THE CONTROL OF CYCLOSPORIN IN TRANSPLANTATION: PHARMACOKINETIC ASPECTS

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PREFACE

The research work described in this thesis was undertaken during my appointments as a Research Assistant in the Department of Materia Medica, and as Staff Pharmacist in the Clinical Pharmacokinetics Laboratory, Stobhill General Hospital. I acknowledge the help and co-operation of all colleagues in the departments of Pharmacy and Materia Medica.

Except where stated, all work presented in this thesis was personally carried out by me. A list of communications and publications presented to Learned Societies or accepted for publication is included. The writing of this thesis is entirely my own work.

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Firstly, I would like to thank my supervisor, Professor Brian Whiting and also Dr. Andrew Kelman and Dr. Joachim Grevel for their help and advice throughout the period of research leading to the presentation of this thesis. I would also like to thank Ms. Catherine Howie for advice on the statistical analysis. I am grateful Professor John Reid for allowing me to use the facilities in his department and also to Dr. Alison Thomson and Ms. Janet Harrigan for their encouragement.

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SUMMARY

Cyclosporin is a relatively new immunosuppressant drug which has been shown to be useful in prevention of graft rejection following organ transplantation. Its major disadvantage is its toxicity, which appears to be related to high concentrations of the drug. Low concentrations are associated with rejection episodes. The aim of cyclosporin dosing is to achieve cyclosporin concentrations which minimise both toxicity and rejection. Individualisation of therapy is necessary since cyclosporin has a narrow 'therapeutic range' and also exhibits wide pharmacokinetic variability. The aims of this work are to quantify the pharmacokinetic variability of cyclosporin in renal and liver transplant patients in an attempt to improve control of therapy.

A review of the literature relating to cyclosporin outlines the background to the use of the drug and also discusses reasons for the pharmacokinetic variability. The analytical technique used to measure cyclosporin concentrations in whole blood (high performance liquid chromatography) is described in detail and validation for the method is provided. Pharmacokinetic and statistical methods utilised are described.

In an initial study, the pharmacokinetics of cyclosporin were investigated in eleven renal transplant recipients. A Bayesian technique was used to estimate the pharmacokinetic parameters of both a one and a two compartment model. This enabled pharmacokinetic parameters to be continually revised for each patient

over a period of two months following transplantation. Revised parameters were used to predict future concentrations for which prediction errors were calculated. Both models were found to consistently under predict later cyclosporin concentrations. Examination of the pharmacokinetic parameter estimates (of the two compartment model since this was considered more appropriate for cyclosporin) showed a decline in both Cl/F and V_1/F. This decline appeared to be exponential over time post-transplant and three mono-exponential models were subsequently investigated.

Possible reasons for the change in pharmacokinetics were investigated by relating Cl/F to various other factors such as biochemical and haematological measurements, or demographic data. Although several of these factors appeared to explain the change in Cl/F, it was not possible to determine whether this was a causative effect or merely an association. Several of the factors studied showed upward or downward trends which may also be related to time after transplantation, and their relationship with time may therefore be spurious. The data available suggested that time post-transplant was the most powerful explanatory variable for the change in Cl/F. Inclusion of further factors in a monoexponential model containing time may improve the model.

The one and two compartment versions of the

Bayesian program used earlier were modified to take into account the change in Cl/F. Thus, four models were available; one compartment with constant Cl/F, one compartment with changing Cl/F, two compartment with constant Cl/F and two compartment with changing Cl/F. The ability of these four models to predict cyclosporin concentrations was evaluated in eighteen renal transplant patients. The evaluation study was based on the prospective collection of data. Results of this study showed a significant improvement in accuracy of prediction when the modified program (either one or two compartment) was used. Precision was poor in all four models and possible reasons for this are discussed.

A further study was carried out in 11 liver transplant patients. The aim of this study was to investigate changes in cyclosporin pharmacokinetics following clamping of the external biliary T-tube. A series of blood samples was collected from each patient following oral and intravenous dosing before and after clamping of the T-tube. Of particular interest was a possible increase in bioavailability related to increased bile flow to the gut following clamping. Analysis of data from the eleven patients in the study showed an increase in bioavailability which was not significant. Two patients with vanishing bile duct syndrome at the time of the clamped study may have complicated the analysis; omission of these two patients resulted in a significant increase in bioavailability at

the time of the clamped study. Whether this increase was due solely to the effect of T-tube clamping, or whether a time dependent factor (as seen in renal transplant patients) was involved, was not clear.

These studies have enabled quantitation of some of the pharmacokinetic variability of cyclosporin occurring in the early period following either renal or liver transplantation. The findings presented in this thesis should lead to better control of cyclosporin therapy in such patients.

CHAPTER 1

GENERAL INTRODUCTION AND BACKGROUND

1.1 INTRODUCTION

Cyclosporin is an immunosuppressant drug which has played a major role in the survival rate of patients with transplanted organs. It was first used clinically in renal transplant patients in 1978 (Calne et al, 1978) and since then it has been administered to various groups of transplant patients, including liver (Starzl et al, 1985), cardiac (Oyer et al, 1983) and pancreatic transplant patients (Dubernard et al, 1988). Its use has also been investigated in prevention of graft versus host disease in bone marrow transplant recipients (Storb et al, 1985) and it may be of benefit in the treatment of auto-immune disease (Dipalma, 1989).

Immunological mechanisms are responsible for graft rejection and some means of modifying the immune response is therefore necessary (Monaco, 1986). Before the introduction of cyclosporin, transplant patients were usually treated with corticosteroids and/or azathioprine, an approach known as "conventional immunosuppression". The introduction of cyclosporin has however, significantly improved the success rate of renal transplantation (European Multicentre Trial Group, 1983) and liver transplantation is now considered the treatment of choice for nearly all causes of liver failure (Gordon et al, 1986).

Cyclosporin, however exhibits several toxic side effects, most notably nephrotoxicity. Efficacy and

toxicity of cyclosporin are not necessarily related to the dose administered, but concentrations of the drug in blood or plasma provide a useful method of minimising both toxicity and graft rejection (Irschik et al, 1984). The blood or plasma concentrations above which cyclosporin is effective and below which toxicity is unlikely constitute the therapeutic range. This range depends on the analytical technique used to measure cyclosporin and on the matrix in which it is measured. This is discussed later in Chapter 1.

Cyclosporin exhibits inter- and intra-patient pharmacokinetic variability resulting in unpredictable concentrations (Newburger and Kahan, 1983). The combination of a relatively narrow therapeutic range and wide pharmacokinetic variability has necessitated frequent monitoring of cyclosporin concentrations.

A method of individualising cyclosporin dosage based on pharmacokinetic principles using measured concentrations would be of great benefit. Single dose pharmacokinetic profiles performed either pre- or post-operatively (Lokiec et al, 1986; Kahan et al, 1986c) take account of differences between individuals but cannot account for important differences which occur within individuals. This latter problem is addressed by Bayesian estimation of pharmacokinetic parameters (Sheiner and Beal, 1982; Kelman et al, 1982), a technique which requires only one or two concentration measurements in each dosing interval and which may be

employed to continually revise pharmacokinetics in individual patients over a period of several weeks. Bayesian estimation is described more fully in Chapter 3.

This thesis involves the study of pharmacokinetic variability in renal and in liver transplant recipients, both within and between patients. This chapter provides a background of the immunological mechanisms involved in destruction of organ transplants and presents the need for immunosuppressant drugs. The advantages and disadvantages of cyclosporin will be discussed in relation to conventional therapy. Cyclosporin pharmacokinetics will be discussed in detail and the aims of the thesis will be outlined.

1.1.1 Immunological Response to Organ Grafting

A transplant between two genetically identical twins (isograft) does not evoke an immune response (Merrill et al, 1956). However, isografts are uncommon and allografts (same species but different genetic constitution) are much more usual. If no attempt is made to modify the immune system of the allograft recipient, rejection of the transplant will occur after a short period of time. An immune response directed against the cells of an allograft occurs because the antigens of the graft are genetically different from those of the recipient.

Antigenic expression on the cell surface of an allograft is genetically controlled by the major histocompatibility complex (MHC) and, in particular the human leukocyte antigen (HLA). Attempts are usually made to match the donor and recipient at the MHC, but even where they are well matched, rejection can still occur (Pfeffer et al, 1988).

Acute allograft rejection is mainly due to cellular immunity, that is immunity involving T-cells without the formation of free antibodies. The second main type of immune response, humoral immunity involving B-cell lymphocytes, is thought to play a smaller part in acute rejection. The rejection process involves a complex sequence of events. Antigens on the allograft are recognised as foreign and trigger the proliferation of activated T-cells from the lymphatic tissue. This leads to the release of cytotoxic T-cells whose function is to damage the cells of the allograft, helper T-cells which allow T-dependent B-cells to respond to certain antigens, and suppressor T-cells which result in feedback suppression of the immune response. The transplanted organ is destroyed by injury to blood vessels, resulting in ischaemia and necrosis.

1.1.2 Immunosuppressant Drugs

Transplant survival depends upon suppression of the immune system and, following transplantation, virtually

all patients receive immunosuppressant drugs. Ideally, the immunosuppressant agent should protect the cells of the allograft while having a minimal effect on the recipient, that is, it should inhibit only the T-cells known to be involved in the rejection process. The following section describes immunosuppressant drugs commonly used.

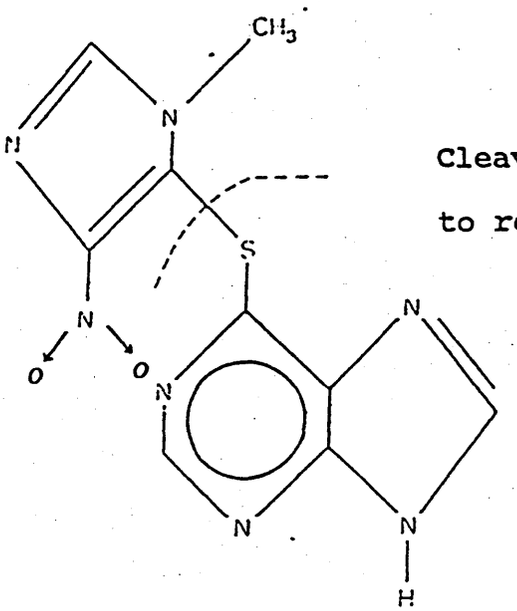
(i) Azathioprine

Azathioprine has now been used for about 30 years. It is a nitro-imidazole derivative of 6-mercaptopurine which is its active component. Six-mercaptopurine is liberated by metabolic cleavage in the liver after absorption (Figure 1.1). It is thought to inhibit T-cell proliferation (Hall, 1982) by interfering with nucleic acid synthesis. Azathioprine therapy however, increases the incidence of infections in the recipient and also has a toxic effect on the bone marrow. Before the introduction of cyclosporin, azathioprine in combination with corticosteroids was the most common means of suppressing the immune system following organ transplantation and is termed "conventional therapy".

(ii) Corticosteroids

Corticosteroids are used in virtually all transplant patients either in combination with azathioprine or, more recently, with cyclosporin. The chemical structures of the corticosteroids, prednisone

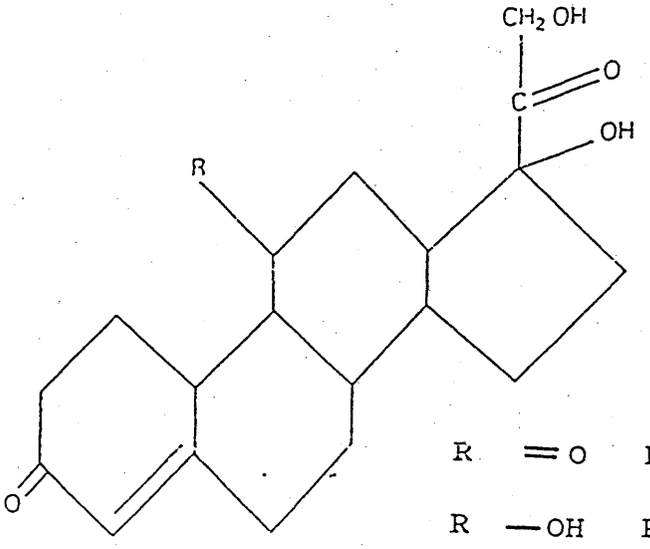
Figure 1.1 Chemical structure of azathioprine



Cleaved at this point

to release 6-mercaptopurine

Figure 1.2 Chemical structure of prednisone and prenisolone



R ═O Prednisone

R —OH Prenisolone

and its active moiety prednisolone, are shown in Figure 1.2. The corticosteroids act by binding to the DNA molecule, followed by increased transcription of mRNA molecules. This leads to alterations in protein synthesis and changes in cell function (Mukwaya, 1988), thus suppressing proliferation and differentiation of T-cells. Steroids can cause several unwanted side effects including growth retardation in children, Cushingoid features, obesity, hypertension and infection. Fortunately, lower doses of steroids can be used when the drug is used in combination with cyclosporin and some steroid side effects can be avoided.

(iii) Cyclophosphamide

Cyclophosphamide was used in the early years of transplantation but is less commonly used nowadays. It is inactive in vitro and is transformed in the liver into active metabolites. The active metabolites interfere with replication of immunologically competent cells by crosslinking to DNA (Yadav et al, 1988).

(iv) Total lymphoid irradiation

Total lymphoid irradiation is a means of immunosuppression limited to the lymphoid system. In animals, it has been shown to produce involution of the lymphoid system followed by preferential regeneration of T-suppressor cells (Slavin et al, 1978).

(v) Orthoclone OKT3

This is a murine monoclonal antibody which acts by inhibition of cytotoxic T-cells (Hirsch et al, 1987). It is used in some centres instead of steroids to reverse acute rejection episodes.

(vi) Antilymphocyte and antithymocyte globulin (ALG and ATG)

ALG and ATG exert a non-specific action against the lymphocytes and are useful in treating sensitised patients, for example those who have had a previous transplant.

(vii) FK 506

FK 506 is a new drug, produced as part of a drug program which was designed to identify fungal metabolites that would inhibit interleukin 2 (IL-2) production (Morris et al, 1989). Preliminary studies suggest that it is useful both as "salvage therapy" in patients with rejection or nephrotoxicity after receiving other immunosuppressant drugs, and as primary immunosuppression in high risk patients (Starzl et al, 1989).

(viii) Cyclosporin

The introduction of cyclosporin as a new class of immunosuppressant drug has played a major role in improving the success rate of kidney, liver, heart,

heart-lung, bone marrow and pancreas transplantation (Beveridge, 1986). Its chemical structure differs markedly from that of earlier immunosuppressant drugs (Figure 1.3). The potent immunosuppressive properties of cyclosporin were reported in 1976 (Borel et al, 1976) and the first clinical trial was carried out in seven renal transplant patients in 1978 (Calne et al, 1978). Cyclosporin was found to differ from earlier immunosuppressant drugs in its selective action on T-lymphocytes (Borel et al, 1977). The following section will outline the mechanism of action of cyclosporin.

1.2 CYCLOSPORIN

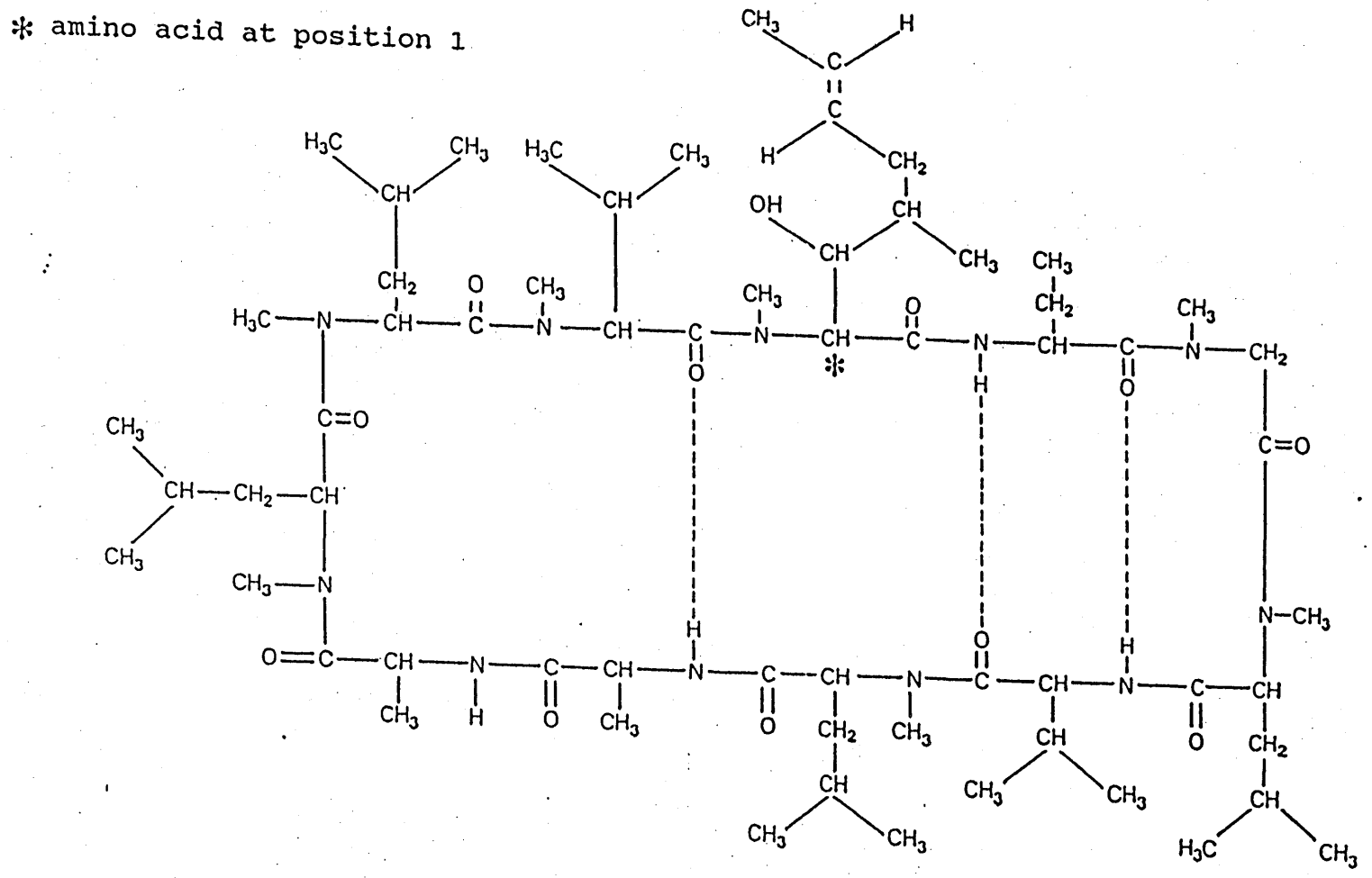
1.2.1 Background

Cyclosporin was fortuitously isolated from a soil fungal culture during a microbiological screening programme (Dreyfuss et al, 1976). Although it was found to have little anti-fungal activity, it showed potent immunosuppressant properties in animals (Borel et al, 1976). Cyclosporin is a cyclic undecapeptide of molecular weight 1202. Ten of the amino acids were already known but the C-9 amino acid on position 1 is unique and appears to be essential for immunosuppression although it is not active on its own (Wenger, 1986).

1.2.2 Mechanism of Action

Cyclosporin has a selective action on T-lymphocytes

Figure 1.3 Chemical structure of cyclosporin



(Borel et al, 1977) and appears to inhibit the release of IL-2 by stimulated helper T-cells (Hess et al 1983). The site of action of cyclosporin is shown in Figure 1.4. Cyclosporin has a sparing effect on suppressor T-cells; there is thus a change in the ratio of suppressor:effector cells (Kupiec-Weglinski, 1984).

At the cellular level it appears that inhibition of IL-2 production by activated helper T-lymphocytes occurs at the level of messenger ribonucleic acid (mRNA) transcription (Elliott et al, 1984); it may be that cyclosporin inhibits calmodulin dependent inducible mRNA transcription (Hess and Colombani, 1986).

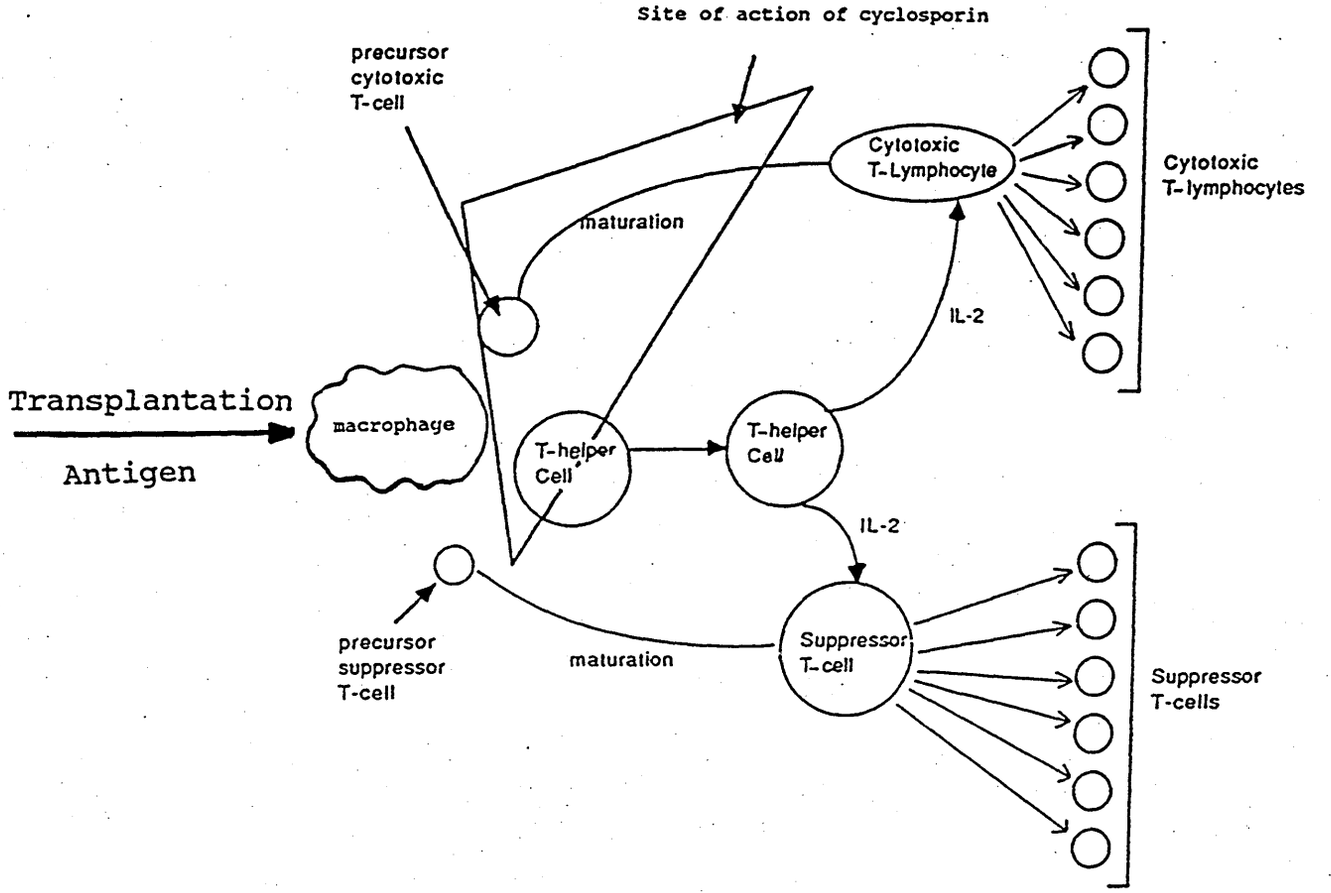
Cyclosporin does not inhibit cytotoxic T-cells which are already formed; the drug is therefore ineffective if treatment is started after antigenic stimulation has occurred (Wish, 1986).

Cyclosporin is thought to have no effect on humoral immunity (Borel et al, 1977) although there is some conflicting evidence on this (Paavonen and Hayry,1980).

1.2.3 Adverse Effects of Cyclosporin

Cyclosporin exhibits several toxic side effects, the most serious of which is nephrotoxicity. Nephrotoxicity occurs both in transplant patients (Calne et al, 1978) and in patients receiving the drug for other reasons (Dijkmans et al, 1987; Tegzess et al, 1988). The most likely site of action of cyclosporin on

Figure 1.4 Diagram of the immune response to organ transplantation and site of action of cyclosporin



From: Hess et al, 1983.

the nephron is the afferent arteriole where it produces vasoconstriction (Kahan, 1989). Nephrotoxicity is characterised by a decrease in fluid output from the proximal tubules, an increase in proximal fractional reabsorption and a moderate decrease in the glomerular filtration rate, probably caused by a reduction in renal blood flow (Dieperink et al, 1987). Animal studies suggest that the renin-angiotensin aldosterone system is involved (McAuley et al, 1987).

Nephrotoxicity is usually heralded by an increase in serum creatinine. However in the case of renal transplantation, it is difficult to determine whether an increased serum creatinine is due to nephrotoxicity or to rejection of the transplanted organ. Nephrotoxicity is thought to be associated with high cyclosporin concentrations and rejection with low concentrations (Irschik et al, 1984). An increase in creatinine due to rejection caused by inadequate cyclosporin levels will generally be reversed by treating with high dose steroids whereas increased creatinine due to nephrotoxicity is not reversed in this way (Taube et al, 1985). The use of ultrasound to detect an increase in cross-sectional area of the kidney (Parvin et al, 1986) or graft biopsy (Thomsen et al, 1987) may help to differentiate between the two conditions. Clinical signs indicating rejection include decreased urine output, increased weight, an increase in body

temperature and a rapid increase in serum creatinine. Nephrotoxicity is usually associated with a slower increase in serum creatinine (Klintmalm et al, 1983). Hepatotoxicity occurs in many patients but usually reverses on dosage reduction (Lorber et al, 1987). Hypertension is also common although the relationship with cyclosporin concentration is less clear (Loughran et al, 1985). Increased plasma LDL concentrations following renal transplantation may be associated with cyclosporin therapy (Raine et al, 1987). Seizures as a result of neurotoxicity may be related to elevated cyclosporin concentrations (Beaman et al, 1985). The incidence of lymphoma associated with cyclosporin is less frequent than that found with conventional immunosuppression; lymphomas which do occur generally respond to reduction in dose or termination of cyclosporin therapy (Starzl et al, 1984).

1.3 PHARMACOKINETICS OF CYCLOSPORIN

1.3.1 Absorption

Cyclosporin is a highly lipophilic compound which can be given orally in an oily suspension or in soft gelatin capsules. Its absorption is slow, variable and incomplete. The time to peak concentration following an oral dose varies between 1 and 8 hours (Ptachcinski et al, 1985b). Bioavailability of orally administered cyclosporin is variable. Values ranging from 5% to 60%

are reported (Ptachcinski et al, 1986). To make the oily solution more palatable it is usually mixed with fruit juice or milk. Oral absorption of the drug is unlikely to be affected by the vehicle (Johnston et al, 1986), although others have observed higher peak concentrations and higher area under the concentration time curve (AUC) when cyclosporin is mixed with milk rather than orange juice (Keogh et al, 1988).

The mechanism of absorption of cyclosporin is unclear. In animal studies, despite its lipophilicity, only about 2% of the drug is absorbed by the lymphatic system (Ueda et al, 1983). It is likely that cyclosporin is absorbed in the small intestine with micelles formed from bile (Lindholm et al, 1988c). Bile is necessary for absorption of cyclosporin in dogs (Ericzon et al, 1987) and animal studies show that poor absorption occurs in hepatic dysfunction, presumably due to lack of bile (Takaya et al, 1987). In liver transplant patients, very low concentrations of cyclosporin are detected after an oral dose during periods of biliary diversion; AUC increases following clamping of the external bile drain (Mehta et al, 1988) probably due to increased absorption (Andrews et al, 1985). Similarly in animal studies, the presence of bile results in approximately three fold increase in bioavailability of cyclosporin in dogs, compared to those with biliary diversion (Venkataramanan et al,

1986b). The concomitant administration of bile salts and cyclosporin to liver transplant patients may overcome this problem (Ericzon et al, 1987).

Pharmacokinetic studies of cyclosporin are usually carried out assuming first order absorption ie assuming that the rate of absorption is proportional to the concentration of drug in the gut (Newburger and Kahan, 1983). Zero order absorption (which assumes a constant rate of absorption independent of the concentration in the gut) and the presence of an "absorption window" in the gut has been proposed (Grevel et al, 1986). The absorption of cyclosporin may be dose dependent, ie at lower doses relatively more of the drug is absorbed (Grevel, 1988; Reymond et al, 1988). Conversely, Ueda et al (1984) have reported that in animal studies the proportion of cyclosporin absorbed increases as dose increases.

The effect of food on the absorption of cyclosporin is controversial. Food is reported to reduce bioavailability (Keown et al, 1982), to have no effect (Keown et al, 1983; Keogh et al, 1988) and to increase bioavailability (Ptachcinski et al, 1985c; Gupta and Benet, 1989). The presence of intestinal dysfunction, such as vomiting or diarrhoea, impairs absorption (Atkinson et al, 1984).

As a result of the various factors discussed above, there is considerable variability between subjects in cyclosporin absorption and bioavailability. In the

first two weeks following renal transplantation a mean (SD) bioavailability of 27.6% (18.1) has been reported, and in 7 of 41 subjects bioavailability was less than 10% (Ptachcinski et al, 1985b). In liver transplant recipients, bioavailability values ranging from 8% to 60% (mean 27%) have been reported (Burckart et al, 1986c).

Of crucial importance is the finding that bioavailability also varies considerably within subjects both in healthy volunteers (Lindholm et al, 1988c) and in renal transplant recipients (Kahan et al, 1983; Odlind et al, 1986). In the case of renal transplantation, it is known that as time progresses post transplant, the dose of cyclosporin must be reduced in order to maintain constant steady state concentrations (Tufveson et al, 1986). Bioavailability has been reported to increase gradually in the early period following renal transplantation (Kahan et al, 1983; Odlind et al, 1986). This has been verified by the finding that the same dose of cyclosporin given at about 3 days and again at about 7 months after a renal transplant results in a greater amount absorbed at the later time (Wilms et al, 1988). Similarly a mean bioavailability of 25.9% (19.4) in the first two weeks post transplant rising to 50.2% (7.9) at 6-12 months has been reported by Kahan et al (1986a).

Various theories for an increase in bioavailability

have been proposed. One possibility is that it is a time dependent change as the patient recovers post operatively (Venkataramanan et al, 1989). As previously discussed, bile is necessary for absorption of orally administered cyclosporin and poor bile flow immediately post-operatively may lead to impaired absorption. In addition, patients with renal failure often have altered gastric pH or gastro-intestinal dysfunction, both of which may impair drug absorption (Venkataramanan et al, 1989) immediately after transplantation.

1.3.2 Distribution

Cyclosporin exhibits multicompartmental behaviour following intravenous administration (Ptachcinski et al, 1987a; Follath et al, 1983). An initial rapid distribution phase, with a half life of about 0.1 hours is followed by a second slower distribution phase with a half life of the order of 1 hour and a terminal phase with an elimination half life of about 16 hours (Follath et al, 1983).

Cyclosporin distributes widely in the body as a result of its lipophilicity. Some reported values for volume of distribution are shown in Table 1.1. High concentrations of the drug have been detected in adipose tissue, liver, intestine, gall bladder, pancreas, kidney, adrenal glands, spleen and lymph nodes (Ried et al, 1983). A recent study has shown similar disposition of cyclosporin in lean and obese patients, suggesting

Table 1.1 Reported pharmacokinetic parameter values for cyclosporin in different groups of patients (measured in whole blood by HPLC).

Patient group	Clearance (l/h/kg)	Volume of dist^{n} (l/kg)	Elim^{n} half life (hours)	Ref
Healthy volunteers	0.234 (0.174-0.330)	1.3 (0.3)	6.2 (4.2-12.6)	(i)
Renal transplant	0.342 (0.038-1.43)	4.54 (3.59)	10.7 (4.5-53.4)	(ii)
Liver transplant	0.308 (0.192-0.456)	-	-	(iii)
Cardiac transplant	0.390 (0.126-0.906)	2.7 (2.0)	6.4	(iv)

Clearance; harmonic mean (range)

Volume of distribution; arithmetic mean (standard deviation)

Elimination half life; harmonic mean (range)

References: (i) Ptachcinski et al, 1987a,
(ii) Ptachcinski et al, 1985b,
(iii) Burckart et al, 1986a,
(iv) Venkataramanan et al, 1985

that the drug is distributed primarily in lean body mass (Yee et al, 1988a). Permeability of the blood/brain barrier is poor (Cefalu and Partridge, 1985) which conflicts with reports of central nervous system toxicity (Beaman et al, 1985). The drug is also distributed into breast milk (Flechner et al, 1985).

The distribution of cyclosporin in blood is both temperature and concentration dependent. At 37^{o}C approximately 58% of cyclosporin is associated with erythrocytes (Lemaire and Tillement, 1982). At lower temperatures in vitro, cyclosporin partitions increasingly into blood cells (Niederberger et al, 1983). The effect of this on the measurement of cyclosporin concentrations is discussed more fully in Chapter 2. Erythrocytes become saturated at concentrations greater that 5000ug/l. There is, however, little evidence for a relationship between haematocrit and the volume of distribution of cyclosporin (Yee et al, 1988c).

Cyclosporin is also highly bound to plasma proteins, particularly the lipoproteins (Lemaire and Tillement, 1982). Any change in lipoprotein concentration may affect binding and thus result in altered disposition of cyclosporin (Lithell et al, 1986). It is possible that some of the variability in pharmacokinetics of cyclosporin can be explained by alterations in clinical parameters that reflect binding of cyclosporin in the blood (Kasiske et al, 1988). One

group has observed increased haematocrit, LDL, HDL, total cholesterol, triglycerides, total protein and albumin post transplant and suggests that the observed changes in pharmacokinetics of cyclosporin are due to changes in binding of the drug (Awni et al, 1989).

1.3.3 Metabolism

Cyclosporin is extensively metabolised in the liver (Maurer, 1985). Nine metabolites have been identified although at least 25 are thought to exist (Venkataramanan et al, 1989). Biotransformation is limited to N-demethylation, hydroxylation and cyclization.

The metabolites may contribute to the immunosuppressant or toxic effects of cyclosporin. Some metabolites do have immunosuppressive activity (Rosano et al, 1987) and concentrations of cyclosporin measured by a non-specific radio-immunoassay (measures both parent drug and some of its metabolites) have been reported to correlate better with nephrotoxicity than those measured by high performance liquid chromatography which detects parent drug only (Holt et al, 1986; Yee et al, 1986). This suggests involvement of some of the detected metabolites.

Cyclosporin is primarily metabolised by the cytochrome P-450 system in the liver and consequently any concomitant drug therapy which induces or inhibits

this system may affect drug concentrations (Section 1.5). Patients with liver dysfunction metabolise cyclosporin more slowly than other groups of patients (Kahan et al, 1986a, Takaya et al, 1988).

1.3.4 Elimination

Cyclosporin metabolites are primarily eliminated via the bile. Less than 2% of an absorbed dose is excreted unchanged by this route (Burckart et al 1986b). Approximately 6% of administered cyclosporin is excreted in the urine, and less than 1% is excreted unchanged in the urine (Wood et al, 1983). Enterohepatic recirculation of parent drug does not occur, although some metabolites are recirculated (Venkataramanan et al, 1985b). Some reported values of clearance of cyclosporin in various groups of patients are shown in Table 1.1.

Elimination of cyclosporin follows first order pharmacokinetic behaviour, although during rapid intravenous infusion (>6mg/kg/hour), elimination may become zero order (Kahan, 1985a). Gupta et al, (1987) have shown a lower clearance at higher doses, but this is unlikely to be of clinical significance.

Cyclosporin is almost completely metabolised in the liver and consequently a deterioration in liver function, as defined by increased bilirubin or transaminase levels, may result in decreased clearance (Kahan et al, 1986a). A non-linear relationship has

been reported between alanine amino transferase (ALAT), which is indicative of hepatocyte damage, and cyclosporin clearance in uraemic patients (Reynolds et al, 1988). The relationship between bilirubin levels and cyclosporin disposition is more difficult to interpret since bilirubin levels may well be an index of liver function and therefore of cyclosporin metabolism, but may also be a measure of bile flow into the gut, an important consideration in the absorption of cyclosporin. In animal studies a significant negative correlation has been reported between serum bilirubin and bioavailability (Takaya et al, 1988) but the same group was unable to show a relationship between bilirubin levels and the clearance of intravenously administered cyclosporin. A study in bone marrow transplant patients showed no correlation between liver function tests and cyclosporin clearance (Yee et al, 1988b).

Renal function does not affect cyclosporin clearance (Follath et al, 1983; Roberts et al, 1986). Reports of a relationship between renal function and cyclosporin clearance are based on results obtained using a non-specific radio-immunoassay (see Chapter 2) (Arnold et al, 1987; Kahan et al, 1986a). However the effect of renal function on cyclosporin disposition is difficult to interpret and it is possible that some factor relating to improved renal function following a successful transplant may cause changing

pharmacokinetics of the drug. The clearance of cyclosporin is not affected by haemodialysis.

Hepatic extraction of cyclosporin is low to moderate (Venkataramanan et al, 1985a) which indicates that its clearance may be altered by changes in intrinsic clearance and blood protein binding, and to a lesser extent by changes in liver blood flow. As indicated earlier, cyclosporin is a lipophilic compound and is highly bound to lipoproteins. There is a close relationship between lipoprotein levels and cyclosporin clearance in uraemic patients awaiting renal transplantation (Lithell et al, 1986; Lindberg et al, 1986), possibly due to a reduction in the fraction of cyclosporin unbound with increasing cholesterol concentration (Legg et al, 1988). The decrease in cyclosporin clearance with age (Kahan et al, 1986a) may be due to increased lipoprotein concentrations which occur in older patients (Yee et al, 1987). Lipid abnormalities are common in patients undergoing dialysis, and transplantation may not overcome this. A significant increase in cholesterol in patients with hypercholesterolaemia has been shown one year post-transplant compared to pre-transplant (Kasiske et al, 1987). This increase may be due to cyclosporin itself (Raine et al, 1987) or to both cyclosporin and prednisolone (Harris et al, 1986 ; Vathsala et al, 1989).

Clearance of cyclosporin in bone marrow transplant

patients is higher than in other groups of transplant patients, possibly due to lower haematocrit levels in the former group (Yee et al, 1988b).

The requirement for lower cyclosporin doses as time progresses following renal transplantation, may be partly due to a decreased elimination with time (Newburger and Kahan, 1983; Habucky et al, 1988).

1.4 EFFECT OF DEMOGRAPHIC FACTORS ON THE PHARMACOKINETICS OF CYCLOSPORIN

Various demographic factors have been found to contribute to the inter-patient pharmacokinetic variability of cyclosporin. The effect of hepatic impairment, renal dysfunction and gastro-intestinal dysfunction have been discussed earlier in this chapter.

Renal transplant patients over 45 years of age have lower clearance and higher volume of distribution than younger patients (Kahan et al, 1986a); females have higher clearance and higher volume of distribution than males.

There appears to be no relationship between the pharmacokinetics of cyclosporin, and haematocrit, haemoglobin, bilirubin, albumin, aspartate transaminase, lactate dehydrogenase or alkaline phosphatase (Grevel et al, 1988a), although a significant relationship between alanine transaminase and clearance has been observed (Grevel et al, 1988a). This relationship is seen

regardless of whether cyclosporin is measured in whole blood by specific high performance liquid chromatography or by non-specific radio-immunoassay. A relationship between alanine transaminase and bioavailability is found only when cyclosporin is measured in serum by non-specific radioimmunoassay.

1.5 DRUG INTERACTIONS

Drug interactions with cyclosporin are of two types: (i) drugs altering the pharmacokinetics of cyclosporin or whose pharmacokinetics are altered by cyclosporin, and (ii) drugs affecting the toxicity of cyclosporin.

(i) Drugs which alter the pharmacokinetics of cyclosporin usually do so by interfering with either the absorption or the metabolism of the drug. Some interactions which have been investigated are discussed below and also listed in Table 1.2.

Metoclopramide has been reported to improve absorption of cyclosporin by increasing gastric emptying, therefore decreasing the time taken for the drug to reach small intestine absorption sites (Wadhwa, 1987).

The calcium channel blockers, diltiazem (Wagner et al, 1989), nicardipine (Bourbigot et al, 1986) and verapamil (Lindholm and Henricsson, 1987) cause increased cyclosporin concentrations. The mechanism of

Table 1.2 Reported pharmacokinetic drug interactions with cyclosporin

Drug	Effect on Cyclosporin Concentrations	Reference
Metoclopramide	Increases	Wadhwa, 1987
Erythromycin	Increases	Ptachcinski et al, 1985d, Gupta et al, 1988.
Diltiazem	Increases	Wagner et al, 1989
Nicardipine	Increases	Bourbigot et al, 1986.
Verapamil	Increases	Lindholm and Henricsson, 1987.
Nifedipine	No effect	Bourbigot et al, 1986.
Steroids	Increase Decrease	Ost et al, 1985. Ptachcinski et al, 1987b
Carbamazepine	Decreases	Lele, 1985.
Phenobarbitone	Decreases	Carstensen et al, 1986.
Phenytoin	Decreases	Freeman et al, 1984; Rowland and Gupta, 1987.
Sodium valproate	No effect	Hillebrand et al, 1987.
Rifampicin	Decreases	Langhoff and Madsen, 1983.
Sulphonamides	Decreases	Jones et al, 1986.
Cimetidine	No effect Increases	Jarowenko et al, 1986. Cockburn, 1986.
Ciprofloxacin	No effect	Tan et al, 1989.

this interaction is probably inhibition of hepatic microsomal drug metabolism (Renton, 1985). This interaction is not seen with nifedipine (Wagner et al, 1989; Bourbigot et al, 1986). It is of note that despite the increase in cyclosporin concentrations caused by diltiazem, nicardipine and verapamil, the glomerular filtration rate is not decreased (Wagner et al, 1989). This suggests that the calcium blocking drugs may have a protective effect on renal function possibly due to their vasodilator effect (Feehally et al, 1987).

Carbamazepine and phenobarbitone cause a decrease in cyclosporin concentrations, possibly due to induction of the cytochrome P450 enzyme system (Carstensen et al, 1986; Lele et al, 1985). Concomitant administration of phenytoin also causes decreased cyclosporin concentrations, but it is not clear whether this is due to decreased absorption (Rowland and Gupta, 1987) or to increased metabolism (Freeman et al, 1984). No such interaction has been reported between cyclosporin and sodium valproate (Hillebrand et al, 1987).

The use of high dose methylprednisolone during rejection episodes may affect cyclosporin pharmacokinetics. Increased cyclosporin concentrations have been seen during concomitant administration of both drugs when cyclosporin concentrations are measured by non-specific radio-immunoassay (Klintmalm and Sawe,

1984; Ost et al, 1985), presumably due to inhibition of cyclosporin metabolism. However, when cyclosporin is measured by specific high performance liquid chromatography, an increase in cyclosporin clearance is seen (Ptachcinski et al, 1987b).

Erythromycin causes increased concentrations of cyclosporin (Ptachcinski et al, 1985d) either by inhibiting its metabolism (Vereerstraeten et al, 1987) or by enhancing its absorption (Gupta et al, 1988).

The interactions with rifampicin and isoniazid (Langhoff and Madsen, 1983), sulphonamides (Jones et al, 1986) and ketoconazole (Ferguson et al, 1982) are probably due to alteration in cyclosporin metabolism. Ketoconazole inhibits metabolism of cyclosporin and its concomitant use may allow lower doses of cyclosporin to be used (First et al, 1989).

Spiramycin has no effect on the pharmacokinetics of cyclosporin (Guillemain et al, 1989; Birmele et al, 1989) suggesting that spiramycin is metabolised by a different isoenzyme than cyclosporin. Similarly, in spite of the effects of ciprofloxacin on pharmacokinetics of other drugs metabolised by the cytochrome P450 enzyme system (Bachmann et al, 1988), ciprofloxacin does not appear to affect the pharmacokinetics of cyclosporin (Tan et al, 1989). Other workers have been unable to detect any effect of norfloxacin on serum cyclosporin trough concentrations (Jadoul et al, 1989).

Cyclosporin itself affects the pharmacokinetics of some drugs. Digoxin toxicity has been observed in heart transplant patients treated with cyclosporin due to altered pharmacokinetics of digoxin (Dorian et al, 1988). The interaction between cyclosporin and prednisolone is mutual as shown by increased prednisolone concentrations when the two drugs are administered concurrently (Ost et al, 1985). Cyclosporin itself has been reported to inhibit the cytochrome P450 enzyme system (Moochala and Renton, 1986).

(ii) The second group of drug interactions includes drugs which are nephrotoxic themselves and may enhance the toxicity of cyclosporin. Both gentamicin and cyclosporin exert a toxic effect on the renal proximal tubule and it has been reported that the total nephrotoxicity when these two drugs are given together is greater than the sum of their individual toxicity when given alone (Whiting and Simpson, 1983). The mechanism by which amphotericin B causes nephrotoxicity is unclear but it has been reported to enhance the toxicity of cyclosporin when given in combination (Kennedy et al, 1983). In animal studies, frusemide has been seen to cause abnormalities of the renal tubules which are more severe when administered at the same time as cyclosporin (Whiting et al, 1984b).

Some drug interactions are beneficial and may

reduce toxicity. Cyclosporin nephrotoxicity appears to be caused by vasoconstriction of the renal arterioles and it has been reported that vasodilators, in particular nifedipine, may have a protective effect on renal function (Dieperink et al, 1986; Feehally et al, 1987).

1.6 MONITORING OF CYCLOSPORIN THERAPY

Pharmacokinetic variability of cyclosporin, coupled with the toxicity of the drug, has necessitated regular monitoring of cyclosporin concentrations in virtually all patients receiving the drug. Until the recent introduction of a specific monoclonal antibody for radio-immunoassay, most transplant centres used a non-specific radio-immunoassay because of the speed and ease of this type of assay. High performance liquid chromatography (HPLC) was the only specific assay available. The equipment required by HPLC and the time taken to perform the assay limited its use in routine clinical laboratories. Analytical methods will be discussed in more detail later in this section and in Chapter 2.

It is generally accepted that measuring of cyclosporin trough concentrations in blood or plasma is useful in minimising toxicity and rejection (Irschik et al, 1984). Similar findings have been reported by Kahan et al (1984). Lower cyclosporin plasma levels in renal

transplant patients with acute rejection have been observed compared to non-rejecting patients (Lindholm et al, 1988b). Furthermore, the same group has reported a significantly lower free fraction of cyclosporin during periods of acute rejection compared to one week earlier (Lindholm et al, 1988a). Others have shown that monitoring of cyclosporin concentrations is useful in prevention of nephrotoxicity in bone marrow transplant recipients but does not help to prevent graft-versus-host disease (Lindholm et al, 1987). Conversely, Rogerson et al (1986) found that measurement of cyclosporin concentrations in renal transplant patients is helpful in determining minimum concentrations to prevent rejection but does not help to prevent nephrotoxicity. It may be that both pharmacokinetic and pharmacodynamic monitoring are useful in optimising cyclosporin therapy (Kahan et al, 1985b).

The measured concentration of cyclosporin is highly dependent on the type of assay used (ie specific or non-specific) and on the matrix in which the drug is measured (ie blood, plasma or serum). Radio-immunoassay is the most commonly used analytical technique as it is relatively simple and fast to perform. However, until recently the antibody used in the radioimmunoassay was non-specific (ie it measured both parent drug and metabolites). HPLC is a specific technique but is too time consuming for most routine laboratories. The introduction of a specific antibody for cyclosporin

should combine the ease of radio-immunoassay with the specificity of HPLC (see Chapter 2). The matrix of analysis is also important due to partitioning of cyclosporin between plasma and blood cells. Whole blood appears to be the matrix of choice as this overcomes methodological problems associated with altered haematocrit and changes in temperature during storage of the blood sample (Shaw et al, 1987).

Most commonly, trough concentrations are measured ie samples are collected immediately before a dose. Table 1.3 lists some proposed therapeutic ranges for cyclosporin concentrations using different analytical methods (24 hour trough samples, renal transplant patients).

Trough cyclosporin concentrations are usually measured because variability in the ratio of parent drug to metabolite is minimised at this time (Robinson et al, 1983). It has been suggested that the use of a sample collected six hours after an oral dose (measured by HPLC) may be more useful than a trough sample in preventing toxicity and rejection (Cantarovich et al, 1988). Recently, an alternative approach to cyclosporin monitoring using a pharmacokinetic strategy has been proposed (Kahan and Grevel, 1988; Grevel et al, 1989) and they suggest that the use of AUC measurements is more useful that single trough concentration measurements. The disadvantage of such an approach is

Table 1.3 Suggested therapeutic ranges for cyclosporin using different analytical techniques (24 hour trough samples, renal transplant patients)

Method	Matrix	Range(ug/l)	Reference
HPLC	Whole blood	100-200	Najarian et al, 1985.
RIA (non-specific)	Whole blood	200-800	Irschik et al, 1984.
RIA (non-specific)	Serum	100-250	Kahan et al, 1984.
RIA (specific)	Whole blood	95-205	Kwan et al, 1987.

the number of samples required (approximately 28 samples in the first 5 days and a further 6 samples three days after any dose change).

An alternative pharmacokinetic approach to dosage adjustment is Bayesian estimation (Sheiner et al, 1979; Sheiner and Beal, 1982; Kelman et al, 1982), which uses only one or two concentration measurements in a dosage interval to estimate the most likely set of pharmacokinetic parameters for an individual patient. This method requires an accurate record of doses and samples but does not require that the patient is at steady state. This technique (described more fully in Chapter 3) has been successfully applied to a number of drugs. Kahan et al (1986b) have reported that Bayesian estimation may be a useful method for estimating cyclosporin pharmacokinetic parameters, but Grevel (1988) cautions that the degree of intra-individual variability may compromise the ability to successfully forecast cyclosporin concentrations.

1.7 AIMS OF THESIS

This thesis presents cyclosporin pharmacokinetic parameters obtained by Bayesian estimation in renal transplant patients and these are used to explore sources of inter- and intra-individual pharmacokinetic variability. A study in patients with liver transplants attempts to quantify the intra-individual variability

generated by the presence or absence of external biliary drainage.

CHAPTER 2

BIOCHEMICAL ANALYSIS

2.1 BACKGROUND

This chapter will discuss the various analytical techniques available to measure cyclosporin concentrations and the advantages and disadvantages of each method. The method used in this thesis will be described and validation of the method will be presented. Throughout this chapter cyclosporin will be described as cyclosporin A in order to differentiate it from the internal standard in the HPLC assay, cyclosporin D.

An early technique used to measure cyclosporin A in plasma and urine was high performance liquid chromatography (HPLC; Niederberger et al, 1980). This method, which uses cyclosporin D as an internal standard, has a limit of detection of 20ug/l but its use in routine clinical practice is limited by the length of time required to analyse samples. Improvements in HPLC methods now involve either extensive sample preparation and relatively simple chromatography (Sawchuck and Cartier, 1981) or more straightforward extraction followed by complex chromatography (Smith and Robinson, 1984). The most important feature of HPLC is its specificity for cyclosporin A.

In an attempt to overcome the disadvantages of HPLC and to provide a method suitable for clinical use, a radioimmunoassay (RIA) was developed (Donatsch et al, 1981). This has a similar limit of detection to the HPLC

method but is faster and requires less outlay on expensive equipment. The problem with RIA is that until 1987, the antibody used in the assay was not specific for cyclosporin A and measured both parent drug and some of its metabolites. In spite of this, its speed, ease of operation and small sample volume requirement meant that this method was favoured in routine laboratories. The use of a trough sample (i.e. a sample taken before a dose) was thought to minimise variability in the ratio of parent drug:metabolites and this was considered adequate for routine patient monitoring (Robinson et al, 1983). However, in patients with liver dysfunction or in liver transplant recipients where cyclosporin A metabolism is disturbed, the use of both HPLC and RIA may be appropriate (Burckart et al, 1986a). In these patients RIA results do not provide an adequate measure of the concentration of cyclosporin A.

The recent development of a specific antibody for cyclosporin A has produced RIA results which are claimed to be comparable to those of HPLC (Schran et al, 1987). Since this assay combines the speed and ease of the non-specific RIA with the specificity of HPLC, it is likely that this will be the method of choice in the future for both clinical and research work. Despite claims that this assay is specific for cyclosporin, recent work has shown that concentrations determined by this assay are 10-20% higher that those obtained by HPLC (Speck et al, 1989).

Little is known, however about the toxic and immunosuppressive properties of the metabolites and some centres may continue to use the non-specific assay as a measure of cyclosporin A metabolism. Another recent development is a fluorescence polarisation immunoassay (Abbott TDx) which is a non-specific assay for cyclosporin A (Schroeder et al, 1988). HPLC continues to be a useful method for large scale pharmacokinetic studies where rapid throughput of samples is not required and where specific measurement of metabolites is of interest.

The choice of sample matrix is controversial since cyclosporin is highly bound to erythrocytes and to plasma proteins. At 37^{o}C, 58% of the drug is bound to erythrocytes, 9% is bound to leukocytes and 33% is distributed within the plasma (Lemaire and Tillement, 1982). At lower temperatures, cyclosporin diffuses from plasma into blood cells (Niederberger et al, 1983). Consequently an apparent fall in cyclosporin plasma concentrations to approximately 50-60% of the concentration at 37^{o}C is seen when sample separation is carried out at 21^{o}C (Follath et al, 1983). An additional problem with the use of plasma as the matrix is that variations in haematocrit will affect the proportion of drug in plasma (Rosano, 1985; Niederberger et al, 1983). As a result, cyclosporin is usually measured in whole blood rather than in plasma (Wenk et

al, 1983). By using whole blood, the effect of changes in temperature and haematocrit can be overcome (Shaw et al, 1987).

2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

As detailed pharmacokinetic studies were the focus of this thesis, all data in Chapters 4, 5 and 7 are based on the HPLC analysis of cyclosporin in whole blood and this section will describe the method used and its validation. Data in chapter 6 are based on the specific RIA measurements and validation of this method is given in Appendix 1.

2.2.1 Chemicals and Equipment

The manufacturers and suppliers of all chemicals, reagents and equipment are listed in Tables 2.1(i) and (ii).

2.2.2 Preparation of Solutions and Standards

(a) Cyclosporin A

5mg of cyclosporin A were dissolved in 10ml methanol. This was then diluted 1:1 with water. A 50:50(v/v) solution of acetonitrile in water was used to prepare solutions of cyclosporin A for spiking standards in whole blood.

Table 2.1 (i) Chemicals and Reagents used in HPLC assay

Chemical	Supplier
Cyclosporin A	Sandoz Ltd, Basle, Switzerland
Cyclosporin D	Sandoz Ltd, Basle, Switzerland.
Far UV Grade Acetonitrile	Fisons plc, Loughborough, Leicestershire.
HPLC Grade Hexane	May and Baker Ltd, Dagenham, England.
HPLC Grade Methanol	May and Baker Ltd, Dagenham, England.

Table 2.1(ii) Equipment used in HPLC assay

Equipment	Model and Supplier
Spherisorb methyl 5um matrix column (5cm x 4.6mm)	Jones Chromatography Ltd, Mid Glamorgan, Wales.
Spherisorb C-18 ODS2 column (15cm x 4.6mm)	HPLC Technology Ltd, Macclesfield, England.
Pre-column filter frit (2um)	Upchurch Scientific, Inc Washington.
Column block heater	Model TC 860 HPLC Technology Ltd, Macclesfield, England.
HPLC pumps (2)	Model 510 Millipore, Waters Chromatography Division, Massachusetts.
Automatic Injector	WISP Model 710B
Valve station	Waters Automated Valve Station
Gradient controller	Model 680
Ultra violet detector	Model SPD-6A Shimadzu Corporation, Kyoto, Japan.
Integrator	Model Chromatopac C-R3A, Shimadzu Corporation, Kyoto, Japan.

(b) Cyclosporin D (internal standard)

5mg of cyclosporin D were dissolved in 10ml methanol and diluted with acetonitrile to give a solution of 500ug/l.

(c) Calibration Standards and Controls

Blank whole blood was spiked with cyclosporin to give calibration standards of 50, 100, 500 and 1000ug/l cyclosporin A in whole blood. Controls of 0, 20 and 200ug/l cyclosporin A in whole blood were also prepared.

(d) Mobile Phases

Acetonitrile and water were filtered and measured individually and then mixed thoroughly in the following proportions.

55:45 acetonitrile:water

70:30 acetonitrile:water

90:10 acetonitrile:water

2.2.3 Methods

(a) Pre-chromatographic Extraction

1ml of whole blood (calibration standard, control or unknown sample) was pipetted into a test-tube, 0.2ml methanol added and vortexed for 5 seconds. 1.2ml

internal standard solution was added to each tube. The tubes were capped, vortexed and incubated at 37°C for two hours. They were then vortexed and centrifuged for 15 minutes. Between 1 and 1.2ml of the upper layer was transferred to a conical glass tube, 1ml of hexane added and vortexed for 30 seconds. These tubes were placed in a -70°C freezer for 10 minutes and then thawed at 37°C for 10 minutes. 1ml of the lower layer was transferred to a clean glass conical tube, 0.3ml water added, vortexed and placed in the 37°C water bath for 10 minutes to evaporate any remaining hexane. The tubes were centrifuged for 5 minutes and 1.2ml supernatant transferred to WISP injection vials.

(b) Chromatography

The HPLC equipment was set up as shown in Figures 2.1(i) and (ii). A 15cm C-18 ODS2 column was used as the analytical column. The pre-column was a Spherisorb methyl 5um cartridge column. A pre-column filter of frit size 2um was placed in front of the pre-column. The pre-column filter and both chromatography columns were placed in the column block heater at 72°C in order to improve peak shape (Bowers and Matthews, 1985). The automated valve station was used to control the direction of flow of mobile phase and to operate the detector and integrator. It had seven switches (as shown in Figure 2.2): the function of each switch is

Figure 2.1 (i) Diagram of equipment used for HPLC analysis of cyclosporin

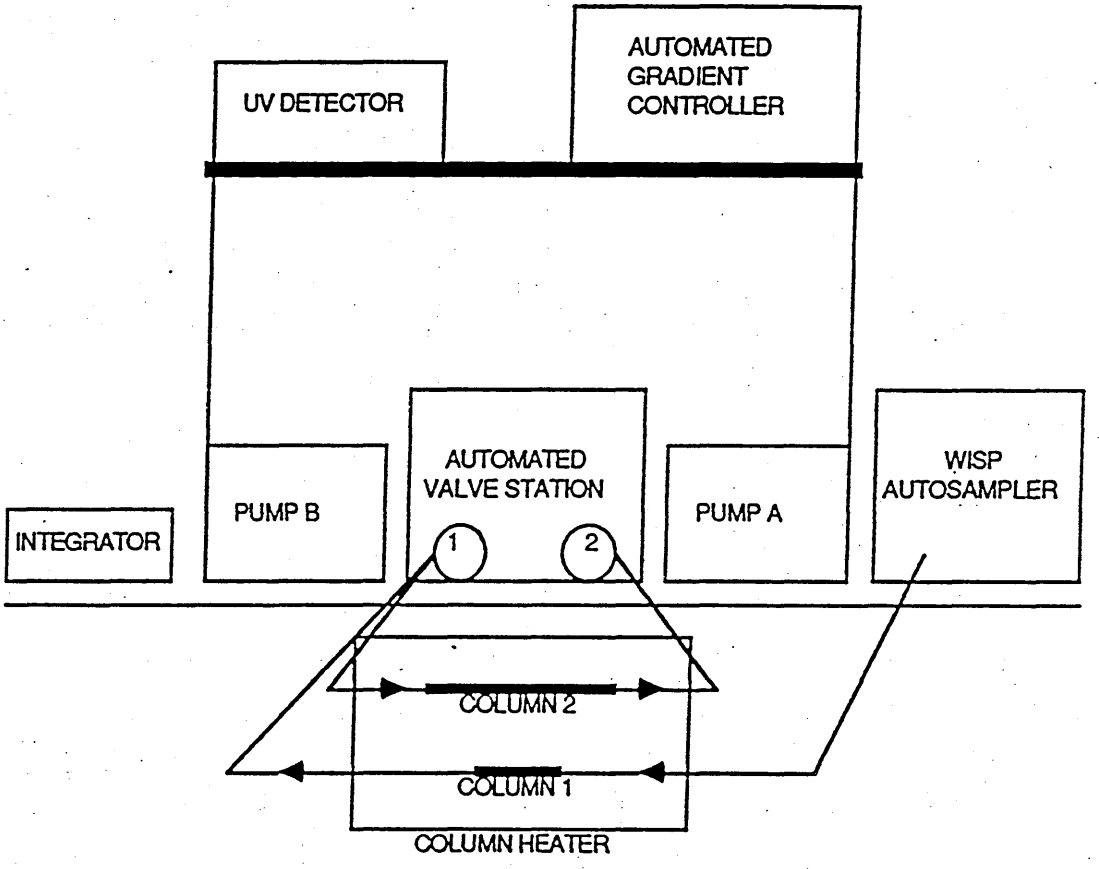
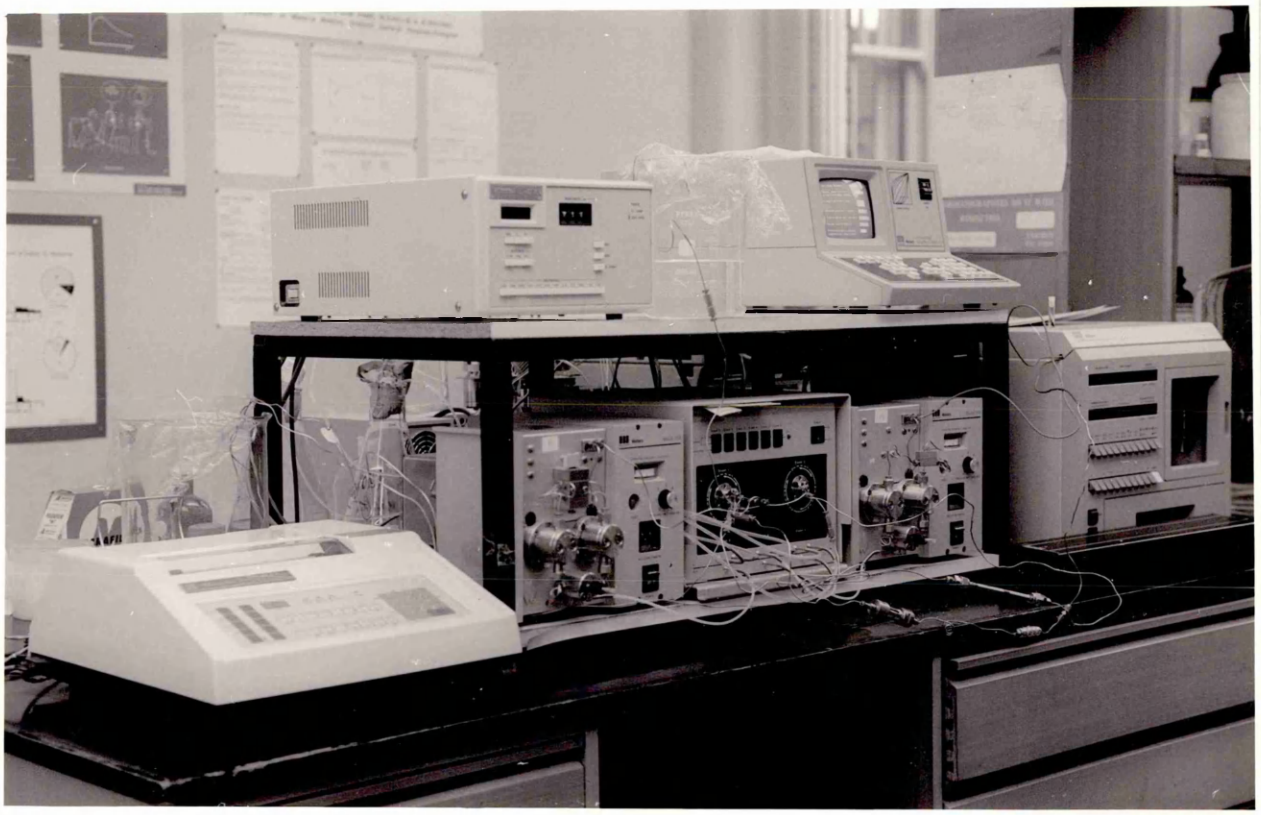


Figure 2.1 (ii) Photograph of equipment used for HPLC analysis of cyclosporin



listed in Table 2.2. The automated gradient controller controlled the timing of the switches on the valve station by use of a Time Program and a Gradient Program.

At the start of each new run the gradient controller was reprogrammed. This was done by altering the set up of the equipment slightly so that eluent from the pre-column passed directly to the integrator. A standard solution containing equal amounts of cyclosporin A and D was injected onto the pre-column in triplicate and the time from the start of the cyclosporin A peak to the end of the cyclosporin D peak calculated. This time period was called the "cutting time" and was entered in the time program of the gradient controller. The time program also controlled auto-zeroing of the detector, starting the integrator and equilibrating the system in preparation for the next injection. The gradient controller had a gradient program to control the flow rate of the mobile phase through the columns. Typical time and gradient programs are shown in Tables 2.3 and 2.4.

2.2.4 Results

(a) Chromatography

Cyclosporin A and D were separated by the method described. The quality of separation was highly dependent on the age of the columns. The pre-column lasted for approximately 300 injections and was replaced when interfering compounds in the blood extract were

Table 2.2 Use of switches on Waters Automated Valve Station

Switch	ON (=1)	OFF (=0)
1	eluent from column 1 is directed onto column 2	eluent from column 1 is directed to waste
2	eluent from column 2 passes through detector	eluent from column 2 is directed to waste
3	mobile phase for column 2 is 55:45	mobile phase for column 2 is 70:30
4	allows switch 5 to function	mobile phase for column 1 is 55:45
5	mobile phase for column 1 is 70:30 if switch 4 is on	mobile phase for column 1 is 90:10 if switch 4 is on
6	Pulse on, off for 0.05 minutes to autozero detector	
7	Pulse on,off for 0.01 minutes to start integrator	

Figure 2.2 Use of the seven switches available on the automated valve station used in HPLC assay

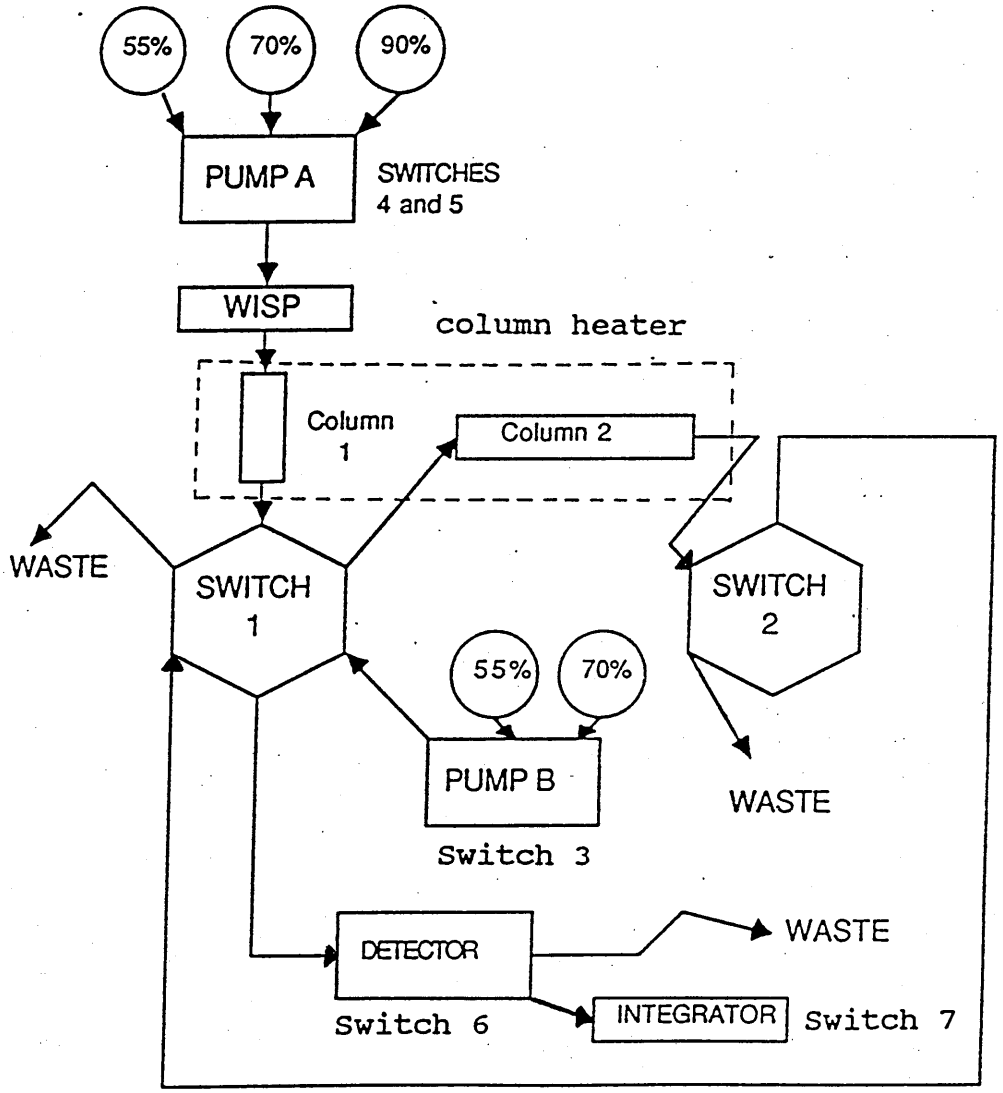


Table 2.3 Typical time program set on Waters Automated Gradient Controller

Time (minutes)	Switch	Action	Result of action
0.01	5	0	
5.00	1	1	CUTTING TIME (determined
10.50	1	0	earlier),
10.51	4	1	90:10 mobile phase to clean column 1.
10.52	2	1	eluent from column 2 directed to detector
10.53	3	0	70:30 mobile phase to column 2 to elute cyclosporin A and D
15.50	6	1	0.05 minute pulse to
15.55	6	0	autozero detector
15.60	7	1	0.01 minute pulse
15.61	7	0	to start integrator
19.00	4	0	55:45 mobile phase to re-equilibrate column 1
26.00	3	1	55:45 mobile phase to re-equilibrate column 2
26.01	2	0	eluent from column 2 directed to waste

NOTE: (i) 0 = off, 1 = on.

(ii) integrator stopped by setting "stop-time" on integrator

(iii) the time of the next injection was determined by the analysis time set on the WISP.

Table 2.4 Typical gradient program set on Waters Automated Gradient Controller

Time (minutes)	Flow rate (ml/min)	Pump A (% of total)	Pump B (% of total)
0.00	2.5	60	40
10.50	2.5	60	40
10.51	4.0	50	50
11.50	5.0	50	50
11.51	5.0	60	40
22.50	5.0	60	40
22.51	6.0	50	50

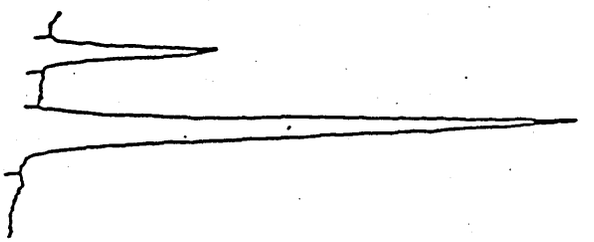
able to pass onto the analytical column with cyclosporin A and D. By replacing the pre-column regularly, the analytical column lasted for approximately 1500 injections. As the analytical column aged, the cyclosporin A and D peaks became broader and were less well resolved from each other. When peak shape and separation became unacceptable (it became difficult to tell where one peak ended and the other began) a new analytical column was installed. The effect of age on the analytical column is shown in Figure 2.3.

(b) Calibration

A calibration line was constructed for each run. Standards of 50, 100, 500 and 1000ug/l cyclosporin A in whole blood were assayed in duplicate. Peak areas of the cyclosporin A and D peaks were measured by the integrator and the ratio of cyclosporin A : cyclosporin D calculated. The calibration line was constructed by plotting the known concentration of the standard on the x-axis and the corresponding ratio on the y-axis. The best fit line of this plot was determined by linear regression and was used to determine concentrations of unknown and quality control samples. A typical calibration line is shown in Figure 2.4. Figure 2.5 shows two typical chromatograms of unknown samples and their concentrations calculated from the calibration line. All unknown and quality control samples were

Figure 2.3 Effect of age of the analytical column on peak shape of cyclosporin A and cyclosporin D

(i) new analytical column (extract of blood sample spiked with 100ug/l cyclosporin A)



(ii) analytical column after approximately 1000 injections (extract of blood sample spiked with 100ug/l cyclosporin A)

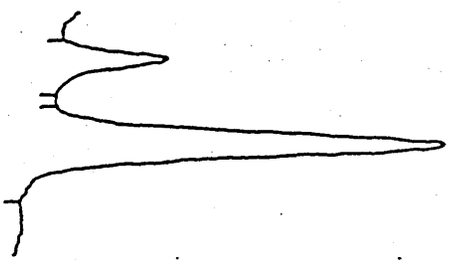


Figure 2.4 Typical calibration line for calculation of concentration of cyclosporin A in unknown and control samples

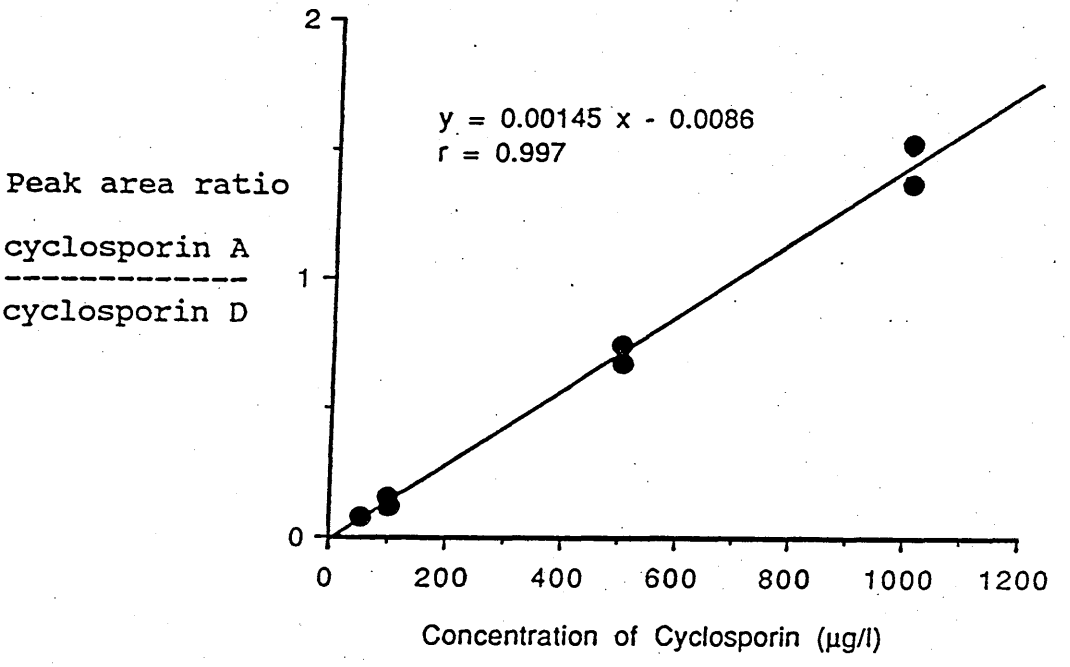
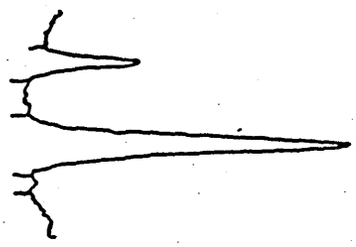
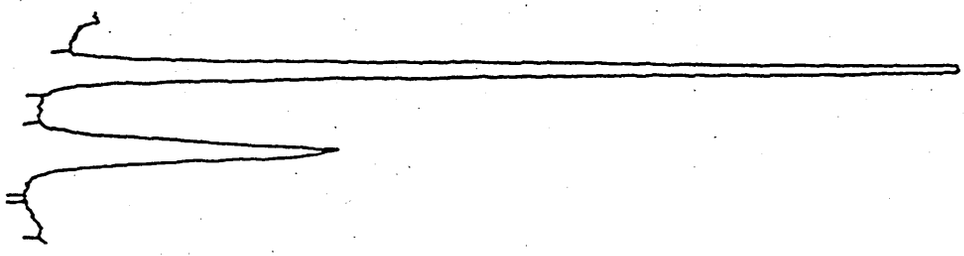


Figure 2.5 Chromatograms of samples containing unknown concentrations of cyclosporin A

(i) calculated to contain 76ug/l cyclosporin A



(ii) calculated to contain 924ug/l cyclosporin A



assayed in duplicate.

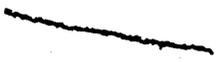
(c) Specificity

Extraction of whole blood samples known to contain no cyclosporin A was carried out to ensure that no constituents of blood interfered with the assay. This was done using a blood sample to which was added the vehicle of the internal standard solution (i.e., no cyclosporin A or D) and this was a "double blank". Another blood sample was extracted in the usual way and was termed a "single blank" (ie contained internal standard only). The double blank check was carried out on blood to be used for preparation of standard calibration controls. The single blank sample was assayed with each run to ensure that specificity for cyclosporin A was being maintained. Chromatograms of these samples are shown in Figure 2.6 (i) and (ii). No peaks co-eluted with cyclosporin A or cyclosporin D in the blank blood used to prepare pooled samples of standards.

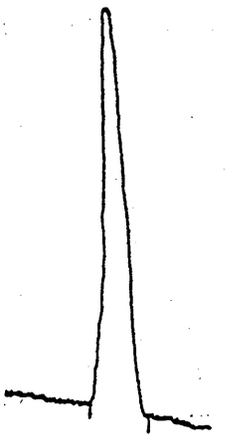
In addition, several drugs which the patients in the study were known to be taking were dissolved in appropriate solvents and added to blank whole blood. The blood used to prepare calibration standards and controls was obtained from patients known to be taking a variety of drugs (other that cyclosporin) and no coeluting peaks were seen.

Figure 2.6 Chromatograms of controls used in HPLC assay

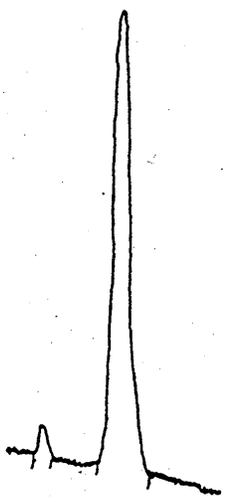
(i) 'double blank' blood extract



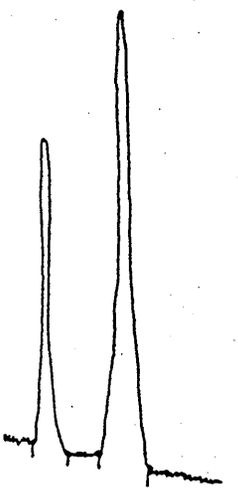
(ii) 'single blank' blood extract



(iii) blood extract spiked with 20ug/l cyclosporin A



(iv) blood extract spiked with 200ug/l cyclosporin A



(d) Limit of Detection

A 20ug/l cyclosporin A control sample was included in each run to ensure that this concentration could always be detected. The lowest detectable concentration was defined as that producing a signal (peak) of five times the baseline noise. A typical chromatogram of this concentration is shown in Figure 2.6 (iii).

(e) Linearity

The assay was validated for linearity over the range 20 to 1000ug/l cyclosporin A. Unknown or quality control samples found to contain concentrations above 1000ug/l were diluted with blank whole blood and re-assayed. Concentrations below 20ug/l were recorded as <20ug/l.

(f) Extraction Efficiency

The percentage of cyclosporin extracted from a blood sample was investigated by comparing peak areas of extracted samples with direct injection of an equivalent amount into the system. The extraction efficiency of the assay for a concentration of 200ug/l cyclosporin A was 56.2%.

(g) Precision and Quality Control

Inter-assay precision was determined by comparison of peak area ratios of 12 consecutive calibration lines

and the results of this are shown in Table 2.5(i). In addition, a quality control sample containing 200ug/l cyclosporin A (Figure 2.6(iv)) was included in each run of the assay. Inter-assay precision calculated from this sample is shown in Table 2.5 (ii).

Intra-assay variability was determined by assaying each calibration standard six times together with six samples from a pool of unknown concentration. In addition the 200ug/l quality control sample was assayed twelve times and intra-assay precision of the calculated concentrations determined. The results of this are shown in Table 2.6(i) and (ii).

(h) External quality control

Specificity of the assay from constituents of blood was determined by running blank samples. It was, however, difficult to prove that none of the cyclosporin metabolites were interfering with the assay since only a few were available commercially. Twelve samples were supplied by the United Kingdom Cyclosporin Quality Assessment Scheme and were assayed by this method and also by an established method at another centre. The results of this are shown in Table 2.7. It can be seen that the results from this centre and the established centre are comparable.

In addition three samples were assayed each month which were also supplied by the same scheme and the

Table 2.5 Inter-assay precision of HPLC assay

(i) Comparison of peak area ratios of calibration standards

Concentration (ug/l)	Coefficient of variation (%)
50	15.5
100	13.6
500	10.3
1000	11.7

(ii) Quality control sample included in each run

Known Concentration (ug/l)	Calculated Concentration (ug/l)	CV (%)	n
200	192	7.3	61

Table 2.6 Intra-assay precision of HPLC assay

(i) Comparison of peak area ratios of calibration standards

Concentration (ug/l)	CV (%)	n
50	5.7	6
100	3.4	6
500	3.6	6
1000	4.4	6

(ii) Calculated concentration of known and unknown cyclosporin pooled samples

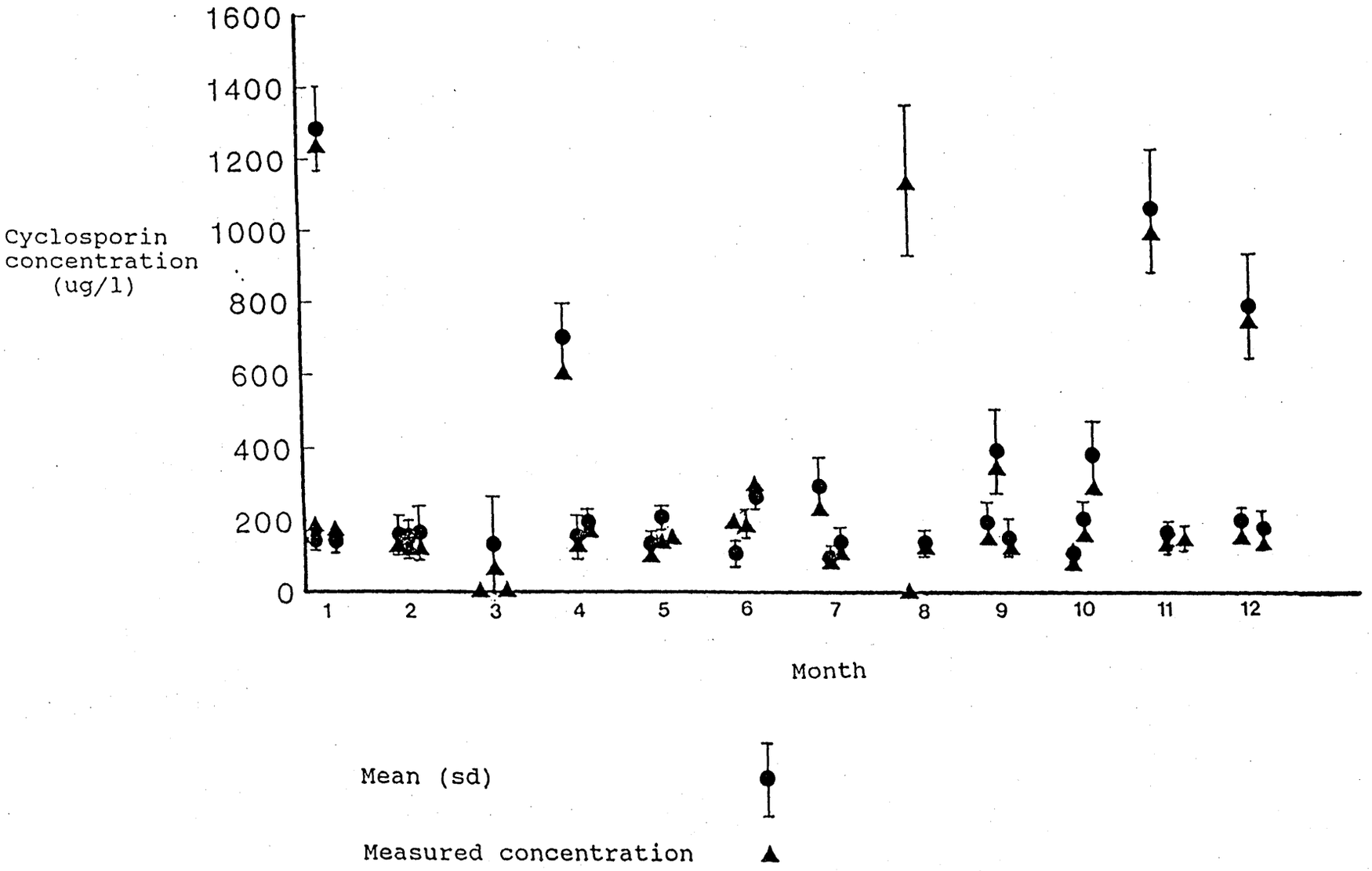
Concentration (ug/l)	Calculated concentration (ug/l)	CV (%)	n
200	204	2.1	12
Unknown	528	3.6	6

Table 2.7 Measured concentrations of 12 unknown samples and comparison of results with an "Established Centre" using the same assay

Sample	Measured (ug/l)	Actual (ug/l)	"Established Centre"(ug/l)
1	439	500	526
2	0	0	0
3	179	unknown	212
4	891	1000	976
5	202	unknown	232
6	240	250	268
7	0	0	0
8	453	500	528
9	175	unknown	280
10	897	1000	1100
11	239	250	272
12	185	unknown	230

results of this are shown in Figure 2.7. No absolute value was known for these samples since, with the exception of samples containing no cyclosporin A, they were usually prepared by pooling patient samples. The results do not therefore give a measure of accuracy. They do, however, provide a comparison with other centres using similar analytical techniques ie measurement of cyclosporin A in whole blood using HPLC. From Figure 2.7 it can be seen that the results from this assay generally fall within one standard deviation of the mean of the other centres. On each occasion where a blank sample (ie containing no cyclosporin A) was included, this was correctly identified.

Figure 2.7 Measured concentrations for external quality control samples and mean (sd) concentrations measured by other centres



CHAPTER 3

DATA ANALYSIS

3.1 INTRODUCTION

In this chapter, the various pharmacokinetic and analytical techniques used throughout this thesis will be discussed. A brief description of relevant pharmacokinetic principles and equations will be followed by a discussion of the statistical methods used. Computer programs used to analyse the data will also be described.

3.2 PHARMACOKINETICS

3.2.1 Background

Pharmacokinetics is the mathematical description of the movement of a drug throughout the body; a series of equations describes the time course of absorption, distribution and elimination of a drug. Such a quantitative description is of great importance because there is often a close relationship between drug concentration and effect. In a clinical setting, knowledge of the pharmacokinetics of a drug is of particular interest for drugs which have a narrow therapeutic range and where efficacy and/or toxicity are closely related to concentration.

3.2.2 Pharmacokinetic parameters

The two most useful parameters to describe the pharmacokinetic behaviour of a drug are clearance (Cl) and volume of distribution (V). Clearance is defined as

the volume of blood, plasma or serum from which drug is completely removed per unit of time and in this thesis is measured in units of litres per hour (l/h). Knowledge of drug input (ie dose) and clearance enables calculation of the average concentration at steady state, $\overline{C}_{ss}$, (Equation 3.1).

$$\overline{C}_{ss} = \frac{\text{Dose rate}}{\text{Clearance}} \qquad \text{Equation 3.1}$$

Volume of distribution relates the amount of drug in the body to the measured concentration and is generally measured in litres (l). Knowledge of volume enables calculation of the concentration of drug immediately following a bolus injection before any elimination has taken place. The ratio of clearance to volume expresses the fractional rate of removal of a drug, ie the elimination rate constant (k_e) (Equation 3.2) the units of which are usually per hour (h^{-1}).

$$k_e = \frac{Cl}{V} \qquad \text{Equation 3.2}$$

3.2.3 Oral absorption

When a drug is administered by a route other than the intravenous route, the rate and extent of absorption will affect the concentration of drug in the systemic circulation. Many drugs, including cyclosporin, are

most commonly given orally. The rate of absorption can be described by a first order absorption rate constant (k_a) which means that the rate of absorption is proportional to the amount of drug at the absorption site (ie the gut in the case of oral administration). The extent of absorption is known as bioavailability (F) and is defined as the fraction of the dose of parent drug reaching the systemic circulation. The oral absorption of a drug is most commonly described by a first order process (as above) but absorption of a few drugs may be better described by an apparent zero order process, ie the rate of absorption is independent of the amount of drug at the site of absorption.

3.2.4 Measurement of Bioavailability

The area under the concentration-time curve (AUC) is a useful method of estimating the extent of absorption of an orally administered drug. Bioavailability can be calculated by comparing the AUC of an oral dose with that of an intravenous dose.

The standard method of measuring AUC is by the linear trapezoidal rule (Gibaldi and Perrier, 1975). AUC is calculated by dividing the concentration-time curve into a series of trapezoids and calculating the area of each of the trapezoids as in Equation 3.3.

Area of trapezoid = $0.5\ (C_i+C_{i+1})(t_{i+1}-t_i)$

Equation 3.3

(for n measured concentrations where i=1,2.....n-1)

C_i is drug concentration at time (t_i).

AUC_o^T is the sum of the trapezoids from time zero to time T ($=t_n$).

When a patient is at steady state, AUC measured from time of administration of the dose to the end of the dosage interval (AUC_0^T) is equal to AUC from time zero to infinity (AUC_0^∞) for a single dose of the drug. If the patient is not at steady state, the influence of previous doses is taken into account by estimation of the terminal elimination rate constant and calculation of the area due to previous doses.

Bioavailability is calculated from equation 3.4

$$F = \frac{AUC_0^\infty\ (oral)}{AUC_0^\infty\ (iv)} \times \frac{Dose(iv)}{Dose(oral)} \qquad \text{Equation 3.4}$$

where AUC_0^∞ expresses the area under the curve from time zero to infinity.

3.2.5 Compartmental models

The concentration-time profile of a drug is commonly represented by a system of compartments. These compartments have no physiological or anatomical meaning; rather they refer to organs or tissues for which rate of uptake and clearance are similar. It is assumed that the concentration of drug is the same

throughout all compartments at equilibrium. The rates of transfer between compartments are assumed to obey first order kinetics. Although compartments have no physiological meaning, they provide a useful method of defining the distribution and elimination processes which contribute to the plasma concentration time profile. Drug concentration in any compartment can be described by a series of differential equations.

The simplest model is the one compartment model which assumes that the body is a homogeneous unit from which drug elimination is first order. If first order absorption is also assumed, then the differential equation describing the pharmacokinetics of the drug is

$$dA/dt = k_a G - k_e A \qquad \text{Equation 3.5}$$

where G is the amount of drug in the gut, k_a is the absorption rate constant, k_e is the elimination rate constant and A is the amount of drug in the body at time t. This is shown diagrammatically in Figure 3.1. Equation 3.5 can be solved by the method of Laplace transformation (Appendix 2) to give Equation 3.6.

$$C = \frac{k_a\ DF}{V(k_a - k_e)} (e^{-k_e t} - e^{-k_a t}) \qquad \text{Equation 3.6}$$

where C is the concentration at time t and D is the oral dose.

A two compartment model can be represented as shown in Figure 3.2 and is described by a biexponential

Figure 3.1 Diagrammatic representation of one compartment model assuming first order absorption

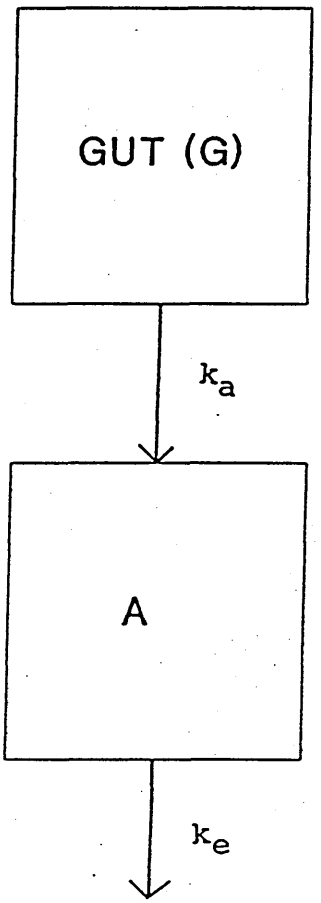
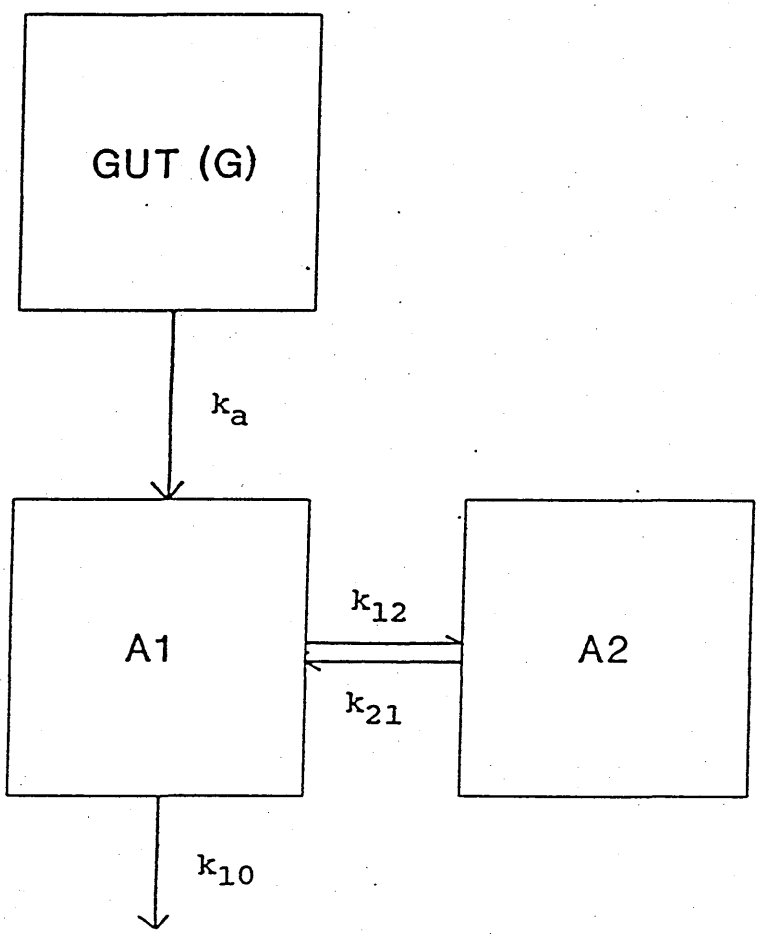


Figure 3.2 Diagrammatic representation of two compartment model assuming first order absorption



equation. This model assumes that there is a central compartment from which the drug distributes into a second compartment. The concentration-time profile for a drug exhibiting two compartment pharmacokinetic behaviour shows an initial decline in concentration which represents both distribution and elimination followed by a second slower decline.

The differential equations which describe this model are

$$dA_1/dt = k_aG - k_{12}A_1 - k_{10}A_1 + k_{21}A_2 \qquad \text{Equation 3.7}$$

$$dA_2/dt = k_{12}A_1 - k_{21}A_2 \qquad \text{Equation 3.8}$$

where A_1 and A_2 are the amounts of drug in compartments 1 and 2 respectively. The rate constants k_{10}, k_{12} and k_{21} represent the rate of elimination from the central compartment, the rate of transfer from the central compartment to the peripheral compartment and the rate of transfer from the peripheral compartment to the central compartment respectively. These equations are also solved by Laplace transformation (Equation 3.9).

$$C = \frac{k_aDF}{V_1}\left(\frac{(k_{21}-\alpha)}{(k_a-\alpha)(\beta-\alpha)}e^{-\alpha t} + \frac{(k_{21}-\beta)}{(k_a-\beta)(\alpha-\beta)}e^{-\beta t} + \frac{(k_{21}-k_a)}{(\alpha-k_a)(\beta-k_a)}e^{-k_a t}\right)$$

Equation 3.9.

where α and β are rate constants describing distribution and elimination and V_1 is the volume of the central compartment. The method of solving these equations by Laplace transformation is given in Appendix 2.

3.3 STATISTICAL TECHNIQUES

3.3.1 General comments

Appropriate statistical techniques were used to analyse the data. Calibration lines in Chapter 2 were calculated by simple linear regression. Standard t tests and analysis of variance were used, and these will be discussed in the relevant areas of the thesis.

3.3.2 Multiple Linear Regression

Simple linear regression assumes a linear relationship between one dependent variable and one independent variable (as in the relationship between drug concentration and peak area ratio in calibration lines described in Chapter 2). Multiple linear regression can be used to describe the relationship between a dependent variable and a number of independent variables and is used in this work to relate changes in pharmacokinetic parameters to several demographic and clinical variables. The multiple linear regression equation is

$$y = a + b_1X_1 + b_2X_2 + ... + b_nX_n \qquad \text{Equation 3.10}$$

where X_1 to X_n are the different independent variables, and a and b_1 - b_n are the parameters of the model. If more than one observation is made in each subject the observations can not be assumed to be independent and the multiple linear regression analysis must also test

for individual subject effects, ie individual intercept parameters (a_i) or individual slope parameters (b_i) in Equation 3.10.

Multiple linear regression is used to determine the parameters of a model and to test the influence of inclusion of various factors in the model. The significance of each factor on the dependent variable is tested by comparing the residual sum of squares obtained when the factor is present or absent. Adding in more parameters to the model will result in reduced residual sums of squares and an apparent improvement in the fit. The aim of multiple linear regression is to obtain the simplest model which adequately explains the variability in the data. Although inclusion of an additional variable may suggest that there is an apparent improvement in the fit, this improvement may not be statistically significant. Significance of adding more variables to the model is tested by using the General Linear Test (F ratio test) as follows

$$F = \frac{RSSQ_r - RSSQ_f \,/\, df_r - df_f}{RSSQ_f \,/\, df_f} \qquad \text{Equation 3.11}$$

r = reduced model

f = full model

df_r = degrees of freedom of reduced model

df_f = degrees of freedom of full model

RSSQ = residual sum of squares

Multiple linear regression can be carried out by

initially using every possible known parameter and testing for a significant reduction in the F value if a parameter is removed (backward stepping). Alternatively the simplest model can be used initially and the significance of adding in parameters one at a time tested (forward stepping). The latter method was used in this thesis.

The fraction of the total variability which is explained by the model is described by the coefficient of determination (R^2).

$$R^2 = 1 - \frac{RSSQ}{SSQ_{total}} \qquad \text{Equation 3.12}$$

The inclusion of further parameters in a model will almost always result in an increase in the value of R^2 as seen by a reduction in the residual sum of squares. It may therefore be helpful to use a stopping rule which takes into account the improvement in R^2 along with the number of parameters in the model. In this thesis, Adjusted R^2 has been used which is calculated as follows

$$\text{Adjusted } R^2 = \frac{(N-1)R^2 - P}{N - P - 1} \qquad \text{Equation 3.13}$$

where N = number of data points and P = number of variables.

3.3.3 Non-linear Regression

When using linear regression the relationship between the dependent and independent variables is

assumed to be linear. The dependent or independent variable may be transformed, eg to a logarithmic scale to obtain linearity. Typically, non-linear regression is used when concentration-time data are collected during the course of a pharmacokinetic study. There is no unique solution to the values of the parameters of the model. Such data are generally analysed by computer, where initial estimates of the parameters are required and these are progressively altered by an iterative procedure until the best set of parameters is obtained. This is the point at which the difference between two successive objective values is less than a pre-defined limit. The best set of parameters is that where minimisation of the objective function occurs.

The simplest objective function is Ordinary Least Squares (OLS) shown in Equation 3.14.

$$\text{Obj (OLS)} = \sum_{i=1}^{n}(c_i - \hat{c}_i)^2 \qquad \text{Equation 3.14}$$

where c_i is the measured concentration and $\hat{c}_i$ is the concentration predicted by the model and its parameters. OLS assumes that the error on each measurement is similar. An alternative is to use Weighted Least Squares (WLS) which weights each observation according to the associated variance of the measurement such that more weight is placed on those points about which there is greater confidence. OLS and WLS are limited by the need to make assumptions about the error on each measurement in advance. An alternative weighting scheme

is extended least squares (ELS) (Sheiner, 1983) which estimates the parameters of the model and also the parameters of a variance model. The objective function used in ELS is shown in Equation 3.15

$$\text{Obj (ELS)} = \sum_{i=1}^{n}(c_i - \hat{c}_i)^2 / v_i + \ln v_i \qquad \text{Equation 3.15}$$

where c_i is an observation which represents the true value, $\hat{c}_i$ and v_i is the variance. The optimal set of parameters is the one which minimises the objective function as before.

The computer program NONMEM (Non-linear Mixed Effects Model) (Beal and Sheiner, 1979) was originally devised to analyse data where only a few measurements are available per subject, but from a large number of subjects. The program uses an extended least squares fitting procedure and estimates the parameters of the chosen pharmacokinetic model and also the parameters of an appropriate variance model which describes the inter- and intra-subject variability of the model.

In the present work NONMEM is used to examine relationships between pharmacokinetic parameters which have already been determined and certain independent variables of interest, such as creatinine concentration or liver function. NONMEM allows both linear and non-linear relationships to be explored whereas multiple linear regression described above assumes a linear relationship between the dependent and independent

variables.

Three input files are required to run NONMEM. These are (i) the data file which contains the data and the independent variables (or covariates), (ii) the PRED file which is a FORTRAN subroutine and defines the structural and variance models and (iii) the control file which contains information on the organisation of data in the data file, the starting values (and upper and lower limits) of the parameters and instructions on the presentation of results, tables and graphs. PRED files used in this thesis are shown in Appendix 3.

The parameters of the structural model, (θ_{ki}) for an individual are represented by the population mean, $\bar{\theta}_k$ plus the deviation from the mean which is relevant to the individual η_{ki} (where η_{ki} represents inter-subject variability), thus

$$\theta_{ki} = \bar{\theta}_k + \eta_{ki} \qquad \text{Equation 3.16}$$

Values of η_{ki} are assumed to be normally distributed with mean of 0 and variance of σ_k^2. Alternatively it can be assumed that the inter-subject variability is proportional to the value of θ which avoids the possibility of 0 or negative parameter values and is therefore more realistic in a physiological setting ie.

$$\ln(\theta_{ki}) = \ln(\bar{\theta}_k) + \eta_{ki} \qquad \text{Equation 3.17}$$

In the PRED files (Appendix 3), the parameters (THETA 1, THETA 2 etc) are defined. The structural model is

the F function. The G functions define the statistical nature of the inter-subject variability and are the first derivatives of the function with respect to each ETA. The H function defines the statistical nature of the intra-subject variability (ϵ_{ij}). The value of ϵ_{ij} is assumed to be normally distributed with a mean of 0 and variance σ^2_ϵ and assumes a constant error model ie no covariance is allowed between η_k and ϵ.

Comparison of Models

Comparison of NONMEM models is based on the objective function. Hierarchical models can be compared using a chi-squared test with degrees of freedom equal to the difference in the number of parameters. For non-hierarchical models, (eg in Section 5.2, where all models have the same number of parameters) model comparison is based on the objective function, on the variances associated with each parameter, and on examination of residual plots.

3.4 BAYESIAN ESTIMATION

In a clinical setting, a blood, plasma or serum sample is often collected from an individual patient for the measurement of a drug concentration, and dosage adjustments made on the basis of that measurement. In order that accurate interpretation of the results may be made, steady state conditions must have been attained

(ie the patient must have been receiving the same dose for at least 5 half lives of the drug). The consequence of any change in dose cannot be assessed pharmacokinetically until the patient is again at steady state. It is likely that in an acute care hospital ward the dose of a drug will be frequently altered, based on clinical requirements and in such situations it is difficult to interpret a single measured concentration. It would be possible to estimate pharmacokinetic parameters for an individual patient using non-linear regression, but this would require collection of many blood samples and is largely impractical.

An alternative is to employ Bayesian estimation. This technique is based on Bayes' Theorem (Wonnacott and Wonnacott, 1977) and the principle of Maximum Likelihood Estimation (Edwards, 1976). Bayesian estimation uses measured concentrations in combination with previously determined population pharmacokinetic parameters and their variances. Revised individual patient parameters are then obtained (Sheiner and Beal, 1982). Bayesian estimation therefore uses both measured concentrations and population pharmacokinetic information to estimate the most likely set of pharmacokinetic parameters in an individual patient. The background to Bayesian estimation is outlined below (Sheiner and Beal, 1982).

A concentration measurement (c_j) is a function of the parameters of the model used (θ_k), independent variables including time (t) and dose (D) and an error

term ϵ_j, which accounts for assay error, model misspecification and random intra-subject variability. This can be expressed as follows

$$c_j = f(\theta_k, D, t) + \epsilon_j \qquad \text{Equation 3.18}$$

The error is assumed to be normally distributed with mean 0 and variance σ_j^2 which is known.

The population parameter estimates ($\bar{\theta}_k$) are obtained from a population study of the particular drug or from published literature. Each individual parameter estimate (θ_k) is defined as the population parameter ($\bar{\theta}_k$) plus a random error (η_k). The random error is assumed to be normally distributed with a mean of 0 and variance equal to the population variance.

The Bayesian procedure minimises the following objective function (Equation 3.19).

$$Obj = \sum_{j=1}^{m} \frac{(c_j - \hat{c}_j)^2}{\sigma_j^2} + \sum_{k=1}^{n} \frac{(\theta_k - \bar{\theta}_k)^2}{\sigma_k^2} \qquad \text{Equation 3.19}$$

where σ_k^2, σ_j^2 and $\bar{\theta}_k$ are pre-defined. The objective function has two components; the first part attempts to minimise the squared errors between observed concentrations and predicted concentrations while the second part of the equation minimises the squared errors between population parameters and revised parameters. Minimisation of this function produces a revised set of parameters and variances which are the most likely set

for that individual patient. When there are no concentration measurements the parameters are those of the population. As concentration measurements become available the population parameter estimates become less important. A time factor is also used which gives less weight to more distant concentrations. This time factor is 15% of the concentration multiplied by 1.01^T where T is time elapsed since the measurement (Peck et al, 1980) i.e.

$$\sigma_j = 1.01^T \times 0.15c_j \qquad \text{Equation 3.20}$$

The use of a Bayesian estimation program has been evaluated for theophylline (Sheiner and Beal, 1982) and digoxin (Whiting et al, 1984a). A package of computer programs which is based on Bayesian theory has been written (Kelman et al, 1982). This package has been adapted for the studies in this thesis. A one compartment model with first order absorption for an orally administered drug is used and estimates k_a, Cl and V. A two compartment version estimates k_a, Cl, V_1, α and β. The program assumes that there is no consistent or underlying change in the parameters throughout the period of drug monitoring. Both the one and two compartment versions of the Bayesian program are evaluated in Chapter 4.

During the course of this work a further version of the Bayesian program was developed. This version allows for a consistent change in pharmacokinetics by allowing

a gradual decrease in the ratio of Cl/F. This version requires that the weighting function above (Equation 3.20) is not used and that equal weight is placed on all concentrations independent of time. The way in which this version of the program works will be described in Chapter 6.

CHAPTER 4

PHARMACOKINETICS OF CYCLOSPORIN IN THE FIRST TWO MONTHS FOLLOWING RENAL TRANSPLANTATION

4.1 INTRODUCTION

This study was designed to examine the pharmacokinetics of cyclosporin in renal transplant patients and to quantify inter- and intra-patient variability. Pharmacokinetic parameters were estimated by a standard Bayesian approach (Section 3.4) and validation of this method is reported.

4.2 PHARMACOKINETIC STUDY

4.2.1 Patients

Twelve consecutive renal transplant recipients were recruited. One patient suffered post-operative complications and was nephrectomized three days after transplantation. The other eleven were studied for sixty days after transplantation. Cyclosporin and low dose steroids were the only regular immunosuppressant drugs given although high dose steroids were occasionally required to treat acute rejection episodes. Patients were excluded from the study if cyclosporin had been administered in a previous transplant. Clinical details of the patients who completed the study are shown in Table 4.1.

4.2.2 Data collection

The demographic and clinical data listed below were recorded for each patient.

(i) Initially age, height, and reason for transplant

Table 4.1 Clinical details of renal transplant patients completing pharmacokinetic study

Patient	Age	Sex	Weight(kg)	Reason for transplant
1	50	M	92	Diabetic nephropathy
2	30	M	62	Chronic pyelonephritis
3	46	F	47	Chronic renal failure
4	55	F	80	Chronic renal failure
5	17	M	57	Chronic renal failure
6	22	M	52	Henoch-schonlein pupura
7	52	M	72	Glomerulonephritis
8	29	M	64	Obstructive uropathy
9	26	M	80	Diabetic nephropathy
10	32	M	80	Glomerulonephritis
11	28	M	56	Obstructive uropathy

were recorded for each patient.

(ii) Throughout the study the following data were recorded for each patient; serum creatinine, total bilirubin, alanine amino transferase, albumin, cholesterol, haematocrit and haemoglobin. In addition periods of vomiting and diarrhoea were noted and patient weight, blood pressure and concurrent drug therapy were recorded over the sixty days of the study.

4.2.3 Cyclosporin therapy

Prior to transplantation each patient received an oral dose of cyclosporin (15mg/kg). The day of transplantation was designated day 0 and following surgery 5mg/kg cyclosporin were administered intravenously over six hours. All subsequent therapy was given orally with the exception of patient 9 on day 10 who received intravenous cyclosporin when he was unable to tolerate oral therapy. Patients remained in hospital for between six and twenty five days after the transplant (mean = 14 days). During this period blood samples for cyclosporin assay were withdrawn immediately prior to each dose (trough) and at least one other sample was collected per dosage interval. Following discharge from hospital, patients attended the renal transplant out-patient clinic, initially daily, then every second day, until towards the end of the study, patients attended only once a week. A trough blood

sample was withdrawn at each visit. Figure 4.1 shows a typical cyclosporin dosing and sampling schedule in one patient. Blood samples were collected in 5ml EDTA tubes and stored at -20^{o}C prior to analysis. An average of fifty samples was collected from each patient. All blood samples were assayed for cyclosporin by the HPLC method described in Chapter 2. Although this assay was time consuming and not generally considered suitable for routine analysis of clinical samples, it was the analytical method of choice for pharmacokinetic studies because of its specificity. Any change in dose in these patients was made on the basis of a non-specific radio-immunoassay which measured both cyclosporin and some cross reacting metabolites.

4.2.4 Data analysis

Using Bayesian estimation (Section 3.4) two pharmacokinetic models were investigated - a one and a two compartment model. The parameter starting values (priors) were obtained from a review of previously published data on cyclosporin (Ptachcinski et al, 1986). Population standard deviations were those recommended by Peck et al (1980) with the exception of volume where the standard deviation was set at 90% (since little information on volume of distribution was available). The starting values and their standard deviations for both models are listed in Table 4.2. The standard

Figure 4.1 Typical cyclosporin dosing and sampling schedule in one patient

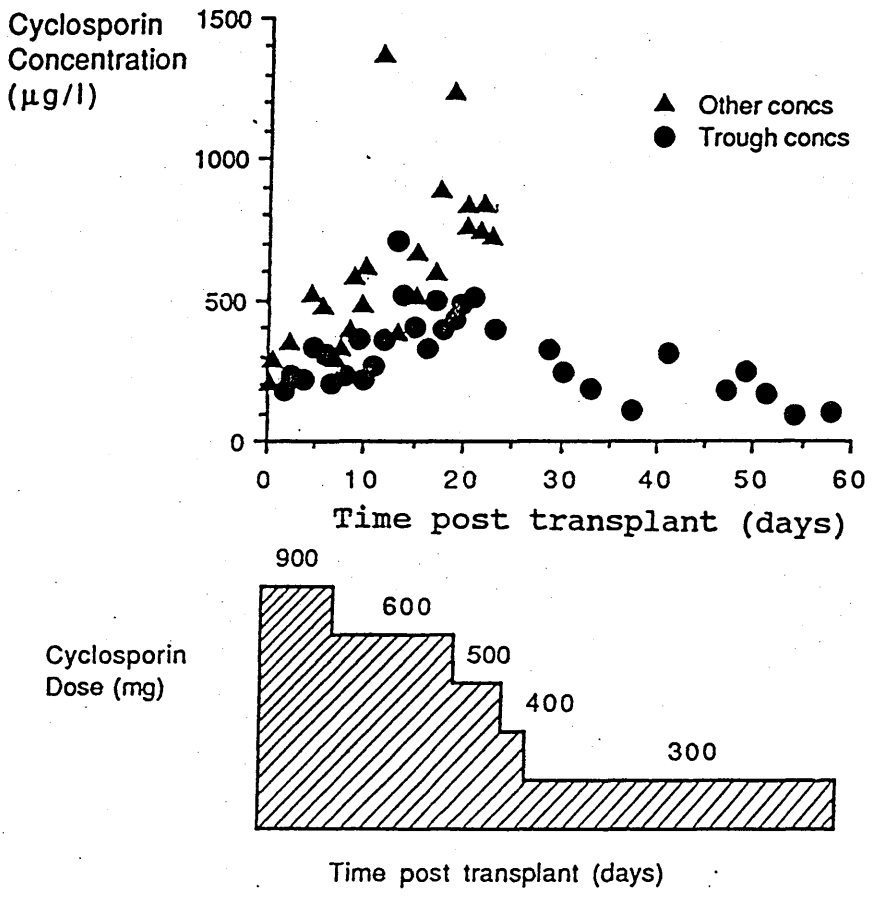


Table 4.2 Estimates of pharmacokinetic parameters (sd)%used in Bayesian estimation

Parameter	1 compartment model	2 compartment model
ka	$0.6h^{-1}$ (50)	$0.6h^{-1}$ (50)
Cl	0.34l/h/kg (50)	0.34l/h/kg (50)
V	4.5l/kg (90)	*0.14l/kg (90)
α		$0.7h^{-1}$ (50)
β		$0.06h^{-1}$ (50)

* V_1 - volume of central compartment

deviation of each concentration was set at 15% of the measured concentration to account for measurement error and model misspecification.

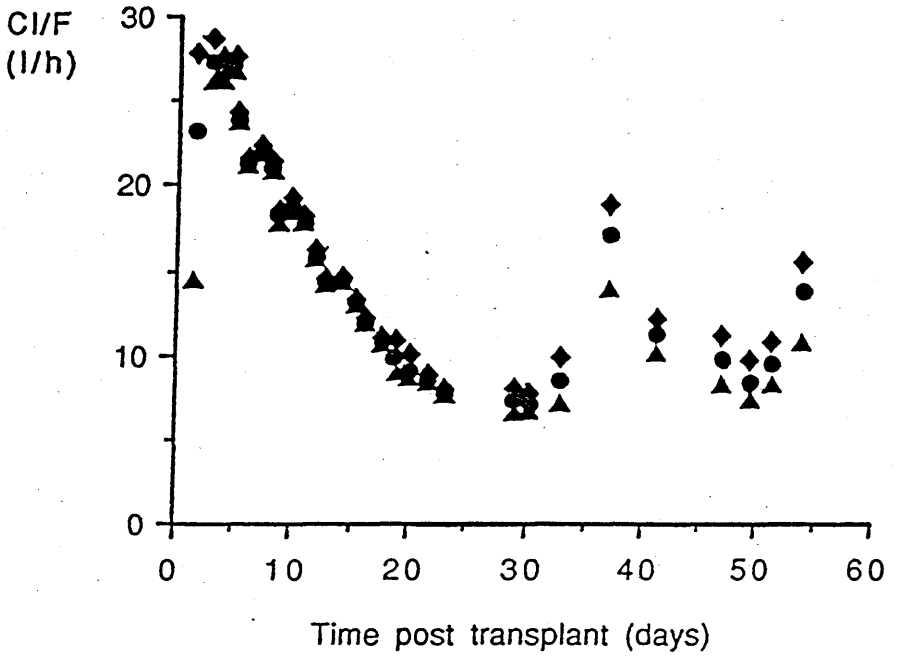
Bayesian parameters were estimated sequentially by first using concentration-time data from day 1 only, then days 1 and 2, days 1, 2 and 3, and so on until days 1 to 60 were used. In this way an average of 33 sets of Bayesian parameters were obtained from each patient. This series of parameter estimates was obtained with the standard program where progressively less weight was assigned to more and more distant measurements. The estimates therefore were biased by the most recent measurements.

4.3 VALIDATION OF BAYESIAN ESTIMATION

The Bayesian method of parameter estimation is initially dependent on the starting values, especially when few concentration measurements are available. The influence of the starting value of clearance was examined by altering the prior estimate by +/- 50%. This was carried out in two patients and the results of this are shown graphically in Figures 4.2 (i) - (ii). The revised estimate on day 1 post-transplant was highly dependent on the prior estimate, due to lack of information from concentration measurements. By day 2 it was seen that the prior estimate was relatively unimportant. More importantly, the same reduction in revised estimates of Cl/F was seen regardless of the

Figure 4.2 Effect on revised estimates when changing the prior estimate of Cl/F by ±50%

(i) Patient 2



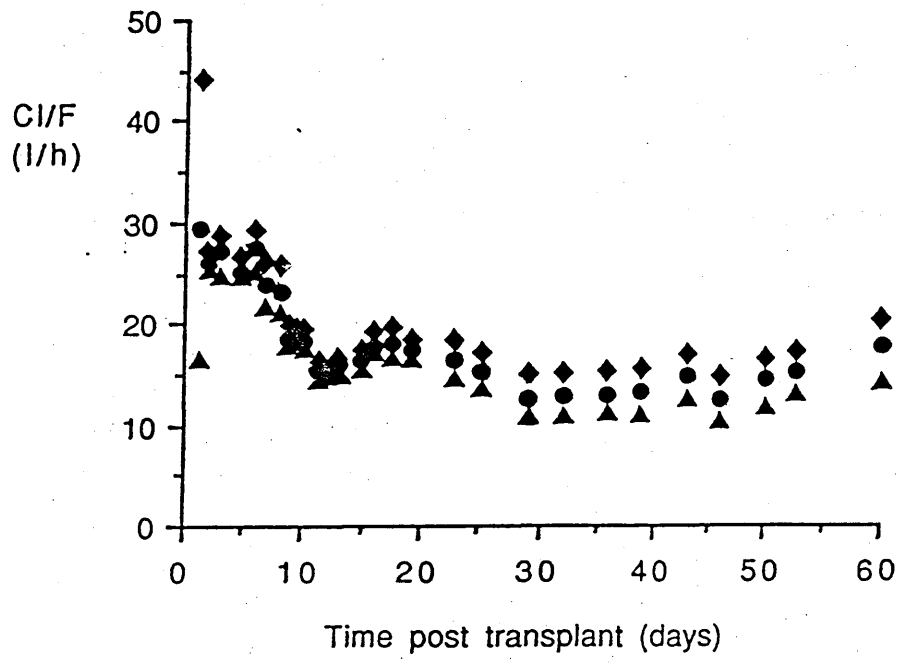
● prior estimate of Cl/F used in data analysis

◆ + 50% of prior estimate

▲ -50% of prior estimate

Figure 4.2 Effect on revised estimates when changing the prior estimate of Cl/F by ±50%

(ii) Patient 4



● prior estimate of Cl/F used in data analysis

◆ +50% of prior estimate

▲ -50% of prior estimate

prior estimate.

A second validation procedure was carried out to explore the influence of the number of concentration measurements in each dosing interval on the pattern of parameter estimates. It was thought that the relatively early rapid accumulation of data might bias later parameter estimates. In the early (inpatient) stage of the study, two or three samples (including a trough sample) were collected daily. At later stages, only trough samples were collected and it was postulated that this might introduce bias. Using trough sample measurements only, the data were divided into 5 day units; 1-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60. Bayesian parameters were obtained using these data and compared to those obtained using all data sequentially. This study was carried out in the same two patients as above. Estimates of Cl/F are shown graphically in Figure 4.3 (i)-(ii). It was seen that dividing the data into 5 day units and using trough samples only did not have an effect on the revised estimates and again the same reduction in clearance estimates was seen.

4.4 RESULTS

4.4.1 Relationship between dose and measured cyclosporin concentrations

Table 4.3 shows cyclosporin doses and trough

Figure 4.3 Effect on revised estimates of Cl/F when using only trough concentrations from a maximum of five days

(i) Patient 2

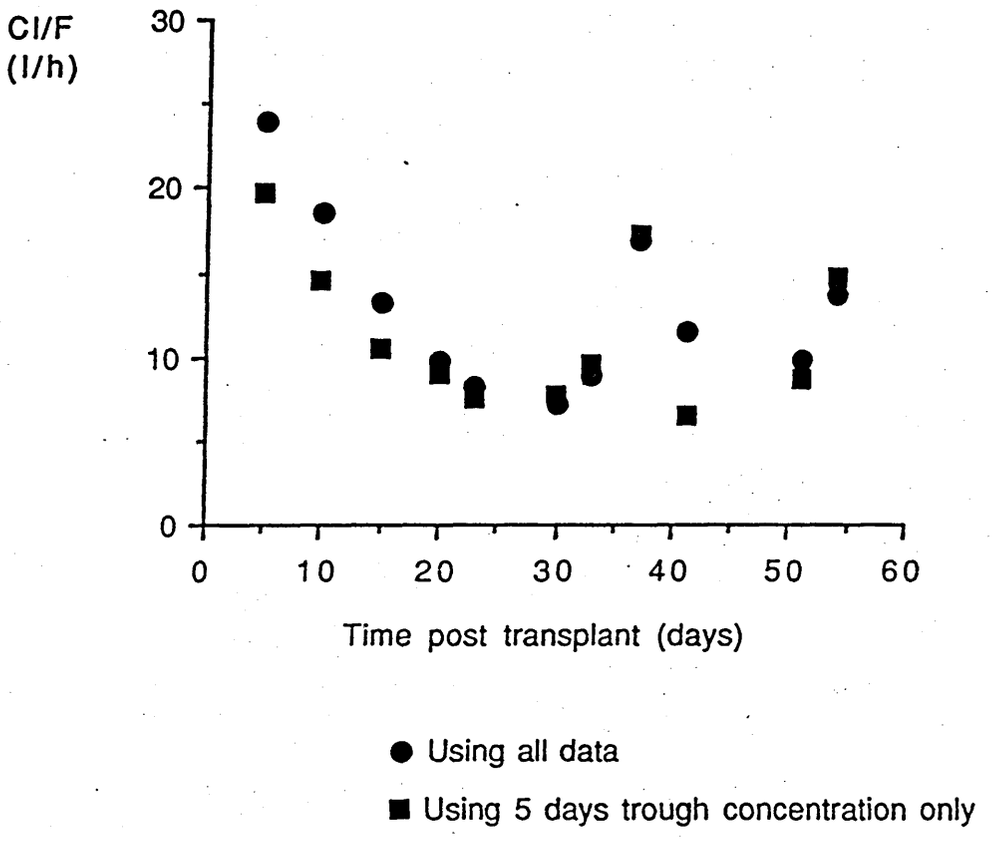


Figure 4.3 Effect on revised estimates of Cl/F when using only trough concentrations from a maximum of five days

(ii) Patient 4

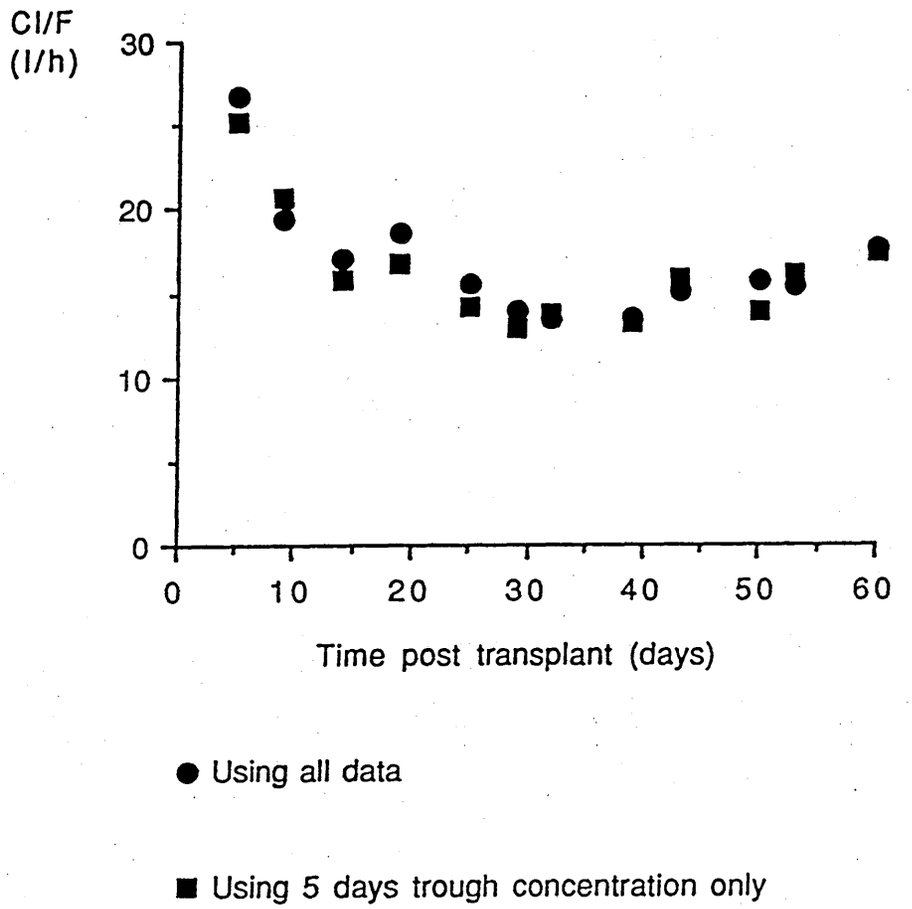


Table 4.3 Measured cyclosporin trough concentrations in eleven renal transplant patients 5 days post-operatively

Patient	Dose (mg/kg)	Dose (mg)	Conc (ug/l)	Conc/dose (ug/l/mg)
1	15	1350	168	0.124
2	15	900	318	0.353
3	15	700	154	0.220
4	15	1150	239	0.208
5	15	850	183	0.215
6	15	750	112	0.149
7	15	1200	409	0.341
8	15	800	51	0.064
9	15	1100	372	0.338
10	15	1200	73	0.061
11	15	850	27	0.032
Mean			191	0.191
SD			129	0.117

concentrations five days after transplant in each of the eleven patients. The mean cyclosporin concentration (SD) was 191 (129)ug/l. When the concentration was adjusted for the actual dose prescribed the mean value (SD) was 0.191 (0.117)ug/l/mg. Figure 4.4 shows graphically the lack of relationship between prescribed dose and resultant trough concentration of cyclosporin. This preliminary analysis demonstrates the poor relationship between dose and measured concentration, and the presence of wide inter-subject variability.

4.4.2 Prediction error analysis

For each set of Bayesian parameter estimates, later concentrations were predicted. The difference between the predicted and observed concentrations (prediction error) was calculated for each patient at various time points throughout the study. Concentration time data from the first five days were used to predict concentrations at days 10, 30 and 60. Ten days' data were used to predict concentrations at days 30 and 60 and 30 days' data to predict to day 60 (Table 4.4). This analysis was carried out for the one and two compartment models.

The prediction error (pe) for each patient at each of the above time points was calculated. The mean prediction error at each of the time points studied was calculated and was compared to zero using the one sample t-test ($p<0.01$). The results of this analysis are shown

Figure 4.4 Cyclosporin doses and trough concentration measurements in eleven renal transplant patients five days post-operatively. All patients had received the same oral dose for four days.

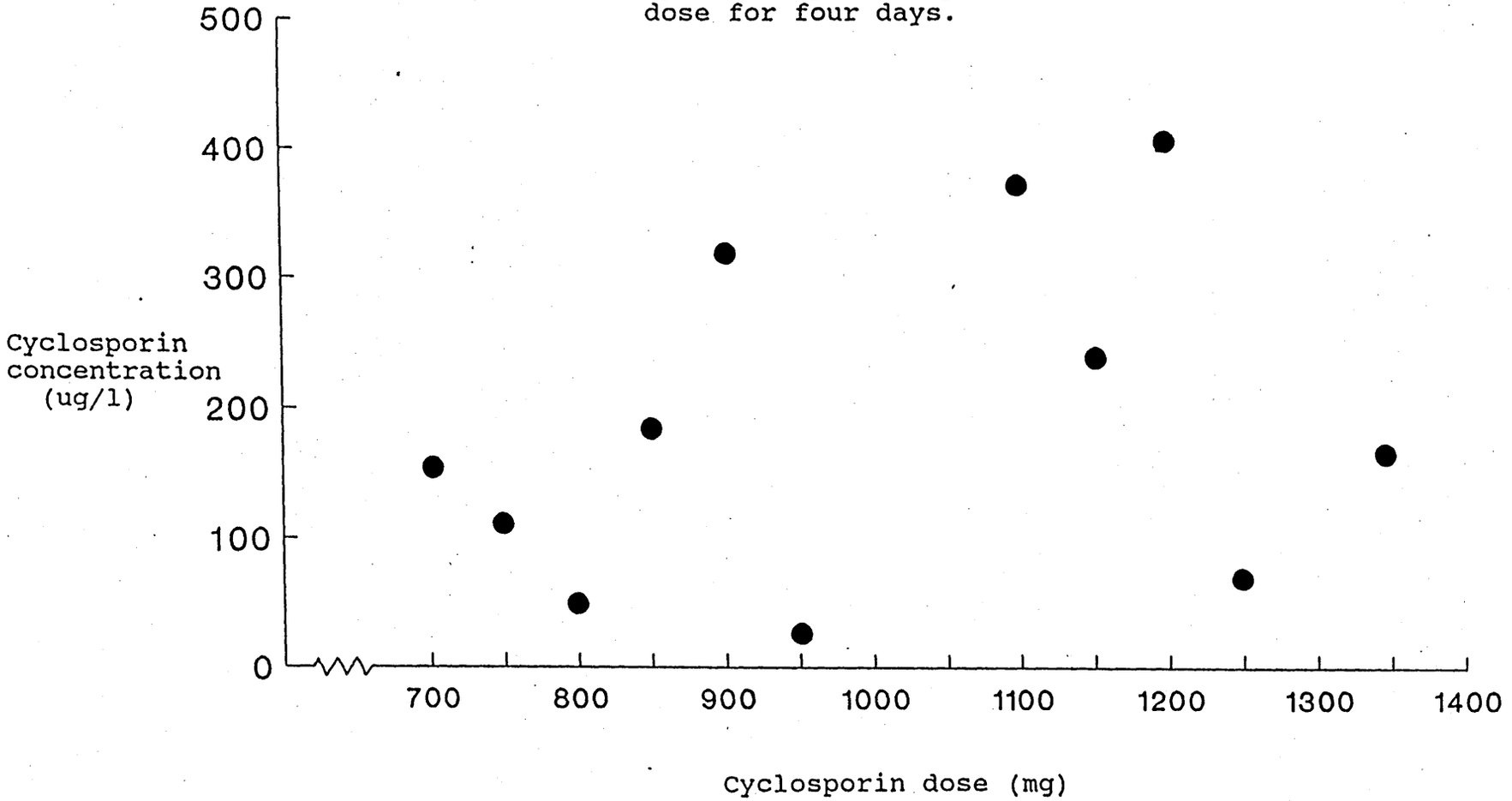


Table 4.4 Data used in calculation of prediction errors

Number of days data used in prediction	Concentrations predicted at days		
5	10	30	60
10		30	60
30			60

in Table 4.5 and in Figure 4.5. It can be seen that a statistically significant bias in prediction was observed at all time points except when 30 days data were used to predict concentrations at day 60.

4.4.3 Pharmacokinetic analysis

Measured cyclosporin concentrations at later time points were consistently higher than those predicted from Bayesian parameter estimates at early time points using either a one or a two compartment model. It was not surprising that the one compartment model produced poor predictions since cyclosporin is known to exhibit multi-compartment distribution characteristics (Ptachcinski et al, 1987a). In addition Kahan et al, (1986b) have reported that a two compartment version of a Bayesian program produced good revised estimates of pharmacokinetic parameters in renal transplant patients. Likewise, the Bayesian approach has been shown to be useful in bone marrow transplant patients assuming no changes occur in the pharmacokinetic parameters (Mentre et al, 1988). Estimated values of Cl/F are shown in Figure 4.6 for the one and two compartment models at various time points throughout the study. Using the paired t test there was no significant difference between the estimates obtained from the two models.

The pharmacokinetic parameters associated with the two compartment model were studied in detail. The

Table 4.5 Results of prediction error analysis

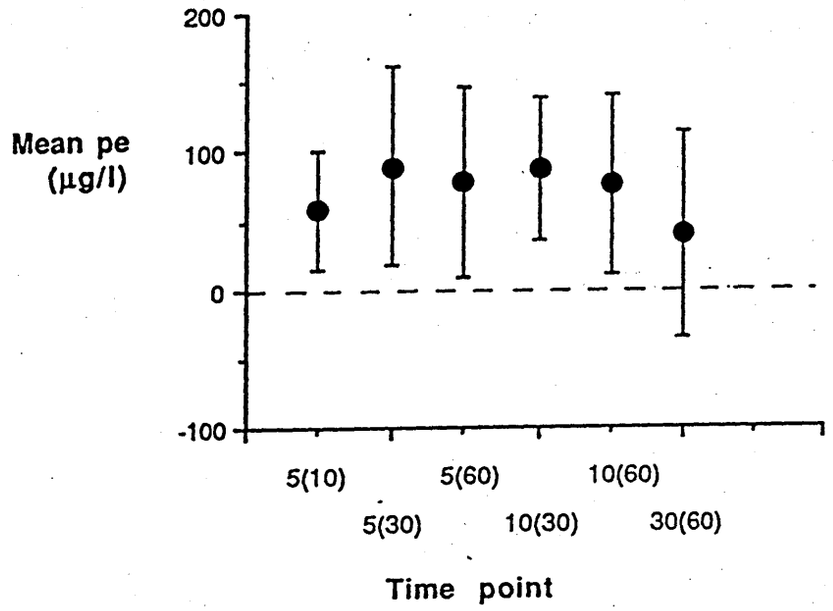
Time point	One compartment mean (sd)	Two compartment mean (sd)
5(10)	*57.1 (42.8)	*51.1 (38.1)
5(30)	*89.7 (71.6)	*80.7 (59.1)
5(60)	*78.3 (68.8)	*71.5 (73.8)
10(30)	*87.1 (51.6)	*72.3 (43.9)
10(60)	*76.0 (65.6)	*65.5 (63.9)
30(60)	39.1 (74.1)	14.8 (78.4)

* denotes significant bias ($p<0.01$)

Prediction error = observed - predicted concentration

Fig. 4.5 Mean pe (± sd) at time points investigated

(i) One compartment model



(ii) Two compartment model

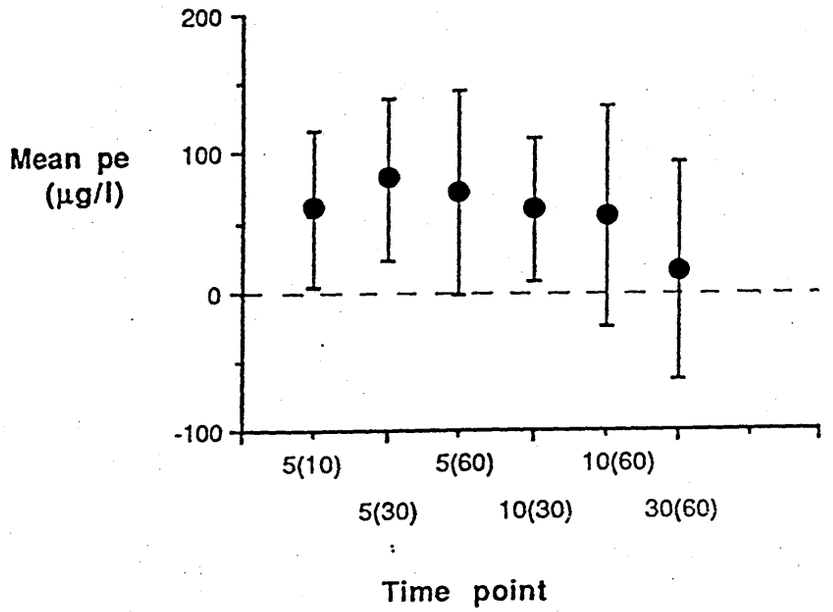
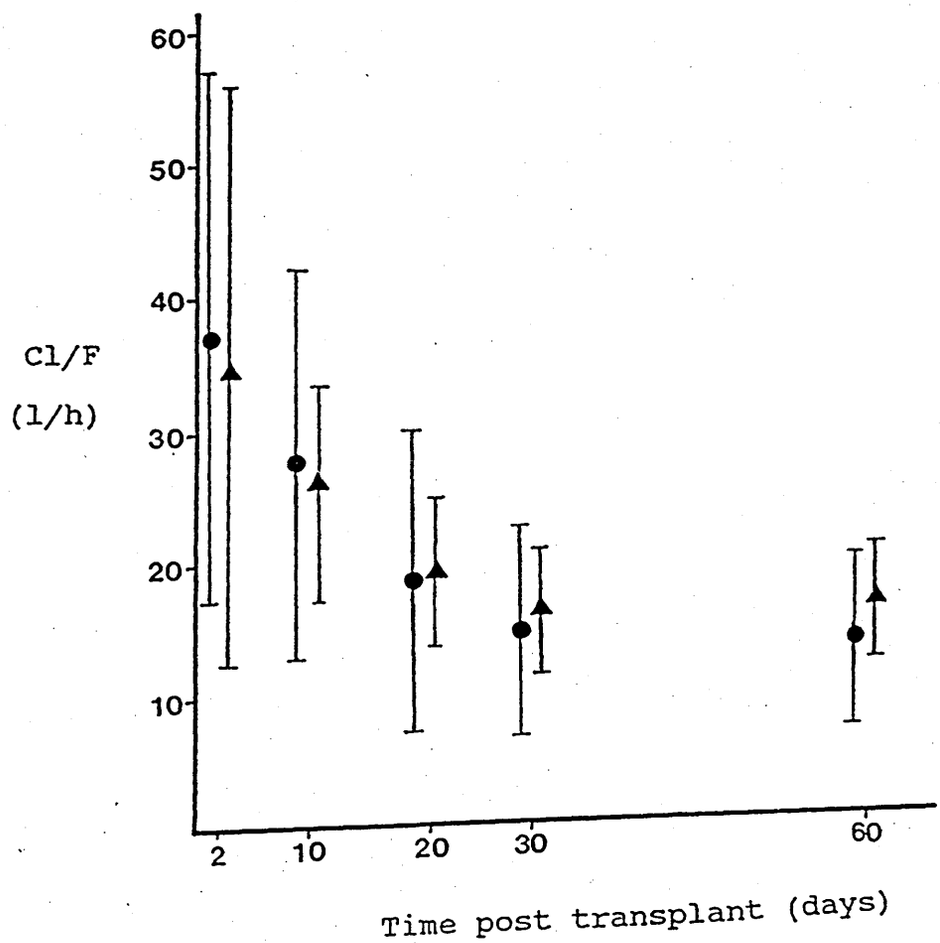


Figure 4.6 Mean estimates of Cl/F (sd) at various time points, using either a one or a two compartment model



● One compartment model

▲ Two compartment model

absorption rate constant (k_a), and the distribution and elimination rate constants α and β were not expected to change much from their prior estimates because the relatively high number of trough concentrations outweighed the information from other samples. Indeed, the evaluation of a Bayesian method by Kahan et al, (1986b) involved fixing these three parameters at constant values. Clearance (Cl) and volume of the central compartment (V_1) did, however, change and Figures 4.7 and 4.8 show the mean estimates (sd) in eleven patients over the time of the study. The Bayesian program did, however, have a fixed value for bioavailability (F) set at 25%, and these graphs (Figures 4.7 and 4.8) actually represent changes in Cl/F and V_1/F respectively. The observed downward trend over time could therefore be due to a decrease in Cl and V_1, an increase in F or a combination of all three.

The Bayesian estimates of Cl/F and V_1/F showed considerable inter-patient variability at any time point. The mean (SD) estimate of Cl/F on day 2 post-transplant was 34.4 (21.6) l/h which decreased to 15.7 (4.2) l/h by day 60. Similarly, V_1/F on day 2 was 137 (128)l which reduced to 44 (13.5)l on day 60.

4.4.4 Relationship between cyclosporin pharmacokinetics and other factors

The biochemical, haematological and clinical data collected throughout the study for each patient (Section

Figure 4.7 Mean successive parameter values of Cl/F (±SD) in the first two months following renal transplantation

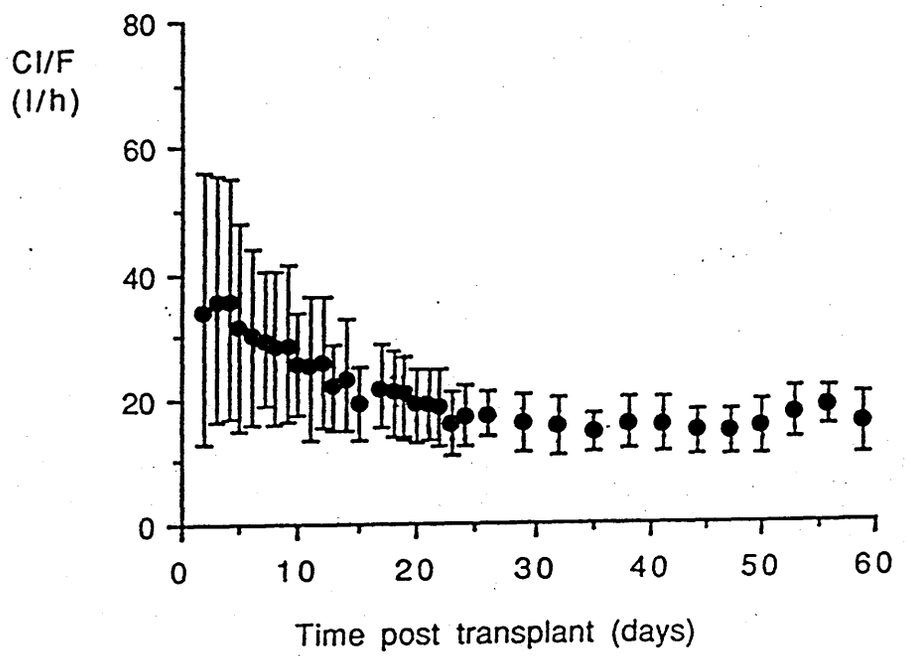
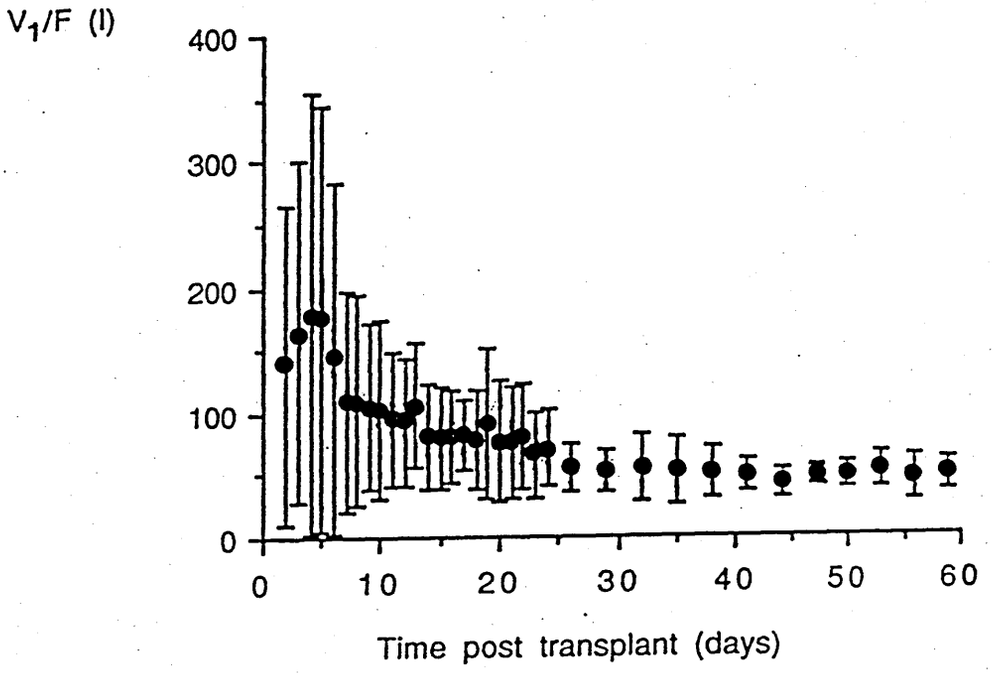


Figure 4.8 Mean successive parameter values of V_1/F (±SD) in the first two months following renal transplantation



4.2.2) were examined in an attempt to correlate changes in these factors with cyclosporin pharmacokinetics following renal transplantation.

(i) Concurrent drug therapy

Many drugs have been reported to interact with cyclosporin as discussed in Chapter 1. All patients in the study were on a variety of drugs. From Table 4.6, it can be seen that few of the patients were on concurrent drug therapy which might affect the pharmacokinetics of cyclosporin (see Section 1.5). Low dose prednisolone, which has been reported to alter cyclosporin pharmacokinetics (Klintmalm and Sawe, 1984; Ost et al, 1985; Ptachcinski et al, 1987) was administered to all patients for the duration of the study. There were not enough cases of administration of other drugs which might potentially affect cyclosporin pharmacokinetics, to provide information on their effect.

(ii) Gastrointestinal dysfunction

It has been established that changes in gastrointestinal function will affect the absorption of cyclosporin (Atkinson et al, 1984). Vomiting and diarrhoea will clearly decrease the amount of cyclosporin absorbed. Vomiting, however had little effect on cyclosporin pharmacokinetics in this study because patients suffering from nausea had the dose withheld for several hours until the nausea had subsided. Acute diarrhoea occurred more often, but as

Table 4.6 Concomitant medication administered to patients in the pharmacokinetic study

Patient	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	1-60	7-16 22-31					4-7	15-21				1	15-60						1-60
2	1-60				16-60		8-15							1-60					1-60
3	1-60	8-17	7-60	18-60	26-49										27-60				
4	1-60		3-4		15-60					1-3	3-6		1-40						
5	1-60		15-60	31-60	31-60														
6	1-60	15-24 52-61		1-40	1	6-11										17-60			
7	1-60		34-37	12-60	17-21 47-48														
8	1-60	12-21		2-60													8-10		
9	1-60	5-14	1-60	22-48					13-21				37-60						
10	1-60	7-16 22-31	1-60																
11	1-60		9-60	1-14	1 3												25-30 19-25	11-16	

A=low dose prednisolone, B=high dose prednisolone, C=Nifedipine, D=Atenolol, E=Frusemide, F=Bendrofluazide, G=Ampicillin, H=Amoxycillin, I=Acyclovir, J=Cefotaxime, K=Cephradine, L=Metoclopramide, M=Temazepam, N=Ranitidine, O=Gaviscon, P=Potassium, Q=Sodium bicarbonate, R=Calcium resonium, S=Insulin.

with data on concurrent drug therapy, there were not enough cases of gastro-intestinal dysfunction in this study to provide conclusive information.

(iii) Biochemical and haematological measurements

It is possible that one or more of the biochemical and haematological factors measured could help to explain the variability in cyclosporin pharmacokinetics. This is investigated fully in Chapter 5.

4.5 DISCUSSION

Work described in this chapter has confirmed previous reports of wide pharmacokinetic variability of cyclosporin both within and between subjects (Kahan et al, 1983; Newburger and Kahan, 1983). It has also been demonstrated that the use of a standard one or two compartment model in a Bayesian parameter estimation system results in biased cyclosporin predictions in the early period following renal transplantation. After approximately one month (day 30), it is possible to make unbiased predictions although precision is still poor. The biased predictions appear to be the result of a change in the pharmacokinetics of cyclosporin within patients, as is shown by graphs of mean (SD) Cl/F and V_1/F over time.

From these data, it is impossible to differentiate F from Cl and V_1. An increase in the absorption of cyclosporin following renal transplantation has been

observed (Kahan et al, 1983; Odlind et al, 1986; Wilms et al, 1988). Alternatively, the biased prediction errors may be a result of increased elimination half life with chronic cyclosporin therapy (Habucky et al, 1988) or a combination of both improved absorption and decreased elimination (Newburger and Kahan, 1983).

The finding of a consistent change in cyclosporin pharmacokinetics over time in the early post-operative period is not unexpected. It is known that the dose of cyclosporin can be gradually reduced during the first year after transplantation (Tufveson et al, 1986). It is likely that changes in patients' physiology in the transition from renal failure to the normal state, may result in a time-related change in one or more of the pharmacokinetic parameters (Venkataramanan et al, 1989).

Various theories have been proposed to explain the changes occurring. Absorption of cyclosporin may undergo a time-dependent change due to factors such as improved gastro-intestinal motility or improved bile flow as time progresses, or due to the presence of post-operative ileus in the early stages. Olive oil (the vehicle for oral cyclosporin) can induce bile production (Guarini and Ferrari, 1985). Since the presence of bile is necessary for the absorption of cyclosporin (Ericzon et al, 1987) it is possible that chronic therapy with either cyclosporin or its vehicle, may improve cyclosporin absorption. Habucky et al (1988) were not

able to show any difference in maximum concentration or in time to achieve peak blood concentration after chronic dosing with either olive oil or cyclosporin. They did however, show an increase in elimination half life.

An alternative theory is based on cyclosporin's ability to inhibit iso-enzymes of the cytochrome P450 system (Moochhala and Renton, 1986) leading to the hypothesis that during chronic therapy, cyclosporin inhibits its own metabolism, thus accounting for the decline in Cl/F.

Various groups have suggested that absorption of cyclosporin is dose-dependent, and as dose is decreased over time post-operatively, a higher proportion of the drug is absorbed (Ptachcinski et al, 1985a; Grevel, 1988), possibly due to limited solubility of the drug in the gastro-intestinal tract (Reymond et al, 1988). However, Wilms et al (1988) showed an increase in F at two time points using the same dose on each study day. Since all patients in this study were initially receiving high doses of cyclosporin which were gradually tapered, the possibility of a dose effect can not be excluded. This is examined in Chapter 5.

Variable binding of cyclosporin to lipoproteins and erythrocytes may explain the results of this chapter. Variability in the lipoprotein profile or haematocrit may contribute to inter-patient variability (Morse et al, 1988). In this study, such changes occur within

patients and may contribute to the intra-patient variability. Changes in cholesterol concentration and in haematocrit will be studied in Chapter 5, along with other biochemical and haematological measurements.

In conclusion, changes in pharmacokinetics of cyclosporin in the early post-operative period have been observed. These changes prevent the use of a standard Bayesian program to accurately predict cyclosporin concentrations. Various theories have been proposed to explain the gradual change in pharmacokinetics, due either to an increase in F and/or a decrease in Cl and V_1. Such changes must be taken into account in any dosage adjustment procedure. The mechanism responsible for the change is still open to speculation and the following chapter will attempt to model this change in pharmacokinetics.

CHAPTER 5

FACTORS INFLUENCING CYCLOSPORIN PHARMACOKINETICS IN RENAL TRANSPLANT PATIENTS

5.1 INTRODUCTION

The results presented in Chapter 4 suggest that the cause of biased prediction errors for cyclosporin is a change in the pharmacokinetics of cyclosporin in the initial post transplant period. Such a change is not entirely unexpected since transplant patients undergo dramatic changes in their physiology in the transition from a disease state (in this case the uraemic state) to a healthy state (Venkataramanan et al, 1989). It is possible that the gradual return to a "normal state" is responsible for the consistent change in pharmacokinetics. A change in pharmacokinetics over time following renal transplantation has been observed by several groups as discussed in Chapter 4. This chapter will discuss the background to the changing pharmacokinetics and will attempt to model this change.

5.2 BACKGROUND

It is possible that time can be used somewhat empirically to describe the changes in pharmacokinetics which occur within subjects after a renal transplant. However, in the transition from the uraemic state to the healthy state, various physiological changes may alter absorption, distribution and elimination of cyclosporin and a change in one or more biochemical or haematological factors may provide a useful index of the change in pharmacokinetics. These factors which may

affect cyclosporin pharmacokinetics are discussed in Chapter 1. The question to be addressed in this chapter is whether time is the most important factor influencing the pharmacokinetics of cyclosporin post transplant, or whether there is a relationship between Cl/F and some other factor. Other variables measured during the course of this study will be investigated in an attempt to identify some of the variability in Cl/F in the early post-operative period.

5.3 TIME DEPENDENT PHARMACOKINETICS

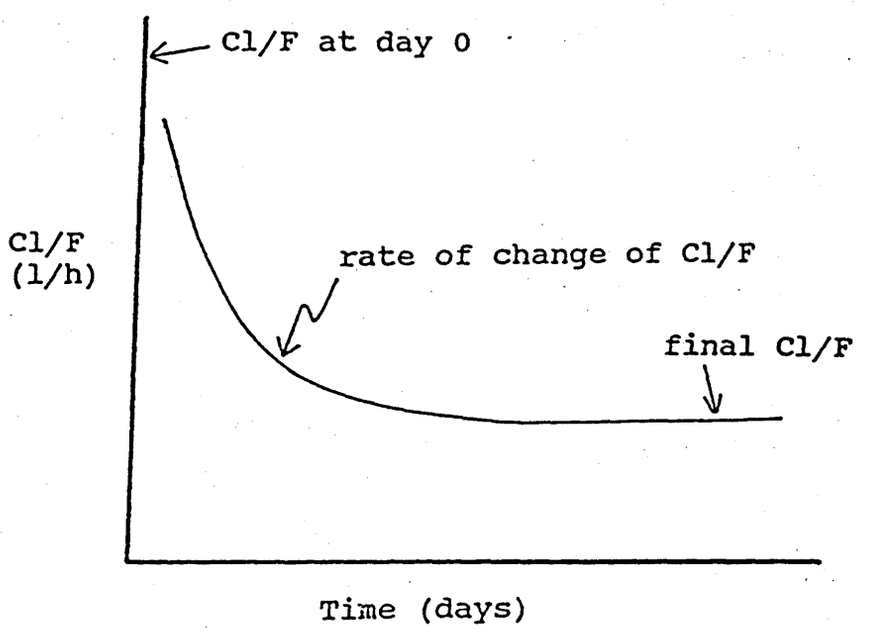
5.3.1 Data Analysis

Examination of the individual patient graphs of Cl/F versus time suggested that an exponential decline in Cl/F might satisfactorily explain the relationship. This can also be seen from the graph of mean values of Cl/F versus time shown in Figure 4.7. On the basis of these graphs, the following three monoexponential models were fitted to the data:

Model 1	$Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3$	Equation 5.1
Model 2	$Cl/F = \theta_1(1-\theta_2 + \theta_2 e^{-\theta_3 t})$	Equation 5.2
Model 3	$Cl/F = \theta_1 - \theta_3 + \theta_3 e^{-\theta_1\theta_2 t}$	Equation 5.3

These models are shown in Figure 5.1. Model 1 assumed that Cl/F declined monoexponentially from an initial value of $\theta_1+\theta_3$ at a rate specified by θ_2 until it reached a baseline value (θ_3). Model 2 assumed that

Figure 5.1 Monoexponential models investigated to describe the change in Cl/F over time



Model 1 $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3$

Model 2 $Cl/F = \theta_1 (1 - \theta_2 + \theta_2 e^{-\theta_3 t})$

Model 3 $Cl/F = \theta_1 - \theta_3 + \theta_3 e^{-\theta_1 \theta_2 t}$

MODEL	1	2	3
Initial Cl/F	$\theta_1 + \theta_3$	θ_1	θ_1
Rate of change	θ_2	θ_3	$\theta_1 \theta_2$
Final Cl/F	θ_3	$\theta_1 - \theta_1 \theta_2$	$\theta_1 - \theta_3$

there was a relationship between the initial value of Cl/F (θ_1) and the total extent of the change in Cl/F ($\theta_1\theta_2$) ie θ_2 was the fractional change in Cl/F. Model 3 assumed a relationship between the starting value of Cl/F (θ_1) and the rate of change in Cl/F ($\theta_1\theta_2$) ie the rate of change was proportional to the initial value.

These three models were investigated using the computer program NONMEM (described in Chapter 3) which allowed estimation of the parameters of each model and simultaneously estimated the variances associated with each parameter. All models were fitted using both additive and proportional errors to estimate the inter-patient variability. The models were non-hierarchical and were compared by examination of the log-likelihood function (the objective function), and by examining the residual plots for abnormal trends. The variances associated with each parameter were also compared.

5.3.2 Results

The results for each model investigated are shown in Tables 5.1 (i) to (vi). The estimated parameters and the objective functions were almost identical, regardless of whether additive or proportional errors were used to estimate the inter-subject variability. Model 1 had the lowest objective function of the three (1255.5 compared with 1274.8 and 1297.2 for Models 2 and 3 respectively). It can be seen that the variances

Table 5.1 NONMEM parameter estimates obtained for three monoexponential models

(i) Model 1 $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3$ (additive errors)

	Parameter	SE
θ_1	36.2	22.0
η_1	1400	1350
CV(%)	103.4	
θ_2	0.147	0.0194
η_2	0.0228	0.0139
CV (%)	102.7	
θ_3	14.3	1.29
η_3	13.5	4.97
CV (%)	25.7	
ϵ	0.0305	0.0109
Obj	1255.5	

Table 5.1 NONMEM parameter estimates obtained for three monoexponential models

(ii) Model 1 $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3$ (proportional errors)

	Parameter	SE
θ_1	36.2	22.0
η_1	1.07	0.508
CV(%)	103.4	
θ_2	0.147	0.0194
η_2	1.05	0.619
CV(%)	102.5	
θ_3	14.3	1.29
η_3	0.0663	0.0268
CV(%)	25.7	
ϵ	0.0305	0.0109
Obj	1255.5	

Table 5.1 NONMEM parameter estimates obtained for three monoexponential models

(iii) Model 2 $Cl/F = \theta_1(1 - \theta_2 + \theta_2 e^{-\theta_3 t})$ (additive errors)

	parameter	SE
θ_1	40.6	7.49
η_1	1190	965
CV (%)	85.0	
θ_2	0.756	0.0709
η_2	0.0476	0.0101
CV (%)	28.9	
θ_3	0.139	0.0130
η_3	0.0238	0.0072
CV (%)	111.0	
ϵ	0.0562	0.0291
Obj	1274.8	

Table 5.1 NONMEM parameter estimates obtained for three monoexponential models

(iv) Model 2 $Cl/F = \theta_1(1-\theta_2 + \theta_2\ e^{-\theta_3 t})$ (proportional errors)

	Parameter	SE
θ_1	40.6	8.82
η_1	0.726	0.548
CV(%)	85.2	
θ_2	0.756	0.0887
η_2	0.0832	0.0659
CV(%)	28.8	
θ_3	0.139	0.0421
η_3	1.24	1.40
CV(%)	111.4	
ϵ	0.0562	0.0453
Obj	1274.8	

Table 5.1 NONMEM parameter estimates obtained for three monoexponential models

(v) Model 3 $Cl/F = \theta_1 - \theta_3 + \theta_3 e^{-\theta_1 \theta 2t}$ (additive errors)

	Parameter	SE
θ_1	49.5	8.57
η_1	1280	1140
CV (%)	72.2	
θ_2	0.00273	0.00053
η_2	3.01×10^{-6}	5.85×10^{-7}
CV (%)	63.3	
θ_3	36.3	13.2
η_3	1200	1120
CV (%)	95.4	
ϵ	0.0327	0.0221
Obj	1297.2	

Table 5.1 NONMEM parameter estimates obtained for three monoexponential models

(vi) Model 3 $Cl/F = \theta_1 - \theta_3 + \theta_3 e^{-\theta_1 \theta_2 t}$ (proportional errors)

	Parameter	SE
θ_1	49.5	11.0
η_1	0.524	0.318
CV (%)	72.4	
θ_2	0.00273	0.00125
η_2	0.400	0.435
CV (%)	63.2	
θ_3	36.3	11.9
η_3	0.914	0.427
CV (%)	95.6	
ϵ	0.0327	0.0378
Obj	1297.2	

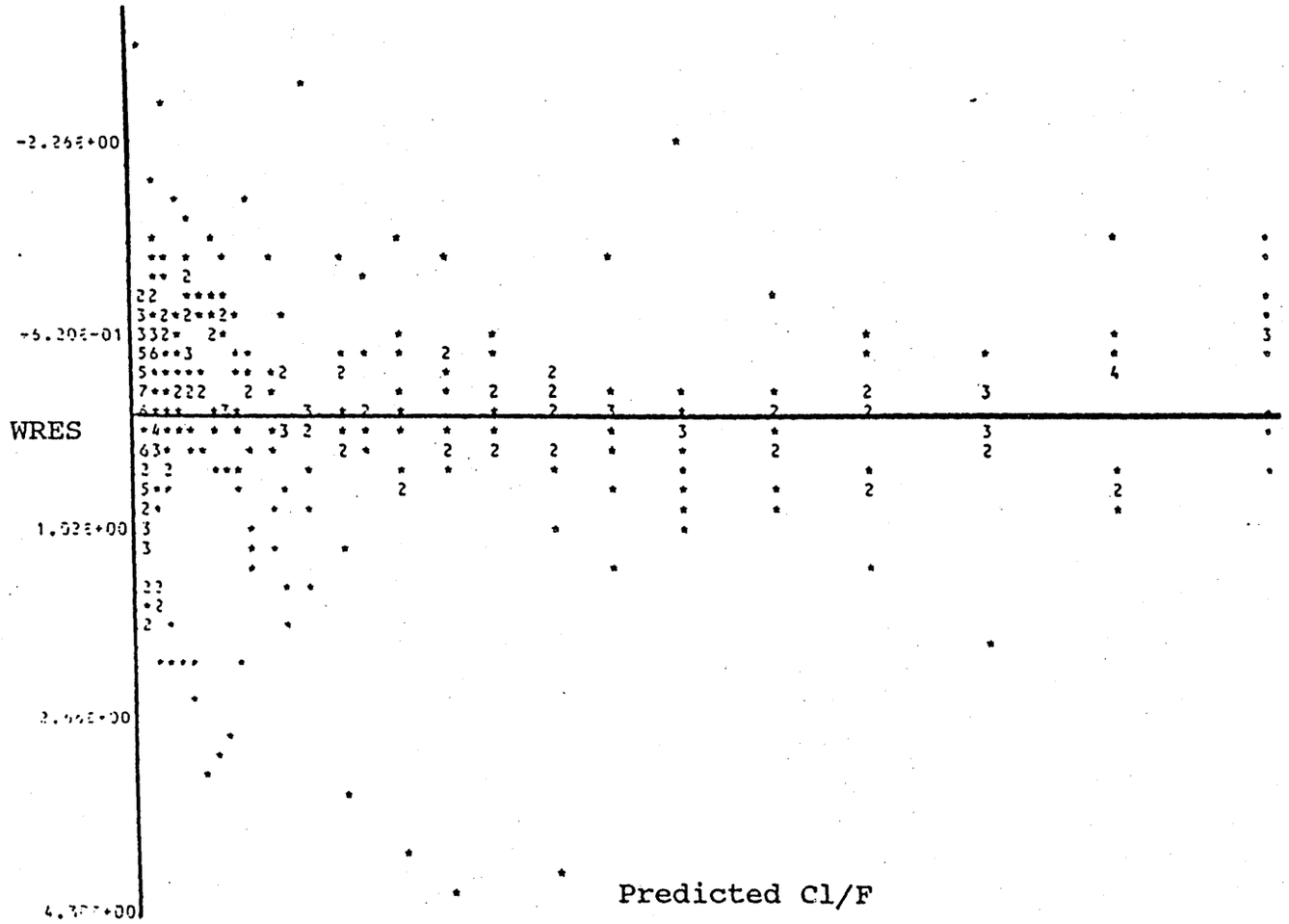
associated with each parameter are large, indicating wide inter-patient variability whichever model is used. There was little difference in the calculated coefficient of variation between the additive error models and the proportional error models. On the basis of the objective function, Model 1 was selected as the best model. Examination of plots of predicted Cl/F versus weighted residual for this model (Figure 5.2(i) and (ii)) showed little difference between the additive and proportional error models. These plots also suggested that it was not an inappropriate model.

Results for model 1 suggested that the initial value of Cl/F was around 50l/h and declined gradually at a rate of $0.147day^{-1}$ to a final Cl/F of approximately 14l/h. From the rate of change, a half life of 4.7 days was calculated which suggested that, on average, the change in pharmacokinetics was complete within three weeks of transplantation. In order to maintain a constant steady state trough concentration during this time period, the dose would have to be gradually reduced. This is in agreement with earlier observations (Tufveson et al, 1986). In this study the dose was gradually reduced to about 40% of its original value.

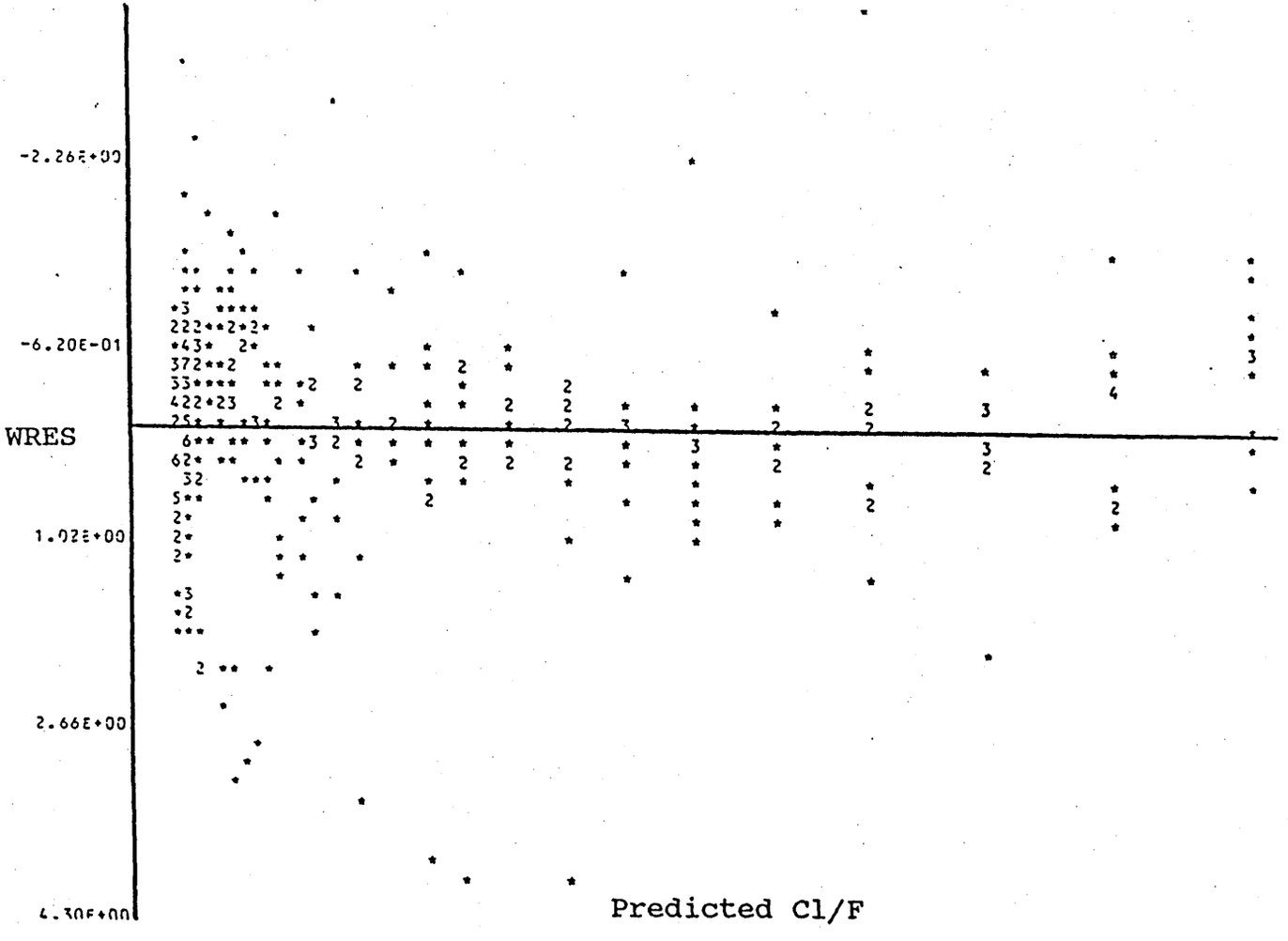
Model 1 has subsequently been used in the modified version of the Bayesian program evaluated in Chapter 6.

Figure 5.2 Graph of predicted value of Cl/F versus weighted residual for Model 1

(i) additive error model



(ii) proportional error model



5.4 RELATIONSHIP BETWEEN BIOCHEMICAL, HAEMATOLOGICAL AND PATIENT DATA AND THE PHARMACOKINETICS OF CYCLOSPORIN

5.4.1 Introduction

Throughout the study described in Chapter 4 various demographic, haematological and biochemical measurements were made. The factors used in this analysis are shown in Table 5.2. There were not enough episodes of gastro-intestinal dysfunction to include in the analysis and few drugs administered concurrently had been implicated in pharmacokinetic interactions with cyclosporin (see Section 4.4.4). The effect of concomitant drug therapy could not therefore be studied.

Along with changes in the pharmacokinetic parameters, marked changes occurred in the other variables measured. Mean values ($\pm$ sd) for each factor of interest are shown in Table 5.3 at various time points throughout the study period. From the data presented in Table 5.3 it can be seen that both pharmacokinetic and biochemical, haematological and demographic measurements showed gradual changes. Cl/F declined by 56% and V_1/F by 67%. Several variables increased; haematocrit 45%, haemoglobin 38%, albumin 13%, and cholesterol 34%. Bilirubin showed no consistent change over the 60 days of the study. ALAT showed no obvious change other than on day 2 when the results may have been biased by two extremely high values. Creatinine concentrations decreased by an

Table 5.2 Biochemical, haematological and demographic variables measured continuously throughout study period

Variable (Abbreviation)	Units
Haematocrit (Hct)	(%)
Haemoglobin (Hb)	(g/100ml)
Albumin (Alb)	(g/l)
Cholesterol (Chol)	(mmol/l)
Bilirubin (Bil)	(umol/l)
Alanine amino transferase (ALAT)	(I.U./l)
Creatinine (Crea)	(umol/l)
Cyclosporin dose (Dose)	(mg)
Patient weight (Wt)	(kg)

Table 5.3 Changes in pharmacokinetic parameters and other variables (mean ± SD) at various time points throughout the study

	Time post transplant (days)				
	2	10	20	30	60
Cl/F	34.4 (21.6)	26.5 (12.1)	18.4 (5.7)	15.5 (4.9)	15.0 (4.3)
V_1/F	136.5 (127.5)	96.9 (66.4)	74.4 (45.7)	54.0 (18.5)	44.5 (12.1)
Haematocrit	22.7 (3.7)	23.3 (3.6)	24.0 (4.5)	26.6 (2.9)	32.9 (2.8)
Haemoglobin	7.9 (1.6)	8.1 (1.3)	8.1 (1.4)	9.1 (0.9)	10.9 (1.0)
Albumin	33.8 (3.8)	33.9 (2.9)	35.0 (4.4)	37.0 (4.4)	38.2 (4.4)
Cholesterol	5.0 (1.1)	5.4 (0.9)	6.5 (1.5)	6.7 (1.8)	6.7 (1.6)
Bilirubin	7.5 (2.7)	9.6 (6.5)	8.4 (3.6)	9.8 (7.1)	6.7 (2.7)
ALAT	*52.3 (68.7)	25.8 (13.2)	21.7 (8.5)	22.1 (9.6)	24.1 (14.6)
Creatinine	810 (399)	520 (386)	353 (307)	234 (121)	154 (46)
Dose	986 (219)	650 (122)	577 (147)	491 (83)	391 (63)
Weight	69 (15)	68 (13)	68 (13)	67 (13)	68 (14)

* Results may be biased by two extremely high values

average of 81% which would be expected in a group of successful renal transplants. Patient weight did not appear to change over the course of the study. Figures 5.3 (i) to (x) show plots of Cl/F versus different variables (covariates) for a representative patient. These plots suggest considerable intra-patient variability in the variables measured.

The relationship between Cl/F and other variables measured during the course of this study has been investigated using two different techniques; (i) General Linear Interactive Modelling (GLIM) and (ii) non-linear Mixed Effects Modelling (NONMEM).

5.4.2 Multiple Linear Regression Analysis

(i) Background

The program GLIM (Baker et al, 1978) was used for the simultaneous analysis of the relationship between Cl/F and various other factors measured, including time post operatively. The background to this technique has previously been discussed in Chapter 3.

(ii) Data analysis

The data were initially analysed assuming that all relationships with Cl/F were linear since (with the exception of time), there was little evidence otherwise. A second analysis was carried out using the logarithm of Cl/F as the dependent variable since Cl/F has been shown to decline exponentially with time.

Figure 5.3 Relationship between variables measured during study and Cl/F in one patient

Figure 5.3(i)

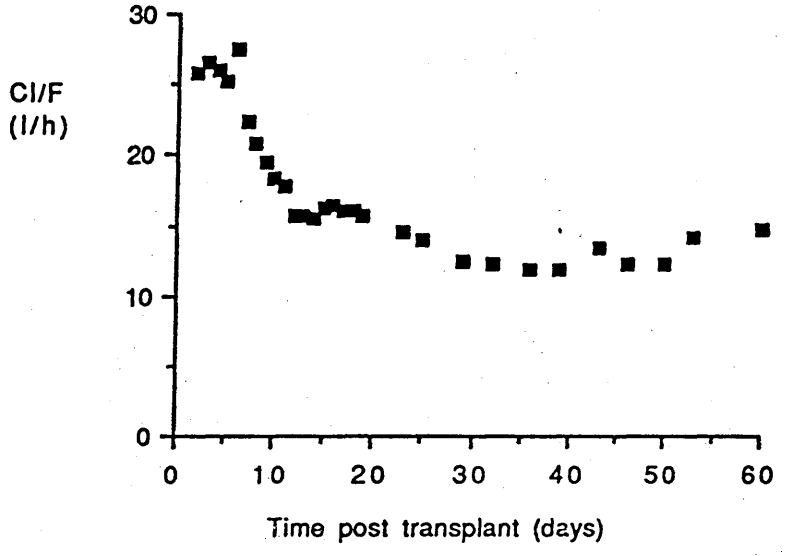


Figure 5.3(ii)

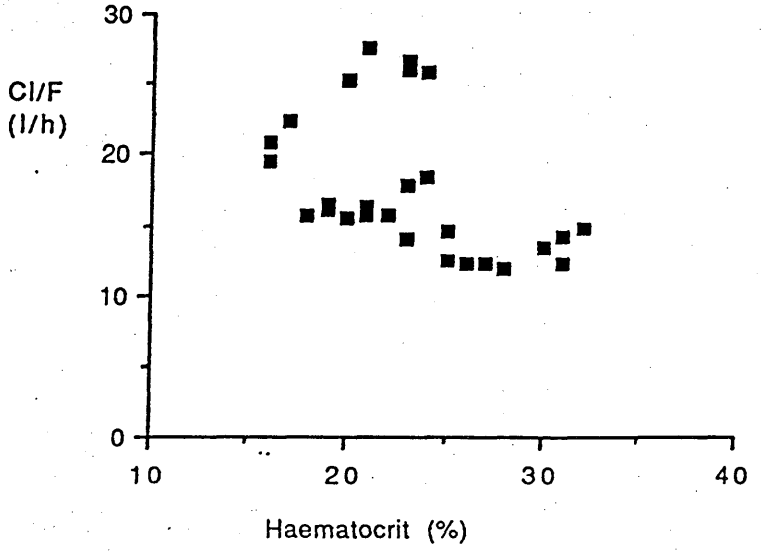


Figure 5.3(iii)

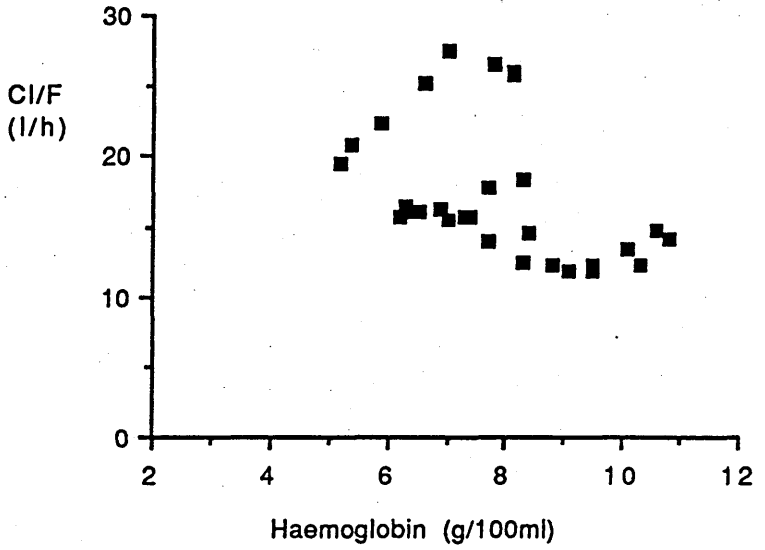


Figure 5.3(iv)

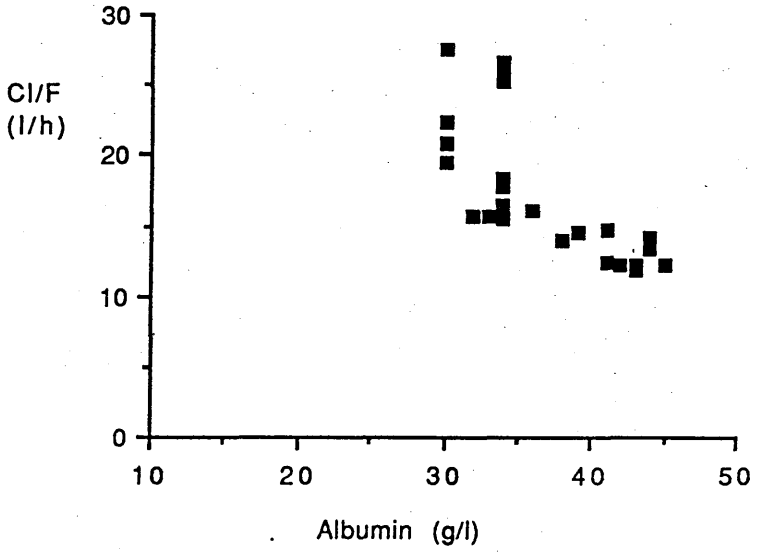


Figure 5.3(v)

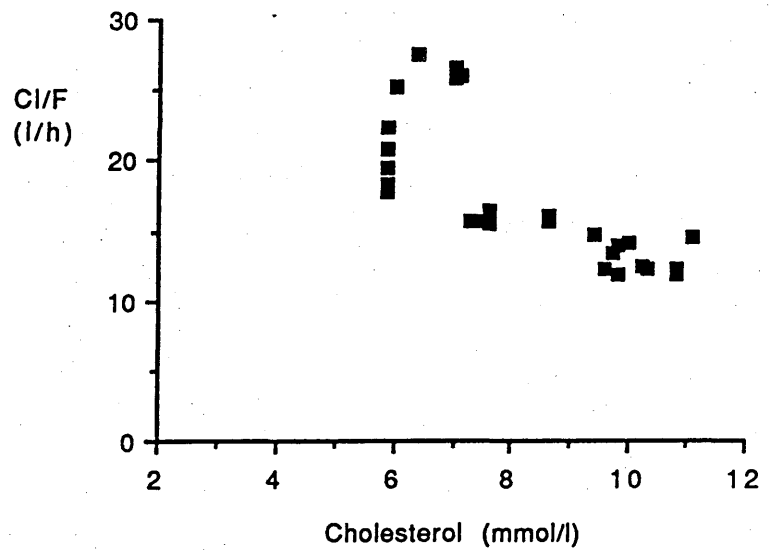


Figure 5.3(vi)

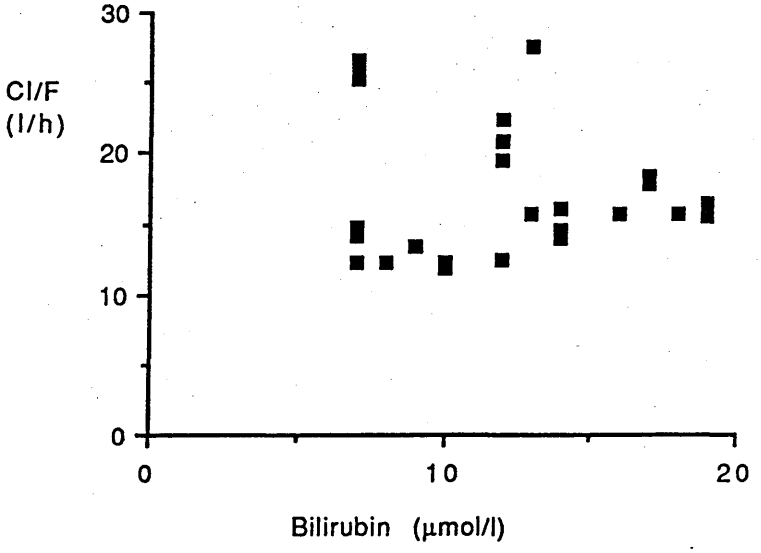


Figure 5.3(vii)

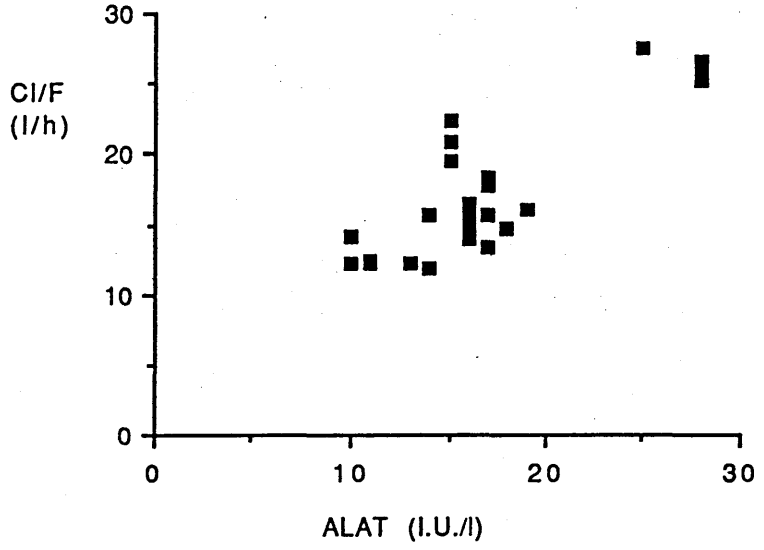


Figure 5.3(viii)

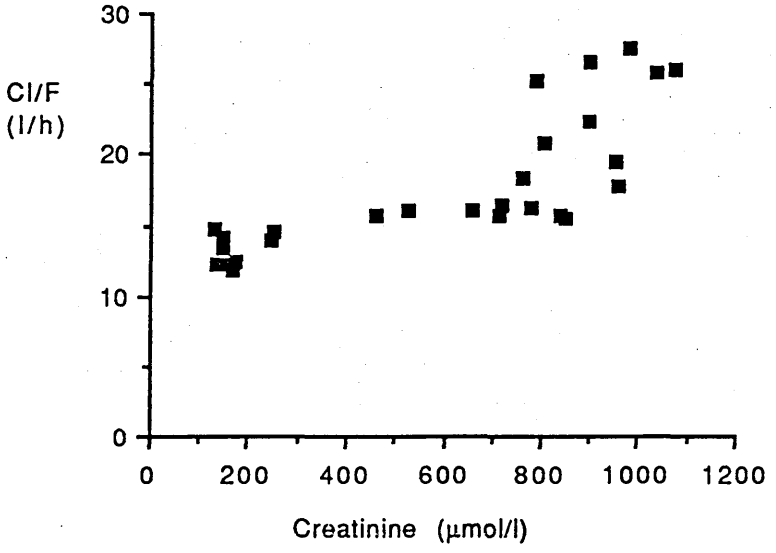


Figure 5.3(lx)

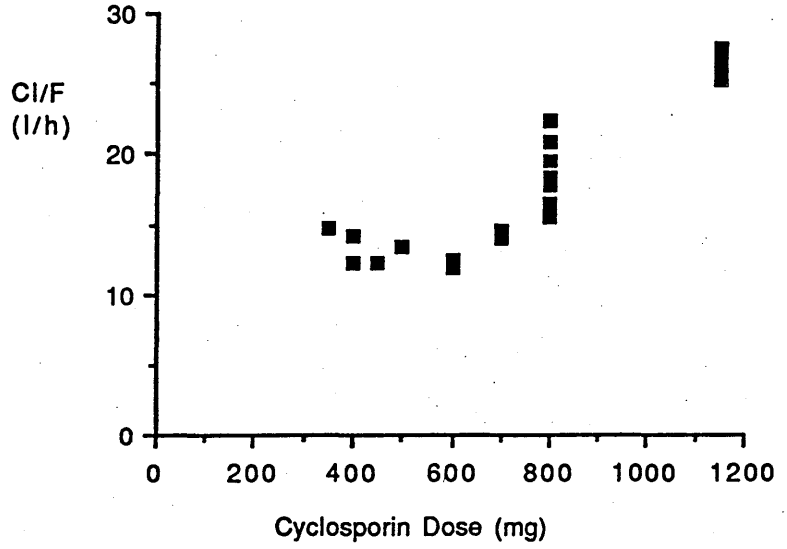
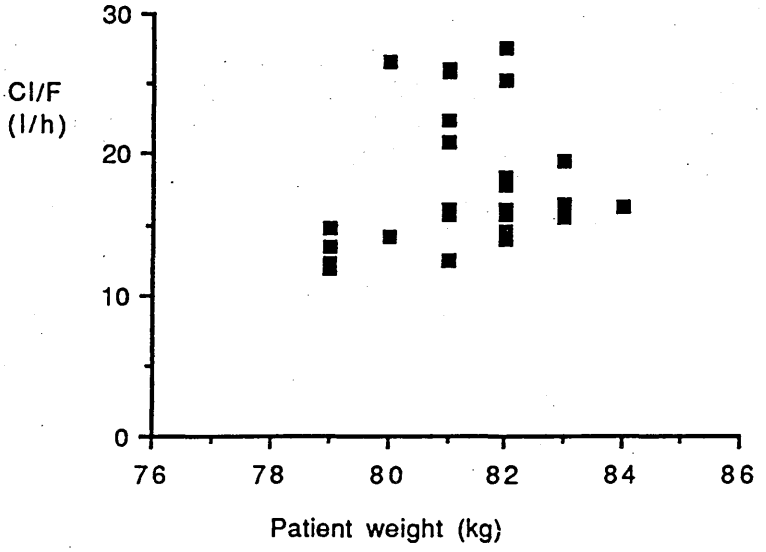


Figure 5.3(x)



The simplest model was initially fitted which incorporated only a subject effect as follows

$$Cl/F = \alpha_i + \epsilon \qquad \text{Equation 5.4}$$

and the residual sum of squares calculated (RSSQ). This model described the data in terms of individual intercepts with slope of zero. Independent variables were then incorporated into the model one at a time using the method of forward stepping for multiple linear regression (Chapter 3). The most significant variable (ie the one that best explained the variability in Cl/F) was determined by calculating the F-to-enter statistic for each variable (Section 3.3); the variable with the highest F-to-enter statistic was incorporated into the model as shown in Table 5.4 (i). At each stage in the forward stepping procedure, any variable with an F statistic which was not significant using F tables ($p<0.001$) was eliminated from the analysis. The level of significance of $p<0.001$ was used since multiple tests were carried out. Assuming no more than fifty tests were performed, this level of significance allowed confidence that any difference was significant at the 5% level. In the first step, dose was the variable with the largest F-to-enter statistic and it was incorporated as follows

$$Cl/F = \alpha_i + \beta_i \text{dose} + \epsilon \qquad \text{Equation 5.5}$$

This model described Cl/F in terms of individual

Table 5.4 Results of multiple linear regression analysis using Cl/F as the dependent variable, where x is the covariate.

(i) $Cl/F = \alpha_i + \beta_i X + \epsilon$

X	SSQ	F	R^2	Sig (p<0.001)
Time	16830	28.6	61.6	Yes
Haematocrit	21480	16.3	51.0	Yes
Haemoglobin	28870	4.9	34.1	Yes
Albumin	20650	18.1	52.9	Yes
Cholesterol	24260	11.2	44.6	Yes
Bilirubin	32220	1.5	26.5	No
ALAT	23860	11.9	45.5	Yes
Creatinine	13260	44.0	69.7	Yes
Dose	12460	48.6	71.6	Yes
Weight	17160	27.5	60.8	Yes

Note: Accepting p<0.001 for individual tests since multiple comparisons are being carried out (see text)

intercepts (α_i) and individual slopes (β_i). The forward stepping procedure was continued, each time adding in the variable with the largest F-to-enter statistic (Tables 5.4 (ii) - (iv)). The analysis was stopped when addition of a further variable did not result in a significant F value. The addition of further variables into a model will always result in an improvement to the model. If the F-to-enter statistic is regarded as a test at every point then the overall confidence will be much less than 95%. A 'stopping rule' was therefore employed which used adjusted R^2 to determine subjectively the point at which no further improvement in the model was seen. Adjusted R^2 was calculated using Equation 3.13. A plot of adjusted R^2 versus the number of parameters is shown in Figure 5.4(i). The choice of an appropriate "stopping point" is a highly subjective procedure and is usually taken to be the point where the graph levels off.

The procedure of forward stepping was repeated using the logarithm of Cl/F as the dependent variable. Multiple linear regression analysis was carried out in the same way as before and the results are summarised in Table 5.5.

(iii) Results

Using the method of forward stepping, a complex model explaining almost 89% of the variability in CL/F was identified. Adjusted R^2 values are shown in

Table 5.4 (ii)

$$Cl/F = \alpha_i + \beta_i dose + \gamma_i X + \epsilon$$

X	SSQ	F	R^2	Sig ($p<0.001$)
Time	8730	11.6	80.1	Yes
Weight	7216	19.8	83.5	Yes
Haematocrit	9952	6.9	77.3	Yes
Haemoglobin	10420	5.3	76.2	Yes
Creatinine	9838	7.3	77.5	Yes
Albumin	10560	4.9	76.9	Yes
ALAT	11090	3.4	74.7	Yes
Cholesterol	9786	7.5	77.7	Yes

Table 5.4 (iii)

$Cl/F = \alpha_i + \beta_i dose + \gamma_i weight + \delta_i X + \epsilon$

X	SSQ	F	R^2	Sig (p<0.001)
Time	6216	4.2	85.8	Yes
Haematocrit	6775	1.7	84.5	No
Haemoglobin	6501	2.9	85.2	Yes
Creatinine	6077	4.9	86.1	Yes
Albumin	6211	4.3	85.8	Yes
ALAT	6710	2.0	84.7	No
Cholesterol	5936	5.7	86.5	Yes

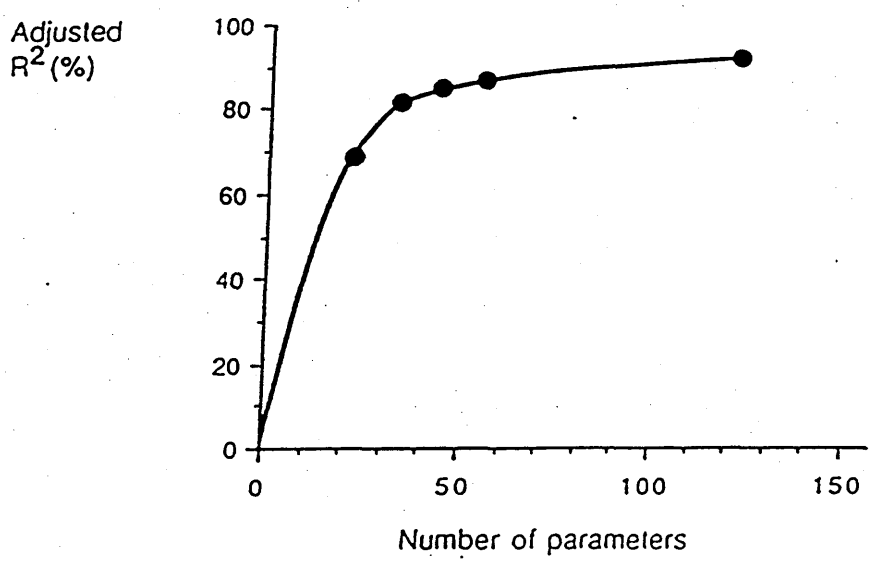
Table 5.4 (iv)

$Cl/F = \alpha_i + \beta_i dose + \gamma_i weight + \delta_i cholesterol + \zeta_i X + \epsilon$

X	SSQ	F	R^2	Sig (p<0.001)
Time	5578	1.6	87.3	No
Haemoglobin	5673	0.6	87.1	No
Creatinine	4909	5.3	88.8	Yes
Albumin	5600	1.5	87.2	No

Figure 5.4 Adjusted R^2 versus number of parameters used in multiple linear regression analysis

(i) dependent variable - Cl/F



(ii) dependent variable - log Cl/F

Figure 5.4 (ii)

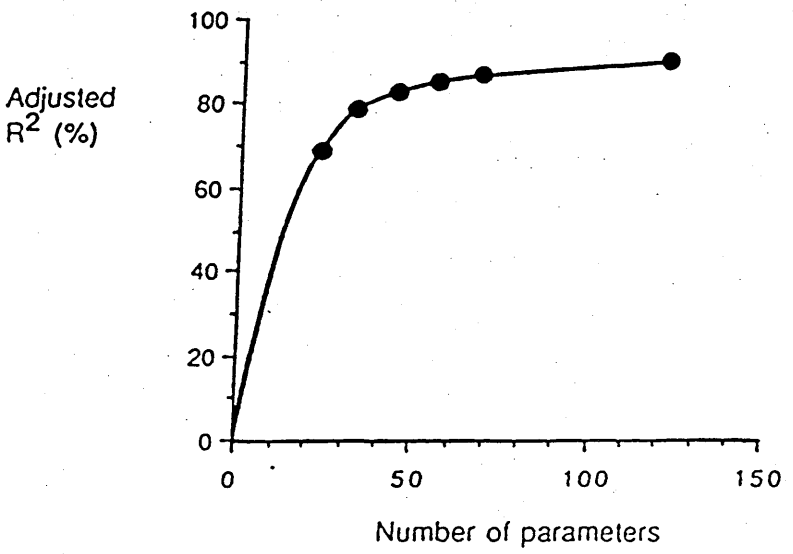


Table 5.5 Summary of results of multiple linear regression analysis using log Cl/F as the dependent variable

$$\log Cl/F = \alpha_i + \beta_i X_1 + \gamma_i X_2 + \delta_i X_3 + \zeta_i X_4 + \eta_i X_5 + \epsilon$$

X	"best" X	F	R^2
X_1	Time	40.1	70.5
X_2	Creatinine	15.7	81.2
X_3	ALAT	6.6	85.0
X_4	Cholesterol	4.7	87.4
X_5	Weight	3.8	89.1

brackets. The most important single factor was dose which alone accounted for 72% (70%) of the variability: creatinine alone accounted for 70% (Table 5.4(i)). As indicated in Chapter 1, inclusion of either of these parameters may be clinically irrelevant. The analysis, however, was continued incorporating dose into the model. Using two covariates, ie dose plus a second variable, the best model contained dose and weight, and explained 84% (82%) of the variability (Table 5.4 (ii)). Addition of cholesterol explained 87% (85%) of the variability (Table 5.4 (iii)) and the further addition of creatinine concentration explained 89% (87%) (Table 5.4 (iv)). No further improvement was gained by adding other factors and the final model was:

$$Cl/F = \alpha_i + \beta_i \text{dose} + \gamma_i \text{weight} + \delta_i \text{cholesterol} + \zeta_i \text{creatinine} + \epsilon$$

Examination of the graph of adjusted R^2 versus the number of variables (Figure 5.4(i)) suggested that little improvement in the model was seen after inclusion of four variables.

Using log Cl/F as the dependent variable, time alone explained almost 71% (69%) of the variability. Further inclusion of creatinine concentration explained 81% (79%), ALAT 85% (83), cholesterol 87% (85%) and patient weight 89% (86%). The best model was therefore

$$\log Cl/F = \alpha_i + \beta_i \text{time} + \gamma_i \text{creatinine} + \delta_i \text{ALAT} + \zeta_i \text{cholesterol} + \eta_i \text{weight} + \epsilon$$

As before, examination of the graph of Adjusted R^2 versus number of variables (Figure 5.4 (ii)) suggested that little improvement in the model was seen after inclusion of four variables.

(iv) Discussion

Multiple linear regression analysis was carried out to identify sources of variability in Cl/F. Initial observation of individual patient plots of Cl/F against individual covariates suggested that all relationships were linear with the exception of time. Multiple linear regression analysis was therefore carried out on two data sets; firstly where all relationships with CL/F were assumed to be linear and secondly where all covariates were linearly related to log Cl/F.

However, although multiple linear regression may be a useful approach in describing variability in the data, several problems became apparent during this analysis.

(i) Inclusion of virtually any of the variables studied explained a considerable amount of the variability in Cl/F and it was impossible to determine whether variability in Cl/F was due to changes in one or more of the covariates or whether it was merely an association due to general recovery post operatively as discussed earlier ie, correlation between the variables was complicating the issue.

(ii) Inclusion of further factors will improve the model. It was therefore necessary to use a "stopping rule" such as adjusted R^2 which described the improvement in the model while taking into account the number of variables in the model. Selection of the appropriate "stopping point" was a subjective procedure and no statistical method of selecting the best value of R^2 was available.

(iii) GLIM does not allow linear and non-linear functions to be fitted simultaneously. The analysis therefore had to be carried out twice; firstly using Cl/F and secondly using log Cl/F.

5.4.3 Non-Linear Mixed Effect Modelling (NONMEM)

(i) Background

Further analysis of the data was carried out using the non-linear regression program NONMEM (Chapter 3), since this technique allowed simultaneous analysis of linear and non-linear relationships with Cl/F.

Multiple linear regression of Cl/F and log Cl/F suggested that there was a relationship between cyclosporin Cl/F and one or more independent variables. Time appeared to be a strong influencing factor in the change in pharmacokinetics post-transplant. However, data analysis described in this chapter suggests that inclusion of a further variable in the model may be appropriate. This possibility was investigated further using NONMEM since this allowed linear and non-linear

covariates to be fitted simultaneously.

(ii) Data analysis

To link with the multiple linear regression analysis described above, a simple linear regression model was investigated initially as shown in Equation 5.6.

$$Cl/F = \theta_2 - \theta_1 X \qquad \text{Equation 5.6}$$

where θ_1 and θ_2 are the parameters of the model and X is the covariate. The PRED file used is given in Appendix 3. The results of this analysis are shown in Table 5.6. Intra-subject variability was explained to a greater or lesser extent by the covariates tested. Variables were compared by examining the objective function and also by comparison of the variances associated with θ_1. The coefficient of variation (CV) associated with θ_1 was lowest when albumin was used as variable and was approximately 15%. In some cases - in particular haemoglobin, ALAT and creatinine concentration - the CV associated with θ_1 was very high (329%, 1203% and 825% respectively) suggesting that none of these variables was useful in explaining variability in Cl/F. The CV of θ_1 when time was incorporated was approximately 50% although the linear model used here may not be appropriate since it has previously been shown in this thesis that Cl/F declines exponentially with time.

In conclusion, using this model the factor which resulted in the lowest variance on θ_1 was albumin,

Table 5.6 Results of NONMEM analysis for simple linear model

$$Cl/F = \theta_2 - \theta_1 X$$

	Time	Weight	Hct	Hb	Crea	Bil	Alb	ALAT	Chol	Dose
θ_1	0.404	-0.307	0.412	-0.219	0.0063	1.02	1.27	0.0144	3.52	-0.032
(SE)	(0.044)	(0.0006)	(0.222	(1.25)	(0.0377)	(0.094	(0.15)	(0.00001)	(1.27)	(0.0031)
η_1	0.0402	0.0061	0.0631	0.520	0.0027	2.55	0.036	0.030	0.454	5.31×10^{-5}
(SE)	(0.019)	(0.002)	(0.044)	(0.453)	(0.0039)	(1.04)	(0.037)	(0.0203)	(0.286)	(0.00152)
CV(%)	49.6	25.4	61.0	329.3	824.8	156.6	14.9	1202.8	19.1	22.8
θ_2	26.1	1.67	35.5	23.5	13.6	19.1	61.8	20.4	42.1	3.72
(SE)	(1.69)	(2.07)	(8.56)	(13.8)	(1.58)	(3.33)	(1.13)	(0.015)	(6.72)	(3.34)
ϵ	0.227	0.0179	0.163	0.159	0.566	0.929	0.247	0.236	0.180	0.0941
(SE)	(0.100)	(0.077)	(0.100)	(0.113)	(0.351)	(0.620)	(0.226)	(0.0880)	(0.133)	(0.0570)
Obj	1713	1849	1879	1902	1782	1896	1768	1874	1787	1616

Hct-haematocrit, Hb haemoglobin, Crea-creatinine, Bil-bilirubin, Alb-albumin.

although it is known that cyclosporin is not highly bound to albumin (Lemaire and Tillement, 1982). The factor which had the lowest objective function was dose. However, as discussed in Chapter 4, the relationship between Cl/F and dose may be spurious. Time post-operatively appeared to be a powerful factor in describing the change in cyclosporin pharmacokinetics and its objective function of 1713 was the second lowest using this linear model. It was shown earlier in this chapter, however, that the relationship between Cl/F and time could be described by a monoexponential equation.

In order to fit the non-linear relationship with time and linear relationships with other covariates, further analysis was carried out using the most appropriate of the three monoexponential relationships between Cl/F and time studied earlier ie (Model 1). Other covariates were added individually (Equation 5.7)

$$Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4 X \qquad \text{Equation 5.7}$$

where t was time post-operatively and X was the additional covariate. An example of the PRED file used for this model is given in Appendix 3.

The results of this analysis are shown in Table 5.7 (i) to (ix). The variances associated with θ_1, θ_2 and θ_3 were still large for each of the models, as shown by the values of η and the calculated coefficient of variation for each parameter.

The models in this analysis were compared to the

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(i) $Cl/F = \theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ x patient weight

Parameter	Estimate	SE
θ_1	24.2	69.5
η_1	1240	739
CV(%)	145.5	
θ_2	0.134	0.0616
η_2	0.0368	0.0211
CV(%)	143.2	
θ_3	26.1	73.1
η_3	39.6	198
CV(%)	24.1	
θ_4	-0.216	0.467
ϵ	0.044	0.300
Obj	1225.5	

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(ii) $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4 \times$ haematocrit

Parameter	Estimate	SE
θ_1	36.2	22.4
η_1	1400	1350
CV(%)	103.4	
θ_2	0.147	0.016
η_2	0.0228	0.0138
CV(%)	102.7	
θ_3	14.3	3.65
η_3	13.5	5.09
CV(%)	25.6	
θ_4	7.04×10^{-3}	0.117
ϵ	0.030	0.011
Obj	1255.5	

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(iii) $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4 \times$ haemoglobin

Parameter	Estimate	SE
θ_1	35.7	22.2
η_1	1400	1290
CV(%)	104.8	
θ_2	0.148	0.014
η_2	0.0236	0.015
CV(%)	103.7	
θ_3	15.4	5.15
η_3	14.0	5.49
CV(%)	24.3	
θ_4	-0.125	0.522
ϵ	0.0306	0.0111
Obj	1254.9	

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(iv) $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4 \times \text{creatinine}$

Parameter	Estimate	SE
θ_1	39.5	11.7
η_1	1050	875
CV(%)	82.0	
θ_2	0.126	0.0240
η_2	0.0110	0.0817
CV(%)	83.2	
θ_3	15.1	1.26
η_3	14.4	5.46
CV(%)	25.1	
θ_4	-0.0051	3.8×10^{-5}
ϵ	0.0287	0.0095
Obj	1244.2	

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(v) $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4 \times \text{bilirubin}$

Parameter	Estimate	SE
θ_1	30.6	20.9
η_1	1390	1270
CV(%)	121.8	
θ_2	0.147	0.0185
η_2	0.0229	0.0140
CV(%)	102.9	
θ_3	14.4	1.32
η_3	13.5	4.94
CV(%)	25.5	
θ_4	-0.0166	0.0841
ϵ	0.0306	0.0107
Obj	1255.4	

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(vi) $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4$ x albumin

Parameter	Estimate	SE
θ_1	36.3	21.4
η_1	1390	1310
CV(%)	102.7	
θ_2	0.146	0.019
η_2	0.0222	0.0142
CV(%)	102.1	
θ_3	12.3	4.86
η_3	13.9	5.17
CV(%)	30.3	
θ_4	0.0511	0.133
ϵ	0.0308	0.011
Obj	1254.7	

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(vii) $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4 \times ALAT$

Parameter	Estimate	SE
θ_1	37.5	20.6
η_1	1470	1340
CV(%)	102.2	
θ_2	0.152	0.0136
η_2	0.0234	0.0140
CV(%)	100.6	
θ_3	15.0	1.43
η_3	12.5	4.46
CV(%)	23.6	
θ_4	-0.0284	0.004
ϵ	0.0298	0.0096
Obj	1245.5	

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(viii) $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4 \times \text{cholesterol}$

Parameter	Estimate	SE
θ_1	33.1	21.7
η_1	1340	1220
CV(%)	110.3	
θ_2	0.148	0.0178
η_2	0.0263	0.0198
CV(%)	109.6	
θ_3	16.7	3.30
η_3	12.2	4.60
CV(%)	20.9	
θ_4	-0.374	0.564
ϵ	0.031	0.011
Obj	1250.6	

Table 5.7 Results of NONMEM analysis incorporating additional variables into the monoexponential model

(ix) $Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4$ x cyclosporin dose

Parameter	Estimate	SE
θ_1	37.4	23.3
η_1	1430	1410
CV(%)	101.1	
θ_2	0.148	0.0192
η_2	0.0218	0.0151
CV(%)	99.8	
θ_3	15.1	1.69
η_3	13.8	2.99
CV(%)	24.6	
θ_4	-0.002	1.44×10^{-4}
ϵ	0.0311	0.018
Obj	1254.7	

monoexponential model (Model 1) using the chi-squared test for the difference between the objective function obtained from fitting the full or reduced models, described in Chapter 3. The results of this are shown in Table 5.8. It can be seen from Table 5.8 that including either patient weight ($p<0.001$), creatinine concentration ($p<0.001$) or ALAT ($p<0.005$) significantly improved the model. Inclusion of patient weight in the model, however, resulted in increased coefficients of variation for each of the parameters, and increased the standard errors associated with the parameter estimates. ALAT appears to be useful since it significantly decreased the objective function, decreased the coefficients of variation and decreased the standard error associated with θ_1 and θ_2. The finding that serum creatinine concentration significantly improved the model was unexpected since renal function has previously been shown to have little effect on the pharmacokinetics of cyclosporin (Follath et al, 1983; Roberts et al, 1986).

The above analyses suggest that time is the single most important factor influencing the pharmacokinetics of cyclosporin post-transplant. However, it was shown in Chapter 4 that predictions made from day 30 post-transplant are not biased. It can be assumed that the time dependent influence is not present after this time. Investigation of the relationship between Cl/F and other

Table 5.8 Hierarchical models tested and differences in their objective functions

Model		cf	ΔObj	P
1	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3$	-	-	-
2	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xWt	1	-30.0	<0.001
3	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xHct	1	0.0	NS
4	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xHb	1	-0.6	NS
5	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xCrea	1	-11.3	<0.001
6	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xBil	1	-0.1	NS
7	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xAlb	1	-0.8	NS
8	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xAlat	1	-10.0	<0.005
9	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xChol	1	-4.9	NS
10	Cl/F= $\theta_1 e^{-\theta 2t} + \theta_3 + \theta_4$ xDose	1	-0.8	NS

variables can be carried out without the effect of time. The following function was fitted as before

$$Cl/F = \theta_2 - \theta_1 X \qquad \text{Equation 5.6}$$

this time using data from days 30 to 60 only. The results of this analysis are shown in Table 5.9. Comparison of the objective functions for each covariate suggested that bilirubin explained most of the variability associated with Cl/F, although inter-subject variability was large. It is of note that creatinine, which significantly improved the monoexponential model above, showed a very large degree of inter-subject variability in this analysis. No single covariate in this analysis appeared to usefully explain the pharmacokinetic variability of cyclosporin.

5.5 DISCUSSION

Work presented in this chapter has attempted to identify the variability in the pharmacokinetics of cyclosporin following renal transplantation. It appears that there is a time related change in Cl/F in the first few weeks after a transplant. However, the influence of other factors cannot be ignored as trends in biochemical and haematological data may be related to the change in kinetics. Some of these factors may help to explain variability.

Inclusion of patient weight as a variable, significantly improved both models investigated by

Table 5.9 NONMEM parameter estimates for simple linear model

$Cl/F = \theta_2 - \theta_1 X$

using data from days 30 - 60 only.

	Weight	Hct	Hb	Crea	Bil	Alb	ALAT	Chol	Dose
θ_1	-0.0513	0.100	0.0463	-0.00124	0.103	-0.0165	-0.0714	0.0738	0.00307
(SE)	0.0686	0.176	0.385	0.00518	0.214	0.162	0.105	0.503	0.00022
η_1	0.00247	0.0139	0.116	0.000503	0.324	0.00938	0.0293	0.339	0.000082
(SE)	0.00077	0.00489	0.0362	0.00018	0.145	0.00331	0.0133	0.143	0.000029
CV(%)	96.9	117.9	735.6	1808.7	552.6	587.0	239.7	788.9	295.0
θ_2	11.2	17.7	15.3	14.2	15.9	14.3	13.6	15.7	16.1
(SE)	(4.06)	(5.19)	(3.74)	(1.51)	(1.42)	(6.05)	(2.02)	(3.21)	(1.32)
ϵ	0.0177	0.0182	0.0179	0.0173	0.0152	0.0171	0.0188	0.0157	0.0174
(SE)	(0.00362)	(0.00315)	(0.00329)	(0.00402)	(0.00294)	(0.00343)	(0.00708)	(0.00263)	(0.00448)
Obj	264.0	268.9	267.6	262.9	260.8	267.2	286.4	263.7	268.3

multiple linear regression and, the inclusion of weight as an additional variable in the monoexponential model resulted in a significant decrease in the objective function. Recent work in uraemic patients, however, suggests that the disposition of cyclosporin is not affected by obesity, despite its lipophilicity and pharmacokinetics of cyclosporin when normalised by ideal body weight are similar in obese and non-obese patients (Flechner et al, 1989). Despite improving the monoexponential model, creatinine concentration is unlikely to have any explanatory power for reasons discussed earlier. The relationship shown with Cl/F is probably spurious. Liver function might have been expected to influence Cl/F: ALAT explained 30% and bilirubin only 4.9% of the variability when analysed by multiple linear regression. Including ALAT as a covariate in the monoexponential model resulted in a significant improvement in the model. The finding that ALAT may be useful in explaining variability in Cl/F in renal transplant patients is in agreement with earlier work (Reynolds et al, 1988).

Some studies have suggested a relative increase in bioavailability as the dose of cyclosporin is decreased: dose was progressively reduced in the present study (normal clinical practice to prevent an increase in trough concentrations). Data collected in this study do not allow any assumptions to be made as to the effect of decreasing dose on cyclosporin pharmacokinetics.

Factors representing binding of cyclosporin either to red blood cells or to plasma proteins may be of interest, since cyclosporin is a low extraction drug (Venkataramanan et al, 1985a) and its clearance is therefore related to the degree of protein binding. A close relationship between lipoprotein levels and cyclosporin clearance in uraemic patients awaiting renal transplantation has been observed (Lithell et al, 1986; Lindberg et al, 1986) and it is postulated that this is due to a reduction in the fraction of cyclosporin unbound with increasing cholesterol concentration (Legg et al, 1988). In addition, clearance of cyclosporin in bone marrow transplant patients is higher than in other groups of transplant patients and this is probably due to lower haematocrit levels in the former group (Yee et al, 1988b). An increase in haematocrit within patients, as seen in this study, might therefore be expected to result in a decrease in cyclosporin clearance. Obvious trends in haematocrit, haemoglobin, albumin and cholesterol were observed. In spite of the finding that these variables were "useful" in the multiple linear regression analysis, they contributed nothing to the monoexponential model.

In conclusion, it would appear that the variability in cyclosporin pharmacokinetics which occurs in the first few weeks following renal transplantation can be largely explained by time. This somewhat empirical

model may in fact reflect changes in other factors since there were correlations between time and the other factors. There remains however, considerable variability between subjects and the following chapter will evaluate a modified Bayesian program which attempts to account for the changes in pharmacokinetics between patients while also taking account of a gradual time-dependent change in pharmacokinetics.

CHAPTER 6

EVALUATION OF A BAYESIAN ESTIMATION PROGRAM INCORPORATING A TIME DEPENDENT CHANGE IN PHARMACOKINETICS FOR RENAL TRANSPLANT PATIENTS RECEIVING CYCLOSPORIN

6.1 INTRODUCTION

The results presented in Chapter 5 showed that the change in Cl/F of cyclosporin following renal transplantation was associated with changes in a number of other factors. It was impossible, however, to determine whether changes in other factors were associated with post operative recovery *per se*, or whether there was a strong relationship between these factors and cyclosporin pharmacokinetics. The analysis showed, however, that post-operative time was the most important factor in determining the value of Cl/F.

The variances associated with each of the parameters in the monoexponential model were large (Model 1, Chapter 5), implying considerable variability between subjects. In this chapter, the incorporation of this model into a Bayesian parameter estimation program is discussed and its ability to predict cyclosporin concentrations in the early post-operative period is evaluated.

6.2 DATA COLLECTION

Twenty two consecutive renal transplant patients were recruited. Of these, the transplant was removed from one patient on day 5 due to surgical complications, one patient had his immunosuppressive therapy changed to azathioprine on day 12, and two patients were unable to complete the out-patient dosing and sampling

questionnaires (described later) necessary for accurate data collection. Eighteen patients completed the study, and data were collected from the time of hospital admission (ie immediately prior to transplant) to six weeks post-operatively. Details of these 18 patients are shown in Table 6.1.

Each patient received a single oral dose of cyclosporin immediately before transplantation (day 0) and all subsequent cyclosporin was administered orally once daily. Patients were asked to take their cyclosporin with the same diluent (milk, fruit juice etc) for the duration of the study. Blood samples were collected twice weekly on average from each patient: cyclosporin concentrations were measured in whole blood using a specific monoclonal radioimmunoassay (CYCLO-Trac SP RIA) described in Appendix 1 (Knepil and McPhillips, 1989).

While in hospital, all cyclosporin dosing and sampling times were recorded accurately, either personally or by a member of the medical or nursing staff. Before discharge, patients were given a diary in which to record the time of daily cyclosporin administration. They also recorded the times of all blood samples collected at out-patient visits and all episodes of vomiting or diarrhoea were carefully recorded by nursing staff (while in hospital) or at home.

No drugs known to affect the pharmacokinetics of

Table 6.1 Clinical details of patients completing the evaluation study.

Patient	Age	Sex	Weight (kg)	Diagnosis
1	49	M	79	Mesingial IgA nephropathy
2	47	M	83	Polycystic kidneys
3	29	M	72	Congenital bladder outflow obstruction
4	37	M	74	Chronic pyelonephritis
5	58	M	76	Pyelonephritis
6	62	M	71	Hypertensive nephrosclerosis
7	45	M	74	Neurogenic bladder
8	50	F	73	Bilateral staghorn calculi
9	50	F	50	Chronic pyelonephritis
10	19	F	40	Focal glomerulosclerosis
11	37	M	69	Diabetic nephropathy
12	36	M	89	Acromegally/nephrotic syndrome
13	19	M	65	Medullary cystic disease
14	42	M	100	Chronic glomerulonephritis
15	38	M	78	Chronic glomerulonephritis
16	56	M	85	Glomerulonephritis
17	21	M	67	Glomerulonephritis
18	15	F	49	Reflux nephropathy

cyclosporin were administered other than prednisolone which all patients received throughout the study.

6.3 BAYESIAN PARAMETER ESTIMATION

6.3.1 Background

All data were analysed using the four versions of a Bayesian program described below. The four models are summarised in Table 6.2 and the parameters estimated by each of the four models are shown in Table 6.3.

Model 1 - this uses a standard one compartment pharmacokinetic model with a weighting scheme which gives progressively less weight to more distant concentration measurements. Any change in clinical status is therefore accounted for by placing greater emphasis on the most recent concentration measurements. The weighting scheme used is

$$\sigma = 1.001^{T} \times 0.15 \times c_j \qquad \text{Equation 6.1}$$

The weighting scheme used in this study is different from that used in the pharmacokinetic study in Chapter 4, since in the present study the time between concentration measurements is greater.

Model 2 - this is a modified version of the one compartment model just described, where the change in Cl/F is also estimated. This model does not incorporate the weighting scheme shown in Equation 6.1 since all concentration measurements are allowed to contribute

Table 6.2 Description of four versions of the Bayesian program investigated in evaluation study

Model	Description
1	One compartment, constant Cl/F
2	One compartment, changing Cl/F
3	Two compartment, constant Cl/F
4	Two compartment, changing Cl/F

Table 6.3 Pharmacokinetic parameters estimated by the four versions of the Bayesian program in the evaluation study

Model 1	Model 2	Model 3	Model 4
ka	ka	ka	ka
Cl/F	Cl/F(0)	Cl/F	Cl/F(0)
V/F	V/F	V_1/F	V_1/F
	delta Cl/F	α	delta Cl/F
	kCl/F	β	kCl/F
			α
			β

where CL/F(0) is Cl/F at day 0,
delta Cl/F is the total change in Cl/F,
kCl/F is the rate of change of CL/F.

equally to the estimation of the rate and extent of change in Cl/F. Cl/F at any time (Cl/F(t)) is modelled using the following equation with the parameters listed in Table 6.3 (Model 2).

$$Cl/F(t) = deltaCl/F \times e^{-kCl/F \times t} + (Cl/F(0)-deltaCl/F)$$

Equation 6.2

Model 3 - similar to Model 1 except that it uses a two compartment pharmacokinetic model.

Model 4 - similar to Model 2 except that it uses a two compartment pharmacokinetic model.

6.3.2 Data Analysis

The data were analysed in two ways as follows;

(i) using only the first three concentrations to estimate Bayesian pharmacokinetic parameters
(ii) using all available data up to any time point to estimate parameters.

(i) Using the first three concentrations

Data collected in the first two weeks post transplant were used to estimate Bayesian parameters for each patient. According to the sampling schedule, two blood samples for cyclosporin measurement were to be collected from each patient per week although this was not always the case. Also, measurements made within 72 hours of a period of gastro-intestinal dysfunction were

omitted. In every patient however, at least three concentrations were available during the first two weeks and these were used to estimate Bayesian parameters and predict concentrations during subsequent weeks 3 and 4 (Time A) and 5 and 6 (Time B), shown diagrammatically in Figure 6.1.

For each predicted concentration, a prediction error was calculated, ie,

prediction error = observed concentration-predicted concentration.

For all patients at each time period (ie Time A and Time B) the mean prediction errors and their standard deviations were calculated and these were compared between all four models to evaluate relative accuracy and precision.

(ii) Using all available data

A second method of data analysis involved using all data up to any particular time point to estimate Bayesian parameters and to predict the "next" concentration. In this way the first three concentrations were used to predict the fourth (during weeks 2-3), the first five concentrations were used to predict the sixth (weeks 3-4) and the first seven concentrations were used to predict the eighth (weeks 4-5). This is shown diagrammatically in Figure 6.2. The difference between this analysis and the previous analysis was that predictions were being made only 3 or

Figure 6.1 Graphical representation of data analysis using first three concentration measurements only

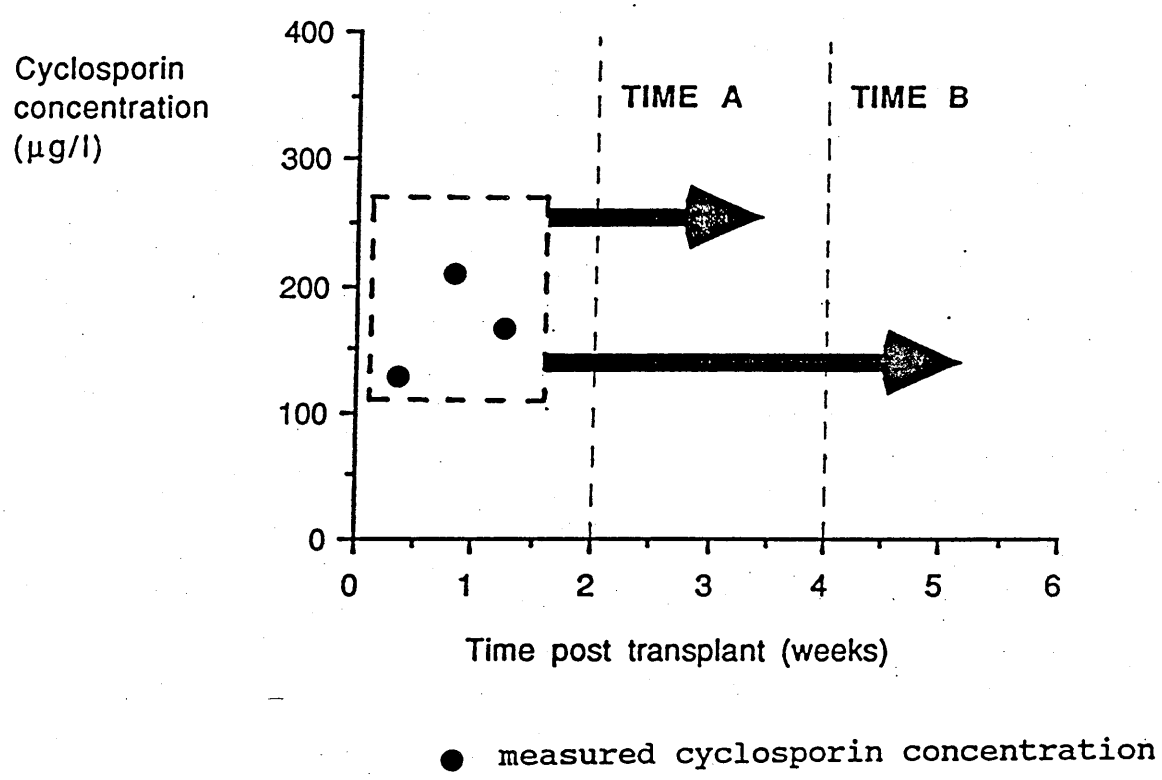
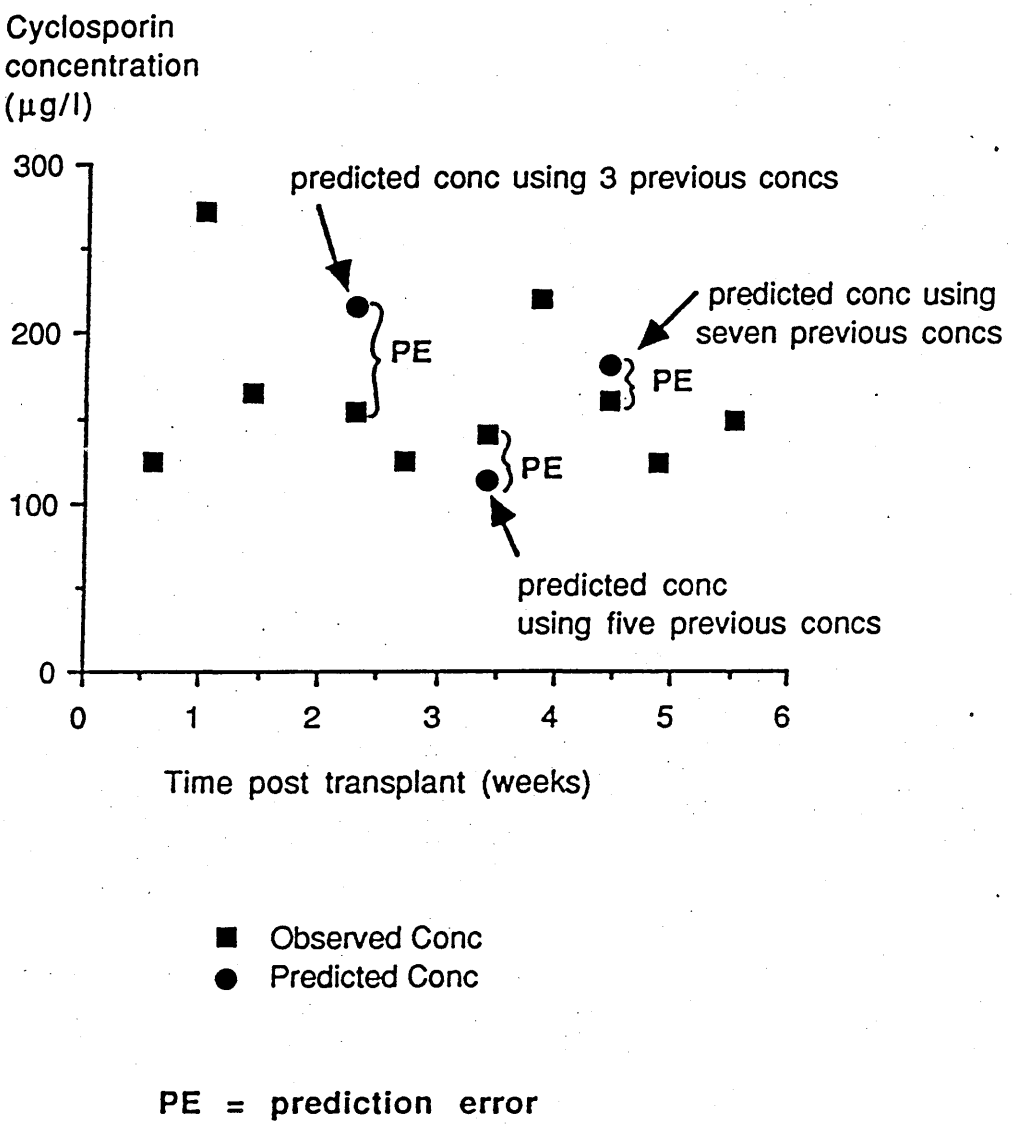


Figure 6.2 Graphical representation of data analysis using all previous data to predict "next" concentration



PE = prediction error

4 days ahead, an approach which is probably more clinically relevant.

6.4 RESULTS

(i) Using the first three concentrations

Figure 6.3 shows observed and predicted concentrations for the four models in one patient (Patient 2). The mean prediction error and standard deviation in each patient during each time period is shown in Table 6.4 (i) - (iv) and a summary of these results is shown in Table 6.5 (i).

Absolute mean prediction errors for each model were compared using Friedman non-parametric analysis of variance. The results of this analysis are shown in Table 6.5(ii): there was no significant difference in the mean prediction error between Models 1 and 3 (the one and two compartment versions of the standard Bayesian program) or between Models 2 and 4 (the one and two compartment versions of the modified Bayesian program) at either Time A or Time B. Models 1 and 2 were, however significantly different from each other at both times as were models 3 and 4. Models 2 and 3 were significantly different. Finally, there was no difference between models 1 and 4 at Time A although they differed at Time B.

The standard deviations of the mean prediction errors were used to assess precision and Table 6.6 (i)

Figure 6.3 Observed and predicted concentrations in one patient (Patient 2) when using first three concentrations only

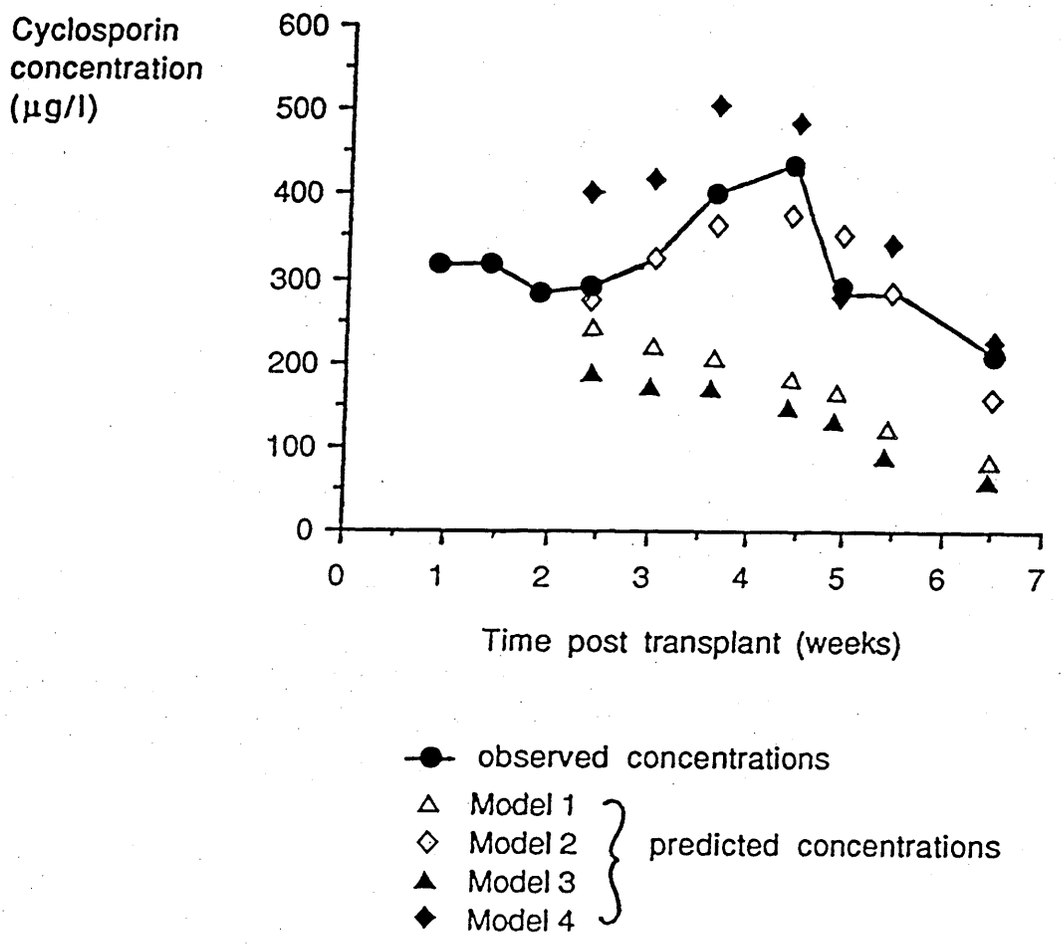


Table 6.4 Mean prediction error (±sd) for each patient at Time A and Time B, when using first three concentrations to revise pharmacokinetic parameters

(i) Model 1 (One compartment,constant Cl/F)

PATIENT	TIME A		TIME B	
	Mean pe	sd	Mean pe	sd
1	16.3	11.8	59.3	55.7
2	115.3	77.8	156.5	74.7
3	66.3	14.7	126.5	52.2
4	52.0	112.5	107.0	29.2
5	26.8	32.3	192.5	40.6
6	63.0	4.2	58.0	64.6
7	168.0	101.9	122.7	55.1
8	77.7	9.3	79.3	17.9
9	51.3	27.4	13.0	51.7
10	102.5	19.4	87.0	56.2
11	-5.0	20.7	22.3	73.5
12	35.0	19.0	35.5	24.9
13	91.3	34.3	141.3	23.8
14	16.8	45.2	120.5	45.0
15	73.1	35.5	55.9	25.1
16	98.0	176.5	197.7	67.5
17	207.3	26.6	154.3	86.7
18	-4.5	37.4	72.3	54.6

Table 6.4 Mean prediction error (±sd) for each patient at Time A and Time B, when using first three concentrations to revise pharmacokinetic parameters

(ii) Model 2 (One compartment,changing Cl/F)

PATIENT	TIME A		TIME B	
	Mean pe	sd	Mean pe	sd
1	2.0	12.2	50.0	54.8
2	23.0	21.3	-20.8	61.6
3	-21.1	23.7	19.8	38.6
4	31.8	72.3	-18.3	38.9
5	1.8	12.9	156.0	42.2
6	-16.0	12.7	-8.3	62.3
7	148.5	103.4	109.7	63.1
8	38.3	4.7	28.3	17.2
9	9.3	42.1	12.5	46.6
10	90.0	21.4	70.5	55.8
11	-16.3	20.1	16.2	72.3
12	-10.3	24.1	-9.8	40.4
13	60.0	28.8	95.3	20.5
14	-15.3	89.5	-25.5	44.7
15	-28.0	38.5	-13.7	28.9
16	-37.3	125.3	52.7	60.5
17	89.5	51.3	-24.8	80.6
18	-42.5	54.4	-20.8	60.0

Table 6.4 Mean prediction error (±sd) for each patient at Time A and Time B, when using first three concentrations to revise pharmacokinetic parameters

(iii) Model 3 (Two compartment,constant Cl/F)

PATIENT	TIME A		TIME B	
	Mean pe	sd	Mean pe	sd
1	51.7	12.5	89.0	58.0
2	161.0	69.3	185.3	80.1
3	85.8	13.7	138.0	51.9
4	85.3	112.3	137.8	30.7
5	55.8	20.2	206.3	38.5
6	100.0	7.1	91.0	63.5
7	189.0	107.2	141.3	59.3
8	78.0	3.0	80.2	17.3
9	58.8	25.8	18.7	51.2
10	75.8	18.1	69.3	54.7
11	-7.0	19.7	22.8	71.9
12	12.0	21.9	12.0	25.7
13	82.3	31.5	132.5	23.4
14	30.8	46.7	128.5	45.0
15	72.0	34.7	56.3	25.3
16	116.0	176.0	213.3	68.0
17	213.3	26.6	158.7	87.2
18	12.0	29.7	94.0	47.5

Table 6.4 Mean prediction error (±sd) for each patient at Time A and Time B, when using first three concentrations to revise pharmacokinetic parameters

(iv) Model 4 (Two compartment,changing Cl/F)

PATIENT	TIME A		TIME B	
	Mean pe	sd	Mean pe	sd
1	53.0	17.0	25.3	65.2
2	-104.3	9.3	-40.8	32.5
3	61.3	14.5	110.8	44.8
4	-51.3	71.0	-20.0	37.3
5	18.8	20.1	176.3	40.6
6	-24.5	30.4	-6.0	53.4
7	161.5	105.8	121.3	64.7
8	77.0	3.6	79.7	17.8
9	45.5	24.3	8.0	52.3
10	23.3	23.1	-20.8	54.9
11	-15.0	19.7	17.8	72.0
12	-13.7	25.6	-39.0	23.9
13	55.0	28.2	88.3	19.6
14	-39.0	50.7	19.5	45.2
15	9.3	47.4	11.4	27.8
16	85.7	172.1	195.3	65.6
17	122.0	24.3	52.5	77.3
18	-3.0	29.7	85.3	44.0

Table 6.5 Comparison of mean prediction errors for models 1 - 4 at Times A and B.

(i) mean (sd)

Model	Time A	Time B
1	69.5 (56.1)	100.1 (55.4)
2	17.1 (51.4)	26.1 (53.1)
3	81.8 (58.9)	109.7 (61.1)
4	25.6 (64.7)	48.1 (70.5)

(ii) Friedman analysis of variance of mean prediction errors.

Model comparisons		Significant difference ($p<0.05$)	
		Time A	Time B
1	2	Yes	Yes
1	3	No	No
1	4	No	Yes
2	3	Yes	Yes
2	4	No	No
3	4	Yes	Yes

Table 6.6 Comparison of standard deviations of mean prediction errors for models 1 - 4 at Times A and B.

(i) mean (sd)

Model	Time A	Time B
1	44.8 (44.8)	49.9 (19.8)
2	42.2 (34.5)	49.4 (17.1)
3	43.1 (45.4)	50.0 (20.2)
4	39.8 (40.9)	46.6 (18.0)

(ii) Friedman analysis of variance of standard deviations of mean prediction errors.

Model comparisons		Significant difference ($p<0.05$)	
		Time A	Time B
1	2	No	No
1	3	No	No
1	4	No	No
2	3	No	No
2	4	No	No
3	4	No	No

shows the mean (sd) of these. The models were compared using Friedman analysis of variance which showed that there was no significant difference in precision between any of the models (Table 6.6(ii)).

(ii) Using all available data

Estimation of Bayesian parameters using all available data was carried out for each of the four models. However, Model 4, the most complex model was not successful when more than three concentrations were used in four of the eighteen patients (patients 3,7,16 and 18). The reasons for this are not clear but it is likely that in these patients variability in concentration-time data was too great to allow estimation of the seven parameters required by model 4. For this reason the present analysis deals only with models 1, 2 and 3. Figure 6.4 shows predicted concentrations using all previous data for the same patient (Patient 2) as shown in Figure 6.3.

The prediction errors at each of the times shown in Figure 6.2 ie concentration 4, concentration 6, and concentration 8, were calculated for models 1 to 3 as shown in Table 6.7 (i) - (iii). Mean (sd) prediction errors are shown in Table 6.8. From this table it can be seen that bias appears to be reduced at each time point (as shown by smaller mean prediction error) using model 2 compared to models 1 or 3.

Absolute prediction errors for the three models

Figure 6.4 Observed and predicted concentrations in one patient (Patient 2) when using all previous data

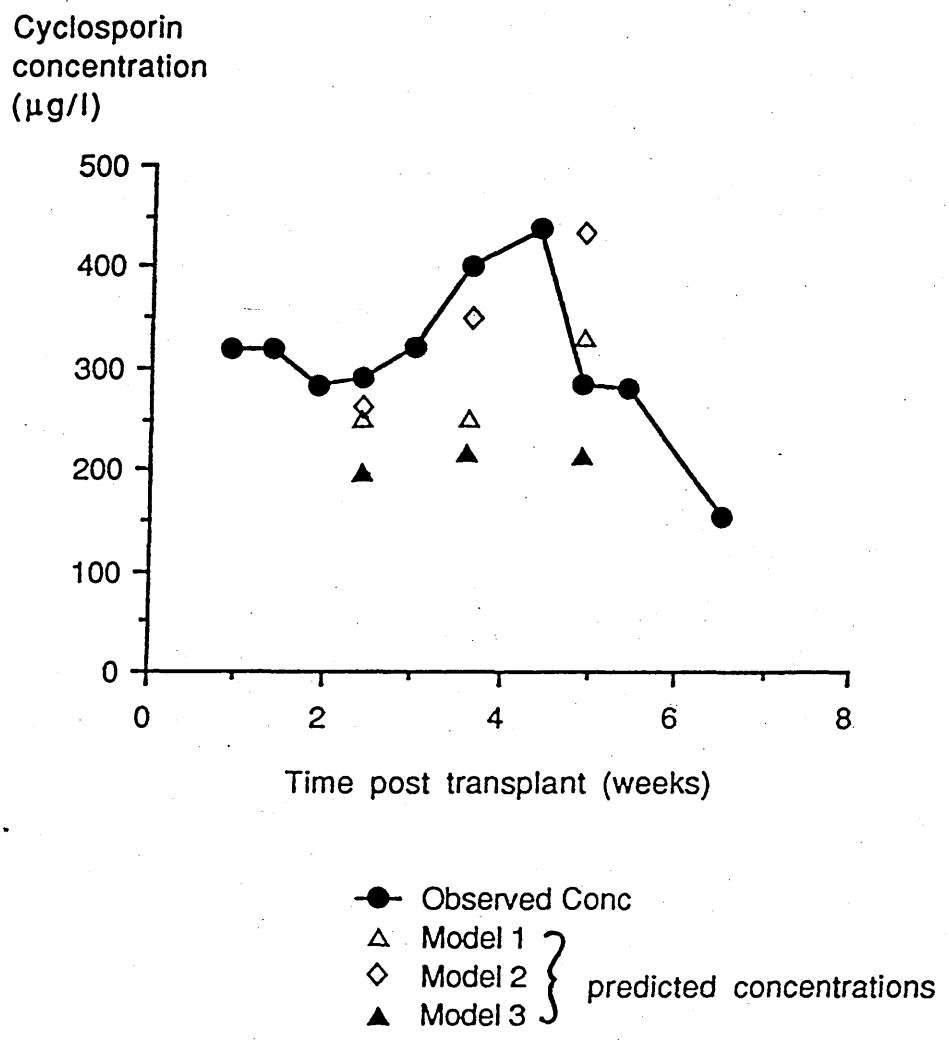


Table 6.7 Prediction errors for each patient when using all previous data for Models 1, 2 and 3.

(i) Concentration 4 (week 3)

PATIENT	Model 1	Model 2	Model 3
1	9	-6	43
2	42	27	96
3	71	12	96
4	-1	72	-12
5	-45	-5	40
6	66	-7	105
7	153	130	181
8	67	40	75
9	58	44	66
10	116	99	83
11	18	6	15
12	54	15	36
13	67	50	64
14	77	24	94
15	55	-67	53
16	-43	-135	-24
17	175	144	181
18	-31	-81	-9

Table 6.7 Prediction errors for each patient when using all previous data for Models 1, 2 and 3.

(ii) Concentration 6 (week 4)

PATIENT	Model 1	Model 2	Model 3
1	39	40	55
2	150	54	189
3	28	11	48
4	61	167	242
5	233	28	68
6	63	63	49
7	56	-46	123
8	59	-3	63
9	10	6	17
10	34	26	62
11	-5	-16	-9
12	-14	-36	-14
13	88	76	99
14	-16	-7	-8
15	-26	-16	34
16	307	300	315
17	218	39	225
18	133	126	141

Table 6.7 Prediction errors for each patient when using all previous data for Models 1, 2 and 3.
(iii) Concentration 8 (week 5)

PATIENT	Model 1	Model 2	Model 3
1	-30	-10	4
2	41	-150	72
3	72	39	84
4	178	115	162
5	106	149	190
6	12	0	59
7	-13	-73	31
8	40	-27	46
9	-65	-73	-58
10	57	37	84
11	8	4	5
12	44	39	41
13	74	44	102
14	106	94	109
15	57	41	59
16	241	12	265
17	38	41	226
18	-13	-51	36

Table 6.8 Mean (sd) prediction errors when using all previous data to predict "next" concentration

Concentration	Model 1	Model 2	Model 3
4	50.4 (60.5)	20.1 (69.7)	65.7 (57.2)
6	78.8 (94.3)	45.1 (83.5)	94.4 (94.1)
8	52.9 (73.3)	12.8 (71.8)	84.3 (81.8)

were compared at each time point using the Friedman analysis of variance (results are shown in Table 6.9). It can be seen that the absolute prediction error associated with concentration 4 was not significantly different between any of the three models although the mean prediction error is less for model 2 than for models 1 or 3 (Table 6.8). The absolute prediction error for the sixth concentration in each patient was not significantly different between the standard and modified versions of the one compartment program although model 3 was significantly poorer than either of the one compartment models. There was no significant difference in absolute prediction error between models 1 and 2 or between models 1 and 3 when the eighth concentration was predicted from the first seven concentrations. Model 2 was significantly better than model 3.

6.5 DISCUSSION

The use of a Bayesian approach to estimate pharmacokinetic parameters has been studied in renal (Kahan et al, 1986b) and in bone marrow transplant patients (Mentre et al, 1988). Both studies assumed that the pharmacokinetics were essentially constant within patients throughout the study. The "standard" approach was examined in Chapter 4 of this thesis and was found to produce biased prediction errors

Table 6.9 Comparison of prediction errors when all previous data are used to predict 'next' concentration.
(Friedman analysis of variance, $p<0.05$).

Concentration	Comparisons		Significant $p<0.05$
	1	2	No
4	1	3	No
	2	3	No
	1	2	No
6	1	3	Yes
	2	3	Yes
	1	2	No
8	1	3	No
	2	3	Yes

due to a time dependent change in Cl/F of cyclosporin in the early post transplant period (Chapter 5). The present chapter has evaluated the use of a modified Bayesian program which takes into account the time dependent change in Cl/F.

A problem in the data analysis in this Chapter was the combined influence of the number of concentration measurements and the time elapsed following transplantation. Mentre et al (1988) suggested the use of two concentration measurements taken at optimum times which vary depending on the weight of the patient. However in the modified program evaluated here, time post transplant was also important. In general, two samples were collected from each patient per week. On several occasions a sample was either not taken or a measurement was omitted due to gastro-intestinal dysfunction. In addition, since these were routinely collected samples, timing of sample collection was not identical between patients. To overcome these problems, three concentrations collected within the first two weeks post transplant were used to estimate parameters.

Models 2 and 4 produced more accurate prediction errors at both time periods than models 1 and 3 respectively. It was of interest that although model 4 (the most complex model) was significantly more accurate than model 1 (the simplest model) at time B, this was not the case at time A where there was no significant difference between the models. It is likely that this

was due to poor parameter estimates in model 4 which had seven parameters compared to three parameters required by model 1.

Neither of the modified programs improved the predictive precision. Several factors could be responsible for this. Cyclosporin is formulated in an oily vehicle as 100mg cyclosporin /ml (Cavanak and Sucker, 1986). In hospital this was measured in a syringe and transferred to a cup by the nursing staff. It is possible that after discharge from hospital, patients were not able to accurately measure the dose of cyclosporin, although it was explained to them before going home. Although the drug was then mixed with fruit juice or milk and the cup rinsed, it was difficult to be sure that the total amount of drug measured out had been swallowed by the patient. It would be interesting to carry out a similar study in patients receiving cyclosporin in soft gelatin capsules. This dosage formulation would enable more accurate administration of cyclosporin and may improve precision of prediction errors.

All patients appeared to understand the importance of taking their cyclosporin regularly and all completed the diaries. Non-compliance was therefore excluded as a source of imprecision.

The times of food intake may have affected the extent of cyclosporin absorption. In hospital,

cyclosporin was administered approximately one hour after the evening meal. Co-administration of cyclosporin with food may either decrease absorption (Keown et al, 1982) or may increase it (Ptachcinski et al, 1985c). Since timing of cyclosporin dosage in relation to meals was not recorded after discharge, it is possible that this was an added source of variability. However the effect of food can not be solely responsible for the poor precision, since up to two fold intra-individual variation in AUC has been seen in fasting, healthy volunteers (Lindholm et al, 1988c).

The second data analysis, where all previous data were used to predict the next concentration, was more clinically relevant. This comparison was carried out for models 1, 2 and 3 as discussed earlier. As before, the problem of number of samples in relation to time post transplant arose. The concentrations to be predicted were therefore within defined time periods using a specific number of concentration measurements. In each case, model 2 resulted in a smaller mean prediction error suggesting that this model was less biased. Friedman analysis of variance of absolute prediction errors showed no significant differences between models 1 and 2 at any of the times. In some instances model 3 was significantly poorer than models 1 and 2. This may be due either to model 3 being an inappropriate model, or to poor prior estimates of the parameters.

The prior estimates used throughout this thesis were taken from the literature (for pharmacokinetic parameters) or from the results in Chapter 5 (for the time dependent parameters). These were the best estimates available.

In conclusion, the use of a modified program improved accuracy but not precision when predictions were made several weeks ahead. Although cyclosporin can be described by a two compartment model, this model showed no advantage. If predictions are to be made only a few days ahead, it was shown that the standard and modified one compartment versions of the program were not significantly different.

CHAPTER 7

EFFECT OF DIVERSION OF BILE FLOW BEFORE AND AFTER CLAMPING OF THE BILIARY T-TUBE ON THE PHARMACOKINETICS OF CYCLOSPORIN IN LIVER TRANSPLANT PATIENTS

7.1 INTRODUCTION

The introduction of cyclosporin has been a major factor in improving the success rate of liver transplantation (Starzl, 1985). As with renal transplantation however, (Chapters 4 and 6), significant pharmacokinetic variability necessitates frequent monitoring of blood cyclosporin concentrations (Burckart et al, 1986a; Venkataramanan et al, 1985c).

Liver transplant patients usually have a biliary T-tube inserted into the common bile duct during surgery. This is to drain bile and prevent blockage of the gall bladder during the recovery period. However, as bile is necessary for the absorption of cyclosporin (Ericzon et al, 1987), external biliary drainage may lead to poor absorption. This is important clinically because increases in trough concentrations after clamping of the T-tube have been reported (Andrews et al, 1985), and a comparative bioavailability study has suggested that absorption improves following clamping (Mehta et al, 1988). It is unlikely that enterohepatic recycling of cyclosporin is involved in increasing bioavailability following T-tube clamping since very little drug is excreted unchanged in the bile (Venkataramanan et al, 1985b).

The aim of the work described in this chapter was to examine the oral and intravenous pharmacokinetics of cyclosporin before and after clamping of the T-tube,

leading to estimates of bioavailability and clearance. Any pharmacokinetic differences before and after clamping will be discussed in relation to demographic data collected during the course of the study.

7.2 METHODS

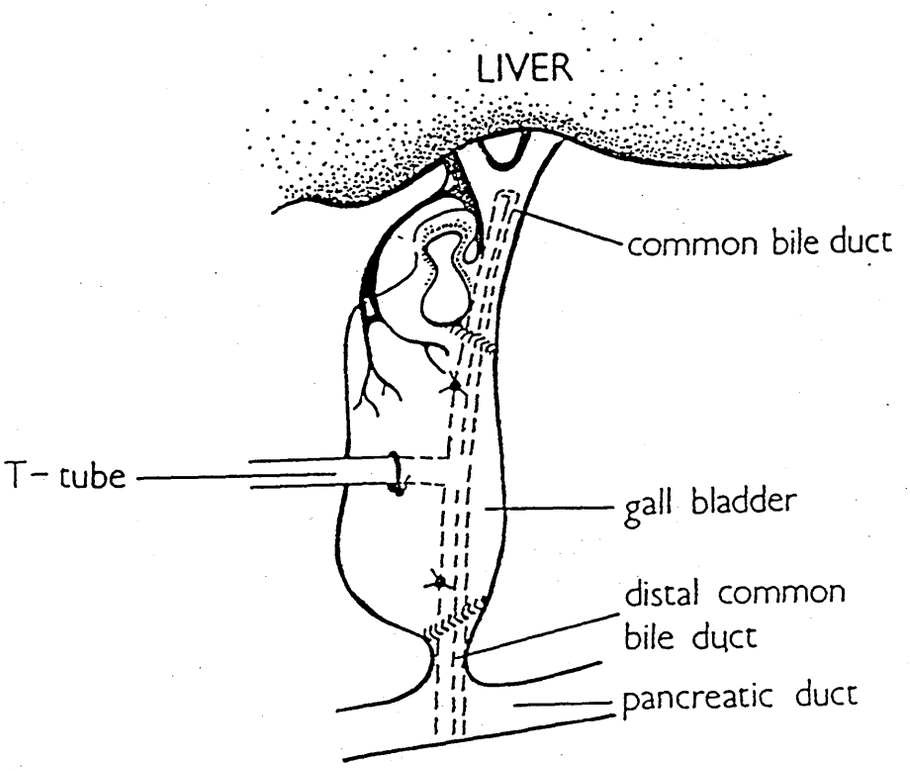
7.2.1 Insertion of Biliary T-tube

During liver transplant surgery a T-tube is inserted into the bile duct of the recipient (Figure 7.1). The function of the T-tube is to allow drainage of bile to an external route in order to minimise the chance of biliary complications or obstruction occurring due to oedema following surgery. The T-tube was inserted about three centimetres into the common bile duct and the emerging long limb secured to the gall bladder wall and the skin of the abdomen. The T-tube is allowed to drain externally (unclamped) or is clamped so that bile flows back to the gut. The tube is typically unclamped for between two and five weeks following surgery. After this the tube is clamped and if no complications arise it is completely removed after eight or twelve weeks (Calne, 1976).

7.2.2 Patients

Thirteen consecutive orthotopic liver transplant patients were recruited. All were receiving cyclosporin as their main immunosuppressant therapy. Two patients died between the unclamped and clamped phases: eleven

Figure 7.1 Diagram showing insertion of biliary T-tube in liver transplant patients



From Calne, 1976.

patients completed the study. Clinical details of the patients studied are shown in Table 7.1.

7.2.3 Cyclosporin Therapy and Blood Sampling

Cyclosporin was administered after an overnight fast, either orally as a solution or infused intravenously in 100ml saline at a constant rate over a two hour period. For each patient, during both the unclamped and clamped phases, an oral and an intravenous study were carried out within 48 hours of each other. The dose of cyclosporin was based on clinical requirements determined by evidence of toxicity or rejection. Oral doses of cyclosporin ranged from 4-14mg/kg orally and 1.5-3.5mg/kg intravenously. All doses, routes and times of administration were accurately recorded by hospital staff during both periods of the study.

Serial blood sampling was carried out following oral and intravenous dosing. Blood samples for measurement of cyclosporin concentrations were collected at 0, 0.5, 1, 2, 2.5, 3, 4, 6, 8 and 10 hours after the start of the intravenous infusion and at 0, 1, 2, 3, 4, 6, 8, 10 and 12 hours after an oral dose. All blood samples were collected in EDTA tubes and frozen at -20^{o}C until required for analysis by HPLC (Chapter 2).

Table 7.1 Clinical details of liver transplant patients completing the study.

Patient	Age	Sex	Diagnosis
1	48	M	Primary hepatic malignancy
2	38	M	Primary hepatic malignancy
3	35	M	Primary sclerosing cholangitis
4	27	F	Chronic active hepatitis
5	48	M	Primary hepatic malignancy
6	39	F	Primary biliary cirrhosis
7	54	F	Primary biliary cirrhosis
8	38	M	Primary hepatic malignancy
9	22	F	Chronic active hepatitis
10	41	M	Primary hepatic malignancy
11	21	F	Acute hepatic failure

7.2.4 Data Collection

At the start of the study the clinical details shown in Table 7.1 were recorded for each patient. Throughout the study, body weight, temperature and blood pressure were observed. In addition, the following biochemical and haematological measurements and clinical observations were made: serum creatinine concentration, bilirubin, alkaline phosphatase (Alp), haematocrit, haemoglobin, albumin concentration and periods of nausea, vomiting or diarrhoea. In addition, initiation or discontinuation of drugs known to affect pharmacokinetics of cyclosporin, or a change in dose of such a drug, was noted.

7.2.5 Data Analysis

The area under the concentration-time curve (AUC_0^t) following either oral or intravenous administrations was calculated using the linear trapezoidal rule (Section 3.2.4). Due to complex dosing schedules of cyclosporin required in the early stages after liver transplantation, it was not possible to assume that steady state conditions existed. AUC from time zero to infinity (AUC_0^∞) was therefore determined by estimating the terminal elimination rate constant (β) from the linear portion of the log concentration versus time curve and using equation 7.1.

$$AUC_0^{\infty} = AUC_0^{t} + \frac{conc(t) - conc(0)}{\beta} \qquad \text{Equation 7.1}$$

This assumed that the terminal elimination phase represented only elimination, and that distribution was complete. Unfortunately, only three concentration measurements were usually available to estimate the terminal elimination rate constant so this could only be an approximation. Bioavailability (F) was then calculated as follows

$$F(\%) = \frac{AUC_{0oral}^{\infty}}{AUC_{0iv}^{\infty}} \times \frac{dose_{iv}}{dose_{oral}} \times 100 \qquad \text{Equation 7.2}$$

Clearance was estimated from intravenous data using the following equation

$$\text{Clearance (l/h)} = \frac{dose(iv)}{AUC_0^{\infty}(iv)} \qquad \text{Equation 7.3}$$

Bioavailability and clearance values before and after clamping of the T-tube were compared using the Wilcoxon Ranked Pairs test.

7.3 RESULTS

At the times of the unclamped and clamped studies no patient had any gastro-intestinal dysfunction. Administration of other drugs did not differ between the two study periods. In addition no patient received any drug known to inhibit or induce hepatic enzymes except for low dose prednisone which was given to all patients

during both study periods.

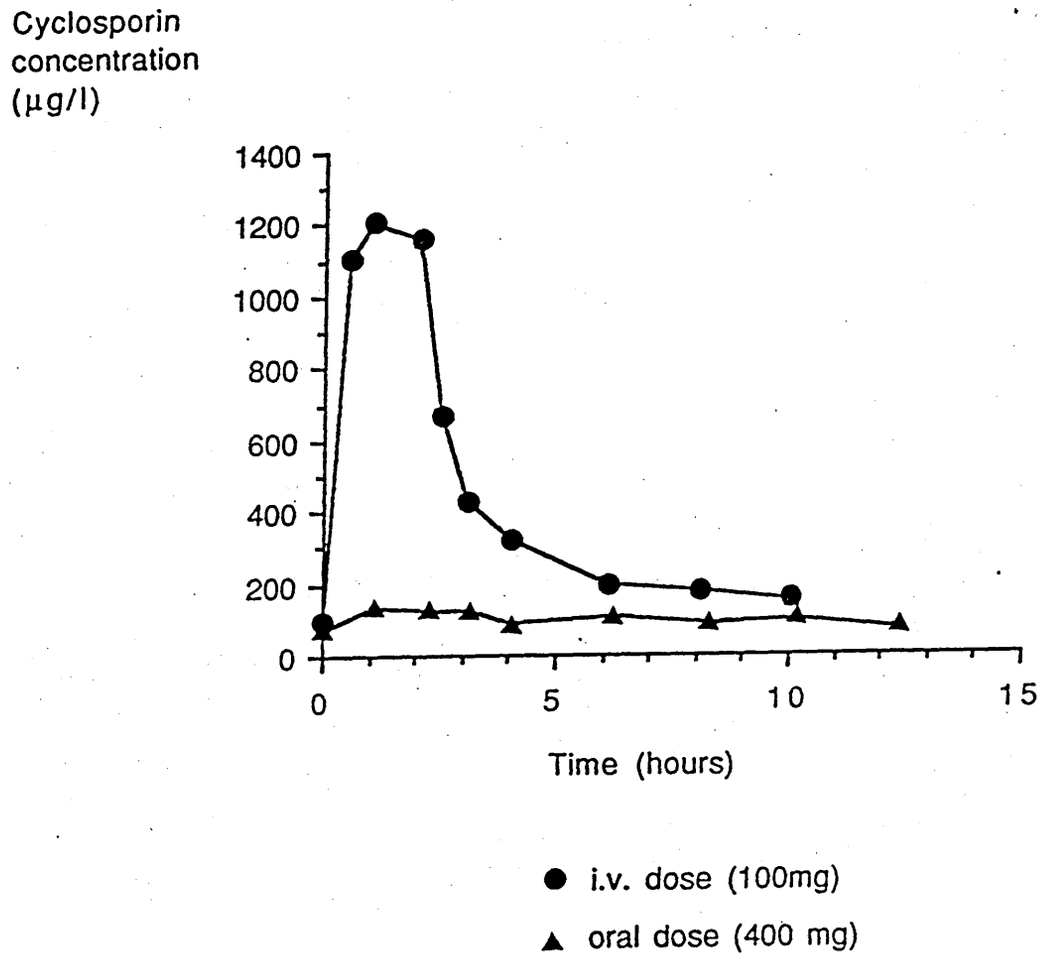
Concentration-time profiles following oral and intravenous dosing before and after clamping of the biliary T-tube are shown for a representative patient (patient 8) in Figure 7.2 (i) and (ii). Both intravenous profiles show high concentrations following the 2 hour infusion period. The oral profiles, however, obviously differ from each other. While the T-tube is unclamped (free bile drainage) the profile is relatively flat. After clamping noticeably higher concentrations are achieved, despite a reduction in the dose.

The oral and intravenous doses administered to each patient and the corresponding estimates of AUC_0^∞ during the unclamped and clamped studies are shown in Tables 7.2 and 7.3 respectively. The dose normalised AUC_0^∞ after oral dosing, and the bioavailability were higher in all patients except for patients 2 and 5. This will be discussed later in this chapter.

Bioavailability and clearance were calculated for each patient using the data in Tables 7.2 and 7.3. The results for this are summarised in Table 7.4. Bioavailability while the T-tube was unclamped ranged from 3.0% to 34.7% with a mean (sd) of 14.5 (11.5). After clamping of the T-tube, bioavailability ranged from 5.3% to 54.5% and the mean (sd) had increased to 24.0 (14.2). Using the Wilcoxon Ranked Pairs test this difference was not significant.

Figure 7.2 Oral and intravenous concentration time profiles before and after clamping of T tube (patient 8)

(i) T tube unclamped



(ii) T tube clamped

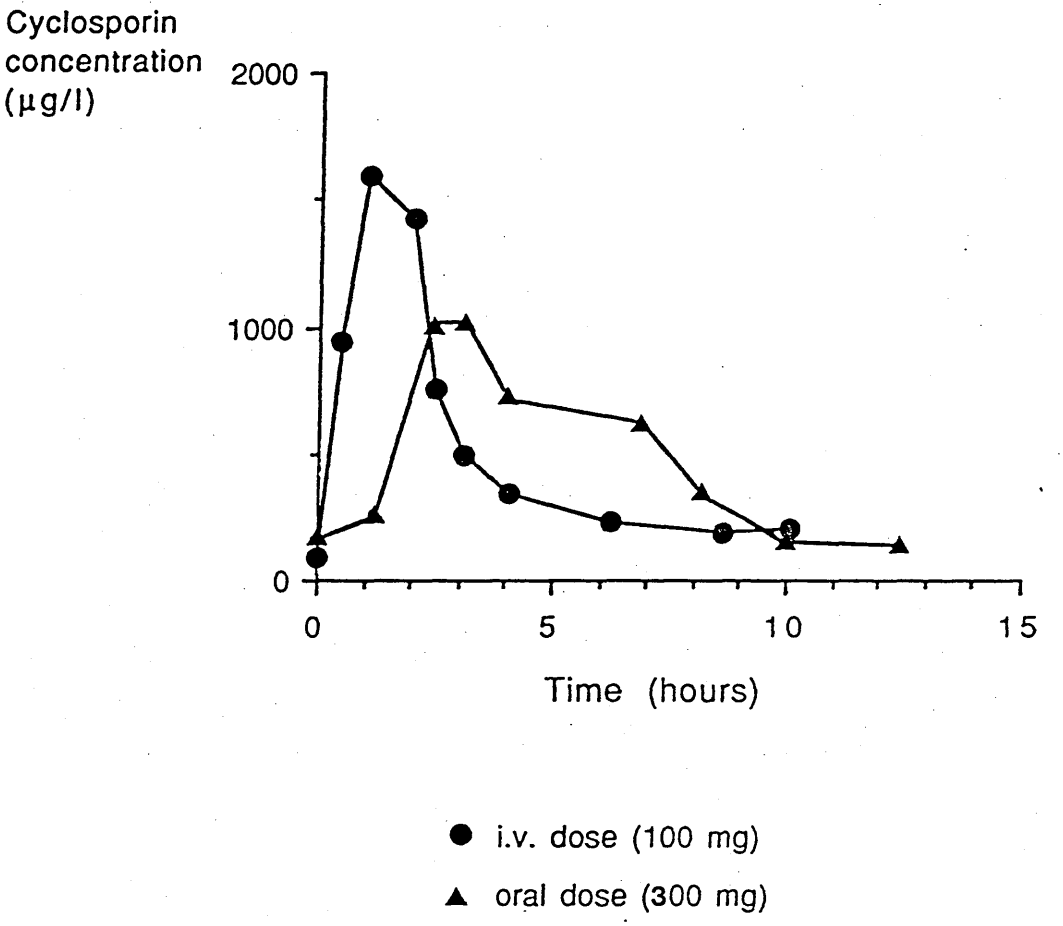


Table 7.2 Dose of cyclosporin administered to each patient and estimates of AUC_0^∞ for oral and intravenous studies when T-tube is unclamped

Patient	Oral			Intravenous		
	dose (mg)	AUC (ug/l.h)	AUC/dose (1/l.h)	dose (mg)	AUC (ug/l.h)	AUC/dose (1/l.h)
1	800	3202	0.004	200	6091	0.031
2	300	8018	0.027	75	5782	0.077
3	400	1783	0.004	100	3222	0.032
4	400	699	0.002	100	5846	0.058
5	400	4569	0.011	100	4393	0.044
6	350	3242	0.009	100	2824	0.028
7	400	658	0.002	100	3445	0.034
8	400	1344	0.003	100	5383	0.054
9	300	881	0.003	100	4465	0.045
10	300	954	0.003	100	6915	0.069
11	400	3098	0.008	120	6500	0.054
Mean			0.007			0.048
sd			0.007			0.016

Table 7.3 Dose of cyclosporin administered to each patient and estimates of AUC_0^∞ for oral and intravenous studies when the T-tube is clamped

Patient	Oral			Intravenous		
	dose (mg)	AUC (ug/l.h)	AUC/dose (1/l.h)	dose (mg)	AUC (ug/l.h)	AUC/dose (1/l.h)
1	800	8190	0.010	200	8627	0.043
2	500	1073	0.002	100	4088	0.041
3	300	2542	0.008	100	4338	0.043
4	500	9538	0.019	150	7516	0.050
5	400	1360	0.003	100	4978	0.050
6	500	7370	0.015	85	2300	0.027
7	500	3202	0.006	100	3842	0.038
8	300	5108	0.017	100	8554	0.086
9	350	4638	0.013	150	5671	0.038
10	600	11178	0.019	165	17457	0.106
11	500	9204	0.018	100	6764	0.068
Mean			0.012			0.054
sd			0.006			0.024

Table 7.4 Estimates of clearance and bioavailability before and after clamping of the T-tube

Patient	UNCLAMPED		CLAMPED	
	Cl(l/h)	F(%)	Cl(l/h)	F(%)
1	32.8	13.1	23.2	23.7
2	13.0	34.7	24.5	5.3
3	31.0	13.8	23.1	19.5
4	17.1	3.0	20.0	38.1
5	22.8	26.0	20.1	6.8
6	35.4	32.8	37.0	54.5
7	29.0	4.8	26.0	16.7
8	18.6	6.2	11.7	19.9
9	22.4	6.6	26.5	35.1
10	14.5	4.6	9.5	17.6
11	18.5	14.3	14.8	27.2
Mean (sd)	23.2 (7.7)	14.5 (11.5)	21.5 (7.7)	24.0 (14.3)

Estimates of clearance ranged from 13.0l/h to 35.4l/h with a mean (sd) of 23.2l/h (7.7) during the unclamped study. During the clamped study, clearance ranged from 9.5l/h to 37.0l/h with a mean (sd) of 21.5l/h (7.7). This difference was compared using the Wilcoxon Ranked Pairs test and was not significant.

The demographic data collected during the course of the study were examined. The data are shown for the unclamped and clamped study in Table 7.5. Measurements made before and after clamping of the T-tube were compared using the Wilcoxon Ranked Pairs test ($p<0.05$) and the results shown in Table 7.6. There were no significant differences in patient weight, albumin, bilirubin, alkaline phosphatase or serum creatinine concentrations between the two study periods. Haematocrit and haemoglobin both increased significantly from the time of the unclamped study to the clamped study ($p<0.05$).

7.4 DISCUSSION

After liver transplantation, most patients receive cyclosporin intravenously in order to overcome the effect of poor absorption of the drug. Low absorption of cyclosporin in these patients is thought to result from the external diversion of bile away from the gut by T-tube (Mehta et al, 1988). This is consistent with knowledge that the presence of bile salts in the gut are necessary for solubilisation and absorption of

Table 7.5 Haematological, biochemical and patient data collected when T-tube was unclamped and clamped

Patient	uc/c	Weight kg	Hct %	Hb g/dl	Alb g/l	Bil umol/l	Alp IU/l	Crea umol/l
1	uc	58	30.4	10.1	35	22	240	107
	c	58	37.8	11.5	36	22	195	126
2	uc	76	32.4	10.8	22	581	603	210
	c	69	36.9	11.8	32	390	1119	79
3	uc	62	35.0	11.4	28	238	425	73
	c	57	40.7	11.8	39	116	991	77
4	uc	47	32.7	10.5	33	55	553	54
	c	46	33.7	11.2	35	33	648	73
5	uc	70	32.9	11.0	35	93	300	90
	c	64	36.7	11.8	29	572	2581	97
6	uc	49	30.0	10.9	44	71	644	72
	c	52	38.1	11.6	40	20	158	72
7	uc	50	26.1	9.0	42	58	197	176
	c	63	34.1	10.9	38	20	337	131
8	uc	57	38.9	13.4	39	78	363	80
	c	60	42.3	13.1	39	39	380	61
9	uc	57	40.2	13.1	48	103	600	54
	c	65	42.9	14.0	46	26	329	50
10	uc	60	39.1	13.1	30	278	374	92
	c	58	45.8	16.0	39	46	135	78
11	uc	54	27.5	9.2	38	72	205	113
	c	54	27.0	9.0	38	38	173	113

uc - unclamped, c - clamped, hct - haematocrit,

hb - haemoglobin, alb - albumin, bil - bilirubin,

alp - serum alkaline phosphatase, crea - serum creatinine

Table 7.6 Comparison of haematological, biochemical and patient data before and after clamping of the T- tube using Wilcoxon Ranked Pairs Test

Variable	Significant p<0.05
Weight	No
Haematocrit	Yes
Haemoglobin	Yes
Albumin	No
Bilirubin	No
Alkaline phosphatase	No
Serum Creatinine	No

cyclosporin (Ericzon et al, 1987). Information on the effect of clamping the T-tube and allowing bile to flow into the gut is of clinical interest because it influences the point at which intravenous therapy can be replaced by oral therapy.

The effect of time post-operatively on the pharmacokinetics of cyclosporin may be of importance in liver transplant patients since such an effect has been observed in renal transplant patients both in this thesis (Chapters 4 and 5) and in previous work (Kahan et al, 1983). Either an increase in bioavailability or a decrease in clearance could cause the increase in trough cyclosporin concentrations in liver transplant patients after clamping of the T-tube reported by Andrews et al (1985). Due to the clinical design of this study, the unclamped study was carried out before the clamped study in all patients and it was therefore not possible to study the effect of time post-operatively. However it is interesting to note that the lowest bioavailability observed during the unclamped study (3.0% in patient 4) was at 10 days post transplant which was the earliest time studied. Similarly the largest increase in bioavailability between the two study days was 28.5% (patient 9), where the clamped study was carried out 162 days after transplant. The effect of time post-operatively can not be ignored, as improving liver function and bile flow will result in improving

absorption over time (Burckart et al, 1986c).

In 11 patients studied in this thesis, the apparent change in bioavailability was from 14.5% to 24.0% although this difference was not significant. Mean clearance decreased from 23.2l/h to 21.5l/h, but again the difference was not significant. However, it was noted that although bioavailability increased in nine patients and decreased in two, there was an important clinical difference between the two groups of patients. The two patients (Patients 2 and 5) in whom bioavailability decreased at the time of the clamped study were suffering from vanishing bile duct syndrome, a condition characterised by a rapid destruction and disappearance of the interlobular bile ducts, associated with chronic rejection and cholestasis. Bile flow into the gut is impaired in these patients (Uchida et al, 1989). The fact that these two patients did not show increased bioavailability after clamping supports the theory that improved bile flow to the gut improves absorption of cyclosporin.

The increased bioavailability in the nine patients with functioning grafts at the time of the clamped study (unclamped mean bioavailability 11.0%, clamped mean bioavailability 28.0%) was probably due to increased bile flow to the gut following clamping. In these nine patients there was no significant change in clearance between the two study periods.

Of the nine patients, one received the same oral

dose during both study periods, six received an increased dose and two received a decreased dose at the time of the clamped study. A dose dependent effect due to limited drug solubility in the gut (Reymond et al, 1988) is therefore unlikely to be responsible for increased bioavailability.

Various biochemical and haematological parameters measured during both study periods were compared (Table 7.6). The number of red blood cells (as measured by haematocrit) and their haemoglobin content increased significantly from the time of the unclamped study to the time of the clamped study. Any change in binding of cyclosporin may affect its disposition (as discussed in Chapter 5 for renal transplant patients). Cyclosporin is highly bound to erythrocytes, and the increase in haematocrit and haemoglobin may represent increased binding of the drug. Since cyclosporin is a drug with low to intermediate extraction (Venkataramanan et al, 1985a), its clearance would be expected to be altered by changes in binding (Yee et al, 1988b). There was, however, no change in clearance as might have been expected if binding of the drug was altered (Kasiske et al, 1988).

Cyclosporin is almost completely metabolised in the liver and it is known that moderate hepatic dysfunction, as determined by elevated serum bilirubin concentration, delays clearance of the drug (Yee et al, 1984). No

change in clearance at the two study periods was detected. It is of interest to note, however, that in the two patients with poor liver function at the time of the clamped study, patient 5 showed only a slight change in clearance and patient 2 actually showed a large increase in clearance between the two study periods.

The findings in this study must be compared to the results discussed earlier in renal transplant patients. Following renal transplant it is known that concentrations of cyclosporin increase (Kahan et al, 1983) and it has been postulated in Chapters 4 - 6 that this is a time dependent change in either clearance, bioavailability or a combination of both factors. The effect of time post transplant may well be a contributory factor and it is likely that improvements in patients' physiology during the recovery post-operatively may alter pharmacokinetics (Venkataramanan et al, 1989). The clinical aspects involved in the study required that the unclamped study was always carried out first. Because of this it was impossible to investigate time as a contributory factor in the change in pharmacokinetics and it cannot be ruled out.

In conclusion, no significant improvement in bioavailability of cyclosporin was observed following clamping of the T-tube in 11 consecutive liver transplant patients. However, two patients with acute rejection of the graft in whom bioavailability decreased, may have biased the data analysis.

The results from this study, suggest that increased cyclosporin concentrations following clamping of the T-tube are not due to changes in clearance of the drug. Further work is required to fully establish the effect of T-tube clamping on the bioavailability of cyclosporin.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

In this thesis, pharmacokinetic variability of cyclosporin has been investigated in renal and liver transplant patients. The variability has been studied both within and between patients, and of particular interest is the variability occurring in the first few weeks following transplantation. Wide pharmacokinetic variability has been observed in the early post-operative period following either renal or hepatic transplantation (Kahan et al, 1983; Burckart et al, 1986a). The aim of this thesis was to quantify pharmacokinetic variability in order to improve the control of cyclosporin dosage adjustment.

Previous work has shown that regular monitoring of trough blood concentrations of cyclosporin is useful in prevention of transplant rejection (associated with low concentrations) and prevention of toxicity (associated with high concentrations) (Kahan et al, 1984). Standard dosage adjustment procedures, however, require that the patient is at pharmacokinetic steady state; in the case of cyclosporin this means that the patient must have been receiving the same dose of drug for at least three days.

A pharmacokinetic approach suggested by Kahan and Grevel (1988) proposes that cyclosporin dosage adjustments are based on individual pharmacokinetic parameter estimates. The disadvantage of such an approach is that it requires collection of many blood samples in each dosage interval from each patient.

An alternative approach is to use Bayesian methods as discussed earlier (Kahan et al, 1986b; Mentre et al, 1988). In this thesis, it was shown that either a one or a two compartment version of a Bayesian program was unable to provide accurate predictions of cyclosporin concentrations in renal transplant patients in the first few weeks after transplantation. Concentrations were consistently under predicted. Examination of estimates of Cl/F and V_1/F showed that these pharmacokinetic parameters declined over time after surgery. This finding supported the frequent observation that immediately after renal transplantation, patients require high doses of cyclosporin and this dose can be gradually decreased (Tufveson et al, 1986). The estimates of Cl/F were investigated in detail; previous reports had suggested that elimination decreases over time (Habucky et al, 1988), that F increases (Kahan et al, 1983; Wilms et al, 1988), or that both bioavailability and elimination are altered (Newburger and Kahan, 1983).

Graphs of Cl/F in individual patients and a graph of mean Cl/F over time in eleven patients suggested an exponential decline over time. Three monoexponential models were therefore investigated which related Cl/F and time (Chapter 5). Time was found to adequately describe the change in Cl/F within patients although there was wide inter-patient variability.

The possibility that some factor other than time was responsible for the change in pharmacokinetics was investigated. Other factors measured throughout the study (eg biochemical or haematological factors) showed upward or downward trends which may have contributed to the variability in Cl/F. The change in these other factors was probably due to the dramatic changes in physiology that these patients undergo following successful renal transplantation (Venkataramanan et al, 1989). These factors were investigated using multiple linear regression techniques (GLIM) and non-linear mixed effects modelling (NONMEM). However, it was impossible to determine whether the trends in these other variables were responsible for the change in Cl/F or whether it was a spurious association. Time post transplant was the most powerful explanatory variable. Even if one of the other factors had proved more useful than time in explaining the change in Cl/F, the problem of predicting that factor in an individual patient would then have been a difficulty. However, further work to establish if there is a relationship between some biochemical or haematological variable and cyclosporin pharmacokinetics would be of great interest. Such work would require collection of data from a considerably larger number of patients.

The most appropriate monoexponential model investigated (Model 1, Chapter 5), was incorporated into a modified Bayesian program to allow a gradual change in

Cl/F. This modified program estimated three parameters related to Cl/F; namely Cl/F(0) which was the value of Cl/F extrapolated back to zero time, kCl/F which was the rate of change in Cl/F, and deltaCl/F which was the total change in Cl/F. Substituting individual estimates of these parameters into Equation 5.1 enabled estimation of Cl/F(t) ie Cl/F at time t. This modification to the program was carried out for both the one and two compartment versions of the standard program.

A further group of renal transplant patients was recruited for evaluation of this modification. Four versions of a Bayesian program were evaluated; these were standard versions using either a one or a two compartment pharmacokinetic model and one and two compartment versions which had been modified to take into account the change in Cl/F. The ability of each version to predict cyclosporin concentrations was investigated. Future concentrations were predicted as far as four weeks. Although predicting as far ahead as this was not representative of the clinical use of such a program, it did provide useful information about accuracy and precision. It was seen that the one and two compartment versions of the modified program significantly improved the accuracy of the predictions but did not improve precision. Further analysis of the data, predicting concentrations only a few days ahead of time showed no significant difference in prediction

errors between a modified and a standard version of the one compartment program. This was probably due to the fact that only a small change in Cl/F would have taken place in the few days between estimation of pharmacokinetic parameters and the predicted concentration. The poor precision seen in all models may not have allowed detection of a small improvement in accuracy. However since the analysis predicting several weeks ahead of time showed the modified version to predict more accurately, it appears that this is a more appropriate model for cyclosporin. Poor precision of the prediction errors was observed and may have been due to one or more factors. Although non-compliance was eliminated as a possible source of imprecision, some of the patients may have had difficulty after discharge in measuring the correct dose of cyclosporin to take. Possibly some patients did not mix the cyclosporin and diluent adequately and some of the drug may have remained on the wall of the cup. Further evaluation of Bayesian estimation would be of interest in patients taking the more recent formulation of cyclosporin in soft gelatin capsules since this would eliminate this source of variability. Another factor which may have contributed to the poor precision was the timing of the dose of cyclosporin in relation to meals. There is considerable controversy as to the effect of food on cyclosporin pharmacokinetics (see Section 1.3.1) and a possible effect can not be ruled out. From the data

available it was not possible to differentiate changes in biochemical or haematological measurements from the effect of time. It is possible, however, that one or more of these factors may have contributed to the change in Cl/F. Possibly the inclusion of patient weight, serum creatinine concentration or ALAT in a future model may improve the precision. Further investigation of the relationship between cyclosporin pharmacokinetics and demographic variables is required.

A study was carried out in liver transplant patients with the aim of identifying changes in the pharmacokinetics of cyclosporin, in particular bioavailability, following clamping of the biliary T-tube. No significant difference in either bioavailability or clearance of cyclosporin was seen. However, two patients who had deteriorating liver function with severe cholestasis and vanishing bile duct syndrome at the time of the post clamped study may have complicated the results. Omitting these two patients on the basis of this diagnosis resulted in a significant increase in bioavailability. It is possible that improved liver function at the time of the clamped study or a time dependent change as seen in renal transplant patients is involved in improved absorption.

In conclusion, this thesis has examined pharmacokinetics of cyclosporin in renal and liver transplant patients and has quantified some of the

variability observed. A modified version of a Bayesian program has been developed which takes into account some of the intra-individual variability in renal transplant patients. However, further work is required to improve the precision of the prediction errors. Development of a version of a Bayesian program suitable for use in liver transplant patients would be of future interest.

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APPENDICES

Appendix 1. Specific radioimmunoassay used to measure cyclosporin concentrations in Chapter 6.

Cyclosporin concentrations in Chapter 6 were measured by a specific radioimmunoassay method (RIA) in the Department of Biochemistry at the Western Infirmary, Glasgow. The method uses a monoclonal antibody which is specific for cyclosporin (Quesinaux et al, 1987) and an iodine-125 labelled cyclosporin derivative as the ligand. Results obtained using this assay are comparable to results from HPLC methods similar to that used in this thesis (Schrann et al, 1987). The assay was supplied in kit form by the manufacturers (CYCLO-Trac SP RIA Whole Blood, Incstar Corporation). Analysis was carried out using the method recommended by the manufacturers in the insert supplied with the kit except in the preliminary extraction step where 1000ul of methanol were used instead of the recommended 400ul. Validation of the method was as follows.

Accuracy

Accuracy was assessed by spiking whole blood with three known concentrations of cyclosporin and assaying these samples in triplicate. Results of this are given in Table A1.1.

Precision

Between day precision was assessed by repeat analysis of quality assurance samples supplied by the manufacturers. Results of this are given in Table A1.2.

Specificity

Specificity of the antibody for cyclosporin has been extensively tested by the manufacturers and was not included in this validation.

Sensitivity

The lower limit of detection of the assay was assumed to be that of the lowest calibration standard which was 20ug/l.

Table A1.1 Assessment of accuracy of specific radio-immunoassay

Spiked concentration	% Accuracy
1000ug/l	90
500ug/l	94
250ug/l	92

Table A1.2 Assessment of between day precision of specific radio-immunoassay

QC no.	n	Mean concentration	CV(%)
1	24	113.6ug/l	9.9
2	24	363.1ug/l	6.4

Appendix 2 Method of Laplace Transformation used to solve differential equations in Chapter 3 (Gibaldi and Perrier, 1975).

GLOSSARY

D	Dose
F	Bioavailability
k_a	First order absorption rate constant
t	Time after dose
X	Amount
Conc	Concentration
V	Volume
Cl	Clearance
k	First order rate constant

Subscripts;

1	Central compartment
2	Peripheral compartment
e	Elimination

MODEL 1 One compartment model with first order absorption

Differential equations

$$dG/dt = -kaG \qquad \text{Eq 1}$$

$$dX/dt = kaG - keX \qquad \text{Eq 2}$$

Equation 1 is solved as follows;

$$sL(G) - Go = -kaL(G) \quad \text{where Go=Dose (D) at t=0.}$$

$$L(G) = D/(s + ka) \qquad \text{Eq 3}$$

Equation 2 is solved;

$$sL(X) - Xo = kaL(G) - keL(X) \quad \text{where Xo = 0 for first dose}$$

$$L(X)(s+ka) = kaL(G) \qquad \text{Eq 4}$$

$$L(X) = \frac{kaL(G)}{(s+ka)} = \frac{kaD}{(s+ka)(s+ke)} \qquad \text{Eq 5}$$

Take inverse Laplace Transforms of Equation 5

$$X = \frac{kaD}{ka-ke}\left(e^{-ket} - e^{-kat}\right) \qquad \text{Eq 6}$$

$$Conc = X/V = \frac{kaD}{V(ka-ke)}\left(e^{-ket} - e^{-kat}\right) \qquad \text{Eq 7}$$

MODEL 2 Two compartment model with first order absorption

Differential equations

$$dX_1/dt = k_aG + k_{21}X_2 - k_{12}X_1 - k_{10}X_1 \qquad \text{Eq 8}$$

$$dX_2/dt = k_{12}X_1 - k_{21}X_2 \qquad \text{Eq 9}$$

These differential equations can be solved by the method of Laplace Transformation as before to give

$$\text{Conc} = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-kat} \qquad \text{Eq 10}$$

where

$$A = \frac{F.D.ka(k_{21} - \alpha)}{V_1(\beta-\alpha)(k_a-\alpha)}$$

$$B = \frac{F.D.ka(k_{21} - \beta)}{V_1(\alpha-\beta)(k_a-\beta)}$$

$$C = \frac{F.D.ka(k_{21} - k_a)}{V_1(\alpha-k_a)(\beta-k_a)}$$

and α and β are the hybrid rate constants which relate to k_{12}, k_{21} and k_{10} where

$$\alpha+\beta = k_{12} + k_{21} + k_{10}$$

$$Cl/F = \frac{D}{A/\alpha + B/\beta} \qquad V_1/F = \frac{D}{A + B}$$

Appendix 3 Files used to run NONMEM

(a) Example of a NONMEM control file (Chapter 5).

(b) NONMEM PRED subroutines used in the analysis of Cl/F data (Chapter 5).

(a) Example of NONMEM Control File

```
DATA     ALISON.DATA
ORES     NONMEM.TABLE
****
PROB      EXP MODEL CL REG ON TIME
DATA        1    0 333  13   0
ITEM        1    3   0   0   1
LABL     SUBJ     TIME       CL       WT     CRIT     GLOB     CREA      BIL
LABL     ALAT     CHOL     DOSE      AGE
FORM
(2F3.0,F7.3,2F3.0,F5.1,F5.0,2F3.0,F4.0,F5.1,F5.0,F3.0)
STRC        2    1    1    1    1    1    0    1    0
THTA      -0.02    15.0
LOWR      -1.0     -1.0
UPPR       1.0     90.0
DIAG       0.01
DIAG       0.02
ESTM       01500    3    2    0    0    0    0
COVR       1
TABL       1    1
TABL       3    1    0    2    0    3    0
SCAT       1    8
SCAT      16    3
SCAT      15    3
SCAT      14    3
SCAT      16   14
SCAT      15   14
SCAT      16    2
SCAT      15    2
SCAT      14    2

END OF LISTING OF FILE :GONV02.ALISON(1,*,1).CONSALB

*************************************************
```

(b) NONMEM PRED File for Model

$CL/F = \theta_1 e^{-\theta_2 t} + \theta_3$ (additive errors)

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C
C
C
C
      DIMENSION THETA(3),DATREC(13),H(1),G(3),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,A,B,EAT,XAE,EXPWCH,XCOV
      T=DATREC(2)
      A=THETA(1)
      B=THETA(3)
      XAE=THETA(2)
      EAT=EXPWCH(-XAE*T)
      F=A*EAT+B
      G(1)=EAT
      G(2)=-A*T*EAT
      G(3)=1
      H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

END OF LISTING OF FILE :GONV02.NONMEM(1,*,1).PRED1

****

(b) NONMEM PRED File for Model

$CL/F = \theta_1 e^{-\theta_2 t} + \theta_3$ (proportional errors)

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C
C
C
C
      DIMENSION THETA(3),DATREC(13),H(1),G(3),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,A,B,EAT,XAE,EXPWCH,XCOV
      T=DATREC(2)
      A=THETA(1)
      B=THETA(3)
      XAE=THETA(2)
      EAT=EXPWCH(-XAE*T)
      F=A*EAT+B
      G(1)=EAT*A
      G(2)=-A*T*EAT*XAE
      G(3)=B
      H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

END OF LISTING OF FILE :GONV02.ALISON(1,*,1).PRED1X

****

(b) NONMEM PRED File for Model

$Cl/F = \theta_1(1 - \theta_2 + \theta_2 e^{-\theta 3t})$ (additive errors)

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C                          WF
C
C
C
C
      DIMENSION THETA(3),DATREC(13),H(1),G(3),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,A,B,C,EAT,XAE,EXPWCH,XCO
      T=DATREC(2)
      A=THETA(1)
      B=THETA(2)
      XAE=THETA(3)
      EAT=EXPWCH(-XAE*T)
      F=A*(1-B+B*EAT)
      G(1)=F/A
      G(2)=-A+A*EAT
      G(3)=-A*B*T*EAT
      H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

END OF LISTING OF FILE :GONV02.ALISON(1,*,1).PRED3

****

(b) **NONMEM PRED File for Model**

$Cl/F = \theta_1(1 - \theta_2 + \theta_2 e^{-\theta 3t})$ (proportional errors)

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C
C
C
C
      DIMENSION THETA(3),DATREC(13),H(1),G(3),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,A,B,C,EAT,XAE,EXPWCH,YCOV
      T=DATREC(2)
      A=THETA(1)
      B=THETA(2)
      XAE=THETA(3)
      EAT=EXPWCH(-XAE*T)
      F=A*(1-B+B*EAT)
      G(1)=F
      G(2)=(-A+A*EAT)*B
      G(3)=-A*B*T*EAT*XAE
      H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

END OF LISTING OF FILE :GONV02.ALISON(1,*,1).PRED3X

****

(b) NONMEM PRED File for Model

$Cl/F = \theta_1 - \theta_3 + \theta_3 e^{-\theta_1 \theta 2t}$ (additive errors)

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C
C
C
C
C
      DIMENSION THETA(3),DATREC(13),H(1),G(3),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,A,B,C,EAT,XAE,EXPWCH,XCOV
      T=DATREC(2)
      A=THETA(1)
      B=THETA(3)
      XAE=THETA(2)
      EAT=EXPWCH(-A*XAE*T)
      F=A-B+B*EAT
      G(1)=1-XAE*B*T*EAT
      G(2)=-A*B*T*EAT
      G(3)=-1+EAT
      H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

END OF LISTING OF FILE :GONV02.ALISON(1,*,1),PRED2

****

(b) NONMEM PRED File for Model

$$Cl/F = \theta_1 - \theta_3 + \theta_3 e^{-\theta_1 \theta_2 t} \quad \text{(proportional errors)}$$

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C
C
C
C
      DIMENSION THETA(3),DATREC(13),H(1),G(3),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,A,B,C,EAT,XAE,EXPWCH,XCOV
      T=DATREC(2)
      A=THETA(1)
      B=THETA(3)
      XAE=THETA(2)
      EAT=EXPWCH(-A*XAE*T)
      F=A-B+B*EAT
      G(1)=(1-XAE*B*T*EAT)*A
      G(2)=-A+B*T*EAT*XAE
      G(3)=(-1+EAT)*B
      H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

END OF LISTING OF FILE :GONV02.ALISON(1,*,1).PRED2X

****

(b) NONMEM PRED File for Model

$Cl/F = \theta_2 - \theta_1 X$

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C
C
C
C
      DIMENSION THETA(2),DATREC(13),H(1),G(1),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,A,B,C,EAT,XAE,EXPWCH,XCOV
      T=DATREC(4)
      A=THETA(1)
      B=THETA(2)
      F=B-A*T
      G(1)=-T
      H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

END OF LISTING OF FILE :GONV02.ALISON(1,*,1).PREDSWT

****

(b) NONMEM PRED File for Model

$$Cl/F = \theta_1 e^{-\theta_2 t} + \theta_3 + \theta_4 X$$

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C
C
C
C
      DIMENSION THETA(4),DATREC(12),H(1),G(3),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,A,B,EAT,XAE,EXPWCH,XCOV
      T=DATREC(2)
      XCOV=DATREC(4)
      A=THETA(1)
      B=THETA(3)
      C=THETA(4)*XCOV
      XAE=THETA(2)
      EAT=EXPWCH(-XAE*T)
      F=A*EAT+B+C
      G(1)=EAT
      G(2)=-A*T*EAT
      G(3)=1
      H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

END OF LISTING OF FILE :GONV02.NIVEN(1,*,1?.PRED1WT

*** ********** ****

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