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Gautam Baheti
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POPULATION PHARMACOKINETICS OF CYCLOSPORINE IN
HEART AND LUNG TRANSPLANT PATIENTS

BY

GAUTAM BAHETI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

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OF

GAUTAM BAHETI

APPROVED:

Thesis Committee

Major Professor

Sara Rosenbaum

F. Akhlag

Rulita J

Harold D. Bibb
DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

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ABSTRACT

Pharmacokinetics involves the analysis of plasma concentration and time data to obtain models that summarize the absorption, distribution and elimination parameters of a drug. The population approach to pharmacokinetics involves the estimation of mean pharmacokinetic parameters and their variability within the population.

Cyclosporine is a widely used immunosuppressive agent, and its pharmacokinetic parameters are characterized by a large variation in blood concentrations after oral or intravenous administration. Cyclosporine being a narrow therapeutic index drug is associated with significant consequences if the drug is present in 'sub-therapeutic' or 'supra-therapeutic' concentration. Optimization of therapy is challenging owing to variable pharmacokinetic parameters and narrow therapeutic index.

Population pharmacokinetic approach is used in this study to identify and characterize demographic and pharmacological variables that influence the pharmacokinetics of cyclosporine in lung transplant recipients.

Cyclosporine concentration-time data obtained through a randomized, prospective clinical trial was re-analyzed. A total of 1004 abbreviated cyclosporine profiles were available from 48 patients at 1, 2, 3, 4, 12, 24, 36 and 52 post operative weeks. Population modeling was performed using NONMEM (Version V). A one-compartment model with first-order absorption and elimination was used to model the data. Exponential models were used for inter-individual variation on oral clearance

(CL/F) and volume of distribution (V/F). A proportional model was used for residual error.

Estimates of CL/F and V/F (\pm S.E) were 26.4 (3.7) L/h and 183 (37) L, respectively. Concomitant itraconazole and diagnosis of cystic fibrosis were identified as significant covariates for CL/F. Time post transplant and different formulations were significant when modeled on bioavailability. With this model the estimated coefficients of variation were 18.5% and 49.6% for interpatient variability in CL/F and residual variability, respectively. Patients taking itraconazole were found to have a CL/F of 11.6 (4.3) L/h, 43.9% that of the other patients. Patients with cystic fibrosis had CL/F of 52.3 (6.9) L/h, 50% higher than patients without cystic fibrosis. Relative bioavailability of cyclosporine from Sandimmune® was 87% that of Neoral®.

In conclusion, the covariates which influenced the pharmacokinetics of study population were concomitant itraconazole, diagnosis of cystic fibrosis, time post transplant and different formulation.

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PREFACE

This document was prepared in the format of manuscript plan in accordance to the Graduate School guidelines of the University of Rhode Island. The thesis is divided into two sections.

Section I is composed of two manuscripts. Manuscript I is a general introduction to the topic of research, encompassing the introduction to pharmacokinetics, population pharmacokinetics, cyclosporine and cystic fibrosis. Manuscript II consists of the main body of this thesis, written in a format required for scientific journal submission. Section II contains appendix that includes additional information and details of control file useful in the analysis to understand the work in Section I. A bibliography follows section II in which all sources used as references in this document are cited.

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MANUSCRIPT I

INTRODUCTION

Pharmacokinetics is the study of the relationship between the dose of a drug and the manner in which its plasma concentrations change over time. More specifically, pharmacokinetics involves the analysis of plasma concentration and time data to obtain models that summarize the dose-plasma concentration relationship in terms of the absorption, distribution and elimination parameters of a drug. An understanding of drug's pharmacokinetic characteristics is important for drug development and the determination of safe and effective doses. Pharmacokinetic studies can also be used to investigate the effects of demographical characteristics such as weight, disease status, sex, age etc that may influence the dose-plasma concentration profile (1).

There are broadly two ways of determining a drug's pharmacokinetic characteristics in a population. Firstly, the traditional analysis which involves modeling each individual's concentration-time data to obtain individual pharmacokinetic parameters. Individual parameters are then averaged to determine the population values. Secondly pharmacokinetic analysis can also be conducted using a population approach in which the pharmacokinetic parameters of the population are determined directly.

The traditional approach to pharmacokinetic studies involves taking intensive plasma samples, up to 10-20 per individual, from a small group of subjects or patients. The data from each subject are individually fitted to a pharmacokinetic model (e.g., a one- or two- compartment model) to obtain the individual's pharmacokinetic parameters. Then summary statistics such as the mean and the variance of the group are calculated by pooling each individual's pharmacokinetic parameters. Limitations to the traditional approach are that only a small number of individuals can be studied and the subjects tend to be either healthy volunteers or relatively healthy patients with only a mild form of the disease. Thus the population is generally not representative of the true population to be treated (2).

In contrast to the traditional approach, the population approach to pharmacokinetic modeling often uses sparse data i.e. only a few samples from each subject. The sparse sampling is balanced with study of a large and often diverse study population. The population approach also provides estimates of the inter-individual variability of the pharmacokinetic parameters in the population. Sources of inter-individual variability such as patient age, weight, sex, disease condition, and concomitant medications can be identified and their relationship to the pharmacokinetic parameters quantified. Identification of these factors and the modeling of their relationship to the specific pharmacokinetic parameters is an important component of the population

approach and is valuable in allowing more rational dosage regimens in patients (3). Also the population approach provides estimates of residual or intra-individual error due to random error and model mis-specification. Thus population approaches is particularly useful for the study of intra- and inter-individual variability.

In summary, the population approach to pharmacokinetic analysis is performed to (i) estimate the mean pharmacokinetic (PK) parameters in a patient population, (ii) investigate and identify patient characteristics as sources of variability (covariates) which influence the PK of drug, (iii) estimate the unexplainable interindividual variability, and (iv) estimate the random residual variability (including intraindividual, measurement error).

Population Approach

There are two components to a population pharmacokinetic model. The first part is the structural model, which characterizes the specific pharmacokinetic model and the relationship of the pharmacokinetic parameters to the patient characteristics. The second part is the statistical model, which quantifies unexplainable variability of the data (4).

The population model will be explained using a simple example using one-compartment model for IV bolus input:

$$C_{pj} = \frac{D}{V} e^{-\frac{CL}{V} t_j} \quad (\text{Eq.1})$$

Where C_{pj} is the observation at time j , as a function of dose (D), volume of distribution (V), time (t) and clearance (CL). Note $CL/V = k$. It is recognized that the value of each parameter will vary in a population.

Various models can be used to describe parameter variability. The simplest model is for additive error:

$$CL_i = CL_{pop} + \eta \quad (\text{Eq.2})$$

Where CL_i is the CL in individual 'i' and CL_{pop} is the population mean. The parameter eta (η) represents difference between CL_i and CL_{pop} . Every individual in the population has a specific value for their pharmacokinetic parameter, which will differ from the population typical value due to unexplainable variability, which is quantified by using the parameter eta (η). It is assumed that η is normally distributed with a mean of zero and Standard deviation of ω . Similar models can be used for V .

Additional models can be used to explain the difference between the predicted and measured concentration in an individual. The difference between the predicted and measured concentrations is due to residual or intra-individual variability. Again the simplest model for this type of variability is an additive model, which has the form:

$$C_{pi,j} = C_{pmij} + \varepsilon \quad (\text{Eq. 3})$$

Where ε (Epsilon) represents the difference between the model predicted concentration (C_{pmij}) in individual i at time j and the actual plasma concentration (C_{pij}) in individual i at time j . Again ε is assumed to be normally distributed with a mean of zero and a standard deviation of σ .

Non-linear Mixed Effect Modeling (NONMEM)

The principle objective of modeling pharmacokinetic data is to find parameter values that reduce the difference between the observed data and predicted data (5). The relationship between concentration-time is non-linear; hence non-linear modeling techniques are required to fit a line through the data to obtain parameter estimates. A number of computer software packages have been developed for population analysis and Non-linear Mixed Effect Modeling (NONMEM) developed by Beal and Shiener (6) is most commonly used

software in population studies. It uses a true population approach in that the population parameters are determined in a single stage of analysis (7).

The term mixed effects modeling is used to describe the modeling process since two types of parameters (“effects”) are estimated. The fixed effect parameters are associated with the pharmacokinetic model and the random effect parameters describe the inter- and intra- individual variability associated with the statistical model (8). The fixed effects are represented by the population pharmacokinetic parameters and covariates relating them to patient characteristics. Thus, fixed effects include the dose, clearance (CL) and volume of distribution (V), and coefficients linking physiological factors such as age, weight and creatinine clearance and other factors such as concomitant medications to the pharmacokinetic parameters (3) (8). In NONMEM the fixed effect parameters are usually given the symbol theta.

Random-effect parameters quantify variability in the model arising from interindividual (between subjects) and intra-individual (within subjects) variation (3). Interindividual variability is the seemingly random between subject variability in the pharmacokinetic parameters that cannot be explained in terms of fixed effects. It is important to obtain an estimate of unexplained variability for a new drug because of the safety and efficacy of a drug tends to decrease as the unexplained variability in its pharmacokinetic parameters

increases (4). Intra-individual or residual variability is the unexplainable variability that occurs at the level of an observed plasma concentration. It may arise from the measurement error, model misspecification, and random variation in a patient's pharmacokinetic parameters that can occur over time (3) (8) (9) (10).

NONMEM can be used to derive a population model from randomly collected sparse data. The data from all individuals are pooled into one data set but individuals are still identifiable and this permits different numbers of repeated measures for the individuals. Once a population model has been derived a Bayesian post-hoc step can be invoked to permit the estimation of the pharmacokinetic parameters for each individual in the data set. The ability of NONMEM to take advantage of sparse data makes it ideally suited for the study of those populations where intensive sampling may be difficult and/or unethical, such as the very old, very young or very sick (6). Output from NONMEM includes estimates of mean variances and covariances of the parameters (6).

CYCLOSPORINE

A group of compounds called cyclosporines were isolated from the soil as major secondary metabolites of the fungus *Tolypocladium infatum* Gams (formerly *Trichoderma polysporum*). These substances were initially found to have antifungal activity. Cyclosporine A (CsA) is one of the major metabolites, and it has revolutionized organ transplantation. CsA is a neutral, hydrophobic, cyclic peptide containing 11 amino acids, (Fig 1) having a molecular weight of 1202.1, ($C_{62}H_{111}N_{11}O_{12}$). Rather than acting as a cytotoxic agent, which defined the activity of a number of available immunosuppressive drugs at that time, cyclosporine produces an immunomodulatory effect principally on the helper/inducer (CD4) lymphocytes, which orchestrate the generation of immune response.

Mechanism of Action

The exact mechanism(s) of immunosuppressive action of CsA has not been fully elucidated but appears to mainly involve inhibition of lymphocytic proliferation and function. It has been suggested that immunosuppressive action of cyclosporine results from specific and reversible inhibition of the immunocompetent T-cells in the G₀ (resting) or G₁ (post-mitotic, or presynthetic) phase of the cell cycle (11). CsA suppresses some humoral

immunity but is more effective against T cell-dependent immune mechanism such as those underlying transplant rejections and some form of autoimmunity (12). It preferentially inhibits antigen-triggered signal transduction in T lymphocytes, blunting expression of many lymphokines, including IL-2, as well as expression of antiapoptotic proteins. Cyclosporine forms a complex with cyclophilin, a cytoplasmic receptor protein present in the target cell. This complex binds to calcineurin, inhibiting Ca^{++} -stimulating dephosphorylation of the cytosolic component of NFAT (nuclear factor of activated T-cell) (13). When the cytoplasmic component of NFAT is dephosphorylated, it translocates to the nucleus, where it complexes with nuclear components required for complete T-cell activation, including transactivation of IL-2 and other lymphokines genes. Calcineurin enzymatic activity is inhibited following physical interaction with the cyclosporine/cyclophilin complex. This results in the blockade of NFAT dephosphorylation; thus, the cytoplasmic component of NFAT does not enter the nucleus, gene transcription is not activated, and the T lymphocyte fails to respond to specific antigenic stimulation.

Pharmacokinetics of Cyclosporine

Absorption and Bioavailability

CsA is absorbed in the upper part of the GI tract (14). Oral absorption of cyclosporine is slow and variable. The extent of absorption depends on the individual patient, patient population (e.g., transplant type), post transplant time, bile flow, GI state, and the formulation administered. CsA is a lipophilic drug and was first marketed in an oil-based formulation, Sandimmune® (Novartis Pharmaceutical) in 1983. The absorption of CsA from Sandimmune is associated with marked pharmacokinetic variability with large variation in the area under the time-blood CsA concentration curve (AUC), C_{max} and T_{max} (15). Emulsification of the crude oil-in-water droplet mixture formed on contact with GI fluids by bile salts is necessary before cyclosporine can be absorbed. Thus, the absorption is known to be highly dependent on bile production and early graft dysfunction with poor bile production and the use of external biliary drainage in patients lead to poor cyclosporine absorption (16) (17). The poor and highly variable absorption of the drug from Sandimmune hindered attainment of adequate cyclosporine concentration in the early post operative period (18) (19) and has been shown to be important risk factor of both acute and chronic rejection after organ transplant (20). The oral bioavailability of Sandimmune varies between 1 and 89%, with a mean value

of around 30% (21) (22). As a result of the biliary emulsification step noted above, the extent of absorption of Sandimmune can vary according to the presence of food, bile flow and GI motility.

To overcome the problems of poor and variable absorption of cyclosporine from Sandimmune, a microemulsion formulation Neoral®, was developed (23). This formulation incorporates cyclosporine in a microemulsified concentrate with a surfactant, lipophilic and hydrophilic solvents, and a hydrophilic co-solvent. Using this preparation, cyclosporine is more rapidly absorbed from the gastrointestinal tract so that blood concentrations reach a higher C_{max} within a shorter time than with the Sandimmune (Table 1). It was found that following oral administration of Neoral, the time to peak blood concentration (T_{max}) is 1.5 to 2.0 hours compared to 3.5 hours for Sandimmune (23) (25) (24). The dispersion of the microemulsion formulation within the intestinal tract does not rely on emulsification with the bile salts; hence CsA is absorbed more uniformly from Neoral than from Sandimmune. Furthermore, several studies have also documented a significant reduction in the incidence of acute cellular rejection in Neoral-treated versus Sandimmune-treated *de novo* renal (25) (26) liver,(27) (28) lung, (29) and cardiac (30) transplant recipients. Neoral has shown to reduce the variability in the gastrointestinal absorption of cyclosporine with an average bioavailability 30% higher than that of Sandimmune (17).

Administration with food both delays and decreases absorption. High- and low- fat meals consumed within 30 minutes of administration decreases the AUC by approximately 13% and maximum concentration by 33%. This makes it imperative to individualize dosage regimes.

Distribution

Cyclosporine is distributed extensively outside the vascular compartment. In the body, cyclosporine accumulates mainly in fat-rich organs including liver, adipose tissue and lymph nodes. After intravenous dosing, the steady- state volume of distribution has been reported to be as high as 3 to 5 liters/kg in solid organ transplant recipients. The drug is 98 to 99% bound to plasma proteins, crosses the placenta, and is distributed into human milk. Of the 90 to 98% of circulating cyclosporine bound to plasma proteins, 85 to 90% is carried on lipoproteins. Distribution of the cyclosporine within the whole blood is dose-dependent with 33 to 47% of the cyclosporine being present in plasma, 4 to 9 % in lymphocytes, 4 to 12% in granulocytes and 41 to 58% in erythrocytes (11). The distribution of cyclosporine in blood is highly temperature dependent. It has been reported (31) that at 37°C, 60% of the blood cyclosporine was localized in plasma compared with 46% at room temperature, which may be due to high affinity of cyclosporine for plasma protein,

including lipoproteins, at elevated temperatures (32). Due to this temperature dependency, whole blood is presently the preferred matrix for the therapeutic monitoring of total cyclosporine because storing the blood at different temperatures does not alter the total concentrations in blood (33).

Elimination

In adults with normal renal and hepatic function, the initial elimination half-life has been reported to be an average 1.2 hours, with a terminal elimination half-life of 8 to 27 hours (range 4 to 50 hours) (11). Clearance from the blood is approximately 0.3 to 0.4L/hr/kg in adults undergoing renal or hepatic transplantation, but is slightly lower after cardiac transplantation. Clearance in infants appears to be several times higher than in adults and is approximately doubled in older children (11).

Cyclosporine is extensively metabolized in the liver by the cytochrome-P450 3A (CYP3A) enzyme system and to a lesser degree in the gastrointestinal tract and the kidneys(34). The metabolism of cyclosporine is influenced by liver function. Pre-systemic extraction of cyclosporine is extensive after oral administration and is a major cause of the low bioavailability of CsA. Cyclosporine undergoes restrictive clearance and the pre-systemic extraction primarily occurs in the GI mucosa (14). The metabolism of cyclosporine

molecule involves mainly hydroxylation, demethylation and cyclisation of different amino acids while the cyclic structure remains intact. Major metabolic pathways that have been identified include hydroxylation of the C γ -carbon of two leucine residues, C λ - carbon hydroxylation and cyclic ether formation (with double bond oxidation) in the 3-hydroxy-N, 4-dimethyl-L-2-amino-6-octenoyl group and N-demethylation of the N-methyl leucine residues (11). Oxidation of cyclosporine yields the major metabolites AM1, AM4N and AM9, which account for approximately 70, 21 and 7.5%, respectively of the total AUC of cyclosporine.

Cyclosporine and its metabolites are excreted principally through the bile into the feces, with only approximately 6% being excreted in urine. Only 0.1% of cyclosporine is excreted unchanged in urine (24). Cyclosporine is also excreted in human milk.

Toxicity

The most important and clinically significant side effect of cyclosporine is nephrotoxicity. The other principal adverse reactions to cyclosporine therapy are tremor, hirsutism, hypertension, hyperlipidemia, and gum hyperplasia (14). Nephrotoxicity is limiting and occurs in the majority of patients. Nephrotoxicity is characterized with increased BUN (Blood Urea Nitrogen)

and serum creatinine concentration, and have been observed in 25-32, 38, and 38% of patients receiving the drug for kidney, heart, or liver allografts, respectively. Elevation of BUN and serum creatinine concentrations resulting from cyclosporine therapy appear to be dose related, may be associated with high trough concentrations of the drug, and are usually reversible upon discontinuation of the drug. Mild cyclosporine-induced nephrotoxicity generally occurs within 2-3 months after transplantation (11).

Mild to moderate hypertension also occurs in about 50% of renal transplant recipients who receive cyclosporine and in most cardiac transplant patients receiving the drug. Hypertension generally develops within a few weeks after initiation of cyclosporine therapy and affects both systolic and diastolic blood pressure. Tremors reportedly occur in 12-21, 31, or 55% of the patients with kidney, heart, or liver allografts, respectively who receive cyclosporine. Seizures (particularly when cyclosporine was used in combination with high-dose corticosteroids), headaches, paresthesia, flushing, and confusion have been reported occasionally in patients receiving cyclosporine.

Drug Interactions with Cyclosporine

Cyclosporine is metabolized by CYP3A4 and is a substrate of P-glycoprotein, hence drug interactions exist with agents that inhibit these pathways or are

cleared by these mechanisms. Drugs that induce cytochrome P-450 activity could increase the metabolism of cyclosporine and decrease its concentration in blood. Because of a large number of drugs interacting with cyclosporine, complete avoidance of drug interactions with cyclosporine is very difficult.

Drugs that inhibit cytochrome 3A4 and/or P-glycoprotein and are known to increase cyclosporine concentrations include: calcium channel blockers (verapamil, diltiazem, nicardipine), azole antifungal (fluconazole, itraconazole, ketoconazole), macrolide antibiotics (erythromycin, clarithromycin, troleandomycine), antivirals (indinavir, nelfinavir, ritonavir, saquinavir), steroids (methylpredisolone, oral contraceptive, androgens), psychotropic agents (fluvoxamine, nefazodone), amiodarone, chloroquine, allopurinol, bromocriptine, metoclopramide, cimetidine, grapefruit juice (11)(35).

Drugs that induce cytochrome 3A4 and/or P-glycoprotein and have been found to reduce cyclosporine concentrations include: anti-microbials such as nafcillin, rifampin and rifabutin, anticonvulsants (phenytoin, carbamazepine, phenobarbital, primidone), barbiturates, aminoglutethimide, troglitazone, octreotide, and ticlopidine (35).

Other agents known to cause interactions with cyclosporine are drugs that cause nephrotoxicity when administered alone. These include aminoglycoside

antibiotics, vancomycin, cotrimoxazole (trimethoprim- sulfamethoxazole), amphotericin B, and anti-inflammatory drugs (diclofenac, naproxen, and other non steroidal anti-inflammatory drugs) (35).

Therapeutic Drug Monitoring of Cyclosporine

Cyclosporine's variable pharmacokinetics result in wide variation in the blood concentrations achieved from a given dose of cyclosporine. Consequently dosage optimization is frequently performed using therapeutic drug monitoring. Monitoring is necessary to achieve clinical efficacy and while avoiding toxicity and maintain good tolerability.

Various approaches are used to monitor CsA (36). These include: (i) the measurement of trough concentrations (C₀) (ii) the measurement of the area under the blood concentration versus time curve (AUC), (iii) limited sampling strategies, (iv) monitoring of concentrations at 2 hours post dose (C₂), (v) Bayesian forecasting, and (vi) pharmacodynamic monitoring. Trough (C₀) monitoring is the traditional approach of monitoring and involves measurement of a single trough blood concentration of cyclosporine. It is simple to carry out and is practical for routine clinical analysis. However C₀ has been found to be a poor indicator of total drug exposure and not an accurate predictor of clinical efficacy (37) (38). AUC monitoring is a more precise way to monitor, as it is a

direct measurement of the extent of exposure to drug; it appears to predict clinical outcomes and allows calculation of oral pharmacokinetic parameters (20) (39). However it is impractical for routine clinical use, and is costly and inconvenient to both clinician and patients (37) (38). Limited sampling strategies represent a clinically feasible way to estimate AUC. A regression equation, developed from AUC values in a sample population is used to estimate subsequent AUC by sampling only 2-3 CsA concentrations at optimum times. This method is limited by the validation and predictive power of the equations generated (36). Monitoring of concentrations at 2 hours post dose is considered as the most useful tool for monitoring. It closely correlates with AUC₀₋₄, the period of maximum intra-individual and inter-individual variability, and C-2 monitoring is practical and convenient for clinical setting (40) (41). The Bayesian forecasting, involves the calculation of pharmacokinetic parameters in a patient by blending the patient specific drug concentrations with pharmacokinetic and statistical models that have been established for the particular patient population. However population databases are not generally available for cyclosporine. Finally pharmacodynamic monitoring has also been used and it involves the use of in-vivo markers of immunosuppression. However it is not widely used because the assays are cumbersome, and because of the difficulty in distinguishing rejection from toxicity.

Analytical Instrumentation for Monitoring Drug Concentration

Cyclosporine is a narrow therapeutic index drug with variable pharmacokinetics. Consequently therapeutic drug monitoring is often performed to individualize the dose to ensure optimum immunosuppressive activity. Monitoring is done using whole blood because of temperature dependency in blood-plasma ratio. The assays available for whole blood and their associated therapeutic ranges include monoclonal radioimmunoassay (range 75-325 $\mu\text{g/L}$), monoclonal antibody fluorescence polarization immunoassay (mFPIA) (range 100-400 $\mu\text{g/L}$), polyclonal antibody fluorescence polarization immunoassay (pFPIA)(range 200-800 $\mu\text{g/L}$), enzyme multiplied immunoassay technique (EMIT) (range 75-375 $\mu\text{g/L}$) and high performance liquid chromatography (HPLC) (range 100-325 $\mu\text{g/L}$) (35). HPLC is used as a reference standard method for monitoring cyclosporine concentration against which other analytical methods should be validated.

CYSTIC FIBROSIS

A varied amount of references are available in clinical textbooks (42), World Wide Web (43) and journal articles (44) (45) concerning the clinical physiology of cystic fibrosis. Cystic fibrosis (CF) is an inherited disorder that affects the body's epithelial cells. Under normal circumstances, certain types

of epithelial cells produce mucus and other watery secretions that coat the passageways of the lungs, liver, pancreas, reproductive tract, and intestinal tract. However, in cystic fibrosis inherited CF gene abnormalities cause these epithelial cells to produce secretions that are much thicker than normal, this abnormally thick mucus is due to the faulty transport of sodium and chloride within cells lining the organs.

In the lungs of persons with CF, thickened secretions trap microorganisms and encourage repeated lung infections. In pulmonary disease airways obstruction, impaired mucociliary clearance, bronchiectasis, and chronic infection are characteristic of CF and account for the progressive loss of lung function. This is marked by an average decline in FEV1 (is the forced expiratory volume in 1 second and determines the capacity of a person to breathe out in one second, trying as hard as possible) of roughly 1.5-4% per year. Therapy is directed at airway clearance, treatment of exacerbations, and management of airflow obstruction. Lung transplantation is a final option for those patients with severe progressive pulmonary disease.

Cystic fibrosis has a variety of symptoms. The most common are: very salty-tasting skin; persistent coughing, wheezing or pneumonia; excessive appetite but poor weight gain; and bulky stools. The sweat test is the standard diagnostic test for cystic fibrosis. This simple and painless test measures the amount of salt in the sweat. A high salt level indicates that a person has CF.

The treatment of CF depends upon the stage of the disease and which organs are involved. Antibiotics are also used to treat lung infections and are administered intravenously, orally, and/or medicated vapors, which are inhaled, to improve breathing.

Chronic infection in the airways is present in most patients with CF. The clinical course of disease is marked by periods of stability which are interrupted by exacerbations characterized by increased sputum production, dyspnea, fatigue, weight loss, and decline in FEV1. Exacerbations most often result from bacterial infections. *Staphylococcus Aureus* and *Haemophilus Influenza* are common pathogens in children. The prevalence of *Pseudomonas Aeruginosa* rises during childhood and adolescence so that 80% of CF patients are infected with *Pseudomonas* by age 18. Aggressive treatment of infection is the recommended approach, and in the adult therapy is mainly targeted at *Pseudomonas*. A typical antimicrobial regimen consists of intravenous tobramycin and an anti-pseudomonal penicillin or cephalosporin. Antibiotics are tailored to the results of each individual's sputum culture.

Bilateral lung transplantation remains an option for severe progressive pulmonary disease. An FEV1 of 30 percent predicted, frequent hospitalizations and pulmonary hypertension are generally used in the decision to refer for

transplantation. Although transplantation offers the only potential life saving therapy, the procedure is not without risks as 5-year survival is just under 50%.

Figure 1.1. Chemical Structure of Cyclosporine

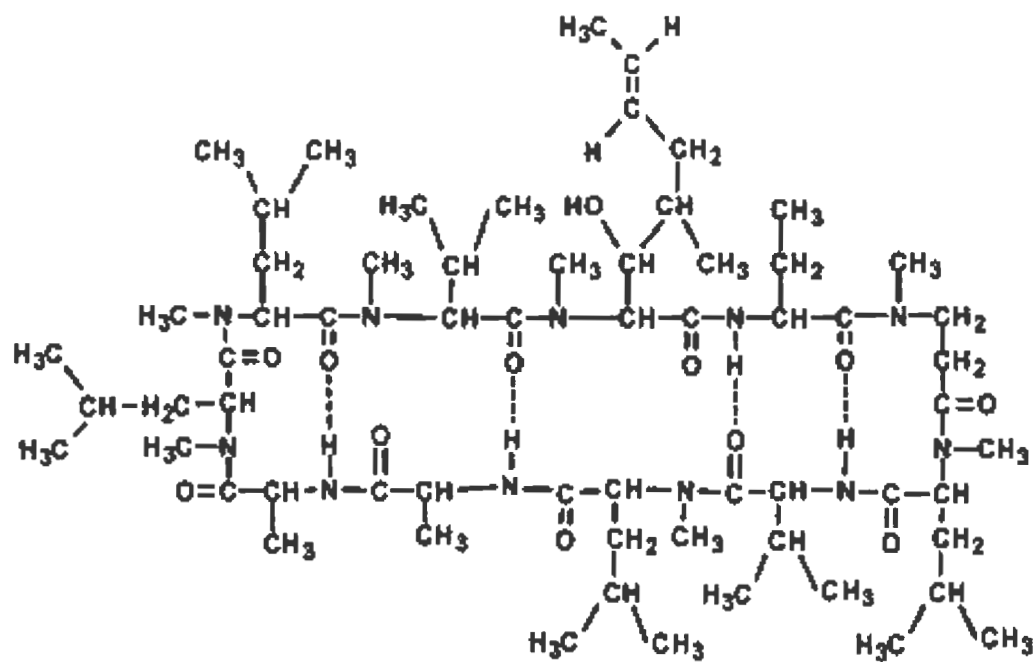


Table 1.1. Comparative pharmacokinetics of Sandimmune and Neoral formulation of cyclosporine

	Neoral	Sandimmune
AUC (µg/L.h)		
a.	18.17	13.29
b.	24.4	16.3
c.	3525	2556
Cmax (µg/L)		
a.	4.09	2.60
b.	6.2	4.7
c.	721	422
Tmax (h)		
a.	1.69	2.7
b.	1.7	4.5
c.	1.5	2.8
Cmin (µg/L)		
a.	0.67	0.55
b.	NA	NA
c.	151	121

Reference: a=Kahan et al (46); b=Keown et al (47); c=Keown et al (25)

AUC shown are at 12-hour intervals.

AUC= area under the blood concentration versus time curve for cyclosporine; Cmax= maximum blood concentration of cyclosporine; Cmin= minimum (trough) blood concentration of cyclosporine; Tmax= time to Cmax

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MANUSCRIPT II

ABSTRACT

Cyclosporine (CsA), a potent immunosuppressive agent has markedly improved the graft survival rate, but owing to its narrow therapeutic index the clinical use of cyclosporine is complicated by large intra- and interindividual variabilities in its pharmacokinetics, and consequently it is necessary to individualize the dose for each patient. Many factors such as patient age, gender, time post transplant, concomitant medication, presence of certain disease conditions like cystic fibrosis (CF), ethnic origin and gastrointestinal status have been believed to influence cyclosporine pharmacokinetics. The population pharmacokinetic analysis is ideally suited to study the variability of CsA pharmacokinetics within the patient population. Additionally, cyclosporine is available in two formulations: Sandimmune, an oil based preparation, and Neoral, a microemulsion formulation, which display different pharmacokinetic profiles.

Non linear mixed effect modeling (NONMEM) was used to perform population modeling of CsA on blood samples obtained from 48 thoracic transplant patients. Samples were collected at weeks 1, 2, 3, 4, 13, 26, 39 and 52 post transplant. In general each individual provided 3 blood samples per visit at approximately time 0, 2 and 6- hours post dose. For the analysis a one-compartment model with first order absorption was used to describe the model.

The value of absorption rate constant (k_a) was fixed due to a limited number of blood samples in the absorption period. An exponential error model was used to describe inter-individual variability in oral clearance (CL/F) and a proportional error model was used for residual variability. Itraconazole and cystic fibrosis were found to be significant covariates for CL/F. The type of formulation and time post-transplant were identified as significant covariates for bioavailability. The final model estimates for CL/F were $26.4(\pm 3.7)$ L/h. In the presence of itraconazole or cystic fibrosis the estimates of clearance were $11.6 (\pm 4.3)$ L/h and $52.3 (\pm 6.9)$ L/h, respectively. The bioavailability of Sandimmune was found to be 87% that of Neoral. During the first four weeks after transplant, in which a linear model was assumed, bioavailability was 64.5% that in subsequent weeks. The volume of distribution was $183 (\pm 37)$ L. The estimates of CV for the final model for interindividual variability on CL/F were 18.5% and for residual variability 49.6%.

INTRODUCTION

Cyclosporine (CsA) is a potent immunosuppressive agent, widely used to prevent graft rejections. Cyclosporine has markedly improved the graft survival rate, but owing to its narrow therapeutic index and its highly variable pharmacokinetics, a whole blood monitoring of CsA concentrations is commonly performed to individualize the dose in patients (1). Cyclosporine was initially formulated under the brand name Sandimmune® as an oil based formulation which successfully reduced the incidence of acute rejection in transplant patients, but this formulation was associated with high inter- and intra- patient variability and poor and variable bioavailability (2)(3), and attainment of adequate CsA level was particularly difficult. A microemulsion formulation of CsA, Neoral® was developed to circumvent some problems associated with Sandimmune (4). Cyclosporine is more rapidly absorbed from Neoral, which has a higher C_{max} and shorter T_{max}.

Due to the presence of marked intra- and inter-individual variability in CsA pharmacokinetics and the serious consequences of plasma concentrations outside the therapeutic range, there is a general consensus that a pharmacokinetic approach be used to optimize therapy (1)(5). Traditionally, trough levels of CsA are monitored (5). However this parameter does not

adequately reflect the overall exposure of the patients to CsA other methods have also been proposed. These include the blood concentration two hours after dose (C₂) and the measurement of area under the concentration-time curve (AUC) (5). However, the latter method requires the collection of a series of blood samples and is impractical in clinical practice. To overcome this disadvantage a Bayesian approach using a limited sampling method in conjunction with a population pharmacokinetic model has been proposed (6).

Many factors are believed to influence cyclosporine pharmacokinetics. Cyclosporine has a highly variable absorption, which is dependent on liver function, bile flow, time post transplant and gastrointestinal status (3). The distribution of CsA is mainly influenced by lipoprotein concentration in plasma. However, age, gender and obesity do not appear to be important factors for distribution. Metabolism of CsA can be influenced by the use of concomitant medication, and other factors such as presence of certain disease conditions like cystic fibrosis, and ethnic origin (3).

Cyclosporine is metabolized primarily by cytochrome P450 3A4 in the liver and small intestine. Cyclosporine is also a substrate of p-glycoprotein (which acts as a counter-transport pump, actively transporting cyclosporine back to the intestinal lumen). Unexpected drug interactions can lead to sub-therapeutic dosing in case of enzyme inducers, or drug toxicity with enzyme inhibitors.

Itraconazole appears to inhibit both cytochrome P450 3A4 and p-glycoprotein. Back et al (7) conducted an in-vitro study using human liver enzymes to examine the ability of antifungal drugs to inhibit the metabolism of CsA. They found out that ketoconazole was the most potent inhibitor of CsA; itraconazole was the next potent, and fluconazole the least potent inhibitor of cyclosporine metabolism. Inhibitory effects of itraconazole have also been demonstrated in-vivo (7). Concomitant administration with CsA increased whole blood or serum concentration of CsA and serum creatinine concentrations.

Cystic fibrosis (CF) affects the mucus and sweat glands of the body and is caused by a defective gene. Thick mucus is formed in the breathing passages in the lungs and this predisposes the person to chronic lung infections. Lung transplant is the treatment of choice for patients with end stage cystic fibrosis. This disease has been found to alter the pharmacokinetics of CsA. These patients exhibit poor absorption that may lead to ineffective immunosuppression and subsequent graft rejection. Patients with CF usually have fat malabsorption due to pancreatic insufficiency and require treatment with pancreatic enzyme supplements (8). Despite such therapy, the absorption of CsA from Sandimmune, which is a lipophilic immunosuppressive agent, has been found to be reduced in CF patients (8). The absorption of CsA from microemulsion formulation Neoral appears less affected by CF (9). Cystic fibrosis patients undergoing heart and lung transplant require a higher dose of

CsA and frequent monitoring of blood level to achieve adequate immunosuppression. In a study by Tan et al. (10) the apparent oral clearance of CsA was found to be about twice as high in patients with CF than in patients without CF. This increase could, however, be caused by the poor bioavailability of the drug which has been suggested in patients with CF by Cooney et al.(11). Other drugs that have shown elevated clearance when patients have CF include theophylline, ciprofloxacin, sulfamethoxazole and ibuprofen (11).

Kahan et al. (12) showed patient's age over 45 years had lower CsA clearance and male and female patients were shown to differ significantly in their ability to clear and distribute CsA. Ethnic background was found to influence the bioavailability of Sandimmune. Lindholm et al., (13) found the bioavailability (F) of CsA to be significantly lower in black patients than in white patients (mean values of $30.9\% \pm 12.3\%$ and $39.5\% \pm 16.5\%$, respectively; $p < 0.001$). The study found that these racial difference in F may contribute to the poorer outcome observed after kidney transplantation in black patients. However in a controlled study done on healthy African American and white volunteers by Stein et al., (14) no difference in pharmacokinetic parameters were found between the ethnic groups for Sandimmune and Neoral. However compared to Sandimmune, Neoral resulted in an approximately 60% higher C_{max} , a 50%

greater AUC, and a 25% lower oral clearance in both African American and white subjects.

An understanding of factors that modify CsA pharmacokinetics, particularly its bioavailability and clearance is important in order to better predict the optimum dose for a patient. This study describes the application of the population approach to pharmacokinetic analysis to the study of the pharmacokinetics of CsA in thoracic transplant patients.

MATERIALS AND METHODS

Study Design:

Population pharmacokinetics of CsA were studied using data obtained from a previously published study (15). A randomized, open-trial study was conducted on heart and lung transplant recipients receiving either Sandimmune or Neoral as immunosuppressive therapy during the first year of transplant conducted at Papworth Hospital, Cambridge, UK. The Local Ethics Committee approved the study and approval from the Institutional Review Board (IRB) at The University of Rhode Island was obtained to re-analyze the data. In brief, blood samples were collected from 48 patients aged 19 to 66. The group consisted of 26 males and 22 females who had undergone either single lung

(18 patients), double lung (9 patients), or both heart and lung (21 patients) transplant. Twenty-one patients received Sandimmune and 27 patients were administered Neoral. Patients with cystic fibrosis (CF) were randomly selected independently because of unusual pharmacokinetic in CF patients. Patients with CF received their daily oral CsA at 8-hour intervals rather than the usual 12-hour interval.

Immediately following transplant, patients received intravenous methylprednisolone and rabbit antithymocyte globulin induced immunosuppressive therapy was given to all the patients, followed by maintenance triple-therapy with oral cyclosporine, azathioprine, and prednisolone. A dose of 50 mg of Sandimmune or Neoral was administered to the patients on the first day of their transplant and increased by 50 mg at each 12-hourly dose until therapeutic trough levels were achieved. Therapeutic goals for the trough levels were 300-400 μ g/L for months 1 and 2 and 200 to 300 μ g/L for months 3 and 12.

Pharmacokinetic Protocol and Analytical Method

The data collected over 12 month period was stored in a computer database. Patients had blood samples drawn for analysis of CsA concentration during clinical follow-up visits at weeks 1, 2, 3, 4, 13, 26, 39 and 52. In general each

individual provided 3 blood samples per visit: at approximately time 0- (C_0), at 2- (C_2) and 6- hours (C_6) post dose.

Blood cyclosporine concentrations were monitored by Dade-Behring Emit 2000 immunoassay (Dade-Behring Diagnostic UK, Ltd.; Milton Keynes, UK). Special care was taken to ensure that the trial database only included cyclosporine measurements taken under steady state conditions.

Data Presentation and Pharmacokinetic Analysis

Clinical, pharmacokinetic, and demographic data including dose of CsA, CsA blood concentrations, age, body weight, time post transplant (TPT), type of formulations given, concurrent medications and disease condition relevant to the population analysis, were extracted from the raw data sets and merged and formatted using Microsoft[®] Excel 2000. The concentration time data were tabulated for completeness and consistency of recorded sampling and dosing time and prepared along with the relevant demographic data for analysis.

The pharmacokinetic analyses were performed using NONMEM (version V, double precision) (S.L. Beal and L.B. Sheiner, NONMEM users guide, NONMEM Project Group, University of San Francisco, San Francisco).

Pharmacokinetic Model

A one-compartment model with first order absorption was used (ADVAN2 and TRANS 2) and was parameterized as the first order absorption rate constant (k_a), apparent clearance (CL/F) and apparent volume of distribution (V/F). Since a limited number of blood samples were collected during the absorption phase, the absorption rate constant (k_a) could not be estimated and was fixed; the fixed values were taken from previously reported population values (16): $1.35h^{-1}$ and $0.25h^{-1}$ for Neoral and Sandimmune, respectively. The relative bioavailability (F) of Sandimmune compared to Neoral was also estimated.

Statistical Model

Additive, proportional or exponential error models were used in developing the population model for interindividual variability in the pharmacokinetic parameters of CL/F , V/F and K_a .

The models were:

$$\theta_i = \theta' + \eta_{\theta i} \quad \text{For additive-error model,}$$

$$\theta_i = \theta' [1 + (\eta_{\theta i})] \quad \text{For proportional-error model and}$$

$$\theta_i = \theta' \exp(\eta_{\theta i}) \quad \text{For exponential-error model}$$

Where θ_i is the estimate for a pharmacokinetic parameter in the i th individual, θ' is the population mean of the pharmacokinetic parameter, and η_{θ_i} represents the random variable with zero mean and variance ω^2 that distinguishes the i th individual pharmacokinetic parameter from the population mean value predicted by the regression model.

Both proportional error model and combined additive- and proportional- error model were used to model residual variability (including intraindividual variability). The equations used are

$$C_{ij} = C'_{ij} (1 + \varepsilon_{1ij}) \quad \text{For proportional error}$$

$$C_{ij} = C'_{ij} (1 + \varepsilon_{1ij}) + \varepsilon_{2ij} \quad \text{For combined proportional- and additive- error models}$$

Where C_{ij} is the observed serum concentration of the i th individual at time j , C'_{ij} is the predicted serum concentration of i th individual at time j , and ε_{1ij} and ε_{2ij} are the component of proportional and additive errors with zero mean and variance σ^2 .

Data Analysis Strategy

The pharmacokinetic and statistical models were evaluated to determine the basic model that best fit the data. A statistically significant decrease ($P < 0.05$)

in the minimum value of the objective function (as measured by the log likelihood difference) was used as the criteria to determine the best model.

Initially, the population pharmacokinetic analysis was conducted without including any covariates in the model (basic model). After a basic model was identified, a model building process was employed to examine the influence of patient covariates on the estimates of pharmacokinetic parameters. The effects of the following patient covariates on CL/F and V/F were evaluated: age, weight, sex, formulation type (Sandimmune or Neoral), presence of cystic fibrosis, type of transplant (single lung, double lung and heart and lung), time post-transplant and use of concomitant medication. Age, weight, time post-transplant were examined as continuous variables. Sex, formulation type, presence of cystic fibrosis and use of itraconazole as a concomitant medication were examined as categorical variables.

A decrease in the minimum value of objective function of 3.841 or greater following introduction of a single covariate into the model was considered statistically significant ($P < 0.05$ with 1 degree of freedom) using the χ^2 distribution if the 95% confidence intervals (CI) for the estimate did not include null value. If the change in the objective function was 3.8 or greater but the 95% CI for the estimate included the null value, the effect of the variable was considered to be of borderline significance and that the covariate was not included in the full model. It was assumed that no significant

interaction between covariate factors existed. If there was an interaction present for an effect that was not significant alone at the $P < 0.05$ level, then the effect would be minor and would not likely to be of clinical significance. Thus, covariates effects were introduced individually and no covariate - covariate interactions were modeled. For the significant covariates the improvement in fit was assessed by the precision of the parameter estimate (standard error of the mean and 95% confidence interval) and by the reduction in interpatient and residual variability. In addition, scrutiny of the scatter-plots of weighted residual (WRES) vs. cyclosporine predicted concentrations (PRED) was another indicator of the goodness of fit in each model.

All significant variables were included in the full model and a backward elimination process was then employed to eliminate covariates from the full model in order to develop the final model. Backward elimination was performed by removal of a covariate from the full model one at a time and increase in the objective function of 6.68 or greater ($P < 0.01$ with 1 degree of freedom) on removal of a covariate from the full model signified that the variable was important, and that covariate was retained in the final model.

RESULTS

All error models (additive, proportional, exponential, and combined additive and proportional models) were tested to account interindividual variability in the pharmacokinetic parameters (CL/F and V/F) and residual error. An exponential model for inter-individual variability in the pharmacokinetic parameters and a proportional error model for residual variability best described the error models. A null value for the 95% confidence interval was observed for interindividual variability in V/F, and hence the error term was deleted in case of V/F for all further data analysis, in accordance with the data analysis strategy.

A scatter plot for observed versus model-predicted cyclosporine concentration is shown in Figure 2. The mean parameter estimates (95% CI are in parentheses) obtained from the analysis of base model is as follows (Table 4): CL/F 23.1L/hr (\pm 3.3 L/hr); V/F 202 liters (\pm 43 liters). The estimates for coefficient of variation (CV) for interindividual variability on CL/F were 32.1% and for residual variability 60.1%.

After the base model was completed, the influence of covariates was studied on both CL/F and V/F individually, and since k_a was kept constant, none of the covariates were estimated for k_a . In the model building process the covariates

were added to the base model one by one and those covariates which were found to have reduced the value of objective function (used as a measure of “goodness of fit”) significantly ($P < 0.05$) when tested against the base model for both CL/F and V/F were considered to be used in the final model building.

Age and type of transplant (single lung, double lung, heart and lung) were not found to be significant covariates for either CL/F or V/F (Table 2). Weight was found to be a significant covariate for CL/F but not V/F. However a null value was observed in the 95% confidence interval of CL/F (Table 2). Thus weight was not considered for the final model, in accordance with the data analyzing strategy.

Time post transplant (TPT) in weeks as a continuous covariate was not found to be statistically significant on CL/F. Analysis of the graph of the post-hoc values of CL/F versus time post transplant revealed a pattern which suggested decrease in CL/F over the first four weeks. Hence, time post transplant was modeled as a covariate for the first four weeks according to the formula $F1 = 1 - \text{Theta} (5) / \text{TPT}$ as shown in appendix. Modeled in this way time post transplant was found to be a significant covariate (Table 2). Estimate of the covariate for TPT on F for the first four occasions was $0.509/\text{TPT} (\pm 0.076)$, hence TPT was used in the final model building process.

In the analysis of the potential influence of gender on CL/F and V/F, male patients were assigned “1” and females “0”. Gender was added as a covariate according to the formula $TVCL = \text{Theta (1)} + \text{Theta (5)} * \text{Sex}$. No significant change was observed in objective function when gender was modeled for V/F. The change in objective function (160.88) was significant ($p < 0.05$) on CL/F and was used for the final model building process. The estimate of CL/F for gender was 28.6L/h for male and for females 18.3 L/h (± 5.2) (Table 2).

Itraconazole was modeled as a categorical variable. A value of “0” was assigned to patients not taking itraconazole and “1” to patients taking itraconazole. On the assumption that itraconazole would reduce the CL/F of CsA, itraconazole was modeled as a negative function according to the formula: $TVCL = \text{Theta (1)} - \text{Theta (5)} * \text{Itra}$. Concomitant itraconazole was found to be a significant covariate for CL/F (Table 2). The estimates for CL/F obtained for patients with itraconazole in their therapy are 11.7 L/h and without itraconazole the estimates for CL/F were 28.0L/h, a decrease in CL/F by 58.2% (Fig 5). The estimates for coefficient of variation obtained for interindividual variability on CL was 31% (Table 2) and residual variability 51.7%. Itraconazole was also studied on V/F and no significant difference in objective function was seen and hence not considered to be used in the final model for V/F.

Cystic fibrosis was evaluated by assigning patients with CF as 1 and patients without CF as 0. Cystic fibrosis was found to be a significant covariate for CL/F (Table 2). The estimates for CL/F when patients have CF and when they do not have CF are 54.4 L/h and 21.7 L/h respectively; evident that presence of CF increase the value of CL/F by 60% when compared to patients without CF. The coefficient of variability for interindividual error on CL/F was 23.7% (Table 2) and residual variability 60.6%. Cystic fibrosis was also analyzed on V/F, but no change in objective function was observed, hence not considered for final model. It was not possible to obtain estimates for cystic fibrosis when studied as a covariate on bioavailability (F), but when the different formulations (Sandimmune and Neoral) were included in the model, CF was found to be a significant covariate for F of Sandimmune and Neoral. However, when CF was included as a covariate for CL/F rather than F (on Sandimmune and Neoral), a more significant result was obtained. The use of CF as a covariate for CL/F resulted in a more significant effect (Table 2) than when it was used as a covariate for F (on Sandimmune and Neoral). It was found that CL/F accounted for a significant change in objective function by 265.80 from the base model; however modeling of CF on F (on Sandimmune and Neoral) was not used in the final model along with CF on CL/F as it was assumed that F would nullify the effect as we had modeled CF on CL/F previously and it was found more significant. It was not possible to model CF on both CL/F and F in the model. However just for the records when the analysis using CF on F

for both Sandimmune and Neoral was done, along with CF on CL/F a null value was obtained in the estimates of 95% CI for both Sandimmune and Neoral, which further confirmed our assumption of not using CF on CL/F and F together. Thus in the final model CF was used as a covariate for CL/F.

Formulation (Neoral or Sandimmune) type was found to be a significant covariate for F. Formulation type was also modeled using categorical variables; Neoral was assigned a variable “0” and Sandimmune “1”. The model had the form $F1 = 1 * \text{Dose} + \text{Theta } (5) * (1 - \text{Dose})$. The estimate of Theta (5) were 1.39 (± 0.45) which showed that the bioavailability of Sandimmune is 72% that of Neoral. The estimate of interindividual variability for CL/F was 30% (Table 2) and residual variability 62.4%.

To summarize, the following were identified as significant covariates for CL/F body weight, gender, cystic fibrosis as disease condition, itraconazole as concomitant medication, type of formulation (Sandimmune or Neoral), and time post transplant (Table 2). The covariates which were not significant were age and type of transplant (single lung, double lung or heart and double lung). Weight had a null value in the estimate and was not included for final model building, in spite of a significant change in objective function. Significant covariates were only found for when analyzed for clearance (CL/F), no

significant change was observed in the value of objective function when covariates were analyzed for volume of distribution (V/F).

Consideration of the above significant covariates during model building also resulted in improvement in the relationship between observed and model-predicted concentration and weighted residuals versus model-predicted concentrations. A reduction in the percentage value of residual random error when compared to the base model also indicated a better model fit (Table 2). A plot of observed and model-predicted concentration and weighted residual versus model predicted for the base model is shown in Figure 2 (a) and (b) respectively.

A full model was then developed containing all the significant covariates. Backward elimination was then performed to identify the covariates for the final model. The criteria for significance was a measure of change in objective function value greater than 6.6 ($p < 0.01$, with one degree of freedom) when a covariate was removed from the model. As a result of this process gender was eliminated from the model. The covariates CF, itraconazole, time post transplant and use of different formulation were retained and added to the final model (Table 3). The final model estimates for CL/F were 26.4(± 3.7), when itraconazole was in therapy the estimates were 11.6 L/h (± 4.3) and when patients had cystic fibrosis 52.3 L/h (± 6.9), as per the final model analysis.

This showed a decrease in CL/F by 58% when patients were on itraconazole without CF, an increase of 51% when patients had CF and no itraconazole in therapy and an increase of 32% when patients had CF and were on itraconazole. For the final model the estimates for different formulation of Sandimmune and Neoral obtained were 1.14 (± 0.18), showing bioavailability of Sandimmune 87% that of Neoral, similarly the estimates for TPT for the first four weeks were 0.355 (± 0.107). The estimates of CV for interindividual variability on CL/F were 18.5% and residual variability 49.6% (Table 3). The final model resulted in a better correlation of predicted versus the observed concentration when compared with the base model, also a better correlation was seen on scrutiny of weighted residual versus predicted concentration for final model when compared with the base model (Figure 6a. and 6b.).

DISCUSSION

The objective of the study was to estimate apparent clearance (CL/F) and apparent volume of distribution (V/F) of CsA in patients who have undergone heart lung transplantation and to identify patient's characteristics that influenced these pharmacokinetic parameters. This would permit more rational dosing of CsA and would assist physicians develop initial dosing regimens in patients who have characteristics known to influence CsA pharmacokinetics.

A one-compartment model best described the data. Others have found that CsA pharmacokinetics are best described by a two-compartment (17). It is likely that the limited number of blood samples especially during the distribution phase made it impossible to fit the data to a two-compartment model.

The estimates of CL/F and V/F obtained for the base model were 23.1 L/hr (\pm 3.3 L/hr) and 202 liters (\pm 43 liters) respectively, which is in good correlation with literature values of 28.1L/h and 280L for oral clearance and volume of distribution respectively (18). It was not possible to include a term for the inter-individual variability of V/F. This is probably because of the paucity of informative data during the initial period following the dose. Consequently, the inter-individual variability in V/F could not be studied.

Gender has been found to influence the CYP3A4 activity. Hunt et al (19) found the hepatic CYP3A4 activity to be 24% higher in females than males. Furthermore, when considered alone gender was found to be significant, ultimately gender was not found to be significant in this study where 54% patient were male.

Time post transplant is thought to affect bioavailability rather than apparent clearance. In a study by Parke and Charles (20) postoperative day was modeled on clearance, but was not found to be statistically significant, but when

modeled on F, a superior fit to the data was obtained with use of mathematical formulae. In this study also time post transplant was found to be significant when it was modeled on F. On plotting a graph (Fig.1) between normalized clearance (clearance in first week divided by clearance in following weeks) and time post transplant it was observed that CL/F decreased in the first four weeks and then stabilize over time, hence it was modeled for the first four weeks post transplant. However to be sure, runs were performed in which CL/F was allowed to decrease over periods of 3 and 12 weeks; however the best results were observed when the CL/F was allowed to decrease over a 4 week period. A mathematical formula was derived which accounted for the first four occasions. Estimates obtained were in agreement with previous studies that states that the bioavailability of CsA decreases in the 2-3 weeks after transplantation and then stabilize (14) (21).

Comparisons of Neoral pharmacokinetics to Sandimmune formulation have been well documented (22) (23). Studies have shown Neoral to be a better predictor of exposure and associated with reduced variability when compared to Sandimmune. In the previously published study (14) conducted on the same patients as this study, Neoral was associated with a higher and more consistent exposure compared to Sandimmune. The lower bioavailability of Sandimmune is in agreement with other studies, although the relative bioavailability of

Sandimmune (0.87) compared to Neoral is somewhat larger than that published in other studies (17) (22) (24).

Cyclosporine absorption and consequently blood concentration may vary significantly according to bile flow, co-administration with food, GI tract motility, renal function, and several drugs that patients may receive after transplantation (25). Several of these factors are particularly important to patients with CF. In CF, hepatobiliary involvement and alterations in bile flow are common, and can change in severity in any individual (26). Bile acid is required for micellar solubilization with Sandimmune, whereas Neoral is a microemulsion that avoids bile salt dependence. In this study, presence of CF as a disease condition increased CL/F when compared to non-CF patients. This observation has also been found by others (27). Patients with cystic fibrosis undergoing lung transplant have been found to absorb Sandimmune cyclosporine poorly (7). Neoral however has shown to provide better absorption and produce higher drug exposure in both heart and lung transplant recipients when compared to Sandimmune (8) (28). In this study eight patients had cystic fibrosis of which 3 patients took Sandimmune and 5 patients took Neoral. It was observed that in the CF patients Sandimmune showed a larger CL/F than Neoral (Fig. 3). This is probably the result of a lower F in patients taking Sandimmune. In a study on CF patients by Reynaud-Gaubert et al, they demonstrated a higher and more reliable bioavailability with less intrapatient

variability from Neoral when compared to Sandimmune (27). In the present study no definite results could be obtained for the change in bioavailability in patients with CF on Sandimmune or Neoral. The relationship of different formulations (Neoral and Sandimmune) on CF and non-CF patients was initially performed. The estimates obtained for CF when patients were on Neoral was 0.52, and 0.193 when on Sandimmune. Similarly the estimates for non-CF patients for Neoral and Sandimmune were 1.85 and 0.193 respectively. However, when this model was added for final model building, a null value obtained, further analysis was not performed as no significant and reliable results could be obtained from the comparison.

The potential for significant drug interactions is well recognized for CsA (3) (16) (29) because it is a substrate for cytochrome P-450 3A4 and P-glycoprotein (30). Itraconazole is a CYP P-450 inhibitor has been shown in previous studies (31) (32) to influence cyclosporine pharmacokinetics by decreasing clearance. This was confirmed in the present study. The population approach to the analysis of pharmacokinetic data is useful for identifying and quantifying clinical significant drug interactions without the need for controlled clinical investigation (33) (34). Using the population approach it is possible to assess the clinical importance of drug interactions in patients administered drugs as part of clinical therapy. However it is difficult to

interpret information that suggests a lack of interaction because of problems assigning the statistical power comparison using a population approach.

A relationship between age and changes in CsA pharmacokinetics was not found to be significant, possibly due to the patient population belonging to a limited age category (range 19-66). Burckart et al (35) did show an increase in clearance in pediatric liver transplant (1-5 years) showing that clearance of CsA may be several times higher in infants and up to twice as high in children than adults, however no study has shown the influence of adult age as a significant covariate on clearance.

There are no reports in literature to support type of transplant affects CsA pharmacokinetics, and this study found no relationship between type of transplant and CL/F. There has been a mixed response in order to ascertain the influence of weight on the pharmacokinetics of CsA. Certain authors (36) found weight not to influence the pharmacokinetics of cyclosporine, but others have found weight to be a significant covariate when modeled on CL/F and V/F(20). In this study weight did have a significant change in objective function for CL/F, however the CI of the estimates for weight involved a null value and hence was not considered significant and was not used for final model building. Weight was not identified as a significant covariate on V/F. It

is possible that the low number of patients and the limited weight range (39.6-88 kg) made it difficult to adequately assess the significance of this covariate.

In conclusion the population pharmacokinetics of cyclosporine in heart and lung transplant recipients was studied. Several patient characteristics such as concomitant itraconazole, presence of cystic fibrosis, different formulation of cyclosporine, and time post transplant were found to influence the pharmacokinetics of cyclosporine. It was found that during the first 4 weeks post transplant oral clearance decreased probably because absorption increased over 4 weeks time, concomitant itraconazole decreased oral clearance by 43.9%, patients with CF has oral clearance higher by 50% than non-CF patients, and the bioavailability of Sandimmune was 87% that of Neoral. The presence of one or all of these covariates has been found to change the pharmacokinetics of cyclosporine and hence care should be taken when selecting a dosing regimen for patients with these characteristics.

Table 2.1. Demographic Characteristic of the Patient Population

Characteristics of the Population N = 48	
Mean Age (years)	42 (range 19-66)
Mean Total Body Weight (Kg)	58.7 (range 39.6-88.0)
Gender	
Male	N = 26
Female	N = 22
Total Samples	1004
Samples per patient †	21 (3-24)
Formulation	
Sandimmune	21
Neoral	27
Cystic Fibrosis	8
Concomitant Itraconazole	11
Cystic Fibrosis and Concomitant Itraconazole	3
Transplant Type	
Single Lung	18
Double Lung	9
Heart and Lung	21

† 3 blood samples were obtained during a dosing interval at 0, 2 and 6 hours after dose. This was repeated for a maximum of 8 times over the course of a year.

‡ Non-cystic fibrosis patients received cyclosporine (25-450mg) every 12-hour
Cystic fibrosis patients received cyclosporine (100-500mg) every 8-hour.

Table 2.2. Summary of Analysis of Covariate Effect Tested on CL/F

Covariates	Model	OBJ	Δ OBJ	% CV	Significant (P=0.05)
Base	TVCL= θ (1)	-456.97	N.A	32.1	N.A.
Itraconazole	TVCL=θ1 + θ5*Itra	-1013.81	556.84	31	Significant
Cystic Fibrosis	TVCL=θ1 + θ5*CF	-739.75	282.02	23.7	Significant
Formulation	F1=1*FORM+θ5*(1-FORM)	-517.67	60.70	30.0	Significant
Gender	TVCL=θ1 + θ5*SEX	-617.85	160.88	33.2	Significant
Time Post Transplant	F1= 1- θ5 / TPT	-680.30	223.33	30.0	Significant
Weight †	TVCL=θ1+ θ5*WT	-549.22	92.24	32.1	Significant
Age	TVCL=θ1+θ5*Age	-456.97	0	32.1	Not Significant
Type of Transplant	TVCL=θ1+θ5*TYPE	-457.35	0	32.1	Not Significant

† Not included in the Final Model since estimate involved a null value in 95% CI
 Itra = Itraconazole. Patients on Itraconazole= 1, patients without itraconazole = 0
 CF = Cystic Fibrosis. Patients with CF=1, patients without CF=0
 FORM= Formulation. Neoral=0, Sandimmune=1
 Male= 1, Female=0
 WT= Weight in kilograms
 TPT= Time post transplant converted in weeks

Table 2.3. Backward Elimination of Significant Covariates to Build the Final Model

	Full Model	No Itra	No CF	No Form	No TPT	No Sex	Final
MOF	-1369.92	-1012.86	-1164.06	-1356.65	-1269.26	-1369.92	-1369.93
θ1 CL/F L/hr	26.4 (±4.7)	20.6 (±4.8)	26.2 (±4.9)	24.2 (±2.7)	29.1 (±5.1)	26.4 (±3.7)	26.4 (±3.7)
θ2 V/FL	182 (±38)	198 (±52)	176 (±40)	168 (±24)	201 (±44)	183 (±37)	183 (±37)
ka θ3 Sand h-1	0.250	0.250	0.250	0.250	0.250	0.250	0.250
ka θ4 Neo h-1	1.35	1.35	1.35	1.35	1.35	1.35	1.35
θ5 Itra	14.8 (±4.5)	NA	14.1 (±4.6)	13.8 (±3.7)	17.1 (±4.6)	14.8 (±4.3)	14.8 (±4.3)
θ6 CF	25.9 (±8.2)	25.9 (±11.8)	NA	23.2 (±6.1)	31.1 (±10.2)	25.8 (±6.9)	25.9 (±6.9)
θ7 Form	1.14 (±0.179)	1.22 (±0.22)	1.11 (±0.187)	NA	1.18 (±0.227)	1.14 (±0.179)	1.14 (±0.179)
θ8 TPT	0.355 (±0.106)	0.434 (±0.118)	0.423 (±0.073)	0.34 (±0.09)	NA	0.355 (±0.106)	0.355 (±0.106)
θ9 Sex	7.6e-007 (±4.80) [✱]	6.80 (±6.9) [✱]	2.02 (±5.45) [✱]	3.3e-011 (±4.68) [✱]	5.3e-005 (±5.43) [✱]	NA	NI
% CV	18.6%	19.2%	28.5%	21.6%	19.5%	18.5%	18.6%
Residual Error	49.6%	56.1%	49.7%	48.9%	52.1%	49.6%	49.6%

✱ denotes null value in the estimates; MOF= Minimum value of Objective function;
Form= Formulation; Neo=Neoral; Sand= Sandimmune; TPT = Time post transplant;

TVCL=THETA(1)-THETA(5)*ITRA+THETA(6)*CF
CL=TVCL*EXP(ETA(1))
TVV=THETA(2)
V= TVV
IF (TPT.LE.4) TVF1=1*DOSE+THETA(7)*(1-DOSE)-THETA(8)/TPT
F1= TVF1
IF (TPT.GT.4) TVF1=1*DOSE+THETA(7)*(1-DOSE)
F1=TVF1

Table 2.4. Comparison of Estimates for Base and Final Model

Parameter (units)	Base Model		Final Model	
	Parameter Estimates (95% CI)	Interpatient Variability CV% (95% CI)	Parameter Estimates (95% CI)	Interpatient Variability CV% (95% CI)
θ1CL/F L/hr	23.1(19.8 – 26.4)	32.1 (24.2 –38.3)	26.4 (22.7 – 30.1)	18.5 (13.6 – 23.4)
θ2 (V/F) L	202 (159 – 245)	NA	183(146-220)	NA
θ3 / θ4 (ka) h ⁻¹				
Sandimmune	0.25 Fixed	NA	0.25 Fixed	NA
Neoral	1.35 Fixed	NA	1.35 Fixed	NA
θ5 Itra	NA	NA	14.8(10.5-19.1)	NA
θ6 CF	NA	NA	25.9(19 - 32.8)	NA
θ7 F	NA	NA	1.14 (0.96 – 1.32)	NA
θ8 TPT	NA	NA	0.355 (0.248-0.462)	NA
Residual error CV	60.1 (50.9 –68.2)	NA	49.6 (45.6-53.2)	NA

Abbreviations: CL/F = clearance; V/F = volume of distribution; ka = absorption rate constant; Itra= Itraconazole; CF=Cystic Fibrosis; F=Bioavailability; NA = not applicable
 $TVCL = \theta_1 - \theta_5 * ITRA + \theta_6 * CF$; $CL = TVCL * \exp(\theta_1)$
 $TVV = \theta_2$; $V = TVV$
 $K = CL/V$
 IF (DOSE.EQ.1) $TVKA = \theta_3$
 $KA = TVKA$
 IF (DOSE.EQ.0) $TVKA = \theta_4$
 $KA = TVKA$
 $S2 = V$
 IF (TPT.LE.4) $TVF1 = 1 * DOSE + \theta_7 * (1 - DOSE) - \theta_8 / TPT$
 $F1 = TVF1$
 IF (TPT.GT.4) $TVF1 = 1 * DOSE + \theta_7 * (1 - DOSE)$
 $F1 = TVF1$

Figure 2.1. Kernel Graph CL_1/CL_x Versus Weeks Post Transplant.

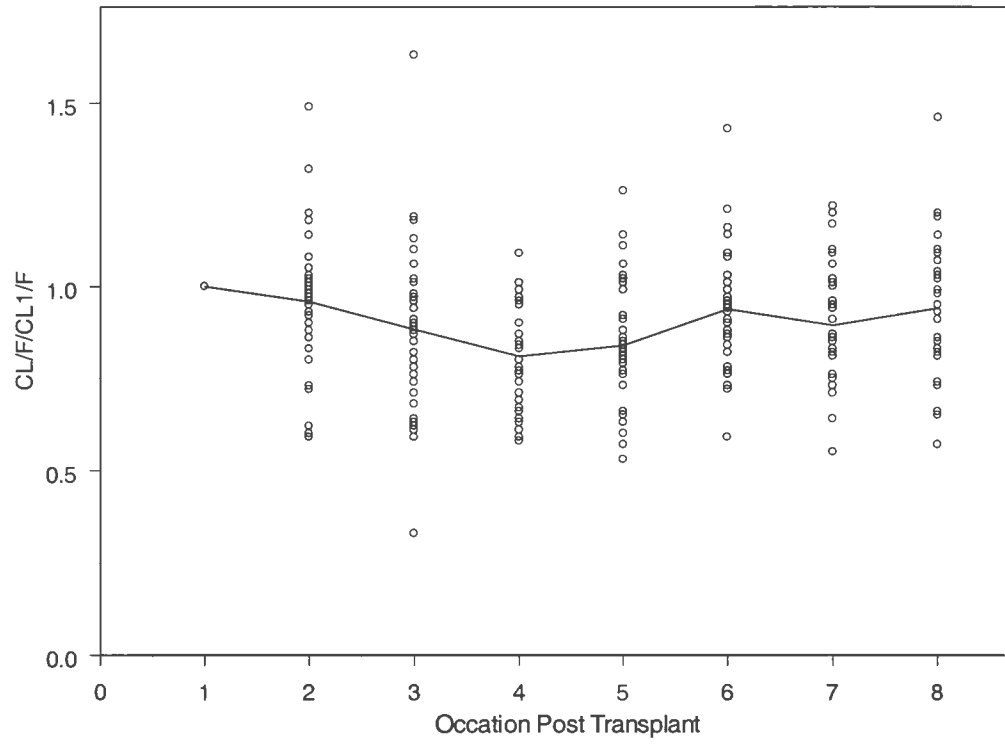


Figure 2.2 (a): Base Model Observed Versus Model-Predicted Concentration (mg/L)

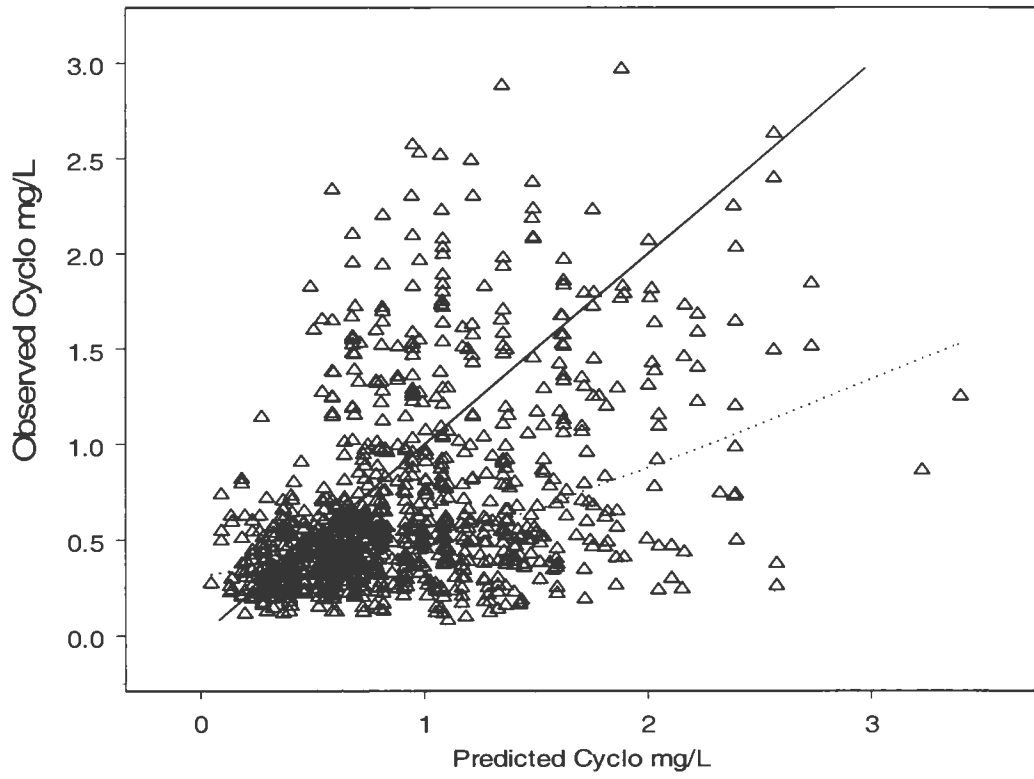


Figure. 2.2 (b): Weighted Residual Versus Model - Predicted Concentration

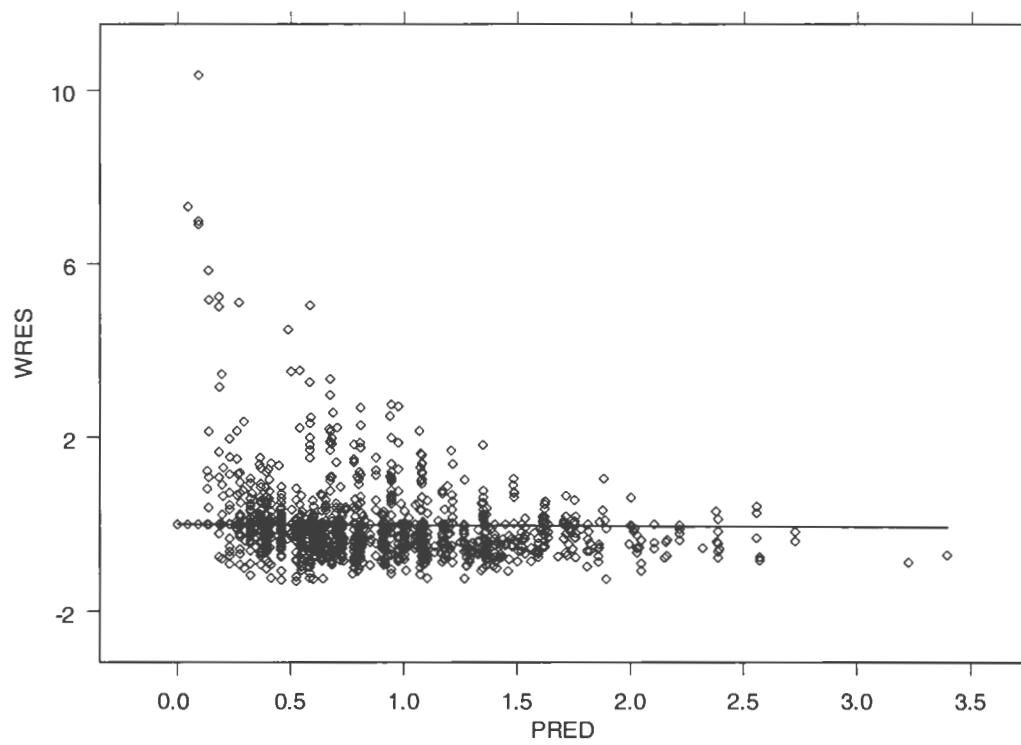
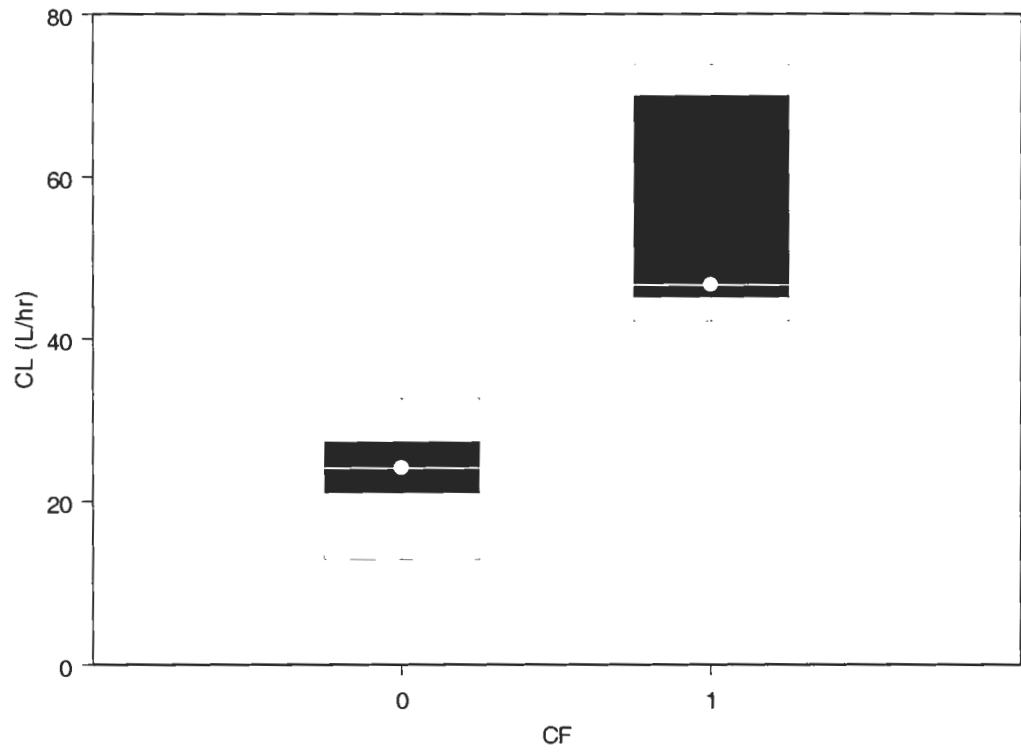


Figure 2.3: Box Plot for Cystic Fibrosis as Covariate



Cystic fibrosis (1) and non cystic fibrosis patients (0)

Figure 2.4: Box Plot for Formulation as Covariate.

Patients on Neoral =0 and patients on Sandimmune =1

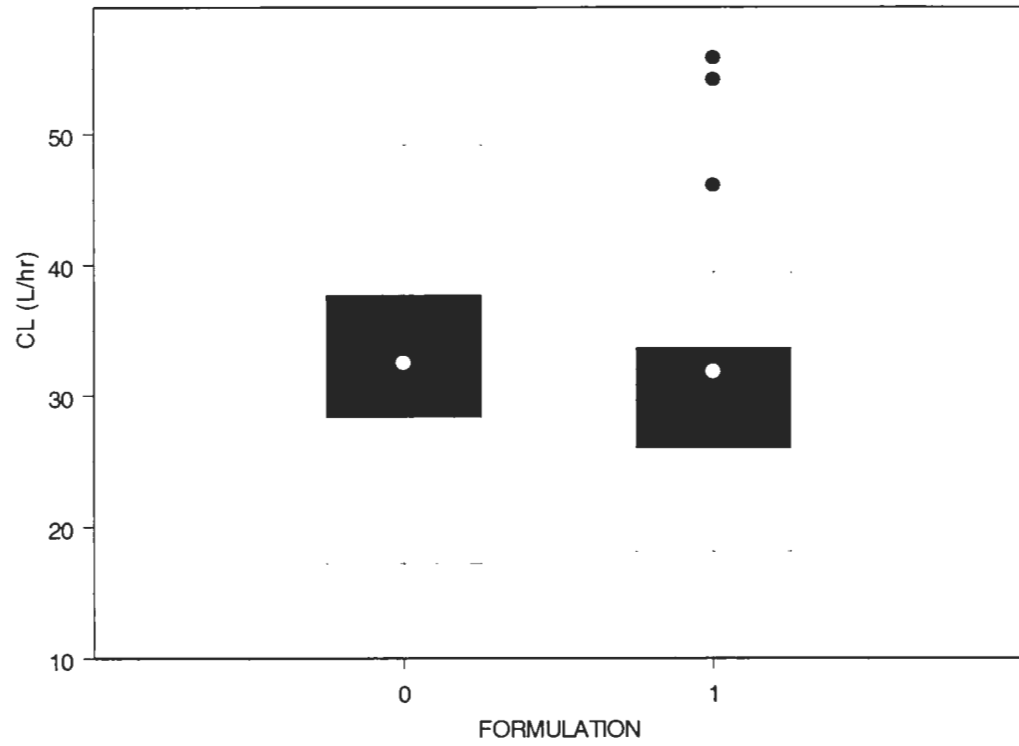


Figure 2.5. Box Plot for Itraconazole as Covariate

Patients on itraconazole are marked 1, and patients not on itraconazole as 0.

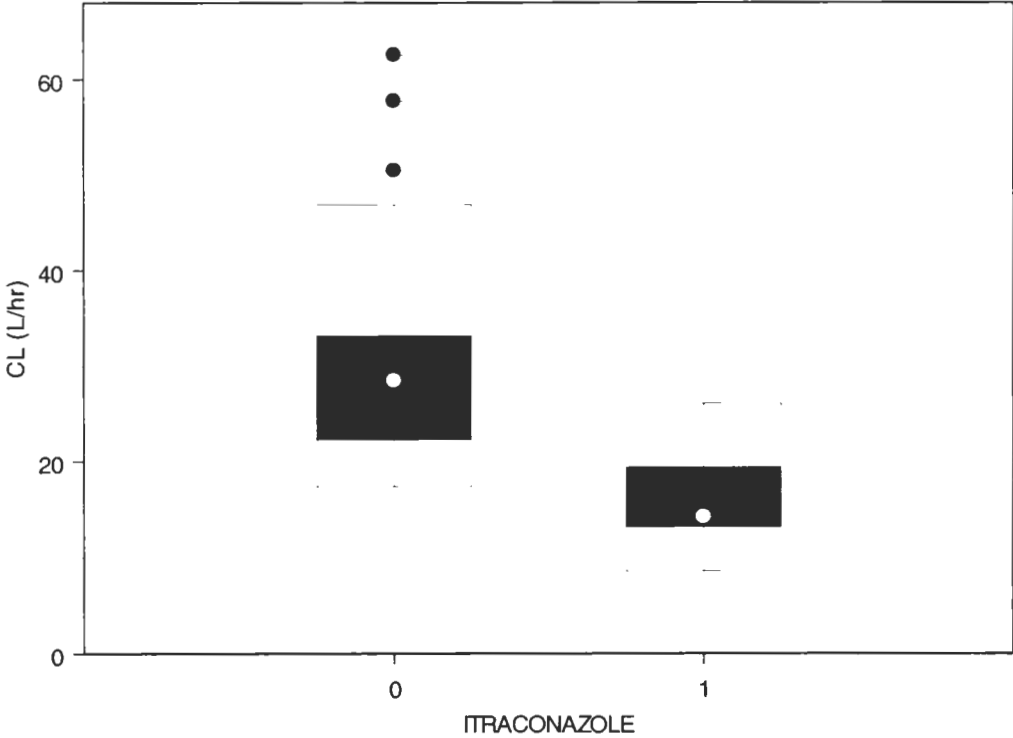


Figure 2.6 (a). Final Model Observed Versus Model - Predicted Concentration

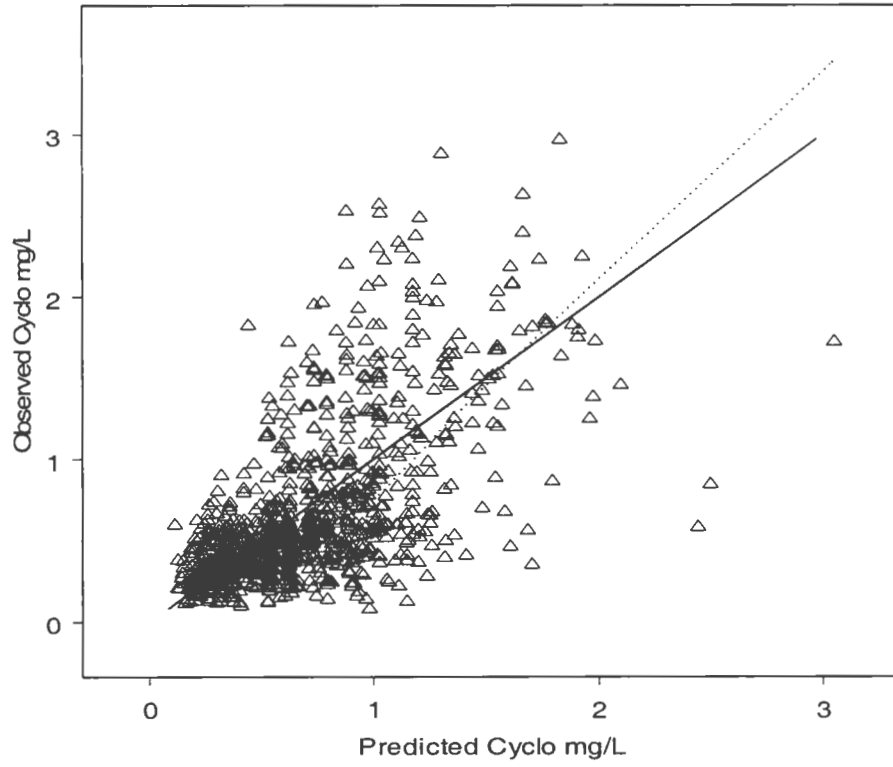
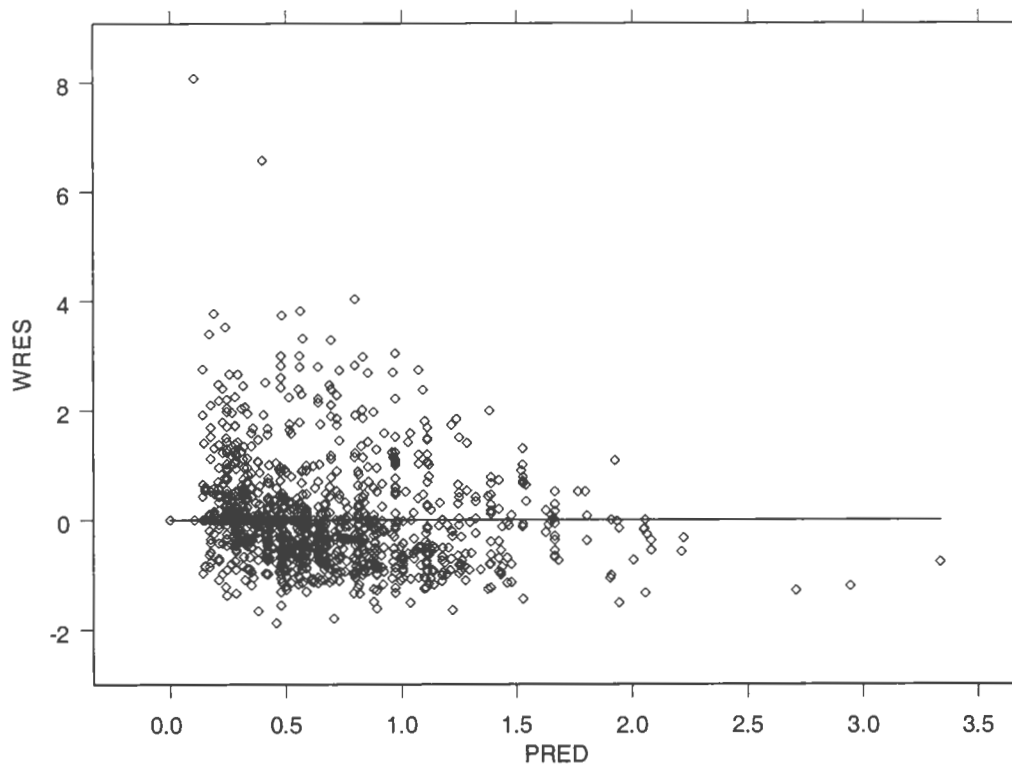


Figure 2.6 (b). Final Model Weighted Residual Versus Model - Predicted Concentration



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APPENDIX A

Figure 3.1. NONMEM Control File for Base Model

```
$PROB RUN# (BASE RUN)
$INPUT C ID TIME AMT DV WT SS DI=II TAD DOSE AGE SEX ITRA CF
      TPT
$DATA 200.CSV IGNORE=C
$SUBROUTINES ADVAN2 TRANS2

$PK
TVCL=THETA (1)
CL=TVCL*EXP (ETA (1))
TVV=THETA (2)
V= TVV
K= CL/V
  IF (DOSE.EQ.1) TVKA=THETA (3)
  KA=TVKA
  IF (DOSE.EQ.0) TVKA=THETA (4)
  KA=TVKA
S2=V

$error
Y=F*(1+ERR (1))

$THETA
(0,105,500) (1,1000,5000) (0.25 FIXED) (1.35 FIXED)

$OMEGA
0.5; [P] INTERIND VAR IN CL

$SIGMA
0.3; [P] PROPORTIONAL COMPONENT

$EST MAXEVAL=5000 PRINT=5 POSTHOC MSF=Base.MSF

$COVARIANCE

$TABLE ID TIME DOSE TAD IPRED CL V NOPRINT ONEHEADER
      FILE=Base.TAB
```

Figure 3.2. NONMEM Control File for Time Post Transplant

```
$PROB RUN# TPT COV
$INPUT C ID TIME AMT DV WT SS DI=II TAD DOSE AGE SEX ITRA CF
      TPT
$DATA 200.CSV IGNORE=C
$SUBROUTINES ADVAN2 TRANS2

$PK
TVCL=THETA (1)
CL=TVCL*EXP (ETA (1))
TVV=THETA (2)
V= TVV
K= CL/V
  IF (DOSE.EQ.1) TVKA=THETA (3)
  KA=TVKA
  IF (DOSE.EQ.0) TVKA=THETA (4)
  KA=TVKA
S2=V
IF (TPT.LE.4) TVF1=1-THETA (5)/TPT
F1= TVF1
IF (TPT.GT.4) TVF1=1
F1=TVF1

$ERROR
  Y=F*(1+ERR (1))

$THETA
(0,105,500) (1, 1000, 5000) (0.25 FIXED) (1.35 FIXED) (0,0.007,0.6)

$OMEGA
0.5; [P] INTERIND VAR IN CL

$SIGMA
0.3; [P] PROPORTIONAL COMPONENT

$EST MAXEVAL=5000 PRINT=5 POSTHOC

$COVARIANCE

$TABLE ID TIME DOSE TAD IPRED CL TPT NOPRINT ONEHEADER
      FILE=TPT.TAB
```


Figure 3.3. NONMEM Control File for Itraconazole as Covariate

```
$PROB RUN# Itra cov
$INPUT C ID TIME AMT DV WT SS DI=II TAD DOSE AGE SEX ITRA CF
      TPT
$DATA 200.CSV IGNORE=C
$SUBROUTINES ADVAN2 TRANS2

$PK
TVCL=THETA (1)-THETA (5)*ITRA
CL=TVCL*EXP (ETA (1))
TVV=THETA (2)
V= TVV
K= CL/V
  IF (DOSE.EQ.1) TVKA=THETA (3)
  KA=TVKA
  IF (DOSE.EQ.0) TVKA=THETA (4)
  KA=TVKA
S2=V

$ERROR
  Y=F*(1+ERR (1))

$THETA
(0,105,500) (1, 1000, 5000) (0.25 FIXED) (1.35 FIXED) (0, 10, 25)

$OMEGA
0.5; [P] INTERIND VAR IN CL

$SIGMA
0.3; [P] PROPORTIONAL COMPONENT

$EST MAXEVAL=5000 PRINT=5 POSTHOC

$COVARIANCE

$TABLE ID TIME DOSE TAD IPRED CL ITRA NOPRINT ONEHEADER
      FILE=Itra.TAB
```

Figure 3.4. NONMEM Control File for Formulation as Covariate.

```
$PROB RUN# FORMULATION
$INPUT C ID TIME AMT DV WT SS DI=II TAD DOSE AGE SEX ITRA CF
      TPT
$DATA 200.CSV IGNORE=C
$SUBROUTINES ADVAN2 TRANS2

$PK
TVCL=THETA (1)
CL=TVCL*EXP (ETA (1))
TVV=THETA (2)
V= TVV
K= CL/V
  IF (DOSE.EQ.1) TVKA=THETA (3)
  KA=TVKA
  IF (DOSE.EQ.0) TVKA=THETA (4)
  KA=TVKA
S2=V
F1=1*DOSE+THETA (5)*(1-DOSE)

$error
Y=F*(1+ERR (1))

$THETA
(0,105,500) (1, 1000, 5000) (0.25 FIXED) (1.35 FIXED)(0,1,5)
$OMEGA

0.5; [P] INTERIND VAR IN CL

$SIGMA
0.3; [P] PROPORTIONAL COMPONENT

$EST MAXEVAL=5000 PRINT=5 POSTHOC

$COVARIANCE

$TABLE ID TIME DOSE TAD IPRED CL NOPRINT ONEHEADER
      FILE=formulation.TAB
```

Figure 3.5. NONMEM Control File for Cystic Fibrosis as Covariate

```
$PROB RUN# CF COV
$INPUT C ID TIME AMT DV WT SS DI=II TAD DOSE AGE SEX ITRA CF
      TPT
$DATA 200.CSV IGNORE=C
$SUBROUTINES ADVAN2 TRANS2

$PK
TVCL=THETA (1) +THETA (5)*CF
CL=TVCL*EXP (ETA (1))
TVV=THETA (2)
V= TVV
K= CL/V
  IF (DOSE.EQ.1) TVKA=THETA (3)
  KA=TVKA
  IF (DOSE.EQ.0) TVKA=THETA (4)
  KA=TVKA
S2=V

$ERROR
  Y=F*(1+ERR (1))

$THETA
(0,105,500) (1, 1000, 5000) (0.25 FIXED) (1.35 FIXED) (0, 35)

$OMEGA
0.5; [P] INTERIND VAR IN CL

$SIGMA
0.3; [P] PROPORTIONAL COMPONENT

$EST MAXEVAL=5000 PRINT=5 POSTHOC

$COVARIANCE

$TABLE ID TIME TAD IPRED DOSE CL CF NOPRINT ONEHEADER
      FILE=CF.TAB
```

Figure 3.6. NONMEM Control File for Final Model

```
$PROB RUN# Final Model
$INPUT C ID TIME AMT DV WT SS DI=II TAD DOSE AGE SEX ITRA CF
      TPT
$DATA 200.CSV IGNORE=C
$SUBROUTINES ADVAN2 TRANS2

$PK
TVCL=THETA (1)-THETA (5)*ITRA+THETA (6)*CF
CL=TVCL*EXP (ETA (1))
TVV=THETA (2)
V= TVV
K= CL/V
  IF (DOSE.EQ.1) TVKA=THETA (3)
  KA=TVKA
  IF (DOSE.EQ.0) TVKA=THETA (4)
  KA=TVKA
S2=V

IF (TPT.LE.4) TVF1=1*DOSE+THETA (7)*(1-DOSE)-THETA (8)/TPT
F1= TVF1
IF (TPT.GT.4) TVF1=1*DOSE+THETA (7)*(1-DOSE)
F1=TVF1

$ERROR
Y=F*(1+ERR (1))

$THETA
(0,105,500) (1,1000,5000)(0.25 FIXED) (1.35 FIXED)(0,0.1,20)(0,5,35)(0.5,2,5)
(0, 0.007, 0.6)

$OMEGA
0.5; [P] INTERIND VAR IN CL

$SIGMA
0.3; [P] PROPORTIONAL COMPONENT

$EST MAXEVAL=5000 PRINT=5 POSTHOC

$COVARIANCE

$TABLE ID TIME DOSE TAD CL ITRA CF TPT NOPRINT ONEHEADER
      FILE=Final. TAB
```

Figure 4.1. Results From the Analysis

a. *Base Model*

MINIMUM VALUE OF OBJECTIVE FUNCTION: -456.975

FINAL ESTIMATE		95% CONFIDENCE INTERVAL			
		%RSE	LBOUND	UBOUND	
THETA					
1	23.1	7.27%	19.8	26.4	
2	202	10.7%	159	245	
3	0.250	
4	1.35	
OMEGA					INTERINDIVIDUAL VARIABILITY
1,1	0.103	21.9%	0.0587	0.147	CV = 32.1%
SIGMA					RESIDUAL VARIABILITY
1,1	0.361	14.8%	0.256	0.466	CV = 60.1%

%RSE is percent relative standard error (100% x SE/EST)

b. *Cystic Fibrosis*

MINIMUM VALUE OF OBJECTIVE FUNCTION: -739.749

FINAL ESTIMATE		95% CONFIDENCE INTERVAL			
	%RSE	LBOUND	UBOUND		
THETA					
1	21.7	7.28%	18.6	24.8	
2	205	10.6%	162	248	
3	0.250	
4	1.35	
5	32.7	21.3%	19.0	46.4	
OMEGA					
1,1	0.0563	25.8%	0.0279	0.0847	INTERINDIVIDUAL VARIABILITY CV = 23.7%
SIGMA					
1,1	0.367	13.7%	0.269	0.465	RESIDUAL VARIABILITY CV = 60.6%

%RSE is percent relative standard error (100% x SE/EST)

c. Itraconazole

MINIMUM VALUE OF OBJECTIVE FUNCTION: -1013.817

		95% CONFIDENCE INTERVAL					
FINAL ESTIMATE		%RSE	LBOUND	UBOUND			
THETA							
1	28.0	6.61%	24.4	31.6			
2	182	6.98%	157	207			
3	0.250			
4	1.35			
5	16.3	13.7%	11.9	20.7			
					INTERINDIVIDUAL VARIABILITY		
OMEGA					CV =		
1,1	0.0959	20.4%	0.0575	0.134		31.0%	
					RESIDUAL VARIABILITY		
SIGMA					CV =		
1,1	0.267	8.20%	0.224	0.310		51.7%	

%RSE is percent relative standard error (100% x SE/EST)

d. Gender

MINIMUM VALUE OF OBJECTIVE FUNCTION: -617.857

FINAL ESTIMATE		95% CONFIDENCE INTERVAL				
		%RSE	LBOUND	UBOUND		
THETA						
1	18.3	14.5%	13.1	23.5		
2	188	9.10%	154	222		
3	0.250		
4	1.35		
5	10.3	38.8%	2.46	18.1		
					INTERINDIVIDUAL VARIABILITY	
OMEGA					CV =	
1,1	0.110	22.7%	0.0610	0.159		33.2%
					RESIDUAL VARIABILITY	
SIGMA					CV =	
1,1	0.319	11.4%	0.248	0.390		56.5%

%RSE is percent relative standard error (100% x SE/EST)

e. Formulation

MINIMUM VALUE OF OBJECTIVE FUNCTION: -517.676

FINAL ESTIMATE		95% CONFIDENCE INTERVAL			
	%RSE	LBOUND	UBOUND		
THETA					
1	29.4	9.56%	23.9	34.9	
2	246	18.2%	158	334	
3	0.250	
4	1.35	
5	1.39	16.6%	0.937	1.84	
OMEGA					
1,1	0.0897	22.3%	0.0505	0.129	INTERINDIVIDUAL VARIABILITY CV = 29.9%
SIGMA					
1,1	0.389	13.9%	0.283	0.495	RESIDUAL VARIABILITY CV = 62.4%

%RSE is percent relative standard error (100% x SE/EST)

f. Time Post Transplant

MINIMUM VALUE OF OBJECTIVE FUNCTION: -680.309

FINAL ESTIMATE		95% CONFIDENCE INTERVAL		
	%RSE	LBOUND	UBOUND	
THETA				
1	20.6	7.52%	17.6	23.6
2	173	9.31%	141	205
3	0.250
4	1.35
5	0.509	7.58%	0.433	0.585
OMEGA				
1,1	0.0901	20.0%	0.0548	0.125
INTERINDIVIDUAL VARIABILITY CV = 30.0%				
SIGMA				
1,1	0.308	14.0%	0.224	0.392
RESIDUAL VARIABILITY CV = 55.5%				

%RSE is percent relative standard error (100% x SE/EST)

g. *Final Model*

MINIMUM VALUE OF OBJECTIVE FUNCTION: -1369.928

		95% CONFIDENCE INTERVAL					
	FINAL ESTIMATE	%RSE	LBOUND	UBOUND			
THETA							
1	26.4	7.08%	22.7	30.1			
2	183	10.4%	146	220			
3	0.250			
4	1.35			
5	14.8	14.7%	10.5	19.1			
6	25.9	13.5%	19.0	32.8			
7	1.14	8.04%	0.960	1.32			
8	0.355	15.3%	0.248	0.462			
					INTERINDIVIDUAL VARIABILITY		
OMEGA					CV =		
1,1	0.0344	28.9%	0.0149	0.0539		18.5%	
					RESIDUAL VARIABILITY		
SIGMA					CV =		
1,1	0.246	7.93%	0.208	0.284		49.6%	

%RSE is percent relative standard error (100% x SE/EST)

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