USE OF THE NONMEM COMPUTER PROGRAM TO PREDICT AN ORAL CYCLOSPORIN DOSE WHEN CHANGING OVER FROM INTRAVENOUS THERAPY

Nicole Webster

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DECLARATION

I, Nicole Webster declare that this research report is my own work. It is being submitted for the degree of Master of Clinical Hospital Pharmacy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

18th day of February, 2000

ABSTRACT

The introduction of cyclosporin has brought about a new era in drug therapy for transplantation and other immune-related diseases. However, cyclosporin therapy has been associated with large inter-individual differences in drug absorption, distribution, metabolism and elimination making it impossible to establish fixed dose regimens based on body weight. Recently, a new oral formulation called Sandimmune Neoral, also known as Neoral, was introduced showing a more consistent absorption profile, improved dose linearity and enhanced relevance of cyclosporin blood levels.

This is a retrospective pilot study conducted at the Garden City Clinic on 13 de novo renal transplant patients and the aims were:

- to establish the relative bioavailability between the IV and the oral dose of cyclosporin,
- to calculate population pharmacokinetic parameters from routinely taken trough levels using the NONMEM program,
- to investigate the influence of factors such as weight and gender on pharmacokinetic parameters.

This information could then be applied to arrive at a formula for the smooth changeover from IV to oral therapy.

For the IV cyclosporin doses population mean values of total body clearance (Cl) and volume of distribution (Vd) were estimated to be 22.4 L/h and 167 L respectively, with an inter-individual variation (η) of 48% and 44% respectively. The residual error was 10%. For the oral cyclosporin, they were 52.6 L/h (η = 10%) and 339 L (η = 48%) respectively with a fixed absorption constant (k_a) of 0.7 h^{-1}. The residual error was 10%. Consequently, the relative bioavailability was estimated to be around 43%. More data is needed to confirm this result.

Although this study included only a small number of patients a significant correlation was found between total body clearance of cyclosporin and creatinine clearance. This should be investigated further in a more in-depth study.

Due to the power of NONMEM, we were able to show that it is possible to calculate pharmacokinetic parameters from only cyclosporin trough levels without the need for the extra expense and morbidity of multiple blood level monitoring. This study showed us that it is possible to arrive at a formula to calculate what oral dose is needed for the smooth changeover from IV cyclosporin therapy using NONMEM if more data is available.

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CHAPTER 1

1.0 INTRODUCTION

The renal transplant unit at Garden City Clinic was opened in November 1995. By February 1998, more than 50 transplants had been performed. This is where the research for this study was conducted.

Patients with end stage renal failure either require dialysis to sustain life or, depending on the patients age and any inherent disease, a renal transplant could be considered another option. As a result of the success in solid organ transplantation, the number of transplants and the number of centres where these transplants are being performed, have greatly increased. One-year patient survival rate, as well as the one-year graft survival rate, stands around 80 - 90%, but depends on the individual transplant centre[1].

The development of new drugs such as cyclosporin and OKT3, as well as the development of new surgical techniques, have made this possible[1].

Immunosuppressant drugs are used to prevent loss of the graft due to rejection. The aim of immunosuppression is to alter the recipient's immune response to prevent rejection of the transplanted organ, while sparing the immune system's ability to fight infection and provide surveillance against malignancies[1].

The early post-transplant phase is characterised by unstable graft function and increased alloreactivity, making it essential to achieve high levels of immunosuppression as quickly as possible[2]. There is no consensus on which immunosuppressive regimen is superior and depends on the centre's preference, and the specific organ to be transplanted[1,2]. At this centre a combination of three drugs namely cyclosporin, prednisolone and azathioprine is being used. This is known as triple therapy, and allows smaller doses of each drug to be used[1,2]. The advantage is that the incidence of drug toxicity associated with the large doses needed, if any one of these agents were to be used independently, is significantly reduced. The disadvantage is the difficulty in assessing adverse effects[1].

To ensure adequate blood levels of cyclosporin immediately post-transplant, the intravenous form of cyclosporin is used. After a few days the switch can be made to the oral formulation of the drug.
At the present time, there is no established protocol for a smooth changeover from intravenous to oral dosing. The general guidelines given are that the intravenous dose is a third of the oral dose, because the average bioavailability of the original oral formulation is approximately 30%[2]. Sandimmune Neoral®, commonly referred to as Neoral, is the trade name of a new oral formulation of cyclosporin. The bioavailability for Neoral, the oral preparation used in this study, is known to be a great deal higher than the original one, approximately 59%. It also shows a more consistent absorption profile, improved dose linearity and enhanced relevance of cyclosporin blood levels[3].
It could therefore be possible to calculate a formula for the changeover from IV to oral therapy.

In this study an attempt will also be made to calculate population pharmacokinetic parameters, using only routinely taken trough levels. With Neoral there is a greater correlation between trough levels and total drug exposure, which is an accepted indicator of clinical outcome[3]. The quicker the patient can be stabilised on the correct oral

dose of cyclosporin, the better the long-term survival will be[3]. The more instances of acute graft rejection occur, the greater are the chances of chronic rejection at a later stage[1].

From an economic point of view, there is a potential for cost saving, because less blood samples need to be taken, and once the patient has stabilised on the oral medication, he or she can be discharged.

With the above in mind, we designed this study with the following aims.

1.1 Aim

The aims of this study were:

- to establish the relative bioavailability between the IV and oral dose of cyclosporin,
- to calculate population pharmacokinetic parameters from the routinely taken cyclosporin trough levels,
- to investigate the influence of factors such as weight and gender on the pharmacokinetic parameters in these patients.

1.2 Literature review on cyclosporin

In 1980, when cyclosporin was introduced as a new immunosuppressant, there was a dramatic rise in the number of successful organ transplants being performed[4]. It is a metabolite extracted from the soil fungus *Tolypocladium inflatum* gams[1,2]. It is used extensively for the prevention of allograft rejection in kidney-, liver-, heart-, and bone-marrow transplants, and more recently in the treatment of certain autoimmune diseases[2,4]. Cyclosporin has increased the success rate of transplantation in the elderly, paediatric, diabetic, retransplant and poorly matched patients[2].

The drug has a highly specific mode of action namely:

- it inhibits Interleukin-2 production thereby suppressing the proliferation and generation of T-cytotoxic lymphocytes, but it does not affect T-suppresser cells, which are critical for the promotion of allograft tolerance,
- it prevents gamma-interferon release[1,2].

The resulting advantages over azathioprine and prednisolone are:

- it does not inhibit the chemotactic or phagocytic activity of neutrophils,
- it is not myelosuppressive at immunosuppressive doses,
- the effects are reversible, following cessation of therapy, because it is not lymphocytotoxic[2].

Since the introduction of cyclosporin, there has not only been a notable decrease in the incidence of morbidity associated with graft rejection, but also, due to the fact that not all of the immunocompetent cells are being suppressed, there is a substantially lower risk of serious and fatal infections[2]. There are, however, some problems associated with the use of this drug.

Cyclosporin, also known as cyclosporin A, is a cyclic polypeptide of 11 amino acids, making it relatively insoluble in water, but very soluble in lipids[1,2]. The lipophilic nature of the compound has been the main cause of its poor absorption characteristics, and poor and variable oral availability[1,2]. The reason is, that lipophilic substances cannot be absorbed, unless they are first emulsified[4,5].

This emulsification, which has a direct effect on cyclosporin absorption, and therefore cyclosporin blood levels, is therefore dependant on the availability of bile acids, presence of food, the fat content of the food and the dispersal in the intestinal tract[3,4,5]. The result is wide inter- and intra-patient variability in cyclosporin blood levels[3,4,5].

It has a very narrow therapeutic window, therefore it is imperative that cyclosporin blood levels are consistently maintained within a certain narrow range, for an optimal balance of safety and efficacy[3,4]. If the level is too low, there is a risk of rejection, even graft loss. Conversely if it is too high, there is an increased risk of side effects like tremor, hirsutism, impaired renal function, hepatic dysfunction, gingival hypertrophy and gastro-intestinal disturbances[1,3]. These are responsive to dosage reduction, and are less common with the lower dosages given, when using triple therapy[1].

Cyclosporin levels need to be monitored, because of the narrow therapeutic window, and because of certain toxicities associated with this drug.

It is also necessary, to check for patient compliance[1]. Most commonly, cyclosporin trough levels are used, due to the variable and prolonged absorption profile[1]. Ideally, it would be preferable to take multiple levels in one dosing interval, to be able to measure total drug exposure, which is more predictive of clinical outcome[1]. This is very costly, and too time consuming, and painful for the patient, especially, when this is done on an outpatient basis. Neoral shows a much greater correlation between trough levels and total drug exposure, making this project more feasible[3].

During the early postoperative period, routine daily cyclosporin trough levels should be taken, until the patient is stabilised on the correct oral dose[1].

The recommended pre-dose whole blood cyclosporin level range for renal transplant recipients is 300 - 400 ηg/ml for single drug therapy, and 150 - 250 ηg/ml for triple drug therapy[1]. This is for the first two weeks post-transplant.

Neoral is a new formulation of cyclosporin developed to overcome the variability in the absorption characteristics experienced with the original

formulation[3,5]. The addition of lipophilic and hydrophilic solvents, together with a surfactant, ensures immediate dispersal of the drug in the gut once contact is made with the water based digestive juices[3,4,5]. From this microemulsion the drug is absorbed quicker, and to a greater extent[3,4,5]. The result is an improved, more predictable pharmacokinetic profile, because the emulsification process can take place independently, without the need for the ingestion of a fatty meal to stimulate the secretion of bile[3,4,5].

Cyclosporin therapy is only initiated once adequate graft function, as measured by urine output, has been achieved. The drug has been known to prolong the duration of oliguria, and increase the time needed, until adequate kidney perfusion occurs[1]. The longer it takes before adequate graft function is reached, the worse the long-term prognosis is[1]. In certain cases, it becomes necessary to dialyse the patient, before the kidney is able to perform its function sufficiently[1]. Induction therapy is started with azathioprine and corticosteroids, and cyclosporin is only added, once the urine output has reached an acceptable level. This usually only takes a few hours after the transplant.

Initially, cyclosporin is given intravenously, to be able to achieve optimal levels of immunosuppression as soon as possible[6]. In the early post-transplant period, cyclosporin absorption can be hindered by a postoperative illeus[6].

After one or two days, the patient is eating normally, and the illeus has resolved, the changeover can be made to the oral form of the drug. The sooner this can be achieved, the better, because there are some severe side effects that are associated with the intravenous form of the drug, namely:

- hypersensitivity to polyoxyethylated castor oil, which is an ingredient in the intravenous concentrate, and has been known to cause anaphylactic reactions[2],
- neurotoxicity[2],
- cyclosporin nephrotoxicity[2].

1.3 Pharmacokinetics of cyclosporin

There are large interindividual differences in drug absorption, distribution, metabolism and elimination[6]. This is why fixed dose regimens based on actual or ideal body weight, are not feasible[6].

1.3.1 Absorption

Cyclosporin absorption occurs in the upper small intestine[3]. Poor initial post-transplant bioavailability (less than 25%) has been associated with reduced 1-year graft survival, due to an increase in acute rejection episodes[3]. There are certain types of patients, that display poor cyclosporin bioavailability, namely, newly transplanted liver recipients, paediatric patients, blacks, diabetics, cystic fibrosis patients and patients with cholestasis or gastrointestinal dysfunction such as diarrhoea or short-bowel syndrome[3]. These patients often require higher doses of cyclosporin per kilogram of body weight.

The greatest increase in bioavailability with Neoral, has been seen in the immediate post-transplant period[3]. Peak blood levels are seen 1 to

2 hours after ingestion[3,6]. When using the original oral formulation, peak blood levels are only seen 2 to 4 hours after ingestion[2].

The improved absorption of Neoral necessitates a twice-daily dosing schedule, maybe even three times daily in children, blacks and cystic fibrosis patients[3].

The absorption constant (k_a) is a description of the rate of absorption of the drug from the intestinal tract. The lower the value of this constant is, the quicker is the absorption rate. There is a large variation in the absorption constants that are reported in the literature. Yee and Kennedy report a value of 0.7 h^{-1} [7], while values of 1.28 h^{-1} [8] even 5.69 h^{-1} [9] can be found elsewhere.

1.3.2 Distribution

The distribution of cyclosporin is not only dependent on its physiochemical properties, but is also influenced extensively by the concentration of its biological carriers such as lipoproteins and erythrocytes in blood[10]. Intracellularly, cyclosporin binds to cyclophyllin,

(an immunophyllin), which consequently also influences its distribution[1]. Despite its lipophilicity, cyclosporin is not found in the brain[10]. The drug is highly tissue bound, and is widely distributed with the highest concentrations found in the liver, pancreas and fatty tissues[2]. The average volume of distribution is 4 to 5 L/kg[11]. Another reference quotes an average volume of distribution of 3.5 L/kg[1]. Cyclosporin exhibits two compartment modelling with a significant alpha phase[11].

Cyclosporin in the blood is approximately 20% bound to leukocytes, 40% to erythrocytes, and 40% remains in the plasma mostly bound to lipoproteins[11]. This leaves less than 10% unbound or free drug available[11]. The binding to erythrocytes, however, is temperature and time dependant[1]. The measurement of cyclosporin in plasma can differ by as much as 50%, if the temperature of the plasma is 21° C, instead of 37 °C[1]. When using whole blood samples, there is no need for a separation protocol, and the effects of sample haemolysis are eliminated.

This means that whole blood samples are much more accurate, and reproducible, also, because of the higher concentrations found in blood, compared to plasma[1].

1.3.3. Metabolism and clearance

Cyclosporin undergoes extensive hepatic metabolism, about 99%[2]. The cytochrome P450-3A isoenzyme is found in the intestinal tract and in the liver, and is responsible for presystemic metabolism of cyclosporin[1]. Induction or inhibition of these isoenzymes in both the GI tract and the liver by other drugs, consequently has a significant effect on cyclosporin blood levels[1]. It also follows that drugs that prolong the transit time of cyclosporin in the GI tract, will alter the amount of drug that is absorbed[1].

Cyclosporin has a low extraction ratio, making the unbound fraction in the blood the rate determining factor[11]. Changes in the free fraction of cyclosporin in plasma will therefore influence the clearance rate of the drug from the body[11].

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Increased serum concentrations of total cholesterol, low density lipoproteins and triglygerides occurs within 6 to 18 months in 50% to 80% of patients that received a heart or kidney transplant[1]. In renal transplant patients this can be attributed to the intake of drugs such as diuretics, beta-blockers and also cyclosporin and prednisolone[1]. Cyclosporin may have an independent as well as a synergistic effect with prednisolone on lipid levels[1]. Cumulative doses of prednisolone can increase the hepatic synthesis of very low-density lipoproteins, cause insulin resistance, and hyperinsulinaemia[1]. When the corticosteroid dose is lowered, or therapy is discontinued, the lipid levels seem to normalise again[1].

Numerous metabolites of cyclosporin with little or no immunosuppressant activity have been identified[1,3]. Excretion occurs mainly via the bile, with only 6% urinary excretion of which 0,1% is eliminated unchanged in the urine[3]. Paediatric patients have been shown to have a clearance rate that is approximately twice as high, as that in adults[1].

1.4 NONMEM (NON-linear Mixed Effects Model)

Therapeutic drug monitoring is of great value when drugs have a narrow therapeutic index, or if there are significant consequences associated with therapeutic failure or toxicity, or if a drug displays wide inter- and intra-patient variability[12].

The aim is tc individualise drug dosage regimens, and predict loading and maintenance doses[12]. Population pharmacokinetic modelling offers certain advantages when individualising drug dosage regimens, because it allows a detailed analysis of variability, and an evaluation of factors, which influence responses[13]. Population parameter values are used specifically to estimate individual dosage requirements[13].

Population pharmacokinetics describe the influence of physiological and pathological factors on pharmacokinetics, including any inter-individual variability, and also any residual intra-individual variability[14].

The traditional approach of doing a pharmacokinetic study uses individual responses derived from multiple blood sampling in few

subjects[13]. This is a retrospective study, where we have only few levels per patient. This is why another approach is necessary.

NONMEM is a computer program used for population pharmacokinetic analysis, and it has certain advantages:

- it can be used with routine clinical data, so no extra blood levels have to be taken, which is especially useful, when the study is done on very sick patients,
- it is usable with limited data,
- it accommodates unstructured and unbalanced data,
- it allows for inter-patient and intra-patient variability,
- it calculates the influence of covariates[13].

NONMEM is an abbreviation for NON-linear Mixed Effects Modelling[13]. Mixed effects modelling is a technique of population pharmacokinetic estimation, which allows direct estimation of these parameters in a single stage of analysis applied simultaneously to the data of many individuals[12,14].

There are three different types of pharmacokinetic parameters that are examined, namely, fixed effects parameters, inter-individual and intra-individual random effects parameters[13,14].

Fixed effects parameters (Theta (θ)) measure the typical i.e. median values of the primary pharmacokinetic parameters, namely, clearance (Cl), volume of distribution (Vd), absorption rate constant (K_a) and bioavailability factor (F)[13,14]. These factors can be adjusted according to the effect various patient characteristics or pathophysiological factors such as age, creatinine clearance, weight etc have on these parameters[13,14].

Inter-individual random effects parameters (Eta (η)) describe the difference between an individual's parameter and the mean[11,12]. Intra-individual random effects parameters (Sigma (ε)) describe the remaining variability in the individual, and considers factors such as the patients day to day variability, drug assay variability, inaccurate dosing and blood sampling times and model specification error[13,14].

CHAPTER 2

2.0 METHOD

A list of all patients that received a kidney transplant at Garden City Clinic from November 1995 till February 1998 was obtained from the transplant unit at Garden City Clinic. Permission was obtained to examine these files for the purpose of this study. The whole study was therefore performed retrospectively.

In this chapter the following is going to be discussed:

(a) Patient selection

(b) Data collection

(c) Sample analysis

(d) Data analysis

2.1 Patient selection

2.1.1. Inclusion criteria

The inclusion criteria were:

- patients between ages 14 and 65,
- patients on triple immunosuppressant drug therapy, starting off with prednisolone and azathioprine, and adding cyclosporin once adequate graft function has been achieved,
- cyclosporin therapy started off with the intravenous form of the drug, with the change over to oral therapy occurring after a few days,
- oral therapy with microemulsion formulation Neoral, and at least one whole blood trough level.

2.1.2. Exclusion criteria

The exclusion criteria were:

- patients displaying rejection episodes in the first two weeks after the transplant ,
- patients with cystic fibrosis,

- patients receiving cytochrome P450 enzyme inducing or inhibiting drugs while on cyclosporin e.g. ketoconazole, erythromycin, phenobarbitone, carbamazepine, rifampicin or cimetidine.

2.2 Data collection

The demographic, clinical and laboratory data were extracted from the patient files, and entered into a data collection sheet. (An example of the data sheet can be seen in appendix A)

All cyclosporin intravenous and oral doses were recorded with the times they were administered. The corresponding trough levels were also documented accordingly.

The intravenous form of the drug is administered over 4 hours by slow intravenous infusion. There are two different dosing regimens that are used at Garden City Clinic, either 50mg eight hourly or 100mg twelve hourly. The concentrate is mixed with 200mls normal saline in a glass bottle, because the concentrate contains polyoxyethylated castor oil, which can cause phthalate stripping from plastic[2]. The whole blood

trough levels are usually taken approximately an hour before the morning dose is administered.

Neoral is available in 25mg and 100mg capsules. The dose is given twice daily with breakfast and with dinner, although it is not necessary to take it with a meal[3]. The whole blood trough level is taken approximately an hour before the morning dose is given.

When the venous blood sample is taken in the morning, serum creatine, urea and potassium values are measured as well. Increased serum levels of creatinine, urea and potassium levels are seen during acute rejection episodes, and can also be an indication of nephrotoxicity[1,2]. Transient acute renal dysfunction is the most common type of nephrotoxicity experienced with cyclosporin, and is rapidly fully reversible with a dose reduction[1]. A slight increase in serum creatinine and urea levels is seen from baseline with long term cyclosporin therapy[2].

Age, weight, sex and race of the patients were also entered into the data collection sheet.

2.3 Sample analysis

2.3.1. Cyclosporin whole blood level analysis

Venous blood samples are taken routinely from all the patients in the transplant unit at Garden City Clinic approximately an hour before the morning cyclosporin dose is administered. Various tests are performed, including a whole blood cyclosporin level.

Currently, the assays being used to measure cyclosporin levels in blood are:

- high performance liquid chromatography (HPLC),
- fluorescence polarisation immunoassay (FPIA),
- radioimmunoassay (RIA) with polyclonal or monoclonal antibodies,
- enzyme immunoassay technique (EMIT)[1].

HPLC, monoclonal FPIA and RIA, and EMIT, are specific for the parent compound of cyclosporin alone[1]. The cross-reactivity with metabolites is minor[1]. The polyclonal FPIA and RIA methods measure the parent

drug, as well as the metabolites, and the levels are found to be more than 15% higher, compared to the monoclonal assays[1].

The assay method should be easy to perform, have a rapid turn-around time, and have a high level of consistency[1]. HPLC is the most accurate, and shows no cross-reactivity with metabolites, but takes a very long time to complete[1].

The laboratory at the centre uses the Abbot TDx assay, which measures cyclosporin in whole blood by means of mFPIA. The advantages of this assay method are:

- easy and simple sample preparation,
- for 20 samples it only takes about 30 minutes for the run,
- highest between run degree of precision when compared to other assay methodologies[1].

The sensitivity is 95% for samples containing > 25 ng/ml of cyclosporin[15].

A trough sample is desirable for measurement of cyclosporin with this assay. A minimum whole blood specimen of 150 µl with heparin or

EDTA is required[15]. A pre-treatment step must be performed on each cyclosporin blood sample, as well as the calibrators, and the controls[15]. The pre-treatment step minimises interference from endogenous protein-bound fluorescent compounds, and consists of the addition of Solubilisation Agent and Whole Blood Precipitation Agent[15]. This dissolves the cells and precipitates the protein in the sample[15]. After centrifugation, a clear supernatant liquid is obtained, on which the actual assay is performed[15]. A minimum of 150 µl of clear supernatant is required to perform the assay[15].

2.4 NONMEM data analysis

The population pharmacokinetic analysis was done using Double Precision NONMEM 77- version II level 1.4 together with the PREDPP package (ADVAN 1, ADVAN 2, TRANS 2 and SS2).

Both the IV and oral data were fitted with a one compartment linear pharmacokinetic model. For the oral data, a one compartment linear pharmacokinetic model with first order absorption was implemented by choosing the ADVAN 2 subroutine provided in the NONMEM-PREDPP load module.

The use of the subroutine TRANS 2 makes it possible to estimate the pharmacokinetic parameters clearance (Cl) and volume of distribution (Vd)[14].

K_e is the elimination rate constant and can be calculated from Cl and Vd:

$$\text{Where} \qquad K_e = \frac{Cl}{Vd}$$

For the IV data, a one compartment linear pharmacokinetic model was implemented by choosing the ADVAN 1 subroutine provided in the NONMEM-PREDPP load module. As with the oral data, subroutine TRANS 2 was used to estimate the pharmacokinetic parameters of clearance (Cl) and volume of distribution (Vd).

2.4.1 Statistical model

A log-normal distribution was assumed to describe the variability of the pharmacokinetic parameters. The error models for inter-individual variability in the jth individual were:

$$\ln Cl_j = \ln \text{ true } Cl_j + \cap Clj \quad \text{(additive)}$$

Or $$Cl_j = \text{true } Cl_j * \exp(\cap^{Clj}) \quad \text{(exponential)}$$

And similarly,

$$Vd_j = \text{true } Vd_j * \exp(\cap^{Vdj})$$

$$Ka^j = \text{true } Ka_j * \exp(\cap^{Kaj})$$

where $\cap$ has a mean value equal to zero and a covariance matrix Ω. Residual variability in the ith concentration of the jth individual was modelled as follows :

$$\text{measured } c_{ij} = \text{predicted } c_{ij} * \exp(\varepsilon_{ij})$$

where ε has a mean value equal to zero and variance of σ^2.

The standard error (SE) of the parameters Cl, Vd and K_a (absorption rate constant) and the variances (SE_{var}), σ^2 and Ω are estimated by NONMEM. The standard error of the inter- and intra-individual variability was approximated using the following equation:

$$SE = (\cap \text{ or } \varepsilon + SE_{var})^{0.5} - (\cap \text{ or } \varepsilon)^{0.5}$$

and expressed as a percentage.

The intravenous and oral data was evaluated separately.

The first step was to find a model that best described the intravenous data. The aim was to find the fixed parameters of clearance (Cl) and volume of distribution (Vd) represented by θ_1 and θ_2 respectively. The next step was to see if weight had any influence on (Cl) and (Vd), first separately and then on both together. The effect of serum creatinine on clearance was evaluated in the following run. Lastly, it was checked if race and gender had any bearing on the clearance of cyclosporin.

The approach to the oral data was almost the same. First, it was found the model that best fit the data, and calculated the parameters of clearance and volume of distribution. Due to the fact that it did not have any cyclosporin blood levels in the absorptive stage, because all the levels recorded were trough levels, the data had to be tested with a selection of k_a values from the literature to see which value best described our data.

The next step was to see if weight had any influence on clearance and/or volume of distribution. Then we corrected the clearance value for weight and serum creatinine, also individually and then together. Lastly, we tried to find a correlation between race and clearance as well as gender and clearance.

2.4.2 Criteria for testing superiority of one model over another[16].

- Each NONMEM run provides in its output a value of its minimum objective function (MOF), which is 2 times the negative logarithm of the likelihood function. The difference in the value of the objective function (DMOF) obtained for the general and the constrained model is approximately chi square distributed with degrees of freedom equal to the number of fixed parameters minus one. Therefore, for two fixed parameters a DMOF of 3.8 or more indicates a statistically significant ($p<0.05$) improvement in the fit of the data and suggests that the constrained model should be accepted,
- a decrease in the inter-individual variance (η) is another criteria used in determining whether the hypothesis should be rejected or not,

- a lack of correlation between parameters by inspection of the correlation matrix of the estimates provided in the NONMEM output,
- small standard errors of estimates,
- weighted residuals, which are randomly scattered around zero when plotted against the predicted concentration,
- improvement of scatterplots in general.

CHAPTER 3

3.0 RESULTS

The records of 47 kidney transplant patients were examined. These patients had received a kidney transplant at the Garden City Clinic during November 1995, when the transplant unit was opened, to February 1998. Of the 47 files examined only 13 could be used that conformed to the criteria.

Unfortunately, it was impossible to extend the study period to be able to include more patients and to get clearer and more meaningful results, due to the drug regimen being changed to include new immunosuppressant drugs.

Most patients had to be excluded from this pilot study because of the concurrent use of cytochrome P450 enzyme inducing or inhibiting drugs with cyclosporin.

The data presented in this study is retrospective clinical data. The result is that the data is unstructured i.e. that the same amount of data

is not available for each patient. Occasionally, laboratory data was missing out of the patient file consequently certain cyclosporin levels could not be included in the study. This is the great advantage of NONMEM because we still managed to calculate pharmacokinetic parameters and find certain correlations even though the data was scarce and sometimes incomplete.

3.1 Demographic data analysis

All patients were between 14 and 65 years of age, the youngest being 14 and the oldest 54 with an average age of 36,46 years. Of the 13 patients included in the study, 11 (84,62%) were male and 2 (15,38%) were female.

There were 4 black (30,77%) and 9 white (69,23%) patients in the study. One black patient was female.

Figure 3.1 The gender and race distribution of the study population

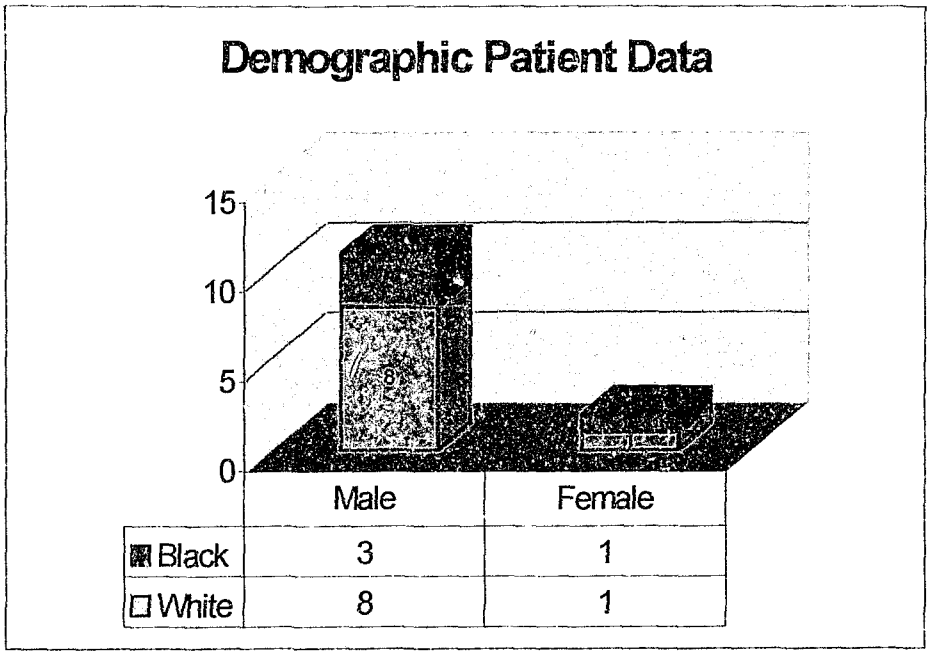

Table 3.1 Individual patient demographic data

PATIENT ID	SEX	RACE	AGE IN YEARS	WEIGHT IN KG.
1	M	W	34	75
2	M	W	14	34
3	M	B	50	69.9
4	M	B	45	102
5	F	B	37	57.5
6	M	W	25	70
7	F	M	54	51.5
8	M	W	43	61
9	M	W	14	31
10	M	W	24	94
11	M	W	53	64
12	M	B	47	80
13	M	W	34	63

3.2 Intravenous data analysis

There were 26 trough levels recorded while the patients were on intravenous cyclosporin. There were no intravenous cyclosporin levels available for patient number 9. Three patients had one recorded trough level, for six patients there were two levels recorded while on intravenous cyclosporin therapy, one patient had three trough levels and two patients had four recorded trough levels.

Table 3.2 The influence of different factors on the clearance and volume of distribution of IV cyclosporin as calculated with NONMEM

MODEL	MOF	DMOF	θ_1	η_1	θ_2	η_2	ϵ
(1) θ_1 = Cl (L/h) θ_2 = Vd (L)	185	0	22.4	48%	167	44 %	10%
(2) Cl =θ_1*((1+0.00156* (wt-68)) (L/h/kg) Vd = θ_2 (L)	185	0	22.6 (wt) 0.00156	47%	164	46%	10%
(3) Cl = θ_1 (L/h) Vd = θ_2*((1+0.0000195* (wt-68)) (L/kg)	185	0	22.4	48%	167 (wt) 0.0000195	43%	10%
(4) Cl =θ_1*((1+0.00232*(wt-68)) (L/h/kg) Vd =θ_2*((1+0.002*(wt-68)) (L/kg)	185	0	22.4 (wt) 0.00232	46%	166 (wt) 0.002	44%	10%
(5) Cl =θ_1*((1+0.0128*(Clcr (ml/min)-40)) (L/h) Vd = θ_2 (L)	177	8	20 (Clcr) 0.0128	26%	116	$2.6x10^{-13}$	10%
(6) Cl = θ_1 * RACE	185	0	22.4	48%	167	45%	10%
(7) Cl =θ_1* GENDER	185	0	22.4	48%	167	44%	10%

MOF - minimum objective function
DMOF - *difference in the value of the objective function*
η - inter-individual variation
ϵ - residual intra-individual variability
Clcr - creatinine clearance
wt - weight
Vd - volume of distribution
Cl - total body clearance

Table 3.3 Hypothesis testing for clearance and volume of distribution for IV data

Hypothesis	Model	DMOF	p-value	Conclusion
Did weight influence clearance?	1 + 2	0	p>0.05	No
Did weight influence volume of distribution?	1 + 3	0	p>0.05	No
Did weight influence clearance and volume of distribution?	1 + 4	0	p>0.05	No
Did creatinine clearance influence clearance?	1 + 5	8	P<0.05	Yes
Did race influence clearance?	1 + 6	0	p>0.05	No
Did gender influence clearance?	1+ 7	0	p.0.05	No

The general model calculated a clearance value of 22.4 L/h and a Vd value of 167 L with a MOF of 185.

The objective function (MOF) did not decrease significantly with the inclusion of weight, race or gender on clearance and/or volume of distribution.

However, when serum creatinine was included in clearance, the reduction of the main objective function (DOMF) by 8 points was significant. This resulted in a decrease in the inter-individual variation of clearance (η_1) from 48% to 26%.

3.3 Oral data analysis

There were 100 cyclosporin trough levels that were collected from these 13 patients while on Neoral.

A k_a of 0.7 h^{-1} best fit our data because it resulted in the lowest MOF (main objective function) value of 446, with an inter-individual variation of 46%. When we used a k_a of 1.27 h^{-1} the result was a substantial rise in the inter-individual variation to 93% with an MOF of 449. A k_a of 5.4 h^{-1} was associated with an unacceptable rise in the inter-individual variation to 313% with an MOF of 470.

Table 3.4 The influence of different factors on the clearance and volume of distribution of Neoral as calculated with NONMEM

MODEL	MOF	DMOF	θ_1 Cl	η_1	θ_2 Vd	η_2	ϵ
(1) θ_1 = Cl (L/h) θ_2 = Vd (L) k_a = 07 (h^{-1})	446	0	52.6	10%	339	48%	10%
(2) Cl =θ_1*((1+0.00206* (wt-68)) (L/h/kg) Vd = θ_2 (L) k_a=0.7 (h^{-1})	444	2	51.8 (wt) 0.00206	7%	332	44%	10%
(3) Cl = θ_1 (L/h) Vd =θ_2*((1+2.24x10^{-13} * (wt-68) (L/kg) K_a = 0.7 (h^{-1})	446	0	52.6	33%	339 (wt) 2.34x10^{-13}	48%	10%
(4) Cl =θ_1*((1+0.00210* (wt-68)) (L/h/kg) Vd =θ_2*((1+0.000175* (wt-68)) (L/kg) K_a = 0.7 (h^{-1})	445	1	51 (wt) 0.00210	7%	331 (wt) 0.000175	44%	10%
(5) Cl =θ_1*((1+0.00146* (wt-68))*((1+0.00613* (Clcr (ml/min)-40)) (L/h) Vd = θ_2 (L) k_a = 0.7 (h^{-1})	437	9	51.7 (wt) 0.00146 (Clcr) 0.00613	15%	356	44%	10%
(6) Cl =θ_1*((1+0.00634* (Clcr(ml/min)-40) (L/h) Vd = θ_2 (L) k_a = 0.7 (h^{-1})	437	9	51.9 (Clcr) 0.00634	14%	357	49%	10%
(7) Cl = θ_1 * RACE k_a = 0.7 (h^{-1})	446	-	52.6	10%	339	48%	10%
(8) Cl = θ_1 * GENDER k_a = 0.7 (h^{-1})	446	-	52.6	10%	339	48%	10%

MOF - minimum objective function
DMOF - difference in the value of the objective function
η - inter-individual variation
ϵ - residual intra-individual variability
k_a - absorption rate constant
Clcr - creatinine clearance
wt - weight
Vd - volume of distribution
Cl - total body clearance

Table 3.5 Hypothesis testing for clearance and volume of distribution for Neoral

Hypothesis	Model	DMOF	p-value	Conclusion
Did weight influence clearance?	1 + 2	0	p>0.05	No
Did weight influence volume of distribution?	1 + 3	2	p>0.05	No
Did weight influence clearance and volume of distribution?	1 + 4	1	p>0.05	No
Did weight and serum creatinine influence clearance?	1 + 5	9	P<0.05	Yes
Did serum creatinine influence clearance?	1 + 6	9	P<0.05	Yes
Did race influence clearance?	1 + 7	0	p>0.05	No
Did gender influence clearance?	1 + 8	0	p>0.05	No

The general model calculated a clearance value of 52,6 L/h and a Vd value of 339 L with a fixed k_a of 0.7 h^{-1}.

Again, as was the case with the intravenous data, the objective function did not decrease statistically significantly when clearance was corrected for weight, race or gender.

Although the DMOF was only 2 when clearance was corrected for weight, there was a notable reduction in the inter-individual variation (η_1) from 10% to 7%.

The MOF decreased significantly (DMOF = 9), when clearance was corrected for creatinine clearance (Clcr), more than with weight alone.

CHAPTER 4

4.0 DISCUSSION

This study was conducted with only 13 patients and the results can therefore only be seen and discussed as trends. However, when the values of clearance and volume of distribution for both the intravenous and the oral data were compared to the literature values[17], they compared favourably. The average weight of the patients included in this study was 70.26 kg.

Table 4.1 Comparison of the pharmacokinetic parameters calculated by NONMEM with the literature[17]

PARAMETER	CALCULATED WITH NONMEM	LITERATURE VALUE	FOR A 70KG PERSON
Cl for IV cyclosporin	22.4 L/h	0.318 L/h/kg	22.26 L/h
Vd for IV cyclosporin	167 L	1.3 L/kg	91 L
Cl for Neoral	52.6 L/h	0.768 L/h/kg	53.76 L/h
Vd for Neoral	339 L	4.3 L/kg	301 L

The slight difference in the Vd values especially in the intravenously administered cyclosporin could have several explanations.

1. This is a retrospective study, and it was very difficult to ascertain the exact times of administration of the drug from the patient charts. This obviously makes a big difference, when one is using such a sensitive program such as NONMEM.

2. The intravenous data was also very scarce, and the more data one has with NONMEM, the more accurate the result will be.

3. A study was done on the pharmacokinetics of cyclosporin in pre-kidney transplant patients, where the Vd was found to be 30% to 100% higher than in healthy individuals[18]. This is most probably due to the decreased protein binding increasing the free fraction of cyclosporin in the plasma, because of the uraemic state of these patients[18]. The patients that were on intravenous cyclosporin in our study had a few days, sometimes only hours before, been just such patients. This might explain the slightly higher Vd than the one quoted in the literature[17].

4. There was also a large day to day fluctuation in the patients' weight, which most probably is due to fluid being either retained, possibly due to corticosteroid therapy, or excreted by inducing a diuresis of

the extra water. This will certainly also influence the volume of distribution. It is difficult to explain that the inclusion of weight as a variable on Vd did not decrease the MOF significantly. It could be due to the influence of protein binding, which was not considered in this study.

The values for clearance compared favourably with the literature values for both the IV and the oral doses. Just as with the volume of distribution, there was only a small reduction in the MOF value when clearance was corrected for weight, but there was a decrease in the inter-individual variation as well. In this study the patient's different daily weights were used. It could be that there was too much daily fluctuation in the weight to be able to find a correlation. More data is necessary to investigate this further. It is also important to do more cyclosporin blood levels with the same weight.

These values for clearance and volume of distribution were calculated using a k_a of 0.7 h^{-1}. This is a relatively low k_a, which would most probably be due to the quicker absorption rate seen with Neoral[3].

A study was conducted in China where population pharmacokinetic parameters of cyclosporin A in renal transplant patients were calculated also using the NONMEM system, estimated a k_a of 1.28 h^{-1} with an inter-individual variation of 75,10%[8]. This study was done before the advent of Neoral and therefore the original oral formulation of cyclosporin was used which should result in a higher k_a value because of the longer time needed for absorption to occur.

Both the IV and the oral data showed that there was a significant decrease in the MOF when clearance was adjusted for serum creatinine.

A study conducted on renal transplant patients in Japan found a significant correlation between the area under the concentration versus time curve (AUC) of cyclosporin A and creatinine clearance[19]. In this study the traditional oral cyclosporin formulation was used where there is only little correlation between total drug exposure and trough levels. Neoral shows a much improved correlation between trough levels and total drug exposure i.e. AUC[3]. There is a possibility, if they had used Neoral in this study, they might have also found a correlation between creatinine clearance and trough levels.

The explanation most likely, is the fact that nephrotoxicity is one of the most common adverse effects experienced with this drug[1]. Acute cyclosporin-induced nephrotoxicity is most frequently seen in the first few months after the transplant because the doses and blood levels are highest and are being adjusted at this time[1]. A rise in serum creatinine, potassium, uric acid and cyclosporin is associated with this syndrome[1]. The pathophysiology is not quite clear, but seems to be related to the glomerular hypoperfusion that is induced secondary to the vasoconstriction of the afferent glomerular arteriole[1]. One possible explanation for this is, that cyclosporin alters the balance of prostacyclin and thromboxane A_2 in renal cortical tissue, and increased thromboxane A_2 results in renal vasoconstriction[1]. This is dependent on the dose of cyclosporin[1]

More recent data indicate that renal function, measured as 1/Scr, declines initially when patients are treated with cyclosporin[1]. The renal function stabilises after approximately six months to one year without a further decline[1].

Unfortunately, no correlation could be found between race and clearance. The explanation most probably lies in the scarcity of the

data. However, it has been found in a recent study conducted in U.S.A. on white and black paediatric liver transplant patients, when converting from the original formulation to the microemulsion, that the increase in bioavailability was much greater in the black compared to the white patients, when they were stratified by age[20]. This might also apply to adult black patients, reducing the difference in bioavailability between black and white transplant patients.

Bioavailability (F) is defined as the rate as well as the fraction of the parent compound that reaches the systemic circulation[1]. We have established a clearance of 22.4 L/h for the IV cyclosporin and a clearance of 52.6 L/h for Neoral. The amount of parent compound in the blood at steady state (cp_{ss}) is calculated by:

$$Cp_{ss} = \frac{F * Dose}{Cl * 24\ hours}$$

Therefore if

$$\text{Bioavailability} = \frac{Cp_{ss\ oral}}{Cp_{ss\ IV}}$$

$$\text{Then} \quad \text{Bioavailability} = \frac{Cl_{ss\ IV}}{Cl_{ss\ oral}} = \frac{22.4}{52.6}$$

$$= 0.43$$

The average bioavailability of Neoral in this study is proposed to be 43%. This is quite low when the average bioavailabity of cyclosporin from the microemulsion formulation is supposed to be around 60%.

It is reasonable that this could be due to an interaction occurring with one of the many other drugs that are taken daily by these patients. It is also reasonable that the type of food ingested could also influence the bioavailability, because the interaction with food is reduced, not eliminated with Neoral[3]. It is also important to remember that the k_a value was fixed. This influences the clearance value and consequently also the bioavailability. Further investigation is necessary to draw a final conclusion from this result.

It is however possible to test this bioavailability in another study. One could use this value as a conversion factor to work out what dose is needed of Neoral, once the patient has been stabilised on a particular dose of the IV cyclosporin:

$$\text{Dose of Neoral} = \frac{\text{Dose of IV cyclosporin}}{0.43}$$

Careful monitoring of blood levels is necessary to see if the same trough levels can be achieved as with the IV cyclosporin formulation.

However, a study conducted in Valencia, Spain used NONMEM to find a model that best described cyclosporin pharmacokinetic parameters in renal transplant patients in the first two months post-transplant[21]. Their findings suggest that during this early post-transplant period the time-dependant Michaelis-Menton pharmacokinetic model could be used to individualise the microemulsion cyclosporin dose[21].

Due to the side-effects experienced with the IV preparation, the newest approach is to start the patients on Neoral straight away[22]. The rationale behind this is the improved intestinal absorption independent of food and bile flow with more consistent blood cyclosporin

concentrations of Neoral[22]. They found that in the absorption of Neoral in the immediate post-transplant period was improved to the extent that target trough levels could be achieved even better and earlier than with the IV formulation[22]. Also, there seems to be an association of freedom from rejection with a high peak cyclosporin blood level (c_{max}) which is more likely to be achieved with Neoral compared with IV cyclosporin[23].

The validity of the NONMEM computer system is widely established and is capable of analysis of sparse unstructured clinical data[9]. It appears to provide the most satisfactory approach to the analysis of population pharmacokinetic data[9]. A larger, more in-depth study is necessary to further elucidate the correlation between serum creatinine levels and cyclosporin trough levels. With more data it will definitely be possible to arrive at a formula that can be used to calculate the dose of Neoral required to sustain satisfactory cyclosporin levels that were reached while on IV cyclosporin.

CHAPTER 5

5.0 Conclusion

The amount of data available for this retrospective pilot study was very restricted, but we still managed to calculate the population pharmacokinetic parameters of Cl and Vd for the IV cyclosporin as well as the oral microemulsion Neoral with NONMEM. These compared favourably with those found in the literature. Therefore it is possible to calculate population pharmacokinetic parameters from routinely taken trough levels. An unexpected correlation was found between the serum creatinine levels and cyclosporin trough levels which needs to be investigated further. A relative bioavailability between the IV and oral cyclosporin of 43% was established but more in depth investigation is necessary to confirm this result.

We succeeded in achieving three of our aims but due to the scarcity of our data we were not able to arrive at a formula to predict the required dose of Neoral when converting from IV cyclosporin therapy. With more data NONMEM would be able to do this.

APPENDIX A

DATA COLLECTION SHEET

Patient Hospital No. & Initial	Sex	Race	Age in Years	Weight in Kg	1. Heamatocrit 2. Erythocyte Count		Serum Potassium	Serum Creatinine	Serum Urea	Dose: Amount of Drug in milligram	Rate of Infusion (for IV)	Dosing Intervals	SS	EVID	DV

SS = 1 for dose of dosing interval
2 second and third dose
3 plasma level

EVID = 1 dose
= 0 plasma level

DV = plasma level
= 0 for dosing

APPENDIX B

Intravenous cyclosporin trough levels

PATIENT ID	DOSE IN MILLIGRAMS PER KG PER DAY	DOSE IN MILLI-GRAM	DOSING INTERVIAL	TROUGH LEVEL ηg/ml	SERUM CREAT-ININE	WEIGHT IN KG
1	4.00	100	8 hourly	61.1	135	75
1	4.11	100	8 hourly	98.4	129	73
2	3.13	50	12 hourly	106.8	312	32
3	2.15	50	8 hourly	403.2	332	69.9
3	0.72	50	24 hourly	70.6	375	69.9
3	2.14	50	8 hourly	97.3	339	70
4	0.95	50	12 hourly	221.4	366	105
5	3.48	100	12 hourly	191.5	122	57.5
5	3.15	100	12 hourly	226.1	120	63.5
6	4.44	100	8 hourly	136.7	263	67.5
6	3.03	100	12 hourly	171.9	192	66
7	2.75	50	8 hourly	609.3	438	54.5
7	2.38	50	8 hourly	376.1	435	63
7	0.81	50	24 hourly	81.8	464	61.5
7	-	-	-	51.8	429	61.5
8	3.28	100	12 hourly	125.5	238	61
8	3.28	100	12 hourly	47.1	190	61
8	4.84	100	8 hourly	875.1	165	62
8	1.59	100	24 hourly	92.9	165	63
10	1.60	50	8 hourly	133.4	294	94
10	1.58	50	8 hourly	224.1	214	95
11	2.34	50	8 hourly	217.5	142	64
11	2.31	50	8 hourly	307.1	117	65
12	1.88	50	8 hourly	305.9	179	80
13	1.59	50	12 hourly	43.9	225	63
13	1.59	50	12 hourly	35.6	186	63

APPENDIX C

Trough levels taken from patients on Neoral

PATIENT ID	DOSE PER KG PER DAY	DOSE IN MILLI-GRAM	DOSING INTERVIAL	TROUGH LEVEL	SERUM CREAT-ININE	WEIGHT IN KG
1	9.59	350	12 hourly	120.3	117	73
1	9.59	350	12 hourly	118.3	125	73
1	9.46	350	12 hourly	115.3	130	74
1	9.46	350	12 hourly	135.2	123	74
1	10.74	400	12 hourly	235.1	149	74.5
1	10.88	400	12 hourly	239.1	149	73.5
1	10.88	400	12 hourly	206.3	137	73.5
2	14.71	250	12 hourly	184.9	223	34
2	14.71	250	12 hourly	437.8	219	34
2	14.71	250	12 hourly	225.1	222	34
2	11.76	200	12 hourly	285.7	202	34
2	11.76	200	12 hourly	272.3	238	34
2	10.29	175	12 hourly	250.9	214	34
2	10.29	175	12 hourly	271.0	217	34
2	8.82	150	12 hourly	271.5	149	34
3	7.14	250	12 hourly	235.0	320	70
3	7.25	250	12 hourly	272.0	336	69
3	6.62	225	12 hourly	249.9	317	68
3	6.82	225	12 hourly	191.2	342	66
3	6.62	225	12 hourly	218.9	337	68
3	6.62	225	12 hourly	230.4	330	68
3	6.82	225	12 hourly	183.3	290	66
4	3.76	200	12 hourly	45.0	266	105.5
4	5.66	300	12 hourly	242.0	205	106
4	5.71	300	12 hourly	156.3	196	105
4	5.83	300	12 hourly	157.7	169	103
4	7.62	400	12 hourly	137.7	182	105
4	8.74	450	12 hourly	374.2	187	103
4	8.78	450	12 hourly	287.6	185	102.5
4	8.78	450	12 hourly	371.9	212	102.5
4	3.90	400	24 hourly	277.8	216	102.5
5	8.06	250	12 hourly	212.6	104	62
5	8.00	250	12 hourly	140.6	115	62.5
5	8.06	250	12 hourly	202.2	115	62
5	9.38	300	12 hourly	285.2	123	64
5	8.59	275	12 hourly	422.0	136	64
5	5.59	275	12 hourly	328.8	124	64

APPENDIC C (Cont.)

Trough levels taken from patients on Neoral

PATIENT ID	DOSE PER KG PER DAY	DOSE IN MILLI-GRAM	DOSING INTERVIAL	TROUGH LEVEL	SERUM CREAT-ININE	WEIGHT IN KG
6	9.23	300	12 hourly	280.8	187	65
6	7.58	250	12 hourly	241.4	207	66
6	7.58	250	12 hourly	239.7	180	66
6	7.58	250	12 hourly	251.7	196	66
6	7.46	250	12 hourly	177.3	184	67
6	7.52	250	12 hourly	161.2	170	66.5
6	7.46	250	12 hourly	254.5	155	67
6	7.46	250	12 hourly	221.2	161	67
7	3.57	100	12 hourly	263.9	363	56
7	2.73	75	12 hourly	80.8	282	55
7	2.70	75	12 hourly	59.8	241	55.5
7	5.41	150	12 hourly	94.2	224	55.5
7	5.45	150	12 hourly	107.8	198	55
7	7.27	200	12 hourly	196.2	196	55
8	9.65	275	12 hourly	206.3	175	57
8	9.65	275	12 hourly	216.3	176	57
8	9.65	275	12 hourly	251.7	161	57
8	9.57	275	12 hourly	215.2	172	57.5
8	9.65	275	12 hourly	239.2	164	57
8	9.65	275	12 hourly	302.8	154	57
9	12.31	200	12 hourly	320.9	79	32.5
9	9.38	150	12 hourly	155.0	56	32
10	4.30	200	12 hourly	12.1	189	93
10	5.38	250	12 hourly	104.6	164	93
10	6.45	300	12 hourly	102.3	163	93
10	6.45	300	12 hourly	159.1	235	93
10	8.60	400	12 hourly	169.9	322	93
10	9.09	450	12 hourly	407.5	391	99
10	7.14	350	12 hourly	243.0	386	98
10	7.00	350	12 hourly	322.1	383	100
10	6.50	325	12 hourly	251.6	308	100
10	6.50	325	12 hourly	192.8	242	100
10	7.14	350	12 hourly	164.9	201	98
10	7.81	375	12 hourly	339.9	181	96
10	7.41	350	12 hourly	253.3	181	94.5
10	7.41	350	12 hourly	305.1	168	94.5
11	7.69	250	12 hourly	554.0	117	65
11	3.73	250	24 hourly	298.8	117	67
11	-	-	-	154.1	110	67
11	7.35	250	12 hourly	302.4	147	68
11	6.34	225	12 hourly	389.1	145	71
11	6.34	225	12 hourly	271.2	120	71
11	5.48	200	12 hourly	581.1	117	73
11	-	-	-	236.6	117	73
11	2.67	200	24 hourly	336.0	110	75
11	5.14	175/200	12 hourly	398.6	108	73
11	4.11	150	12 hourly	483.2	109	73
12	7.14	300	12 hourly	78.6	140	84

APPENDIC C (Cont.)

Trough levels taken from patients on Neoral

PATIENT ID	DOSE PER KG PER DAY	DOSE IN MILLI-GRAM	DOSING INTERVIAL	TROUGH LEVEL	SERUM CREAT-ININE	WEIGHT IN KG
12	7.50	300	12 hourly	62.8	139	80
12	8.97	350	12 hourly	15.1	126	78
12	9.03	350	12 hourly	185.9	136	77.5
12	8.97	350	12 hourly	284.4	137	78
12	8.86	350	12 hourly	215.2	123	79
12	9.33	350	12 hourly	193.9	142	75
12	9.21	350	12 hourly	292.7	139	76
12	8.67	325	12 hourly	221.5	147	75
13	8.33	350	12 hourly	121.3	150	60
13	8.20	250	12 hourly	234.0	118	61
13	8.13	250	12 hourly	227.2	105	61.5
13	9.84	300	12 hourly	266.6	96	61
13	8.33	250	12 hourly	306.3	97	60
13	5.83	175	12 hourly	227.1	97	60
13	5.83	175	12 hourly	245.3	101	60

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