of reason for transplant, type of transplant, concomitant medication, plasma concentration, laboratory examination, and dosing history data relevant to the pharmacokinetic analysis were provided in Microsoft Excel (Excel 2000) spreadsheets. For covariates that were not evaluated every day, values were carried forward in time for that covariate until a new measurement was taken. Two patients had an outlier value for one covariate. The outlier was changed to the last known value for that patient. Thus, on day 20, patient #8 had a cystatin C value changed from 10.9 to 1.46. On day 70, patient #69, had a bilirubin value changed from 210 to 10. Creatinine clearance was calculated (using the Cockcroft-Gault formula) for each record as:

Males: 1.23·(140-age)weight (kg)/serum creatinine (μmol/L)

Females: 1.04· (140-age)weight (kg)/serum creatinine (μmol/L) (57).

Prednisolone has a reported half-life of 2.3-3.5 hours (4). Since it was not possible to ascertain that steady state had been achieved, the dosing history for the five days prior to an observed concentration record was included in the database. A covariate, "dose", was generated to represent the dose that a patient was taking in relation to their corresponding plasma concentrations at hours 0, 2, and 6 post dose.

Total prednisolone concentrations were assayed in this study. The unbound fraction was estimated based on patients' albumin concentrations and a published algorithm, which included values for transcortin and albumin binding capacities and affinity constants

(9;24;31). The first step was to convert the total prednisolone concentrations (ng/ml) to molar concentrations using a molecular weight of 360.4 for prednisolone (21). The values used for the binding capacity (N_tP_t) and affinity constant (K_t) for transcortin were $5.69x10^{-7}$M and $3.01x10^7$ L/M respectively (24). The values used for the albumin binding capacity (N_aP_a) were calculated for each patient using their molar albumin concentrations (molecular weight of albumin=66,300) (18). The value used for the affinity constant (K_a) for albumin was $2.05x10^3$ L/M (24). Again, assuming that $K_aD_f<<1$, there is one prednisolone binding site per molecule of albumin ($N_a=1$), and that prednisolone only binds to transcortin and albumin(23;24;28;29), excel solver can be used to solve for D_f in the following equation:

$$0 = D_t - \frac{N_tP_tK_tD_f}{(1+K_tD_f)} - P_aK_aD_f - D_f$$

where D_t = total prednisolone concentration and D_f= unbound prednisolone concentration (16). The unbound fraction (fu) was then calculated as:

$$fu = \frac{Df}{Dt}$$

Unbound prednisolone plasma concentration (ng/ml) was then calculated as:

Cp unbound prednisolone= Cp total prednisolone·fu.

The AUC for total prednisolone, unbound prednisolone, and total prednisone were determined by the trapezoidal rule (58).

The pharmacokinetic analysis was performed using NONlinear Mixed Effect Model (NONMEM) version 5 level 1.1 double precision on a Pentium IV computer with a Visual Fortran 5.0 compiler (59-62). NONMEM was run using PDx-Pop (v. 1.1) (63)

Data Analysis Strategy.

An approach proposed by Mandema, *et.al.* (1992) (64) was used for the data analysis: 1) a base model was developed for the population, 2) the estimates found during step 1 were used to explore potential covariates with the base model, and 3) a mixed effects model was developed to describe the relationship between the covariates and pharmacokinetic parameters.

Pharmacokinetic and statistical models were evaluated to determine the model that best fit the model dataset. To discriminate between models, the following criteria were used: 1) a decrease in the objective function value (which is proportional to minus twice the log-likelihood of the data) of 3.84 (χ^2 distribution, df=1, $p< 0.05$) or greater following the addition of a single parameter was deemed statistically significant; 2) diagnostic plots (e.g. predicted concentration versus observed concentration data, predicted concentrations overlaying all concentration data, weighted residuals versus predicted concentration values), 3) minimization of variances: reduction of interindividual variances and residual variability, and 4) the Akaike Information Criterion (AIC) (65;66).

Prednisolone Pharmacokinetic Base Model

Initially, a population base model was developed using only the prednisolone data. A one compartment model with first order absorption was used to fit the unbound prednisolone concentration data in order to determine prednisolone's pharmacokinetic parameters prior to the inclusion of the metabolite (prednisone) data. There were not enough concentrations captured during the absorption phase to adequately model the absorption rate constant (k_a). Instead, k_a was determined by using a range of values and finding which model had the lowest objective function value. The range of values for k_a was found by using Excel solver to solve for k_a based on the following equation:

$$T_{max} = 2.303 * \frac{\log(\frac{k_a}{k})}{(k_a - k)}$$

where T_{max}= time at which the peak concentration occurs, k_a= absorption rate constant, and k= the elimination rate constant (57) The elimination rate constant, k, can be represented as:

$$k=0.693/t_{1/2}$$

where $t_{1/2}$= prednisolone's half-life (57). Based on literature values, the Tmax and half-life ranges for prednisolone are 1-2 and 1.8-3.41 hours respectively (4;11;24;28). A range of absorption rate constants were determined by using combinations of the minimum and maximum Tmax and half-life values. Models were generated using each absorption rate constant. The model that produced the smallest objective function value was determined to be the best fit: a one-compartment model with a first-order absorption rate of 2.84 hr^{-1}. The one-compartment model was parameterized as oral

clearance (CL/F), apparent volume of distribution (V/F), and the first-order absorption rate constant (k_a) (NONMEM subroutines ADVAN2 TRANS2).

Analysis of Covariates with Prednisolone Base Model

Once the base prednisolone pharmacokinetic model was obtained, the posthoc Bayesian estimation (FO method) was implemented to obtain the individual parameter estimates to evaluate potential influences of covariates. An exponential error model for interindividual variability and a combined additive and proportional error model for the residual variability were initially assumed for the covariate analysis. For pharmacokinetic parameters CL/F and V/F, the potential influence of covariates on the individual pharmacokinetic parameter estimates were evaluated. Age, weight, and time post transplant were treated as continuous variables. Type of transplant was treated as a categorical variable (0=single lung transplant, 1=double lung and heart transplant, 2=double lung transplant). Gender was treated as an indicator variable (0=female, 1=male). A concomitant medication was considered as present (0= not present, 1=present) if it was taken at any point within five days or on the same day as a concentration value. The following concomitant medications were evaluated as covariates: flucloxacillin, cefotaxime, ceftazidime, imipenem, ciprofloxacin, acyclovir, ganciclovir, amphotericin, itraconazole, lyposomal amphotericin, and septrin. The presence of cystic fibrosis was evaluated as a categorical covariate (0=not present, 1=present). Because menopausal status was not collected in this study, a variable was created that was a marker for women over and under the age of fifty with 0=under 50 and 1=over fifty. Creatinine clearance and cystatin C were evaluated as continuous

covariates and included in the model as markers for renal function. Creatinine clearance, cystatin C, time post transplant, and all concomitant medications were only evaluated on CL/F. Cortisol was evaluated as a continuous variable and was only evaluated on V/F. All other covariates were evaluated on both CL/F and V/F.

Each covariate was added individually to the base model. If the inclusion of the covariate caused a decrease of the objective function value of at least 3.84, the covariate was deemed as being statistically significant. Covariates that were found to be statistically significant from the initial screening were then added simultaneously to the base model to generate the full model. A backward elimination procedure was then performed on the full model. Each covariate was removed one at a time from the full model. If the objective function value decreased by a more conservative value of 7.88 (χ^2 distribution, df=1, $p< 0.005$), the parameter was included in the final model.

Prednisolone and Prednisone Pharmacokinetic Base Model

The prednisone and prednisolone concentration data was simultaneously modeled. The values for both V/F terms (VP/F=apparent volume of distribution for prednisolone and VM/F=apparent volume of distribution for prednisone) were fixed and therefore not estimated. Since there were no statistically significant covariates found on V/F in the covariate analysis, VP/F was fixed to the population average value determined in the prednisolone base model based on unbound prednisolone concentrations (VP/F=420 L). VM/F was fixed at 55 L based on literature values (39). There were a total of four possible elimination processes that could be modeled: clearance of prednisolone that is

not metabolized to prednisone (CPR), clearance of prednisolone that is metabolized to prednisone (CPM), clearance of prednisone that is reconverted to prednisolone (CMM), and clearance of prednisone that is not reconverted to prednisolone (CMR). Model 1 modeled all four rates simultaneously (Figure 2). Because of the difficulty in modeling all four rates, several simplified versions of Model 1 were used: Models 2-8 (Figures 3-9). A summary of the model variations follow.

Model 1: All four elimination terms are included linearly in the model (Figure 2)

Model 1 was a complete model of prednisolone and prednisone, modeling all four elimination processes. CPR, CPM, CMM, and CMR were included in the model linearly. This model was structurally unstable; it was not possible to obtain estimates for any of the individual clearances. There was not enough prednisone information for the software to distinguish between the four elimination processes.

Model 2: No reconversion of prednisone to prednisolone (Figure 3)

Model 2 was a subset of Model 1; one elimination pathway was removed from Model 1. The reconversion of prednisone to prednisolone (CMM) was removed. In this model, all other elimination pathways (i.e. CPR, CPM, and CMR) were modeled linearly. It was thought that by removing the interconversion, a simpler model might provide insight to the pharmacokinetics of prednisone.

Model 3: Removal of the reconversion of prednisone to prednisolone and the elimination of prednisolone by pathways other than metabolism to prednisone (Figure 4)

Model 3 was a subset of Model 2; one of the elimination pathways from Model 2 was removed. It was assumed prednisolone was not metabolized to prednisone (CPR=0). The conversion of prednisone to prednisolone was removed from the model; thus all prednisolone was converted to the metabolite prednisone (CPM) and then eliminated by means other than reconversion to prednisolone (CMR). CPM and CMR were modeled linearly. This model was attempted to see the effect of forcing all prednisolone to be metabolized to prednisone.

Model 4: All prednisone was reconverted to prednisolone (Figure 5)

Model 4 was a subset of Model 1. The clearance of prednisone along pathways other than reconversion to prednisolone (CMR) was set to zero. In this model, all other elimination processes (i.e. CPR, CPM, and CMM) were modeled linearly. This model was attempted because the clearance of prednisone is thought to be much smaller than the other clearance processes (11).

Model 5: Relative value of the interconversion clearances were fixed (Figure 6)

Model 5 was a subset of Model 4. The reconversion of prednisone to prednisolone (CMM) has been reported to be 10 times the rate of conversion of prednisolone to prednisone (CPM) (11). CMM was fixed at 10 times the rate of CPM. CPR and CPM were modeled linearly.

Model 6: Nonlinear metabolism of prednisolone to prednisone (Figure 7)

Model 6 was a subset of Model 4. The conversion of prednisolone to prednisone was treated as a Michaelis-Menton process. CMM and CPR were modeled linearly. It has been proposed that the conversion of prednisolone to prednisone follows a non-linear process, so this model explores the possibility of CPM being nonlinear (2;24).

Model 7: Nonlinear reconversion of prednisone to prednisolone (Figure 8)

Model 7 was a subset of Model 4. The conversion of prednisone to prednisolone was treated as a Michaelis-Menton process. CPM and CPR were modeled linearly. It has been proposed that the reconversion of prednisone to prednisolone may follow a non-linear process, so this model explores the possibility of CMM being nonlinear (2;24).

Model 8: Nonlinear elimination of all prednisolone not metabolized to prednisone

Model 8 was a subset of Model 4. Prednisolone that was not metabolized to prednisone (CPR) was modeled as a Michaelis-Menton process. In this model, the conversion and reconversion of prednisolone and prednisone (CPM and CMM respectively) were modeled linearly. It has been proposed that the renal clearance of prednisolone may follow a non-linear process, so this model explores the possibility of CPR being nonlinear (24).

Statistical Model.

An exponential-error model was used to describe the interindividual variability of the pharmacokinetic parameters. For example:

Exponential model: $\Theta_j = TV\Theta * EXP(\eta_{j,\Theta})$

where $\eta_{j,\Theta}$ is a random variable distributed with a zero mean and variance of ω^2_Θ and $TV\Theta$ is the population mean value for Θ.

Residual variability was modeled separately for prednisolone and prednisone using an additive and proportional-error model:

$$C_{ij} = C_{pred,ij} * (1+\varepsilon_{1ij}) + \varepsilon_{2ij}$$

where C_{ij} is the observed plasma concentration value for the jth individual at time=i, $C_{pred,ij}$ is the model predicted plasma concentration for the jth individual at time=i, ε_{1ij} is a randomly distributed variable with a zero mean and variance of σ^2_1, and ε_{2ij} is a randomly distributed variable with a zero mean and variance of σ^2_2.

RESULTS

Prednisolone and Prednisone Plasma Concentration-Time Data:

The fraction of unbound prednisolone at the various total prednisolone concentrations was estimated using a patient's specific albumin concentrations. The results are shown in Figure 10. The calculated fraction of unbound prednisolone follows an expected curve for saturable protein binding.

Figures 11 and 12 show the observed plasma concentration time data for total and unbound prednisolone respectively. In Figure 13, the total prednisone curve shows that prednisone has a slower elimination since the slope is much shallower between the 2 and 6 hour post dose values than the slope seen in the prednisolone plots. The dose normalized AUC of total prednisolone and unbound prednisolone were plotted versus dose in Figures 14 and 15 respectively. The AUC of total prednisone normalized by prednisolone dose versus dose was plotted in Figure 16. A negative slope was seen for the dose normalized AUC of total prednisolone and total prednisone when plotted versus dose (Figures 14 and 16). A slope of zero was seen for the dose normalized AUC of unbound prednisolone versus dose (Figure 15). The AUC total prednisolone/AUC prednisone and AUC unbound prednisolone/AUC prednisone versus dose were plotted in Figures 17 and 18 respectively. There was a negative slope when AUC total prednisolone/AUC prednisone was plotted versus dose, and a positive slope when AUC unbound prednisolone/AUC prednisone was plotted versus dose. The AUC unbound prednisolone/AUC prednisone versus AUC unbound prednisolone/AUC total prednisolone was plotted and exhibited a positive slope (Figure 19).

Prednisolone Model:

The plasma prednisolone concentration time data was best described using a one-compartment model with a first-order absorption rate of 2.84 hr^{-1}. Interindividual variability was described with exponential error terms for CL/F and V/F. Residual variability was described with a combined additive and proportional-error model. Originally, a base model was developed using total prednisolone concentrations. The

observed versus predicted total prednisolone plasma concentrations have a wide spread around the line of identity (Figure 20). The weighted residuals versus the predicted total prednisolone concentrations have a negative trend. The smaller concentrations are underpredicted while the larger concentrations are overpredicted (Figure 21). When the unbound prednisolone concentrations were modeled, the diagnostic plots showed a better fit. As a result, all further analyses were conducted on unbound prednisolone concentrations.

Results from the unbound prednisolone concentration analysis are shown in Figures 22-23 and Table 4. In Figure 22, observed unbound prednisolone concentrations were more clustered around the line of identity though still underpredicted. The weighted residual plot showed that larger values of predicted unbound prednisolone concentrations are no longer overpredicted, but the smaller concentrations still had large variability (Figure 23). The base model results for the unbound prednisolone dataset are given in Table 4. CL/F and V/F were found to be 17.2 L/h (0.302 L/h/kg) and 416 L (7.298 L/kg) respectively.

Analysis of Covariates with Prednisolone Base Model.

Table 5 summarizes the effect of the individual addition of each covariate for CL/F and V/F. Sex, ciprofloxacin, septrin, amphotericin, imipenem, and cystic fibrosis were found to be statistically significant when included individually in the base model for CL/F. No statistically significant covariates for V/F were identified. Additionally, cefotaxime, albumin, age, type of transplant, flucloxacillin, acyclovir, dose, and creatinine clearance

on CL/F and albumin, age, dose, and sex for V/F had significant changes in the objective function value. While these covariates did have objective function value changes >3.84, the 95% confidence interval for the covariate parameter estimate included the null value and therefore the covariates were not considered in further model development. Time post transplant was evaluated differently than the other covariates. Each occasion for each patient was treated as having been a separate patient. CL/F was calculated at each occasion and then normalized to that patient's first CL/F value (i.e. change in CL/F from baseline). The normalized clearance was plotted versus time post transplant (Figure 24). No trends were seen in the normalized clearance data; time post transplant was determined to be insignificant. A t-test assuming equal variances for independent samples was also performed to further evaluate itraconazole use. There was no statistical difference seen between the mean dose normalized unbound AUC of prednisolone between itraconazole and non-itraconazole users.

All significant covariates were then combined to make the full model, which consisted of sex, ciprofloxacin, septrin, amphotericin, imipenem, and cystic fibrosis as covariates for CL/F. Backwards elimination was then performed to generate a reduced model. Because there is a limit to the number of characters that can be used in a single line of Fortran code, the backwards elimination analysis was done in 2 steps. Each covariate was removed individually from the first full model (sex, ciprofloxacin, septrin, and amphotericin for CL/F). A covariate was retained in the reduced model if there was a significant decrease in the goodness of fit (i.e., objective function value decreased by 7.88 (χ^2 distribution, df=1, $p< 0.005$). Following the first backwards elimination

procedure, only sex and ciprofloxacin were identified as significant covariates for CL/F. The second full model consisted of sex, ciprofloxacin, imipenem, and cystic fibrosis as covariates for CL/F. Again, backwards elimination was performed in the same manner to generate the final model. Following the second backwards elimination procedure, only sex and ciprofloxacin were identified as significant covariates for CL/F in the final model (Table 6).

Table 7 summarizes the final model results for the unbound prednisolone concentration data. Interindividual variability was best described with exponential error terms for CL/F and V/F. Residual variability was described with a combined additive and proportional-error model. For the final model, lower values were obtained for the %RSE base estimates for CL/F and V/F compared to the base model (14.1 vs 15.9% and 11.1 vs. 14.7% respectively) and the variability estimates, $\omega^2_{CL/F}$, $\omega^2_{V/F}$, σ^2_1, and σ^2_2 (0.020 vs. 0.313, 0.109 vs. 0.390, 0.580 vs. 0.697, and 73.5 vs. 79.5 respectively). The variability of the interindividual variability estimates for $\omega^2_{CL/F}$ and $\omega^2_{V/F}$ were higher for the final model as compared to the base model (135.0 vs. 37.4% and 58.7 vs. 36.7% respectively). Parameter estimate ranges were as follows: CL/F=7-73 L/h and V/F=135-415 L. The observed versus predicted plasma unbound prednisolone concentrations and weighted residual versus predicted plasma unbound prednisolone concentrations were plotted in Figures 25 and 26 respectively. In Figure 25, the observed unbound prednisolone concentrations were better predicted as seen by more of a spread around the line of identity. The model still had difficulty predicting the large unbound prednisolone concentrations (Figure 26).

Prednisolone and Prednisone Pharmacokinetic Base Model.

As explained in the Methods, a pharmacokinetic model for prednisolone and its metabolite, prednisone was developed based on the pharmacokinetic characteristics of these species (Figure 2). Since the amount of prednisone formed from prednisolone was unknown it was not possible to use this complete model. Several simplified versions of the complete model were evaluated as described below.

Model 2: No reconversion of prednisone to prednisolone (Figure 3)

In this model, the reconversion of prednisone to prednisolone (CMM) was ignored. All other elimination processes, the clearance of prednisolone not metabolized to prednisone (CPR), the clearance of prednisolone metabolized to prednisone (CPM), and the clearance of prednisone not reconverted to prednisolone (CMR) were modeled linearly. This model was highly dependent on initial estimates and structurally unstable. With one set of initial estimates, the clearance of prednisolone was pushed through processes not involving prednisone. Thus CPM and CMR were very small. When the initial estimates were slightly changed, the model "flipped" and virtually all prednisolone was then eliminated through metabolism to prednisone (CPM).

Model 3: Removal of the reconversion of prednisone to prednisolone and the elimination of prednisolone by pathways other than metabolism to prednisone (Figure 4)

In this model, all of the prednisolone was assumed to be metabolized to prednisone (CPM). All elimination processes were modeled linearly. This model proved to be structurally sound, but is not in agreement with the known pharmacokinetic characteristics of the drug. A recycled fraction of 0.76 has been reported for the interconversion of prednisolone and prednisone and urine recovery of unchanged prednisolone has been reported at approximately 20% (11).

Model 4: All prednisone was reconverted to prednisolone (Figure 5)

In this model the clearance of prednisone by pathways other than reconversion to prednisolone was removed from the model. Thus all prednisone was reconverted back to prednisolone. All other elimination processes (i.e. CPR, CPM, and CMM) were modeled linearly. This model provided equivalent values for CPM and CMM. Essentially, the model was unable to distinguish between these clearances. The model was unable to account for the interconversion of prednisolone and prednisone.

Model 5: Relative value of the intereonversion clearances were fixed (Figure 6)

According to the literature, CMM is estimated to be about 10 times CPM (11). Thus in this model, CPM was estimated but CMM was fixed to 10 times the value of CPM. All processes were modeled linearly. This model did not adequately describe the metabolite data as seen in diagnostic plots (Figure 27). The predicted prednisone concentration values were not distributed around the line of identity and were underpredicted.

The plots with the AUC for total prednisone indicated that their nonlinearity might be associated with the interconversion of prednisolone and prednisone (Figures 16-19). Models 6 and 7 were attempted to address these concerns. In Models 6 and 7, the clearance of prednisone was assumed to occur only through its reconversion to prednisolone (i.e. CMR was set to zero).

Model 6: Nonlinear metabolism of prednisolone to prednisone (Figure 7)

The conversion of prednisolone to prednisone was assumed to follow Michaelis-Menton kinetics. This model was structurally unstable and it was not possible to obtain estimates for CPR.

Model 7: Nonlinear reconversion of prednisone to prednisolone (Figure 8)

The reconversion of prednisone to prednisolone was modeled using Michaelis-Menton kinetics. This model was structurally unstable. Estimates were obtained for the two prednisolone clearances (CPR and CPM). Initially, the model was able to obtain the Michaelis-Menton estimates for the reconversion of prednisone to prednisolone, but again these estimates were highly sensitive to the initial estimates.

Model 8: Nonlinear elimination of all prednisolone not metabolized to prednisone (Figure 9)

In this model, the clearance of prednisolone, by processes other than metabolism to prednisone, was modeled as a nonlinear process. The conversion and reconversion of prednisolone and prednisone (CPM and CMM respectively) were modeled linearly.

This model was structurally unstable. This model had equivalent values for CPM and CMM. Essentially, the model was unable to distinguish between these elimination processes.

DISCUSSION

The study demonstrated that a population pharmacokinetic modeling approach could be used to model prednisolone concentration-time data from a thoracic organ transplant clinical trial. Additionally, the study demonstrated that it was possible to identify covariates to explain variability in the pharmacokinetic parameters. Sex and ciprofloxacin were found to be significant covariates for CL/F. It was not possible to model prednisolone and prednisone concentration-time data simultaneously.

The data used for this study consisted of total prednisolone concentrations. A negative slope in the dose normalized AUC of total prednisolone versus dose was observed (Figure 14); as dose increased the dose normalized AUC decreased. If total prednisolone concentrations exhibited linear pharmacokinetics, the slope of Figure 14 would have been zero (CL=F·Dose/AUC). The negative trend could be explained by one of two reasons; either CL increased with dose or F decreased with dose. Previous studies have suggested that the nonlinearity in prednisolone's pharmacokinetics is due to saturable protein binding. Furthermore Rose *et.al.* (24)developed a model for the protein binding of prednisolone based on prednisolone concentration, albumin concentration and transcortin levels. This model was used to estimate the unbound prednisolone concentrations associated with each total prednisolone concentration for this analysis. In

the model for this analysis, the albumin binding capacity (N_aP_a) was calculated for each patient using their molar albumin concentrations, while the affinity constant (K_a) for albumin was set at $2.05x10^3$ L/M based on the values obtained in the Rose *et.al.* paper (24). Since transcortin concentrations were not available for the patients in the study, the Rose *et.al.* values for the transcortin binding capacity (N_tP_t) and affinity constant (K_t) were used ($5.69x10^{-7}$M and $3.01x10^7$ L/M respectively) (24). Using a population value for the transcortin concentration levels may have introduced bias into the calculation of unbound prednisolone. However, a plot of the dose normalized AUC of unbound prednisolone versus dose had a slope approaching zero (Figure 15), which indicated the pharmacokinetics of unbound prednisolone were linear.

Base models were generated for both total and estimated unbound prednisolone concentrations. In addition to the nonlinearity seen in Figure 14, it appeared that the base model using total prednisolone concentrations was nonlinear based on the negative slope seen in the weighted residuals plot (Figure 21). As a result all subsequent analyses were conducted on the estimates unbound prednisolone concentrations. For the unbound prednisolone concentrations, CL/F and V/F were approximately 0.302 L/h/kg and 7.298 L/kg respectively (mean weight=57 kg). Two previous studies with a control cohort of healthy volunteers found unbound prednisolone CL/F and V/F values ranging from 0.666-0.694 L/h/kg and 1.340-1.610 L/kg respectively (Table 1) (27;30). In studies with kidney transplant patients taking concomitant cyclosporine, total and unbound prednisolone CL/F values ranged from 0.075-0.140 and 0.492-0.510 L/h/kg respectively (Table 1) (38;67-70). In this same population, the total and unbound

prednisolone V/F values ranged from 0.680-0.720 and 1.480-1.600 L/kg (Table 1) (38;70). In studies with kidney transplant patients not taking concomitant cyclosporine, total and unbound prednisolone CL/F values ranged from 0.045-0.171 and 0.315-0.886 L/h/kg respectively (Table 1) (1;15;38;67;68;70;71). These patients had total and unbound prednisolone V/F values ranging from 0.224-0.780 and 1.460-2.100 L/kg respectively (Table 1) (1;38;70;71). The unbound prednisolone CL/F value of 0.302 L/h/kg found in the present study compares favorably to both the transplant patients taking cyclosporine (0.492-0.510 L/h/kg) and those transplant patients not taking concomitant cyclosporine (0.315-0.886 L/h/kg). There is some debate as to whether cyclosporine inhibits the metabolism of prednisolone (38;67;68;70). It has been suggested that cyclosporine is an inhibitor of CYP3A4 (72), though references as to the mechanisms of the inhibition have not been found in a literature search(73). In two separate studies, Ost and Langhoff *et.al.* found that patients taking concomitant cyclosporine had lower total prednisolone clearances than those patients taking concomitant azathioprine (67;68). Frey *et.al.* found no differences between concomitant cyclosporine and azathioprine users in total or unbound prednisolone clearances (38). Rocci *et.al.* found that concomitant cyclosporine use did not affect the total or unbound prednisolone clearances (70). Both Frey *et.al.* and Rocci *et.al.* attributed their different results to the fact that they measured unbound prednisolone concentrations, conducted repeated measurements over a longer interval, and used both intravenous prednisolone and oral prednisone to eliminate confounding from the interconversion process (38;70). The CL/F values found in the present analysis were about 50% of the healthy volunteers and the unbound prednisolone V/F value of 7.298 L/kg were considerably larger than the

reported values for cyclosporine and non-cyclosporine users (1.480-1.600 and 1.460-2.100 L/kg respectively), and about five times larger than the healthy volunteers. There was difficulty in estimating V/F in the present study. Data was only collected at approximately three time points for all patients (0, 2, and 6 hours post dose). Thus, a complete concentration-time profile for the entire population was not captured. Furthermore, owing to the paucity of information in the early period following the dose, assessment of both volume of distribution and k_a were extremely difficult. The k_a was fixed in this study.

In the present analysis, the values for unbound prednisolone oral clearance for males (n=23) and females (n=18) were 1.216 L/h/kg and 0.240 L/h/kg respectively; males had a significantly larger clearance than females. There have been three reported studies that have found sex as a statistically significant covariate for prednisolone CL/F (Table 1) (40;43;44). Meffin *et.al.* and Frey *et.al.* reported that males had a significantly smaller unbound prednisolone clearance than females (Table 1) (40;43). In both of these studies, a small number of subjects were dosed with intravenous prednisolone (Meffin: four males, four females; Frey: eight males, six females). In a different study using oral prednisone, Magee *et.al.* found opposite results; males had a significantly larger (20%) unbound prednisolone oral clearance than females (44). Magee's study had a larger number of subjects (Magee: sixteen males, sixteen females). Additionally, Magee's study controlled for menstrual cycle phase by having each female begin the study at the same point in their menstrual cycle (44). Magee *et.al.* suggested that the reasons for their different results from Meffin *et.al.* and Frey *et.al.* may be attributed to their larger sample

size, different formulation, and that they controlled for the timing of the female menstrual cycle, which made it easier to identify a gender effect. Both CL/F and V/F were smaller in female subjects, implying that women may have a larger bioavailability of prednisone relative to men (44). The present analysis had a similar sample size (23 males; 18 females) to the Magee study. The inability to identify a gender effect for V/F in the present study could be a function of the lack of informative data to adequately estimate V/F. In the present analysis oral contraceptive usage, menstrual cycle phase, and menopause status were not collected. Oral contraceptive usage has been reported to reduce unbound prednisolone clearance (2;4;15;22;27;29;30;38;46). The unbound prednisolone clearance value of 0.240 L/h/kg for females in this study was lower than the 0.467-0.540 L/h/kg values reported elsewhere (Table 1) (27;30). Postmenopausal women reportedly have lower unbound prednisolone clearances than premenopausal women by around 30% (Table 1) (34). Since oral contraceptive use and menopause status was not captured in this study, its effect was unknown.

It has been suggested that young women have approximately 1.4 times the CYP3A4 activity of men (74). Thus, it would be anticipated that females might exhibit larger clearances in CYP3A4 substrate drugs than males. The role, if any, of CYP3A4 in prednisolone elimination is not clear. While some authors have found little effects of CYP3A4 inhibitors on prednisolone clearance (34;36;48;49), another study found total and unbound prednisolone clearances decreased with ketoconazole (13). In the present study, itraconazole was not found to reduce the clearance of unbound prednisolone. However, it is possible that the study did not have sufficient statistical power to

adequately probe the effects of itraconazole. Only seven subjects were taking itraconazole at some point during the study period and these seven subjects provided a total of only fifteen samples during concomitant itraconazole use.

Concomitant ciprofloxacin was also identified as a significant covariate for CL/F; ciprofloxacin use reduced the unbound prednisolone oral clearance (by 48% and 10% in females and males respectively). Unbound clearance values for patients using ciprofloxacin were 0.125 L/h/kg and 1.100 L/h/kg for females and males respectively. Ciprofloxacin is a known inhibitor of CYP1A2 and CYP3A4 activity (75-77). A literature search found no reports of interaction studies for prednisolone and CYP1A2 inhibitors. The results from the present study suggest that CYP1A2 may be a pathway for prednisolone metabolism and that CYP1A2 interaction studies may be warranted.

Other studies have reported that that cystic fibrosis patients have increased total prednisolone clearances (50). In the present study cystic fibrosis was not a significant covariate for CL/F in the final model. However, all of the cystic fibrosis patients (n=6) were male. Thus, it may be impossible to separate the effects of cystic fibrosis and gender in this data set. It is also possible that effects of cystic fibrosis may have elevated the estimates for CL/F in males in the present study. It is interesting to note that the weight normalized values of CL/F tended to be larger in the male cystic fibrosis patients compared to the other male patients (Figure 28).

It proved to be challenging to model prednisolone and prednisone simultaneously. When a complete model that included all elimination processes was used, the model was structurally unstable (Model 1; Figure 2). The amount of prednisone formed from prednisolone was unknown; consequently the estimates of the parameters used in this model were highly unstable. Many variations of Model 1 were used (Models 2-8; Figures 3-9) but they were either structurally unstable or did not adequately describe the data. It is believed that part of the difficulty in modeling the data was due to previously noted nonlinearity in the pharmacokinetics of prednisolone and prednisone. Figure 16 showed that the AUC of total prednisone normalized by prednisolone dose decreased with increasing dose (negative slope), indicating the presence of nonlinear pharmacokinetics. If all clearances of prednisolone had been equally affected by protein binding, increasing the dose of prednisolone simply would have produced a proportional increase in the conversion of prednisone. Assuming linear pharmacokinetics of prednisone, this scenario would have produced a slope of zero in Figure 16. Alternatively, if the conversion of prednisolone to prednisone was more sensitive to protein binding than the other clearances, then as the dose of prednisolone increased, the fraction converted to prednisone would have increased disproportionally. Again assuming linear pharmacokinetics of prednisone, a positive slope would have been expected in Figure 16. Instead, the relationship seen (negative slope) could occur through two possible mechanisms: the conversion of prednisolone to prednisone may be a saturable process and/or the elimination of prednisone may be nonlinear. The reason for the saturable metabolism of the conversion of prednisolone to prednisone is unknown, though it has been proposed that it may be attributable in part to a saturation

of 11-β-hydroxydehydrogenase, the enzyme responsible for the interconversion of prednisolone to prednisone (10;20). Nonlinearity with prednisone was noted in Figure 18. AUC total prednisolone/AUC prednisone versus dose produced a negative slope (Figure 17). When correcting for prednisolone's nonlinearity by using unbound prednisolone concentrations instead of total prednisolone concentrations, AUC unbound prednisolone/AUC prednisone versus dose produced a positive slope (Figure 18). Knowing that unbound prednisolone exhibited linear pharmacokinetics, it was the nonlinearity of total prednisone driving the increase in slope on this plot.

The concentration-dependent binding of prednisolone may affect the interconversion process. If the interconversion of prednisone and prednisolone depended solely on the concentration of unbound prednisolone, the ratio of unbound prednisolone to prednisone would remain constant, regardless of the unbound fraction of prednisolone in plasma (slope=zero). Instead, a positive slope was seen in Figure 19; interconversion is not solely dependent on protein binding, other factors (e.g enzyme inhibition) must influence the interconversion process (24).

In order to model prednisolone and prednisone concentration time data simultaneously, future clinical trials should be designed to address some of the issues encountered in this analysis. More concentration values are needed during the absorption time period (specifically concentration data in between the 0-2 hour post dose interval). The additional data would have allowed one more parameter in this model to have been estimated rather than fixed from literature values and would have permitted better

estimation of V/F. If unbound prednisolone concentrations are not measured directly in patients, than both albumin and transcortin should be measured in order to permit a more accurate estimation of the unbound fraction of prednisolone. Information regarding oral contraceptive usage, menstrual cycle phase, and menopause status would provide a better understanding of the impact of these factors in the pharmacokinetics of prednisolone. If possible, populations that are anticipated to have altered pharmacokinetics should be stratified to address potential confounders; in this trial, all cystic fibrosis patients were male. Dosing patients with prednisone, in addition to prednisolone, would enable the estimation of the pharmacokinetic parameters of prednisone.

In conclusion, this study has shown that population pharmacokinetic models could be used to model prednisolone plasma concentration time data obtained in a thoracic organ transplant population dosed with oral prednisolone. A one-compartment model with a fixed first order rate of absorption was used to describe the unbound prednisolone concentration versus time data. Sex and ciprofloxacin for CL/F were found as significant covariates. It was not possible to adequately model prednisolone and prednisone concentration time data simultaneously. The results of this study may provide help to better dose thoracic transplant patients with oral prednisolone.

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Table 1. Summary of Prednisolone Pharmacokinetics in Various Populations

First Author (Reference)	Population	Total CL (L/h/kg)	Unbound CL (L/h/kg)	Total VD (L/kg)	Unbound VD (L/kg)
Stuck (14)	Elderly	---	0.400†	---	---
	Young	---	0.640	---	---
Meffin (40)	Male	0.164†	0.620†	0.669	1.546
	Female	0.193	0.752	0.676	1.543
Frey (43)	Female – before phenytoin	0.171†	0.693†	0.650	1.690
	Female – after phenytoin	0.248	0.103	0.620	1.590
Frey (43)	Male- before phenytoin	0.148†	0.650	0.562†	1.550
	Male- after phenytoin	0.210	0.640	0.839	1.540
Magee (44) ¥	White Males	0.178	0.788	0.590	2.180
	Black Males	0.172	0.778	0.613	2.183
	White Females	0.181	0.644	0.453	1.650
	Black Females	0.165	0.554	0.586	1.580
Meffin (30)	Control	0.169†	0.694†	0.684†	1.340
	OC users	0.081	0.467	0.550	1.290
Frey (27)	Control	0.174†	0.666†	0.640†	1.610
	OC users	0.096	0.540	0.540	1.710
Boekenoogen (29)	Control*	0.170†	0.876†	0.818†	2.770
	OC users*	0.082	0.550	0.605	1.840
Imani (47)	Control*	0.168†	---	---	---
	Diltiazem users*	0.139	---	---	---
Harris (34)	Premenopausal	0.150†	0.996†	0.580	2.520
	Postmenopausal	0.110	0.696	0.430	1.790
Ost (67)	Transplant - Cyclosporine	0.110-0.140†			
	Transplant- Azathioprine	0.150-0.160			
Ost (1) – no cyclosporine	Cushingoid	0.160			
	Non-Cushingoid	0.150			

Gambertoglio (71) – no cyclosporine	Transplant Control	0.100	0.644	0.460	2.03
	Transplant-Anticonvulsant	0.171†	0.886†	0.470	1.53
Langhoff (68)	Transplant – Cyclosporine	~0.114			
	Transplant-Azathioprine	~0.144†			
Bergrem (15) – no cyclosporine	Cushingoid*	0.045†	0.273†	0.229	---
	Non-cushingoid*	0.051	0.315	0.224	---
Frey (38)	Transplant - Cyclosporine	0.140	0.492	0.680	1.480
	Transplant-Azathioprine	0.132	0.499	0.610	1.460
Rocci (70)	Transplant – Pre-Cyclosporine	0.160	0.620	0.780	2.100
	Transplant – Post-Cyclosporine	0.130	0.510	0.720	1.600
Jusko (69) – with cyclosporine	Transplant – Pre Sirolimus	0.094			
	Transplant – Post Sirolimus	0.075			

* Mean weight of 70kg used

† P-value <0.05 or smaller for the population comparison

¥ P-value <0.01 for comparison of gender. Race and gender by race comparisons were not significant.

Abbreviations: VD=volume of distribution, CL=clearance, OC=oral contraceptive

Table 2. Characteristics of Thoracic Organ Transplant Patients Evaluated in the Population Pharmacokinetic Analysis of Prednisolone

Characteristic	
Total number of patients	41
Male	23
Female	18
Age (years)	42.9 (range=19-66)
Weight (kg)	57.0 (range=37-85)
Transplant type	
Heart and lung	19
Double lung	7
Single lung	15
Cystic fibrosis	6
Acyclovir	8
Amphotericin	6
Cefotaxime	10
Ceftazidime	17
Ciprofloxacin	14
Flucloxacillin	24
Ganciclovir	33
Imipenem	4
Itraconazole	7
Lyposomal amphotericin	4
Septrin	31

Table 3. Example of Partial Prednisolone Dosing History: Patient #4

Patient	Day Post Transplant	Total Daily Oral Prednisolone Dose(mg)	Prednisolone a.m. Dose (mg)	Prednisolone p.m. Dose (mg)	Methylprednisolone Dose (mg)
4	0	0	0	0	0
4	1	50	25	25	0
4	2	45	25	20	0
4	3	40	20	20	0
4	4	35	20	15	0
4	5	30	15	15	0
4	6	25	15	10	0
4	7	20	15	5	0
4	8	15	15	0	0
4	9	30	15	15	0
4	10	15	15	0	0
4	11	.	0	0	1000
4	12	.	0	0	1000
4	13	.	0	0	500
4	14	50	25	25	0
4	15	50	25	25	0
4	16	40	20	20	0
4	17	35	20	15	0
4	18	30	15	15	0
4	19	25	15	10	0
4	20	20	15	5	0
4	21	15	15	0	0
4	22	10	10	0	0
4	23	10	10	0	0
4	24	.	0	0	1000
4	25	.	0	0	500
4	26	.	0	0	500
4	27	60	30	30	0
4	28	55	30	25	0
4	29	50	25	25	0
4	30	45	25	20	0
4	31	40	20	20	0
4	32	35	20	15	0
4	33	30	15	15	0
4	34	25	15	10	0
4	35	20	15	5	0
4	36	15	15	0	0
4	37	10	10	0	0
4	38	10	10	0	0

Table 4. Base Model for the Pharmacokinetics Model of Prednisolone

Parameter	Estimated Value	%RSE	95% Confidence Interval
CL/F (L/h)=Θ_1	17.2	15.9	11.8,22.6
V/F (L)= Θ_2	416	14.7	296,536
k_a (h^{-1})= Θ_3	2.84†	--	--
$\omega^2_{CL/F}$	0.313	37.4	0.084,0.542
$\omega^2_{V/F}$	0.390	36.7	0.110,0.670
σ^2_1	0.697	22.8	0.385,1.010
σ^2_2	79.5	50.8	0.3,159.0

† k_a was fixed at 2.84 h^{-1}

Abbreviations: %RSE=percent relative standard error, CL/F = oral clearance, V/F = oral volume of distribution, ka= first-order absorption rate constant, $\omega^2_{CL/F}$ =interpatient variability of CL/F, $\omega^2_{V/F}$ = interpatient variability of V/F, σ^2_1 =variance of proportional portion of residual error, σ^2_2 =variance of additive portion of residual error.

A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Table 5. Analysis of Individual Covariates for Unbound Prednisolone Base Model

Covariate Analysis	Objective Function Value	Change in Objective Function Value
Base Model – No covariates	3554.588	
Sex for CL/F	3405.003	-149.585
Cefotaxime for CL/F *	3487.214	-67.374
Ciprofloxacin for CL/F	3490.455	-64.133
Septrin for CL/F	3512.221	-42.367
Albumin for V/F *	3544.546	-30.991
Albumin for CL/F *	3525.231	-29.357
Amphotericin for CL/F	3526.041	-28.547
Imipenem for CL/F	3528.633	-25.955
Age for CL/F *	3533.241	-21.347
Cystic fibrosis for CL/F	3540.647	-13.941
Type for CL/F *	3541.016	-13.572
Flucoxacillin for CL/F *	3542.368	-12.220
Acyclovir for CL/F *	3543.551	-11.037
Age for V/F *	3545.029	-9.559
Dose for V/F *	3546.328	-8.260
Sex for V/F *	3546.738	-7.850
Dose for CL/F *	3547.350	-7.238
Creatinine clearance for CL/F *	3547.753	-6.835
Lyposomal amphotericin for CL/F	3553.567	-1.021
Type for V/F	3553.859	-0.729
Weight for CL/F	3553.942	-0.646
Weight for V/F	3554.257	-0.331
Cystic fibrosis for V/F	3554.343	-0.245
Cystatin C for CL/F	3554.588	0
Itraconazole for CL/F	3555.423	0.835
Ganciclovir for CL/F	3558.203	3.615
Ceftazidime for CL/F	3636.749	82.161
Cortisol for V/F **	---	---

Bold covariates were deemed statistically significant.

* 95% confidence interval of parameter estimate includes the null value.

** The addition of cortisol as a covariate in the base model caused model instability. The diagnostic plots showed that cortisol did not add any further improvement in the fit of the model and therefore was not included in any further model development.

Abbreviations: CL/F = oral clearance, V/F = oral volume of distribution, type=type of transplant.

A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Table 6. Backward Elimination for the Full Model of Unbound Prednisolone

Removed Covariate	Objective Function Value	Change in Objective Function Value	p-Value
Sex, ciprofloxacin, septrin, amphotericin for CL/F			
No covariate removed	3439.156	--	--
Sex	3464.470	25.314	< 0.005
Ciprofloxacin	3445.645	6.489	< 0.005
Septrin	3370.275	-68.881	NS
Amphotericin	3469.252	30.096	< 0.005
Sex, ciprofloxacin, amphotericin for CL/F			
No covariate removed	3370.275	--	--
Sex	3466.281	96.006	< 0.005
Ciprofloxacin	3399.395	29.120	< 0.005
Amphotericin	3376.170	5.895	NS
Sex, ciprofloxacin, imipenem, cystic fibrosis for CL/F			
No covariate removed	3365.838	--	--
Sex	3619.883	254.045	< 0.005
Ciprofloxacin	3376.681	10.843	< 0.005
Imipenem	3368.618	2.780	NS
Cystic fibrosis	3375.064	9.226	< 0.005
Sex, ciprofloxacin, cystic fibrosis for CL/F			
No covariate removed	3368.618	--	--
Sex	3633.179	264.561	< 0.005
Ciprofloxacin	3387.505	18.887	< 0.005
Cystic fibrosis	3376.167	7.549	NS
Sex, ciprofloxacin for CL/F			
No covariate removed	3376.167	--	--
Sex	3490.455	114.288	< 0.005
Ciprofloxacin	3405.003	28.836	< 0.005

Abbreviations: CL/F = oral clearance, NS=not significant.

A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Table 7. Final Model for Prednisolone Alone Dataset

Parameters	Estimated Value	%RSE	95% Confidence Intervals
CL/F (L/h)=Θ_1 + Θ_4·sex+ Θ_5·cipr			
Θ_1	13.7	14.1	9.9,17.5
Θ_4	55.6	11.6	43.0,68.2
Θ_5	-6.6	29.8	-10.5,-2.8
V/F (L)= Θ_2	281	11.1	220,342
k_a (h^{-1})= Θ_3	2.84 †	--	--
$\omega^2_{CL/F}$	0.020	135	-0.033,0.073
$\omega^2_{V/F}$	0.109	58.7	-0.016,0.234
σ^2_1	0.580	18.6	0.368,0.792
σ^2_2	73.5	33.7	24.9,122.0

† k_a was fixed at 2.84 h^{-1}

Abbreviations: %RSE=percent relative standard error, CL/F = oral clearance, V/F = oral volume of distribution, ka= first-order absorption rate constant, $\omega^2_{CL/F}$ =interpatient variability of CL/F, $\omega^2_{V/F}$ = interpatient variability of V/F, σ^2_1 =variance of proportional portion of residual error, σ^2_2 =variance of additional portion of residual error, cipr=ciprofloxacin use.

Sex: 0=female, 1=male
Ciprofloxacin: 0=no concomitant ciprofloxacin use, 1=concomitant ciprofloxacin use

A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Figure 1. Distribution of Percent (%) of Concentration Samples versus Prednisolone Dose (mg)

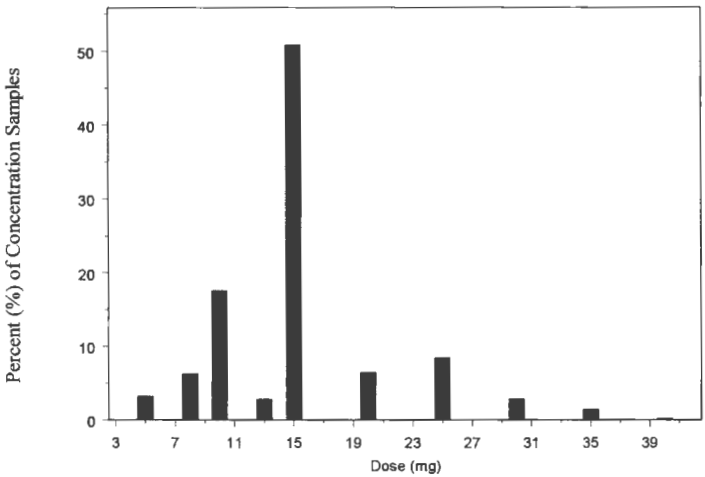

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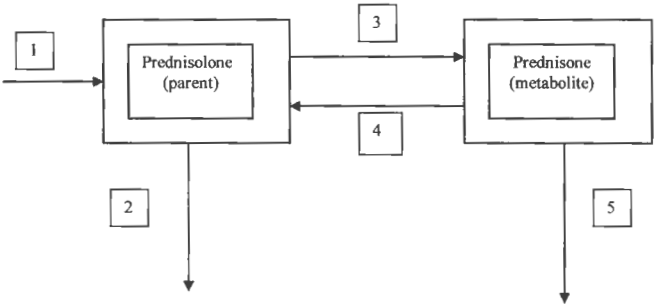
Figure 2. Prednisolone (Parent) and Prednisone (Metabolite) Pharmacokinetic Model

1: All four elimination terms are included linearly in the model



1) Fixed first order absorption rate constant

2) Clearance of prednisolone that is not metabolized to prednisone = CPR

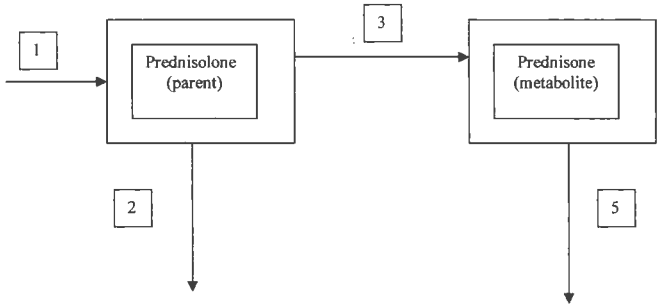
3) Clearance of prednisolone that is metabolized to prednisone = CPM

4) Clearance of prednisone that is reconverted to prednisolone =CMM

5) Clearance of prednisone that is not reconverted to prednisolone =CMR

Figure 3. Prednisolone (Parent) and Prednisone (Metabolite) Pharmacokinetic Model

2: No reconversion of prednisone to prednisolone



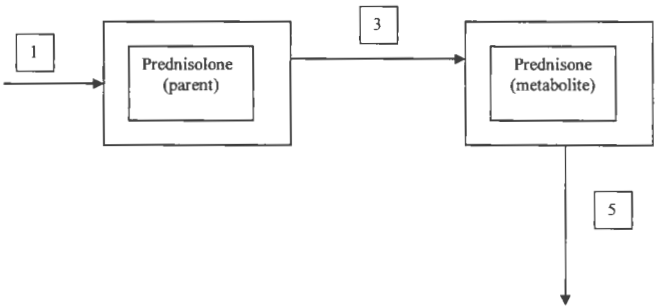
1) Fixed first order absorption rate constant

2) Clearance of prednisolone that is not metabolized to prednisone = CPR

3) Clearance of prednisolone that is metabolized to prednisone = CPM

5) Clearance of prednisone that is not reconverted to prednisolone =CMR

Figure 4. Prednisolone (Parent) and Prednisone (Metabolite) Pharmacokinetic Model 3: Removal of the reconversion of prednisone to prednisolone and the elimination of prednisolone by pathways other than metabolism to prednisone



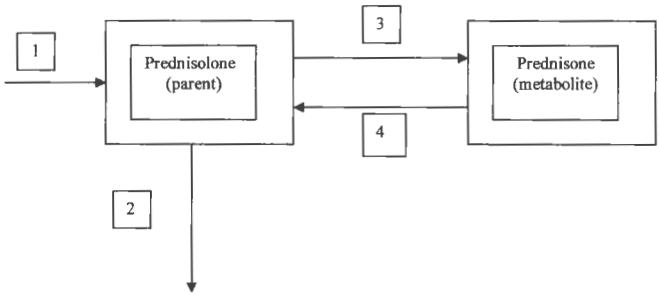
1) Fixed first order absorption rate constant

3) Clearance of prednisolone that is metabolized to prednisone = CPM

5) Clearance of prednisone that is not reconverted to prednisolone =CMR

Figure 5. Prednisolone (Parent) and Prednisone (Metabolite) Pharmacokinetic Model

4: All prednisone was reconverted to prednisolone



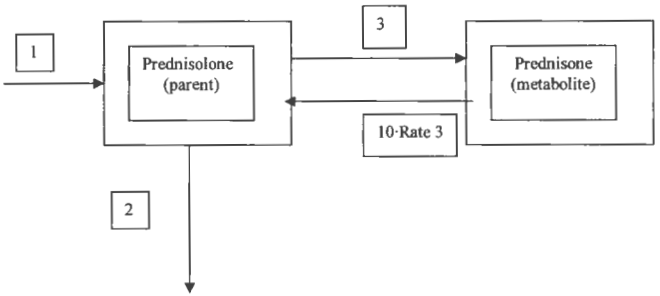
1) Fixed first order absorption rate constant

2) Clearance of prednisolone that is not metabolized to prednisone = CPR

3) Clearance of prednisolone that is metabolized to prednisone = CPM

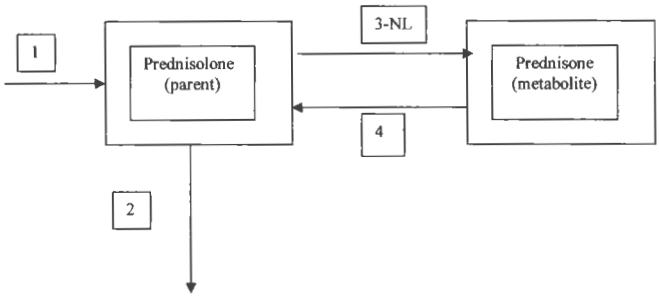
4) Clearance of prednisone that is reconverted to prednisolone =CMM

Figure 6. Prednisolone (Parent) and Prednisone (Metabolite) Pharmacokinetic Model 5: Relative value of the interconversion clearances were fixed



1) Fixed first order absorption rate constant
2) Clearance of prednisolone that is not metabolized to prednisone = CPR
3) Clearance of prednisolone that is metabolized to prednisone = CPM
4) Clearance of prednisone that is reconverted to prednisolone =CMM fixed to be 10·CPM

Figure 7. Prednisolone (Parent) and Prednisone (Metabolite) Pharmacokinetic Model 6: Nonlinear metabolism of prednisolone to prednisone



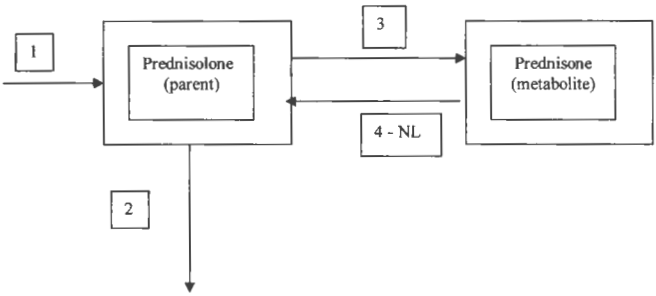
1) Fixed first order absorption rate constant

2) Clearance of prednisolone that is not metabolized to prednisone = CPR

3) Clearance elimination of prednisolone that is metabolized to prednisone = CPM

4) Clearance of prednisone that is reconverted to prednisolone =CMM

Figure 8. Prednisolone (Parent) and Prednisone (Metabolite) Pharmacokinetic Model 7: Nonlinear reconversion of prednisone to prednisolone



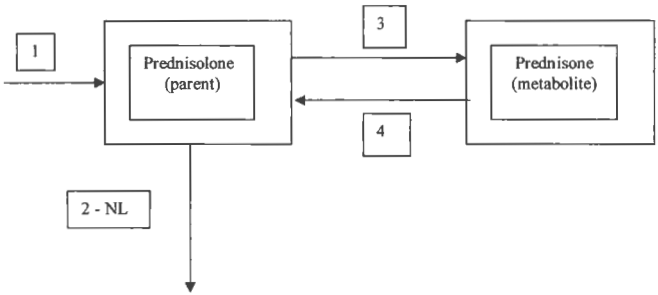
1) Fixed first order absorption rate constant

2) Clearance of prednisolone that is not metabolized to prednisone = CPR

3) Clearance of prednisolone that is metabolized to prednisone = CPM

4) Nonlinear clearance of prednisone that is converted to prednisolone =CMM

Figure 9. Prednisolone (Parent) and Prednisone (Metabolite) Pharmacokinetic Model 8: Nonlinear elimination of all prednisolone not metabolized to prednisone



1) Fixed first order absorption rate constant
2) Nonlinear clearance of prednisolone that is not metabolized to prednisone = CPR
3) Clearance of prednisolone that is metabolized to prednisone = CPM
4) Clearance of prednisone that is reconverted to prednisolone =CMM

Figure 10. Fraction of Unbound Prednisolone Concentration versus Total Prednisolone Concentration (ng/ml) Individual Values for Albumin

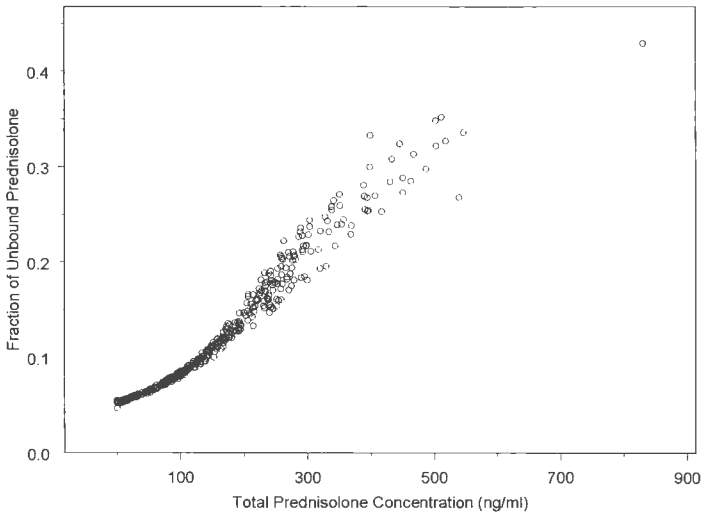


Figure 11. Observed Total Prednisolone Concentration (ng/ml) versus Time Post Dose (minutes)

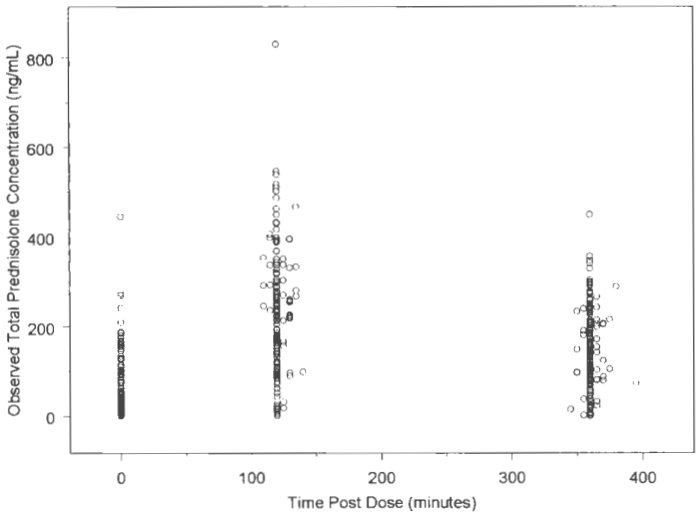


Figure 12. Observed Unbound Prednisolone Concentration (ng/ml) versus Time Post Dose (minutes)

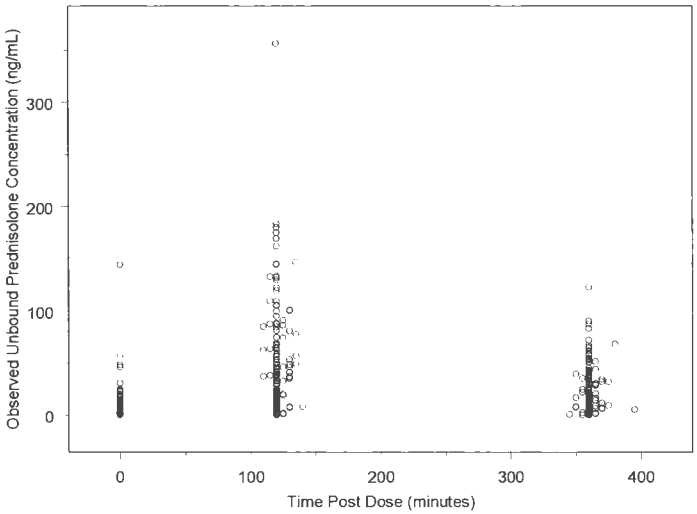


Figure 13. Observed Total Prednisone Concentration (ng/ml) versus Time Post Dose (minutes)

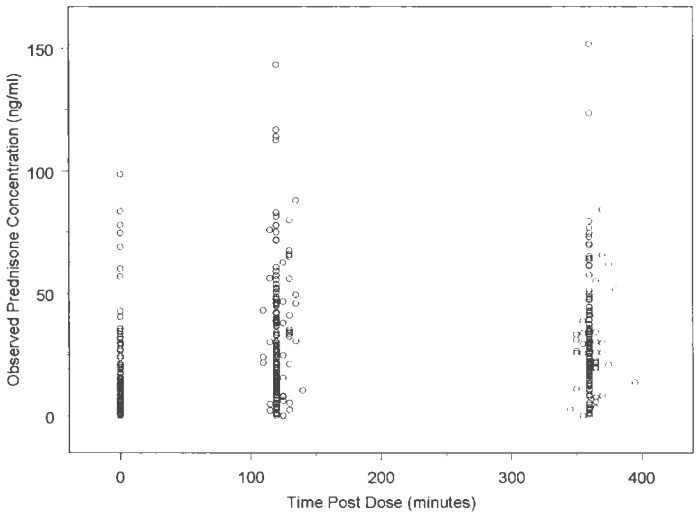


Figure 14. Dose Normalized AUC Total Prednisolone (ng*hr/ml/mg) versus Dose (mg)

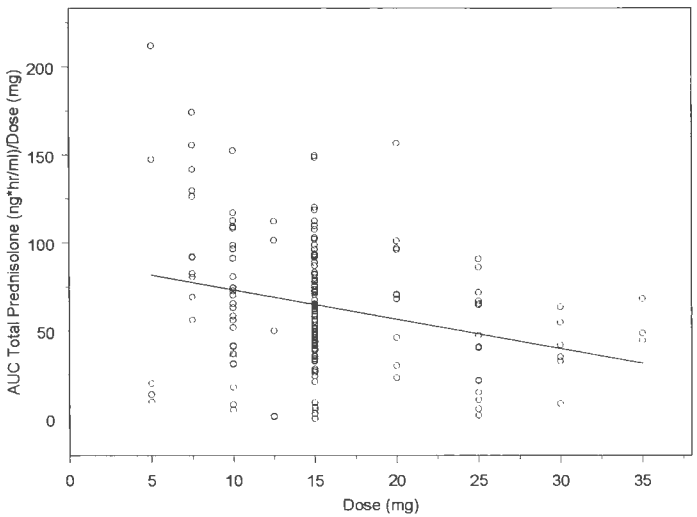


Figure 15. Dose Normalized AUC Unbound Prednisolone (ng*hr/ml/mg) versus Dose (mg)

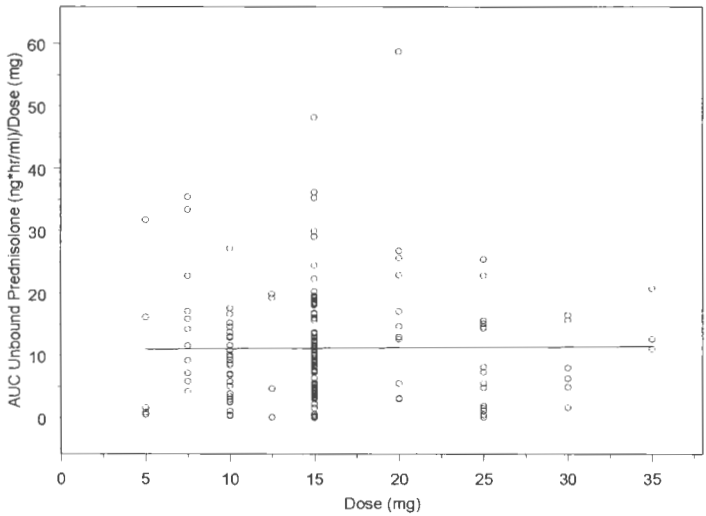


Figure 16. AUC Total Prednisone (ng*hr/ml/mg) Normalized by Prednisolone Dose versus Dose (mg)

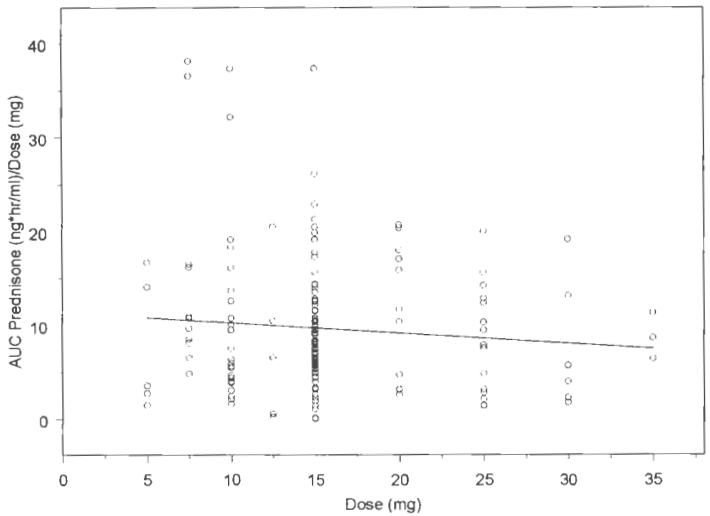


Figure 17. AUC Total Prednisolone/AUC Total Prednisone versus Dose (mg)

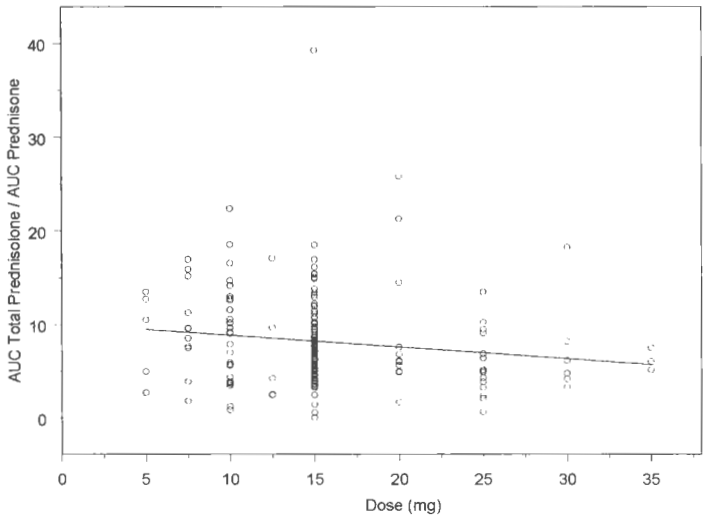


Figure 18. AUC Unbound Prednisolone/AUC Total Prednisone versus Dose (mg)

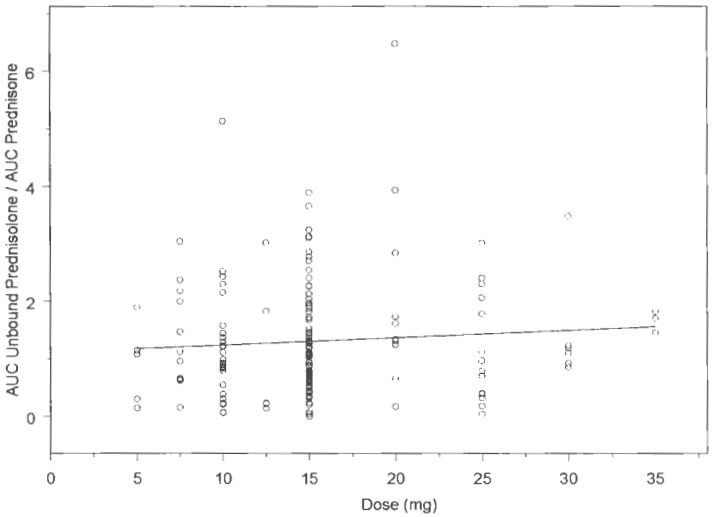


Figure 19. AUC Unbound Prednisolone/AUC Total Prednisone versus AUC Unbound Prednisolone/AUC Total Prednisolone

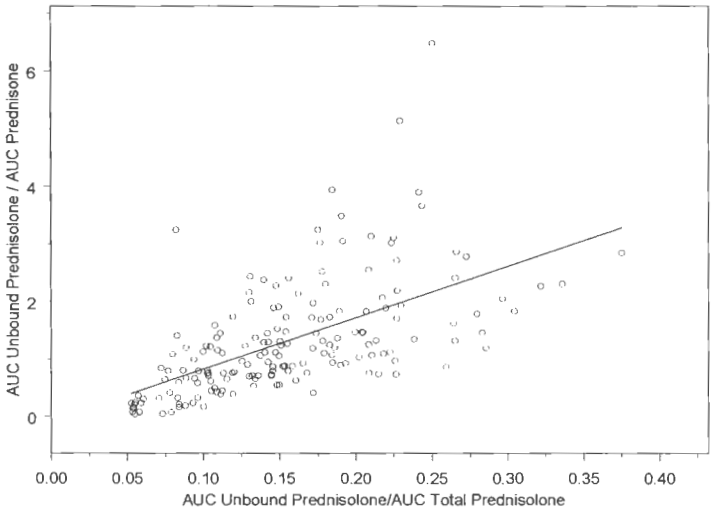
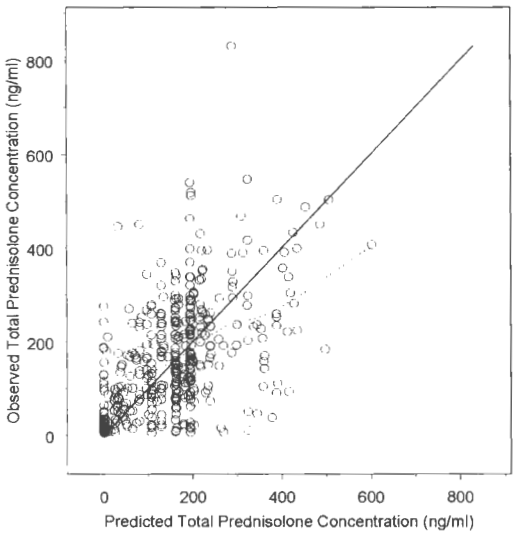
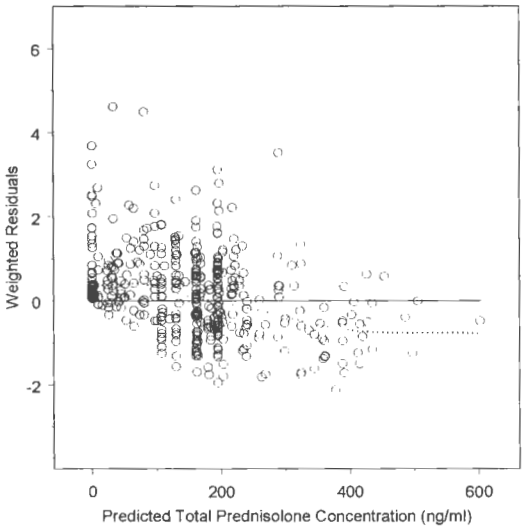


Figure 20: Observed Prednisolone Plasma Concentration versus Predicted Prednisolone Plasma Concentration Values for the Total Prednisolone Concentration Base Model



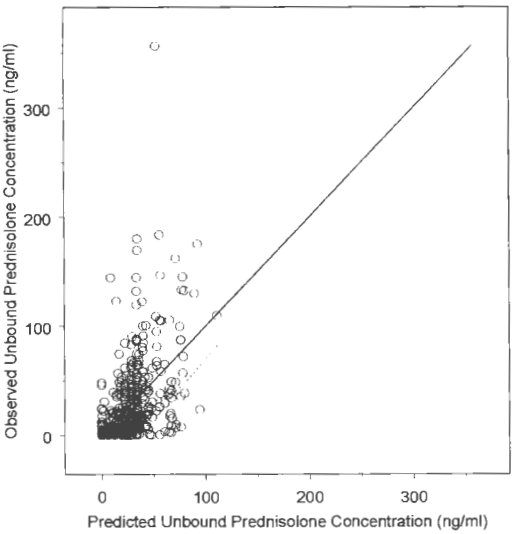
A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Figure 21: Weighted Residuals versus Predicted Prednisolone Plasma Concentration Values for the Total Prednisolone Concentration Base Model



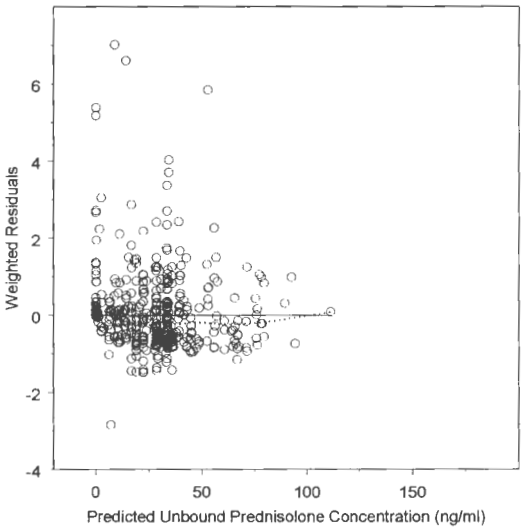
A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Figure 22. Observed Prednisolone Concentration versus Predicted Prednisolone Concentration Values for the Unbound Prednisolone Base Model



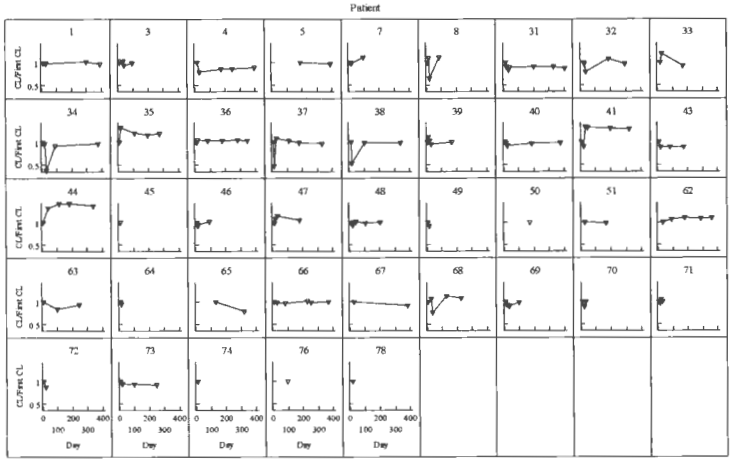
A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Figure 23. Weighted Residuals versus Predicted Prednisolone Concentration Values for the Unbound Prednisolone Base Model



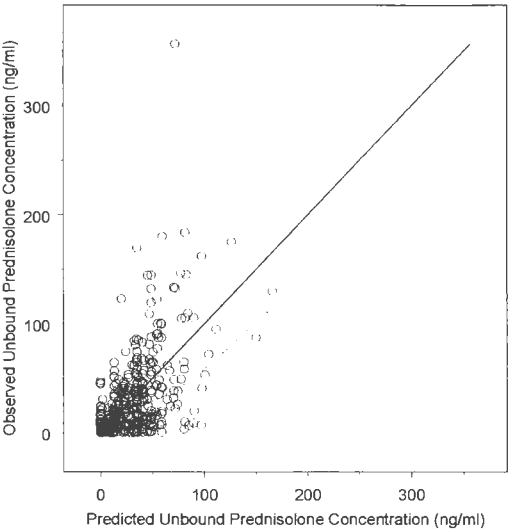
A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Figure 24. Normalized Clearance versus Time Post Transplant (Day) for Each Patient Using Unbound Prednisolone Base Model Clearance Estimates



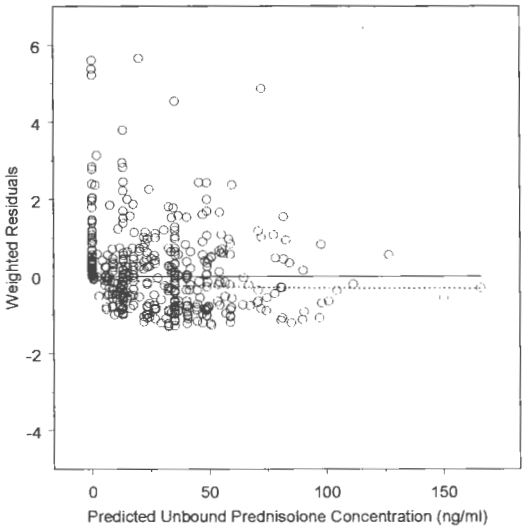
A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Figure 25. Observed Prednisolone Plasma Concentration versus Predicted Prednisolone Plasma Concentration Values for the Final Unbound Prednisolone Alone Model



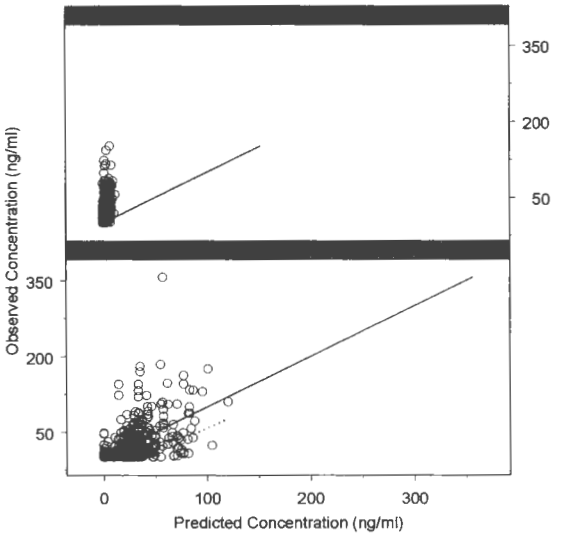
A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Figure 26. Weighted Residuals versus Predicted Prednisolone Plasma Concentration Values for the Final Prednisolone Unbound Alone Model



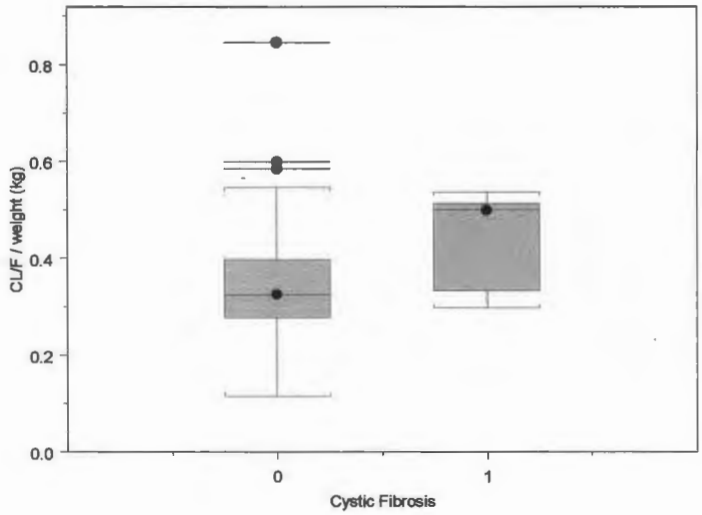
A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

Figure 27. Observed versus Predicted Unbound Prednisolone and Total Prednisone Concentrations for Model 5



CMT 2.00= Unbound Prednisolone Concentrations
CMT 3.00= Total Prednisone Concentrations

Figure 28. CL/F versus Cystic Fibrosis for Males not Using Ciprofloxacin (Final Prednisolone Alone Model)



0= no cystic fibrosis; 1= cystic fibrosis

no cystic fibrosis: n=17; cystic fibrosis: n=6

Abbreviations: CL/F = oral clearance

A one compartment model with a fixed first order absorption rate constant (k_a=2.84 hr^{-1}), exponential interindividual variability, and a combined proportional and additive residual error were used.

SUMMARY OF CONCLUSIONS

The population approach to pharmacokinetic analysis has become a common tool in the drug development process. Two major advantages of this type of analysis are 1) the ability to pool data from a population from which it might otherwise be difficult to collect information and 2) the ability to model sparse data. Thus, this approach can be used to reduce the number of clinical trials that need to be conducted in order to obtain alternate dosing information for sub-populations.

Azithromycin Model:

The azithromycin model was an example of an analysis that pooled data from multiple clinical trials. Dosing information in various sub-populations of the pediatric patients was analyzed without having to conduct more clinical trials. The objective of this analysis was to develop a population pharmacokinetic model for 58 pediatric patients taking azithromycin in four separate clinical trials. A two compartment model with parallel zero-order and first-order absorption was found to best fit the data. When standardized by the mean weight, the parameter values generally compared well for the pediatric patients compared to values found in the adult population. Because of the richness of the sample times collected in these studies, the FOCE approach was used to determine the final model. Weight was found to be a significant covariate for both CL/F and V2/F. The final model was an improvement over the base model as seen by reductions in %RSE on parameter estimates, reductions in interindividual variability, a reduction in the residual variability, and an improvement in the diagnostic plots. While

the final model was an improvement, there was still bias and imprecision present in the model. Additional model development pursuing a three compartment model may address the bias and imprecision.

The final azithromycin model found in this analysis supports the current weight adjusted dosing guidelines for azithromycin.

Prednisolone Model:

The prednisolone model was an example of an analysis that utilized sparse data collected as a secondary endpoint in a clinical trial. The objective of this analysis was to develop a population pharmacokinetic model from 41 thoracic organ transplant patients dosed with prednisolone. Unbound prednisolone concentrations were estimated and found to follow linear pharmacokinetics. A one compartment model with a fixed absorption rate constant of 2.84 hr^{-1} was found to best fit the unbound prednisolone concentration time data. Sex and concomitant ciprofloxacin use were found to be significant covariates for CL/F. The final model was an improvement over the base model as seen by reductions in %RSE on parameter estimates, a reduction in the residual variability, and an improvement in the diagnostic plots. The prednisolone and prednisone concentration data were simultaneously modeled using final parameter estimates from the prednisolone alone model and literature values for the V/F terms. Many models were developed, but they all proved to be inadequate because of lack of robustness or lack of clinical meaningfulness with our understanding of prednisolone/prednisone pharmacokinetics.

APPENDIX A

The following text provides additional information on the pharmacokinetics of azithromycin.

The following figures provide additional information on the modeling process that was employed in Manuscripts II and III.

Figures 1-7 contain NONMEM control streams. For azithromycin, NONMEM was run using the command prompt in MS-DOS (Figures 1-4). For prednisolone, NONMEM was run using PDx-Pop (Figures 5-7). Figure 8 contains information regarding S-Plus box and whisker plots.

Azithromycin Pharmacokinetics

Absorption

Oral azithromycin has a bioavailability (F) of approximately 37% (1). In a study of twelve patients that had ileostomies, it was found that slow or incomplete absorption was the most important limitation on the bioavailability of azithromycin, as opposed to acid degradation or extensive first-pass metabolism (2).

Azithromycin has a rapid rate of absorption with a peak concentration (Cmax) occurring around 2-3 hours post dose (Tmax) (1;3).

Distribution

Azithromycin exhibits a rapid distribution into tissues. Azithromycin is actively transported into cells and then slowly released into the extracellular fluid compartments (4). There are significantly higher azithromycin concentrations in tissues than in plasma or serum (10- to 100-fold) (1;4-6).

Serum protein binding is low and variable. A bound fraction of 0.5 has been observed for serum concentration ranges of 0.02-0.05 mg/L, and 0.7-0.12 for ranges of 0.5-2.0 mg/L; lower concentrations of azithromycin exhibit greater protein binding (1;4;5). Azithromycin binds predominately to α_1-acid glycoprotein (1).

The volume of distribution of azithromycin has been reported as being 23-33 L/kg (3-7).

Metabolism

Metabolism is not a major route of elimination for azithromycin (2;4;8;9). When azithromycin is metabolized, the primary route of metabolism is hepatic demethylation (10). Unlike other macrolide antibiotics, there has been no evidence of cytochrome P450 induction or inhibition by azithromycin (9;11;12).

Excretion

Azithromycin is principally eliminated via the liver (5). The major route of elimination is through biliary excretion, predominantly as unchanged drug (1;2;8;9). Over 50% of drug related material in the bile is unchanged azithromycin (10).

Urinary excretion of unchanged azithromycin appears to be a minor route of elimination (<6%) (5;9;10). The renal clearance of azithromycin is in the range of 6-11.34 L/h (3;9;10). The plasma clearance of azithromycin is in the range of 37.8-39.9 L/h (3;5;7;13;14).

Azithromycin has a half life around 55-70 hours (1;4;5;7;14). The apparent steady-state volume of distribution (~30 L/kg) and plasma clearance (~38 L/h) suggest that the long half-life is due to extensive distribution and subsequent release of drug from tissues rather than to an intrinsic inability to clear azithromycin (5;7;13;14).

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(6) Physicians' Desk Reference. *Zithromax for Oral Suspension*, 300mg, 600mg, 900mg, 1200mg. www.pdr.net . Date accessed 11-5-2001.

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(14) Rapp RP. *Pharmacokinetics and Pharmacodynamics of Intravenous and Oral Azithromycin: Enhanced Tissue Activity and Minimal Drug Interactions.* Annals of Pharmacotherapy 1998; 32:785-793.

Figure 1. NONMEM Control File for Base Azithromycin Model – FO Method

```
$PROB AZITHROMYCIN PEDS 80% Additive Error - RUN BASE PEDS MODEL
$INPUT ID AMT TIME DATE ADDL II DV RATE
$DATA NOZERO.CSV IGNORE=C
$SUBROUTINE ADVAN4 TRANS4
$PK
TVCL=THETA(1)
TVV2=THETA(2)
TVQ=THETA(3)
TVV3=THETA(4)
TVKA=THETA(5)
TVR1=THETA(6)

CL=TVCL*EXP(ETA(1))
V2=TVV2
Q=TVQ*EXP(ETA(2))
V3=TVV3*EXP(ETA(3))
KA=TVKA*EXP(ETA(4))
R1=TVR1
S2=V2

$ERROR
 IPRED = F
 IRES  = DV - IPRED
 Y = F*(1+EPS(1))

$THETA
(10,100,300) ;CL
(100,200,500); V2
(50,150,400) ;Q
(1000,2900,4000) ;V3
(0,1,5) ;KA
(50,100,300);R1

$OMEGA 0.25 0.25 0.25 0.25
$SIGMA 0.25

$ESTIMATION MAXEVAL=9999 PRINT=5 POSTHOC METHOD=0
$COVARIANCE
$TABLE ID AMT TIME CL V2 V3 Q KA R1 RES WRES PRED
IPRED IRES ETA1 ETA2 ETA3 ETA4 NOPRINT FILE=CLV3KAQ.T01
```

Figure 2. NONMEM Control File for Full Azithromycin Model – FO Method

```
$PROB AZITHROMYCIN PEDS 80% Additive Error - RUN BASE PEDS MODEL
W/ COVARIATES

$INPUT ID AMT TIME DATE ADDL II DV RATE RACE=DROP AGE HT=DROP
WT ANE=DROP CA=DROP PN=DROP NAU=DROP COL=DROP ALB AMI
CAP=DROP  CEFA=DROP CEFR DIG=DROP DIP=DROP DOP FEN=DROP
LAS=DROP  MOR NYS=DROP SUL=DROP TIC VAN VER=DROP

$DATA NOZEROCO.CSV IGNORE=C

$SUBROUTINE ADVAN4 TRANS4
$PK

TVCL=THETA(1)+THETA(7)*(WT)+ THETA(8)*(ALB)
TVV2=THETA(2)
TVQ=THETA(3)+THETA(11)*(DOP)
TVV3=THETA(4)+THETA(9)*(CEFR)+THETA(10)*(WT)
TVKA=THETA(5)+THETA(12)*MOR
TVR1=THETA(6)

CL=TVCL*EXP(ETA(1))
V2=TVV2
Q=TVQ*EXP(ETA(2))
V3=TVV3;*EXP(ETA(3))
KA=TVKA*EXP(ETA(3))
R1=TVR1
S2=V2

$ERROR
 IPRED = F
 IRES  = DV - IPRED
 Y = F*(1+EPS(1))

$THETA

(30,50,90) ;CL
(100,200,400); V2
(50,100,300) ;Q
(800,1000,2000) ;V3
(1,2,4) ;KA
(50,100,200);R1
(0,1,5) ; WT
(-40, -30, -10) ;ALB
```

Figure 2. NONMEM Control for Full Model (con't)

```
(-800,-500,-300) ; CEFR
(0,30,50) ; WT
(1); DOP
(.0001); MOR

$OMEGA 0.25 0.25 0.25 ;0.25
$SIGMA 0.25

$ESTIMATION MAXEVAL=9999 PRINT=5 POSTHOC METHOD=0
$COVARIANCE
$TABLE ID AMT TIME CL V2 V3 Q KA R1 RES WRES PRED
IPRED IRES ETA1 ETA2 ETA3 NOPRINT FILE=MORKA.T01
```

Figure 3. NONMEM Control File for Final Azithromycin Model – FO Method

```
$PROB AZITHROMYCIN PEDS 100% data - Exp Error
$INPUT ID WT ALB CEFR DOP=DROP AMT TIME DATE ADDL II RATE DV
$DATA VALIDATE.CSV IGNORE=C
$SUBROUTINE ADVAN4 TRANS4
$PK
TVCL=THETA(1)+THETA(7)*(WT)+ THETA(8)*(ALB)
TVV2=THETA(2)
TVQ=THETA(3)
TVV3=THETA(4)+THETA(9)*(CEFR)+THETA(10)*(WT)
TVKA=THETA(5)
TVR1=THETA(6)

CL=TVCL*EXP(ETA(1))
V2=TVV2
Q=TVQ*EXP(ETA(2))
V3=TVV3*EXP(ETA(3))
KA=TVKA*EXP(ETA(4))
R1=TVR1
S2=V2

$ERROR
 IPRED = F
 IRES  = DV - IPRED
 Y = F*(1+EPS(1))

$THETA
(0,50) ;CL
(100,200,500); V2
(50,150,400) ;Q
(1000,2900,4000) ;V3
(0,1,5) ;KA
(50,100,300);R1
(0,50) ; WT
(1) ;ALB
(100) ; CEFR
(10) ; WT

$OMEGA 0.25 0.25 0.25 0.25
$SIGMA 0.25
$ESTIMATION MAXEVAL=9999 PRINT=5 POSTHOC METHOD=0
$COVARIANCE
$TABLE ID AMT TIME CL V2 V3 Q KA R1 RES WRES PRED WT ALB CEFR
IPRED IRES ETA1 ETA2 ETA3 ETA4 NOPRINT FILE=VALIDATE.T01
```

Figure 4. NONMEM Control File for Final Azithromycin Model – FOCE Method

```
$PROB AZITHROMYCIN PEDS 100% data – FOCE Method
$INPUT C ID WT ALB CEFR DOP=DROP AMT TIME DATE ADDL II RATE DV
$DATA 002.CSV IGNORE=C
$SUBROUTINE ADVAN4 TRANS4
$PK
TVCL=THETA(1)+THETA(7)*(WT)
TVV2=THETA(2)
TVQ=THETA(3)
TVV3=THETA(4)+THETA(8)*(WT)
TVKA=THETA(5)
TVR1=THETA(6)
CL=TVCL*EXP(ETA(1))
V2=TVV2
Q=TVQ
V3=TVV3
KA=TVKA*EXP(ETA(2))
R1=TVR1
S2=V2

$ERROR
 DEL=0
 IF(F.EQ.0) DEL=1
 W=F
 IPRED=F
 IRES=DV-IPRED
 IWRES=IRES/(W+DEL)
 Y=F+F*EPS(1)

$THETA
(10,30,50) ;CL
(100,200,300); V2
(50,150,300) ;Q
(600,1000,1500) ;V3
(.5,2,3) ;KA
(50,100,250);R1
(0,5,10) ; WT
(10,20,40) ; WT
$OMEGA 0.5 0.5
$SIGMA 0.25
$ESTIMATION MAXEVAL=9999 PRINT=5 POSTHOC NOABORT METHOD=1
$COVARIANCE
$TABLE ID AMT TIME CL V2 V3 Q KA R1 RES WRES PRED WT IPRED IRES
ETA1 ETA2 NOPRINT FILE=FOCE.T01
```

Figure 5. NONMEM Control File for Base Prednisolone Model

```
;Model Desc: linear code parent (KA=2.84, tmax=1, thalf=3.41 all data W/ FUALB)
;Project Name: pred
;Project ID: GM00-001

$PROB RUN# 228 (base model ka=2.84)
$INPUT C ID DATE TIME DVT=DROP SRT2 AMT2=DROP AMT II ADDL EVID
CMT FU2=DROP FU DV

$DATA 005.CSV IGNORE=C

$SUBROUTINES ADVAN2 TRANS2 SS2

$PK

        TVCL=THETA(1)
        TVV=THETA(2)
        TVKA=THETA(3)

        CL=TVCL*EXP(ETA(1))
        V=TVV*EXP(ETA(2))
        KA=TVKA
  S2=V

$ERROR
 DEL=0
 IF(F.EQ.0) DEL=1
 W=F
 IPRED=F
 IRES=DV-IPRED
 IWRES=IRES/(W+DEL)

  IF(CMT.EQ.2) THEN
   Y=F+F*EPS(1)+EPS(2)
  ENDIF

$THETA
   (0,10,100);CL
   (50,100,1000);VP
   (2.84 FIXED)

$OMEGA
 0.25 ;[P] INTERIND VAR IN CL
 0.25 ;[P] INTERIND VAR IN VP
```

```
$SIGMA
 0.1 ;[P]
 0.1 ;[A]

$EST MAXEVAL=9999 PRINT=5 METHOD=0 POSTHOC
$COVARIANCE
$TABLE ID CL V ETA1 ETA2 RES WRES DV NOPRINT ONEHEADER
FILE=228.TAB
$TABLE ID CL V ETA1 ETA2 RES WRES DV NOPRINT ONEHEADER
FILE=patab228.TAB
$TABLE ID CL V ETA1 ETA2 RES WRES DV NOPRINT ONEHEADER
FILE=cotab228.TAB
$TABLE ID CL V ETA1 ETA2 RES WRES DV NOPRINT ONEHEADER
FILE=catab228.TAB
$TABLE ID CL V  ETA1 ETA2 RES WRES DV NOPRINT ONEHEADER
FILE=sdtab228.TAB
```

Figure 6. NONMEM Control File for Final Prednisolone Model

```
;Model Desc: parent FUALB SEX,CIPR ON CL, CEFO(DROP),
GANC(DROP),ITRA(DROP),ACYC(DROP) ON V
;Project Name: BACKPFUALB
;Project ID: JJ001

$PROB RUN# 736 (PARENT SEX,CIPR ON CL)
$INPUT C ID DATE OTPD=DROP NTPD=DROP TIME PDN=DROP PDL=DROP
DVT=DROP COR=DROP TOT=DROP SDOS=DROP DOSE=DROP SORT=DROP
PD1=DROP PD2=DROP AMT2=DROP AMT II ADDL EVID CMT TYPE
AGE=DROP SEX PDG=DROP WT=DROP GTT0=DROP GTT2=DROP
WBC=DROP HB=DROP NEUT=DROP LYM=DROP MONO=DROP BAS=DROP
EOS=DROP CRT=DROP ALB=DROP AUCM=DROP AUCP=DROP AUCC=DROP
AZA=DROP
MPD=DROP FLU=DROP CEFO CEFT=DROP IMIP=DROP CIPR ACYC GANC
AMPH=DROP ITRA LAB=DROP SEPT=DROP FU=DROP FUA=DROP
REA=DROP
CREA=DROP CCL2=DROP BILI=DROP A1=DROP CSY2=DROP SCRE=DROP CF
CRCL=DROP CSYC=DROP DV MENO=DROP

$DATA 007.CSV IGNORE=C

$SUBROUTINES ADVAN2 TRANS2 SS2

$PK
 TVCL=THETA(1)+THETA(4)*SEX+THETA(5)*CIPR
 TVV=THETA(2)
 TVKA=THETA(3)

 CL=TVCL*EXP(ETA(1))
 V=TVV*EXP(ETA(2))
 KA=TVKA
 S2=V

$ERROR
 DEL=0
 IF(F.EQ.0) DEL=1
 W=F
 IPRED=F
 IRES=DV-IPRED
 IWRES=IRES/(W+DEL)

  IF(CMT.EQ.2) THEN
   Y=F+F*EPS(1)+EPS(2)
```

```
  ENDIF


$THETA
 (10,20,60);CP
 (100,400,800);VP
 (2.84 FIXED)
 (0,10);SEX
 (-10);CIPR

$OMEGA
 0.25 ;[P] INTERIND VAR IN CP
 0.25 ;[P] INTERIND VAR IN VP

$SIGMA
 0.1 ;[P]
 0.1 ;[A]

$EST MAXEVAL=9999 PRINT=5 METHOD=0 POSTHOC NOABORT
$COVARIANCE
$TABLE ID CL V ETA1 ETA2 SEX CIPR ITRA DATE RES WRES DV NOPRINT
ONEHEADER FILE=736.TAB
$TABLE ID CL V ETA1 ETA2 SEX CIPR ITRA  DATE RES WRES DV NOPRINT
ONEHEADER FILE=patab736.TAB
$TABLE ID CL V ETA1 ETA2 SEX CIPR ITRA  DATE RES WRES DV NOPRINT
ONEHEADER FILE=cotab736.TAB
$TABLE ID CL V ETA1 ETA2 SEX CIPR ITRA  DATE RES WRES DV NOPRINT
ONEHEADER FILE=catab736.TAB
$TABLE ID CL V  ETA1 ETA2 SEX CIPR ITRA  DATE RES WRES DV NOPRINT
ONEHEADER FILE=sdtab736.TAB
```

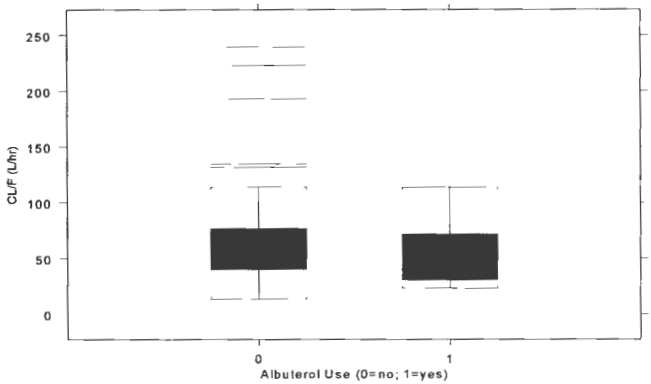
Figure 7. NONMEM Control File Example Using Nonlinear Rates for Prednisolone and Prednisone Simultaneous Model – Model 6

```
;Model Desc: p&m - 2c model -non linear k23, linear k32
;Project Name: METABOLITE
;Project ID: GM00-001

$PROB RUN# 521 (PARENT AND METABOLITE)
$INPUT C ID DATE OTPD=DROP NTPD=DROP TIME PDN=DROP PDL=DROP
DVT=DROP CORT=DROP TOT=DROP SDOS=DROP DOSE SORT=DROP
PD1=DROP PD2=DROP AMT2=DROP AMT II ADDL EVID CMT TYPE
AGE=DROP SEX PDG=DROP WT=DROP GTT0=DROP GTT2=DROP
WBC=DROP HB=DROP NEUT=DROP LYM=DROP MONO=DROP BAS=DROP
EOS=DROP CRT=DROP ALB=DROP AUCM=DROP AUCP=DROP AUCC=DROP
AZA=DROP
MPD=DROP FLU=DROP CEFO CEFT=DROP IMIP CIPR=DROP ACYC
ANC=DROP AMPH=DROP ITRA LAB=DROP SEPT=DROP FU=DROP
FUA=DROP UREA=DROP CREA=DROP CCL2=DROP BILI=DROP A1=DROP
CSY2=DROP SCRE=DROP CF=DROP CRCL=DROP CSYC=DROP DV

$DATA 010.CSV IGNORE=C

$SUBROUTINES ADVAN6 TOL=3

$MODEL NPARAM=7 NCOMP=3
     COMP=(GUT, DEFDOSE)
     COMP=(PARENT,DEFOBS)
     COMP=(METAB)

$PK
;CPM=THETA(1)*EXP(ETA(1))
 CPR=THETA(1)*EXP(ETA(1))
  VP=THETA(2)*EXP(ETA(2))
 CLM=THETA(3)*EXP(ETA(3))
  VM=THETA(4)*EXP(ETA(4))
 VMAX=THETA(5)*EXP(ETA(5))
  KM=THETA(6)*EXP(ETA(6))

  S2=VP
  S3=VM

      K12=THETA(7)
;     K23=CPM/VP
      K32=CLM/VM
```

```
      K20=CPR/VP

$DES
 DADT(1)=-K12*A(1)
 DADT(2)=K12*A(1)-K20*A(2)-VMAX*A(2)/(KM*VP+A(2))+K32*A(3)
 DADT(3)=VMAX*A(2)/(KM*VP+A(2))-K20*A(3)

$ERROR
 DEL=0
 IF(F.EQ.0) DEL=1
 W=F
 IPRED=F
 IRES=DV-IPRED
 IWRES=IRES/(W+DEL)

  IF(CMT.EQ.2) THEN
   Y=F+F*EPS(1)+EPS(2)
  ENDIF
  IF (CMT.EQ.3) THEN
   Y=F+F*EPS(3)+EPS(4)
  ENDIF

$THETA
   (0,2,30);CPR
   (420 FIXED);VP
   (0,50,100);CLM
   (55 FIXED);VM
  (0,6000);VMAX
 (0,15000);KM
   (2.84 FIXED)

$OMEGA
 0.001 ;[P] INTERIND VAR IN CPR
 0.3 ;[P] INTERIND VAR IN VP
 0.008 ;[P] INTERIND VAR IN CLM
 0.008 ;[P] INTERIND VAR IN VM
 0.001 ;[P] ] INTERIND VAR IN VMAX
 0.001 ;[P] ] INTERIND VAR IN KM

$SIGMA
 0.1 ;[P]
 0.1 ;[A]
 0.1 ;[P]
 0.1 ;[A]
```

```
$EST MAXEVAL=9999 PRINT=5 NOABORT POSTHOC
$COV
$TABLE ID VMAX KM CPR VP CLM VM K32 K20 ETA1 ETA2 ETA3
ETA4 RES WRES DV CMT IPRED IRES IWRES NOPRINT ONEHEADER
FILE=521.TAB
$TABLE ID  VMAX KM CPR VP CLM VM K32 K20 ETA1 ETA2 ETA3
ETA4 RES WRES DV CMT IPRED IRES IWRES NOPRINT ONEHEADER
FILE=patab521.TAB
$TABLE ID  VMAX KM CPR VP CLM VM K32 K20 ETA1 ETA2 ETA3
ETA4 RES WRES DV CMT IPRED IRES IWRES NOPRINT ONEHEADER
FILE=cotab521.TAB
$TABLE ID  VMAX KM CPR VP CLM VM K32 K20 ETA1 ETA2 ETA3
ETA4 RES WRES DV CMT  IPRED IRES IWRES NOPRINT ONEHEADER
FILE=catab521.TAB
$TABLE ID  VMAX KM CPR VP CLM VM K32 K20 ETA1 ETA2 ETA3
ETA4 RES WRES DV CMT IPRED IRES IWRES NOPRINT ONEHEADER
FILE=sdtab521.TAB
```

Figure 8. Explanation of S-Plus Box Plots using Manuscript II Figure 5



The lower and upper lines of the box designate the 25 and 75th percentile respectively.

The whiskers of the plot are the nearest value not beyond a standard span from the quartiles. The standard span is calculated as 1.5·interquartile range.

Lines outside of the box and whiskers are outliers.

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