ECONOMICALLY BENEFICIAL DRUG INTERACTIONS WITH CYCLOSPORIN AND TACROLIUMUS - CLINICAL STUDIES IN RECIPIENTS OF KIDNEY AND LIVER TRANSPLANTS

By

T. E. JONES B.Pharm, Dip. Ed.

Department of Clinical and Experimental Pharmacology

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List of Abbreviations:

AUC area under concentration-time curve
AxSYM Brand name of Abbott analyser
CsA cyclosporin
CD controlled diffusion
CV coefficient of variation
CYP3A4 isoenzyme 3A4 of the cytochrome P450 enzyme superfamily
DTZ diltiazem
DADTZ deactyldiltiazem
DMDTZ demethyldiltiazem
DMDADTZ demethyldeacetyldiltiazem
EMIT enzyme multiplied immunoassay
FPIA Fluorescence polarisation immunoassay
HPLC high performance liquid chromatography
ICZ itraconazole
KCZ ketoconazole
L litre
MEIA Microparticulate enzyme immunoassay
Tmax time to maximum concentration
TRM tacrolimus
µg microgram
µmol micromole
mL millilitre
mo month
tds three times a day
bd twice a day
pa per annum
p p value
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- the nursing staff who drew so many of the blood samples in these studies.
Declaration:
I hereby declare that this thesis contains no material which has been used for the award of any other degree or diploma in any university and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the text. I also hereby consent to allow this thesis to be available for photocopying and loan if applicable.

29/03/01

Terence E Jones
The following publications have resulted from work contained within this thesis:


6) Jones TE. The use of other drugs to allow a lower dosage of cyclosporin to be used. Clin Pharmacokinetic 1997; 32: 357-367 (invited article)


8) Morris RG, Jones TE, Mathew TH. Diltiazem disposition and metabolism in renal transplant recipients. Ther Drug Monit 1998; 20: 365-370
The following presentations at scientific meetings have also resulted from the work contained in this thesis:


9) Morris RG, Jones TE, Westley IS. Time course of the inhibition of cyclosporin or tacrolimus metabolism by diltiazem. International Association of Therapeutic Drug Monitoring and Clinical Toxicology meeting, Cairns, September 1999. Published in Ther Drug Monit 1999; 21(4): 437

ABSTRACT

Abstract:

The studies contained in this thesis comprise three separate clinical studies in organ transplant recipients, an extension clinical study and two surveys of Australasian transplant units. The overall aims of the studies presented are to examine fundamental questions regarding the clinically and economically important pharmacokinetic interaction between diltiazem and cyclosporin, an interaction widely utilised in organ transplantation.

The first survey demonstrated, that diltiazem is widely employed as a cyclosporin-sparing agent by some transplant physicians but not by others. It also showed that the reasons for using cyclosporin-sparing agents was not based upon hard data but was more likely to be influenced by familiarity with the agents and/or philosophical reasons. The magnitude of the savings afforded by the coprescription of diltiazem approximated AUD $7 million in 1995/6.

The second survey demonstrated that the use of diltiazem provided benefits in the early post transplant period in the form of reduced need for dialysis and immunosuppressive drugs and this data provides a strong argument in favour of the routine coprescription of diltiazem in adult kidney transplant recipients. It also examined the effects of using different blood cyclosporin concentrations on markers of efficacy and adverse effects which allowed recommendations for therapeutic ranges for cyclosporin in kidney transplantation.
ABSTRACT

The first clinical study demonstrated considerable interpatient variability in both magnitude of cyclosporin-sparing effect and the dose of diltiazem needed to produce the effect. Importantly, a significant effect was demonstrated with doses of diltiazem that were lower than those currently employed. The potential for cyclosporin to interact with diltiazem’s metabolism was also investigated and three different patterns of diltiazem kinetics were seen in kidney transplant recipients treated with cyclosporin. Although interpatient plasma diltiazem concentrations varied considerably, these appeared to have little bearing on the magnitude of the cyclosporin-sparing effect.

The extension clinical study demonstrated the folly of switching formulations without proof of bioequivalence. The ‘controlled diffusion’ formulation of diltiazem was shown to interact differently with cyclosporin than conventional release formulation diltiazem in several transplant recipients.

The second clinical study failed to demonstrate an interaction between cyclosporin and diltiazem in a patient who did have a considerable cyclosporin-sparing effect with itraconazole, despite using a higher than usual dose of diltiazem.

The final clinical study examined the potential tacrolimus-sparing effect of diltiazem in kidney and liver transplant recipients. Interestingly, there appeared to be a difference in tacrolimus-sparing effect between these two transplant types where a clinically significant sparing effect was noted in kidney transplant recipients but a less marked effect was observed in liver transplant recipients.
ABSTRACT

These data should assist the development of soundly based policies that will ensure a benefit exists before a sparing agent is coprescribed and that the lowest effective dose of sparing agent is used.
ABSTRACT

Synopsis

The advent of cyclosporin (CsA) improved organ transplant success rates but increased the costs of maintaining these transplants and introduced a new set of adverse effects (including nephrotoxicity and hypertension). CsA-sparing agents were advocated by the late 1980s primarily as a means of curbing costs, but how widely these were used and what drugs were used, was not known. No formal dose response studies had been performed with any sparing agent and the reliability of the interaction had also not been studied.

A number of drugs had been shown to increase blood CsA concentrations via interactions with the cytochrome P450 isoenzyme CYP3A4 (and/or the P-glycoprotein drug efflux pump). Potentially useful CsA-sparing agents included DTZ, verapamil and ketoconazole (KCZ) while grapefruit juice had also been shown to elevate blood CsA concentrations. In addition to the economic benefits, therapeutic benefits (including reduced nephrotoxicity) had also been demonstrated with some agents in some transplant types.

Much of the literature on interactions with CsA and of the use of CsA-sparing agents was derived from limited data and there was limited data on interpatient variability in sparing effect. No dose-response relationship studies had been conducted and this was presumably the reason for the doses of CsA-sparing agents being the same as those that applied to the approved indications for these drugs. There was no data on the extent of
use of CsA-sparing agents by different Australasian transplant centres, nor whether their use affected therapeutic ranges that were utilised by these centres.

A survey of Australasian transplant physicians demonstrated that CsA-sparing agents (especially DTZ) were widely used (Chapter 2). Considerable variability existed with some centres using CsA-sparing agents routinely in all patients while in other centres, they were not used at all. Different patterns of use were noted, where the majority of heart, lung and kidney transplant recipients were prescribed CsA-sparing agents, but only a minority of liver and pancreas transplant recipients were. The decision to use these agents appeared to be based upon local factors since centres located in close proximity to each other had widely different utilisation rates. Factors affecting the decision included prescriber familiarity (surgeons appeared less likely to use them), consideration of drug regimen complexity and philosophy rather than specific data relating to their use. Several physicians observed that perceived benefits afforded by the use of DTZ needed to be balanced against the potential for adverse outcomes, both direct and indirect e.g. via increased complexity of drug regimen with resulting poor compliance.

The economic benefits resulting from the use of CsA-sparing agents accrued to the funding body (the Commonwealth government when CsA was used for organ transplantation). However, the Commonwealth government did not advocate this use and had not granted approval for any specific drug for a CsA-sparing indication while the respective pharmaceutical manufacturers were either ambivalent or hostile to their use. Hence, individual physicians and transplant units would be the most likely targets for litigation in the event that an adverse outcome resulted from this (non-approved) use.
ABSTRACT

Interestingly, there was no evidence that the use of CsA-sparing agents affected the therapeutic range for blood CsA concentrations reported by clinicians and, despite comments that assay methodology should affect the range used, this was not evident from the data collected. There was great disparity in therapeutic ranges quoted which was evident not only across organ transplant types (which may reflect the life-threatening consequences of rejection of transplanted hearts and livers versus the less disastrous effects of rejecting a kidney) but also within the same organ transplant type.

In Chapter 3, data from almost the entire Australasian cadaver kidney transplant population was analysed to assess the clinical sequelae of using different therapeutic ranges for CsA and also of using DTZ. Earlier findings that DTZ use reduced the need for dialysis in the first week and month post transplantation were confirmed. DTZ use appeared not to reduce the frequency of rejection episodes post transplant, but this may be because the marker used (methylprednisolone use) was insensitive. DTZ use was associated with a reduced muromonab use, suggesting that the severity of rejection episodes was reduced in this period. Importantly, these therapeutic benefits did not occur as a consequence of higher blood CsA concentrations induced by DTZ.

No association was found between blood CsA concentration and either the need for dialysis or the likelihood that serum creatinine concentrations would fall sooner in the post transplant period. This may be a type II error, but it is consistent with earlier findings in similar patient populations (Nankivell BJ, et al. 1994. Mahalati K, et al. 1999) and is one of the reasons for the current interest in sparse sampling AUC monitoring.
DTZ use was associated with a reduced need for additional antihypertensive agent use at 3, 6 and 12 months post transplantation. This accords with the approved indication for DTZ but is contrary to earlier findings from a prospective study in an Australian kidney transplant population. The reduced need for additional antihypertensive therapy was confirmed when drug doses were taken into account by way of a scaling system.

Serum creatinine concentration was used as a marker of nephrotoxicity, one of the more ironic adverse effects of CsA. Creatinine concentration was also used as a marker of efficacy since rejection reduces functioning kidney mass. It was perhaps not surprising therefore that no relationship was found between this marker (serum creatinine concentration) and blood CsA concentration at 3, 6 and 12 months post transplantation (Chapter 3).

When data from virtually the entire adult Australasian kidney transplant population was examined for evidence of benefit or harm resulting from either high or low blood CsA concentrations, there was little to find that would assist the fine-tuning of current therapeutic ranges. A range of 100-225µg/L for the early post transplant period and 100-200µg/L for the later post transplant period reflects current practice and is recommended for trough blood CsA concentration in the medium to long term.

The study reported in Chapter 4 provides the basic dose-response relationship on the interaction between CsA and DTZ in adult kidney transplant recipients. There was considerable interpatient variation in response to DTZ with respect to elevation of blood CsA concentrations. Mean CsA AUC(0-24h) increased even after the lowest dose of
ABSTRACT

DTZ used (10mg/day). The mean rate of increase slowed after 30-60mg/day but continued up to the maximum dose tested (180mg/day). Interestingly, morning only doses of DTZ (<60mg/day) affected both morning (0-12h) and evening (12-24h) CsA AUCs equally. This proves the interaction between DTZ and CYP3A4 (and/or P-gp) persists after DTZ is removed from plasma.

This data shows that a CsA-sparing effect occurs with lower DTZ doses than those currently used for many patients (60mg thrice daily). Lower DTZ doses should reduce the frequency of adverse effects and allow its use as a CsA-sparing agent where conventional doses might be contraindicated. Because of the considerable interpatient variability observed, a CsA-sparing effect must be demonstrated in each patient (via blood CsA concentration monitoring both before and after the introduction of DTZ). One caveat is that therapeutic benefits demonstrated for DTZ have occurred following the use of 'conventional' doses (≥180mg/day) and these may not occur at lower doses.

It was shown in Chapter 2 that many centres switched from conventional release DTZ to the 'controlled diffusion' (CD) formulation in the absence of evidence of bioequivalence with respect to CsA-sparing effect. The folly of this switch was shown in Chapter 5 when patients were given 180mg CD formulation DTZ and the CsA-sparing effect compared to 90mg (conventional release) given twice daily. Group data showed no significant difference, but there were individual falls in CsA AUC(0-12) between 30-60% in 3 of the 8 patients while one experienced an increase of 36%. Interestingly, one patient had unusually low plasma DTZ concentrations following the use of conventional release DTZ followed by a surprising increase when the CD formulation was given.
ABSTRACT

Notwithstanding this increase in plasma DTZ concentrations, the CsA-sparing effect was largely unaffected and there was no evidence of increased therapeutic effect. The CD formulation appeared not to perform according to the manufacturer’s specifications since mean DTZ AUC(0-24) fell by >30% and the anticipated am:pm ratio of 0.67 was not observed. This failure to perform may have affected the CsA-sparing activity and conversely, may have been caused by the coprescription of CsA to the study participants. Patients might be better served by changing the dosing regimen (to once or twice daily) of conventional release DTZ (which simplifies the dosage regimen), rather than switching to CD formulation DTZ.

In Chapter 6, the relative potencies of two CsA-sparing agents, DTZ and the antifungal agent itraconazole (ICZ), was studied in a patient with a single lung transplant. CsA AUC(0-24) increased significantly when ICZ was co-prescribed (compared to when no CsA-sparing agents were given) but no increase was apparent when DTZ was co-prescribed with CsA. Despite widespread use as a CsA-sparing agent, this shows that DTZ does not always increase CsA concentrations, despite the higher than usual dose being used in this study (240mg/day). It is therefore recommended that, where DTZ is prescribed for its economic benefit, the interaction should be proven and not assumed, in every case.

In Chapter 7, DTZ kinetics were reported in adult kidney transplant recipients taking routine CsA. Three different kinetic patterns were observed. One patient had a ‘subnormal’ AUC for DTZ and all its metabolites which was consistent with poor bioavailability while the other patient had a ‘normal’ AUC for parent DTZ and primary
metabolites (DMDTZ and DADTZ) but a higher AUC for the secondary metabolite DMDADTZ which suggests a different metabolic pathway. Both patients demonstrated a significant CsA-sparing activity across the DTZ dose range studied however and, combined with the strong statistical association with parent DTZ (compared to metabolites), this suggests that DTZ is the interacting moiety. DTZ dose was as good a predictor of CsA-sparing effect as either Cmax or AUC and hence there was no point in monitoring DTZ kinetics.

In Chapter 8, the interaction between tacrolimus (TRM) and DTZ was studied in recipients of kidney (n=2) and liver transplants (n=2). This study was undertaken because the literature was equivocal regarding this interaction and it was considered important to define the nature of the interaction before DTZ was advocated as a TRM-sparing agent. DTZ exerted a clinically significant TRM-sparing effect in some organ transplant recipients. The increase in AUC(0-24) for TRM was more marked in the 2 kidney transplant recipients than the liver transplant recipients. The magnitude of the increase determined by trough (C₀) concentrations was similar to that demonstrated by AUC(0-24). Whether the difference was due to functional differences in the liver (the organ transplanted) or whether this is a manifestation of wider interpatient variability in the transplant population remains to be determined.

These data will assist the formulation of rationally based policies for CsA (and TRM) sparing agents which will reduce unnecessary exposure to potentially toxic drugs and help simplify drug regimen design such that any effect on compliance is minimised.
INTRODUCTION

Chapter 1  INTRODUCTION and LITERATURE REVIEW

While it is possible to live for many years with the aid of one or more of the various forms of dialysis, for most patients with end stage kidney failure, kidney transplantation offers the best hope of leading a 'normal' lifestyle. Kidney transplantation involves the surgical removal of a single kidney from a donor and its subsequent implantation into a recipient. Kidneys are numerically the most commonly transplanted organ in Australia where the first operations were conducted in the early 1960s. The source of the transplanted kidney is usually a cadaver, although since most individuals have two kidneys and a healthy life is possible with only one, it is relatively commonplace for the donor to be a living relative of the recipient. This especially applies to kidney transplantation in many overseas countries where religious beliefs prevent the use of cadaver organs. In these situations, living (usually related) donors are the only source of kidneys. One of the most important hurdles in kidney transplantation is the problem of rejection of the transplanted kidney by the immune mechanisms of the recipient.

The immune mechanisms comprise a complex series of cellular and humoral processes that are meant to protect the individual by recognising and destroying invading 'pathogens'. Because many of these invaders contain similar structures (ie enzymes, proteins etc) to the host, it is vital that the immune mechanisms are able to detect 'self' from 'non-self' such that the attack can be directed against the invader. This recognition of self is achieved by the expression of antigens that are present on the surface of most cells. While antigens are made from the same (amino acid) base units, the arrangement of these units is specific for each individual and the potential number of different arrangements is enormous. The consequence of this for practical purposes is each individual will recognise every other individual's tissues are 'foreign'. Cyclosporin impairs the ability of the immune system to destroy 'foreign tissue' (see section 1.2).
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The immune system can be likened to armies where the antigens are the flags that are used to distinguish between friend and foe and the different components of the immune system can be likened to the various specialist units of the army.

Because they carry antigens that identify their origin, transplanted organs will be recognised as foreign and rejected by the recipient unless the immune mechanisms are suppressed. While immunosuppression can be achieved with non-drug therapy (including radiation and a variety of antibodies), the long term immunosuppression currently required for the maintenance of organ transplantation is primarily achieved via drugs. The drugs used in earlier decades (primarily prednisolone and azathioprine) were relatively non-specific and hence the ability of the immune system to respond to foreign bacteria and/or cancer cells was impaired along with the ability to reject the transplanted organ. The most important advance in recent times has been the development of more selective drugs including cyclosporin (CsA) (Morris PJ. 1996. Diasio RB, et al 1996).

CsA is currently the major immunosuppressive drug used to prevent organ transplant rejection. It is also used in a variety of autoimmune disorders including rheumatoid arthritis, psoriasis and steroid resistant asthma (Keown PA. 1990).

CsA was obtained from the fungi Trichoderma polysporum and Cyclindrocarpon lucidum Booth which were isolated from soil samples from Wisconsin, U.S.A. and the Hardanger Vidda area of Norway in 1970 (Lindholm A. 1991, Borel JF 1981b). The latter of these fungi was excluded as a commercial source of CsA because it would not grow in submerged culture and the former fungus became the focus of attention. The original name was found to be taxonomically incorrect and the fungus was therefore renamed Tolypocladium inflatum Gams (Borel JF 1981b). More recently, this fungus has been renamed Beauveria nivea (Personal communication, Rosalind Tindale, Novartis
Pharmaceuticals, North Ryde, NSW, Australia). During testing for potential antimicrobial properties, CsA was shown to exert a more selective immunosuppressive action than existing immunosuppressive agents (Borel JF. 1981a, Borel JF. 1981b) and in particular, bone marrow function was spared. CsA’s mechanism of action was shown to be selective, affecting primarily T helper cells. After success in a variety of animal organ transplantation models, CsA was first used in the late 1970s in human transplantation (kidney, pancreas and liver) (Calne RY, et al. 1978a) either in addition to, or as an alternative to, existing immunosuppressive agents (including prednisolone and azathioprine) (Calne RY et al. 1978b, Calne RY, et al. 1979). While studies have proven that CsA is an effective immunosuppressive drug when used alone (Hall BM, et al. 1988. Wood AJ. 1982), it is more often used in combination (often in lower doses) with other immunosuppressive agents (Keown PA, 1990. Morris PJ. 1996. Kunz R, et al. 1997).

1.1. Chemical structure:
CsA has a non polar, cyclic, undecapeptide structure (see Fig 1.1) with a molecular weight of 1202. CsA is only marginally water soluble (0.004%w/w) although it is soluble in fats and organic solvents (partition coefficient 120:1 octanol:water) (Shaw LM et al 1987). Other similar peptides are produced by the same fungus including cyclosporin G which exhibits immunosuppressive activity and is thought to be less toxic (Shaw LM, et al. 1987). Although trialed clinically, cyclosporin G has to this time not become commercially available.

1.2. Mode of Action:
Foreign antigens on transplanted organs are recognised by specialist cells (including macrophages and B cells) which internalise the antigens, process them and then express
INTRODUCTION

the resulting peptides on the surface of the cell (see Fig 1.2). These processed antigens are then presented to T helper cells which, in response to the contact, produce cytokines. One of these cytokines is interleukin II (IL2) which increases T cell multiplication and differentiation. The net result is the production of large numbers of cytotoxic T cells which destroy those foreign cells bearing the particular antigen. This T cell portion of the immune mechanism is the main mechanism whereby transplanted organs are rejected and it is therefore vital to the success of all organ transplantation that this portion of the recipient's immune system is effectively suppressed (Harlan DM, et al. 1999).

Fig 1.1 Chemical structure of cyclosporin showing ring shape.

Within the T cell, CsA binds to an intracellular protein receptor (an 'immunophillin' called cyclophilin) and this complex then binds to the enzyme, calcineurin (Monaco AP, et al. 1999). Calcineurin is required for calcium dependent transcription of those genes that produce various cytokines including IL2. In the presence of CsA, intracellular calcineurin is not activated and hence, IL2 production is inhibited (see fig 1.7). The consequence of this is that T cell multiplication and differentiation is impaired (Faulds D, et al. 1993). Although it is derived from a completely different microorganism (Streptomyces tsukubaensis) which was isolated from the opposite side of the Earth
(Japan), the recently developed immunosuppressive drug tacrolimus, has an almost identical mode of action to CsA despite binding to a completely different intracellular immunophyllin (FK binding protein) (Kelly PA, et al. 1995) - (see below).

**Figure 1.2 diagram of T cell role and effects of immunosuppressive drugs**

1.3. Pharmacokinetics

Many studies (Shaw LM, et al. 1987. Kahan BD, et al. 1995b. Min DI, et al. 1996. Lown KS, et al. 1997) have demonstrated large interpatient and intrapatient variability in a variety of kinetic parameters of CsA, including at least one study in stable renal transplant recipients which found variability in kinetic parameters over the course of the day - ie chronopharmacokinetic variability (Ohlman S, et al. 1993). The nonpolar structure of CsA renders it highly lipid soluble and this has hampered the ability to formulate acceptable commercial products. The original formulation, a 'drink solution', was a simple solution of CsA in olive oil which was unpalatable to many patients. This formulation was replaced by a more complex capsule formulation containing maize oil.
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and alcohol (Sandimmun®, Sandoz Aust, later Novartis) which was more acceptable but this formulation has been replaced by a technologically more advanced one - a ‘microemulsion preconcentrate’ (Neoral®, Novartis). This latest formulation contains emulsifying agents that are aimed at reducing the reliance on the presence of bile for absorption thereby improving absorption from the gastrointestinal tract (Drewe J, et al. 1992a).

1.3.1 Absorption
Drewe et al (1992) showed that absorption of CsA occurs predominantly from the jejunum (Drewe J, et al. 1992b) when they instilled an emulsion of CsA at various locations along the gastrointestinal tract in 10 volunteers. Absorption (expressed as mean ± sd) from the jejunum (AUC = 4344 ± 2754μg.h/L), exceeded that from the duodenum (AUC = 2837 ± 1391μg.h/L), which exceeded that from the ileum, (AUC = 1474 ± 783μg.h/L) while absorption from the descending colon (AUC = 1377 ± 2731μg.h/L) was least. Variability in absorption was greatest when CsA was administered to the colon when 7 of the 10 subjects had absorption <3% of the same dose given orally while the other 3 had 37%, 190% and 299% of the same dose given orally. It is unlikely that this variability resulted from problems with study design since subjects were similar, meals were standardised, subjects served as their own controls, a monoclonal assay method was used and hence metabolite cross reactivity should not have been a major problem (especially with the single dose design). Since CsA is fat soluble, it is more likely that variability in the ability to solubilise the drug (viz presence of bile) was the cause of the variability.

That the small bowel is the major site for absorption of CsA is also supported by a study in paediatric liver transplant recipients (Whittington PF, et al. 1990) which noted that the
dose of CsA required to maintain therapeutic blood CsA concentrations was almost ten times that required for adults on a mg/kg basis. These authors demonstrated that the extent of absorption of CsA correlated closely with the length of small bowel which increases with growth during childhood.

Systemic absorption following oral administration of both liquid and capsule formulations of CsA has been noted to be poor and highly variable, varying from as little as 2% to as much as 92% (Venkataramanan R, et al. 1985. Jacqz-Agrain E, et al. 1994). A number of physiological factors have been reported to affect both the rate and extent of absorption of CsA including the presence of bile in the gastrointestinal tract (Grevel J. 1986. Lindholm A, et al. 1990). Bile flow may be reduced by disease (e.g. cholestasis) or by surgical drainage and the poorest absorption is seen in liver transplant recipients where external drainage of bile is required in the early postoperative period (Venkataramanan R, et al. 1985. Burckart GJ, et al. 1986). CsA absorption is so markedly impaired by these states that parenteral administration may be required for prolonged periods until bile flow can be restored (Burckart GJ, et al. 1986. Trull AK, et al. 1993. Levy G, et al. 1994. Friman S & Backman L. 1996. Spencer CM, et al. 1997).

This poor absorption is perhaps not surprising, since CsA is a fat soluble drug which requires solubilisation before it can be absorbed and this is the reason behind the most recent modification to the formulation which attempts to reduce the reliance on bile flow. The administration of bile salts with CsA has been shown in one early study to increase CsA’s AUC (Lindholm A, et al. 1990). In this study, 11 volunteers were given single doses (6mg/kg) of CsA on separate occasions, with and without food and bile salts. Blood CsA concentrations were measured by HPLC and a significant 25% mean increase in AUC was observed (9078 ±2140 vs 7283±2122µg.h/L). There was considerable intersubject variability in response ranging from 121% increase to a 9% decrease in AUC. In addition, 2 of the 11 volunteers had a decrease in AUC (-1% and -9%) that is
consistent with the well described variability in CsA kinetics. The study was well
designed (standardised meals, random order of treatments, HPLC assay method etc) and
hence provides reasonable evidence of an effect of bile acid on CsA absorption in some
circumstances.

1.3.2 Distribution and Metabolism

CsA has a large volume of distribution (approximately 13L/kg) and is distributed widely
in the body. In whole blood, the majority of the CsA present is found in red blood cells
(approximately 50%) and leucocytes (10-20%). The remainder of the CsA in blood is
found bound to lipoproteins (Diasio RB & LoBuglio AF. 1996).

The cyclic ring structure of CsA is relatively resistant to metabolism in the body but the
side chains are extensively metabolised to more than 30 metabolites (Holt DW, et al.
has been shown to be the major enzyme responsible for CsA metabolism (Combalbert J,
P450 enzymes was originally based upon the substrate metabolised (e.g. nifedipine
oxidase) but has now changed to one based upon the amino acid sequence of the enzyme
Slaughter RL, et al. 1995). The ‘cytochrome P450’ part of the name is derived from the
ability of this enzyme family to absorb light at the 450 nanometre wavelength.
Subdivision into ‘families’ is denoted by an Arabic number and requires that all members
of the family have ≥40% amino acid homology. For enzymes that have ≥55% amino
acid homology, the next subdivision (into ‘subfamilies’) is denoted by a capital letter.
The final subdivision currently recognised is denoted by an Arabic number (Watkins PB.
1992) and at this level of discrimination, CYP isoenzymes have been shown to have
≥85% amino acid homology (Kolars JC, et al. 1994). In a recent study comparing the
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effects of chemical inhibitors of four CYP enzymes, it was noted that there are differences in inhibitory specificity between rat derived enzymes and those derived from humans (Eagling VA, et al. 1998). It is thus possible that structural differences exist between CYP enzymes that are currently thought to be a single isoenzyme.

While the liver was thought to be the major source of drug metabolising enzymes (and was the major target for research into cytochrome P450 enzymes), it has more recently been recognised that CYP enzymes are also abundant in human enterocytes (Tjia JF, et al. 1991). They are not uniformly distributed throughout the gastrointestinal tract but rather, are concentrated in the small intestine (Kolars JC, et al. 1994. Krishna DR, Klotz U. 1994) (see Fig 1.3). Cytochrome P4503A4 is the predominant enterocyte enzyme although there is significant intersubject variability in both intestinal and hepatic activity (Henricsson S & Lindholm A. 1988. Kolars JC, et al. 1991a. Ketter TA, et al. 1995).

Figure 1.3 Immunoperoxidase staining from human jejunum showing CYP3A4 concentration in the tip of the villus –from Kolars et al 1994
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While it was initially thought that metabolism of CsA occurred primarily in the liver (Faulds D, et al. 1993), the importance of intestinal metabolism was established in 1991 when researchers found metabolites of CsA in portal blood following instillation of CsA into the small bowel of 2 patients during the anhepatic phase of liver transplantation (Kolars JC, et al. 1991b). These authors were among the first to suggest that intestinal metabolism might contribute to CsA's poor bioavailability and that drug interactions might also occur at this site. Other workers have also provided evidence that CsA is metabolised in the gastrointestinal tract prior to absorption. In an early study (Hoppu K. et al 1991), CsA was administered orally and intravenously to 20 children requiring kidney transplantation. The study design included the administration of both oral and intravenous CsA and blood sampling over a 24h period to allow the calculation of AUC. Samples were assayed by two different assay methods – a ‘specific’ and non-specific radioimmuno-assay that allowed an estimation of the degree of metabolism of CsA. Non-specific assays give higher values because they cross-react to a greater extent with CsA’s metabolites than ‘specific’ assays and hence the ratio of non-specific to specific assays is an index of the degree of metabolism. The ratio of non-specific to specific AUC after oral administration was 1.96 (range 1.4-2.7) while after intravenous administration, it fell to 1.43 (range 1.1-2.0) (p=0.0001), indicating greater metabolism following oral administration. Because the ratio of non-specific to specific AUC after oral administration decreased as clearance increased among the subjects, the authors speculated that this implied pre-hepatic metabolism. This study had a number of limitations including subject heterogeneity. In particular, there was a wide age range of ages (1.1 – 16.8 years) and different diseases, including some children (n=12) with nephrotic syndrome. This disease is commonly associated with hyperlipidaemia (Witztum JL 1996) which thus has the potential to affect CsA’s volume of distribution (since CsA is highly bound to lipoproteins - see above). Also, a wide variety of kinetic profiles including three distinct patterns of absorption has been described in this very
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population (Jacqz-Agrain E et al. 1994). A further limitation was the assumption that the ‘specific’ immunoassay method measured only parent CsA. The authors attempted to verify the specificity of this method by also analysing some samples via an HPLC assay method. However, only 8 samples (from a total of 440 samples drawn for the study) were compared by the 2 methods and hence the ratio of 1.16 (RIA:HPLC) value quoted is suspect. Notwithstanding this limitation, because this study design involved only a single dose, it would be unlikely that metabolite concentrations would exceed those of parent CsA and hence the assumption may have been valid. The study design could not however exclude the possibility that the difference in clearance was caused by hepatic first pass metabolism.

In another study, 6 healthy volunteers were given fixed doses of CsA (10mg/kg orally and 3mg/kg intravenously) with or without rifampicin (Hebert MF, et al. 1992). Clearance of CsA was increased from 0.3 to 0.42L/h/kg by the coprescription of this enzyme inducing drug, while bioavailability dropped from 27% to 10%. These authors concluded that this fall in bioavailability was best explained by induction of intestinal cytochrome P450 enzymes which was markedly greater than the induction of hepatic enzymes. Data from this study was used in a detailed pharmacokinetic analysis discussed below (Wu CY, et al. 1995).

Interestingly, the ratio between more specific and less specific assays has been used as a guide to liver activity in liver transplant recipients (see Chapter 2). Given the importance of presystemic metabolism and wide intersubject variability in intestinal CYP3A4 activity, it is possible that this might explain much of the intersubject variability in absorption following oral CsA administration.
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A later report applied pharmacokinetic principles to determine the relative contributions of gastrointestinal and hepatic extraction to overall bioavailability (Wu CY, et al. 1995). Assuming that intestinal extraction of CsA was minimal after intravenous administration, the authors compared results from 3 interaction studies they had previously reported. These studies involved the administration of CsA, both orally and intravenously before and after the administration of another drug that was known to affect CsA's metabolism. The drugs in question were rifampicin (an enzyme inducer), or ketoconazole and erythromycin (both enzyme inhibitors). By setting upper and lower estimates on the extent of absorption of CsA, the authors were able to calculate gut extraction ratio, fraction of oral dose absorbed through the gut and effect of interacting drug on gut extraction. The results of these calculations were that gut extraction ratio exceeded hepatic extraction ratio and that the effect of enzyme inhibitors is more marked in the gut than in the liver. These authors further noted that the fraction of administered CsA that is absorbed into the gut wall from the Sandimmun formulation was high (approximately 86%) and that metabolism in the gut wall was the major factor responsible for the poor oral bioavailability of CsA. It was necessary for the authors to make some assumptions including one that administration of each of the interacting drugs did not affect the extent of absorption of CsA into the gut wall. This is reasonable since dosing of CsA and interacting drugs were separated by 10-12 hours, but may be flawed since erythromycin can affect gastric emptying (Janssens J, et al. 1990). A further assumption was that rifampicin induced enzymes in the gastrointestinal wall to the same extent as in the liver. Because the relative concentration of rifampicin in the intestinal lumen is higher, this assumption may be flawed. These concerns are relatively minor and do not detract significantly from this well presented and cogently argued work from highly respected authors.
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CsA is extensively metabolised to 3 primary metabolites (AM1, AM4N and AM9) and more than 20 subsequent metabolites (Fig 1.4) (Shaw LM, et al. 1990). Pharmacological activity, both beneficial and toxic, has been attributed to some of these metabolites (Kunzendorf U, et al. 1989. Kunzendorf U, et al. 1988. Lucey MR, et al. 1990. Freed BM, et al. 1987). Metabolites are excreted via the bile with little or no parent CsA being excreted via the kidneys.

![Diagram of metabolic fate of CsA]

1.3.3 Excretion

Very little CsA is excreted via the kidneys and therefore dosage reductions are not required for renal impairment. The majority of administered CsA is excreted via the bile, primarily as metabolites and dosage reductions may be required when liver function is impaired (Diasio RB & LoBuglio AF. 1996).
1.4. Drug Interactions

Many drugs have been reported to either elevate or lower blood CsA concentrations and most of these interactions can be explained either via inhibition or induction of intestinal and/or hepatic CYP3A4 (Wu CY, et al. 1995). Some authors however have proposed alternative mechanisms including alterations to volume of distribution to account for these interactions with CsA (Tjia JF, et al. 1989. Wagner K, et al. 1989. Shaw MA et al. 1987). The uncertainty was caused primarily because concentrations of secondary CsA metabolites were not reduced (as would be expected by inhibition of enzymes that produce them). The currently accepted explanation for the increased (rather than decreased) concentration of some secondary CsA metabolites is that the interaction occurs at multiple levels, reducing both the metabolism of CsA and that of its primary metabolites.

Established enzyme inducers including rifampicin, phenytoin and phenobarbitone have been shown to decrease blood CsA concentrations, while drugs which have been reported to increase blood CsA concentrations include erythromycin, verapamil, diltiazem, nicardipine, ketoconazole and itraconazole (Holt DW, et al. 1994). Interestingly, interacting agents are not confined to prescription drugs - e.g. grapefruit juice has been noted to interact with a number of drugs that are metabolised by CYP3A4, including CsA (Min DI, et al. 1996. Edwards DJ, et al. 1999). Drugs that altered blood CsA concentrations were initially seen as relatively contraindicated (Dieperink H & Moller J. 1982) for patients receiving CsA because of the enhanced potential for toxicity and/or rejection. Once the economic potential was realised however, deliberate coprescription of drugs which allowed the dose of CsA to be reduced (while maintaining blood concentrations within the therapeutic range) was soon advocated (Leibbrandt DM & Day RO 1992. Wadhwa NK, et al. 1987. Valentine H, et al. 1992). These drugs are thus called 'CsA-sparing agents'.
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1.4.1 Cyclosporin-sparing agents

CsA is substantially more expensive than earlier immunosuppressive drug regimens based on prednisolone and azathioprine. In Australia, the annual acquisition costs for these older drugs was approximately $400.00 per patient, which is approximately one twentieth the acquisition cost for CsA (based on 4mg/kg/day at $7/100mg Neoral® capsules). The significance of these acquisition costs for CsA was recognised in the design of a multicentre Australian study conducted in the mid 1980s (Hall BM, et al. 1988). The trial design included one arm where, partly for financial reasons, kidney transplant recipients were switched (at three months post transplantation) from CsA only immunosuppression to ‘conventional’ immunosuppression with azathioprine and prednisolone. A detailed analysis of drug acquisition costs (including intravenous methylprednisolone and antilymphocyte gammaglobulin therapy used for acute rejection) of providing CsA, as part of triple immunosuppressive therapy to 84 kidney transplant recipients, was undertaken at Westmead Hospital, Sydney in 1991 (Barclay PG, et al. 1992). These authors used actual patient data to determine the costs of providing different immunosuppressive drugs regimens when patients were switched from triple therapy using CsA to dual therapy (prednisolone and azathioprine alone) at 3 months post transplant. The annual savings were shown to be $2350 per patient. This study did not however include the costs associated with therapeutic drug monitoring and, since this is only an issue with CsA based regimens, the financial impact of using CsA is larger than this sum indicates.

Reports describing economically advantageous interactions with CsA vary from serendipitous findings in individual patients (where drugs have been prescribed for therapeutic purposes to patients already receiving CsA) (Dieperink H, et al. 1982. Ferguson RM, et al. 1982) through single dose prospective studies in volunteers to long term, prospective studies in transplant recipients (Macdonald P, et al. 1992.)
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Chrysostomou A, et al. 1993. Keogh A, et al. 1995). These latter studies have usually described the degree of CsA dosage reduction, at a set time post transplantation, realised by coprescribing the CsA-sparing agent. In these studies, as in the clinical setting, dosage requirements of CsA have been adjusted according to the results of a single (morning) trough blood CsA concentration. Trough concentration monitoring to determine drug-drug interactions is suboptimal, especially where the interaction occurs in the intestinal wall. Because interactions at this site affect absorption more than clearance, it is important that full AUC monitoring is performed rather than limited (trough) sampling. These studies also provide limited data on the interaction between CsA and the interacting drug by virtue of the limited doses of interacting drug used. These studies have also used doses of CsA-sparing agents that are the recommended doses for the traditional therapeutic indication and hence there is little data available on the effects of different (especially lower) doses. Agents that have been studied in some detail for their CsA-sparing activity are considered below:

1.4.1.1 Grapefruit juice

Grapefruit juice was serendipitously shown to increase the bioavailability of some dihydropyridine calcium channel blockers when it was used as a diluent for alcohol in a drug interaction study (Bailey DG, et al. 1991). It has also been shown to interact with other drugs including CsA (Ducharme MP, et al. 1993), and caffeine (Fuhr U, et al. 1993). The active agent, although not identified, is probably naringin or its aglycone metabolite, naringenin. Naringin is the flavonoid glycoside that is responsible for the bitter taste of grapefruit which is not present in orange juice (Bailey DG, et al. 1991).

There is evidence in the literature that the site of interaction is within the intestine. In one study of 6 hypertensive males, grapefruit juice increased bioavailability (expressed as
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AUC) of orally administered felodipine by 284% (range 164%-469%) when compared to the same dose taken with water (Bailey DG, et al. 1991). The increase in AUC largely resulted from an increase in Cmax (13 ± 2nmol/L increasing to 29 ±3nmol/L) since elimination half-life was not affected (3.0h vs 2.3h - not significant). This contrasted to the effect of erythromycin, which increased both Cmax and half-life, which the authors concluded indicated that grapefruit juice interacted only within the intestine but erythromycin interacted at the intestinal and liver levels. A similar increase in bioavailability of nifedipine (134% - range 108-169) in 6 healthy males was reported in the same paper. In this study, the same dose of nifedipine (10mg) was taken either with grapefruit juice or water and, once again, the increase in AUC was primarily due to an increase in Cmax (250±42 nmol/L vs 222±54nmol/L). Interestingly this study also demonstrated a reduction in half-life of nifedipine caused by grapefruit juice (2.2 ± 0.1h vs 1.8 ± 0.2, p<0.05). In a study in 10 healthy volunteers, CsA was administered by both oral and intravenous routes both with and without grapefruit juice (Ducharme MP, et al. 1995). The authors noted an increase in both Cmax and AUC for CsA when it was administered with grapefruit juice via the oral route when compared to administration without grapefruit juice and no alteration in pharmacokinetic parameters when CsA was administered intravenously. The authors therefore concluded that the effect of grapefruit juice was to inhibit intestinal metabolism of CsA. In another study in 12 kidney transplant recipients, grapefruit juice was shown to increase Cmax of CsA which the authors ascribed to a transient effect, probably upon gut enterocyte CYP3A rather than hepatocyte CYP3A (Hollander AAMJ, et al. 1995).

The use of grapefruit juice as a cyclosporin-sparing agent has been both advocated (Ducharme MP, et al. 1993) and cautioned against (Johnston A & Holt DW. 1995) because of the increased variability in a number of pharmacokinetic parameters (especially Cmax) and uncertainty about the nature of the interacting species. The long
term reliability of the interaction in transplant recipients has also not been verified and, since the source of the interacting species is a seasonal food, there is likely to be variability in content of active species (and hence in extent of interaction) with time. This additional source of variability might therefore aggravate the already considerable intrapatient variability in blood CsA concentrations which would increase the difficulty of maintaining blood CsA concentrations within the therapeutic range and increase the potential for toxicity or rejection.

1.4.1.2. Ketoconazole (and related azole antifungal agents)

KCZ was one of the first drugs shown to elevate blood CsA concentrations (Ferguson RM, et al. 1982) when, in the early 1980s, it was prescribed for its antifungal activity to a kidney transplant recipient. A dramatic increase in trough blood CsA concentrations was noted (149 to 2828μg/L) over a 40 day period despite modest CsA dose reductions (from 900 to 800mg/day). Of particular concern was an associated significant increase in serum creatinine concentration (1.1 to 3.6mg/dL) which was attributed to CsA toxicity. The interaction was confirmed later that same year when a bone marrow transplant recipient was prescribed KCZ in a similar dose (200mg/d) and both CsA trough concentrations (<400 to 1225ng/mL) and serum creatinine rose sharply (<140 to 450μmol/L) (Dieperink H & Moller J. 1982). These authors reported that the interaction was 'hazardous' and recommended caution if these two drugs must be coprescribed.

Later in the 1980s, researchers from Cincinnati prospectively studied the interaction between KCZ and CsA in a small group of kidney transplant recipients (First MR, et al. 1989. First MR, et al. 1991. First MR, et al. 1993). One of the aims of these researchers was to demonstrate the magnitude of the cost savings that could be achieved
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by this coprescription. KCZ was shown to be a potent CsA-sparing agent, allowing doses of CsA to be reduced by more than 80% after 1 year which, the authors speculated, if applied to the entire transplant population in the U.S.A., would translate into savings in excess of US$100 million per year. Concerns over the safety over this use of KCZ were partially addressed by this group who found that renal function and both patient and graft survival were similar to those transplant recipients who were given usual doses of CsA without KCZ. A similar decrease in CsA dose requirements was observed in a study of 43 heart transplant recipients where 23 were randomised to receive 200mg/day KCZ while the remaining 20 did not receive it (Keogh A, et al. 1995). These authors also noted annual savings in excess of $5,000 per patient and therapeutic benefits in the form of reduced infection episodes and less rejection in the KCZ treated patients.

Another report (Girardet RE, et al. 1989) attested to the apparent safety of the combination, albeit in a single heart transplant recipient, who received both drugs for a 3.5 year period without apparent adverse effects. There is evidence in the literature that the magnitude of the interaction between KCZ and CsA increases with time (First MR, et al. 1993). In this report of 43 kidney transplant recipients, the required CsA dose was reduced from 92mg/day 1month after the introduction of KCZ to 55mg/day at 36months. This is greater than the usual fall in dosage of CsA required to maintain therapeutic blood CsA concentrations in transplant recipients that has been attributed to an increase in CsA bioavailability. These authors noted that the coprescription of KCZ with CsA was of particular importance where the transplant recipient was underinsured and thus required to pay for CsA. Another study in a small group of liver and kidney transplant recipients demonstrated similar reductions in CsA dosage which averaged 22% (liver transplant) and 41% (kidney transplant) at 1 week post transplant increasing to 70% (liver transplant) and 67% (kidney transplant) at 3 months post transplant. The potential
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savings to the US health system was estimated at only US$4 million pa in this report which is a much lower value than other estimates (First MR, et al. 1993. Sobh M, et al. 1995). The reason for this lower value is not entirely clear from this limited report but the costs of providing KCZ were higher than expected ($1386 pa) and these offset some of the savings from reduced CsA dosage (Odocha O, et al. 1996).

The strategy of deliberate coprescription of KCZ with CsA has been questioned on several grounds (Frey FJ. 1990). These grounds can be broadly divided into pharmacokinetic and economic. Pharmacokinetic concerns include the increase in interpatient variability in CsA kinetics (variability in Cmax increasing from 5 to 11 fold and dose range of CsA required to maintain therapeutic blood CsA concentrations increasing from 3 to 4 fold) (First MR, et al. 1989). Economic concerns include overstating the benefit derived from CsA-sparing agent use by virtue of the omission of the costs associated with additional monitoring. There is also an increased potential for adverse effects (especially hepatotoxicity) associated with the use of KCZ which would have a negative impact on the overall saving. Another concern noted by several authors (Frey FJ. 1990. Schweizer RT, et al. 1990. Didlake RH, et al. 1988. Kiley DJ, et al. 1993) is the impact on compliance when a complicated regimen (typically comprising >6 drugs) is further complicated by the addition of the CsA-sparing agent. The potential for harm by the use of CsA-sparing agents has been dramatically demonstrated when one transplant recipient inadvertently ran out of KCZ. The fall in blood CsA concentration that occurred as a result of the withdrawal of KCZ resulted in rejection (First MR, et al. 1989).

Some kidney transplant recipients prescribed KCZ have also been prescribed calcium channel blocking drugs including nifedipine, diltiazem or verapamil for the treatment of hypertension. The CsA dosage required to maintain therapeutic blood CsA
concentrations in this group taking the two CsA-sparing agents was 5% lower on average (First MR, et al. 1993) than that required for those patients taking only KCZ. This suggests that KCZ may act at a different site to calcium channel blocking drugs or that the effects of calcium channel blocking drugs are additive to those of KCZ.

In an in vitro study (Back DJ & Tjia JF. 1991), the relative potencies of the imidazole antifungal drugs KCZ, itraconazole, fluconazole and the unrelated terbinafine as CsA-sparing agents were studied. KCZ was found to be the most potent of these drugs. The concentration of KCZ required to reduce ‘cyclosporin hydroxylase’ activity by 50% (IC50) was 0.24µmol/L, the IC50 for itraconazole was 2.2µmol/L, for fluconazole and terbinafine, the IC50 was >100µmol/L. The clinical significance of the interaction between CsA and itraconazole has been confirmed in a kidney transplant recipient given itraconazole (200mg/d) for a cryptococcal infection in whom the mean blood CsA concentration doubled (Kwan JTC, et al. 1987). A similar finding was observed in a heart transplant recipient given 200mg/d itraconazole and whose trough blood CsA concentrations trebled as a consequence (Trenk D, et al. 1987). While this interaction is thus of considerable clinical significance for the CsA treated patient with a fungal infection, itraconazole is an unlikely CsA-sparing agent because virtually all of the savings achieved by the reduced CsA dosage would be spent on providing itraconazole.

1.4.1.3. Calcium channel blocking drugs
Diltiazem and verapamil were shown to inhibit hepatic enzymes in an in vitro study on aminopyrine metabolism (Renton KW. 1985). The enzyme responsible for CsA metabolism was subsequently shown to also metabolise nifedipine in vitro (Combalbert J, et al. 1989). Interestingly, this dihydropyridine calcium channel blocking drug does not appear to affect blood CsA concentrations in vivo to any significant degree (Wagner K,

1.4.1.3.1. Verapamil
The interaction between CsA and verapamil has been demonstrated in vitro (Henricsson S & Lindholm A. 1988) and confirmed in a small number of organ transplant recipients. In a retrospective review of 5 kidney transplant recipients (Lindholm A & Henricsson S. 1987) verapamil, in doses ranging from 120-320mg/day, was associated with a significant rise in trough, blood CsA concentrations. From the figure published in this letter to the editor, the mean value rose from approximately 500ng/mL to approximately 1100ng/mL, although specific concentrations and details of assay methodology were not stated. In a South Australian report (Robson RA, et al. 1988), a kidney transplant recipient prescribed verapamil 80mg twice daily for hypertension had an increase in trough blood CsA concentration from 350 to 1054µg/L which fell back to 409µg/L when nifedipine was substituted. Another case report of a kidney transplant recipient demonstrated that the interaction may be dose dependent since no interaction was observed with a dose of verapamil of 240mg/day (for the treatment of hypertension) but, when the dose was increased to 360mg/day, the trough blood CsA concentration rose from 244µg/L to 1090µg/L some 3 months later (Maggio TG & Bartels DW. 1988). There have been no prospective controlled studies to this time which confirm the safety and reproducibility of this interaction in organ transplant recipients.
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1.4.1.3.2. Diltiazem

The pharmacokinetic interaction between CsA and diltiazem (DTZ) was first observed in the mid 1980s when it was prescribed for prophylaxis of angina pectoris (Pochet JM & Pirson Y. 1986) in a patient receiving CsA. Within 3 days of commencing DTZ, blood CsA concentrations had risen approximately three fold while serum creatinine had risen from 120 to 140µmol/L. These values returned to baseline within 4 days of stopping DTZ but, upon rechallenge some 2 months later, even greater increases were observed (CsA concentration rose approximately five fold and creatinine rose from 130 to 170µmol/L). The effect was once again reversible upon stopping DTZ. DTZ was also used in the mid 1980s in prospective trials in both dog and human kidney transplant recipients.

In a series of studies, researchers aimed to demonstrate that, by calcium channel blockade, DTZ might prevent the increase in renal vascular resistance from CsA therapy and thus reduce post transplant, ischaemic, acute renal failure (Wagner K & Neumayer HH. 1985. Neumayer HH & Wagner K 1986. Oppenheimer F et al. 1992). In one human kidney transplant study where results appear to have been published twice by the authors (Wagner K & Neumayer HH. 1985. Neumayer HH & Wagner K. 1986) DTZ was added to the solution which perfused the kidney prior to transplantation as well as being given orally to the recipient post transplant. It has also been noted that DTZ administration orally post transplant (ie without adding it to the kidney perfusion solution) also reduces the incidence of delayed graft function (Neumayer HH & Wagner K. 1986. Oppenheimer F, et al. 1992). Compared to controls, (primary graft function 59%), DTZ treated kidney transplant recipients had better primary graft function (90%) and a corresponding reduction in the need for haemodialysis in the post transplant period. This benefit was seen despite higher blood CsA concentrations in the treatment group which would have been expected to increase the incidence of nephrotoxicity. The
authors concluded therefore that DTZ exerted a protective effect against CsA induced nephrotoxicity. A similar reduction in frequency of delayed kidney graft function has been reported by other researchers, most notably in an Australian study in human kidney transplant recipients (Chrysostomou A, et al. 1993). These authors prospectively studied 113 recipients of kidney transplants from both Adelaide and Melbourne in whom oral DTZ was given post transplantation in a randomised fashion to 53 recipients while it was withheld from the remaining 60. The control group suffered more primary non-function (16 vs 5) and vascular rejection (14 vs 3) than the DTZ treated group while there were no apparent differences in renal function (as measured by serum creatinine concentration) or the incidence of graft failure (4 vs 3) at any interval up to 2 years post transplantation. While these authors postulated that a reduction in CsA associated hypertension might also be observed in the DTZ-treated patients, the trial did not demonstrate any such difference. Interestingly, these authors concluded that, at a dose of 60mg administered thrice daily, DTZ did not appear to exert an antihypertensive effect in this population.


A recent paper (McLachlan AJ &Tett SE. 1998) has confirmed the extent of the interactions observed previously between CsA and DTZ, itraconazole, KCZ and the combined use of DTZ and KCZ in cardiothoracic transplant recipients. These authors
used the results of routine trough blood samples used for therapeutic drug monitoring (for which details of concurrent drug therapy were available) and compared the CsA dose-rate to the trough CsA concentration at steady-state via a linear mixed effects modelling approach. They concluded that DTZ reduced the CsA dose by 29%, itraconazole by 49%, KCZ by 77% and combined DTZ and KCZ by 84%.

1.4.1.3.3. Mibefradil
This recently developed calcium channel blocker was withdrawn by its manufacturers shortly after its launch due (in part) to interactions with drugs via the CYP3A4 isoenzyme (personal communication, 5.6.98, Managing Director, Roche Products Pty Ltd, Dee Why, Australia). Mibefradil was shown to increase the incidence of rhabdomyolysis in patients taking ‘statins’ which are metabolised via this enzyme and in an unpublished study (Peters J, et al. 1995), to significantly elevate blood CsA concentrations. In this single centre, open label study, 6 stable kidney transplant recipients maintained on CsA were given 50mg mibefradil daily for 5-6 days. A mean increase of 103.7% (range 43-196%) in Cmax of CsA was observed along with a similar increase in AUC(0-11) of 128.5% (range 83-232%). From this very limited data set, the manufacturers recommended that the dose of CsA should be halved upon commencement of mibefradil.

1.5. Economic considerations relating to the prescription of CsA-sparing agents
Anecdotal evidence suggests that there is considerable variability in the use of CsA-sparing agents around the world. In developing countries, the cost of CsA, combined with meagre incomes, often precludes the use of CsA in transplantation. A similar situation is seen in some western countries where transplant recipients are sometimes (viz
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uninsured patients) required to pay for this expensive immunosuppressive drug (First MR, et al. 1993. Curtis JJ, et al. 1986). In Australia, the Commonwealth government meets the bulk of the costs of providing CsA for transplant recipients through the ‘Section 100’ scheme which was set up in 1991 (primarily to cope with the costs of providing CsA). State governments (via public hospitals) pay only for the CsA that is consumed by inpatients and where CsA is used for non approved uses (as defined by the ‘Section 100’ scheme). This represents only a small percentage of total CsA use and hence State governments derive little economic benefit from the use of CsA-sparing agents. Indeed, any benefit that is derived from the use of CsA-sparing agents is offset by the costs of providing the sparing agent. Whether the practice of using CsA-sparing agents changed with the introduction of the ‘Section 100’ scheme is not known.

1.6. Effect of taking CsA with food

Before the advent of CsA-sparing agents, other strategies were advocated to reduce the required dose (and hence cost) of CsA. One of the most widely studied was the effect of taking CsA with or without food. In a single dose study in volunteers (Gupta SK, et al. 1990), the effect of taking CsA with a high fat meal not only resulted in an increase in bioavailability (79% compared to 21% when taken with a low fat meal) but also increases in both clearance and volume of distribution. A similar increase in bioavailability has been noted in renal transplant recipients in whom taking CsA with a standard hospital breakfast increased bioavailability by 61% compared to taking CsA in the fasting state (Ptachcinski RJ, et al. 1985). Unfortunately the literature is conflicting, with other reports showing the opposite effect. In an Australian study (Keogh A, et al. 1988) involving 10 heart transplant recipients, researchers examined the effect of giving CsA with a standard hospital breakfast versus taking the same dose in the fasting state. Mean data for the group showed no significant alteration in CsA AUC although there
was considerable intersubject variability (range -21% to +55%). Because of this confusion as to the effects of taking CsA with food, most authorities recommended that patients be consistent in the way they take CsA in relation to meals (Honcharik N. 1991. Morris RG. 1994).

1.7. Analytical problems

Much of the variability in results from early kinetic work on CsA was due to the significant problems associated with analytical methodology (Shaw LM, et al. 1987). High pressure liquid chromatography (HPLC) was one of the earliest assay methodologies but it is laborious and expensive, and generally not suited to routine therapeutic drug monitoring. Despite the availability of more easily performed assays however, one laboratory in Australia has routinely used the HPLC assay method since the mid 1980s (Morris RG. 1994). Early immunoassays utilised antibodies that were not specific for parent CsA but cross-reacted to varying extents with many of CsA's metabolites. Studies have been conducted in which results from non-specific assays were compared to those from more specific assays and, by inference, any change in the ratio of the results suggests an alteration in the metabolic fate of CsA (Sabate I, et al. 1989). Non-specific assays have largely disappeared but one interesting use remains - as an indicator of the metabolic capacity of the transplanted liver (see Chapter 2). While there is still some variability in the degree of cross-reactivity between commercially available immunoassays, the extent of this cross-reactivity with currently marketed immunoassays is significantly less and inter-laboratory variability has been reduced by the creation (and adoption) of consensus statements prepared by professional groups (Shaw LM, et al. 1990. Shaw LM, et al. 1987. Morris RG, et al. 1994. Oellerich M, et al. 1995).
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A number of other factors have been shown to affect the result of CsA assays including the sample matrix that is analysed, sample storage temperature and type of anticoagulant used. Consensus recommendations have addressed these issues also and, although they have not been universally adopted (Morris RG. 1994), more recent pharmacokinetic studies are more reliable than those from a decade earlier. While it is tempting to believe that analytical problems have been resolved, one recent development suggests that they may continue to be a source of concern into the future. This development is the AxsYM/FPIA assay which, the manufacturers claim, gives results that are closer to the value reported by specific assays (e.g. HPLC) than the older TDx/FPIA assay. Since this newly released assay method uses the same antibody as the older (monoclonal TDx/FPIA) assay method but reports a different value (Morris RG. 2000), it appears that a 'correction factor' has been used. Given the potential for variability in metabolite concentrations in the clinical setting, it is possible that this factor may on occasion reflect more closely the specific parent CsA concentration but at other times be less accurate than the older assay method. Because of the reality of commercial pressures, it is important to remain vigilant about assay methods.

1.8. Dose Response Relationships

Before a drug is approved for use in human medicine, it is customary to perform dose-response studies that define the dosage range over which the drug exerts the effect in question. In the case of CsA-sparing agents, studies have been performed only for the therapeutic indication(s) for which the drug is marketed (viz for DTZ, anti-anginal or antihypertensive uses). To this time, pharmaceutical manufacturers have not sought a CsA-sparing indication for any drug and the doses that are currently used are those approved for the marketed indication(s). Pharmaceutical companies are unlikely to perform the necessary dose-response studies for the CsA-sparing indication for two
reasons. Firstly, the market afforded by this indication is small and secondly, there are both ethical and legal concerns over using a drug primarily for an economic purpose (personal communication Dr H Smith, Medical Director, ICI Aust, manufacturer of ‘Cardizem’ brand of diltiazem, 1995).

While formal studies in humans are lacking, there are indications from the literature that the interaction between CsA and the various sparing agents is dose dependent and hence the doses used currently may not be appropriate. In one animal study (Myre SA, et al. 1991) on 7 adult mongrel dogs, the dose-response relationship for the interaction between CsA and KCZ covering five dosages (ranging from 1.25 - 20mg/kg/d) was studied. The dogs were divided into two groups, the first (n=3) received 6mg/kg/d CsA and the second (n=4) received reduced doses (3 or 1.5mg/kg/day) to allow for the increased blood CsA concentrations that were anticipated as a result of the use of KCZ. The first group received KCZ at doses of 0, 1.25 and 2.5mg/kg/d while the second group received 5, 10 and 20mg/kg/day. The authors used the ratio between HPLC and FPIA/TDx assay methodologies to indicate the extent of metabolism of CsA and found that this was highly correlated with KCZ dose ($r=0.9980$, $p<0.0001$ ). The effect was not significant until at least 1.25mg/kg/d and little additional effect was evident at KCZ doses above 10mg/kg/d.

There is evidence that the interaction between verapamil and CsA is also dose dependent (as noted earlier) where, in one kidney transplant recipient, no effect on blood CsA concentrations was noted until the dose was increased from 240mg/d to 360mg/d (Maggio TG & Bartels DW 1988).

There is evidence that the interaction between CsA and grapefruit juice is not present in all patients (Min DI, et al. 1996). In this study in 10 kidney transplant recipients, 240mL
INTRODUCTION

of grapefruit juice was given in a randomised crossover fashion with the morning dose of CsA. In 6 patients, CsA AUC(0-12h) was observed to increase (mean 51%, range 23-93%) but in the remaining 4, no increase was observed. Interestingly, this study did not demonstrate an increase in Cmax although a significant prolongation of Tmax was noted (5.4h vs 2.8h).

There are suggestions in the literature that the interaction between DTZ and CsA occurs at lower doses than those currently used (180mg/day in South Australia). One group using 60mg bd (Wagner K & Neumayer HH. 1985. Brockmoller J, et al. 1990) noted a 40% increase in CsA AUC in kidney transplant recipients. In a retrospective study of calcium channel blocker use in patients receiving CsA (Sketris IS, et al. 1994), CsA dosage requirements were reduced by approximately 30% in a group of 13 patients given DTZ even though 2 of these 13 were given a dose of ≤90mg/day. Unfortunately no reason was given for the lower DTZ dose and the CsA dose reductions for these 2 patients were not given separately. In a study on 69 heart transplant recipients (Valentine H, et al. 1992), DTZ was given to 32 patients starting at 30mg thrice daily during the third or fourth week post transplantation. Trough CsA concentrations were significantly higher (223 vs 175μg/L) despite CsA doses being significantly lower (4.3mg/kg/day vs 5.9mg/kg/day) at '1-2' months post transplantation. The dose of DTZ was increased to 60mg three times a day and to the target dose of 120mg three times a day as tolerated. While the data quoted for blood CsA concentration and DTZ dose are imprecise (mean DTZ dose was 90mg at 1mo and 195mg at 3mo), it suggests a significant increase in blood CsA concentration occurred with the 30mg thrice daily dose.
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1.9. Other areas of uncertainty

Most of the studies concerning the interaction between CsA and drugs which reduce the required dose of CsA have been performed using conventional release formulations. There has been a tendency over recent years for pharmaceutical companies to produce extended release formulations of those drugs which have short half lives and therefore need to be prescribed more than twice a day if blood concentrations are to be maintained. This has been particularly evident in the calcium channel blocking drug group (examples include nifedipine, diltiazem and verapamil) and has been done to improve patient compliance by simplifying the dosage regimen. While these formulations have been shown to be as effective as the conventional release formulations in the control of hypertension, there are reasons to suspect that their efficacy as CsA-sparing agents may be different. As noted earlier, CsA is absorbed primarily from the upper gastrointestinal tract and there is evidence that the gut is an important site for CsA’s metabolism (Kolars JC, et al. 1991b, Hoppu K, et al. 1991). DTZ was marketed in two extended release formulations, ‘Cardizem SR’ and ‘Cardizem CD’ (ICI Aust, Melbourne, Australia). The latter ‘controlled diffusion’ formulation has replaced the former but, rather than providing a slow, continuous release of drug, this formulation is designed to release DTZ in two bursts, the first delivering 60% of the dose in the first 12h and the second burst delivering the remaining 40% in the last 12h (personal communication, ICI Aust). Because of the nature of gastrointestinal transit, the second burst will probably occur at a site well below the principal site of absorption of CsA. If the interaction between CsA and DTZ is a simple competitive inhibition of CYP3A4 occurring largely at the intestinal wall site, this second burst of DTZ may not affect intestinal CYP3A4 to the same extent and hence the effect on blood CsA concentrations may be less marked or absent.

If the second burst of DTZ has a lesser effect on the absorption of the evening dose of CsA, there are implications for total exposure to CsA. Since CsA dosage adjustments
are usually made following morning trough concentration monitoring (ie following the evening AUC), the reduced effect of the evening DTZ burst will result in a relatively higher CsA dose being prescribed. The consequence of this is that daytime CsA AUC will be increased relative to the evening CsA AUC and hence total daily exposure to CsA will be greater. Differences in CsA-sparing effect have been noted to occur when brands of sustained release DTZ are changed (Cooke CE. 1994), although specific details of brand and magnitude of difference were not noted in this letter and the author was not able to be contacted to provide these details.

1.10. Secondary benefits versus adverse effects
CsA-sparing agents have the potential to produce secondary therapeutic benefit as well as adverse effects. The therapeutic benefits attributed directly to the use of DTZ include reduced post operative acute tubular necrosis in kidney transplant recipients and reduced atherosclerosis in heart transplant recipients (Schroeder JS, et al. 1993). DTZ is also seen as having a 'modest' adverse effect profile, in contrast to KCZ whose adverse effects (especially hepatotoxicity) are of greater concern (Lewis JH, et al. 1984). In addition, KCZ appears to have minimal secondary therapeutic benefits and this combination (of limited benefit and potential for toxicity) is probably the reason for the limited enthusiasm for using KCZ as a CsA-sparing agent in Australia. Indeed, despite the evidence (albeit limited) regarding the safety of the combination from overseas groups, the use of KCZ in Australia has, on occasion, been actively discouraged by ethics bodies and pharmaceutical companies (personal communication-A.Keogh see Chapter 2).
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1.11. Cytochrome P450 or P-glycoprotein?
As noted earlier, explanations other than an effect on CYP3A enzymes have been proposed to explain the interactions between CsA and other drugs. The principal alternative explanation in the past has been an alteration to volume of distribution but more recently, a totally different explanation has been proposed. This explanation is the P-glycoprotein drug efflux pump.

In the late 1980s, a novel mechanism was discovered which was shown to be important for the development of resistance to chemotherapeutic drugs by a variety of cancer cell lines (Yahanda AM, et al. 1992).

The development of resistance in tumour cell lines that were previously sensitive is one of the most disconcerting aspects of cancer chemotherapy. The nature of the acquired resistance is particularly disturbing since it sometimes involves the development of resistance to drugs the tumour line has not been exposed to. The gene responsible is called the mdr1 (multiple drug resistance) gene and the product of this gene is a 170 kd membrane glycoprotein called P-glycoprotein (P-gp). Using a monoclonal antibody, this glycoprotein was found to be concentrated in a number of specific sites within the body including liver, pancreas, kidney, colon and jejunum (Thiebaut F, et al. 1987). Cell types which contained P-gp were noted to be those on epithelial surfaces which were in contact with an excretory route. P-gp is also known to be expressed by a variety of tumour lines that arise from these epithelial tissues and, following exposure to chemotherapeutic drugs, the expression of P-gp has been shown to increase. In one study of newly diagnosed lymphomas, only 2% of cells expressed P-gp but this figure rose to 64% in previously treated, resistant tumour cells (Miller TP, et al. 1991).
P-gp is structurally related to active transport pumps found in some bacteria and it has been postulated that it affords protection from chemotherapeutic drugs by transporting them out of tumour cells before they can cause harm (Sonneveld P, et al. 1994). As shown in Fig 1.5, the location of P-gp and CYP3A enzymes (in gut wall) are very similar and in particular, both occur in high concentrations within cells of both the liver and intestine. Both P-gp and CYP3A enzymes have the potential to prevent drugs crossing the intestinal mucosae and entering the bloodstream. There would appear to be a potential for cooperation between P-gp and CYP since the genes responsible for producing CYP3A enzymes and P-gp are located adjacent to each other on chromosomes 21 (Kiyoshi I, et al. 1992. Callen DF, et al. 1987). However, in a more recent study examining the relative concentrations of P-gp and CYP3A4 in small bowel biopsies taken from kidney transplant recipients, there was no apparent correlation between small bowel CYP3A4 concentration and P-gp activity (Lown KS, et al. 1997). As shown in Table 1.1, the similarities between CYP enzymes and P-gp extend further
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than biological role since many of the drugs that act as substrates or inhibitors for a variety of CYP enzymes also modulate P-gp activity (Wacher VJ, et al. 1995). These drugs include the calcium channel blocking drugs DTZ and verapamil, and the immunosuppressant drugs CsA and TRM. Both CsA and verapamil have been shown to reduce P-gp activity and to thereby reverse the resistance that occurs to some chemotherapy regimens (Sonneveld P, et al. 1994. Miller TP, et al. 1991. Yahanda AM, et al. 1992. Lum BL, et al. 1992).

P-gp has also been recently identified in endothelial cells of the blood brain barrier (Miyama T, et al. 1998. Rao VV, et al. 1999) where it is thought to regulate the influx and efflux of endogenous substances as well as a variety of drugs. It has been suggested that coprescription of drugs which reduce P-gp activity might result in elevated concentrations of drugs including CsA and tacrolimus in the brain (Hebert MF & Lam AY. 1999).

Because of the close proximity of the P-gp and CYP3A systems within the gastrointestinal tract and their similar potential for induction and/or inhibition by a number of drugs, it is difficult to apportion the effect of altered CsA absorption caused by the coprescription of another drug to either system. Using the erythromycin breath test as a marker of liver CYP3A4 activity and taking small bowel biopsies from kidney transplant recipients, one group of researchers (Lown KS, et al. 1997) attempted to apportion the relative contributions of each system to the overall interpatient variability in clearance of orally administered CsA. The erythromycin breath test uses a radioactive methyl group which is cleaved from the erythromycin molecule by CYP3A4 and after absorption, radiolabelled carbon dioxide appears in expired air. The researchers assumed that intestinal CYP3A4 does not contribute to demethylation of erythromycin and using forward logistic regression, they were not able to attribute any of the interpatient
variability in either peak blood CsA concentration or clearance following oral
administration to intestinal CYP3A4, but were able to apportion a significant percentage
to both liver CYP3A4 concentrations and intestinal P-gp!

Table 1.1 Substrates for and Inhibitors of both CYP3A and P-gp (adapted from Wacher
VJ, et al. 1995)

<table>
<thead>
<tr>
<th>CYP3A substrate</th>
<th>P-gp (I= inhibitor, S=substrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>I</td>
</tr>
<tr>
<td>Lignocaine</td>
<td>I</td>
</tr>
<tr>
<td>Quinidine</td>
<td>I</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>I</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>I</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>S,I</td>
</tr>
<tr>
<td>Felodipine</td>
<td>I</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>S,I</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>I</td>
</tr>
<tr>
<td>Verapamil</td>
<td>S,I</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>S</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>S,I</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>S,I</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>S</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>I</td>
</tr>
</tbody>
</table>

Because of the similarities in consequence between P-gp and CYP3A4, the clinical
relevance of which system or other is responsible for drug interactions with CsA appears
at this time to be of little import. If modalities become available which will allow either
system to be selectively affected, this will inevitably change.
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1.12. Tacrolimus

Tacrolimus (TRM) was isolated in 1984 by workers from the Fujisawa Pharmaceutical Co from the bacterium *Streptomyces tsukubaensis*, so named because it was obtained from the Japanese town, Tsukuba, some 30km north of Tokyo. Despite the geographical and biological differences, there are many similarities between CsA and TRM, including solubility, mode of action, pharmacokinetics, drug interactions, adverse effects and cost.

1.12.1 Physical Properties and Mode of Action

TRM is a large, macrolide, lactone antibiotic (Fig 1.6) with a 23 member ring structure (Tanaka H, et al. 1987). Like CsA, it has a large molecular weight (804) and it is practically insoluble in water and n-hexane but freely soluble in other organic solvents including methanol, chloroform and acetone (Honbo T, et al. 1987).

![Figure 1.6 Structure of Tacrolimus](image-url)
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TRM binds to an intracellular binding protein (called 'FKBP' after the drug's investigational 'name' 'FK506') which is different to the site that CsA binds to, but following binding, the effect is almost identical (see Figure 1.7). After binding to their respective intracellular binding proteins, the complex binds to calcineurin. After the antigen presenting cell delivers the processed antigen to the T cell, a rise in intracellular calcium occurs. This calcium is bound to calmodulin which then binds to calcineurin. The next step in the process is the stimulation by calcineurin of the nucleus which produces IL-2 (and other cytokines). When calcineurin is blocked by the respective CsA or TRM complex, IL-2 production is prevented. Since IL-2 is needed to upregulate T cell production and differentiation, the ability of T cells to destroy the antigen bearing cell is impaired (see Fig 1.2). As noted earlier, T cells are the most important part of the immune system with respect to organ rejection and the effect of TRM is selective for this system. Despite the similarities in mode of action between TRM and CsA, there are important differences in clinical outcome, especially with respect to reversing acute rejection where TRM has been shown to be effective whereas CsA is not (Kelly PA, et al. 1995).

The first reports of the use of TRM in human medicine occurred in 1989 when researchers from Pittsburgh reported on its use in liver, kidney and pancreas transplantation (Starzl TE, et al. 1989). Until recently, TRM was approved for use in liver transplantation only in Australia although it has been widely used overseas in kidney transplantation. The enthusiasm for using TRM for kidney transplantation is very much dependent upon its inclusion under the Section 100 scheme when the Commonwealth government pay the acquisition costs. TRM had not been included in this scheme by late 1999 and hence the extent of use of TRM for kidney transplantation in Australia was limited to this time. Success rates with TRM in liver transplantation are generally better...
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than comparable data using CsA, while success rates in kidney transplantation are similar to those obtained using CsA (Kelly PA, et al. 1995).

Figure 1.7 Diagram of intracellular sites of action of CsA and TRM
FKBP = Tacrolimus binding protein, CsABP = Cyclosporin binding protein (cyclophilin). The CsA/TRM-binding protein complex binds to calcineurin, preventing Ca from binding, which then prevents IL-2 production in the nucleus. IL-2 = Interleukin II is released from the T cell nucleus into the environment. IL-2 attaches to receptors on the T cell surface (●), resulting in multiplication and differentiation of cytotoxic T cells

1.12.2 Pharmacokinetics of TRM

Like CsA, TRM’s oral bioavailability is relatively poor (mean 29%) and it exhibits considerable interpatient variability (range 5-67%), (Venkataramanan R, et al. 1995. Kelly PA, et al. 1995). Despite possessing similar solubilities and molecular weight to
CsA, TRM is not dependent upon the presence of bile in the gastrointestinal tract for absorption. Hence TRM exhibits acceptable oral bioavailability even in the early postoperative liver transplant period when bile is diverted externally (Jain AB, et al. 1990. Spencer CM, et al. 1997).

TRM is extensively metabolised, principally via CYP3A4 (Sattler M, et al. 1992) and, while data is limited, there are indications that TRM interacts to a similar extent with many drugs that also interact with CsA. In one volunteer (n=6) study (Floren LC, et al. 1997), oral and intravenous doses of TRM were given before and after 12 days of treatment with oral KCZ. The authors demonstrated a doubling of bioavailability (14±5% increasing to 30±8%) following the use of KCZ. In a retrospective review of drug charts and other medical records, it was concluded by one group of workers that nifedipine reduced the daily TRM requirement by 31% (Seifeldin RA, et al. 1997) in liver transplant recipients. This study showed a trend (not statistically significant) toward a lower dose of TRM when 60mg daily, rather than 30mg daily, nifedipine was used. Unfortunately the study design (retrospective review) and limited data set (22 nifedipine treated subjects and 28 untreated subjects only) limited the ability to define the interaction which the authors concluded would require a controlled prospective study to define more fully.

In an in vitro study using human liver microsomes (Christians U, et al. 1996), 15 drugs were shown to inhibit the metabolism of TRM including the calcium channel blocking drugs verapamil and nifedipine. Interestingly, DTZ was not shown to affect TRM's demethylation in this study although the authors noted that results from such in vitro studies do not necessarily imply the same effect will be observed in vivo.
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In another report (Regazzi MB, et al. 1996), the systemic interaction between DTZ and TRM was defined in 4 piglets. Following 48h of constant intravenous infusion of TRM, an intravenous infusion of DTZ was commenced and maintained for 72h. Blood concentrations of TRM (pre and post DTZ infusion) were compared and clearance of TRM was shown to be reduced from 5.0L/h/kg to 1.2L/h/kg by DTZ. While this study provides useful information, extrapolation to human medicine would be premature, partly because of the species difference and partly because the oral route of administration in used in clinical practice involves another variable, viz the potential for the interaction within the intestine.

1.12.3 Adverse Effects of TRM

TRM shares a similar adverse effect profile to CsA, especially nephrotoxicity and hypertension. Nephrotoxicity has been reported to occur in 18-42% of liver transplant recipients and 44% in kidney transplant recipients (Yamaguchi Y. 1991). In a retrospective study of 128 consecutive renal transplant recipients, the incidence of nephrotoxicity (defined as an increase in serum creatinine that responded to lowering the dose of TRM and the absence of histopathological changes of acute rejection on biopsy) was found to be 17% (Katari SR, et al. 1997). It is likely that the frequency of adverse effects (including nephrotoxicity) reported from these early studies will be higher than the frequency that will be encountered after more experience is gained and optimal therapeutic windows established. Nonetheless, nephrotoxicity and hypertension are two adverse effects that may be ameliorated by the use of antihypertensive agents including calcium channel blocking drugs. It is thus likely that, as with CsA, one or other members of the calcium channel blocking group of drugs will be routinely coprescribed with TRM.
1.12.4 Cost of provision of TRM

TRM is similarly priced to CsA with each 1mg capsule costing approximately AUD$4.00. The dosage required to maintain blood TRM concentrations within the currently accepted therapeutic range (5-20μg/L) appears to vary markedly within a range of 4mg to 20mg daily and hence the annual acquisition cost of providing TRM will often exceed $8,000 per patient.

TRM was included in the ‘Section 100’ scheme for liver transplant immunosuppression in 1997 but this approval was not extended to cover kidney transplantation until January 2000. The enthusiasm for using TRM in kidney transplantation has thus been tempered because the individual transplant unit/hospital was responsible for the costs of provision until it was included in the above scheme. It is reasonable to expect that the usage of TRM will increase following the lifting of this financial burden from individual transplant units.

1.13. Confounding biological factors affecting drug metabolism

Hepatic CYP 450 enzyme activity is not constant and it has long been recognised that a variety of diseases can affect drug metabolism via this enzyme system. Thus theophylline’s metabolism was shown to be affected by viral respiratory illness in the 1970s (Chang KC, et al. 1978) and more recently, nitrendipine’s kinetics were shown to be affected by acute febrile illness (Soons PA, et al. 1992). In this study, 10 patients with febrile illnesses were given nitrendipine, a dihydropyridine analogue of nifedipine during a febrile illness and again at least 6 weeks after their recovery. This data was compared to that from a similar group of patients who were given the β1 selective beta blocker, bisoprolol. These two drugs were chosen because nitrendipine is completely metabolised via the CYP3A enzyme family while bisoprolol is partly excreted unchanged.
in the urine. As expected, most patients showed an elevation of blood concentration of both enantiomers of nitrendipine during the infectious period but, because renal function was not affected, changes to clearance of bisoprolol were smaller and not detectable in this study.

One group of researchers demonstrated that infusing recombinant interleukin 6 into rats resulted in a reduction in hepatic microsomal CYP concentrations (Chen YL, et al. 1992). In an elegant study, these same researchers applied this knowledge to a study of 6 bone marrow recipients receiving constant intravenous infusions of CsA (Chen YL, et al. 1994). In all patients there was a significant increase in blood concentration of parent CsA (3.6 fold relative to day 2 of infusion) and the AM1 metabolite (2.3 fold relative to day 2 of infusion) while AM9 metabolite concentrations were low or undetectable (all being measured by HPLC). The times at which blood concentrations of CsA and AM1 increased were correlated with a prior peak in IL6 concentrations. The authors concluded that IL6 directly affected metabolism of both CsA and its AM1 metabolite and, given the difficulty of the assay for IL6, that this could best be monitored by measuring the more easily measured marker of inflammation, C-reactive protein (CRP).

1.14. Summary

CsA is currently the most important immunosuppressive drug used to prevent rejection of transplanted organs. TRM has an established place in the maintenance of liver transplants and will be more widely used in kidney transplantation now that the financial burden to transplant units has been removed by inclusion in the ‘Section 100’ scheme in Australia. Both drugs have narrow therapeutic margins and the dosage regimen of both drugs is adjusted according to the results of blood concentration monitoring. Both drugs are metabolised via the CYP 3A4 isoenzyme and a number of significant drug
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interactions have been described which result in either an increase in blood CsA/TRM concentrations (with potential for toxicity) or a decrease in these concentrations (with potential for rejection). Because CsA is substantially more expensive than former immunosuppressive drugs, deliberate coprescription of drugs which elevate blood CsA concentrations (CsA-sparing agents) has been advocated so as to allow a reduction in CsA dosage while maintaining blood CsA concentrations within the therapeutic range. The deliberate use of drugs which reduce the dose of TRM has not been advocated in the literature to this time, but given the similarity in adverse effect profile between CsA and TRM and the magnitude of the acquisition costs associated with the use of both these drugs, it is likely that TRM-sparing agents will be advocated at some time soon.

Although CsA-sparing agents have been routinely used for years and their use appears widespread, there is little data on how they are used, when they are used, when they are not used and why they are not used. The nature of the interactions between CsA and those drugs which are used as sparing agents is not well described, especially with respect to the dose-response relationship, the reliability of the interaction, the time-course of the interaction, the effect of changing formulations of sparing agent and the effect on CsA metabolite profiles. It is unlikely that TRM-sparing agents are widely used since less is known about the extent and reliability of interactions between TRM and any of the drugs which might be considered as sparing agents. Given that CsA sparing agents have been used for such a long time, this lack of knowledge is disturbing and it is highly desirable that such data be made available before contemplating the use of drugs as TRM-sparing agents.

Having identified these areas where knowledge is lacking, it is thus appropriate to investigate these and other similar areas in order to optimise the use of CsA, TRM and
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drugs which are used as sparing agents. In particular, it is important to conduct studies that will:
- identify the current usage of CsA-sparing agents by Australasian transplant physicians,
- identify why some centres may not use such agents
- examine the relationship between DTZ dose and increase in both blood CsA and TRM concentration in organ transplant recipients,
- examine the clinical sequelae of using DTZ on parameters of efficacy and toxicity of CsA and TRM.

1.15. Aims
1. To ascertain the frequency of use of CsA-sparing agents, the doses and formulations used, and savings achieved by their use in Australasian organ transplant recipients. This will be achieved by surveying transplant centres identified from the ANZDATA registry database that has details of all transplant centres performing organ transplantation in Australia and New Zealand.

2. To ascertain the therapeutic ranges for CsA that are clinically relevant to transplant physicians (as opposed to the ranges quoted by laboratories) in Australasia and to assess the effect that the use of CsA-sparing agents has on these ranges. This data will be obtained by surveying Australasian transplant centres in the same way as for Aim 1.

3. Where CsA-sparing agents are not routinely used, to ascertain the reasons for not using them in the Australasian setting. This data will be obtained by surveying Australasian transplant centres in the same way as for Aim 1.
4. To assess the therapeutic benefit of using DTZ in kidney transplant recipients with respect to affecting early graft function and blood pressure control. This data will be obtained by surveying Australasian transplant centres in the same way as for Aim 1.

5. To define the dose-response relationship for DTZ with respect to its interaction with CsA in a prospective trial in stable kidney transplant recipients.

6. To determine whether the controlled diffusion formulation of diltiazem affects the extent of the interaction with CsA or TRM.

7. To define the dose-response relationship for DTZ with respect to its interaction with TRM in a prospective trial in kidney and liver transplant recipients.

1.16. Hypotheses to be tested:
1. That the doses of DTZ currently used are greater than those required to produce a significant CsA-sparing effect for a significant number of transplant recipients.

2. That coprescribing DTZ with TRM results in an increase in blood TRM concentrations within the range of DTZ doses that are able to be used clinically and that the use of DTZ as a TRM-sparing agent is clinically acceptable.

3. That the controlled diffusion formulation of DTZ given once daily interacts with CsA, or TRM, differently and to a lesser extent than conventional release formulations.
CHAPTER 2

CHAPTER 2:

Survey of Australasian transplant centres to ascertain current practices regarding use of CsA-sparing agents, therapeutic ranges used and assay methodology.

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CHAPTER 2

2.1 Introduction:

Since its introduction in the early 1980s, CsA has become the major immunosuppressive drug used in organ transplantation and while it has improved graft survival, it has significantly increased the cost of maintaining transplanted organs. CsA is approximately twenty times more expensive than the immunosuppressive drugs it replaced (prednisolone and azathioprine) - typical CsA costs for an adult transplant are AUD$8,000 pa. (based on 4mg/kg/day Sandimmun® capsules at $7/100mg).

CsA was initially difficult to manage because of poor and variable oral bioavailability, the 'drink solution' adhered to plastic surfaces, the injection leached plasticisers from administration lines, there appeared to be limited correlation between blood concentration, efficacy and/or toxicity, little was known about drug interactions and therapeutic drug monitoring was difficult because most assay methodologies were not specific for parent CsA, were expensive and laborious. Some of these issues have been partially or completely resolved. Formulation refinements have reduced variability in bioavailability while the advent of rapid and relatively selective CsA immunoassays have facilitated therapeutic drug monitoring which now plays an important part in the management of CsA therapy.

In the mid 1980s it was noted that some drugs reduce CsA metabolism resulting in elevated blood CsA concentrations and consequent toxicity (Pochet JM & Pirson Y. 1986. Wagner K & Neumayer HH. 1985). Drugs which have been shown to elevate blood CsA concentrations include DTZ, KCZ and erythromycin, while the enzyme inducers rifampicin and phenytoin decrease blood CsA concentrations. In the late 1980s it was suggested that this interaction could be exploited in part to contain the ever growing cost of transplantation (Neumayer HH, Wagner K. 1986). The two drugs most frequently cited as 'CsA-sparing' agents are DTZ and KCZ. DTZ is thought to be more commonly used in Australia and New Zealand, but KCZ is thought to be more
commonly used in countries where the transplant recipient is required to pay for CsA because without the large dose reductions afforded by the coprescription of KCZ, CsA would not be affordable. While many transplant patients maintained on CsA have a therapeutic indication for DTZ (eg. hypertension and angina), and while there is some evidence that DTZ improves transplant kidney function (Epstein M. 1992. Chrysostomou A, et al. 1993. Vasquez EM & Pollak R. 1995) many are prescribed DTZ purely for its economic benefit. This is the first time that drugs have been advocated primarily for an economic purpose and thus it raises many ethical concerns. There is little published data on the Australasian usage of CsA-sparing agents and so this survey of Australian and New Zealand transplant centres was undertaken to ascertain current practices.

2.2 Aims:
To determine the extent to which CsA-sparing agents are used by Australian and New Zealand organ transplant centres, to determine which agents and what dosage regimens are used and why these agents are used by some but not all centres. To ascertain the assay methodologies and therapeutic ranges used by organ transplant centres and the utility of therapeutic drug monitoring.

2.3 Methods:
Questionnaires (appendix 1) were mailed to the directors of 40 heart, kidney, liver, lung and pancreas transplant centres identified from the Australia and New Zealand Dialysis and Transplant - ANZDATA -registry in late 1995/early 1996. The questions asked included the number and type of organ transplants performed, whether CsA-sparing agents were routinely used, which agent/s were used and in what dosages, what savings were associated with this use and reasons for not using CsA-sparing agents. Questions
regarding the frequency of administration of CsA, assay methodology and therapeutic ranges were also asked.

2.4 Results:
Centres were considered discrete for analytical purposes according to type of organ transplanted viz a single adult heart & lung transplant centre was counted as two centres (1 heart and 1 lung). The survey was restricted to centres performing transplantation and thus excluded referral centres that may provide ongoing care for transplant recipients once stabilised. Questionnaires were returned from 37 centres (Appendix 2), with the following breakdown: 24 kidney, 4 heart, 3 lung, 4 liver and 2 pancreas transplant centres.

Table 2.1 Response to question 1, *Approximately how many transplants do you perform each year?*:

<table>
<thead>
<tr>
<th>Tx type</th>
<th>10 OR LESS</th>
<th>11 - 20</th>
<th>21 - 30</th>
<th>31 OR MORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEART</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>LIVER</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>LUNG</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>PANCREAS</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Of the 6 centres reporting 10 or fewer kidney transplants per year, 5 were paediatric centres. Ten adult kidney transplant centres reported performing 20 or less transplants per year. Only 4 centres reported performing 50 or more kidney transplants per year. Queensland and South Australia had only 1 adult kidney transplant centre, but there were at least 7 centres in NSW, 5 in Victoria and 4 in New Zealand. Five adult kidney
transplant centres in NSW and 3 in Victoria reported performing 20 or less transplants per year. All centres performing transplants were situated in the capital city of their respective state with the exception of one centre performing adult kidney transplants which was situated in Newcastle.

Table 2.2 Response to question 2, *In what percentage of patients do you use CsA sparing-agents?:*

<table>
<thead>
<tr>
<th>Tx type</th>
<th>ALL</th>
<th>MOST</th>
<th>SOME</th>
<th>NONE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEART</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>13</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>LIVER</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>LUNG</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>PANCREAS</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Because the number of transplants performed varies considerably between units and the numbers of transplant recipients receiving CsA-sparing agents was only grouped into one of 4 categories (viz. 'all', 'most', 'some' or 'none'), it was necessary to make the following approximations. It was assumed that within the category 'most', 2/3 of transplant recipients receive CsA-sparing agents and within the category 'some', 1/3 of transplant recipients receive them. Using this approximation, 85% of heart, 71% of kidney, 66% of lung, 29% of pancreas and 6% of liver transplant recipients routinely receive DTZ.

The response to question 3, *What CsA-sparing agent do you use?* showed that DTZ was the most frequently used CsA-sparing agent. All 28 centres which reported using CsA-sparing agents (in all, most or some transplant recipients) used DTZ while four centres routinely used KCZ and one centre (heart transplant) had used both agents concurrently.
The only other agent reported in the survey which was used routinely as a CsA-sparing agent was cimetidine, which was coprescribed with DTZ in 1 lung transplant centre.

Answers to question 4, *What dose and frequency of CsA-sparing agents is used?*, showed considerable variation in DTZ doses with 2 centres using 60mg per day, 22 using between 120mg and 180mg per day while 4 centres used between 180mg and 240mg per day. The most frequently prescribed DTZ dosage regimens were 60mg (conventional formulation) thrice daily by 15 centres and 180mg ‘controlled diffusion’ (Cardizem CD® ICI Aust) once each morning by 9 centres. Correcting for number of transplants performed, 60mg thrice daily and 180mg CD each morning were equally popular (49% vs 51%) within the adult kidney transplant community. For all other transplant recipients, the 60mg thrice daily DTZ regimen was more popular than the 180mg CD each morning regimen (93% vs 7%). The dose of KCZ reported was 100 - 200mg/day and the cimetidine dose was 400mg twice daily.

Response to question 5, *How long after transplantation are CsA-sparing agents started?*, most reported commencing therapy within the first week (23 centres) while 5 centres waited for longer periods (up to 26 weeks) post transplantation. Fifteen centres reported starting CsA-sparing agents on the first day after transplantation.

<table>
<thead>
<tr>
<th></th>
<th>1986 or before</th>
<th>1987-8</th>
<th>1989-90</th>
<th>1991-2</th>
<th>1993 or since</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLZ</td>
<td>1 centre</td>
<td>5 centres</td>
<td>9 centres</td>
<td>9 centres</td>
<td>4 centres</td>
</tr>
<tr>
<td>KCZ</td>
<td>-</td>
<td>-</td>
<td>1 centre</td>
<td>1 centre</td>
<td>2 centre</td>
</tr>
</tbody>
</table>

Three of the four adult kidney transplant centres performing 50 or more transplants per year started using CsA-sparing agents before 1991.
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Answers to question 7, *How frequently is CsA administered each day?*, showed that the majority of centres used a twice daily CsA regimen although two centres (1 kidney, 1 pancreas) used a once daily regimen and three centres sometimes used a thrice daily regimen. Two of the five paediatric transplant centres reported using thrice daily CsA regimens in some patients.

Responses to question 8, *What values do you use as a therapeutic range for CsA?*, were divided into three separate periods; the period soon after transplantation, an intermediate period and in the long term. Twenty eight centres responded that they used different ranges for all three periods, 6 centres reported using only two discrete ranges while 3 centres reported using only one range irrespective of time post transplantation. Data from the adult kidney transplant population are presented in Table 2.4 and the corresponding data for paediatric kidney transplantation are presented in Table 2.5. In order to rank the ranges in tables 2.4 and 2.5, the mid-point of each range was taken for comparison. Hence two ranges with the same mid-point value are quoted in the highest range for the early and middle periods post transplantation.

Table 2.4 Highest and lowest CsA therapeutic ranges (ranked by mid-point value) reported for adult kidney transplant recipients:

<table>
<thead>
<tr>
<th>Period post transplant</th>
<th>lowest range(µg/L)</th>
<th>highest range(µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early period</td>
<td>80-250</td>
<td>300-400 or 200-500</td>
</tr>
<tr>
<td>Middle period</td>
<td>130-170</td>
<td>200-400 or 250-350</td>
</tr>
<tr>
<td>Late period</td>
<td>50-150</td>
<td>225-300</td>
</tr>
</tbody>
</table>

Where more than one range was identified, the values given for the early period after transplantation were always higher than the middle which were higher than the late period. The mean of the mid-point values quoted for CsA therapeutic ranges for early,
middle and late periods after transplantation from heart (425, 288, 206\mu g/L respectively) and lung (417, 283, 225\mu g/L) transplant centres, although fewer in number, were higher than the corresponding ranges quoted from kidney (270, 202, 151\mu g/L), pancreas (325, 213, 150\mu g/L) or liver (244, 206, 138\mu g/L) transplant centres.

Table 2.5 Highest and lowest CsA therapeutic ranges (ranked by mid-point value) for paediatric kidney transplant recipients

<table>
<thead>
<tr>
<th>Period post transplant</th>
<th>lowest range(\mu g/L)</th>
<th>highest range(\mu g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>early period</td>
<td>100-150</td>
<td>300-500</td>
</tr>
<tr>
<td>middle period</td>
<td>75-100</td>
<td>200-400</td>
</tr>
<tr>
<td>late period</td>
<td>50-75</td>
<td>150-300</td>
</tr>
</tbody>
</table>

The frequency that values were used as therapeutic ranges are presented for each of the three periods post adult kidney transplantation in Figures 2.1, 2.2 and 2.3.

Figure 2.1 Frequency distribution of CsA therapeutic range for the early period post adult kidney transplantation. The light shaded bars represent the bottom end of the range and dark shaded bars the upper limit of the ranges quoted.
Figure 2.2 Frequency distribution of CsA therapeutic range for the middle period post adult kidney transplantation. The light shaded bars represent the bottom end of the range and dark shaded bars the upper limit of the ranges quoted.

Figure 2.3 Frequency distribution of CsA therapeutic range for the late period post adult kidney transplantation. The light shaded bars represent the bottom end of the range and dark shaded bars the upper limit of the ranges quoted.
A supplementary, telephone question was asked of respondents after some suggested that the assay method would affect the therapeutic range used. This question (which assay methodology was used?) shows that the FPIA/TDx® (Abbott Diagnostics, Chicago, USA) assay was the most prevalent assay method in both Australia and New Zealand (22 centres). Two centres used HPLC, 8 used enzyme multiplied immunoassay (EMIT®, Syva, San Jose, USA) and 5 used a radioimmunoassay (CYCLO-Trac-SP®, Incstar, Minnesota USA). Mid point values quoted for the bottom and top of the therapeutic range for adult kidney transplantation for each of the three time periods (early, middle and late post transplantation) for the less specific assay method (FPIA/TDx®) vs more specific methods (EMIT®, HPLC & CYCLO-Trac-SP®,) are presented in Figures 2.4 – 2.9.

![Graph](image)

*Figure 2.4 Frequency distribution of the lower value of the therapeutic ranges quoted for the early period adult kidney post transplantation. Lighter shaded bars represent values from units using the less-specific (FPIA/TDx) assay methodology, dark shaded bars represent units using the more specific (EMIT, HPLC & CYCLO-Trac-SP®,) assay methodology.*
CHAPTER 2

Figure 2.5 Frequency distribution of the higher value of the therapeutic ranges quoted for the early period adult kidney post transplantation. Lighter shaded bars represent values from units using the less-specific (FPIA/T Dx) assay methodology, dark shaded bars represent units using the more specific (EMIT, HPLC & CYCLO-Trac-SP®) assay methodology.

Figure 2.6 Frequency distribution of the lower value of the therapeutic ranges quoted for the middle period adult kidney post transplantation. Lighter shaded bars represent values from units using the less-specific (FPIA/T Dx) assay methodology, dark shaded bars represent units using the more specific (EMIT, HPLC & CYCLO-Trac-SP®) assay methodology.
Figure 2.7 Frequency distribution of the higher value of the therapeutic ranges quoted for the middle period adult kidney post transplantation. Lighter shaded bars represent values from units using the less-specific (FPIA/TDx) assay methodology, dark shaded bars represent units using the more specific (EMIT, HPLC & CYCLO-Trac-SP®) assay methodology.

Figure 2.8 Frequency distribution of the lower value of the therapeutic ranges quoted for the late period adult kidney post transplantation. Lighter shaded bars represent values from units using the less-specific (FPIA/TDx) assay methodology, dark shaded bars represent units using the more specific (EMIT, HPLC & CYCLO-Trac-SP®) assay methodology.
Figure 2.9 Frequency distribution of the higher value of the therapeutic ranges quoted for the late period adult kidney post transplantation. Lighter shaded bars represent values from units using the less-specific (FPIA/TDx) assay methodology, dark shaded bars represent units using the more specific (EMIT, HPLC & CYCLO-Trac-SP®) assay methodology.

The mid point values from the therapeutic ranges quoted by users of the EMIT® assay were lower than those reported by users of the FPIA/TDx® assay for the early (259 vs 276μg/L) and mid periods post transplantation (196 vs 206μg/L). The values for the late period post transplant were virtually identical (157 vs 156μg/L). These differences were not statistically significance (Mann-Whitney test, p values 0.633, 0.541 and 0.74 for the early, mid and late period post transplant).

In response to question 9, Are there specific reasons for not using CsA-sparing agents?, the wish to avoid polypharmacy was noted by respondents from 7 centres (2 paediatric), ethical concerns were noted from 3 centres while one centre reported that a locally conducted study had concluded that DTZ was of little economic value in the long term (although it was still routinely used in some patients in a low dose).
In answer to the final question, 'What reduction in dose of CsA do you feel you achieve?', respondents estimated the savings in CsA dosage facilitated by the use of sparing agents. These varied from 20% (6 centres) through 30-40% (20 centres) to 50-60% (2 centres) with DTZ. Those centres quoting a lower value were more likely to use a lower dose (120mg or less per day in 3 of these 6 centres) while those quoting the highest value were more likely to use a higher DTZ dose (including one paediatric unit using 180mg/day).

KCZ was reported to reduce CsA dose by more than 70% in five centres and one centre reported the combined use of DTZ and KCZ reduced CsA dose by 80-90%. The reduction in CsA dose afforded by the use of cimetidine was unknown.

2.5 Discussion:
This survey, like all surveys, has limitations. In particular, the views obtained were those of the respondent rather than a consensus of all practitioners in the unit. Because the respondent was usually the Director of the transplant unit, it is however likely that this is the predominant view and the most authoritative available. The approximation used to assess the number of transplant recipients taking CsA-sparing agents is another limitation. The respondent was not asked for actual numbers because it was anticipated that the extra work involved might have reduced the willingness to complete and return the questionnaire. Hence findings derived from these data (especially the estimated annual cost savings) should be viewed with some caution. The times post transplantation (early, middle and late) were also not specified but left to the respondent. This question was deliberately formulated to gauge whether practitioners considered a gradual reduction in blood CsA concentration was appropriate as concerns over chronic toxicity exceeded the concern of rejection. The question did not ask if the ranges were officially
quoted by the unit and hence the answer might have been different if another member of staff (and especially a member of the analytical laboratory) was asked.

This survey has highlighted a number of differences between Australian and New Zealand transplant centres with regard to the dosage and formulation of the most frequently prescribed CsA-sparing agent (DTZ), the therapeutic ranges quoted and assay methodologies employed. Perhaps the most important of these is whether CsA sparing agents should be used at all.

2.5.1 To Use CsA-sparing agents Or Not?
Nine centres surveyed do not use CsA-sparing-agents at all and a further six centres only use them for 'some' patients. Heart (4/4 use in all or most), lung (2/3 use in all and 1/3 in some) and kidney (14/24 use in all or most) transplant centres are more likely to use CsA sparing agents than liver or pancreas transplant centres. The data for CsA-sparing agent use by liver transplantation centres (only 1 out of 4 centres surveyed used these agents in only 'some' patients) and pancreas transplantation centres (one unit used CsA-sparing agents in all patients while the other used DTZ only when it is indicated for a therapeutic purpose) is interesting because the absorption of CsA has been reported to be decreased following abdominal surgery and if bile flow or liver function is impaired (Lindholm A. 1991). The consequence of this is that to achieve comparable blood CsA concentrations, CsA dosages must be higher in recipients of liver and pancreas transplants than those used in renal transplant recipients and hence the need for agents which might allow a lower dose to be administered is arguably greater.

Compliance has been shown to be affected by the number of drugs and/or doses taken each day (Schweizer RT, et al. 1990. Kiley DJ, et al. 1993) and thus the prescription of
any additional drug may reduce compliance. This was the reason given by 7 respondents for not using CsA-sparing agents.

Most of the data on DTZ's role as a CsA-sparing agent has come from studies of recipients of kidney transplants but this survey highlights the lack of consensus in this population within Australia and New Zealand almost 10 years after this was first advocated. The routine use of CsA-sparing agents early in the post transplant period (23 out of 28 centres using CsA-sparing agents commence them in the first week post transplantation) indicates that the primary reason is for economic rather than therapeutic benefit. Hence the reason for this lack of uniformity might be related to the different funding difficulties that affect states or institutions. This survey has shown that Australian kidney transplant centres who do not routinely use CsA-sparing agents are sometimes situated close to centres who routinely use it in all patients, which suggests that the decision to use CsA-sparing agents is not made at a state level but within individual transplant units. Also one respondent noted that the decision to not use CsA-sparing agents was based upon "local reasons for wishing to delay cost savings" which operated at the time of the survey. Another possibility is that centres performing more transplants have a greater financial incentive to use CsA-sparing agents. Results from this survey support this hypothesis since 3 of the 4 kidney transplant centres performing 50 or more transplants per year use DTZ routinely (all or most patients) while only one does not (used only in some patients).

2.5.2 Who benefits from the use of CsA-sparing agents?
In 1991, the Commonwealth government assumed funding for CsA for organ transplantation in Australia under the 'section 100' scheme. Since this time therefore, neither individual transplant units, the hospitals of which they are a part, nor State governments have gained financially by using CsA-sparing agents. Indeed, individual
transplant units, hospitals and/or states are financially poorer because this practice requires them to provide the CsA-sparing-agent (which is not covered by the section 100 scheme). Interestingly therefore, while three of the four kidney transplant units who perform 50 or more transplants per year started using DTZ as a CsA-sparing agent before 1991, the fourth centre (where DTZ is used in only some patients) started this practice in 1993, i.e., after the 'section 100' scheme was in operation. In New Zealand, the costs are assumed by the individual transplant unit, hospital and health authority and thus there is a direct economic benefit to the prescribers of CsA-sparing agents. Not surprisingly perhaps, only one of the five New Zealand respondents (3 kidney, 1 heart, 1 lung) to this survey reported not routinely using CsA sparing agents.

It is interesting that in neither country does the transplant recipient benefit financially from the reduced dose of CsA and indeed, is required to pay an additional fee for the CsA-sparing agent. Transplant recipients are unlikely to be involved in the decision to use CsA-sparing agents and may not even be aware of the reasons for their prescription.

This is the first time in Australia or New Zealand that a drug has been widely used for a purely economic purpose and it is only because of the magnitude of the cost of CsA that this is done. With the loss of patent protection (which has already expired in Australia), it is possible that cheaper generic brands will become available and the economic pressure to use CsA-sparing agents may lessen. Another factor which may affect the rate of usage of CsA-sparing agents shown in this survey is the more recent availability of an improved oral formulation (Neoral® Sandoz) which has been shown to produce more reliable and better absorption but at a similar price to the older formulation. If the action of CsA sparing agents is (at least) in part due to improved bioavailability, the magnitude of their effect may well be decreased with this formulation.

Since the Commonwealth government is the economic beneficiary of the use of CsA-sparing agents in Australia and since this body has the responsibility for approving
indications for use of drugs, it seems appropriate that the Commonwealth should actively participate in (if not lead) the debate over their use. It is worth noting therefore that neither DTZ nor KCZ are approved for use as CsA-sparing agents in Australia or New Zealand and their respective manufacturers do not promote this use. The manufacturers of CsA have long opposed the use of CsA-sparing agents, but awareness of their use is widespread within the medical community and it is unlikely that regulatory authorities in either Australia or New Zealand could be ignorant of this practice. Based upon the 35% savings estimated in the Australian kidney transplant setting (Chrysostomou A, et al. 1993), this survey indicates that if the practice of using CsA-sparing agents were abruptly stopped, the costs of providing CsA would increase by approximately AUD$7 million per year in Australia (the cost for providing CsA under the 'section 100' arrangement was approximately AUD$28 million for the fiscal year 1995-1996).

2.5.3. To Use DTZ or KCZ

This survey shows that the decision as to whether DTZ or KCZ is used as the CsA-sparing agent does not depend upon cost factors alone since KCZ is substantially more potent in this regard but is only used by four transplant centres. The safety of using DTZ as a CsA-sparing agent in transplant patients has been established by clinical trials involving many patients (Chrysostomou A, et al. 1993. Wagner K, et al. 1988. Neumayer HH, Wagner K. 1986), one of which was conducted in Australia. While there is data on the safety of using KCZ as a CsA-sparing agent (Keogh A, et al. 1995), experience with this latter agent is more limited. The limited use of KCZ reported in this survey is probably due to a combination of the reported adverse effects and the minimal (if any) additional therapeutic benefit which, for the majority of transplant centres, outweigh the increased dollar saving. In particular, hepatotoxicity (including toxic hepatitis) has been reported with KCZ and one authoritative review notes that the risks are thought to increase with the duration of therapy (Tester-Dalderup CBM. 1992). The
safety or otherwise of using KCZ as a CsA-sparing agent in transplant recipients will not be established until more experience is obtained and thus it is of interest that one of the respondents suggest that such trials may be difficult to perform in Australia due to pressures exerted by pharmaceutical companies, ethics committees and/or drug committees (Personal communication, A Keogh, 1995).

2.5.4. Dosage regimen of DTZ
The threefold variation in DTZ dose (60 to 180mg/day) across paediatric centres and the fourfold variation across adult centres (60 to 240mg/day) is interesting since the interaction between DTZ and CsA was initially noted in patients taking 'conventional' doses of DTZ for therapeutic purposes. Because of the serendipitous nature of the finding that DTZ reduced the required dose of CsA and the lack of dose-response data in the literature at the time of this survey, it is not surprising that usual anti-anginal or anti-hypertensive DTZ dosage regimens (60mg thrice daily or 180mg CD formulation taken each morning) were the most frequently reported in this survey. The recommended dose of DTZ in Australia is 180mg – 360mg/day but no centre reported using doses higher than 240mg per day. This is interesting given the incidence of hypertension following some types of organ transplantation which might sometimes warrant higher DTZ doses and which might then afford a greater CsA-sparing effect.

2.5.5. Formulation Effects
There has been a trend in Australia to switch hypertensive patients from conventional release (tablet) DTZ formulation to the modified release, CD capsule formulation, principally for compliance reasons. Twelve Australian transplant centres reported using the CD formulation routinely as a CsA-sparing agent whereas, despite its availability, no New Zealand transplant centre used this formulation. This is of interest since, at the time
of this survey, there was evidence that CsA was absorbed from the upper gastrointestinal tract (Drewe, J, et al. 1992) and also some evidence that the interaction between DTZ and CsA occurs in the enterocyte (Hebert MF, et al. 1992). Modified release formulations will inevitably release some of their DTZ lower down the gastrointestinal tract, perhaps beyond the site of the interaction. There is thus cause to suspect that modified release formulations of DTZ might interact to a lesser extent than conventional release formulations. At the time of this survey, there was no data in the literature on the effect of changing DTZ formulation although it had been noted that altering the brand of DTZ could result in variable effects on blood CsA concentrations (Cooke CE. 1994).

Also of interest is that two centres use conventional (ie non slow release) formulations of DTZ given only once daily. Because of its short residence time in both gastrointestinal tract and plasma (the manufacturer's product information states DTZ is completely absorbed and has a half-life of 3.5 hours), it is likely that, unless DTZ acts as an 'enzyme poison', the effect of such a regimen will be to elevate primarily the daytime blood CsA concentration-time profile. Since CsA dosage modifications are usually based upon morning trough concentrations, a single morning DTZ dose combined with twice daily CsA dosing would produce higher overall CsA concentrations (and potential toxicity).

2.5.6. CsA therapeutic range
The questionnaire asked which concentrations are used as a therapeutic range for CsA for the early period after transplantation, a middle and late period. These periods were not defined and since they were usually the response of the director of the transplant unit, they therefore do not necessarily reflect the 'official' ranges quoted by the assay laboratory. Most respondents (28) identified three different ranges, 6 identified two discrete ranges and the remaining 3 identified only one range. Where more than one range was identified, the values given for the early period after transplantation were
always higher than the middle or late period. This reflects the view that the immunological challenge of a transplanted organ decreases with time, while the incidence of adverse effects caused by CsA increases with time.

Higher CsA therapeutic ranges were reported from heart and lung transplant centres than from kidney, pancreas or liver transplant centres. This may reflect either the immunological nature of the heart or lung, the dire consequences of rejection for these organs, or a lack of knowledge on the efficacy of lower CsA concentrations in heart and/or lung transplantation.

While therapeutic ranges reported from units performing heart, lung, liver and pancreas transplants were similar for each organ type, there was considerable disparity in ranges reported from kidney transplant centres. This was especially evident from paediatric centres where therapeutic ranges quoted from two of the five centres surveyed were lower than corresponding ranges for adult kidney transplant centres, while the ranges quoted from the other three centres were on the higher side of the adult ranges quoted. Indeed, the lowest range quoted for the early period after transplantation from paediatric centres (100-150µg/L) is lower than the highest range quoted for the period late after transplantation (150-300µg/L) despite the same assay methodology being used (Table 2.5).

The highest and lowest values reported for the late-period range for adult kidney transplantation are disparate and do not even overlap. The highest range reported is 225-300µg/L while the lowest is 50 -150µg/L (Table 2.4). There was a similar difference in the values used for the therapeutic range for the early period following kidney transplantation where the highest range reported was 300-400µg/L and the lowest was 80 -250µg/L. The consequences of rejection of transplanted organs are such that it is undoubtedly logical to err on the higher side of the blood concentration range
CHAPTER 2

initially (especially for heart and lung transplantation) and, to reduce the impact of drug toxicity, to aim for a lower value in the long term. Data in the literature supports a relationship between blood CsA concentration and efficacy and toxicity (Dunn J, et al. 1990. Lindholm A & Kahan BD. 1993) but this remains somewhat controversial (Tsunoda SM & Aweka FT. 1996) and relates primarily to adult kidney transplantation. This survey shows a lack of consensus on this aspect of CsA therapy in Australia and New Zealand, and more work is needed to define a therapeutic range for CsA if indeed one can be defined.

Similar variability with respect to therapeutic ranges (and assay methodology) has been noted by other authors (Morris RG. 1994, Morris RG. et al, 1994. Holt DW, et al. 1994. Oellerich M, et al. 1995) but, given the relative homogeneity within the Australasian kidney transplant community, the magnitude of this variability is perhaps surprising.

2.5.7. CsA assay methodology and monitoring

Because clinicians (rather than laboratories) were surveyed, these results do not necessarily reflect the popularity of any assay method. Thus one hospital performing heart, lung and kidney transplantation that used the same laboratory for all CsA assays, was recorded in this survey as three separate users of the same assay method. Similarly, laboratories that might have provided CsA assay services solely to referral centres were not included.

The type of CsA assay used and in particular, its specificity for parent CsA, should affect the values used for the therapeutic range used since less specific assays cross-react to a greater extent with CsA metabolites and thus have higher total values than assays which measure only parent CsA. This was observed anecdotally by several respondents to the
original questionnaire and was the reason for the additional (verbal) question regarding assay method used. There is evidence that some metabolites of CsA may exert an immunosuppressive action (Kunzendorf U, et al. 1989) but more recent reviews have cast doubt on this (Oellerich M, et al. 1995). The degree of metabolite cross reactivity with the most popular (FPIA/TDx®) assay noted in this survey is greater than other, rapid assays (EMIT®, CYCLO-Trac-SP®) but much less than earlier, non-specific assays. Hence it is unlikely that metabolite cross reactivity is the reason for the popularity of this assay which is more likely to be related to logistical issues including availability of this analyser and its ease of use.

High pressure liquid chromatography (HPLC) is considered to be the ‘reference standard’ assay method because it measures only parent CsA, while the fluorescence polarisation immunoassay (FPIA/TDx®, Abbott Laboratories) assay methodology has been shown to be the least specific assay of those quoted in this survey (Dusci LJ, et al. 1992). Different assay methodologies have been used at various times in Australia (Morris RG. 1994) and individual units have adopted and changed assay methods (and therapeutic ranges) independently of each other. This has the potential to cause confusion, especially where patients move from one centre to another for ongoing care. Guidelines have been published for CsA therapeutic monitoring in Canada (Shaw LM, et al. 1990), in the USA (Shaw LM, et al. 1987), in the UK (Holt DW, et al. 1994) and Australia (Morris RG, et al. 1994) which would, if adopted, eliminate this problem.

Ranges quoted by users of more specific assay methods (HPLC, CYCLO-Trac-SP® and EMIT®) were generally lower than those quoted by users of the FPIA/TDx® assay. The EMIT® assay was the most frequently used ‘more specific’ assay (6 adult kidney transplant centres) while the FPIA/TDx® assay was the most frequently used ‘less specific’ assay (15 adult kidney transplant centres). Comparison of the mean values for
CsA ranges from these two assay methodologies (FPIA/TDx® vs EMIT®) from adult kidney transplant centres are as follows:

- for the early period post transplantation, 276μg/L (FPIA/TDx®) vs 259μg/L (EMIT®)
- for the intermediate period, 206μg/L (FPIA/TDx®) vs 196μg/L (EMIT®)
- for the late period post kidney transplant, 156μg/L (FPIA/TDx®) vs 157μg/L (EMIT®)

As noted earlier, the differences between these mean values are not statistically different (Mann Whitney test). Since several respondents noted assay method should affect the range used, this finding is somewhat surprising.

Interestingly, one liver transplant centre used both monoclonal (relatively specific) FPIA/TDx® and polyclonal (non-specific) FPIA/TDx® assays where the difference was used as a guide to the metabolic activity of the transplanted liver. Because of this cross reactivity, this centre quoted the highest overall therapeutic range (200 - 800μg/L). This was excluded from other analyses because it was used primarily as a guide to liver metabolic activity.

The sole kidney transplant centre using the HPLC assay method reported therapeutic ranges which were lower than the average (mean values for the early and middle periods 175μg/L and late period 125μg/L) but are not the lowest single range reported. The highest individual value for a CsA therapeutic range (excluding the polyclonal FPIA/TDx® assay mentioned earlier) was 400-600μg/L which is the range used for the early period following heart or lung transplantation in one centre. Despite several respondents reporting that therapeutic ranges should be modified to account for differences in assay specificity, these results suggest that the lack of consensus over CsA therapeutic range owes little to the assay method used. These data therefore suggests
that increasing the level of uniformity with respect to assay methodology will not necessarily translate into less variability in values quoted for CsA therapeutic ranges.

2.5.8. Frequency of dosing for CsA

Only two adult transplant centres use a once daily CsA dosing regimen, all others use two or more times per day. Once daily administration results in higher peak blood CsA concentrations and lower trough concentrations than equivalent twice daily regimens. Since CsA therapeutic monitoring is based on trough concentrations, adoption of a lower trough range would be appropriate for centres using a once daily CsA dosing regimen. An alternative approach was adopted by the two centres using a once daily regimen, namely the use of a 15 hour (rather than 24 hour) post dose blood CsA concentrations. The values quoted for CsA therapeutic ranges from these two centres (mean values for the early, middle and late period being 300, 225 and 150μg/L respectively) were comparable with those from centres using a twice daily regimen.

Two of the five children's transplant centres surveyed prescribed CsA three times a day. This regimen has presumably been adopted because of the higher clearance values seen for CsA in children (Jacqz-Aigrain et al. 1994) and the reduced surface area of bowel which has been shown to correlate with CsA absorption and dosage requirements (Whittington PF, et al. 1990). Thrice daily CsA regimens should result in higher trough and lower peak values than for a comparable twice daily regimen and hence thrice daily CsA ranges (which are based upon trough concentrations) should be higher. Data from this survey shows the opposite effect. The mean of the values quoted for the three post transplant periods (early, middle and late) for the two centres which sometimes use thrice daily CsA regimens are 150, 88 and 81μg/L. The comparable mean values for the three centres utilising twice daily CsA regimens are 358, 250 and 167μg/L. These mean trough values are much higher (indeed, the ranges barely overlapped at any period post
transplantation) for the twice daily CsA regimen centres which magnifies the increased exposure to CsA by those centres using twice daily CsA regimens.

2.5.9 Numbers of transplants performed at each centre
There was considerable disparity between Australian States with respect to the numbers of transplant centres offering adult kidney transplants and hence the numbers of transplants performed by each centre each year. In particular, New South Wales (seven centres) and Victoria (five centres) had the majority of centres and while these are the most populous States, they are not geographically the largest. Only one centre in each state performed 50 or more transplants per year with the exception of Western Australia, where the two centres performing kidney transplantation performed 25 or less per year. Ten adult kidney transplant centres reported performing 20 or less transplants per year, four of these were in New South Wales and three in Victoria.

With the exception of one centre in Newcastle, all centres performing adult kidney transplants are located in the capital city of their respective states and hence the number of centres appears to be unrelated to ease of access for patients and/or relatives. Queensland has only one centre which is located in Brisbane, a very long distance from expanding population centres in northern Queensland.

Questions asked in this survey did not allow any conclusions to be drawn regarding the issue of optimal numbers needed to maintain satisfactory levels of expertise. However, while kidney transplantation is a relatively routine procedure nowadays, it is probable that there is a minimum number of procedures that need to be performed each year both to justify the expertise that is required and to maintain the standard of that expertise.
Precisely how individual centres came to provide adult kidney transplant services is a matter of historical fact but presumably, it was not planned by a central authority. Given the concentration of centres in Sydney and Melbourne, and the distances some patients must travel, there could be advantages in rationalising both the numbers and location of these centres.

2.6. Conclusions

This survey has demonstrated considerable variability within Australia and New Zealand in relation to dosage regimens of the CsA-sparing agent, DTZ. This variability probably results from the paucity of data on the interaction. There is also considerable variability in both assay methodologies and values quoted for therapeutic ranges for CsA which are not explained by differences in assay specificity. Some of the clinical ramifications of these differences are discussed in Chapter 3.
Chapter 3

Survey of outcomes of Australasian kidney transplant recipients for the first 12 months post transplantation to ascertain the effect of blood CsA concentration and/or DTZ use on measures of organ rejection and/or CsA toxicity.

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3.1 Introduction

The first experience with CsA in organ transplantation occurred in the late 1970s when it was demonstrated to have powerful immunosuppressive action in a variety of animal models involving a number of different transplant organ types (Green CJ & Allison AC. 1978. Calne RY, et al. 1978b). In these early studies, it was noted to be more potent than existing agents and appeared to have a very modest adverse effect profile, especially when compared to existing immunosuppressive drugs. Initial use in human transplant medicine often included other immunosuppressive drugs (Calne RY, et al. 1979). Unfortunately, the effect of this combination of CsA with existing immunosuppressive drugs was to greatly increase mortality via serious infections and/or tumours (especially lymphomas) in transplant recipients. Because of this experience and the desire to avoid the long-term adverse effects of corticosteroids, the focus turned to using CsA as the sole immunosuppressive agent.

Early experience was also hampered by lack of information on optimal dosage, primitive formulations with unknown bioavailability and availability of a suitable assay methodology. Doses of CsA were higher than those used today (initially 25mg/kg/day administered by intramuscular injection) with the consequence that adverse effects including nephrotoxicity, hepatotoxicity and hirsutism, albeit relatively mild, were frequently seen (Calne RY, et al. 1978a).

Some years later, assays were developed but early methodologies were unsatisfactory from a number of perspectives including complexity and cost of HPLC assays, and the capacity of antibody based immunoassays to cross-react with metabolites of CsA. These non-selective assays yielded results which were 3-5 fold greater than existing HPLC or current immunoassay methods (Tredger JM, et al. 1988. Zylber-Katz E & Granit L. 1989. Schran HF, et al. 1987). This delayed the construction of therapeutic ranges that
would eventually assist clinicians to prescribe CsA in a manner that maximised therapeutic benefits while minimising toxicity (Shaw LM, et al. 1987). Assay methodologies have improved substantially (especially with respect to immunoassay cross-reactivity with CsA metabolites) and are sufficiently easy to perform such that daily assay services are available to most clinicians using CsA. This improvement has allowed therapeutic ranges for CsA to be refined and therapeutic monitoring has become an integral part of CsA therapy for most indications. Notwithstanding these technical advances, it has been observed that there is only limited agreement about the blood CsA concentrations that should be used for the therapeutic range for any given indication (Shaw LM, et al. 1987. Shaw LM, et al. 1990. Morris RG. 1994. Holt DW, et al. 1994).

Therapeutic ranges should not remain static but should evolve as drug therapies evolve. With CsA therapy, evolution has seen the focus change from add-on therapy (noted above) through monotherapy (because of concerns over corticosteroid adverse effects and increased risk of malignancies) to the more recent dual, triple (or more) regimens. The aims of combination therapy with two or more immunosuppressive drugs are to minimise the frequency and severity of adverse effects by using lower doses of each individual agent while reaping the benefits of additive (or synergistic) immunosuppression. Typically, azathioprine or the newly introduced mycophenolate mofetil are coprescribed with CsA in combination regimens (sometimes with prednisolone). Protocol changes which involve lower doses of CsA of this type should be accompanied by a downward shift in desired therapeutic ranges, but this is not evident with CsA. The shift from monotherapy with CsA to dual or triple drug regimens occurred at a time when therapeutic ranges were at an early stage of evolution and hence
it is not surprising that there are no comparative data on therapeutic ranges for CsA when used as monotherapy or when used as a part of dual/triple therapy.

Discord in the values used for CsA therapeutic ranges was evident in an Australasian survey conducted in 1995-6 as outlined in Chapter 2. Results from this survey revealed a marked variability in therapeutic ranges quoted for CsA by the directors of individual transplant units. Differences were evident not only across organ transplant types, as has been reported previously (Holt DW, et al. 1994) and might be expected, but also within the same transplant organ type. The magnitude of this variability in CsA therapeutic ranges exceeded that which might be anticipated as a consequence of variability in metabolite cross-reactivity of the different assay methodologies employed at this time. This was surprising considering the relative uniformity of Australasian transplant funding arrangements, kidney availability, quality of care, etc. and the amount of research conducted into CsA at the time this survey was conducted.

Within the same organ transplant type, the variability in CsA therapeutic ranges was greatest for the paediatric kidney transplant population. There are however, only limited number of these transplants performed each year and there is considerable variability within this population with respect to ages of donors and recipients, etc. It is probable therefore that any analysis of this population examining clinical sequelae resulting from differences in therapeutic ranges would prove inconclusive due to a lack of statistical power. There was also large variability in CsA therapeutic ranges quoted by directors of adult kidney transplant centres. The adult kidney transplant population is larger in number and hence any effect of using different therapeutic ranges for CsA might be evident in this group.

Therapeutic ranges usually comprise 2 numbers, the lower value being the minimum concentration for efficacy and the higher value being the point above which efficacy is
only marginally increased but toxicity increases significantly. Markers of efficacy of CsA include frequency and severity of transplant rejection which can be measured in a number of ways including the need for dialysis and/or additional immunosuppression (methylprednisolone, muromonab or other antibody preparations) in the early postoperative period and/or graft survival. Since CsA causes hypertension and renal impairment, markers of CsA toxicity which might be investigated include blood pressure and/or the use of antihypertensive drugs, and plasma creatinine concentrations.

DTZ has been shown to interact with CsA such that the average dose of CsA for a population of organ transplant recipients can be reduced by approximately 35% while maintaining blood CsA concentrations within accepted therapeutic ranges. If this interaction were purely pharmacokinetic (ie, to alter parent blood CsA concentrations only), limited or no alteration to the CsA therapeutic range would be warranted. This was a finding from the survey outlined in Chapter 2 where no transplant centres used a different CsA therapeutic range for patients given DTZ. However, if DTZ exerts effects other than elevating parent CsA blood concentrations (including altering active CsA metabolite concentrations), or if immunological processes were affected, as has been noted with both DTZ and verapamil (Weir MR. 1991. Carozzi S, et al. 1995), an entirely different therapeutic range might be warranted for patients coprescribed DTZ.

DTZ has been shown to confer therapeutic benefit to recipients of kidney transplants in the form of reduced need for post transplant dialysis and immunosuppressive therapy (Chrysostomou A, et al. 1993). The authors of this prospective, randomised study involving two Australian adult kidney transplant centres also demonstrated that DTZ coprescription reduced the required dose of CsA by 35% and that, despite its marketed indication for hypertension, coprescription of DTZ had no apparent effect on lowering blood pressure or reducing the need for antihypertensive medication at 3 months post transplantation. These authors concluded that it was ineffective as an antihypertensive
agent at the dose used (180mg/day). There were several shortcomings with this trial, including the failure to control for therapeutic practices at the two sites (other than the use of DTZ) and the use of different assay methodologies and therapeutic ranges (one centre used an FPIA/TDx polyclonal assay with a target therapeutic range of 400-800µg/L, while the other centre used a more specific assay methodology (EMIT) with a therapeutic range quoted as 80-250µg/L). Blood CsA concentrations were also higher in DTZ treated patients at most times post transplant and hence the benefit observed with DTZ may have resulted merely from increased CsA exposure. It is thus difficult to determine if differences in outcome in this study were due to a pharmacological effect of DTZ or other factors including blood CsA concentration. To address these shortcomings, another prospective, double blind, placebo controlled, randomised trial controlling for all factors other than DTZ use might be conducted.

In Chapter 2, it was demonstrated that the use of DTZ is widespread, but not universal within the Australasian kidney transplant setting and that the decision to not use DTZ is not made for medical reasons. Given the similarity in recipient and donor population, standards of medical care, etc. within the Australasian transplant community, comparing outcomes for transplant recipients receiving DTZ with those that do not receive it, might shed some light on the potential for therapeutic benefit conferred by DTZ. Data analysis from this population would need to control for any effect which was attributed to individual centres or other practices and focus on the effects of using DTZ and/or the effect of blood CsA concentrations on markers of efficacy and toxicity.

Since outcomes of kidney transplantation are affected by source of kidney (viz cadaver or living donor) and previous transplant history, these factors should also be controlled for in a study which aims to identify differences in outcome that are caused by different blood CsA concentrations and/or use of DTZ.
3.2 Aims

- To determine whether blood CsA concentration in the early post transplant period is a predictor of efficacy (immune suppression) as indicated by the need for post transplant dialysis, additional immunosuppression or early graft function, and of toxicity (hypertension and nephrotoxicity) in first time recipients of cadaver kidney transplants.
- To determine whether blood CsA concentrations at 3, 6 and 12 months post transplantation correlate with markers of efficacy (indicated by plasma creatinine concentration) or toxicity (indicated by systolic and diastolic blood pressures and numbers and doses of antihypertensive drugs)
- To determine the values of blood CsA concentration that correlate with these outcomes and hence devise (or refine) a therapeutic range for CsA for adult recipients of cadaver kidney transplants in Australasia.
- To confirm earlier observations that DTZ has a beneficial therapeutic effect on markers of kidney function post transplantation including a reduced need for dialysis and use of additional immunosuppressive drugs (methylprednisolone, muromonab, etc.)
- To confirm that DTZ does not exert an antihypertensive effect (determined by systolic and diastolic blood pressures and use of antihypertensive drugs) in kidney transplant recipients at the doses used when coprescribed for its CsA-sparing effect

3.3 Methods

All kidney transplant centres identified from the first survey (obtained from the ANZDATA registry) were contacted and asked to complete a single A4 sheet questionnaire (Appendix 1a) for each primary recipient of a cadaver kidney transplant. To coincide with the previous survey, the same 12 month period was chosen (viz for transplants performed between 1.7.94 and 30.6.95). Data was collected for the first 12 month period post transplantation. Questions were designed to assess both efficacy and toxicity of CsA. Questions aimed at determining efficacy included plasma creatinine
concentrations at various times in the first 12 months and need for dialysis and additional anti-rejection drugs (methylprednisolone, muromonab or antithymocyte gammaglobulin) in the early post transplantation period. Questions aimed at assessing CsA toxicity included blood pressure, use of antihypertensive drugs and plasma creatinine concentration at various times in the first 12 months post transplantation. These data were compared to blood CsA concentration and use of DTZ.

3.3.1 Statistical methods
Analysis of relationships between trough blood CsA concentrations and various outcomes were performed using CsA concentrations as both continuous variables and categorised into three groups (<125µg/L, 125-199µg/L and >200µg/L) and coded to reflect risk relative to the lowest concentration group. These values were selected because they represented high, medium and low values obtained from the earlier survey discussed in Chapter 2. This analysis was undertaken to provide data to assist the definition of an optimal therapeutic range. Logistic regression was used to examine the relationship between blood CsA concentration and the need for dialysis, allowing for clustering by centre. Logistic regression, allowing for clustering by centre was also used to examine the relationship between blood CsA concentration and plasma creatinine concentrations at two levels, >150µmol/L and >200µmol/L. Clustering by centre utilises robust standard errors to allow for differences between centres and similarities among patients within a centre.

The relationship between taking DTZ and binary outcome variables such as the need for dialysis, use of methylprednisolone (a marker of rejection), use of OKT3 (a marker of more severe rejection) and use of antihypertensive drugs were also examined using logistic regression, allowing for clustering by centre. The relationship between use of
CHAPTER 3

DTZ and non-normally distributed variables including blood CsA concentration and number of anti-hypertensive drugs used were examined using Wilcoxon Rank Sum test.

Data was examined using STATA release 6.0 (Stata Corp, College Station, Texas, U.S.A.). Significance was assumed at p<0.05.

3.4 Results

Data was obtained on 240 adult recipients of first time, cadaver kidney transplants. Data was not complete for every patient and hence each analysis shows the number of data points available. Only one small hospital (performing <10 transplants/year) failed to respond to the original questionnaire and hence this data set represents >95% of all cadaver kidney transplants conducted during the period of interest.

There was no apparent correlation between blood CsA concentration on days 1, 2 or 3 post transplant or the mean of these concentrations and need for dialysis in the first week post transplantation (Table 3.1)

Table 3.1 Correlation between blood CsA concentration and need for dialysis in first week post transplantation corrected for clustering by centre

<table>
<thead>
<tr>
<th></th>
<th># of observations</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsA conc on day 1</td>
<td>36</td>
<td>0.995</td>
<td>0.984-1.006</td>
</tr>
<tr>
<td>CsA conc on day 2</td>
<td>89</td>
<td>0.997</td>
<td>0.989-1.004</td>
</tr>
<tr>
<td>CsA conc on day 3</td>
<td>101</td>
<td>1.002</td>
<td>0.997-1.007</td>
</tr>
<tr>
<td>Mean CsA conc (day 1-3)</td>
<td>147</td>
<td>1.001</td>
<td>0.997-1.005</td>
</tr>
</tbody>
</table>
CHAPTER 3

This apparent lack of correlation was also evident when blood CsA concentrations were
categorised into low (<125µg/L = category 1), medium (125-199µg/L = category 2) and
high concentrations (≥200µg/L = category 3) on days 1, 2 or 3 post transplant or mean
of these concentrations (also expressed as a category) and the need for dialysis in the
first week post transplantation (Tables 3.2.1 – 3.2.4).

Table 3.2.1 Correlation between blood CsA concentration (day 1) expressed as a
category and the need for dialysis in the first week post transplant corrected for

<table>
<thead>
<tr>
<th>CsA category</th>
<th># needing dialysis/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>6/20 (30%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>1/7 (14%)</td>
<td>0.39</td>
<td>0.07-2.30</td>
</tr>
<tr>
<td>Category 3</td>
<td>1/9 (11%)</td>
<td>0.29</td>
<td>0.03-2.92</td>
</tr>
</tbody>
</table>

Table 3.2.2 Correlation between blood CsA concentration (day 2) expressed as a
category and the need for dialysis in the first week post transplant corrected for

<table>
<thead>
<tr>
<th>CsA category</th>
<th># needing dialysis/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>6/26 (23%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>3/21 (14%)</td>
<td>0.56</td>
<td>0.09-3.53</td>
</tr>
<tr>
<td>Category 3</td>
<td>4/42 (10%)</td>
<td>0.35</td>
<td>0.06-2.03</td>
</tr>
</tbody>
</table>
Table 3.2.3 Correlation between blood CsA concentration (day 3) expressed as a category and the need for dialysis in the first week post transplant corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># needing dialysis/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>5/24 (21%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>4/25 (16%)</td>
<td>0.72</td>
<td>0.11-4.81</td>
</tr>
<tr>
<td>Category 3</td>
<td>10/52 (19%)</td>
<td>0.91</td>
<td>0.19-4.34</td>
</tr>
</tbody>
</table>

Table 3.2.4 Correlation between blood CsA concentration (mean days 1-3) expressed as a category and the need for dialysis in the first week post transplant corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># needing dialysis/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>8/44 (18%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>6/38 (16%)</td>
<td>0.84</td>
<td>0.23-3.06</td>
</tr>
<tr>
<td>Category 3</td>
<td>11/65 (17%)</td>
<td>0.92</td>
<td>0.27-3.12</td>
</tr>
</tbody>
</table>

There was also no relationship between blood CsA concentrations categorised as above on days 1, 2 or 3 post transplant or mean of these concentrations (also expressed as a category) and the need for dialysis in the first month post transplantation (Tables 3.3.1 – 3.3.4).
CHAPTER 3

Table 3.3.1 Correlation between blood CsA concentration (day 1) expressed as a category and the need for dialysis in the first month post transplant corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># needing dialysis/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>6/20 (30%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>1/7 (14%)</td>
<td>0.39</td>
<td>0.07-2.29</td>
</tr>
<tr>
<td>Category 3</td>
<td>1/9 (11%)</td>
<td>0.29</td>
<td>0.03-2.92</td>
</tr>
</tbody>
</table>

Table 3.3.2 Correlation between blood CsA concentration (day 2) expressed as a category and the need for dialysis in the first month post transplant corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># needing dialysis/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>8/26 (31%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>3/21 (14%)</td>
<td>0.38</td>
<td>0.07-2.12</td>
</tr>
<tr>
<td>Category 3</td>
<td>4/42 (10%)</td>
<td>0.24</td>
<td>0.04-1.35</td>
</tr>
</tbody>
</table>

Table 3.3.3 Correlation between blood CsA concentration (day 3) expressed as a category and the need for dialysis in the first month post transplant corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># needing dialysis/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>6/24 (25%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>4/25 (16%)</td>
<td>0.57</td>
<td>0.10-3.41</td>
</tr>
<tr>
<td>Category 3</td>
<td>10/52 (19%)</td>
<td>0.71</td>
<td>0.16-3.14</td>
</tr>
</tbody>
</table>
Table 3.3.4 Correlation between blood CsA concentration (mean days 1-3) expressed as a category and the need for dialysis in the first month post transplant corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># needing dialysis/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>10/44 (23%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>6/38 (16%)</td>
<td>0.64</td>
<td>0.18-2.27</td>
</tr>
<tr>
<td>Category 3</td>
<td>11/65 (17%)</td>
<td>0.69</td>
<td>0.19-2.47</td>
</tr>
</tbody>
</table>

There was no apparent correlation between blood CsA concentration on days 1, 2 or 3 (or mean of these concentrations) and early graft function (as expressed by plasma creatinine concentration remaining above 200μmol/L by day 7) post transplant (Table 3.4).

Table 3.4 Correlation between blood CsA concentration and plasma creatinine concentration staying >200μmol/L by day 7 corrected for clustering by centre

<table>
<thead>
<tr>
<th></th>
<th># of observations</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsA conc day 1</td>
<td>35</td>
<td>0.995</td>
<td>0.986-1.003</td>
</tr>
<tr>
<td>CsA conc day 2</td>
<td>88</td>
<td>0.999</td>
<td>0.996-1.003</td>
</tr>
<tr>
<td>CsA conc day 3</td>
<td>100</td>
<td>1.000</td>
<td>0.996-1.004</td>
</tr>
<tr>
<td>mean (day 1-3) CsA conc</td>
<td>144</td>
<td>1.000</td>
<td>0.997-1.003</td>
</tr>
</tbody>
</table>

This apparent lack of correlation was also evident when blood CsA concentrations were categorised (as before) on days 1, 2 or 3 post transplant or mean of these concentrations (also expressed as categories) and early graft function (expressed as plasma creatinine staying >200μmol/L by day 7 post transplantation) (Tables 3.5.1-3.5.4).
Table 3.5.1 Correlation between blood CsA (day 1) concentration by category and plasma creatinine concentration staying ≥200μmol/L by day 7 corrected for clustering by centre

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># with Creat&gt;200/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>9/19 (47%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>1/7 (14%)</td>
<td>0.19</td>
<td>0.03-1.01</td>
</tr>
<tr>
<td>Category 3</td>
<td>1/9 (11%)</td>
<td>0.14</td>
<td>0.01-1.40</td>
</tr>
</tbody>
</table>

When these data are analysed as 2 categories (category 1 vs category 2 and 3), the difference is statistically significant (Odds ratio = 0.16, p=0.003).

Table 3.5.2 Correlation between blood CsA (day 2) concentration by category and plasma creatinine concentration staying ≥200μmol/L by day 7 corrected for clustering by centre

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># with Creat&gt;200/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>8/26 (31%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>6/21 (29%)</td>
<td>0.90</td>
<td>0.19-4.20</td>
</tr>
<tr>
<td>Category 3</td>
<td>10/41 (24%)</td>
<td>0.23</td>
<td>0.20-2.66</td>
</tr>
</tbody>
</table>

Table 3.5.3 Correlation between blood CsA (day 3) concentration by category and plasma creatinine concentration staying ≥200μmol/L by day 7 corrected for clustering by centre

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># with Creat&gt;200/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>9/23 (39%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>7/25 (28%)</td>
<td>0.61</td>
<td>0.17-2.15</td>
</tr>
<tr>
<td>Category 3</td>
<td>13/52 (25%)</td>
<td>0.52</td>
<td>0.19-1.45</td>
</tr>
</tbody>
</table>
CHAPTER 3

Table 3.5.4 Correlation between blood CsA (mean days 1-3) concentration by category and plasma creatinine concentration staying ≥200μmol/L by day 7 corrected for clustering by centre

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># with Creat≥200/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>14/42 (33%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>11/38 (29%)</td>
<td>0.82</td>
<td>0.31-2.18</td>
</tr>
<tr>
<td>Category 3</td>
<td>17/64 (27%)</td>
<td>0.72</td>
<td>0.31-1.69</td>
</tr>
</tbody>
</table>

There was no significant relationship between blood CsA concentration on days 1, 2 or 3 or the mean of these concentrations (expressed as categories) and the use of methylprednisolone (Tables 3.6.1 – 3.6.4) in the early post transplant period.

Table 3.6.1 Correlation between blood CsA concentration (expressed as a category) on day 1 and use of methylprednisolone

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># given methylprednisolone/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>14/20 (70%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>5/7 (71%)</td>
<td>1.07</td>
<td>0.25-4.55</td>
</tr>
<tr>
<td>Category 3</td>
<td>5/9 (56%)</td>
<td>0.54</td>
<td>0.10-2.85</td>
</tr>
</tbody>
</table>

Table 3.6.2 Correlation between blood CsA concentration (expressed as a category) on day 2 and use of methylprednisolone

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># given methylprednisolone/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>12/26 (46%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>13/21 (62%)</td>
<td>1.90</td>
<td>0.62-5.78</td>
</tr>
<tr>
<td>Category 3</td>
<td>30/42 (71%)</td>
<td>2.92</td>
<td>0.98-8.68</td>
</tr>
</tbody>
</table>
Table 3.6.3 Correlation between blood CsA concentration (expressed as a category) on day 3 and use of methylprednisolone

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># given methylprednisolone/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>13/24 (54%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>11/25 (44%)</td>
<td>0.66</td>
<td>0.24-1.83</td>
</tr>
<tr>
<td>Category 3</td>
<td>28/52 (54%)</td>
<td>0.99</td>
<td>0.38-2.54</td>
</tr>
</tbody>
</table>

Table 3.6.4 Correlation between mean (days 1-3) blood CsA concentration (expressed as a category) and use of methylprednisolone

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># given methylprednisolone/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>24/44 (55%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>20/38 (53%)</td>
<td>0.93</td>
<td>0.27-3.19</td>
</tr>
<tr>
<td>Category 3</td>
<td>36/65 (55%)</td>
<td>1.03</td>
<td>0.35-3.08</td>
</tr>
</tbody>
</table>

Also, there was no significant relationship between blood CsA concentration on days 1, 2 or 3 or the mean of these concentrations (expressed as categories) and the use of muromonab in the early post transplant period (Tables 3.7.1 – 3.7.4).

Table 3.7.1 Correlation between blood CsA concentration (expressed as a category) on day 1 and use of muromonab

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># given muromonab/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>6/20 (30%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>1/7 (14%)</td>
<td>0.39</td>
<td>0.13-1.18</td>
</tr>
<tr>
<td>Category 3</td>
<td>2/9 (22%)</td>
<td>0.66</td>
<td>0.18-2.41</td>
</tr>
</tbody>
</table>
CHAPTER 3

Table 3.7.2 Correlation between blood CsA concentration (expressed as a category) on day 2 and use of muromonab.

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># given muromonab/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>3/26 (12%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>6/21 (29%)</td>
<td>3.07</td>
<td>0.84-11.25</td>
</tr>
<tr>
<td>Category 3</td>
<td>6/42 (14%)</td>
<td>1.28</td>
<td>0.43-3.82</td>
</tr>
</tbody>
</table>

Table 3.7.3 Correlation between blood CsA concentration (expressed as a category) on day 3 and use of muromonab.

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># given muromonab/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>2/24 (8%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>4/25 (16%)</td>
<td>2.10</td>
<td>0.57-7.74</td>
</tr>
<tr>
<td>Category 3</td>
<td>7/52 (13%)</td>
<td>1.71</td>
<td>0.21-13.65</td>
</tr>
</tbody>
</table>

Table 3.7.4 Correlation between mean (days 1-3) blood CsA concentration (expressed as a category) and use of muromonab.

<table>
<thead>
<tr>
<th>Category CsA</th>
<th># given muromonab/Total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>6/44 (14%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>6/38 (16%)</td>
<td>1.19</td>
<td>0.58-2.44</td>
</tr>
<tr>
<td>Category 3</td>
<td>8/65 (12%)</td>
<td>0.89</td>
<td>0.39-2.05</td>
</tr>
</tbody>
</table>

There was no significant relationship between the use of DTZ on day 1 or 2 and blood CsA concentration (day 1-3) (Table 3.8)
Table 3.8 Correlation between use of DTZ (on or before day 2) and blood CsA concentration (days 1-3), showing no difference between the groups (p=0.242).

<table>
<thead>
<tr>
<th></th>
<th>CsA concentration (days 1-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># used DTZ</td>
</tr>
<tr>
<td>No of observations</td>
<td>80</td>
</tr>
<tr>
<td>Median value</td>
<td>178 (μg/L)</td>
</tr>
<tr>
<td>Minimum value</td>
<td>43 (μg/L)</td>
</tr>
<tr>
<td>Maximum value</td>
<td>566 (μg/L)</td>
</tr>
</tbody>
</table>

There was no significant difference in mean blood CsA concentration (days 1-3) between the group using DTZ and the group not using DTZ (p=0.242, Wilcoxon Rank Sum test)

There was a significant relationship between the use of DTZ on or before day 2 and early graft function (expressed as the need for dialysis in the first week and first month) and severity of rejection (expressed as use of muromonab -Orthoclone®, Janssen-Cilag, Australia) in the first week post transplantation (Table 3.9, Figure 3.1). There was no apparent relationship between the use of methylprednisolone, which is a marker of the incidence of rejection episodes.
Table 3.9  Relationship between the use of DTZ on or before day 2 and the need for dialysis (first week and first month), use of methylprednisolone or OKT3 in the first week post transplantation controlled by clustering for centre.

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Use DTZ (n=119)</th>
<th>Not used DTZ (n=89)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysis in 1st week</td>
<td>9 (8%)</td>
<td>23 (26%)</td>
<td>0.23</td>
<td>0.06-0.94</td>
<td>0.04</td>
</tr>
<tr>
<td>Dialysis in 1st month</td>
<td>10 (8%)</td>
<td>25 (28%)</td>
<td>0.23</td>
<td>0.06-0.87</td>
<td>0.03</td>
</tr>
<tr>
<td>use of methylprednisolone</td>
<td>48 (40%)</td>
<td>52 (58%)</td>
<td>0.48</td>
<td>0.14-1.60</td>
<td>0.23</td>
</tr>
<tr>
<td>use of OKT3</td>
<td>11 (9%)</td>
<td>19 (21%)</td>
<td>0.38</td>
<td>0.16-0.86</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Fig 3.1  Odds ratio and 95% CI for use of DTZ in first 2 days post transplant and markers of early graft function, frequency and severity of rejection.

There was no apparent relationship between systolic or diastolic blood pressures at either 3, 6 or 12 months and the use of DTZ at any time post transplantation (Table 3.10)
Table 3.10 Effect of DTZ use at any time post transplantation and systolic and diastolic blood pressures at 3, 6 and 12 months post transplantation corrected for clustering by centre (linear regression)

<table>
<thead>
<tr>
<th></th>
<th>Used DTZ</th>
<th>Did not use DTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>3 month systolic BP</td>
<td>120</td>
<td>138.3 (18.0)</td>
</tr>
<tr>
<td>3 month diastolic BP</td>
<td>120</td>
<td>81.9 (9.6)</td>
</tr>
<tr>
<td>6 month systolic BP</td>
<td>117</td>
<td>138.9 (18.2)</td>
</tr>
<tr>
<td>6 month diastolic BP</td>
<td>117</td>
<td>81.1 (10.2)</td>
</tr>
<tr>
<td>12 month systolic BP</td>
<td>113</td>
<td>139.1 (18.1)</td>
</tr>
<tr>
<td>12 month diastolic BP</td>
<td>113</td>
<td>80.7 (10.0)</td>
</tr>
</tbody>
</table>

Figure 3.2. Relationship between use of antihypertensive drugs at 3 months post transplantation and use of DTZ. Wilcoxon Rank Sum test shows use of DTZ was associated with a reduced need for additional antihypertensive drugs (p=0.002).
There was a significant relationship (Wilcoxon Rank Sum test) between the use of DTZ at any time and the need for additional antihypertensive drug use at 3 months ($p=0.002$), 6 months ($p=0.037$) and 12 months ($p=0.009$) post transplantation (Figs 3.2 - 3.4).

**Fig 3.3** Relationship between use of antihypertensive drugs at 6 months post transplantation and use of DTZ. Wilcoxon Rank Sum test shows use of DTZ was associated with a reduced need for additional antihypertensive drugs ($p=0.037$).

**Fig 3.4** Relationship between use of antihypertensive drugs at 12 months post transplantation and use of DTZ. Wilcoxon Rank Sum test shows use of DTZ was associated with a reduced need for additional antihypertensive drugs ($p=0.009$).
The above analysis does not take into account the doses of antihypertensive drugs used. In order to distinguish between the use of low doses of multiple antihypertensive drugs and higher doses of fewer antihypertensive drugs, and to allow for the blood pressure lowering effect of drugs which had an alternative indication, a rating system was devised thus:

Where the dose of a drug indicated for use in hypertension was at the low to mid range (see Table 3.11) a value of 1 was assigned. If the dose employed was above this range, a value of 1.5 was assigned the therapy. Where a drug was used which had a different indication, but was known to exert a blood pressure lowering effect (see list below), a value of 0.5 was assigned this therapy. Where no information was available on dose of antihypertensive drug used, a value of 1 was assigned. These calculations were made at the 3, 6 and 12 month periods.

Other drugs which were not used primarily for an antihypertensive effect but which are known to exert antihypertensive effects attracted a score of 0.5. These included frusemide, sorbide nitrate, hydrochlorothiazide/amiloride and verapamil.

There was also a statistically significant relationship (Wilcoxon Rank Sum test) between the use of DTZ and the use of antihypertensive drugs according to the scaling system outlined above at 3 months (p=0.002), 6 months (p=0.026) and 12 months (p=0.020) (Figures 3.5, 3.6 and 3.7).
Table 3.11 Scaling system for antihypertensive drug therapy used in kidney transplant recipients at 3, 6 and 12 months post transplantation.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>DOSE LOW-MEDIUM (Scale 1)</th>
<th>DOSE HIGH (Scale 1.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>&lt;100mg/day</td>
<td>≥100mg/day</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>&lt;10mg/day</td>
<td>≥10mg/day</td>
</tr>
<tr>
<td>Captopril</td>
<td>&lt;75mg/day</td>
<td>&gt;75mg/day</td>
</tr>
<tr>
<td>Cilazopril</td>
<td>&lt;2.5mg/day</td>
<td>&gt;2.5mg/day</td>
</tr>
<tr>
<td>Clonidine</td>
<td>&lt;300microgram/day</td>
<td>&gt;300microgram/day</td>
</tr>
<tr>
<td>Enalapril</td>
<td>&lt;15mg/day</td>
<td>≥15mg/day</td>
</tr>
<tr>
<td>Felodipine</td>
<td>≤10mg/day</td>
<td>&gt;10mg/day</td>
</tr>
<tr>
<td>Fosinopril</td>
<td>&lt;20mg/day</td>
<td>&gt;20mg/day</td>
</tr>
<tr>
<td>Labetalol</td>
<td>&lt;200mg/day</td>
<td>&gt;200mg/day</td>
</tr>
<tr>
<td>MethylDopa</td>
<td>&lt;500mg/day</td>
<td>&gt;500mg/day</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>&lt;200mg/day</td>
<td>≥200mg/day</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>&lt;15mg/day</td>
<td>&gt;15mg/day</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>≤60mg/day</td>
<td>&gt;60mg/day</td>
</tr>
<tr>
<td>Perindopril</td>
<td>≤4mg/day</td>
<td>&gt;4mg/day</td>
</tr>
<tr>
<td>Prazosin</td>
<td>≤10mg/day</td>
<td>&gt;10mg/day</td>
</tr>
<tr>
<td>Propranolol</td>
<td>&lt;240mg/day</td>
<td>≥240mg/day</td>
</tr>
<tr>
<td>Ramipril</td>
<td>≤5mg/day</td>
<td>&gt;5mg/day</td>
</tr>
<tr>
<td>Verapamil</td>
<td>≤240mg/day</td>
<td>&gt;240mg/day</td>
</tr>
</tbody>
</table>
Figure 3.5 Relationship between scaling system score for use of antihypertensive drugs at 3 months post transplantation and use of DTZ. Wilcoxon Rank Sum test shows DTZ use was associated with a reduced need for additional antihypertensive drugs ($p=0.002$).

Figure 3.6 Relationship between scaling system score for use of antihypertensive drugs at 6 months post transplantation and use of DTZ. Wilcoxon Rank Sum test shows DTZ use was associated with a reduced need for additional antihypertensive drugs ($p=0.026$).
Figure 3.7 Relationship between scaling system score for use of antihypertensive drugs at 12 months post transplantation and use of DTZ. Wilcoxon Rank Sum test shows DTZ use was associated with a reduced need for additional antihypertensive drugs \((p=0.020)\).

There was no apparent correlation between blood CsA concentration at 3, 6 or 12 months post transplantation and the likelihood that plasma creatinine concentration would be \(\geq 150\mumol/L\) at the corresponding time (Tables 3.12.1-3.12.3).
CHAPTER 3

Table 3.12.1  Relationship between blood CsA concentration and the likelihood that plasma creatinine concentration ≥150µmol/L at 3 months post transplantation corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># with creat≥150/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10/23 (43%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20/65 (31%)</td>
<td>0.58</td>
<td>(0.35,0.95)</td>
</tr>
<tr>
<td>3</td>
<td>37/105 (35%)</td>
<td>0.71</td>
<td>(0.33,1.52)</td>
</tr>
</tbody>
</table>

Table 3.12.2  Relationship between blood CsA concentration and the likelihood that plasma creatinine concentration ≥150µmol/L at 6 months post transplantation corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># with creat≥150/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12/35 (34%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14/57 (25%)</td>
<td>0.62</td>
<td>(0.35,1.12)</td>
</tr>
<tr>
<td>3</td>
<td>27/93 (29%)</td>
<td>0.78</td>
<td>(0.35,1.73)</td>
</tr>
</tbody>
</table>

Table 3.12.3  Relationship between blood CsA concentration and the likelihood that plasma creatinine concentration ≥150µmol/L at 3 months post transplantation corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># with creat≥150/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18/43 (42%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24/72 (33%)</td>
<td>0.69</td>
<td>(0.47,1.02)</td>
</tr>
<tr>
<td>3</td>
<td>17/62 (27%)</td>
<td>0.52</td>
<td>(0.27,1.03)</td>
</tr>
</tbody>
</table>

This analysis was repeated for plasma creatinine concentration ≥200µmol/L at 3, 6 or 12 months and no statistically significant relationship was found (Tables 3.13.1 – 3.13.3).
Table 3.13.1 Correlation between blood CsA concentration (by category) at 3 months post transplant and plasma creatinine concentration being $\geq 200\mu$mol/L corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># with creat $&gt; 200$/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/23 (13%)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>4/65 (6%)</td>
<td>0.37</td>
<td>(0.07, 1.86)</td>
</tr>
<tr>
<td>3</td>
<td>10/105 (10%)</td>
<td>0.60</td>
<td>(0.12, 2.88)</td>
</tr>
</tbody>
</table>

Table 3.13.2 Correlation between blood CsA concentration (by category) at 6 months post transplant and plasma creatinine concentration being $\geq 200\mu$mol/L corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># with creat $&gt; 200$/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7/35 (20%)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>6/57 (11%)</td>
<td>0.39</td>
<td>(0.16, 0.94)</td>
</tr>
<tr>
<td>3</td>
<td>11/93 (12%)</td>
<td>0.44</td>
<td>(0.16, 1.20)</td>
</tr>
</tbody>
</table>

Table 3.13.3 Correlation between blood CsA concentration (by category) at 12 months post transplant and plasma creatinine concentration being $\geq 200\mu$mol/L corrected for clustering by centre

<table>
<thead>
<tr>
<th>CsA category</th>
<th># with creat $&gt; 200$/total (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/43 (14%)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>12/72 (17%)</td>
<td>1.07</td>
<td>(0.51, 2.24)</td>
</tr>
<tr>
<td>3</td>
<td>7/62 (11%)</td>
<td>0.68</td>
<td>(0.38, 1.21)</td>
</tr>
</tbody>
</table>

There was no significant relationship between the use of DTZ and blood CsA concentrations at 3, 6 or 12 months (Table 3.14).
Table 3.14 Relationship between blood CsA concentration and the use of DTZ at 3, 6 and 12 months post transplantation

<table>
<thead>
<tr>
<th></th>
<th>Used DTZ</th>
<th>Not used DTZ</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># Median (Range)</td>
<td># Median (Range)</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>120 197 (96,623)</td>
<td>69 227 (56,525)</td>
<td>0.387</td>
</tr>
<tr>
<td>6 months</td>
<td>115 194 (54,821)</td>
<td>64 226 (20,669)</td>
<td>0.188</td>
</tr>
<tr>
<td>12 months</td>
<td>112 167 (49,958)</td>
<td>60 189 (78,760)</td>
<td>0.281</td>
</tr>
</tbody>
</table>

Table 3.15 shows the percentages of kidney transplant recipients whose blood CsA concentrations fell below certain values at 3 time points post transplantation. At 3 months post transplantation, 5% of blood CsA concentrations fell below 99µg/L, at 6 months 5% fell below 92µg/L and at 12 months 5% fell below 97µg/L. At 3 months post transplant, 55% of blood CsA concentrations fell below 224µg/L and 72% fell below 283µg/L. At 6 months post transplant, 55% fell below 213µg/L and 72% below 253µg/L while at 12 months post transplant, 55% fell below 188µg/L and 72% below 237µg/L. Hence, 50% of transplant recipients had blood CsA concentrations between 99µg/L and 224µg/L at 3 months post transplant, and 67% had concentrations which fell between 99 and 283µg/L. At 12 months, 50% of blood CsA concentrations fell between 97µg/L and 188µg/L and 67% fell between 97µg/L and 237µg/L.

Table 3.15. Cut-off blood CsA concentrations at 3, 6 and 12 months post transplantation.

<table>
<thead>
<tr>
<th>% of patients</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>99µg/L</td>
<td>92µg/L</td>
<td>97µg/L</td>
</tr>
<tr>
<td>55%</td>
<td>224µg/L</td>
<td>213µg/L</td>
<td>188µg/L</td>
</tr>
<tr>
<td>72%</td>
<td>283µg/L</td>
<td>253µg/L</td>
<td>237µg/L</td>
</tr>
</tbody>
</table>
3.5 Discussion:
The categories for blood CsA concentration (<125µg/L, 125-199µg/L and ≥200µg/L) selected for these analyses were chosen from the survey findings (Chapter 2) as representative of low, medium and high concentrations used for adult kidney transplantation in Australasia. Additional analyses were performed using blood CsA concentration expressed in tertiles (values were <140µg/L, 140-222µg/L and >223µg/L). These analyses (for likelihood that plasma creatinine would be ≤200µmol/L and need for dialysis in the first month post transplantation) provided similar results and did not alter the conclusions.

The failure to reach statistical significance for analyses in this study does not necessarily mean that correlations do not exist, since the same conclusion would result from too few data (Type 2 error). Data was available from virtually the entire Australasian adult, first cadaver, kidney transplant population for analysis but there were few blood CsA concentrations in the first 3 days post transplantation. It was not surprising therefore that the data did not support a relationship between the three categories of blood CsA concentration (<125µg/L, 125-199µg/L and ≥200µg/L) and the need for dialysis in the first week post transplantation. Assuming 30% of the lowest category and 10% of either category 2 or 3 would require dialysis in the first week, in order to detect a significant difference (β=0.2 and α=0.05), there would need to be 60 patients in each category. Data available for analysis included only 44 in the first category, 38 in the second and 65 in the third category. It was surprising that there were so few blood CsA concentration data available (only 36 on day 1 post transplant and 147 over the first 3 post operative days) given the availability of the assay and the importance of immunosuppression to prevent rejection. Some transplant recipients were not commenced on CsA immediately post transplant and hence blood CsA concentration monitoring in the first 3 days was not performed. Not all transplant recipients received CsA on the day after transplant. One limitation to these findings is that the time of commencement of CsA was not specifically
asked in the questionnaire but where it was stated, it was assumed that the blood CsA concentration was zero prior to this time and hence the data was available for analysis.

A similar limitation was seen when the relationship between blood CsA concentration and the likelihood that serum creatinine concentration would be elevated (either >150µmol/L or >200µmol/L) at 3, 6 or 12 months. The data available to detect a difference between category 1 (blood CsA concentration <125µg/L) and either category 2 (125-199µg/L) or category 3 (>200µg/L) allowed only 50% - 70% power (assuming 30% of those in category 1 and 10% in the other categories would have elevated serum creatinine concentrations and α=0.05).

The relationship between blood CsA concentration on day 1 (expressed as categories) and likelihood that serum creatinine concentration would remain above 200µmol/L by day 7 was interesting. When analysed as category 2 vs category 1 or category 3 vs category 1, the relationship was not statistically significant (Table 3.5.1) but when categories 2 and 3 were combined and then compared to category 1, the difference became significant (p=0.003). This outcome implies that blood CsA concentrations >125µg/L (ie. categories 2 and 3) on day 1 reduce the risk that creatinine concentrations will remain elevated by day 7 (ie, a desirable outcome). It may however be just a statistical artefact. Before interpreting this data, it is important to note that:

- the relationships between blood CsA concentration on days 2 or 3, as well as the mean of blood CsA concentrations on days 1-3 and likelihood that serum creatinine concentration would be less than 200µmol/L by day 7 were not statistically significant (Tables 3.5.2 - 3.5.4).
- the number of observations available for the day 1 analysis was small (9 in category 1 and 1 each in category 2 and category 3).

These suggest that the finding of significance in the relationship on day 1 is unlikely to be real and should be ignored.
Further limitations of these analyses include the use of methylprednisolone as an indicator of the occurrence of rejection and plasma creatinine concentration as a marker of both nephrotoxicity and rejection. It was apparent from the distribution of the use of methylprednisolone that, in some transplant recipients, there were additional reasons (probably standard protocol) for use in the early post transplant period. Hence the use of methylprednisolone is an imperfect marker of rejection. The use of plasma creatinine concentration as a marker of kidney rejection is also suboptimal because high creatinine concentrations might result from either rejection (presumed increased risk at lower blood CsA concentration) or CsA induced nephrotoxicity (presumed increased risk at higher blood CsA concentration). Similarly, using plasma creatinine concentration as a marker of CsA induced nephrotoxicity is suboptimal. This may explain the lack of statistical significance for the association between use of DTZ and need for methylprednisolone post transplantation and also for the association between blood CsA and serum creatinine concentrations at 3, 6 and 12 months post transplantation.

The finding that the blood CsA concentration in the immediate post transplant period is not useful for predicting acute rejection has been reported previously in an Australian kidney transplant population (Nankivell BJ, et al. 1994). These authors noted that “...the majority of patients who reject do so at therapeutic CsA levels”. They did however note that blood CsA concentrations >400ng/mL were 88% predictive and concentrations >500ng/mL were 95% predictive for excluding acute rejection as a cause of worsening creatinine concentrations. While the assay method used was relatively specific for parent CsA, the CsA dosage and blood sampling schedule (once daily with blood concentration determined at 12-14h after the dose) restricts the ability to extrapolate to twice daily CsA regimens used by most centres. Indeed, the authors recommended individual transplant units construct their own therapeutic ranges to account for variability in dosing regimen, sampling time and other differences. The use of DTZ was not noted as a factor but, in the survey discussed in Chapter 2, this hospital was one where DTZ was
used 'sometimes' (ie, neither in all nor none) post transplant and hence its use may have further confounded these findings.

The inability of CsA trough concentration monitoring to predict outcomes has led some authors to propose that AUC monitoring is a better tool to guide dosing (Kahan BD, et al. 1995a. Grevel J, et al. 1989. Mahalati K, et al. 1999). Full AUC monitoring is time consuming and expensive and hence a ‘limited sampling’ strategy has been advocated to estimate AUC. One recent study (Mahalati K, et al. 1999) studied the relationship between trough CsA concentrations or estimated CsA AUC and clinical outcomes (acute rejection and CsA induced nephrotoxicity). Their findings from 156 kidney transplant recipients included the observation that AUC was more closely associated with both nephrotoxicity and rejection than trough concentration alone during the first 90 days post transplant. Patients in that study used the microemulsion formulation of CsA which has been shown to have improved absorption characteristics compared to the older ‘Sandimmun’ formulation. This is significant since they monitored blood CsA concentrations over the first four hours only which is the time that concentrations from the older formulation were more likely to vary due to poorer and more variable absorption. Unfortunately their findings were complicated by variability in the immunosuppressive regimens (3 very different protocols), the use of DTZ in some but not all patients and the high therapeutic range (trough concentrations 250-400µg/L days 1-3, reducing to 200-300µg/L during the second and third months) which resulted in greater exposure (AUC(0-12) 9500-11500µg.h/L) than that demonstrated in the study in kidney transplant recipients reported in Chapters 4 & 5. In that study, the maximal CsA AUC was seen at the 180mg/day DTZ dose (conventional release) when the mean AUC(0-12) was 5301µg.h/L (Table 5.2). Also worthy of note was the apparent lack of benefit of DTZ use in the early post transplant period – a contrary finding to that of the present study and of an earlier study in Australian kidney transplant recipients (Chrysostomou A, et al. 1993). In the present study, the apparent lack of influence of
trough CsA concentration (which varied from <100µg/L to >500µg/L Table 3.14) on clinical outcomes at 3, 6 and 12 months post transplant suggests that AUC monitoring in the long term is unlikely to improve clinical outcomes significantly.

These data confirm that DTZ confers a therapeutic benefit in the early post transplant period as indicated by the reduced need for dialysis. There was no apparent relationship between DTZ use and the need for methylprednisolone which suggests that the frequency of rejection episodes are not reduced by the use of DTZ (if the use of methylprednisolone is an efficient marker of rejection). However, there was a relationship between the use of DTZ and a reduced need for muromonab which may indicate that rejection episodes are milder when transplant recipients are prescribed DTZ. Similar beneficial therapeutic effects have been noted before - in an Australian study discussed earlier (Chrysostomou A, et al. 1993), DTZ was noted to exert a beneficial therapeutic effect (reduced need for dialysis and other immunosuppressive drugs post transplantation). Unfortunately, in this study, kidney transplant recipients who received DTZ had higher blood CsA concentrations at the first week and first month post transplant and hence this therapeutic benefit might not be due to the prescription of DTZ alone. In the present study DTZ use was not associated with higher blood CsA concentrations in the early post operative period (Table 3.8) and hence the beneficial therapeutic effects are likely to be as a direct consequence of DTZ use. Similar therapeutic benefits in the form of a reduction of delayed graft function in the immediate post transplantation period have been noted when verapamil was given to recipients of cadaver kidney transplants (Dawidson I & Roth P. 1991).

It was not surprising that DTZ use was not associated with a higher blood CsA concentration at 3, 6 or 12 months post transplant (Table 3.14) since doses are adjusted to keep blood CsA concentration in the therapeutic range. This was also a finding in the earlier Australian study (Chrysostomou A, et al. 1993) where the use of DTZ allowed
the daily dose of CsA to be reduced by approximately 35%. Respondents to the questionnaire were not asked about the doses of CsA used and hence relative savings are not quantifiable from the present study.

Blood pressure control (both systolic and diastolic blood pressure at any time post transplantation) was similar in those kidney transplant recipients given DTZ to those not given it (Table 3.8). This is not surprising since this is one parameter that is regularly monitored and kept within acceptable limits by the use of antihypertensive drugs. The data does however demonstrate that the use of DTZ was associated with a reduced need for additional antihypertensive drugs (Figure 3.2, 3.3 and 3.4). This conflicts with the earlier observations (Chrysostomou A, et al. 1993) made in a similar Australian transplant population. The reason for this difference may be due to the larger population in the present study since the Chrysostomou study suggested that DTZ reduced the need for additional antihypertensive drug use but the difference failed to reach statistical significance.

In order to further explore the relationship between DTZ use and antihypertensive effects, a scaling system was devised to overcome the bias associated with using numbers of antihypertensive drugs used. Using crude numbers of antihypertensive drugs ignores the effect of drug dose on blood pressure and also the blood pressure lowering effect of other drugs which are prescribed for non-antihypertensive indications. The relationship between DTZ use and each of these scales at 3, 6 and 12months were significant (Figures 3.5 - 3.7) and very similar to the relationship between DTZ use and actual number of antihypertensive drugs used. These data therefore confirm that DTZ exerts an antihypertensive effect in this patient population where the dose most commonly employed in Australia during this time was 180mg per day given either as one conventional release tablet (60mg) thrice daily or as one 180mg CD capsule each morning. Both dosage regimens are approved for the treatment of hypertension in
Australia, albeit this is the lowest dose recommended (the maximum daily dose recommended is 360mg per day).

Given the therapeutic benefits that have been confirmed in the present study, it would seem wise to routinely prescribe DTZ in the immediate post transplant period for all kidney transplant recipients (except where there is a clear contraindication). While lower doses of DTZ have been shown to exert a significant CsA-sparing effect (see Chapter 4), therapeutic benefits have only been demonstrated with 'conventional' doses of DTZ. Therefore, until more data are available demonstrating therapeutic benefits at lower DTZ doses, the recommended dose of DTZ should be 180mg/day.

In the event that blood pressure control warranted additional antihypertensive drugs, it would seem reasonable to first increase the dose of DTZ (ie rather than starting another drug) at least to 240mg per day. This could be administered either as 120mg conventional release tablets given twice daily with the CsA, or as a single CD capsule given once daily. The effect of this dosage increase on drug regimen complexity would be minimal and there is at least some potential for further CsA dose reduction (see Chapter 4) and hence potential for greater financial savings over the 180mg/day dose.

Data from this study suggest that there is no therapeutic benefit to be gained from increasing blood CsA concentration above a 'reasonable' value. Although there was no demonstrable toxicity from higher blood CsA concentrations, using lower therapeutic ranges would reduce the costs associated with CsA therapy. These costs are of such a magnitude that it was one of the reasons for initially proposing that sparing drugs be coprescribed. Reducing daily dosage also has the potential to improve compliance (by reducing the number of capsules taken each day). Most respondents to the earlier questionnaire (Chapter 2) used at least 2 therapeutic ranges and hence the data were analysed to determine where the majority of blood CsA concentrations lay at the 3 and
12 month periods post transplant. As noted in Table 3.15, 50% of blood CsA concentrations at 3 months post transplant fell between 99µg/L and 224µg/L while 67% fell between 99µg/L and 283µg/L. At 12 months post transplant, 50% of blood CsA concentrations fell between 97µg/L and 188µg/L while 67% fell between 97µg/L and 237µg/L. From these data, it would seem reasonable to adopt an early post transplant period CsA therapeutic range of 100µg/L - 225µg/L (or 100µg/L - 285µg/L for higher risk patients). Similarly, a reasonable late period post transplantation CsA therapeutic range is 100µg/L - 200µg/L (or 100µg/L - 240µg/L for higher risk patients). Because these data are derived from the clinical setting, these proposed ranges are de facto what happens in the Australasian, adult kidney transplant setting.

3.6 Conclusions
This study failed to show a significant relationship between blood CsA concentration at any time post kidney transplantation and markers of efficacy (eg. reduced need for dialysis or additional immunosuppression) or toxicity (eg. the likelihood that serum creatinine concentration would remain above 150µmol/L). While data from almost the entire Australasian kidney transplant population was available, this may be due to lack of data or it may be as a consequence of the adequacy of the chosen variables to reflect these outcomes. In particular, the use of methylprednisolone as a marker of rejection episodes post transplantation was flawed by the apparent use by some centres of this immunosuppressive drug routinely.

The finding that DTZ use was associated with a reduced need for dialysis and muromonab use post transplantation supported earlier findings from the Australian kidney transplant population and is a strong argument in favour of its routine prescription. Contrary to earlier findings, this study demonstrated that DTZ did exert an antihypertensive effect in this population and since antihypertensive drug use was
widespread (>75% of patients not taking DTZ were prescribed one or more antihypertensive drugs at 3mo post transplantation), the routine coprescription of DTZ is logical.

Blood CsA concentration data provided at 3, 6 and 12mo post transplantation revealed that 50% of kidney transplant recipients’ concentrations fell between 100μg/L and 225μg/L at 3 months and between 100μg/L and 190μg/L at 12 months. In addition, 67% of blood CsA concentrations fell between 100μg/L and 290μg/L at 3 months and between 100μg/L and 240μg/L at 12 months post transplantation. The following therapeutic ranges are thus recommended:

-100μg/L - 225μg/L (or 100μg/L - 285μg/L for patients deemed to need greater immunosuppression) for the early period post transplantation.

-100μg/L - 200μg/L (or 100μg/L - 240μg/L for patients deemed to need greater immunosuppression) for the late period post transplantation.

These values reflect the clinical situation that applied at the time of this study and are lower than many in use at that time (see Chapter 2). The introduction of the microemulsion formulation of CsA ('Neoral') and use of mycophenolate mofetil (instead of azathioprine) have complicated the issue of therapeutic range construction. Given the improvement in bioavailability associated with the former and enhanced immunosuppression associated with the latter, it would seem reasonable to consider using even lower values for CsA therapeutic range in the current environment.

Data provided in this and the previous Chapter provides valuable background data on the practices associated with the use of CsA-sparing agents in Australasia. Because these data are derived from populations, they are limited by virtue of not being able to identify individual patient responses. Clinical studies into the use of DTZ as a CsA-sparing agent
in individual transplant recipients are needed to identify variability in response – these are
the subject of the next 5 Chapters.
Chapter 4

Diltiazem-Cyclosporin pharmacokinetic interaction - dose response relationship

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4.1 Introduction

As noted in Chapter 1, CsA has become the most important immunosuppressive agent used for the prevention of organ transplant rejection. The use of CsA has resulted in better 12 month graft survival but this improvement has come at a significant financial cost and with a new set of adverse effects. Partly for economic and partly for therapeutic reasons, deliberate coprescription of other drugs with CsA has been advocated (First MR, et al. 1993. Keogh A, et al. 1995. Wagner K, et al. 1988. Leibbrandt DM & Day RO. 1992). CsA is metabolised by a cytochrome P450 isoenzyme (CYP3A4) in both liver (Kronbach T, et al. 1988. Combalbert J, et al. 1989) and enterocyte (Kolars JC, et al. 1991. Wu CY, et al. 1995) and many drug interactions occur via this isoenzyme. In particular, KCZ and DTZ are inhibitors of CYP3A4 (Slaughter RL & Edwards DJ. 1995) and both have been shown to elevate blood CsA concentrations.

In vitro studies using human liver enzymes have shown large inter-individual variation in CsA metabolic capacity (Kronbach T, et al. 1988. Combalbert J, et al. 1989. Henricsson S & Lindholm A. 1988). It is therefore possible that the pharmacokinetic interaction between CsA and drugs which affect its metabolism (including CsA-sparing agents) will also show significant interpatient variability. The current study was therefore designed to investigate the effect of DTZ on blood CsA concentrations in individual transplant recipients. DTZ was chosen because, as demonstrated in Chapter 2, it is the most widely prescribed CsA-sparing agent in Australia and New Zealand.

As noted in Chapter 1, fluctuating concentrations of endogenous IL-6 are thought to affect drug metabolising capacity. For this reason blood C-reactive protein concentrations will be ascertained on each study day to monitor this variable.

4.2 Aims
The principal aim of this study was to define the dose-response curve for the interaction between DTZ and CsA in renal transplant recipients maintained on this immunosuppressive drug.. A secondary aim was to ascertain whether DTZ is the interacting moiety or whether either of its metabolites interact by comparing the correlation between blood concentrations of DTZ and/or those of its three major metabolites with changes in blood CsA concentrations.

4.3 Methods
Eight stable renal transplant recipients maintained on CsA (Sandimmun® capules, Novartis, formerly Sandoz Australia) (but not on DTZ) consented to take part in this pharmacokinetic study which was approved by The Queen Elizabeth Hospital’s Human Ethics Committee (Appendix 3). Entry criteria included stable trough blood CsA
concentrations on routine monitoring for ≥3 months and plasma creatinine concentrations which were stable for ≥3 months and ≤180μmol/L. Patient details are shown in Table 4.1. Exclusion criteria included: plasma creatinine concentration >180μmol/L, known allergy to DTZ, sick sinus syndrome, hypotension, severe congestive heart failure, acute myocardial infarction and/or pulmonary congestion.

On each 24h study day, serial blood samples were taken from an indwelling venous catheter at times 0 (pre dose) and 1, 2, 3, 4, 6 and 12h after both morning and evening CsA doses, making a total of 13 blood samples spanning 2 CsA dosing periods. Incremental doses of DTZ were given between study days which were ≥2 weeks apart to allow the interaction between CsA and DTZ to stabilise and steady-state blood CsA concentrations to be achieved. The sequence of DTZ doses was 0, 10, 20, 30, 60mg in the morning followed by 60mg and 90mg taken twice daily. DTZ doses less than 30mg were taken in the form of a 10mg capsule manufactured from commercially available tablets by the Hospital's Pharmacy Department, while doses of 30mg or more were taken in the form of commercially available, conventional release 60mg tablets (Cardizem®, ICI Australia). The DTZ content of the manufactured capsules was verified by HPLC assay (see Chapter 7). Each DTZ dose was taken with the usual morning and/or evening CsA dose (Sandimmun® capsules, Novartis, formerly Sandoz, Australia) and food was not consumed for at least 1h before or after CsA dosing. CsA dose reductions were planned, if needed, to maintain blood CsA concentrations within the therapeutic range used by this hospital (80-250μg/L) and the resultant AUC was corrected by the same factor (viz dose-normalised AUC) to allow for the dosage change. Apart from DTZ, no drug which was known to interfere with CsA metabolism (including erythromycin and KCZ), grapefruit juice, or any drug whose metabolism might be affected by DTZ (especially terfenadine and similar antihistamine drugs) were allowed during the course of the study.
Patency of the venous catheter was maintained by instilling 1.5mL heparinised saline (10units/mL) after each 7mL blood sample was withdrawn. To prevent contamination from the heparinised saline, the first 2mL of blood withdrawn was discarded. Blood samples were immediately stored at 0°C (on ice) and transferred to a freezer (-20°C) as soon as practicable and within 8h. Whole blood CsA concentrations were measured by enzyme immunoassay (EMIT® Behring/Syva, San Jose, California) which is relatively specific for parent CsA (Dusci LJ, et al. 1992. Steimer W. 1999). AUCs were calculated using the logarithmic trapezoidal method (Appendix 4).

Plasma DTZ, demethylldiltiazem (DMDTZ), desacetyldiltiazem (DADTZ) and demethyldesacetyldiltiazem (DMDADTZ) concentrations were determined using an HPLC method developed as part of this study and described in Chapter 7. The assay had an intra-assay CV(%) of <11 to <3% at concentrations of 2.5 to 100µg/L, respectively (Morris RG, et al. 1996).

The relationship between the daily dose of DTZ and both change in CsA AUC(0-24) and change in CsA trough concentrations (12h and 24h) were modelled using the sigmoid E_{max} method. The curve was generated by entering the change from baseline AUCs into the sigmoid Emax equation (Appendix 5) using GraphPad PRISM software (v 2.0, Intuitive Software for Science, San Diego, USA), forcing the line through zero at time zero.

CsA AUC prior to DTZ administration was defined as E0 and the increase in effect at daily DTZ doses of 30, 60 120 and 180mg were defined as E30, E60, E120 and E180. Morning CsA AUCs were compared to evening AUCs by paired Student-t test and the 5% probability level was assigned to indicate significance. Relationships between change in CsA AUC(0-24) and DTZ determinants were analysed by least squares ordinary
CHAPTER 4

regression, with differences between the 8 subjects at 7 levels of DTZ dose grouped and treated as categorical variables within the analysis of variance (SYSTAT 1990, ver 5.03, SYSTAT Inc, Illinois, USA). The repeated measures ANOVA design was also used to confirm associations with each trial measurement forming the dependent variable within the model design.

Plasma C-reactive protein concentrations from the first blood sample on each study day were assayed by a monoclonal antibody agglutination method (Behring, Frankfurt, Germany).

4.4. Results:

Patient demographics are shown in Table 4.1.

Table 4.1. Patient characteristics and concurrent drug therapy

<table>
<thead>
<tr>
<th>sex</th>
<th>age</th>
<th>wt (kg)</th>
<th>graft duration (mo)</th>
<th>other drug therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>30</td>
<td>98</td>
<td>27</td>
<td>Azat, Pred, Praz, Aten, Ceph</td>
</tr>
<tr>
<td>M</td>
<td>57</td>
<td>77</td>
<td>75</td>
<td>Azat, Rani, Ceph</td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>90</td>
<td>65</td>
<td>Azat, Frus, Nife, Ceph, Amox</td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>91</td>
<td>86</td>
<td>Praz, Aten, Azat</td>
</tr>
<tr>
<td>F</td>
<td>64</td>
<td>53</td>
<td>104</td>
<td>Fol, Simv, Frus</td>
</tr>
<tr>
<td>M</td>
<td>27</td>
<td>62</td>
<td>130</td>
<td>Aten, Amox</td>
</tr>
<tr>
<td>F</td>
<td>47</td>
<td>63</td>
<td>71</td>
<td>Nife, Aten, Azat, Enal</td>
</tr>
<tr>
<td>F</td>
<td>60</td>
<td>87</td>
<td>73</td>
<td>Azat, Praz, Aten, Acitr, Sulin</td>
</tr>
</tbody>
</table>

Abbreviations:

Acitr=Acitretin, Amox=Amoxycillin, Aten=Atenolol, Azat=Azathioprine,
Ceph=Cephalexin, Enal=Enalapril, Fol=Folic Acid, Frus=Frusemide, Nife=Nifedipine,
Praz=Prazosin, Pred=Prednisolone, Rani=Ranitidine, Simv=Simvastatin, Sulin=Sulindac
Data from 56 AUCs(0-24) were collated (8 patients at 7 dose increments) and analysed. CsA AUCs from 6 study periods were excluded from the analysis because of either insufficient data to define the CsA AUC and/or patient vomited shortly after the CsA dose was administered. In this latter case, there was doubt about the fraction of both CsA and DTZ absorbed and hence the corresponding AUCs. These omitted data points were as follows: Patient 1, DTZ dose 30mg/d; patient 4, DTZ dose 120mg/d; patient 5, DTZ dose 30mg/d; patient 6, DTZ dose 60mg/d and 180mg/d; patient 7, DTZ dose 120mg/d.

CsA dosage reductions were made on three occasions, in two patients, to maintain concentrations within the therapeutic range accepted by this hospital (80-250µg/L). In patient 4, CsA dose was reduced from 175mg to 150mg twice daily one week prior to the 90mg twice daily DTZ study day. In patient 5, CsA dose was reduced from 125mg to 100mg twice daily, 12 days prior to the 90mg twice daily DTZ study day. The dose of CsA was reduced further in patient 5 (to 75mg twice daily) one week prior to the 180mg CD DTZ study day (see Chapter 5). As noted above, the CsA pharmacokinetic data was adjusted by the same factor (viz dose-normalised AUC) to allow for these CsA dose reductions.

Group data show that an increase in CsA AUC(0-24) (Fig 4.1, Table 4.2) and CsA 24h trough concentration (Fig 4.2, Table 4.2) occurred following the lowest dose of DTZ used (10mg mane). The Sigmoid Emax fitted line shows that the rate of increase in these parameters slowed after the dose of DTZ was increased beyond 30-60mg mane but continued to increase to the maximum DTZ dose administered (180mg/d).
Figure 4.1 Fitted curve created by Emax model for change in CsA AUC(0-24) expressed as percentage over baseline v DTZ dose (mg/day). Individual patient data points are overlayed (n=7-8).

Figure 4.2 Fitted curve created by Emax model for change in CsA 24h trough concentration expressed as percentage over baseline v DTZ dose (mg/day). Error bars show mean ±SEM (n=7-8).
Table 4.2. Increase in CsA AUC(0-24) and trough blood CsA concentrations (12h and 24h) derived from the sigmoid Emax curve for DTZ doses within the range studied (n=8).

<table>
<thead>
<tr>
<th>E value</th>
<th>Increase in CsA AUC(0-24) (%)</th>
<th>Increase in CsA trough 12h (%)</th>
<th>Increase in CsA Trough 24h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E30</td>
<td>28</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>E60</td>
<td>39</td>
<td>62</td>
<td>69</td>
</tr>
<tr>
<td>E120</td>
<td>52</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>E180</td>
<td>61</td>
<td>87</td>
<td>88</td>
</tr>
</tbody>
</table>

E30 = Increase in CsA AUC(0-24) predicted for 30mg DTZ daily  
E60 = Increase in CsA AUC(0-24) predicted for 60mg DTZ daily  
E120 = Increase in CsA AUC(0-24) predicted for 120mg DTZ daily  
E180 = Increase in CsA AUC(0-24) predicted for 180mg DTZ daily

Fig 4.3 shows that individual patient responses in CsA AUC(0-24) to increasing DTZ dose was variable, both in dose of DTZ required to produce an increase in CsA AUC(0-24) and in the magnitude of the increase in CsA AUC(0-24).

CsA trough (12h and 24h) and AUC(0-24) responses for clinically relevant DTZ doses are given in Table 4.2. These data (expressed as E30, E60, E120 and E180 from the fitted sigmoid Emax curve) also show an early increase in effect with a plateau at doses above 60mg/d DTZ for all sets of data. CsA AUC(0-24) data show a 28% increase while the 0-12h trough and 12-24h trough data each show a 49% increase at a DTZ dose of 30mg. Increasing DTZ dose from 30mg/d to 180mg/d (a 6 fold increase in dose) resulted in only a 2.1 fold increase in CsA AUC(0-24) and a 1.8 fold increase in both 0-12h trough and 12-24h trough concentrations.
Figure 4.3 Individual patient data showing change in CsA AUC(0-24) expressed as percentage over baseline v DTZ dose (mg/day). Discussion of patients 7 and 8 data appears in text.

Figure 4.4 shows the effect of a single morning dose of DTZ (10mg - 60mg) on CsA AUC(0-24), CsA AUC(0-12) and CsA AUC(12-24). This shows that, despite the short half-life of DTZ in plasma (3.9±1.3h – see Chapter 7), the increase in morning AUC(0-12) was virtually identical to the increase in evening AUC(12-24).

There was a linear relationship between plasma concentration of DTZ and its 3 major metabolites over the entire DTZ dose range administered (see Chapter 7).

Subject 7 exhibited a substantially lower DTZ AUC(0-24) than the other 7 subjects over the entire dosage range, the ratio at 180mg/d DTZ compared to group data being 0.047 (Table 4.3). While this subject also demonstrated a somewhat lower CsA-sparing effect.
over the entire DTZ dosage range (Fig 4.3), the magnitude of the CsA sparing effect was not reduced to the same extent, the ratio (compared to group data) for increase over baseline CsA AUC(0-24) at 180mg/d DTZ being 0.63. Subject 8 exhibited a ‘normal’ DTZ AUC(0-24) but a substantially larger AUC(0-24) for both DMDTZ and DMDADTZ over the entire DTZ dosage range (Table 4.3). Despite this atypical metabolite pattern, the CsA-sparing effect noted in this subject was consistent with group data over the entire DTZ dosage range (Fig 4.3).

Figure 4.4. Change in CsA AUC(0-12) (closed circles), change in CsA AUC(12-24) (open circles) and change in CsA AUC(0-24) (filled squares) v DTZ dose(mg/day). Data expressed as absolute increase (µg.h/L) over baseline. Mean data (n=8)
Table 4.3. Ratio of AUC for DTZ and its metabolites for individual subjects 7 and 8 compared to group (n=8) data. DTZ dose = 180mg/d

<table>
<thead>
<tr>
<th></th>
<th>DTZ</th>
<th>DMDTZ</th>
<th>DADTZ</th>
<th>DMDADTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 7</td>
<td>0.047</td>
<td>0.64</td>
<td>0.33</td>
<td>0.16</td>
</tr>
<tr>
<td>Subject 8</td>
<td>0.82</td>
<td>1.0</td>
<td>2.71</td>
<td>6.00</td>
</tr>
</tbody>
</table>

The relationship between increase in CsA AUC(0-24) with both DTZ dose, AUCs for DTZ and its 3 metabolites, and peak plasma DTZ concentration for the group are shown in Table 4.4. All DTZ variables tested except DMDADTZ AUC(0-24) had a significant relationship with the change in CsA AUC (p<0.0001). The strongest correlation between the DTZ indices tested (ie. the best predictor of) and increase in CsA AUC(0-24) was the AUC(0-24) of the DMDTZ metabolite. The dose of DTZ used was also strongly correlated with increase in CsA AUC(0-24) and this correlation was better than the correlation between parent DTZ AUC(0-24) and CsA AUC(0-24).

Table 4.4. Relationship between a range of DTZ determinants and percentage change in CsA AUC(0-24) (compared using stratified least squares ordinary regression analysis).

<table>
<thead>
<tr>
<th></th>
<th>DTZ dose (mg/d)</th>
<th>DTZ AUC</th>
<th>DTZ peak conc (µg/L)</th>
<th>DMDTZ AUC</th>
<th>DADTZ AUC</th>
<th>DMDADTZ AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squared multiple r</td>
<td>0.531</td>
<td>0.451</td>
<td>0.40</td>
<td>0.591</td>
<td>0.435</td>
<td>0.24</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.046</td>
</tr>
</tbody>
</table>

DTZ= diltiazem; DMDTZ=demethyl diltiazem; DADTZ=deacetyl diltiazem, DMDADTZ=demethyl deacetyl diltiazem. AUCs expressed as (µg.h/L)
C-reactive protein results are given in Table 4.5. Only 5 of the 55 values exceeded the upper limit of normal for our laboratory and these elevations were not as high as has been shown to greatly affect CsA metabolism (Chen Y, et al. 1994).

Table 4.5. C-reactive protein concentrations (mg/L) for CsA-DTZ dose ranging study

<table>
<thead>
<tr>
<th>Pat Number</th>
<th>Study1</th>
<th>study2</th>
<th>study3</th>
<th>study4</th>
<th>study5</th>
<th>Study6</th>
<th>study7</th>
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<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>3.1</td>
<td>3.1</td>
<td>5.9</td>
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<td>6.7</td>
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</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>5.3</td>
<td>10.1</td>
<td>7.9</td>
<td>24.7</td>
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<td>3.1</td>
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</tr>
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<td>5.2</td>
<td>3.5</td>
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</tr>
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<td>6</td>
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<td>30.9</td>
<td>19.6</td>
<td>5.8</td>
<td>9.7</td>
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<td>3.9</td>
</tr>
<tr>
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<td>2.5</td>
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<td>2.5</td>
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<tr>
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<td>7</td>
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<td>7.1</td>
<td>9.4</td>
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<td>9.7</td>
</tr>
</tbody>
</table>

Normal range for C-reactive protein values is <10mg/L

4.5 Discussion

While the dose-response relationships for the approved indications (anti-anginal and antihypertensive) of DTZ have previously been determined, the relationship between DTZ dose and extent of the interaction with CsA has not. Since the pharmacokinetic interaction between CsA and DTZ was noted with established antianginal/antihypertensive doses of DTZ, it is not surprising that these regimens are the most frequently employed throughout Australasian transplant units (Chapter 2) when DTZ is employed as a CsA-sparing agent.
The aim of this pharmacokinetic interaction study was to define the dose-response relationship between CsA and DTZ. Surprisingly, even the lowest dose of DTZ used (10mg/d) increased CsA AUC(0-24) significantly (mean±sem increase 14.7±14.7%, p=0.039). The maximum DTZ dose used in this study was restricted to 180mg/d to reduce the potential for unwanted pharmacological effects. In an extension study (see Chapter 5), one subject who needed additional antihypertensive therapy was given a higher dose (240mg/d) of the CD formulation of DTZ.

Applying the sigmoid Emax model to our data, the predicted maximum effect (Emax) occurred at a DTZ dose greatly in excess of that used in this study. It has been noted that estimates of Emax and EC50 (dose required to achieve half the maximal effect) are inaccurate when the greatest measured effect is less than 95% of the estimated Emax (Dutta S, et al. 1996). These authors recommended that, instead of reporting unreliable estimates, data descriptors be presented which are taken from within the range of doses tested. Hence, values for both change in CsA trough concentrations and AUC(0-24), at clinically relevant DTZ doses, are presented in Table 4.2. Importantly, these data demonstrate that a clinically significant CsA-sparing effect is evident at doses of DTZ much lower than those currently used (180mg/d) for the majority of transplant recipients (see Chapter 2). Although DTZ is considered to be relatively safe, the use of lower doses should reduce the frequency and severity of adverse effects while also allowing its use as a CsA-sparing agent in situations where a pharmacological effect might be unwanted.

On three occasions, CsA dose was reduced because the blood CsA concentration rose above the therapeutic range accepted by this hospital. This effect of DTZ on CsA’s kinetics was anticipated, and the respective CsA kinetic data used for calculations (AUCs and trough concentrations) were increased proportionately to ‘normalise’ the data for
dose. This simple method of normalisation is widely applied but presumes drug kinetics to be linear over the dose range in question. There is some evidence to suggest that this may not apply for the Sandimmun® formulation of CsA, especially when other drugs are coprescribed. Sandimmun® absorption kinetics over the dose range used are noted by the manufacturer to be linear when plasma was the matrix studied but non-linear when whole blood was the matrix (Sandimmun® product information, Sandoz). Absorption has been noted to be best described as first order and zero order by different authors (Grevel J. 1986) and one report (where the oral solution formulation of CsA was used) describes three different patterns of absorption in just 42 children (Jacqz-Agrain E, et al. 1994). DTZ is thought to affect CsA’s intestinal CYP3A4 (and/or P-gp) and hence to increase the extent of CsA’s absorption. DTZ thus complicates the absorption process and thus it may affect the relationship between CsA dose and blood CsA concentration. There are no data on the linearity of CsA absorption when DTZ is coprescribed. Further complications in the early Sandimmun® data include the well described intrapatient variability in absorption associated with this formulation and cross reactivity of CsA metabolites with the assays used. The use of this method of normalisation is thus a limitation to the study findings but, given that this was only required on three occasions, it is unlikely that the data would be greatly affected.

While there is evidence that DTZ improves transplanted kidney function (Chrysostomou A, et al. 1993. Epstein M. 1992. Vasquez EM, et al. 1995. Wagner K, et al. 1987) and while hypertension is frequently observed in CsA-treated organ transplant recipients, as noted in Chapter 3, not all transplant physicians are convinced that DTZ should be routinely used. This is the first time that a drug has been widely prescribed primarily for an economic purpose and as such there are significant ethical concerns that should be addressed. Not the least of these concerns is that the optimal dosage of the 'sparing agent' should be defined.
As reported with earlier *in vitro* studies, the results from this *in vivo* study also demonstrate considerable interpatient variability in the dose of DTZ required to elevate blood CsA concentrations and in the magnitude of the increase. One potential source of variability is fluctuating levels of cytokines (including interleukin-6). Threefold increases in blood CsA concentrations have been observed in bone marrow transplant recipients (Chen YL, et al. 1994) following elevations in blood interleukin-6 concentrations. In the present study, C-reactive protein (a marker of cytokine activity) concentrations were only marginally elevated and in only a few patients (Table 4.5) and hence it is unlikely that fluctuating interleukin-6 concentrations contributed to the interpatient variability observed in this study.

CsA is metabolised by CYP3A4 within enterocytes (Kolars JC, et al. 1991a. Wu CY, et al. 1995) and the effect of DTZ on CsA metabolism might therefore largely occur as a result of inhibition of this presystemic metabolism. In order to maximise any local effect that might occur in the enterocyte, DTZ was administered at the same time as CsA in this study. Because of the design employed in this study, data is available on the effect of single, morning only doses of DTZ in the range 10mg - 60mg. Interestingly, the results demonstrate that these, single, morning doses affected morning and evening pharmacokinetic profiles to a similar extent (Fig 4.3). This was somewhat unexpected since absorption of DTZ from the gut was rapid (Tmax = 2.8±1.4h) and plasma half-life of DTZ was short (3.9±1.3h) (data from Chapter 7). In the simplest model of drug action, the effect wanes after 3 – 4 half-lives when the concentration of drug in plasma (which is a reflection of the concentration at the receptor site) falls below 10% of the peak concentration. If this model of action applies to the CsA-sparing effect of DTZ, its effect would be expected to be less evident (or even absent) during the second CsA dosing interval when compared to the first. Because this was not observed, it is likely that the mechanism of action of DTZ is more complex than simple competitive inhibition.
of CYP3A4. It is not known if DTZ remains within the enterocyte but this lack of effect of timing of DTZ is of considerable clinical significance since, as noted in Chapter 2, thrice daily DTZ regimens are widely employed in Australasia. It therefore follows that not only can the dose of DTZ be significantly reduced for many patients, but the frequency of administration could also be simplified to a morning only regimen without reducing CsA-sparing efficacy.

Many transplant centres have switched from conventional release DTZ to the modified release 'controlled diffusion' (CD) formulation of DTZ administered once daily (see Chapter 2). The principal attraction of this formulation is the reduced frequency of administration and thus, improved compliance. However, the prolonged CsA-sparing action of DTZ observed in the present study suggests that there is no need to change DTZ formulations to achieve an improvement in compliance. The lowest dosage available in the CD formulation (180mg DTZ in each capsule) is more than that required to significantly elevate blood CsA concentrations for most patients. Because of this lack of low-end dosage flexibility, the CD formulation is probably inferior to the conventional DTZ formulation for CsA-sparing purposes. Additionally, there are reasons for expecting that the CD formulation may result in an inferior CsA-sparing effect (discussed in Chapter 5).

The low AUC of DTZ and correspondingly lower CsA-sparing effect exhibited by subject 7 (Table 4.3 & Fig 4.2) suggests that parent DTZ is the interacting moiety. This hypothesis is also supported by the above average AUC for both DADTZ and DMDADTZ but typical CsA-sparing effect in subject 8.

If the primary target of DTZ is CYP3A4 within the enterocyte, then peak blood DTZ concentration may more closely reflect the extent of the metabolic interaction than either
DTZ AUC or DTZ dose. The results (Table 4.4) from the DTZ time-concentration profiles do not support this hypothesis. A highly significant relationship (p<0.0001) was demonstrated between change in CsA AUC(0-24) and DTZ dose, DTZ peak concentration, DTZ AUC(0-24), DMDTZ AUC(0-24) and DADTZ AUC(0-24). Since peak concentration of DTZ and AUCs of DTZ metabolites are related to the dose of DTZ, this is perhaps not surprising. Of the DTZ and/or metabolite parameters examined, all showed strong relationships (p<0.0001) with increases in CsA AUC(0-24), with the exception of the AUC of DMDADTZ (p=0.046). While the relationship between DMDTZ AUC(0-24) and CsA AUC(0-24) was marginally better than the relationship between DTZ dose and CsA AUC(0-24) (Table 4.4), the insubstantial magnitude of the difference combined with the difficulty in determining DMDTZ AUC are strong arguments in favour of simply using DTZ dose as the most practicable predictor of CsA-sparing effect for clinical purposes.

Intensive blood CsA concentration monitoring required to define the 24h AUC is inconvenient, laborious and expensive, and hence the relationship between increase in trough blood CsA concentrations and CsA AUC(0-24) was examined. The relationship showed a similar increase (Fig 4.2) commencing with the lowest dose of DTZ used and plateauing out at doses above 60mg/d DTZ. The magnitude of the response was different however, where the change in both 12h and 24h trough CsA concentrations suggested the CsA-sparing effect was greater than that shown by the increase in CsA AUC(0-24) (Table 4.2). Assuming CsA AUC(0-24) is a better guide to therapeutic efficacy, clinicians should not reduce the daily CsA dose to the same extent that the increase in CsA trough concentration suggests. If the dose were reduced by the same factor, the total exposure to CsA (ie. CsA AUC(0-24)) would be reduced. This provides a sound argument to adopt a higher therapeutic (trough concentration) range for CsA when DTZ is used as a CsA-sparing agent. However, as noted in Chapter 3, there is a
marked lack of concordance with respect to CsA therapeutic ranges within the Australasian transplant community and this does not seem to have translated into any discernible effect on either benefit or toxicity. It is thus unlikely that any modification to existing CsA therapeutic ranges is warranted given the relatively modest difference between the changes to CsA trough concentration and CsA AUC(0-24).

There are only limited data in the literature on the time course of the interaction between DTZ and CsA but several authors have noted that it is non-competitive (Brockmoller J, et al. 1990. Wagner K, et al. 1989. Tortorice KL, et al. 1990.). It has also been observed that a significant effect on blood CsA concentrations are evident after a 7 day period (Brockmoller J, et al. 1990). In the present study, a minimum 2 week interval between study days was selected partly because of concerns about restoration of blood volume in the subjects and partly because this was thought to be sufficient for the interaction to stabilise and blood CsA concentrations to reach their new steady state.

DTZ has a half-life of approximately 4h which is consistent with steady state blood DTZ concentrations being attained by the end of the first day. There were thus an additional 13 day period for the interaction to develop but it is conceivable that the effect was not maximal at this time, especially if factors other than mere blood concentration were important. The incremental increase in DTZ dose design was adopted because the study was undertaken in stable kidney transplant recipients and this study design reduced the risk that dramatic alterations to CsA AUC would be effected. Since the order of doses of DTZ were not randomised, a 'period effect' cannot therefore be excluded.

4. Recommendations

The following recommendations are drawn from the study findings. In patients where DTZ is indicated primarily for its economic benefit (viz to reduce the cost of providing
CsA), either CsA AUC(0-24) or trough blood CsA concentration (12h and/or 24h) should be determined prior to prescribing DTZ. Following a suitable stabilisation period (eg. 2 weeks) on each dosage increment, blood CsA concentrations should be remeasured. For practical reasons, the initial dose of DTZ should be 30mg (half a conventional tablet) each morning followed by 60mg mane and then 60mg twice daily depending upon the clinical situation and magnitude of response. DTZ doses should be given at the same time as the CsA doses to lessen the impact of drug regimen complexity on compliance. If there is no clinical indication for DTZ and no increase in blood CsA concentrations are seen at this dose, it would seem prudent to discontinue DTZ. In Chapter 5, data is presented which suggests higher doses of DTZ will produce a greater CsA-sparing effect but in Chapter 6, data is presented which suggests that higher doses of DTZ do not necessarily result in a CsA-sparing effect.

For transplant recipients currently receiving DTZ in a dose of \( \geq 180 \)mg/day for its CsA-sparing effect, any dosage reduction should be undertaken with caution because the potential for harm resulting from blood CsA concentrations falling below the therapeutic range is significant. If the dosage regimen is thrice daily, it should first be simplified to a twice daily regimen after which time the DTZ dosage should be reduced by 30-60mg decrements every 2 weeks, aiming for a final dose of 60mg/day. In this situation it would seem prudent to monitor blood CsA trough concentrations more frequently (eg. weekly). Where there is a clinical indication for DTZ, it should be prescribed in the most appropriate dose for the indication and the effects on blood CsA concentrations (either trough concentrations or AUC estimations) monitored. Where patients are maintained on 180mg/d DTZ as a CsA-sparing agent (as is commonplace in Australasia) and additional therapeutic benefit is wanted, there would seem to be no reason not to increase the dose of DTZ (to 240mg or higher) as warranted with the same blood CsA concentration monitoring as outlined above.
Chapter 5

A clinical study to compare the effect of changing DTZ formulation, from conventional release tablet to extended delivery capsule, on the pharmacokinetic interaction between CsA and DTZ in kidney transplant recipients.

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5.1 Introduction

Diltiazem (DTZ) is an anti-hypertensive and anti-anginal agent which has been shown to inhibit the metabolism of the expensive immunosuppressive drug, cyclosporin (CsA) via the cytochrome P450 isoenzyme CYP3A4 (Kronbach T, et al. 1988. Pichard L, et al. 1990) and/or via the P-gp drug efflux system (Lown KS, et al. 1997).

As described in Chapter 2, this interaction is widely exploited by Australasian transplant centres to allow a lower dose of CsA to be prescribed while maintaining whole blood CsA concentrations within the therapeutic ranges accepted by the respective transplant units. The pharmacokinetic interaction study described in Chapter 4 confirmed the observations of several authors (Chrysostomou A, et al. 1993. Patton PR, et al. 1994. Tortorice KL, et al. 1990. McLachlan A & Tett S. 1998. Leibbrandt DM & Day RO. 1992) that significant savings are afforded by this deliberate coprescription. The dollar savings to the Australian health care system were estimated to be approximately AUD$7 million per annum in Australia in 1995-6 (Chapter 2). Several authors have noted that, in addition to this economic benefit, DTZ also affords therapeutic benefits (Epstein M. 1992. Chrysostomou A, et al. 1993. Morales JM, et al. 1994). These benefits were confirmed in the early post kidney transplant period in the Australasian setting in the study presented in Chapter 3. The therapeutic savings afforded by the use of DTZ (less need for post transplant dialysis etc) have not been quantified and are outside the scope of this thesis. In such a pharmacoeconomic analysis, the cost of provision of DTZ will need to be discounted because >75% transplant recipients require one or more antihypertensive agents (Figs 3.5-3.7) for the treatment of hypertension.

One of the factors which has directed trends in antihypertensive therapy in recent years has been the issue of suboptimal patient compliance with antihypertensive medication. The pharmaceutical industry has responded by producing newer agents with longer half-lives and ‘sustained release’ formulations (designed to release the drug continuously over
an extended time period) of older agents, both of which allow less frequent administration. This trend has been especially evident with the calcium channel blocker group of drugs and sustained release formulations of verapamil, nifedipine and DTZ are now commonly prescribed. At the time of this study, DTZ was marketed in two different formulations with extended-release characteristics, one of which was a ‘controlled diffusion’ (CD) formulation. This is not a ‘conventional’ sustained release formulation since the contents are not constantly released but rather, this formulation is designed to deliver DTZ in two bursts, the first (40%) over the first 12h period and the second (60%) over the next 12h period. While sustained release formulations have proven efficacy in the control of angina and/or hypertension, their effect on another widely used indication - that of CsA-sparing agent - has not been studied.

In Chapter 2 it was demonstrated that many Australasian transplant centres had made the switch to the CD DTZ formulation and hence the present study was undertaken to consider the effect of switching from conventional to CD formulation DTZ in a group of stable renal transplant recipients.

5.2 Methods
This study was conducted as an extension of the dose-escalation study described in Chapter 4. Briefly, Ethics of Human Research Committee (see Appendix 3) approval was gained and 8 stable renal transplant recipients were enrolled in a dose-escalation study using conventional formulation DTZ. Patient details are given in Table 4.1. Inclusion criteria included stable trough blood CsA concentrations on routine monitoring for ≥3 months, stable renal function for ≥3 months and plasma creatinine concentration <180μmol/L. Exclusion criteria (see Chapter 4) included consumption of any medication which was known to interfere with the kinetics of CsA (especially erythromycin and KCZ) or grapefruit juice, or the use of drugs whose metabolism might be adversely affected by DTZ (especially terfenadine).

The first study day in this trial was the final study day in the dose escalation study described in Chapter 4. The DTZ dose taken on this day (and for the previous 13 days) was 90 mg twice daily of the conventional release DTZ formulation, Cardizem®, (ICI Australia). As outlined in Chapter 4, DTZ was taken at 12h intervals, each dose being taken with the respective CsA dose (Sandimmun® capsules, Sandoz, Australia). After this study day, subjects were switched to the CD formulation of DTZ at the same dose (180mg, Cardizem CD®, ICI Australia) given with the morning CsA dose. The second study day occurred 2 weeks later and on both study days, no food was allowed within 1h of taking either CsA (and hence DTZ) doses and subjects received the same meals. Serial blood samples were drawn pre-dose, as well as 1, 2, 3, 4, 6, and 12 hr following
both morning and evening CsA doses and the area under the blood CsA concentration versus time curve (AUC) for each CsA dose was determined using the log trapezoidal method (Appendix 4). Whole blood CsA concentrations were determined using a specific (<10% cross-reactivity with CsA metabolites) monoclonal, whole-blood enzyme immunoassay (EMIT®, Behring/Syva, San Jose, USA). The precision of the method in this laboratory was ±11.9% at a concentration of 86µg/L and ±7.1% at a concentration of 409µg/L (Sallustio BC, et al. 1997). Samples with concentrations exceeding the top calibration standard (500µg/L) were diluted according to the manufacturer’s protocol, viz by diluting the supernatant following precipitation with methanol/water and reassayed. Plasma DTZ concentrations were determined using a reverse phase HPLC with UV detection assay method as described in Chapter 7. Each blood sample was divided into two aliquots, one for CsA assay (into an EDTA collection tube) and the other for DTZ and its metabolites (into a lithium heparin collection tube) and hence the timing of the samples for the DTZ assay was identical to those used for the CsA assay. The results were compared by the Wilcoxon Signed-rank test and a 5% level assigned as indicating significance.

5.3 Results
Data for 1 subject (Patient 6) were excluded because of vomiting shortly after CsA administration which virtually eliminated the expected absorption phase of the CsA concentration-time curve. The results for the remaining 7 subjects are shown in the tables and figures.

There was considerable interpatient variability (in both magnitude and direction of CsA AUC) when changing from conventional to the CD formulation of DTZ and this difference was most marked following the morning CsA dose (Table 5.1). Three of the 7 subjects exhibited reductions in CsA AUC(0-12h) of 30-60% while one showed a 36%
increase in AUC with the CD formulation. The AUC following the evening CsA dose did not show the same degree of variability between subjects, with a range from 19% lower to 26% higher CsA AUC. There was a mean fall of 16.5% in CsA AUC(0-12) while the mean CsA AUC(12-24) remained unchanged (a mean increase of 0.4%) following the change to DTZ formulation. Neither of these changes was statistically significant (p=0.18 and p=0.98 respectively).

Fig 5.1. Individual patient and mean (n=7) CsA AUC(0-12) and CsA AUC(12-24) (μg.h/L) for the 90mg twice daily conventional formulation DTZ followed by 180mg once daily CD formulation DTZ.
CHAPTER 5

Table 5.1 CsA AUC(µg.h/L) data following morning and evening CsA doses and the percentage difference in CsA AUC between the DTZ conventional release (90mg twice daily) and controlled diffusion (180mg once daily) formulations.

<table>
<thead>
<tr>
<th>Patient No</th>
<th>AUC(0-12)</th>
<th></th>
<th>AUC(0-24)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>90mg bd</td>
<td>180mg CD</td>
<td>% diff</td>
<td>90mg bd</td>
<td>180mg CD</td>
</tr>
<tr>
<td>1</td>
<td>5493</td>
<td>4680</td>
<td>-15.0</td>
<td>4586</td>
</tr>
<tr>
<td>2</td>
<td>4842</td>
<td>6568</td>
<td>+35.7</td>
<td>4925</td>
</tr>
<tr>
<td>3</td>
<td>3225</td>
<td>2979</td>
<td>-7.6</td>
<td>5289</td>
</tr>
<tr>
<td>4</td>
<td>6177</td>
<td>2555</td>
<td>-58.5</td>
<td>6119</td>
</tr>
<tr>
<td>5</td>
<td>8006</td>
<td>4894</td>
<td>-38.9</td>
<td>6487</td>
</tr>
<tr>
<td>7</td>
<td>5122</td>
<td>5200</td>
<td>+1.5</td>
<td>5096</td>
</tr>
<tr>
<td>8</td>
<td>4247</td>
<td>2846</td>
<td>-33.0</td>
<td>3398</td>
</tr>
<tr>
<td>Mean</td>
<td>5301</td>
<td>4245</td>
<td>-16.5</td>
<td>5128</td>
</tr>
<tr>
<td>s.d</td>
<td>1515</td>
<td>1490</td>
<td>30.7</td>
<td>1015</td>
</tr>
</tbody>
</table>

Tables 5.2 - 5.4 show plasma DTZ AUC(0-12), AUC(12-24) and AUC(0-24) (µg.h/L) compared to the corresponding CsA AUC data following consumption of 180mg DTZ as either conventional (90mg twice daily) or CD (180mg once daily) formulation.

As noted in Chapter 4, CsA dose remained unchanged for 6 of these 7 patients but in patient 5, the daily dose was reduced from 200mg/d to 150mg/d before the second study day because of concerns over CsA toxicity. The CsA AUC data have been adjusted to allow for this dose alteration. As can be seen in Tables 5.2 - 5.4, patient 5 experienced a 14% fall in DTZ AUC(0-12), a 45% fall in DTZ AUC(12-24) and a 29% fall in DTZ AUC(0-24) following the switch from conventional formulation to CD formulation DTZ.
CHAPTER 5

Table 5.2. CsA AUC(0-12) and DTZ AUC(0-12) data and difference between these data when 180mg/d DTZ was administered as conventional release formulation (90mg twice daily) followed by CD formulation (180mg once daily).

<table>
<thead>
<tr>
<th>Patient No</th>
<th>CsA AUC(0-12) μg.h/L</th>
<th>DTZ AUC(0-12) μg.h/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90mg bd 180mg CD</td>
<td>% diff</td>
</tr>
<tr>
<td>1</td>
<td>5493 4680</td>
<td>-15.0</td>
</tr>
<tr>
<td>2</td>
<td>4842 6568</td>
<td>+35.7</td>
</tr>
<tr>
<td>3</td>
<td>3225 2979</td>
<td>-7.6</td>
</tr>
<tr>
<td>4</td>
<td>6177 2555</td>
<td>-58.5</td>
</tr>
<tr>
<td>5</td>
<td>8006 4894</td>
<td>-38.9</td>
</tr>
<tr>
<td>7</td>
<td>5122 5200</td>
<td>+1.5</td>
</tr>
<tr>
<td>8</td>
<td>4247 2846</td>
<td>-33.0</td>
</tr>
<tr>
<td>Mean</td>
<td>5301 4245</td>
<td>-16.5</td>
</tr>
<tr>
<td>s.d</td>
<td>1515 1490</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3 CsA AUC(12-24) and DTZ AUC(12-24) data and difference between these data when 180mg/d DTZ was administered as conventional release formulation (90mg twice daily) followed by CD formulation (180mg once daily).

<table>
<thead>
<tr>
<th>Patient No</th>
<th>CsA AUC(12-24) μg.h/L</th>
<th>DTZ AUC(12-24) μg.h/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90mg bd 180mg CD</td>
<td>% diff</td>
</tr>
<tr>
<td>1</td>
<td>4586 5064</td>
<td>+10.4</td>
</tr>
<tr>
<td>2</td>
<td>4925 6208</td>
<td>+26.1</td>
</tr>
<tr>
<td>3</td>
<td>5289 4473</td>
<td>-15.4</td>
</tr>
<tr>
<td>4</td>
<td>6119 6157</td>
<td>+0.6</td>
</tr>
<tr>
<td>5</td>
<td>6487 5398</td>
<td>-16.8</td>
</tr>
<tr>
<td>7</td>
<td>5096 5903</td>
<td>+15.8</td>
</tr>
<tr>
<td>8</td>
<td>3398 2788</td>
<td>-18.7</td>
</tr>
<tr>
<td>Mean</td>
<td>5128 5141</td>
<td>+0.4</td>
</tr>
<tr>
<td>s.d</td>
<td>1015 1211</td>
<td></td>
</tr>
</tbody>
</table>
Table 5.4. CsA AUC(0-24) and DTZ AUC(0-24) data and difference between these data when 180mg/d DTZ was administered as conventional release formulation (90mg twice daily) followed by CD formulation (180mg once daily).

<table>
<thead>
<tr>
<th>Patient No</th>
<th>CsA AUC(0-24) µg.h/L</th>
<th>DTZ AUC(0-24) µg.h/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90mg bd 180mg CD % diff</td>
<td>90mg bd 180mg CD % diff</td>
</tr>
<tr>
<td>1</td>
<td>10079 9745 -3.3</td>
<td>4412 2944 -33.3</td>
</tr>
<tr>
<td>2</td>
<td>9767 12776 +30.8</td>
<td>9344 3532 -62.2</td>
</tr>
<tr>
<td>3</td>
<td>8514 7452 -12.5</td>
<td>4874 2385 -51.1</td>
</tr>
<tr>
<td>4</td>
<td>12296 8713 -29.1</td>
<td>3246 3136 -3.4</td>
</tr>
<tr>
<td>5</td>
<td>14494 10292 -29.0</td>
<td>4811 3442 -28.5</td>
</tr>
<tr>
<td>7</td>
<td>10219 11104 +8.7</td>
<td>256 2082 +713.3</td>
</tr>
<tr>
<td>8</td>
<td>7644 5633 -26.3</td>
<td>3497 2736 -21.8</td>
</tr>
<tr>
<td>Mean</td>
<td>10430 9388 -10.0</td>
<td>4349 2894 -33.5</td>
</tr>
<tr>
<td>s.d</td>
<td>2311 2369</td>
<td>2713 534</td>
</tr>
</tbody>
</table>

DTZ AUC(0-12) and AUC(12-24) following the use of the CD formulation were 1576 ± 419 µg.h/L vs 1318 ± 183 µg.h/L. This is not consistent with the manufacturer’s claim that the CD formulation releases 40% of the dose during the first 12 hours and the balance during the next 12 hours. In 6 of the 7 patients, individual patient data also failed to show twin peaks which were anticipated from the ‘double dump’ design.

In order to test the hypothesis that DTZ concentrations in the gastrointestinal tract are better represented by the early part of each concentration-time curve, the same comparison was made with DTZ AUC(0-3) and DTZ AUC(12-15) (ie. the first 3 hours of both morning and evening profiles). These data are presented in Tables 5.5. They demonstrate that both AUC(0-3) and AUC(12-15) were greater when conventional DTZ formulation was given than when CD formulation was given.
Table 5.5. DTZ AUC(0-3) and DTZ AUC(12-15) for DTZ doses of 180mg/day given either as 90mg (conventional release) twice daily or 180mg (CD formulation) once daily.

<table>
<thead>
<tr>
<th>Pt No</th>
<th>DTZ AUC(0-3) (µg.h/L)</th>
<th>% diff</th>
<th>DTZ AUC(12-15) (µg.h/L)</th>
<th>% diff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90mg bd</td>
<td>180mg CD</td>
<td></td>
<td>90mg bd</td>
</tr>
<tr>
<td>1</td>
<td>736</td>
<td>314</td>
<td>-57.3</td>
<td>382</td>
</tr>
<tr>
<td>2</td>
<td>1444</td>
<td>399</td>
<td>-72.4</td>
<td>1438</td>
</tr>
<tr>
<td>3</td>
<td>1116</td>
<td>223</td>
<td>-80.0</td>
<td>366</td>
</tr>
<tr>
<td>4</td>
<td>319</td>
<td>472</td>
<td>+48.0</td>
<td>496</td>
</tr>
<tr>
<td>5</td>
<td>768</td>
<td>398</td>
<td>-48.2</td>
<td>669</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>254</td>
<td>+568.4</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>794</td>
<td>235</td>
<td>-70.4</td>
<td>411</td>
</tr>
<tr>
<td>Mean</td>
<td>745</td>
<td>328</td>
<td>-56.0</td>
<td>540</td>
</tr>
<tr>
<td>sd</td>
<td>467</td>
<td>97</td>
<td></td>
<td>441</td>
</tr>
</tbody>
</table>

In one subject (patient 3), additional antihypertensive therapy was indicated after the second study day and hence the dose of DTZ was increased to 240mg, taken as the CD formulation, once daily. Two weeks later, the study was repeated with the same blood sampling as had occurred in other study days. Figure 5.2 shows the change in CsA AUC(0-24) which accompanied the administration of DTZ over the dosage range 0-180mg for the group (n=8) taken from Chapter 4. Overlaying this curve is the change in CsA AUC(0-24) (over the entire DTZ dose range) for patient 3 who was given 180mg and 240mg DTZ as the CD formulation. Table 5.6 shows the change from baseline CsA AUC(0-24) and trough CsA concentration over the DTZ dose range 0 – 240mg where the last two doses were taken as the CD formulation.
Figure 5.2. Change from baseline (expressed as a percentage) in CsA AUC(0-24) with increasing doses of DTZ (mg/day). Group data (n=8) taken from Chapter 4. Overlaying this curve is the individual data for patient 3 over the DTZ dose range 0-240mg. Closed circles represent DTZ given as conventional tablet formulation and closed squares as the CD formulation.

Table 5.6. Change (expressed as percentage) over baseline in morning CsA trough concentration (C24) and CsA AUC(0-24) with DTZ dose (mg/day) in patient 3.

<table>
<thead>
<tr>
<th>DTZ dose (mg/d)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>180 (CD)</th>
<th>240 (CD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C24</td>
<td>0</td>
<td>123</td>
<td>62</td>
<td>80</td>
<td>58</td>
<td>108</td>
<td>104</td>
<td>75</td>
<td>146</td>
</tr>
<tr>
<td>AUC(0-24)</td>
<td>0</td>
<td>7</td>
<td>8</td>
<td>17</td>
<td>20</td>
<td>38</td>
<td>12</td>
<td>-2</td>
<td>53</td>
</tr>
</tbody>
</table>

These data demonstrate that the magnitude of the change in trough CsA concentration always exceeded the magnitude of change in CsA AUC(0-24).
The effect of the 30% increase in dose of DTZ (from 180mg/d to 240mg/d) in patient 3 was a jump in the percentage increase over baseline for both CsA morning trough (75% to 146%) and CsA AUC(0-24) (-2% to 53%) (Table 5.6). These data demonstrate that the CsA-sparing effect of DTZ is not necessarily maximal at the 'conventional' dose of 180mg/day used in Australasia (Chapter 2).

Table 5.7 shows the apparent Tmax for DTZ for the 90mg (conventional release) given twice daily (n=7). These data demonstrate a mean time to peak concentration of 2.7h following the morning DTZ dose and 3.0h following the evening DTZ dose.

Table 5.7. Apparent Tmax for DTZ following morning and evening doses of 90mg (conventional release) DTZ.

<table>
<thead>
<tr>
<th>Pt No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>7</th>
<th>8</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax (am)</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>Tmax (pm)</td>
<td>3.75</td>
<td>2</td>
<td>4.25</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>3.0</td>
</tr>
</tbody>
</table>

5.4 Discussion

This study compared the CsA-sparing effect of the same daily dose of DTZ given as two different DTZ formulations (conventional release and CD). As noted in Chapter 2, at the time of this study, the CD formulation was used by approximately 51% of Australasian organ transplantation recipients. While not asked of respondents to the questionnaire, the reason for the switch to this formulation is presumably because the once daily dosing schedule simplifies the medication regimen and improves compliance. Poor patient compliance has been noted to jeopardise transplant outcome and compliance deteriorates as drug regimen complexity increases (Didlake RH, et al. 1988. Kiley DJ, et al. 1993. Burke JF, et al. 1994. Schweizer RT, et al. 1990).
As noted above, this study was conducted in kidney transplant recipients who had completed an escalating dose regimen of conventional formulation DTZ, where the final dose was 90mg twice daily. While statistical variability was reduced by virtue of each patient serving as his/her own control, it was not possible to effect a randomised crossover design with appropriate washouts between doses and hence a period effect cannot be excluded. These data need also be considered in the light of the well established interpatient and intrapatient variability in CsA pharmacokinetics which was observed in Chapter 4 and which has been described by others in the absence of complications associated with the coprescription of metabolic inhibitors (Ohlman S, et al. 1993. Grevel J. 1986. Gupta SK, et al. 1990. Kahan BD, et al. 1995b).

The manufacturer of CsA (Novartis, formerly Sandoz, Australia) has replaced the older Sandimmun® formulation with a ‘microemulsion preconcentrate’ formulation of CsA (Neoral®) which is designed to reduce the dependence on bile for CsA absorption and hence reduce the variability in oral absorption. Interpatient variability has been shown to be lessened by this formulation (Drewe J, et al. 1992a) and hence it is possible that the magnitude and/or reliability of the CsA-sparing effect might also be affected. However, it has been estimated by one group of researchers who have extensively studied absorption of CsA from the gastrointestinal tract (Wu CY, et al. 1995), that the extent of absorption of CsA from the older Sandimmun® formulation was already very high (86%) and that bioavailability was more likely to be affected by intestinal CYP3A4 activity than CsA formulation. Therefore, the change to the newer CsA formulation may not result in any marked change in CsA kinetics in the presence of CsA-sparing agents.

The precise site(s) and mechanism of this metabolic interaction between CsA and DTZ remains to be completely elucidated but the upper intestine is one likely site. CYP3A4 enzyme activity is high in the upper intestine and is approximately half the CYP3A4 activity of the liver on a tissue weight basis (de Waziers I, et al. 1990. Watkins PB.
Several authors have postulated that large percentages of orally administered CsA might be metabolised by CYP3A4 in enterocytes (Watkins PB. 1992. Wu CY, et al. 1995). Further support for a significant role of the intestine was provided by the pharmacokinetic interaction between CsA and erythromycin (a substrate for and inhibitor of CYP3A4). In a study in six renal transplant recipients, bioavailability of orally administered CsA increased from 36% to 60% following oral erythromycin (Gupta SK, et al. 1989). However, when CsA was administered intravenously under the same circumstances, there was only a modest (13%) decrease in clearance. Other researchers have suggested that intestinal P-gp is more likely to be responsible for reduced CsA absorption than CYP3A4 (Lown KS, et al. 1997). However, since P-gp is also located in the proximal intestine, this reinforces the importance of this site as the most likely site for the interaction.

The magnitude of the interaction between CsA and DTZ at this upper intestinal site should be greatest if the concentration of DTZ at this site was maximal at the time that CsA arrived. The concentration of DTZ at intestinal mucosal sites were not measured in this study but the design required that conventional release DTZ formulation was to be taken at the same time as CsA doses in order to maximise the interaction. The CD formulation DTZ was also taken with the (morning) dose of CsA but, because it is designed to release the second ‘dump’ 12 hours after the first, it was anticipated that this might occur at more distal intestinal sites. If so, when the CD formulation was taken, the concentration of DTZ at the upper intestinal site of absorption of CsA would be less for the evening CsA dose than for the morning CsA dose. Individual time-concentration profiles for the CD formulation of DTZ provides information on the blood concentration throughout the day (Tables 5.2 - 5.4 and Chapter 7) but this does not necessarily reflect upper intestinal concentrations since absorption of DTZ is unlikely to be confined to upper intestinal sites.
While there was no consistent reduction in CsA AUC(12-24), the explanation may lie in the performance of the CD formulation. The ratio of DTZ AUC(0-12) to AUC(12-24) when the CD formulation was taken (Tables 5.2 and 5.3), was 1.20, not the 0.67 (40:60) anticipated from the manufacturer’s claims for the CD formulation. Also, with the exception of one subject, plasma concentrations of DTZ did not exhibit the dual peaks which were expected from the design of the CD formulation. In addition, the CD formulation resulted in an AUC(0-24) which was 33.5% lower (Table 5.4) than the same dose taken as conventional release formulation. The CD formulation thus failed to perform as expected, although the data does confirm that DTZ was being released from the capsule and that absorption was still occurring during the second 12h period of the study day. Given the nature of gastrointestinal tract transit times, it is likely that much of the 12-24h absorption occurred below the primary site of CsA’s absorption, namely the upper intestine (Drewe J, et al. 1992b).

Mean CsA AUC(0-12) fell 16.5% (Table 5.2) following the switch from conventional release DTZ to the CD formulation. The corresponding value for CsA AUC(12-24) was a rise of 0.4% (Table 5.3). DTZ AUC(0-12) fell by 31.9% following the switch while the AUC(12-24) fell by a similar amount, namely 35.3%. The lesser CsA-sparing effect following the morning dose of CsA might thus be explained by the fact that less DTZ was present in the gastrointestinal tract (and hence was absorbed into the bloodstream) during the first 12 hours. Despite the fall in DTZ AUC(12-24) of 35.3% after switching to the CD formulation, there was no similar fall in CsA AUC(12-24). This suggests that the presence of DTZ in the gastrointestinal tract in the second 12 hour period after taking the CD formulation had less impact on the absorption of CsA. This is consistent with the proposition that during the 12-24h period, DTZ was absorbed from lower gastrointestinal sites while CsA was in the upper intestine. That there was any CsA-sparing effect at all can be explained with the persistence of the interaction following conventional release DTZ formulation as described in Chapter 4.
Conventional release DTZ was rapidly absorbed from the gastrointestinal tract at the 90mg twice daily dose. Apparent Tmax values following the am dose was 2.7h and following the evening dose apparent Tmax was 3h (Table 5.7). The early part of any concentration-time curve is dominated by drug absorption while the latter part of the same curve is dominated by excretion processes. Hence the early part of the curve should more accurately reflect the amount of drug in the gastrointestinal tract than the whole AUC. Since Tmax for conventional release DTZ was $\leq$3h, the same comparisons between change in CsA AUC(0-12) and CsA AUC(12-24) were made with DTZ AUC(0-3) and DTZ AUC(12-15) (ie the absorption part of both morning and evening DTZ curves) for 180mg DTZ given either as 90mg (conventional release) twice daily or as 180mg (CD formulation) given in the morning. Five patients experienced falls in DTZ AUC(0-3) and 4 experienced falls in DTZ AUC(12-15) (Table 5.5) when switched from conventional (rapid release) to CD formulation. The mean fall for DTZ AUC(0-3) was 56% while the corresponding fall for DTZ AUC(12-15) was 26%. Patient 7 demonstrated an atypical response from the change to CD formulation with an increase in DTZ AUC(0-3) of 568% and in DTZ AUC(12-15) of 1228%. The mean fall would have thus have been greater had the data from patient 7 been excluded.

Comparing data from tables 5.2, 5.3 and 5.5, it is apparent that neither DTZ AUC(0-3) nor AUC(12-15) provide better explanations of the changes in CsA AUCs than do DTZ AUC(0-12) or AUC(12-24). There are two potential explanations for this observation; the first being that AUC(0-3) is a poor correlate of intestinal DTZ concentrations and the second explanation is that intestinal DTZ concentrations are a poor predictor of changes to CsA AUC. However, as demonstrated in Chapter 4, at a dose of 180mg DTZ daily, the CsA-sparing effect was plateauing off and hence the magnitude of the fall in either set of DTZ AUC data would be expected to be greater than the corresponding effect on CsA AUC data.
Also of interest in this regard was the very low DTZ AUC(0-12), AUC(12-24) and hence AUC(0-24) demonstrated by patient 7 with the conventional release formulation DTZ (Tables 5.2 – 5.4). Similarly, DTZ AUC(0-3) and (12-15) were also much lower than the mean values (Table 5.5) for the group. Lower than expected values for DTZ AUC(0-12) and AUC(12-24) were also observed with previous doses of DTZ used in the dose escalation study (Chapter 4). Surprisingly, the switch to CD formulation of DTZ resulted in a massive 640% increase in DTZ AUC(0-12), a similar 810% increase in DTZ AUC(12-24) and hence a 713% increase in DTZ AUC(0-24). The corresponding increases for CsA AUC(0-12) (1.5%), CsA AUC(12-24) (15.8%) and CsA AUC(0-24) (8.7%) were much less, suggesting that the CsA-sparing effect was unrelated to plasma DTZ concentrations in this patient. The 568% increase in DTZ AUC(0-3) and 1228% increase in DTZ AUC(12-15) are similarly much larger than the modest increases in CsA AUC. Once again, the failure of DTZ AUC(0-3) and AUC(12-15) to predict changes to CsA AUC in this patient can be explained by the poor predictive value of AUC(0-3) to reflect intestinal DTZ concentrations or that intestinal DTZ concentrations (at this dose of DTZ) are poorly correlated with changes to CsA AUC.

Patient 5 experienced a fall in DTZ AUC(0-12) of 14%, in DTZ AUC(12-24) of 45% which resulted in a fall in DTZ AUC(0-24) of 29% when switched from conventional release DTZ (90mg taken twice daily) to CD formulation (180mg taken in the morning). This patient also had a reduction in dose of CsA (from 100mg twice daily to 75mg twice daily) between study days (CsA data were corrected for this reduction). CsA has been proven to act as a felodipine-sparing agent (Madsen JK, et al. 1996) and since both these calcium channel blockers are metabolised by CYP3A4, there is potential for CsA to also act as a DTZ-sparing agent. Because of the design of the studies which comprise this thesis, this hypothesis has not been tested. If this were the case, the reduced CsA dose (25% lower) in patient 5 might explain the 29% fall in DTZ AUC(0-24). While this is one potential explanation, it is worth noting that 3 other patients (whose CsA dose was
unchanged) experienced greater falls in DTZ AUC(0-24) than patient 5 (Table 5.4) and hence it is more likely that the explanation lies in poor performance of the CD DTZ formulation.

The decision to switch from conventional formulation DTZ (administered thrice daily) to CD formulation (administered once daily) by many Australasian transplant centres (see Chapter 2) has presumably been made for compliance reasons. Given the variability resulting from this switch to CD formulation and especially the falls of ≥30% in CsA AUC(0-24) demonstrated by 3 patients observed in the present study, it would seem more sensible to change to a 90mg conventional release DTZ formulation regimen which required DTZ to be given twice daily, with each dose of CsA. Since CsA is dosed twice daily in adult kidney transplant recipients (Chapter 2), this should improve compliance, maximise proximal intestinal DTZ concentrations and hence optimise the CsA-sparing effect.

The opportunity to increase DTZ dose from 180mg to 240mg in patient 3 provided further insight to that gleaned in Chapter 4 into the effect of increasing DTZ dose on CsA-sparing activity. The further increase in CsA AUC(0-24) (-2% over baseline to 53% over baseline) and trough CsA concentration (75% over baseline to 146% over baseline) shows that the CsA-sparing activity is not maximal at a DTZ dose of 180mg daily in some subjects. Considerable interpatient variability in CsA kinetics, consistent with the data on variability in intestinal CYP3A activity (Lown KS, et al. 1994), were demonstrated in Chapter 4 and this will be further explored in Chapter 6.

5.5 Conclusions
Changes in CsA AUC(0-12h), AUC(12-24h) and AUC(0-24) were observed when changing from conventional release formulation DTZ to CD formulation DTZ. While
these changes were statistically insignificant across the group, individual changes were clinically significant.

The CD formulation DTZ failed to perform as expected with a decrease in DTZ AUC(0-24) of 33.5% relative to the same dose taken as conventional release formulation, more being absorbed in the first 12 hour period and only one patient demonstrating a ‘twin peak’ concentration-time profile anticipated from the manufacturer’s description of the formulation.

If compliance is the reason for considering a switch from conventional release to CD formulation DTZ, the patient may be better served by changing from conventional release formulation given thrice daily to the same formulation given twice daily, where each DTZ dose is given with the corresponding CsA dose.
Diltiazem does not always increase blood cyclosporin concentration – a clinical study in a lung transplant recipient

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6.1 Introduction

Since its introduction in the early/mid 1980s in Australia, CsA has become the major immunosuppressive drug used for the prevention of transplant organ rejection. While its use has resulted in improved graft survival figures, it has been complicated by a new set of adverse effects and interactions with other drugs. CsA is metabolised by CYP3A4 and several important drug interactions have been observed including interactions with DTZ, KCZ, itraconazole (ICZ) and erythromycin which all impair metabolism and elevate blood CsA concentrations, and rifampicin and phenytoin which induce metabolism and significantly reduce blood CsA concentrations. In the mid 1980s, deliberate coprescription of drugs which increased blood CsA concentrations were advocated in an attempt to contain the costs of transplantation (Neumayer HH & Wagner K. 1986. Kohlaw K, et al. 1988. Brockmoller J, et al. 1990). As noted in Chapter 2, approximately 70% of kidney transplant recipients and 66% of lung transplant recipients are routinely prescribed DTZ in Australia and New Zealand.

Many patients prescribed CsA will have a clinical indication for the use of DTZ (angina and/or hypertension). Data presented in Chapter 3 demonstrated that DTZ improves renal transplant outcomes in the early post operative period, a finding reported in an earlier Australasian kidney transplant study (Chrysostomou A, et al. 1993). In Chapter 2 it was demonstrated that many transplant recipients were routinely prescribed DTZ prior to the availability of this evidence and that the routine prescription was done primarily for the economic benefits that accompany the CsA dose reduction. This is the first time that a drug has been widely prescribed for an economic rather than a therapeutic purpose but, despite the widespread use of DTZ as a CsA-sparing agent, there were only limited pharmacokinetic data on the interaction prior to the study described in Chapter 4. In that study, the dose-response curve for the interaction between CsA and DTZ was explored in stable kidney transplant recipients and large interpatient differences in both the magnitude of the interaction and the dose of DTZ required to produce a sparing effect were demonstrated. While the mean increase in CsA AUC (or trough) observed in Chapter 4 was consistent with the previously reported figure of 35%, there was considerable interpatient variability in response with some patients experiencing greater and some patients lesser increases.

There have been no systematic studies which compare the CsA-sparing effect of different drugs in the same patients. Hence the opportunity to study the CsA-sparing effect of both itraconazole and diltiazem in a lung transplant recipient was welcomed when the clinical situation allowed the study to be undertaken.

6.2 Methods

A 49 year old Caucasian female received a single lung transplant for cigarette smoking related emphysema in July 1994. She was commenced on ICZ (200mg twice daily) 4
months later for its CsA sparing action but in August 1995 this antifungal agent was withdrawn because it was thought to be contributing to adverse effects including ankle oedema and hypokalaemia. After a period of 2 weeks without this known CsA sparing agent, DTZ was commenced both for its antihypertensive activity and its potential for minimising nephrotoxicity. Pharmacokinetic studies were conducted on 3 occasions, first when the patient was taking ICZ, second when not taking any CYP3A4 modifying drug and third when taking DTZ in a dose of 240mg given as the controlled (CD) diffusion formulation.

On the day of the first study, 200mg ICZ (Sporanox®, Janssen-Cilag, Australia) was given in the morning with CsA (Sandimmun®, Novartis, formerly Sandoz Australia) and 13 serial blood samples were taken from an indwelling catheter. Sample times were 0 (pre dose) and at times 1, 2, 3, 4, 6 and 12 hours after both the morning and evening CsA doses. This allowed the estimations of the area under CsA concentration vs time curve for the 24 hour period (AUC(0-24)) spanning two doses of CsA. AUCs were calculated using the log-linear trapezoidal rule (Appendix 4). Patency of the catheter (dead space 0.3mL) was maintained by instilling 1.5mL of heparinised saline (15 units heparin) following collection of each 7mL blood sample. The first 2mL of blood was discarded to prevent contamination by the heparinised saline. Samples were immediately refrigerated (0°C - 4°C) before transporting to the laboratory where they were frozen prior to assay. Because DMDTZ has been shown to be unstable at -20°C (Bonnefous JL, et al. 1992), samples for DTZ analysis were stored at -80°C.

Similar kinetic studies were performed 2 weeks later when the patient was receiving no CsA sparing agents and finally 8 weeks later when stabilised on an extended release formulation of DTZ (Cardizem CD® ICI Australia). DTZ was commenced on the morning after the second study day at a dose of 180mg (CD formulation) each morning
and increased to 240mg (because of inadequate control of hypertension) 11 days later and 6 weeks before the final study day. Each DTZ dose was taken in the morning with the corresponding CsA dose. As discussed in Chapter 5, this formulation is not a true 'sustained release' formulation of DTZ but one where the manufacturer claims that approximately 40% of the drug is released in the first 12 hours and the remaining 60% over the second 12 hour period.

Whole blood CsA concentrations were measured by enzyme immunoassay (EMIT®, Behring/Syva, San Jose, California) which is relatively specific for parent CsA (Steimer W. 1999). Intra- and inter-assay CVs ranged from 6.6 to 13.3% at CsA concentrations of 78µg/L and 360µg/L (Morris RG, et al. 1992). DTZ concentrations were determined by an HPLC assay described in Chapter 7. ICZ concentrations were measured by a previously reported HPLC method (Badcock NR. 1990).

6.3 Results
Apart from CsA, other ‘baseline’ drugs consumed throughout the study (indication) included prednisolone and azathioprine (to inhibit rejection), acyclovir and co-trimoxazole (prophylaxis against herpes virus/pneumocystis carinii), prazosin and captopril (hypertension), omeprazole (gastric reflux), calcium carbonate (prophylaxis for osteoporosis), glipizide (hyperglycaemia), betaxolol eye drops (glaucoma), frusemide (oedema), budesonide (airway inflammation) and salbutamol (bronchodilatation). During study day 1 amoxycillin (500mg three times a day) was also taken, during study day 2, paracetamol (1g four times a day) and spironolactone (50mg twice daily) were taken and during study day 3, ceftriaxone (1g once a day) was taken in addition to the baseline drugs.
Table 6.1 shows the relevant pharmacokinetic variables for the three 24 hour studies. Study 2 (when no CsA sparing agents prescribed) was taken as the baseline. Because CsA dosage adjustments were made to maintain the blood CsA concentration within the therapeutic range, AUCs were dose-normalised for the first study by multiplying by the ratio of daily CsA dose. The CsA AUC(0-24) increased by 2.78 times when ICZ was used concurrently which, if the kinetics were linear corresponds to a decrease in daily CsA dose required of 64% (if dose x is required to give an AUC of Y, then dose of 0.36x is required to give an AUC of 2.78Y). The CsA AUC(0-24) fell when DTZ was used concurrently which, using the same assumption, corresponds to an increase in CsA dose required of 14%). Average trough CsA concentrations (evening and following morning) showed a similar effect - when ICZ was prescribed there was a 440% increase and after stabilisation on DTZ, a 17% fall occurred.

Table 6.1 Cyclosporin pharmacokinetic parameters in a single lung transplant recipient studied on 3 occasions

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs and doses</td>
<td>CsA (50mg twice daily) &amp; ICZ (200mg twice daily)</td>
<td>CsA (100mg twice daily)</td>
<td>CsA (100mg twice daily) &amp; DTZ (240mg 'CD' in the morning)</td>
</tr>
<tr>
<td>AUC(0-24) μg.h/L</td>
<td>8159</td>
<td>5863</td>
<td>5069</td>
</tr>
<tr>
<td>Dose normalised AUC(0-24) ratio</td>
<td>2.78</td>
<td>1</td>
<td>0.86</td>
</tr>
<tr>
<td>Average CsA trough conc (μg/L)</td>
<td>162</td>
<td>73</td>
<td>61</td>
</tr>
</tbody>
</table>
Plasma DTZ concentrations are presented in Fig 6.1. DTZ AUC(0-24) was lower than expected from data on kidney transplant recipients discussed in Chapter 7.

DTZ AUC(0-24) following the use of the CD formulation (240mg taken with the morning dose of CsA) was 1617μg.h/L. The DTZ concentration-time profile produced values for AUC(0-12) of 1052μg.h/L, AUC(12-24) of 564μg.h/L and hence a ratio of am to pm AUCs of 1.87.
ICZ concentrations are presented in Figure 6.2. These are consistent with significant absorption and concentrations were above those recommended for effective antifungal activity (Cheymol G & Poirier JM. 1999. Begg EJ, et al. 1999).

Figure 6.2 Itraconazole plasma concentration-time curve following administration of 200mg dose (at steady state) given in the morning 4h before the CsA dose.

6.4 Discussion:
This study was conducted in only one individual lung transplant recipient and hence conclusions drawn from it cannot be extrapolated to other lung transplant recipients. Nonetheless, this study has demonstrated that ICZ is a potent CsA-sparing drug in at least some individuals, increasing CsA AUC(0-24) by a factor of 2.78 in this lung transplant recipient, which is consistent with a dose reduction of 64%. Data in the
literature regarding interactions between ICZ and CYP enzymes is conflicting. Case reports attesting to a sparing action of this magnitude have been published regarding a heart transplant recipient (Trenk D, et al. 1987.) and a kidney transplant recipient (Kwan JTC, et al. 1987) but there have also been clinical reports of no interaction occurring between ICZ and CsA (Novakova I, et al. 1987). In this latter report, CsA blood concentrations and daily doses (mg/kg/day) from 14 bone marrow transplant recipients given ICZ for a therapeutic indication were compared to those same data from 20 similar patients who were not given ICZ. The lack of difference between these two populations in that report may be explained by the use of parenteral CsA (since the interaction between CsA and ICZ may occur principally in the intestine) and/or the non-specific radioimmunoassay used which cross reacts with metabolites of CsA. In another early report (Shaw MA, et al. 1987) where interactions between ICZ and other drugs were examined in rats, the authors concluded that the interaction between CsA and ICZ was unlikely to be via the liver cytochrome P450 enzyme system but may be via an increase in absorption or change in distribution. In another, in vitro study, using human liver microsomes (Back DJ & Tjia JF. 1991), ICZ was noted to inhibit CsA’s metabolism but to a much lesser extent than the structurally related drug, KCZ. In a more recent report, ICZ was shown to increase circulating concentrations of terfenadine, a pro-drug that is normally completely converted to its active metabolite by intestinal CYP3A4 (Kvisto KT, et al. 1994). One potential outcome of this inhibition of intestinal CYP3A4 is cardiotoxicity (prolongation of QT interval) resulting from parent terfenadine.

The increase in CsA dose requirement when on DTZ (study 3) relative to baseline (study 2) suggest that DTZ reduced CsA AUC - the opposite effect of that anticipated and widely reported. There are alternative explanations however. The first is that these data merely reflect the considerable intrapatient variability in CsA kinetics observed in Chapters 4 and 5 and widely reported previously (Ohlman S, et al. 1993. Grevel J. 1986.
Gupta SK, et al. 1990. Kahan BD, et al 1995b). Another explanation is that the apparent decrease in dose normalised AUC(0-24) may have resulted from a 'carry-over' effect from the first study day when the patient was receiving ICZ. If the time course of the interaction between CsA and ICZ was such that blood CsA concentrations had not fallen to their new steady state, they would be artificially elevated during study 2 and hence any CsA-sparing effect afforded by the use of DTZ on the third study day would have been masked. ICZ had a half-life of 15.7h in this study which is consistent with data reported in the literature (21±6h - Goodman & Gilman, 1996) and hence the 2 week washout period between study days 1 and 2 should have been sufficient to effectively clear the drug from the bloodstream, allow regeneration of enzyme and a new steady state to be achieved. There is little data in the literature on the time required for the pharmacokinetic interaction between CsA and the various sparing agents to develop, but observations in a single heart transplant recipient given ICZ suggested that the interaction with this agent may not reach its peak for several weeks and may take > 4 weeks to return to baseline following cessation of ICZ (Trenk D, et al. 1987). This finding must be interpreted with caution because a polyclonal assay method was used for CsA monitoring and hence there is the potential for significant cross-reactivity with metabolites of CsA and the single patient studied. In Chapter 4, it was demonstrated that the duration of CsA-sparing effect of DTZ exceeded that which would be expected merely from its half-life when a single morning dose of conventional release DTZ affected CsA kinetics over a 24h period. Limited data are presented in Chapter 8 which suggest that the interaction between DTZ and tacrolimus is stable by the end of 2 weeks. If these data are accurate, the interaction between DTZ and both CsA and TRM should follow a similar time course since they are both thought to occur via CYP3A4 (or P-gp). The interaction with ICZ may take longer to develop and longer to abate (after discontinuation of ICZ) however and hence the possibility remains that the 2 week
interval between studies 1 and 2 in the present study was not sufficient for the effect of ICZ to be completely reversed.

On the first study day a lower CsA dose was used in order to maintain blood CsA concentrations within the therapeutic range accepted by this hospital. This effect of ICZ and DTZ on CsA’s kinetics was anticipated, and the respective CsA kinetic data used for calculations (AUCs and trough concentrations) were adjusted proportionately to ‘normalise’ the data for dose. As noted in Chapter 4, this simple method of normalisation is widely applied but presumes drug kinetics to be linear over the dose range in question. There is some evidence to suggest that this may not apply for the Sandimmun® formulation of CsA, especially when other drugs are coprescribed. Sandimmun® absorption kinetics over the dose range used are noted by the manufacturer to be linear when plasma was the matrix studied but non-linear when whole blood was the matrix (Sandimmun® product information, Sandoz). Absorption has been noted to be best described as first order and zero order by different authors (Grevel J. 1986) and one report (where the oral solution formulation of CsA was used) describes three different patterns of absorption in relatively small population of 42 children (Jacqz-Agrain E, et al. 1994). CsA-sparing agents are thought to exert much of their sparing activity via intestinal CYP3A4 (and/or P-gp) and hence to increase the extent of CsA’s absorption. DTZ thus complicates the absorption process and may thus affect the linearity of the relationship between CsA dose and blood CsA concentration. There are no data on the linearity of CsA absorption when DTZ is coprescribed. Further complications in the early Sandimmun® data include the well described intrapatient variability in absorption associated with this formulation and cross reactivity of CsA metabolites with the assays used. The use of this method of normalisation of AUCs is thus a limitation to the study findings but, in the absence of a greater understanding and
given the clinical imperative of maintaining blood CsA concentrations within the therapeutic range, it was unavoidable.

The lack of observed CsA-sparing effect in this patient even at the higher DTZ dose (240mg) than usually used in Australia (Chapter 2) could not be explained by other drug interactions. Drugs taken on each study day (in addition to baseline drugs taken on all study days) are noted in the Results section. None of these drugs are thought to interact with CYP3A4 and/or P-gp and hence to interact pharmacokinetically with CsA.

One potential explanation for the lack of effect by DTZ on CsA AUC(0-24) was the lower than anticipated DTZ AUC(0-24) following the 240mg dose (CD formulation) in this lung transplant recipient. Despite using a 30% higher dose (240mg/d vs 180mg/d), the DTZ AUC(0-24) in the current study was lower than the mean value (2894μg.h/L) obtained from the 7 kidney transplant recipients given DTZ (180mg/d) in the study described in Chapter 5. This value was also lower than any individual value obtained from the kidney transplant group. At the same daily DTZ dose (180mg/d as CD formulation), mean DTZ AUC(0-12) observed in the 7 stable kidney transplant recipients presented in Chapter 5 (1576μg.h/L) was higher than the DTZ AUC(0-12) (1052μg.h/L) observed in the current patient. The difference in DTZ AUC(12-24) was larger. Mean DTZ AUC(12-24) observed in Chapter 5 (1318μg.h/L) was higher than the value obtained from the current patient (564μg.h/L). DTZ AUC(0-12), AUC(12-24) and hence AUC(0-24) from the current patient are also substantially lower than the values obtained from the sole kidney transplant recipient (patient 3) given 240mg DTZ as the CD formulation (Chapter 5). Respective values for DTZ AUC(0-12), DTZ AUC(12-24) and DTZ AUC(0-24) are 5396μg.h/L, 6235μg.h/L and 11631μg.h/L for that kidney transplant recipient. Nonetheless, DTZ AUC values observed in the current patient are higher than the AUC values obtained from one kidney transplant patient (number 7)
when taking 180mg/d DTZ as the conventional release tablet (Chapter 5). In this patient, DTZ AUC(0-12), AUC(12-24) and hence AUC(0-24) were 146µg.h/L, 110µg.h/L and 256µg.h/L. Despite these low DTZ AUC values, this kidney transplant recipient experienced a modest 39% increase over baseline CsA AUC(0-24) (Chapter 4) at the 180mg/day dose (conventional release formulation). Switching to the CD formulation of DTZ (at 180mg/d) resulted in a further 9% increase in CsA AUC(0-24) but a much larger increase in DTZ AUC(0-24) (713%) (Chapter 5). The lower DTZ AUC data is probably not the reason behind the lack of a significant CsA-sparing effect in the current patient.

The shape of the DTZ concentration-time profile (Fig 6.1) was unexpected from a formulation that its manufacturer’s claim releases DTZ in two phases, 40% being released in the first 12 hours and the remaining 60% in the following 12 hours. While not being a typical ‘sustained release’ formulation, the peak exhibited in the first few hours was unexpected and this resulted in a DTZ AUC(0-12) of 1052µg.h/L while the DTZ AUC(12-24) was 564µg.h/L and hence a ratio of 1.87 (not the 0.67 expected). A similar ratio (1.20) of am to pm DTZ AUCs was also noted in the study presented in Chapter 5.

As noted in Chapter 5 and further discussed in Chapter 7, CsA is an inhibitor of CYP3A4 and has been shown to be a felodipine-sparing agent. Since the site of the interaction is thought to be in the upper intestine, the reason for this unexpected peak in DTZ during the first 12 hours may result from CsA inhibiting intestinal CYP3A4 (or P-gp). Since the CD formulation of DTZ would be expected to release its second portion at a lower intestinal site, the evening dose of CsA would not be physically present at this intestinal site when DTZ was being released and hence might not increase the absorption. Since this is a single observation, this explanation is speculative and given the failure of the
180mg CD formulation of DTZ to perform as expected in Chapter 5, it is probable that this unexpected profile in the current study is a similar failure to perform as expected.

The results of this study support the conclusion from Chapter 4 that it is important not just to presume, but to demonstrate that a benefit exists when DTZ is prescribed solely for its CsA-sparing effect. This can be achieved either by monitoring the full CsA AUC(0-24) or more easily, by single (usually trough) monitoring both before and after the introduction of the CsA-sparing agent. The change in CsA AUC(0-24) observed in this study was numerically less than the change in trough blood CsA concentration (278% vs 440% increase for ICZ - Table 6.1). This was also a finding of the DTZ dose-escalation study described in Chapter 4. As noted there, it is assumed that CsA AUC(0-24) is a better guide to overall exposure and hence to therapeutic efficacy. Clinicians should thus be cautioned against reducing CsA dosage in line with CsA trough concentration data since this would result in a decrease in overall CsA exposure (as measured by CsA AUC(0-24)). One of the ways that this could be handled in the busy clinic is to adopt a higher therapeutic (trough concentration) range for CsA when CsA-sparing agents are used. However, as noted in Chapter 3, there is a marked lack of concordance with respect to CsA therapeutic ranges within the Australasian transplant community and this does not seem to have translated into any discernible effect on either therapeutic benefit or toxicity. It would thus appear that modifications to existing CsA therapeutic ranges to account for the use of CsA-sparing agents are attempting to bring a degree of finesse to a situation which does not warrant it.

While a similar lack of effect has not been reported for any other CsA-sparing agent, it is likely that not all patients will benefit from the use of all agents. It would therefore seem prudent to stabilise all patients on CsA before introducing any sparing agent and to prove that a sparing effect is occurring by monitoring the effect on blood CsA concentrations.
Similar caution should also be observed when changing brands or formulations of either CsA or sparing agent. In Chapter 5, the effects of changing formulation of DTZ from conventional to controlled delivery formulation were presented. Despite using the same daily dose of DTZ, switching to the CD formulation of DTZ resulted in clinically significant changes to CsA AUC for some transplant recipients. Another example of a formulation change which might result in alterations to CsA-sparing effect is the newer, microemulsion formulation of CsA (Neoral®, Novartis, formerly Sandoz Australia). Since CsA is absorbed primarily from the upper gastrointestinal tract (Drewe J, et al. 1992) and there is evidence that the interaction between CsA and CsA-sparing agents occurs at this site (Kolars JC, et al. 1991b. Watkins PB. 1992. Wu CY, et al. 1995), altering the release characteristics of CsA or the sparing agent might affect the extent of interaction. Indeed, there has been one report of an altered CsA-sparing effect resulting from a change in brand of DTZ (Cooke CE. 1994). Data in this 'letter to the editor' were limited and, despite attempts to contact the author, the brands used are not known, adequacy of compliance and whether other drugs had been started or stopped are also not known. Hence the veracity of this claim remains to be confirmed.

There are serious ethical concerns surrounding the use of a drug for a purely economic purpose, but there can be no justification for the use of a drug if, in addition to having no clinical benefit, it also fails to provide an economic benefit.
Chapter 7

Diltiazem disposition and metabolism in kidney transplant recipients receiving cyclosporin

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7.1 Introduction


CsA is metabolised via the cytochrome P450 isoenzyme, CYP3A4 and DTZ has been noted to reduce the extent of this metabolism with the consequence that blood CsA concentrations rise (Kronbach T, et al. 1988. Pichard L, et al. 1990). The CYP3A4 isoenzyme occurs both in the liver and in enterocytes, where it is the predominant CYP enzyme of the proximal intestine with activity approaching one-third that of hepatocytes (Krishna DR & Klotz U. 1994. Kolars JC, et al. 1992. Hoppu K, et al. 1991. Tjia JF, et al. 1991. Peters WH & Kremer PG. 1989. de Waziers I, et al. 1990. Watkins PB. 1992). In addition, as noted in Chapter 1, P-gp has also been implicated as an alternative interacting species. Interestingly, P-gp is also located in the upper intestine and DTZ has been shown to reduce the activity of this 'drug efflux pump', reinforcing the importance of the upper intestine as the site of the interaction between DTZ and CsA.

In Chapter 2 the widespread use of DTZ as CsA-sparing in Australasia was described. Significant economic benefits have been shown to result from this coprescription since the dosage of CsA can be reduced by approximately 35% (Chrysostomou A, et al. 1993. Tortorice KL, et al. 1990. Patton PR, et al. 1994. McLachlan A & Tett S. 1998. Leibbrandt DM & Day RO. 1992). In the dose-response study described in Chapter 4, these savings were confirmed (albeit with great interpatient variability) and in Chapter 2,
they were estimated to be $7 million in 1995/6. In addition, several authors have noted that DTZ exerts beneficial therapeutic effects in kidney transplant recipients (Epstein M. 1992. Morales JM, et al. 1994. Chrysostomou A, et al. 1993. Neumayer HH & Wagner K. 1986. Oppenheimer F, et al. 1992). These benefits were confirmed in Australasian kidney transplant recipients in the study described in Chapter 3. In addition to these benefits to kidney transplant recipients, other therapeutic benefits have been attributed directly to the use of DTZ including reduced coronary artery atherosclerosis in heart transplant recipients (Schroeder JS, et al. 1993).

The metabolism of DTZ is complex. It has been shown to be converted to 3 major metabolites, N-demethyl-diltiazem (DMDTZ), deacetyl-diltiazem (DADTZ), and demethyl-deacetyl-diltiazem (DMDADTZ) (Yeung PKF, et al. 1990. Yeung PKF, et al. 1993). Whilst most attention to date has focussed on the effect that DTZ has on CsA's metabolism, there has been little attention on the effect that CsA might have on DTZ's metabolism. It was speculated in Chapter 5 that the reduced DTZ AUC exhibited by one patient after switching from conventional release to CD formulation DTZ might have been as a consequence of a dose reduction in CsA that was necessitated by the study. Both CsA and DTZ are metabolised by CYP3A4 and both drugs have been shown to antagonise the action of this important drug metabolising enzyme. CsA has been shown to inhibit the metabolism of midazolam in vitro although this effect could not be demonstrated in vivo (Treiber GLG, et al. 1990). The design of this study did not allow volunteers (kidney transplant recipients taking CsA to prevent rejection) to act as their own controls but instead, data was compared to historic kinetic data from 'normal' individuals. There were few subjects (n=9) in this study and large variability in midazolam clearance (mean 414mL/min, range 218-556) was reported. It is thus possible that CsA did affect midazolam's clearance but the trial did not have the necessary power to identify the fact. CsA has also been shown to elevate blood
concentrations of another calcium channel blocking drug, felodipine (Madsen JK, et al. 1996) (Cmax increased by 151% and AUC by 58%) in a single dose study involving 12 volunteers.

7.2 Aims:
The principal aim of this study is to examine the effect on DTZ’s kinetics by giving increasing doses of DTZ to 8 stable kidney transplant recipients maintained on CsA. A secondary aim is to compare the effect on DTZ’s kinetics of changing the formulation of DTZ from ‘conventional release’ (in a dose of 90mg twice daily) with the ‘controlled diffusion’ formulation (in a dose of 180mg given in the morning).

7.3 Methods
This study was conducted in tandem with the dose-response study discussed in Chapter 4. Eight stable kidney transplant recipients (patient details are given in Table 4.1) consented to take part in this study which was approved by the Hospital’s Ethics of Human Research Committee (Appendix 3). Inclusion criteria (detailed in Chapter 4) included stable trough blood CsA concentrations on routine monitoring for ≥3 month, stable renal function for ≥3 months with plasma creatinine ≤180μmol/L. Exclusion criteria are also described in Chapter 4 and included use, throughout the study period, of other medications known to interfere with either CsA’s or DTZ’s metabolism or the use of drugs whose metabolism might be affected by DTZ to the patient’s detriment (eg. terfenadine).

Patients were studied before the introduction of DTZ and after a minimum interval of 2 weeks at each DTZ dosage level. DTZ doses employed were 0, 10, 20, 30, 60mg taken
with the morning CsA (Sandimmun® capsules, Novartis - formerly Sandoz - Australia) dose followed by 60 and 90mg taken twice daily (with respective CsA doses) and finally 180mg CD formulation of DTZ (Cardizem CD®, ICI, Australia) given with the morning CsA dose. DTZ doses <30mg/day were taken as 10mg capsules made by the Hospital’s Pharmacy Department and assayed by the HPLC assay outlined below. Doses ≥30mg were taken as conventional release 60mg tablets (Cardizem®, ICI, Australia). CsA doses were taken at 12 hourly intervals and at least 1h away from food. On each study day, thirteen blood samples were drawn from an indwelling venous catheter (kept patent with heparinised saline, 15units/1.5mL) over a 24h period. Sample times were 0 (pre-dose) and at 1, 2, 3, 4, 6 and 12hr after both morning and evening doses of CsA which allowed the calculation of both morning and evening AUCs for CsA and DTZ. Heparinised samples for DTZ assays were collected and refrigerated (0-4°C) before transfer to the laboratory where the plasma fraction was separated by centrifugation at 4°C and subsequently stored at -80°C until analysed, because DMDTZ has been shown to deteriorate at -20°C (Bonnefous JL, et al. 1992). Plasma DTZ and each of the metabolite concentrations were determined using a specific reverse-phase HPLC-UV method as follows:

7.3.1 Stock Solutions

Stock solutions (100mg base/L in glass distilled deionised water) were prepared for DTZ, DADTZ, DMDTZ (fumarate salt) and DMDADTZ (HMR, formerly Marion Merrell Dow Research Institute, Cincinnati, Ohio, USA). These solutions were serially diluted to provide solutions of 10mg/L and 1mg/L. A stock solution of desipramine hydrochloride (Novartis, formerly Ciba Geigy Ltd, Sydney, Australia) was prepared in the same way and used as the internal standard. Solutions were stored at -20°C between assays. Solvents for use in the HPLC analysis were of analytic reagent grade (BDH
Laboratory Supplies, Poole, England) and phosphate buffer was Univar grade (Ajax Chemicals, Auburn, NSW, Australia).

7.3.2 Plasma Extraction
Calibration standards were prepared in 1.0mL human plasma which had been shown to be free of interference in the chromatographic system. Concentrations of DTZ, DADTZ, DMDTZ and DMDADTZ used for the preparation of standard curves were 10, 50, 100, 250, 500 and 750µg/L. Patient samples (1.0mL) and quality control samples were aliquoted into 15mL screw capped extraction tubes in parallel with the calibration curve samples. Each tube was spiked with 50µL internal standard, basified with 100µL Na₂HPO₄ (50mM, pH=7.5) and briefly vortex mixed. Diethylether (5mL) was added before capping and shaking horizontally for 20min at 100rpm. Phases were separated by centrifugation (3000rpm for 10min) followed by snap freezing in a dry ice/ethanol bath. The organic phase was decanted into a conical tube containing 100µL hydrochloric acid (50mM) and vortex mixed for 1min. Phases were once again separated by snap freezing in an ethanol/dry ice bath and the ether phase was discarded. The acidified extract was transferred to autosampler tubes and 50µL injected into the HPLC system.

7.3.3 Chromatography
Reverse phase chromatography was performed isocratically using a mobile phase comprising acetonitrile and Na₂HPO₄ (40mM, pH=5.5) in a ratio of 1:3. This was pumped at 1.0mL/min (Spectra Physics P4000) through a 5µm reverse phase column (Lichrocart, RP-SelectB, 10cm x 4mm id, part #50829, E.Merck, Darmstadt, Germany). The column was maintained at 40°C and the eluted substances detected by UV absorption at 215nm (Spectra Physics, model AS2000). Aliquots of 50µL of extracted
samples were injected using an autosampler (Spectra Physics, model AS3000) and quantification provided by software data system (SpectraSystem ver 1.2, Spectra Physics).

7.3.4 Analytical and Statistical considerations
Assay performance (precision and accuracy) was assessed by extracting replicates of plasma containing DTZ and its three metabolites in concentrations of 2.5, 10 and 100µg/L within a single run (n=6) and at 75 and 350µg/L between runs (n=6). The robustness of the assay was assessed by application to samples from 11 non-selected kidney transplant recipients who were taking DTZ along with a variety of other drugs (including aspirin, atenolol, azathioprine, cephalexin, CsA, frusemide, insulin, nifedipine, prazosin, prednisolone, ranitidine and/or sorbide nitrate). These patient samples were received by the laboratory for routine therapeutic drug monitoring of other drugs (usually CsA) and where DTZ was noted as being taken as part of 'other drug therapy' on the assay request form.

The area under the 24h plasma concentration versus time curves, AUC(0-24), for DTZ and 3 metabolites for each of the 7 DTZ dosages were determined using the log trapezoidal method (Appendix 4).

The protocol allowed for dosage reductions of CsA (to reduce the potential for toxicity) and aimed at maintaining trough blood CsA concentrations within the therapeutic range adopted by our laboratory (80-250µg/L). This was planned since increasing DTZ doses were expected to increase blood CsA concentrations. In practice, only 2 patients required reductions in CsA dosage because their blood CsA concentrations approached or exceeded this therapeutic range. Patient 4 had the daily CsA dosage reduced from 175mg twice daily to 150mg twice daily following study 6 and patient 5 had two such
reductions from 125mg twice daily to 100mg twice daily after study 6 and to 75mg twice daily after study 7.

7.4 Results
Inter and intra assay CVs for parent DTZ and its 3 metabolites were ≤11.3% and ≤3.1% at concentrations of 2.5 and 100μg/L respectively (Morris RG, et al. 1996).

In patients 1 to 6, parent DTZ and metabolite AUC(0-24) profiles were similar, the order of magnitude being: DTZ > DMDTZ > DADTZ > DMDADTZ for all DTZ doses studied. Individual data are shown in Tables 7.2 – 7.5 and mean data are presented in Figure 7.1. Patients 7 and 8 displayed different profiles. In patient 7, DTZ AUC(0-24) was substantially lower than the corresponding values obtained from patients 1-6 throughout the entire DTZ dosage used (Table 7.2 – 7.5, Figure 7.2). This patient’s DTZ metabolite AUCs exceeded parent DTZ AUC and, despite being lower than the grouped data (n=6), they were similarly ranked (i.e. DMDTZ>DADTZ>DMDADTZ).

Patient 8 displayed a different profile with a consistently high AUC(0-24) for the DMDADTZ metabolite which was followed in order by DTZ, DADTZ and DMDTZ (Table 7.2 – 7.5, Figure 7.3). The DTZ AUC(0-24) was similar in magnitude to that of patients 1 to 6.
Table 7.2. Individual and mean (n=6) AUC(0-24) data for DTZ over the DTZ dosage range 0-180mg (conventional release formulation)

<table>
<thead>
<tr>
<th>DTZ dose (mg/d)</th>
<th>Pt 1</th>
<th>Pt 2</th>
<th>Pt 3</th>
<th>Pt 4</th>
<th>Pt 5</th>
<th>Pt 6</th>
<th>Pt 7</th>
<th>Pt 8</th>
<th>Mean (pts 1-6)</th>
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<td>0</td>
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<td>0</td>
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<tr>
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<td>152</td>
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<td>162</td>
<td>182</td>
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<tr>
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<td>336</td>
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<td>344</td>
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<td>771</td>
<td>349</td>
<td>968</td>
<td>852</td>
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<td>1205</td>
<td>1289</td>
<td>1548</td>
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<td>913</td>
<td>1657</td>
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<tr>
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<td>3123</td>
<td>1754</td>
<td>3618</td>
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Table 7.3. Individual and mean (n=6) AUC(0-24) data for DMDTZ over the DTZ dosage range 0-180mg (conventional release formulation)

<table>
<thead>
<tr>
<th>DTZ dose (mg/d)</th>
<th>Pt 1</th>
<th>Pt 2</th>
<th>Pt 3</th>
<th>Pt 4</th>
<th>Pt 5</th>
<th>Pt 6</th>
<th>Pt 7</th>
<th>Pt 8</th>
<th>Mean (pts 1-6)</th>
</tr>
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<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>N/A</td>
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<td>28.0</td>
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<td>13.8</td>
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<td>148</td>
<td>111</td>
<td>112</td>
<td>180</td>
<td>206</td>
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<td>87.2</td>
<td>160</td>
</tr>
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<td>201</td>
<td>198</td>
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<td>1370</td>
<td>913</td>
<td>1321</td>
<td>1402</td>
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</tbody>
</table>
Table 7.4. Individual and mean (n=6) AUC(0-24) data for DADTZ over the DTZ dosage range 0-180mg (conventional release formulation)

<table>
<thead>
<tr>
<th>DTZ dose (mg/d)</th>
<th>DADTZ AUC(0-24) μg.h/L</th>
<th>Mean (pts 1-6)</th>
</tr>
</thead>
<tbody>
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<td>0</td>
<td>0 0 0 0 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>N/A 13.7 30.1 16.5 31.7</td>
<td>75.0</td>
</tr>
<tr>
<td>20</td>
<td>77.1 83.6 55.3 33.2 80.1</td>
<td>173 84.8</td>
</tr>
<tr>
<td>30</td>
<td>73.3 66.5 117.6 49.6 219</td>
<td>238 121.4</td>
</tr>
<tr>
<td>60</td>
<td>202 194 156 145 416 336</td>
<td>581 241</td>
</tr>
<tr>
<td>120</td>
<td>474 412 295 557 1268 573</td>
<td>1543 596</td>
</tr>
<tr>
<td>180</td>
<td>642 557 660 399 2029 877</td>
<td>2820 861</td>
</tr>
</tbody>
</table>

Table 7.5. Individual and mean (n=6) AUC(0-24) data for DMDADTZ over the DTZ dosage range 0-180mg (conventional release formulation)

<table>
<thead>
<tr>
<th>DTZ dose (mg/d)</th>
<th>DMDADTZ AUC(0-24) μg.h/L</th>
<th>Mean (pts 1-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 0 0 0 0 0 0 0</td>
<td>0</td>
</tr>
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<td>9.67</td>
</tr>
<tr>
<td>20</td>
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<td>626 38.9</td>
</tr>
<tr>
<td>30</td>
<td>53.2 34.8 56.9 27.1 182</td>
<td>964 83.3</td>
</tr>
<tr>
<td>60</td>
<td>138 89.7 106 268 328 72.7</td>
<td>1964 167</td>
</tr>
<tr>
<td>120</td>
<td>377 214 183 404 917 251</td>
<td>5230 391</td>
</tr>
<tr>
<td>180</td>
<td>563 241 355 241 1687 447</td>
<td>8792 589</td>
</tr>
</tbody>
</table>
Mean AUC data DTZ and metabolites (n=6)

Figure 7.1. Mean (n=6) AUC(0-24) data for DTZ, DMDTZ, DADTZ and DMDADTZ over the DTZ dosage range 0-180mg (conventional release formulation).

AUC DATA, patient 7

Figure 7.2. AUC(0-24) data for DTZ, DMDTZ, DADTZ and DMDADTZ over the DTZ dosage range 0-180mg (conventional release formulation) for Patient 7.
CHAPTER 7

Figure 7.3. AUC(0-24) data for DTZ, DMDTZ, DADTZ and DMDADTZ over the DTZ dosage range 0-180mg (conventional release formulation) for Patient 8.

For patients 1-6, the relationship between DTZ dose and AUC(0-24) was linear over the dosage range 0-180mg/day (conventional release formulation) as shown in Figure 7.4. DTZ metabolite AUC(0-24) data were also linear over the same DTZ dosage range (Figs 7.5-7.7)
Figure 7.4. Mean (n=6) DTZ AUC(0-24) (μg.h/L) over the DTZ dose range 0-180mg. 
$r^2=0.9981$

Figure 7.5. Mean (n=6) DMDTZ AUC(0-24) (μg.h/L) over the DTZ dose range 0-180mg.  
$r^2=0.9988$
Figure 7.6. Mean (n=6) DADTZ AUC(0-24) (µg.h/L) over the DTZ dose range 0-180mg. \( r^2=0.9963 \)

Figure 7.7. Mean (n=6) DMDADTZ AUC(0-24) (µg.h/L) over the DTZ dose range 0-180mg. \( r^2=0.9975 \)
The extension study with DTZ CD formulation (180mg taken in the morning) was designed to allow comparison between conventional release DTZ formulation (90mg taken twice a day with each CsA dose) and this extended release formulation at the same daily dose. Figure 7.8 shows individual DTZ concentration-time profiles following use of this CD formulation. In addition to the general inter-patient variability in DTZ profile, 7 of the 8 patients displayed a single 'peak' with an apparent Tmax ranging from 5 to 8 hr post-dose. Only one patient (#6) demonstrated the 2 peaks anticipated from this formulation (Tmax values of approximately 6 and 18 hr).

**Figure 7.8.** Individual patient 24h plasma DTZ concentration-time profiles (n=8) following 180mg CD formulation. This shows that only one patient demonstrated the expected twin peak of diltiazem.

Figure 7.9 and Tables 5.2 - 5.4 show individual patient DTZ AUCs resulting from the use of 2 formulations of DTZ (conventional release and controlled diffusion), each at the same daily dose of 180mg. Six of the eight patients experienced a fall in DTZ AUC(0-
the mean drop (n=8) being of 33.5%. Similar falls in AUC were experienced across the day - DTZ AUC(0-12) fell by 31.9% and DTZ AUC(12-24) fell by 35.3% following the change to CD formulation at the same daily dose of DTZ.

![DTZ AUC(0-24h)](image)

**Figure 7.9.** AUC(0-24) data comparing 180mg DTZ daily (n=8) given as conventional release formulation (open bars) with CD formulation (closed bars). These data show that 6 of the 8 patients had a fall in AUC with the CD formulation.

Interestingly, patient 7 who had a substantially lower DTZ AUC throughout the dose escalation part of the study with conventional release DTZ, had a remarkable increase in AUC when switched to the CD formulation. Fig 7.9 and Tables 5.2 - 5.4 show an increase in DTZ AUC(0-12) of 640%, AUC(12-24) of 810% and AUC(0-24) of 713%. Absolute values for the DTZ AUCs with the CD formulation from patient 7 are still below the mean for the group as a whole, but the difference is much smaller than when conventional release DTZ formulation was taken.
As comparative studies using intravenous DTZ were not performed, absolute oral bioavailability could not be determined.

No adverse effects attributable to DTZ were noted throughout the study and one patient was able to discontinue atenolol (prescribed for control of hypertension) when the DTZ dose was 120mg/d.

7.5 Discussion

As well as being used widely in the treatment of hypertension and angina, DTZ is widely used as a CsA-sparing agent. As noted in Chapter 2, more than 70% of Australasian kidney transplant recipients were routinely prescribed DTZ in 1995/6. The increase in blood CsA concentrations resulting from DTZ administration (which allows a CsA dose reduction of approximately 35%) has been described by others previously (Chrysostomou A, et al. 1993. Tortorice KL, et al. 1990. Patton PR, 1994. McLachlan A & Tett S. 1998. Leibbrandt DM & Day RO. 1992) and was explored in detail in an Australian kidney transplant population presented in Chapter 4.

each study day and the situation was complicated by the coprescription of CsA, a known inhibitor of CYP3A4.

In the present study there appeared to be 3 different responses to DTZ. Patients 1-6 all had similar kinetic outcomes from the DTZ dose escalation, while patients 7 and 8 exhibited very different responses. Since the dose of CsA remained unchanged (with the exception of 2 dose reductions noted above) and at steady state (patients had been receiving CsA for years - Table 4.1), any metabolic effect from CsA should have been constant. Examination of the kinetic data for DTZ and its metabolites over the 0-180mg dose range (conventional formulation) do not reveal anything that would suggest CsA was having a variable influence on DTZ (or metabolite) kinetics. Indeed, the relationship between DTZ dose and AUCs of DTZ and its metabolites were remarkably linear with $r^2$ values >0.996 (Figs 7.5 – 7.8). Data in the literature of dose-linearity is mixed. One group of workers report clinically insignificant non-linearity in volunteers but only two dosage increments (60mg and 120mg) were used (Hoglund P & Nilsson LG. 1989). Another study demonstrated non-linear disposition in a short study (24-48h) following intravenous infusion (Dias VC, et al. 1992). One group of authors published two papers apparently from the same group (n=13) of American male volunteers and in the first publication, reported dose linearity (Kinney EL, et al. 1981) but in a subsequent publication (Zehs RF, et al. 1982) reported non-linearity! In the first publication, a regression coefficient ($r$=0.99) value was quoted when the average of 3 doses (60mg, 90mg and 120mg) and an assumed zero were used. In the second paper, this relationship was described as non-linear but a regression coefficient was not reported. It is also worth noting that although 12 volunteers took the first dose, only 4 agreed to take the 120mg dose, the remainder dropped out due to adverse effects and difficulty with venipuncture. Both papers reported substantial interpatient variability in plasma DTZ concentrations.
Patient 7 exhibited a very different DTZ profile over the dosage range 10-180mg/d (conventional release formulation). In particular, DTZ AUC(0-24) was considerably lower than the mean of the 6 ‘normals’ (ratio 0.049 at the 180mg/d dose) (Table 7.). While metabolite AUCs were also lower than the ‘normals’, the degree of difference was considerably less. At the same 180mg/d DTZ dose, the ratio of DMDTZ AUC(0-24) compared to the 6 ‘normals’ was 0.651, DADTZ AUC(0-24) ratio was 0.426 and DMDADTZ AUC(0-24) ratio was 0.407. This suggests that DTZ was metabolised to primary (and perhaps secondary metabolites) before absorption into the bloodstream.

In patient 8, the magnitude of the secondary metabolite (DMDADTZ) AUC(0-24) exceeded that of parent DTZ and primary metabolites (DMDTZ and DADTZ) across the whole DTZ dose range. AUC(0-24) for parent DTZ was marginally lower than the mean of the 6 ‘normal’ patients which suggests absorption of parent DTZ was ‘normal’ but that subsequent metabolism differed, allowing the secondary metabolite to be the dominant species. One explanation for this difference is an enhanced conversion of parent to primary and thence to secondary (DMDADTZ) metabolite. This metabolite has been noted to have a longer half life than either parent DTZ or its primary metabolites (Yeung PKF, et al. 1990. Yeung PKF, et al. 1993. Hoglund P & Nilsson LG 1989) and hence accumulation would occur. An alternative explanation is that this patient had ‘normal’ metabolism of parent and primary metabolites but a delayed clearance of secondary metabolite.

Apart from CsA therapy (common to all 8 patients), no other drugs were ingested which might account for this discrepancy (Table 4.1). Diet is also unlikely to be the explanation as the meals consumed were controlled throughout the study and it has been shown that food does not influence the bioavailability of DTZ in either conventional or slow release preparations (Du Souich P, et al. 1990), albeit in a single dose study where
the maximum daily dose was 120mg/d. Another potential confounding factor might be IL6 concentrations which, when elevated, have been shown to affect the metabolism of theophylline (Chang KC et al. 1978) and CsA (Chen YL, et al. 1994). It is unlikely that this could cause variation in DTZ metabolism in this trial since C-reactive protein (CRP - a marker of IL6 concentrations) concentrations were monitored on each study day and only two results were found to be marginally elevated (Table 4.5).

As noted in Chapter 5, the CD formulation of DTZ did not perform as anticipated from the manufacturer’s description of the formulation characteristics. The ratio of DTZ AUC(0-12) to AUC(12-24) when the CD formulation was taken (Tables 5.2 and 5.3), was 1.20, not the 0.67 (40:60) anticipated from the manufacturer’s claims. Also, with the exception of one subject, plasma concentrations of DTZ did not exhibit the dual peaks which were expected from the design of the CD formulation. Switching to the CD formulation of DTZ resulted in an AUC(0-24) which was 33.5% lower (Table 5.4) than the same dose taken as conventional release formulation. These data suggest that the CD formulation of DTZ was performing more like a ‘typical’ sustained release formulation where drug was continually released (and absorption was occurring) throughout both 12h study periods of each study day.

Because of the needs of the study population for immunosuppression, it was not possible to study the absorption characteristics of the CD formulation in the absence of CsA. As noted earlier, CsA has been shown to inhibit intestinal CYP3A4 (and/or P-gp) and thereby acts as a felodipine-sparing agent (Madsen JK, et al. 1996). It is thus possible that CsA also acts as a DTZ-sparing agent and affects the apparent performance of the CD formulation. While this was not evident with conventional formulation DTZ, this may explain some of the anomalies experienced with the CD formulation. Given the nature of gastrointestinal tract transit times, it is likely that the release of DTZ from the
CD formulation over the 12-24h period (following morning only CD dosing) would occur well below the site where the evening dose of CsA was released. If CsA inhibited intestinal CYP3A4 (and/or P-gp), DTZ AUC(0-12) should be increased more than AUC(12-24). Since the ratio of DTZ AUC(0-12) to AUC(12-24) exceeded the anticipated figure of 0.67, this may be the explanation. For this explanation to be valid however, there should be a greater fall in DTZ AUC(12-24) when switching from conventional formulation to CD formulation (because any local DTZ-sparing effect of CsA would be reduced or absent). Data from Tables 5.2 and 5.3 show that mean DTZ AUC(0-12) fell by 31.9% while mean DTZ AUC(12-24) fell by a similar value (35.3%) following the switch from conventional release DTZ given twice daily to CD formulation given in the morning only. These data therefore suggest that the presence of CsA at any particular site within the gastrointestinal tract was unimportant to DTZ’s absorption and that the ‘abnormal’ ratio of 1.2 was merely a failure of the CD formulation to perform as expected.

In patient 7, both morning and evening DTZ AUCs were similarly increased (Tables 5.2 and 5.3) following the switch to CD formulation. AUC(0-12) for DTZ increased from 146µg.h/L to 1081µg.h/L while the AUC(12-24) increased from 110µg.h/L to 1001µg.h/L. The reason for this massive increase is far from clear but one potential explanation relies on the competing effects of metabolism and/or inactivation, and absorption at specific sites within the gastrointestinal tract. The stomach provides a model of such a site where inactivation is favoured over absorption since gastric acid is highly reactive (thus favouring metabolism or inactivation) while the surface area is small (and hence the capacity for absorption is reduced). If conventional release formulation DTZ released most of the drug at this site, much would be destroyed before absorption could occur and hence blood concentrations would be relatively low. Because the CD formulation provides a slower release of DTZ (as demonstrated in Fig 7.8), most of the
DTZ (and all of that released during the 12-24h period) would escape this destruction and hence both am and pm DTZ AUCs would be increased. It should be noted that this is purely speculative however, especially since DTZ is not acid labile. A variation on this speculation is that this patient might have had an unusually high amount of intestinal CYP3A4 which was confined to a short section of upper intestine. If this section of intestine was situated such that conventional release formulation DTZ released all of its DTZ at or above this site, then abnormally high levels of metabolism might occur and systemic absorption of parent DTZ would be low. There is some support for this hypothesis in the ratio of parent DTZ to metabolites. For patients 1-6, DTZ is the predominant species (Fig 7.1) while for patient 7, DTZ is the minor species in blood (Fig 7.2). This reversal of parent to metabolite ratio would be expected if intestinal metabolism was unusually high and if these metabolites were subsequently absorbed into the bloodstream.

As noted in Chapter 5, switching from conventional to CD formulation of DTZ in this patient group had an unpredictable effect upon the CsA AUC. While mean data showed no change, individual patients experienced both increases and decreases that could well be clinically significant. The lack of correlation between change in DTZ AUC and effect on CsA AUC (mean data (n=7) showed a fall of 33.5% in DTZ AUC(0-24) versus a fall of only 10% in CsA AUC(0-24)) (Table 5.4) was most notable in patient 7 where the huge increase (713%) in DTZ AUC(0-24) was accompanied by a very modest increase (8.4%) in CsA AUC(0-24). In Chapter 6 an absence of any CsA-sparing effect (despite using a higher dose of the CD formulation) was observed in a lung transplant recipient.

There was also no obvious relationship between DTZ dose and/or blood DTZ concentrations and anti-hypertensive response. As noted earlier, atenolol was stopped in one patient due to a combined effect of low blood pressure and bradycardia. These
effects are to be expected from a combination of β-blocker and DTZ which both have negative inotropic and chronotropic effects. Interestingly, patient 7 did not experience any adverse effects following the switch from conventional to CD formulation DTZ despite the huge (713%) increase in DTZ AUC(0-24). This data supports earlier observations of large interpatient variability in plasma DTZ concentrations which suggest that antihypertensive efficacy is not related to plasma concentrations. Hypertension is a common finding in kidney transplant recipients (>70% were prescribed ≥1 antihypertensive drug at 3 months post transplant in the survey described in Chapter 3) and is a focus of treatment with one or more of the vast array of drugs currently approved to treat this problem. While earlier data (Chrysostomou A, et al. 1993) suggested that DTZ does not exert an antihypertensive effect in kidney transplant recipients, data presented in Chapter 3 demonstrates that, at DTZ doses of 180-240mg/d, DTZ does exert a clinically significant antihypertensive effect in this population.

7.6 Summary

Only small numbers of patients were enrolled in this study (n=8) but this was sufficient to describe three different patterns of DTZ handling by humans. The most common pattern was that of DTZ being the predominant species in blood followed by DMDTZ, DADTZ and DMDADTZ. One patient demonstrated very low AUCs for DTZ (and to a lesser extent for its metabolites) when conventional release DTZ was administered but ‘normal’ AUCs when CD formulation DTZ was given. The low AUCs following conventional release DTZ is difficult to explain but interestingly, did not appear to affect CsA sparing activity however. Another patient demonstrated ‘typical’ parent DTZ concentrations but markedly elevated DMDADTZ concentrations which might be as a result of a different metabolic pathway for the secondary metabolite.
Over the dosage range studied (up to 180mg/d), kinetics were linear for DTZ and its metabolites DMDTZ, DADTZ and DMDADTZ. Because all patients were taking CsA, it was not possible to determine whether CsA affected DTZ kinetics as has been demonstrated for felodipine.

The CD formulation of DTZ did not perform as expected from the manufacturer’s description of the release characteristics. There was evidence of a ‘double-dump’ profile in only 1 patient and overall AUC(0-24) fell by >30%. The explanation for this is unknown but appears to be formulation related rather than due to concurrent CsA (or other drug) administration.
Preliminary pharmacokinetic study of the dose-response relationship for the interaction between TRM and DTZ in kidney and liver transplant recipients.

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8.1 Introduction

Tacrolimus (TRM) is a powerful immunosuppressive drug that was granted marketing approval in Australia for preventing organ transplant rejection in 1991. As noted earlier, it is structurally unrelated to CsA, binds to a different intracellular receptor and was isolated from a different micro-organism from the opposite side of the world. Notwithstanding these differences, its mode of action, adverse effect profile and a number of physical and pharmacokinetic parameters are remarkably similar.

In particular, TRM is insoluble in water, has a relatively poor oral bioavailability, is metabolised by the cytochrome P450 isoenzyme CYP3A4 (Venkataramanan R, et al. 1995. Kelly PA, et al. 1995. Sattler M, et al. 1992), is nephrotoxic and it is also very expensive.

While experience with this agent is more limited than with CsA, a number of interactions have been reported with drugs that affect the CYP3A4 isoenzyme including drugs which have been demonstrated to affect blood CsA concentrations. In particular, the coprescription of KCZ has been shown to elevate blood TRM concentrations (Floren LC, et al. 1997). Evidence in the literature supporting an interaction with DTZ is conflicting. DTZ has been reported not to interact with TRM either in vitro (Christians U, et al. 1996) or in limited report (abstract only), in human liver transplant recipients (Teperman L, et al. 1996). Other reports however suggest there is an interaction. In a retrospective review of 128 kidney transplant recipients, one group of researchers noted that coprescription of DTZ with TRM in one patient required a 66% TRM dose reduction in order to keep blood TRM concentrations within acceptable limits (Katari SR, et al. 1997). DTZ and TRM have also been reported to interact in animals (Regazzi MB, et al. 1996). In this study, TRM was infused into 4 pigs and after 48h, DTZ was also infused intravenously. Blood TRM concentrations during the DTZ infusion rose
significantly (from $10.0 \pm 3.9\mu g/L$ to $42.4 \pm 10.4\mu g/L$) and mean clearance fell
(5.0L/h/kg to 1.2L/h/kg) when compared to the DTZ free period. This short report
failed to give details of the timing of samples and, although stating that TRM
concentrations were at steady-state, it is possible that some of the increase in blood
TRM concentration may have been due to failure to reach steady-state. This report is
also of interest because it suggests that the interaction occurred in the liver since both
drugs were administered by intravenous infusion.

Oral bioavailability of TRM is less affected than CsA by the presence of bile in the
gastrointestinal tract (Jain AB, et al. 1990. Spencer CM, et al. 1997) and it has therefore
found a special place in liver transplantation where bile is diverted externally in the
immediate postoperative period. As noted earlier, in these settings, CsA may need to be
given intravenously for extended periods because of poor absorption following oral

The adverse effect profile of TRM is also similar to CsA. In particular, hypertension
nephrotoxicity (Katari SR, et al. 1997) are well described. These were the principal
reasons for first investigating the use of DTZ as a CsA-sparing agent and hence there is
potential for therapeutic benefit from coprescribing DTZ (a calcium channel blocking
drug with an approved indication in hypertension) with TRM. Coprescription of DTZ
with TRM has not yet been advocated, but in the absence of any contrary evidence,
given the economic and therapeutic benefits afforded the coprescription with CsA (as
discussed in Chapter 2) and the similarity in adverse effect profile and cost, it seems
likely that it will.
Coprescription of DTZ with CsA was well established before evidence (Chapter 4) became available on the dose-response relationship for the interaction. By this time, protocols had become established and prescribers have been reluctant to alter the DTZ regimens they had become familiar with (personal communication, A/Prof T Mathew, The Queen Elizabeth Hospital, Woodville, South Australia). Therefore, it is highly desirable to have evidence that an interaction occurs and to have data on its reliability, and magnitude of the interaction, before routine coprescribing of DTZ with TRM starts.

8.2 Aims

The principal aim of this study is to determine the dose-relationship between DTZ and TRM in kidney and liver transplant recipients maintained on this immunosuppressive drug. Liver transplant recipients have been included for two reasons; firstly because few kidney transplant recipients are currently taking TRM and secondly to determine whether there are differences between these two populations, given the importance of the liver as a metabolic organ. The reason that TRM is not widely used in the kidney transplant area is not because of pharmacological concerns, but rather an economic/political one. At the time of this trial, TRM was not included in the ‘Section 100’ scheme when used for kidney transplantation (liver transplantation was the only approved indication under this scheme). Not being listed under this scheme means that individual hospitals and State governments pay for TRM when used for kidney transplantation and hence the alternative agent, CsA, is preferred since it is available free of charge to these bodies.

The second aim of the trial was to investigate the effect of DTZ formulation changes – specifically, the controlled diffusion (CD) formulation - in organ transplant recipients who demonstrated a TRM-sparing effect with conventional DTZ formulation.
8.3 Patients and Methods

Two stable kidney transplant recipients and two stable liver transplant recipients maintained on TRM (but not on DTZ) consented to take part in this pharmacokinetic study which was approved by the Ethics of Human Research Committee of The Queen Elizabeth Hospital and the Committee on Clinical Investigation, and Clinical Drug Trials Committee of the Flinders Medical Centre. Entry criteria included:

- stable blood TRM concentrations on routine monitoring for ≥3 months
- serum creatinine concentrations and liver function tests which were stable for ≥3 months and ≤150% of the upper limit of normal.

Exclusion criteria included: unstable plasma creatinine concentration, elevated liver function tests, known sensitivity to DTZ, sick sinus syndrome, hypotension, severe congestive heart failure, history of acute myocardial infarction and/or pulmonary congestion.

On each 24h study day, serial blood samples were taken from an indwelling venous catheter at times 0 (pre dose) and 1, 2, 3, 4, 6 and 12h after both morning and evening TRM doses, making a total of 13 blood samples spanning 2 TRM dosing periods. Incremental doses of DTZ were given between study days which were separated by ≥2 weeks to allow the interaction between TRM and DTZ to stabilise and steady-state blood TRM concentrations to be achieved. The sequence of DTZ doses were 0, 10, 20, 30, 60mg in the morning followed by 60mg and 90mg taken twice daily and finally 180mg CD formulation. DTZ doses less than 30mg were taken in the form of a 10mg capsule manufactured from commercially available, conventional release, tablets (Cardizem®, ICI Australia) by the Hospital's Pharmacy Department and assayed using the HPLC assay outlined in Chapter 7. Doses of ≥30mg were taken in the same form of commercially available, conventional release tablets except for the final study period when CD formulation (Cardizem CD®, ICI, Melbourne, Australia) was given. Each
DTZ dose was taken with the usual morning and/or evening TRM dose (Prograf® capsules, Janssen-Cilag Pty Ltd, Lane Cove, Australia) and food was not consumed for >1h before or after TRM dosing. TRM dose reductions were planned, if needed, to maintain blood TRM concentrations within the therapeutic range used by this hospital (5-20μg/L) and in such an event, the resulting AUC was to be dose-normalised (ie adjusted by the same factor as the dosage). Apart from DTZ, no drug which was known to interfere with TRM (or CsA) metabolism was permitted during the course of the study, nor was any drug permitted whose metabolism might have been affected by DTZ (especially terfenadine).

Patency of the venous catheter was maintained by instilling 1.5mL heparinised saline (15units heparin) into the venous catheter after each 8mL blood sample was withdrawn. To prevent contamination of sample from the heparinised saline, the first 1.5mL of blood withdrawn was discarded. Blood samples were immediately stored at 0-4°C and transferred to a freezer (-20°C) as soon as practicable (≤8 hours). Whole blood TRM concentrations were measured by microparticulate enzyme immunoassay (Tacrolimus 2, MEIA®) on the IMx analyser (Abbott Diagnostics, Chicago USA) which is relatively specific for parent TRM (Cogill JL, et al 1998). AUCs were calculated using the log trapezoidal method (Appendix 4).

TRM assays were performed after each study day to allow immediate dosage reductions and guard against clinical sequelae resulting from prolonged high TRM concentrations. Because these assays were therefore performed over a period of several months, the possibility of inter-assay variability was a potential issue, especially for an assay where the machinery does not allow manual adjustment of baseline. Consequently, three trough samples (C0, C12 and C24h) from each patient on each study day were subsequently assayed on the same day, at the completion of the study. Since this negated inter-assay
variability, where there was $\geq 10\%$ difference between the two results, all samples from that patient's study day were repeated and the new values accepted.

Plasma DTZ, demethyldiltiazem (DMDTZ), deacetyldiltiazem (DADTZ) and demethyldeacetyldiltiazem (DMDADTZ) concentrations were determined using the HPLC method described in Chapter 7.

Plasma C-reactive protein concentrations from the first blood sample on each study day were assayed by an immunonephelometric method (ARRAY 360CE, Beckman Instruments, Ca, USA).

8.3.1 Statistics
Because of the limited numbers of study participants and the potential for differing outcomes because of the nature of their transplanted organ, data are presented as individual, rather than grouped data.

8.4 Results
Patient demographics and concurrent drug therapy for the four study participants are shown in Table 8.1.
Patient 3 (liver transplant recipient) was withdrawn from the study shortly after the 120mg DTZ dose study because of the development of bradycardia, a side effect thought related to the use of DTZ. Patient 4 was withdrawn after the 90mg twice daily (conventional formulation) DTZ because there was little apparent increase in TRM AUC(0-24). Data from 29 study days were thus available for analysis.

The repeat analyses of trough blood TRM concentrations (C0, C12 and C24) from each study day were assayed on one day (using the same calibration curve) after completion of the trial. On four occasions these repeat analyses differed by ≥10% from the analysis performed after the particular study day. Three of these occurred in Patient 2 (DTZ dosages 30mg, 60mg and 120mg/day) and one occurred in Patient 3 (at the 30mg DTZ dosage). The repeat analysis values for Patient 2 were higher than the original values obtained while the repeat analysis values for Patient 3 were lower. The repeat analysis values were accepted as the most reliable because of the lesser risk of between-run analytical variability.
CHAPTER 8

The relationship between the daily dose of DTZ and change in TRM AUC(0-24), morning TRM trough concentration (24h) and mean of the two observed trough (12h and 24h) concentrations for each of the study participants are presented in Table 8.2 and Figures 8.1, 8.2 and 8.3. Data from the 2 kidney transplant recipients (Patients 1 and 2) show that an increase in TRM AUC(0-24) occurred following the 20mg dose of DTZ (26% and 67%). The maximum increase in TRM AUC(0-24) occurred at the maximum DTZ dose used (90mg twice daily) when the increase in TRM AUC(0-24) was 48% and 177%.

The response from the two liver transplant recipients (Patients 3 & 4) was different however. Table 8.2 and Figure 8.1 shows that the TRM AUC(0-24) fell below the baseline value at some lower DTZ doses and only increased above the baseline value after the 60-120mg DTZ dose.

Table 8.2. Change from baseline (%) for TRM AUC(0-24) (µg.h/L) and trough blood TRM concentrations (12h and mean of 12h and 24h) (µg/L) for the 2 kidney (Patients 1 & 2) and 2 liver (Patients 3 & 4) transplant recipients with increasing doses of DTZ.

<table>
<thead>
<tr>
<th>DTZ daily dose (mg)</th>
<th>% increase in TRM AUC(0-24)</th>
<th>% increase in TRM trough 24h</th>
<th>% increase in TRM mean trough (12 and 24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt no</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
</tr>
<tr>
<td>10</td>
<td>-7  -8  0  12</td>
<td>30  -26  6  33</td>
<td>0  -20  9  23</td>
</tr>
<tr>
<td>20</td>
<td>26  67  -17  -27</td>
<td>61  40  -37  -44</td>
<td>19  60  -17  -40</td>
</tr>
<tr>
<td>30</td>
<td>17  55  -10  -12</td>
<td>59  33  -27  -9</td>
<td>25  43  -18  -13</td>
</tr>
<tr>
<td>60</td>
<td>47  69  6  -18</td>
<td>87  56  2  19'</td>
<td>55  58  1  -9</td>
</tr>
<tr>
<td>120</td>
<td>24  103  18  1</td>
<td>43  76  15  21</td>
<td>21  85  20  15</td>
</tr>
<tr>
<td>180</td>
<td>48  177  X  22</td>
<td>92  133  X  55</td>
<td>65  157  X  28</td>
</tr>
<tr>
<td>180CD</td>
<td>53  97  X  X</td>
<td>97  77  X  X</td>
<td>58  96  X  X</td>
</tr>
</tbody>
</table>

X = patient withdrawn
Figure 8.1 Change from baseline (%) TRM AUC(0-24) (µg h/L) for the 2 kidney transplant recipients (closed square and closed diamond) and two liver transplant recipients (closed triangle and closed circle) and mean of all four values (solid line) with increasing doses of DTZ (mg/day).

Figure 8.2 Change from baseline (%) TRM trough (C24) concentration (µg/L) for the 2 kidney transplant recipients (closed square and closed diamond) and two liver transplant recipients (closed triangle and closed circle) and mean of all four values (solid line) with increasing doses of DTZ (mg/day).
CHAPTER 8

Figure 8.3 Change from baseline (%) TRM mean trough (C12 & C24) (μg/L) for the 2 kidney transplant recipients (closed square and closed diamond) and two liver transplant recipients (closed triangle and closed circle) and mean of all four values (solid line) with increasing doses of DTZ (mg/day).

Figure 8.4 Relationship between daily DTZ dose (mg/day) for doses 0-60mg and change from baseline TRM AUC(0-12) (μg.h/L) (open diamonds), TRM AUC(12-24) (μg.h/L) (open squares) and TRM AUC(0-24) (μg.h/L) (open triangles). Data presented are means for the four study subjects.
The mean change in TRM AUC(0-12), AUC(12-24) and AUC(0-24) for DTZ doses ≤60mg/day are shown in Fig 8.4. Both AUC(0-12) and AUC(12-24) contributed approximately equally to the AUC(0-24).

The relationship between daily DTZ dose and plasma DTZ AUC(0-24) are shown in Figure 8.5. The relationship between daily DTZ dose and DTZ metabolite AUC(0-24) are shown in Figures 8.6 - 8.8. These data show an approximately linear relationship between plasma concentration of DTZ and its three major metabolites over the DTZ dose range administered. This is consistent with those DTZ data from the CsA-DTZ interaction study data reported in Chapter 7.

![Figure 8.5. DTZ AUC(0-24) (μg.h/L) for increasing doses of DTZ (0-180mg/day) for two recipients of kidney transplants (closed square and closed diamond) and two recipients of liver transplants (closed triangle and closed circle) with mean of all four shown as the solid line.](image-url)
Figure 8.6. DMDTZ AUC(0-24) (μg.h/L) for increasing doses of DTZ (0-180mg/day) for two recipients of kidney transplants (closed square and closed diamond) and two recipients of liver transplants (closed triangle and closed circle) with mean of all four shown as the solid line.

Figure 8.7. DADTZ AUC(0-24) (μg.h/L) for increasing doses of DTZ (0-180mg/day) for two recipients of kidney transplants (closed square and closed diamond) and two recipients of liver transplants (closed triangle and closed circle) with mean of all four shown as the solid line.
Patient 3 (closed triangles), a liver transplant recipient exhibited a different DTZ and metabolite profile to the other 3 patients. In particular, DMDTZ and DMDADTZ concentrations were generally lower (Figures 8.6 and 8.8) while the DADTZ concentrations were higher (Figure 8.7), at the 120mg/day dose (the highest dose used). These differences were much less marked than those seen in the study with CsA (Chapter 7) however.

C-reactive protein results are given in Table 8.4. Only 1 of the 23 values exceeded the upper limit of normal for our laboratory (10mg/L).
Table 8.4 C-reactive protein concentrations for the first blood sample of each study day 
(normal value <10mg/L)

<table>
<thead>
<tr>
<th>Pt</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>180'CD'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td>&lt;1</td>
<td>NA</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
<td>NA</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2</td>
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<tr>
<td>3</td>
<td>7</td>
<td>&lt;1</td>
<td>4</td>
<td>NA</td>
<td>&lt;1</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>&lt;1</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>26</td>
<td>8</td>
<td>X</td>
</tr>
</tbody>
</table>

NA = not available. X= study not performed

On 2 occasions kidney transplant recipients inadvertently did not follow the correct DTZ and TRM dosage protocol and hence the data from these study days were not included in the primary analysis. The errors were:

- Patient 1 did not increase the DTZ dose as intended, in the interval between the 120mg and 180mg study days. Nevertheless, the study was performed on the set day, 2 weeks after the previous 120mg study and after taking the first 180mg (taken as 90mg twice daily) DTZ dose. The study was repeated 2 weeks later, after taking 180mg/day as set out in the protocol. Data was thus available from both study days (ie, day 1 and day 14 at a DTZ dose of 180mg/d).

- Patient 2 inadvertently omitted the evening TRM dose on study day 7 (DTZ dose 90mg twice daily) and hence the study was repeated 2 weeks later. The data (especially TRM AUC(0-12)) from the 2 days were available for comparison (viz day 14 and 28 for DTZ at the 180mg/d dose).

This provided an opportunity to investigate the effect of length of time taken before the TRM-sparing effect was maximal, albeit in a limited number of subjects. Comparative data for Patients 1 and 2 where either DTZ or TRM doses were not taken correctly are
shown in Table 8.5. Data from the first day is presented without brackets, data from the repeated studies (2 weeks later) are presented in brackets.

Table 8.5 Change (%) from baseline in TRM variables with day 2 data given in brackets

<table>
<thead>
<tr>
<th>Patient</th>
<th>TRM AUC (0-24)</th>
<th>TRM AUC (0-12)</th>
<th>TRM AUC (12-24)</th>
<th>TRM trough (24h)</th>
<th>TRM mean trough (12h &amp; 24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.4 (47.5)</td>
<td>20.8 (45.4)</td>
<td>43.8 (50)</td>
<td>77 (91.8)</td>
<td>47.2 (65.5)</td>
</tr>
<tr>
<td>2</td>
<td>125 (177)</td>
<td>146 (155)</td>
<td>102 (201)</td>
<td>112.8 (132.6)</td>
<td>125.9 (156.8)</td>
</tr>
</tbody>
</table>

AUC data for DTZ and its metabolites on these 2 days are presented in Table 8.6

Table 8.6 DTZ and metabolite AUC(0-24) (μg.h/L) on day 1. Day 2 data in brackets

<table>
<thead>
<tr>
<th>Patient</th>
<th>DTZ AUC(0-24)</th>
<th>DMDTZ AUC(0-24)</th>
<th>DADTZ AUC(0-24)</th>
<th>DMDADTZ AUC(0-24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1834 (2639)</td>
<td>881 (1057)</td>
<td>299 (468)</td>
<td>284 (465)</td>
</tr>
<tr>
<td>2</td>
<td>4162 (4096)</td>
<td>1563 (1371)</td>
<td>645 (627)</td>
<td>469 (439)</td>
</tr>
</tbody>
</table>

The effect of changing from 90mg DTZ (conventional release formulation) given twice daily to 180mg CD formulation given once daily is shown in Tables 8.7 and 8.8 for Patients 1 and 2.
Table 8.7. Comparison of DTZ and metabolite AUC(0-24) (µg.h/L) for DTZ given as 90mg conventional release tablet twice daily with 180mg CD given once daily data in brackets

<table>
<thead>
<tr>
<th>Patient</th>
<th>DTZ AUC(0-24)</th>
<th>DMDTZ AUC(0-24)</th>
<th>DADTZ AUC(0-24)</th>
<th>DMDADTZ AUC(0-24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2639 (1481)</td>
<td>1057 (649)</td>
<td>468 (265)</td>
<td>465 (288)</td>
</tr>
<tr>
<td>2</td>
<td>4096 (1941)</td>
<td>1371 (686)</td>
<td>627 (286)</td>
<td>439 (221)</td>
</tr>
</tbody>
</table>

Table 8.8 Comparison of change from baseline (%) TRM AUC and trough concentrations for DTZ given as conventional release tablets 90mg twice daily with 180mg CD formulation given once daily data in brackets

<table>
<thead>
<tr>
<th>Patient</th>
<th>TRM AUC (0-12)</th>
<th>TRM AUC (12-24)</th>
<th>TRM AUC (0-24)</th>
<th>TRM trough (24h)</th>
<th>TRM trough (mean 12 &amp; 24h)</th>
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<tbody>
<tr>
<td>1</td>
<td>45.4 (42.3)</td>
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<td>47.5 (53.3)</td>
<td>91.8 (96.7)</td>
<td>65.5 (57.7)</td>
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<tr>
<td>2</td>
<td>154.5 (96.7)</td>
<td>201 (96.3)</td>
<td>176.5 (96.5)</td>
<td>132.6 (76.7)</td>
<td>156.8 (96.3)</td>
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8.5 Discussion:
Since there was such variability in the data and the number of study participants available was small, most data are presented individually rather than grouped via the sigmoid Emax method used in Chapter 4.

This study supports earlier observations that DTZ significantly increases blood TRM concentrations in some organ transplant recipients. The magnitude of the TRM-sparing effect appears to vary considerably between individuals, but was of a similar magnitude to the CsA-sparing effect demonstrated in Chapter 4. At a dose of 120mg, an increase in CsA AUC(0-24) of 52% (from the sigmoid Emax curve) was observed and a 62%
increase at 180mg/day. By comparison, the mean increase in TRM AUC(0-24) at 120mg DTZ daily in the current study was 36% (n=4) and at 180mg, the mean increase in TRM AUC(0-24) was 82% (n=3). The two recipients of kidney transplants had clinically (and economically) significant increases in TRM AUC(0-24) but the two liver transplant recipients had lesser increases which did not become evident until higher doses of DTZ (≥120mg/d) were administered. The lesser TRM-sparing effect was not due to lower plasma DTZ concentrations since one of the two liver transplant recipients had higher DTZ concentrations than the two kidney transplant recipients while the other had values that were similar to those values of the kidney transplant recipients (Figure 8.5).

Why the 2 liver transplant recipients behaved differently to the 2 kidney transplant recipients is not immediately apparent. The lack of effect of DTZ on TRM dosage requirements in liver transplant recipients has been noted before (Teperman L. 1996) but transplant recipients in that study were only given 90mg DTZ daily. In addition, that study used trough TRM concentrations only and compared data from only 7 patients given DTZ with 7 not given DTZ. The failure to find an effect may thus have been due to study design or simply a type II error. Given the similarity in kinetics of DTZ and its metabolites in both kidney and liver transplant recipients (Figures 8.5-8.8), it is unlikely that liver function is the differentiating factor since both drugs are metabolised by the same enzyme and hence this would be expected to result in different DTZ kinetics.

Mean AUC(0-24) for DTZ (at 90mg twice daily dosage) was similar for patients in this study to those in the previous CsA-DTZ study (Chapter 4) (3934±1299µg.h/L vs 4403±2708µg.h/L). Mean AUC(0-24) for each of the three DTZ metabolites were also similar at this DTZ dosage (DMDTZ: 860±263µg.h/L vs 1322±372µg.h/L; DADTZ: 717±305µg.h/L vs 787±576µg.h/L; DMDADTZ: 712±450µg.h/L vs 540±495µg.h/L). These data suggest that neither TRM nor CsA have an effect on DTZ metabolism or alternatively, they both affect DTZ metabolism to a similar extent.
Patient 3 was withdrawn from the study after the 120mg/day DTZ dose because of the development of bradycardia, a well described adverse effect with this calcium channel blocking drug. At the time this patient had AUC(0-24) for DTZ, DMDTZ and DMDADTZ which were below the mean of the other three patients, but a higher value for DADTZ AUC(0-24) (872µg.h/L) than the mean for the other 3 study participants (312µg.h/L). The possibility that the DADTZ metabolite might be responsible for this cardiac adverse effect was thus considered. Since patient 4 had a higher DADTZ AUC(0-24) at the 180mg/day DTZ dose (1057µg.h/L) and, in the previous CsA/DTZ study in kidney transplant recipients (Chapter 4), 2 patients also had higher values for DADTZ AUC(0-24) (2029 and 877µg.h/L) at the same DTZ dose and none of these patients developed bradycardia, it seems unlikely that the DADTZ metabolite was responsible.

Blood pressure was well controlled in all patients throughout the study and one (liver transplant recipient) was able to discontinue one antihypertensive drug (40mg enalapril daily) when the DTZ dose was 120mg. No other adverse effects were noted.

As noted earlier, one of the reasons for interpatient (and intrapatient) variability in metabolic capacity is fluctuating levels of cytokines (especially interleukin-6) (Chen YL, et al. 1994). In the present study, C-reactive protein (a marker of IL6 activity) concentrations were within the normal range for our laboratory (<10mg/L) with the exception of 1 value (Table 8.4). This value (26mg/L seen in Patient 4 at the 120mg/day DTZ dose) is nonetheless much lower than those concentrations proven to interfere with drug metabolism (Chen YL, et al 1994) and hence it is unlikely that fluctuating interleukin-6 concentrations contributed to the interpatient variability observed in this study.
TRM is metabolised by CYP3A4 within enterocytes (Floren LC, et al. 1997. Venkataramanan R, et al. 1995. Spencer CM, et al. 1997) and hence much of the TRM-sparing effect of DTZ could result from inhibition of this, presystemic metabolism. In order to maximise this effect and to reduce the complexity of the drug regimen, DTZ was administered at the same time as TRM. DTZ concentrations in the enterocyte should therefore be near to maximal as TRM was passing this site. Interestingly, the change in morning TRM AUC(0-12) was similar to the change in evening TRM AUC(12-24) at DTZ doses given in the morning only (ie ≤60mg/day) (Figure 8.4). Since these DTZ doses were given only with the morning dose of TRM, this confirms the observation made with the earlier CsA/DTZ study (Chapter 4), that DTZ affects TRM concentrations for longer than would be predicted from the DTZ half-life in plasma.

As noted in Chapter 4, this apparent lack of importance of the timing of DTZ is of considerable clinical significance since thrice daily DTZ regimens have been commonly employed in Australasia (Chapter 2) when DTZ is used as a CsA-sparing agent. It is therefore likely that similar regimens would be employed if DTZ were advocated as a TRM-sparing drug.

Intensive blood TRM concentration monitoring required to define AUC(0-24) is inconvenient, laborious and expensive, and hence we examined the relationship between increase in trough blood TRM concentrations (both C24 and the mean of C12 and C24) and TRM AUC(0-24) at different DTZ dosages. Data from Table 8.2 show that mean changes in trough (C12 and C24) values were marginally better than mean change in C24 but both were a good approximation of mean change in TRM AUC(0-24). Data from Patient 1 however showed that change in TRM C24 was greater than the change in TRM AUC(0-24) at all dosages of DTZ. If TRM dosage adjustments were made on the basis
of the C24 value (as usually occurs when dosage adjustments are made in the clinic), this patient would have had a reduction in TRM dosage which would have led to a decrease in overall TRM exposure. The mean change in trough TRM (C12 and C24) concentrations were much closer to the change in TRM AUC(0-24) and hence this would appear to be a more reliable value on which to base dosage reductions.

For reasons of practicality, this study used a stepwise increase in DTZ dosage given for a period of ≥2 weeks in kidney or liver transplant recipients who were stabilised on TRM. Since the doses of DTZ were not randomised, a 'period effect' cannot be excluded. Another potential source of error could be that the magnitude of the interaction was not maximal at the 2 week interval set between study days. There is limited data from this study which supports the hypothesis that 2 weeks was sufficient. On the two occasions when kidney transplant recipients failed to follow either the correct DTZ or TRM dosage protocol, the studies were repeated 2 weeks later. While limited, data is thus available for Patient 1 on both day 1 and day 14 of the 90mg DTZ taken twice daily and for Patient 2, data is available (TRM AUC(0-12)) for day 14 and day 28 at the same DTZ dosage. Data from the first of these two study days was not included in the primary analysis.

Data from Table 8.5 shows that both TRM AUC(0-12) and AUC(12-24) increased such that AUC(0-24) increased from 31% to 48% over baseline in the 2 week interval for Patient 1. A similar effect was noted on TRM trough concentrations where the mean of 12h & 24h concentrations increased from 47% to 66% over baseline in the 2 week interval (Table 8.5). This suggests that the TRM-sparing effect of DTZ is not maximal on day 1. DTZ AUC(0-24) also increased in the 2 week interval (1834 to 2639μg.h/L) (Table 8.6) as did the three DTZ metabolite AUCs. This is also consistent with DTZ not reaching steady-state on the first day after a 50% dose increase.
Data for Patient 2, where day 14 and day 28 at the DTZ dose of 90mg taken twice daily is available shows that TRM AUC(0-12) did not change in the 2 week interval (146 vs 155 µg.h/L) (Table 8.5). Because the evening TRM dose was omitted, neither AUC(12-24) nor AUC(0-24) can be used as the comparator. As expected, there was no increase in DTZ AUC(0-24) nor AUC(0-24) for the three DTZ metabolites (Table 8.6), suggesting that the TRM-sparing effect of DTZ was indeed maximal by day 14.

The effect of switching from conventional release DTZ formulation to CD formulation (at the same 180mg daily dose) was a fall in DTZ AUC(0-24) and corresponding AUC for the three DTZ metabolites for both kidney transplant recipients (Table 8.7). DTZ AUC(0-24) fell by 44% in Patient 1 (2639 to 1481µg.h/L) and 53% in Patient 2 (4096 to 1941µg.h/L). The magnitude of these falls values being greater than the mean fall observed in the 8 kidney transplant recipients studied previously. In that study, the mean (±SD) AUC(0-24) for 180mg DTZ conventional formulation was 4403 ± 2708µg.h/L while for the CD formulation, AUC(0-24) was 3172 ± 907µg.h/L (a fall of 28%) (Chapter 7). This surprising result confirms the earlier observation in the CsA/DTZ study that the CD formulation of DTZ has a poorer bioavailability than conventional release tablet formulation of DTZ.

Changing the formulation of DTZ from conventional tablet to CD capsule had variable effects on TRM-sparing activity. In Patient 1, there was an increase over baseline AUC(0-24) from 48% to 53% but in Patient 2, there was a fall from 177% to 97% (Table 8.8). Similar variability was noted in the previous study with CsA/DTZ in kidney transplant recipients (Chapter 4) and hence physicians should be cautious before switching DTZ formulations merely for compliance reasons.
8.6 Conclusions

Because TRM was not widely used in kidney transplantation at the time of study and there are relatively few liver transplants performed, there were limited numbers of transplants available for this study. Nonetheless, because considerable variability in TRM-sparing activity was demonstrated in this study, it seems prudent to demonstrate that a sparing effect exists in each and every transplant recipient before committing them to a prolonged period of DTZ use. Changes in the mean of 12h and 24h trough blood TRM concentration appears to be a good approximation of changes in TRM AUC(0-24) and is easier to perform. For practical reasons, the initial dose of DTZ should be 60mg (conventional release tablet) each morning followed by 60mg twice daily depending upon the clinical situation and magnitude of TRM-sparing response. DTZ doses should be given with TRM doses. If there is no clinical indication for DTZ and no increase in blood TRM concentrations are seen at this dose, DTZ should be discontinued. There would seem to little point in switching patients from conventional release DTZ formulation to the CD formulation.
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Summative discussion and directions for future research
Summative discussion:

The work contained in this thesis was not intended to either provide support for or discourage the use of CsA (or TRM) sparing agents. Rather, studies were undertaken to explore the extent of their use, the reasons for this use and identify issues that might affect the decision to use them. Prior to publishing the studies contained in this thesis, there was only limited data available to transplant physicians prescribing CsA-sparing drugs that supported this practice. The lack of data (especially dose-response relationship data) did not appear to be a deterrent to the use of these agents, given their widespread use by Australasian transplant physicians identified in Chapter 2.

Physicians can usually assume that such data is available, even if they have not personally sighted it, because it is normally provided by pharmaceutical companies as a routine part of the new drug development process. However, pharmaceutical companies have not been involved in the development of drugs which are intended for CsA (or TRM) sparing indication and hence it is perhaps not surprising that this data has not been available to prescribers. Also, since regulatory bodies have not been formally approached to approve this new indication, it is perhaps not surprising that this data has not been demanded and this situation appears unlikely to change. Indeed, if the withdrawal from sale of the calcium channel blocking drug mibefradil (a more powerful CsA-sparing drug than DTZ) is any guide, pharmaceutical companies are not enthusiastic about promoting drugs which have the potential for such significant drug interactions.

When first advocated in the mid 1980s, there was a sound basis (cost savings and potential therapeutic benefit) for studying the role of potential sparing agents in the transplant population, but early studies were limited in nature and provided relatively few answers. There were also a number of quite new concerns associated with their use (especially the ethics of using a drug for primarily an economic reason) and these
concerns, coupled with the lack of data, has caused many physicians to refrain from using CsA (or TRM) sparing drugs.

The work contained in this thesis has added to the body of knowledge that underpins the use of CsA (or TRM) sparing agents and will allow prescribing physicians to make a more informed decision about whether to use them or not. While numbers are limited, the dose-response relationship has been studied in kidney, liver and lung transplant recipients and considerable variability in response has been noted (Chapters 4, 5, 6 and 8). Clinically significant reductions in CsA (and TRM) dosage have been demonstrated in these studies but at doses that are much lower than those often used for this indication in transplantation. The apparent lack of CsA-sparing effect observed with DTZ in one transplant recipient who demonstrated a significant CsA-sparing effect with ICZ reported in Chapter 5 provides a strong argument for proving, rather than assuming, that a benefit exists. Since a primary focus is cost saving, it is important to note that this need to prove that a benefit exists demands additional effort and hence additional costs which inevitably erodes the economic benefit achieved.

The acceptability of using changes in morning blood CsA or TRM trough concentrations (the usual clinical practice) to prove a sparing effect has been raised as an issue in this thesis because studies involving both CsA and TRM (Chapters 4 and 8) demonstrated a greater effect on trough concentrations than the respective AUCs. Although there has been some work in this area, the issue of the best sampling time/s which correlate with efficacy has not been resolved when CsA (or TRM) are used without sparing agents. AUCs have traditionally been regarded as the best determinant of drug exposure and hence of efficacy but there are good examples elsewhere in medicine where this does not apply. In particular, it does not apply to aminoglycoside antibiotics where peak plasma concentration has been shown to correlate better than trough concentration with efficacy and this awareness has lead to a fundamental change in practice from thrice daily
regimens to less frequent administration of larger doses. If similar studies with CsA (and TRM) demonstrate that AUCs correlate better with efficacy than trough concentrations when sparing agents are used, then more extensive monitoring will be mandatory. This will affect the enthusiasm for using sparing agents since the costs of establishing that a benefit exists (which is borne by the hospital system) will escalate accordingly.

It has been demonstrated in this thesis that the ratio between trough concentration and AUC is altered by the use of sparing agents. Hence there is concern about relying on trough concentration monitoring for adjusting CsA (or TRM) doses. Setting a higher target trough concentration may resolve this concern but this would only be possible if there was little inter and intrapatient variability between AUC and trough concentrations – something that would need to be explored in larger populations. Because different sparing agents might affect the ratio of trough concentration to AUC differently, it is likely that different therapeutic ranges might be needed for both CsA and TRM when different sparing agents were used. This potential for different therapeutic ranges is based solely upon different pharmacokinetic interaction factors and ignores the potential for differing pharmacodynamic interactions between either CsA or TRM and the sparing agent. If such pharmacodynamic interactions could be quantified, further adjustments might be made to the target therapeutic (trough) blood concentration.

It was somewhat surprising that data collected for this thesis (Chapter 3), demonstrated considerable differences between transplant units in such a fundamental area as the therapeutic range for blood CsA concentrations used to maintain even the same type of organ transplant. The use of CsA-sparing agents appeared not to affect the CsA therapeutic range used by transplant physicians although there is potential for these to be lowered due to an immunosuppressive action by some (DTZ) sparing agents (Carozzi S, et al. 1995). While this might allow the development of different therapeutic ranges where these drugs are coprescribed (noted above), the apparent inability to develop
uniform therapeutic ranges in the relatively uncomplicated environment that applied at the time of the study does not auger well for this to happen given the complexity involved.

A significant reduction in the rate of occlusion of coronary arteries has been demonstrated when DTZ is coprescribed with CsA in heart transplant recipients (Schroeder JS, et al. 1993. Keogh A, et al. 1995). This is a strong argument in favour of its routine coprescription and is almost certainly the reason that these patients were almost always prescribed DTZ in the survey (Chapter 2). This thesis has confirmed earlier findings in the Australian adult kidney transplant population of therapeutic benefits in the form of reduced need for dialysis or additional immunosuppressive drugs associated with the coprescription of DTZ in the early days post kidney transplantation. This is also a strong argument in favour of this coprescription at least in the first few weeks post kidney transplantation. An argument in favour of long term coprescription of DTZ is the frequent diagnosis of hypertension when CsA is used in organ transplantation. Contrary to earlier findings, data presented (Chapter 3) in this thesis support the usefulness of DTZ as an antihypertensive drug and hence a therapeutic benefit exists in most cases. It is important to note that these therapeutic benefits were observed with higher doses of DTZ than those needed to spare the dose of CsA (demonstrated in Chapter 4) and hence a therapeutic benefit cannot be assumed if lower doses of DTZ are employed. If lower doses of DTZ were also shown to exert a therapeutic benefit, most of the ethical concerns surrounding its coprescription would be resolved.

While DTZ has a potentially significant role as a CsA-sparing agent, the magnitude of the sparing effect afforded by KCZ is potentially of greater importance, especially in countries where the costs of providing CsA preclude its use. In Australasia, KCZ has not been widely used because it is perceived to be a more toxic (especially hepatotoxic)
drug and its use has been vigorously opposed by some regulatory bodies (personal communication, A Keogh, St Vincent’s Hospital, Sydney, 1995). It has however been used in limited numbers of transplant recipients for extended periods without apparent ill effects and in some countries, it is routinely coprescribed. Dose-response studies of the type performed in this thesis are unlikely to be performed in these countries however because of a lack of expertise and analytical equipment etc. If this data is to be provided therefore, either a change in attitude will be required in Australia (to allow detailed study of the interaction) or appropriate funding will need to be arranged to allow these studies to be performed in developing countries. In addition to defining optimal KCZ doses that afford a CsA-sparing effect, it would also be important to document the safety (or otherwise) of the coprescription, especially since the risk of hepatotoxicity is potentially greater in such countries due at least in part to Hepatitis B and C infection.

The magnitude of the savings afforded by the use of DTZ (as a CsA sparing drug) was estimated at AUD$7 million in 1995. This saving is made by the authority which provides the funding (the Commonwealth government in Australia) which is a powerful argument for this body to play a central role in directing studies into the use of CsA (or TRM) sparing agents. It is interesting to note that many centres started using these agents after the introduction of the ‘Section 100’ scheme (1991) in Australia when this saving, previously made by State governments, began to accrue to the Commonwealth government. The cost of providing the CsA-sparing drugs remains a State government, hospital and individual transplant recipient responsibility however. The Commonwealth government is also charged with the responsibility for approving the marketed indications for drugs within Australia, a reactive role that is normally activated when a pharmaceutical company applies for marketing approval. Despite widespread knowledge of the use of CsA (or TRM) sparing drugs, none have been granted marketing approval and given the relatively small profit that might be generated, it is unlikely that pharmaceutical companies will make such an application. Because no single organisation
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has assumed responsibility for studying the interaction between CsA (or TRM) and the various sparing agents, it is likely that many issues surrounding the coprescription will remain unanswered. Given these factors, it is logical that the Commonwealth government should take the central role in this process, initiating studies which will provide the required information, financing these studies and subsequently approving the indications. It might be argued that Commonwealth government is currently shirking its responsibilities because it is the sole beneficiary of the savings made. It is also worth noting that the most likely targets for litigation in the event of an untoward effect resulting from the use of CsA (or TRM) sparing agents will remain State governments, and/or individual hospitals until sparing agents are officially approved.

One of the more interesting findings from this series of studies is that doses of DTZ up to 60mg/day (which were taken with the morning CsA dose only), affect both morning (0-12h) and evening (12-24h) CsA AUCs equally. This finding demonstrates that the interaction between DTZ and CYP3A4 (and/or P-gp) persists after the drug is removed from plasma. Since patient compliance has been demonstrated to be an important cause of graft failure (Didlake RH, et al. 1988) this information can be directly applied to drug regimen simplification. Many transplant physicians switched patients from conventional release DTZ tablets to the controlled diffusion capsules at the time of the survey (Chapter 2) presumably for this reason.

This thesis has also highlighted the potential dangers of changing formulations without first studying the effects of change. In Chapter 5, 3 patients experienced falls in CsA AUC(0-12) between 30-60% and another experienced an increase of 36% when switched from conventional tablet to CD formulation DTZ in the same dose. Changes in formulation to both CsA and TRM have been made by the respective manufacturers over recent years and while bioequivalence studies have been performed, these have not focussed on the CsA (or TRM) sparing action. The interaction studies performed in this
thesis were performed with the older (Sandimmun®) formulation of CsA and Cardizem® brand of DTZ hence the magnitude and reproducibility of the sparing effects might be different with different formulations or brands. Interestingly, brand changes with DTZ are almost inevitable in the current drug supply systems where contracts for drugs are changed on a regular basis in hospitals and brand price premiums encourage alternative generic prescribing in the community. The potential for different CsA (or TRM) sparing effects should be factored into any decision to change brands and those charged with the responsibility for making these decisions should be cognisant of this potential problem.

The dose-response relationship study described in this thesis for the interaction between CsA and DTZ was published in 1997, approximately a decade after the coprescription was first used. Because the data became available only after policies had been formulated, it is less likely that the data would affect prescribing practice. It is preferable to have access to such basic information before policies are constructed and for this reason, the interaction between DTZ and the newer immunosuppressive drug TRM was investigated (Chapter 8) in a preliminary study in kidney and liver transplant recipients.

While a number of questions have been addressed by these studies, many remain unanswered or have arisen as a result of these studies. These include:

- Is CYP3A4 the major target for CsA-sparing agents or is P-gp more important. Research into this aspect will require agents that are able to selectively affect one system without affecting the other. This is of clinical importance since P-gp has been found in the blood brain barrier and may serve a protective role at this site. Drugs which impair P-gp may thus increase brain concentrations of a variety of drugs with both beneficial and adverse effects.

- What is the relative contribution of gastrointestinal and hepatic CYP3A4 (and/or P-gp) in metabolism of CsA and TRM
-How are CsA (and TRM) metabolite profiles altered by the use of sparing agents and do these changes have any clinical sequelae?

-Is AUC monitoring the best predictor of immunosuppressive outcome or is trough concentration a better predictor? As noted above, research into this area is of great importance since AUC monitoring is both inconvenient and expensive. Such studies should also investigate the potential for creating a new target therapeutic ranges for trough concentrations where organ transplant recipients are prescribed CsA (or TRM) sparing drugs.

-Other sparing agents need to be similarly studied. As noted earlier, the magnitude of the costs associated with the use of CsA and TRM precludes their use in many transplant recipients (especially in developing countries). Strategies which reduce the dose (and hence cost) are thus more important in such countries and, because it is a more powerful interactor, the dose-response relationship between KCZ and both CsA and TRM should be established as soon as possible.

-Is the difference in TRM-sparing activity between kidney transplant and liver transplant recipients (noted in Chapter 8) due to the nature of the transplanted organ or is it another manifestation of the variability observed earlier?

-Is the observed performance failure of the CD formulation of DTZ due to the concomitant use of CsA? Is this confined to organ transplant recipients or is it more widespread?

-How widespread is the poor absorption of DTZ seen in Patient 7 in Chapter 7? While it did not appear to unduly affect CsA-sparing activity, does this poor absorption result in poor antihypertensive and/or anti-anginal activity?

-Is there a relationship between blood CsA concentration and graft function in the early days post transplantation? Data available for analysis in Chapter 3 was too limited to answer this question and given the potential for variable practices with respect to other immunosuppressive drugs (especially immune system antibodies), this would require a carefully designed, multicentre, clinical trial. This is a topical
issue at present and some units have demonstrated less frequent rejection episodes when sparse sampling AUCs for CsA are used (Mahalati K, et al. 1999) in preference to trough blood concentration. The AUCs being advocated are very high compared to those measured in Chapters 4 and 5 (which were many months post transplant however) and hence the potential for adverse effects (especially infection and cancer risk) must also be quantified for this approach.


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APPENDICES

Appendix 1:
Questionnaire to heads of transplant units regarding use of cyclosporin-sparing agent use

Appendix 1a:
Questionnaire to heads of kidney transplant units to ascertain data on individual patients for the first 12 months post transplantation

Appendix 2:
Transplantation centres and responders to questionnaires

Appendix 3:
Ethics of Human Research submission for cyclosporin-diltiazem dose response study

Appendix 3a:
Ethics of Human Research submission for tacrolimus-diltiazem dose response study

Appendix 4:
Log-trapezoid rule used to calculate AUC

Appendix 5:
Sigmoid Emax equation used to derive population estimates from individual renal transplant data (Chapter 4)
Appendix 1. Questionnaire to heads of transplant units regarding use of cyclosporin-sparing agent use

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<tbody>
<tr>
<td>(1) Approximately how many transplants do you perform each year?</td>
<td>LIVER</td>
</tr>
<tr>
<td>(2) In what % of patients do you use Cyclosporin (CSA) sparing agents?</td>
<td>ALL</td>
</tr>
<tr>
<td>(3) What CsA sparing agents do you use?</td>
<td>DILT</td>
</tr>
<tr>
<td>(4) What dose of CsA sparing agent do you use? (Please state mg and frequency, e.g. 60mg TDS.)</td>
<td>DILT</td>
</tr>
<tr>
<td>(5) How long after transplantation do you start CsA sparing agents</td>
<td>DAYS/</td>
</tr>
<tr>
<td>(6) When did you start using these agents?</td>
<td>DILT</td>
</tr>
<tr>
<td>(7) How frequently is CsA administered per day (1x, 2x etc.)?</td>
<td>EARLY</td>
</tr>
<tr>
<td>(8) What values do you use as a Therapeutic Range for CsA?</td>
<td>YES</td>
</tr>
<tr>
<td>(9) Are there specific reasons for not using CsA sparing agents?</td>
<td>YES</td>
</tr>
<tr>
<td>(10) What reduction in dose of CsA do you feel you achieve?</td>
<td>DILT</td>
</tr>
</tbody>
</table>

Name: ________________________________
Telephone: ____________________________
Facsimile: ____________________________
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Appendix 1a. Questionnaire to heads of kidney transplant units to ascertain data on individual patients for the first 12 months post transplantation

For primary cadaver renal transplant recipients who received their graft in the period 1.7.94 to 30.6.95.

<table>
<thead>
<tr>
<th>HOSPITAL:</th>
<th>HOSPITAL U.R. NO.:</th>
</tr>
</thead>
</table>

Date of transplant ( = Day 0):

Data aimed at defining early efficacy:

<table>
<thead>
<tr>
<th>Creatinine and cyclosporin (CsA) concentrations: (highest value where more than one)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>9</td>
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<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs used to treat rejection in 1st month: (please indicate no. of doses/courses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solumedrol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of dialyses in 1st week:</th>
<th>in 1st month:</th>
</tr>
</thead>
</table>

When was CsA started post transplant? (hours/days)

When diltiazem started post transplant? (hours/days/weeks)

Data aimed at defining long term toxicity:

<table>
<thead>
<tr>
<th>Creat. and CsA concentrations, blood pressure and antihypertensive drug* therapy:</th>
</tr>
</thead>
<tbody>
<tr>
<td>at 3 months</td>
</tr>
<tr>
<td>Creat.</td>
</tr>
<tr>
<td>CsA</td>
</tr>
<tr>
<td>BP</td>
</tr>
<tr>
<td>Drugs*</td>
</tr>
</tbody>
</table>

*Antihypertensive drugs are to include only those which are prescribed to treat hypertension.

<table>
<thead>
<tr>
<th>Drugs prescribed for other indications, but known to lower blood pressure:</th>
</tr>
</thead>
<tbody>
<tr>
<td>at 6 months</td>
</tr>
<tr>
<td>-------------</td>
</tr>
</tbody>
</table>

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Appendix 2. Transplantation centres and responders to questionnaires:

Alfred Hospital, Vic; Director of Nephrology, Prof N Thomson
Alfred Hospital, Vic; Mr D Esmore, Director, Heart & Lung transplant service
Auckland Hospital, New Zealand; Director of Nephrology, Dr P Doak
Austin Hospital, Victoria; Director of Nephrology, Dr J Dawborn
Austin Hospital, Victoria; Director, Liver Transplant Unit, Mr R Jones
Christchurch Hospital, New Zealand; Director of Nephrology, Dr R Bailey
Concord Repatriation Hospital, NSW; Director of Nephrology, Dr C George
Flinders Medical Centre, SA; Director Liver transplant Unit, Mr R Padbury
Greenlane Hospital, NZ, Director, Cardiothoracic Surgical Unit, Mr D Haydock
John Hunter Hospital, NSW; Transplant Coordinator, Ms A Stein
Monash Medical Centre, Vic; Kidney and Pancreas Transplant Unit (Adult), Dr I Main
Monash Medical Centre, Vic; Director of Nephrology (Paediatric) Dr M McIver
Prince Henry Hospital, NSW; Director of Nephrology, Prof G MacDonald
Princess Alexandra Hospital, QLD; Director of Nephrology, Dr J Petrie
Princess Alexandra Hospital, QLD; Liver Transplant Unit, S Lynch
Princess Margaret Hospital for Children, WA; Director of Nephrology, Dr I Hewitt
Royal Alexandra Hospital for Children, NSW, Director of Nephrology, Dr E Hodson
Royal Childrens Hospital, Vic; Director, Renal Unit, Dr C Jones
Royal Melbourne Hospital, Vic; Director of Nephrology, Prof R Walker
Royal North Shore Hospital, NSW; Nephrology Unit, J Mahoney
Royal Perth Hospital, WA; Director of Nephrology, Dr M Thomas
Royal Perth Hospital, WA, Cardiopulmonary Transplant unit, Nurse Consultant, Mrs D O'Shannessy
Royal Prince Alfred Hospital, NSW; Director of Nephrology, Prof D Tiller
Royal Prince Alfred Hospital, NSW, Director, Liver Transplant Unit, Ass Prof G McCaughan
St Vincent's Hospital, Vic; Director of Nephrology, Dr B Murphy
St Vincent's Hospital, NSW; Director of Nephrology, Dr J Hayes
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St Vincent's Hospital, NSW; Cardiopulmonary Transplant Unit, Dr A Keogh
Sir Charles Gairdner, WA; Director of Nephrology, Dr B Hutchinson
The Queen Elizabeth Hospital, SA; Director of Nephrology, Dr T Mathew
Wellington Hospital, New Zealand; Director of Nephrology, Dr P Hatfield
Women's and Childrens Hospital, Adelaide; Director, Dr K Jureidini
Westmead Hospital, NSW; Director of Nephrology, Dr J Chapman
St Georges Hospital, NSW, Head, Dept of Nephrology, Prof J Whitworth
Sir Charles Gairdner, WA, Director, Liver Transplant Unit, Prof W Reid
Waikato Hospital, NZ, Director of Nephrology, Dr M Wallace
Appendix 3. Ethics of Human Research submission for cyclosporin-diltiazem dose response study

**Title:**
THE INFLUENCE OF DILTIAZEM DOSE AND FREQUENCY ON THE METABOLISM OF CYCLOSPORIN

**Investigators:**
Mr T. Jones, Pharmacy Dept
Mrs S. Goldsworthy, Pharmacy Dept
Dr R. Morris, Dept of Clin Pharmacology
Dr W. James, Renal Unit
Dr T. Mathew, Renal Unit

**Aim:**
To study the effect of varying doses of diltiazem on the metabolism of cyclosporin A.

**Background:**
Cyclosporin (CyA) is a complex molecule which is extensively metabolized in the body. Several drugs have been shown to reduce the extent of this metabolism and thus elevate CyA blood levels. In order to maintain blood levels within the accepted 'therapeutic range', the dose of CyA must be reduced in these circumstances.

Because CyA is particularly expensive (approximately $10,000 per annum per patient), the concurrent use of metabolic inhibitors can substantially reduce the net cost of this important immunosuppressive agent and this is widely practised throughout the world. Among the drugs which have been shown to reduce the metabolism of cyclosporin, diltiazem has been exploited by most transplant centres because of its limited adverse effect profile and indications in hypertension and ischaemic heart disease (disorders frequently encountered in renal transplant recipients). Coprescribing diltiazem with CyA results in reductions of approximately 30% in CyA dosage and total drug cost at T.Q.E.H. (diltiazem is relatively cheap).

The metabolic interaction was initially noted anecdotally in CyA treated patients who were prescribed diltiazem for hypertension +/- ischaemic heart disease and subsequent trials have confirmed this interaction using 'traditional' anti-hypertensive/antianginal doses (viz 90mg per day to 360mg per day). Since the indication for diltiazem in many CyA patients is a purely metabolic one (viz. to reduce the dose of CyA ), and since there is no dose ranging data available on this interaction, it is pertinent to examine the nature of the metabolic interaction in...
greater detail. This information should allow the selection of the minimum diltiazem dosage and frequency consistent with reducing CyA dose (and thus cost) while reducing the risk of adverse effects from excessive doses of diltiazem.

The enzymatic nature of the CyA - diltiazem interaction is not well understood but has been reported to be 'non competitive'. There is thus the potential that the effect on the enzyme persists after diltiazem is removed from the bloodstream and its antihypertensive and antianginal effect have dissappeared. This may allow once (or twice) daily diltiazem dosing instead of the current thrice daily regimen which should improve patient compliance.

Because cyclosporin's metabolism is complex and its metabolites may possess activity (immunosuppressive and/or toxic), the study will entail measurements of both parent cyclosporin AND total metabolite levels (specific and non-specific immunoassay methodology). These levels will also be related to any observed changes in toxicity +/- immunosuppressive effect.

Methodology: Subjects: 8 renal transplant patients who are NOT currently receiving diltiazem will be enrolled.

Inclusion Criteria:
- males or females age between 18 years and 65 years
- stable renal function over 3 months
- plasma creatinine level less than 150micromol/L
- stable cyclosporin dose and plasma cyclosporin level within the range 80 - 150 over at least 4 weeks.
- signed consent form

Exclusion Criteria:
- patients with cyclosporin levels less than 80 or greater than 150
- known contraindication to diltiazem including:
- documented idiosyncrasy or hypersensitivity, sick sinus syndrome, hypotension (systolic B.P. less than 90mm Hg), severe congestive cardiac failure, acute myocardial infarction and pulmonary congestion
- unwillingness or inability to understand/comply with requirements as stated in information sheet
Withdrawal Criteria:
- development of any of the above exclusion criteria
- rise in plasma creatinine level of 30micromol/L (or greater) from baseline value that cannot be explained by other factors (eg dehydration)
- patient request
- development of any adverse event thought to be caused by diltiazem or due to interference with the metabolism of CyA
- use of any drug known to affect CyA metabolism (including erythromycin, co-trimoxazole, ketoconazole)

Volunteer payment/accommodation:
Patients will be accommodated in ward 3A, Bay 1 for the duration of each of the study days. This will be from approximately 7am to 9am (a total of 26 hours).
Four patients will be accommodated in this bay at each time at intervals of 2 weeks. Beds and meals will be provided by the hospital (paid for from study funds).
Payment ($50.00) will be made for each day of study with a completion bonus ($200.00) being offered for the last study day. Given the 26 hour stay, this amount is merely an honorarium and we do not should not itself induce volunteers to participate.

Diltiazem dose escalation
1) Commence diltiazem at dose of 5mg mane - monitor CyA and diltiazem levels
2) Increase diltiazem dose to 10mg mane and repeat monitoring
3) Continue diltiazem dose escalation as follows - 20mg mane then 30mg mane, then 60mg mane then 90mg mane.

*Parent CyA levels will be monitored routinely and will guide dosage increments such that a balance can be struck between the need to define the dose-response curve and undue imposition on the patient.*

4) The temporal nature of the CyA metabolism (ie whether there is a chronopharmacokinetic effect) and the CyA - diltiazem interaction will be determined by blood sampling over two sequential CyA doses (ie over a 24 hour period) and if results indicate a short term effect on cyclosporin levels, a twice daily diltiazem dosage regimen will be implemented.
Blood level monitoring:
1) Trough blood CyA levels will be determined 5 - 7 days after each diltiazem increment to ensure CyA levels do not rise dramatically. Prior to each diltiazem dosage increment, a 24 hour CyA dose-interval kinetic profile will be determined (blood levels taken at 0 hour, 1 hour, 2 hour, 3 hour, 4 hour, 8 hour and 12 hours post dose for 2 doses.
2) Whole blood CyA levels will be determined by specific EMIT assay and metabolite levels calculated by subtracting this value from the total (ie parent drug and metabolites) determined by a non specific immunoassay.
3) Diltiazem levels will be measured prior to the next dose increment at times 0 hour, 1 hour, 2 hours, 3 hours, 4 hours, 8 hours and 24 hours. These levels will be determined from the same blood sample as the CyA blood levels.
4) Diltiazem assay will be performed by HPLC.
5) Blood samples will be taken by trained R.N.s who will be on duty on 3C. The proximity of ward 3A will facilitate this sampling and staffing levels on 3C will be increased to cover the anticipated needs of the group (funds will be provided from study funds).
6) Blood samples will be taken from an indwelling catheter inserted by an experienced medical officer and patency maintained by flushing with heparinized saline (as per agreed protocol). Samples will be stored in an EDTA tube and split into a whole blood sample (for CyA assay) and plasma sample (for diltiazem assay) after centrifuging. Clinical Chemistry staff will spin and freeze the blood.

Cyclosporin dosage alterations:
1) Cyclosporin level increases will be closely monitored and if they exceed the upper limit of the therapeutic range (250), the CyA dose will be reduced by an appropriate decrement.
2) Plasma creatinine levels that rise more than 30 micromole/L above baseline and that are thought to be consistent with CyA toxicity will be treated by reducing CyA dosage in a similar fashion.
Ethical Issues

The combined use of diltiazem and CyA is widespread and has not been shown to increase the risk of adverse events experienced by the patient. Indeed, it has been suggested that diltiazem might reduce some of the nephrotoxic adverse effects of CyA. Patients will be recruited from the renal transplant population who are not receiving diltiazem, primarily because they received their transplant before the combined use of CyA and diltiazem became popular.

Close monitoring of CyA levels will allow dosage reductions to be made where necessary which will ensure that the levels do not exceed those considered safe. (An alternative approach is reduce the diltiazem dose in patients already receiving the combination and increase the dose of cyclosporin in order to maintain the levels in the normal range. This approach is considered more dangerous in that it exposes the patient to the possibility that cyclosporin levels may fall to a point where immunosuppression fails and rejection may ensue).

Involvement in this trial will require more blood samples being performed than would otherwise occur but such sampling is well established and is not considered to be a major ethical issue.

The requirement to be available for regular blood sampling over a 24 hour period for each diltiazem dosing increment (initially on 6 occasions over a 3 month period) will be clearly explained to the patients and a payment as outlined above will be made.

Drugs:

Diltiazem is marketed in Australia for the treatment of ischaemic heart disease and hypertension. Since it is being used in this trial to reduce the metabolism of CyA, this will require a CTN application. The pharmacy dept. will manufacture capsules containing 5mg and 10mg diltiazem either from tablets or from raw material supplied by the drug company. These will be manufactured and assayed according to the Code Of Good Manufacturing Practice.

Drug company (I.C.I.) support has been sought for purchase of drug, assay kits, payment to volunteers, boarding & meal costs etc as outlined above.
Analysis:

The object of the trial is to relate the dose and/or plasma level of diltiazem to the dose and blood level of CyA and metabolites. Patients will be their own controls and individual and average data will be analysed to determine the magnitude of any observed effect. Results will be reported in an appropriate refereed journal.
THE QUEEN ELIZABETH HOSPITAL
PATIENT CONSENT FORM
THE INFLUENCE OF DILTIAZEM DOSE AND FREQUENCY ON THE METABOLISM OF CYCLOSPORIN

I, the undersigned ........................................ hereby consent to participate in the above research project.

I acknowledge that the nature, purpose and effects of the trial so far as they affect me have been fully explained to my satisfaction and my consent is given voluntarily.

The detail of the procedures proposed including the length of time, frequency of blood monitoring and indication of discomfort have been explained.

I acknowledge that the trial may not be of direct benefit to me.

I have been given the opportunity to have a family member/friend present while the project was explained to me and have received a copy of the 'Patient Information' sheet.

I am informed that although the results of the trial will be published, no information will be divulged which could in any way identify me.

I understand that I am free to withdraw from the trial at any time and that my future treatment at this hospital will not be prejudiced in any way by this action. If I decide to withdraw from the trial, I understand that my cyclosporin levels will need to be monitored and the dosage adjusted to prevent rejection of my kidney.

SIGNED:........................................
ADDRESS:........................................
DATE:..................

WITNESS SIGNATURE:........................................
WITNESS NAME (print):........................................
DATE:........................................
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Appendix 3a. Ethics of Human Research submission for tacrolimus-diltiazem dose response study.

Title: Defining the dose-response relationship for the pharmacokinetic interaction between Tacrolimus and Diltiazem

Investigators: Mr T Jones B.Pharm, Dip.Ed
Dr R Morris PhD
Dr T Mathew MBBS, FRACP

Aim: 1)To define the minimum dose of diltiazem which affects tacrolimus kinetics and the duration of the interaction.
2)To study the effect of giving increasing doses of diltiazem to renal transplant recipients who are treated with tacrolimus.
3)To study the effect of coprescribing diltiazem with tacrolimus on clinical outcomes including hypertension and renal function in kidney transplant recipients.

Background: Tacrolimus is a recently developed immunosuppressive drug that is approved for preventing organ transplant rejection. It shares many similarities with cyclosporin (CsA) including being very expensive, having a similar mode of action and similar drug interactions. Many of these drug interactions are mediated by the cytochrome P450 isoenzyme, CYP3A4, which is found in both liver and gastrointestinal tract.

One of the most important of the drug interactions with CsA is with diltiazem (DTZ) where this latter drug reduces the metabolism of CsA resulting in elevated blood concentrations. The result of prescribing these two drugs concurrently is that the dose of CsA can be reduced by approximately 35% while maintaining the blood concentrations within the therapeutic range(1,2). Drugs which reduce the required dose of CsA in this way are called 'cyclosporin sparing agents'. The routine coprescription of CsA sparing agents has been commonplace throughout Australasia for many years and it was estimated in 1996 that their coprescription saved the Commonwealth government $7 million(3). While the routine prescription of DTZ is largely done for economic reasons, there is evidence from both overseas(4) and local(2) kidney transplant centres that DTZ has therapeutic benefits in that it reduces the extent of kidney damage that frequently results from the use of CsA.

The doses of DTZ that have been employed hitherto for this CsA sparing effect are the standard antihypertensive/antianginal doses

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(180mg/day) that were established many years ago. We have recently demonstrated however that both the necessary dose and the frequency of administration are considerably less when DTZ is employed for its CsA sparing effect(3).

Many drugs which have been shown to interact with CsA have also been observed to interact with tacrolimus and while the evidence is limited, it appears that DTZ reduces the metabolism of tacrolimus and hence reduces the dose required to prevent transplant rejection. DTZ may thus be a 'tacrolimus sparing agent'. This has not been systematically studied in a transplant population however and hence we propose studying this interaction prospectively for both its economic and clinical benefit.

Methodology: The methodology used in this study is based upon the that used in the study undertaken in 1994/5 (ref no 60/92) into the interaction between CsA and DTZ. Eight stable renal transplant recipients who are treated with tacrolimus will be enrolled into this pharmacokinetic study.

Inclusion criteria: Stable renal function over 3 months with serum creatinine <0.18mmol/L. Stable blood tacrolimus concentration over 1 month with concentrations being <10mcg/L.

Exclusion criteria: Unstable renal function and/or creatinine concentration ≥0.18mmol/L. Unstable blood tacrolimus concentration and/or concentrations outside the above range. Documented idiosyncracy or hypersensitivity to DTZ, sick sinus syndrome, hypotension (systolic BP< 90mm Hg), severe congestive heart failure, acute myocardial infarction, pulmonary congestion. Documented difficulty in accessing venous blood. Documented allergy or hypersensitivity to heparin. Use of other drugs which are known to interfere with metabolism of tacrolimus or drugs which might be affected by the use of DTZ.

Diltiazem dose escalation: Incremental doses of DTZ will be given at fortnightly intervals starting with 10mg through 20mg, 30mg and 60mg given in the morning followed by 60mg and 90mg given twice daily. A further study using DTZ 180mg 'CD' capsule given once in the morning will take place at the completion of the conventional formulation phase. DTZ doses less than 30mg will be taken in the form of capsules which will be manufactured by the hospital's pharmacy. 
APPENDICES

department. Doses of DTZ \( \geq 30\)mg will be taken in the form of conventional release tablets.

Blood concentration monitoring:
Each study day will commence at 7.30am with the insertion of an indwelling catheter into a convenient forearm vein. Blood samples (8mL) will be drawn over 24h via this catheter, the patency of which will be enhanced by the instillation of 1.5mL heparinised saline (15u) after each blood sample is drawn. Blood will be divided into two, one for tacrolimus assay and the other for DTZ assay.
Times of blood sampling are 0, 1, 2, 3, 4, 6 and 12h after both morning and evening tacrolimus doses. Thus a total of 13 blood samples will be drawn each study day making a total volume of 104mL each fortnight. The first sample of each day will also be used to check for thrombocytopenia (a side effect of heparin), anaemia (as a result of blood loss) and C-reactive protein. This latter parameter is a marker for infections which may alter tacrolimus' metabolism.

Drug Assays: DTZ assay will be performed by HPLC using a method established in this laboratory(5). Tacrolimus assay will be performed using the MEIA method which has recently been established in this hospital.

Tacrolimus dosage alterations:
In the event that morning, trough blood tacrolimus concentration rises beyond the upper value of the therapeutic range (10mcg/L), an appropriate dosage reduction will be made to facilitate its return to within the range.

Ethical issues: The use of a drug for an economic rather than a therapeutic purpose is of ethical significance. DTZ has been widely and routinely used for this purpose for many years by Australasian transplant centres and the evidence suggests that the coprescription of DTZ confers a therapeutic benefit(2). Hypertension is common in the post transplant period and the majority of transplant recipients receive one or more antihypertensive drugs - DTZ is indicated for this purpose and in many transplant recipients therefore, it will serve a therapeutic purpose. These therapeutic benefits observed when DTZ is coprescribed with CsA may also occur with tacrolimus since it shares many of CsA's adverse effects and hence a secondary endpoint of the trial is to monitor appropriate clinical endpoints. Should any patient suffer any adverse clinical effect as a result of coprescribing DTZ, he/she will be withdrawn immediately and appropriate action taken. The intensity of monitoring involved in this study will reduce the risk that adverse effects may become severe and, if the experience gained from the CsA-
DTZ interaction study are a guide, patients may benefit from the intensity of monitoring (eg optimising blood pressure control).

While CsA has improved graft survival, it has increased the cost of immunosuppression - CsA is approximately 10 times more expensive than previous immunosuppressive drugs. The use of CsA sparing agents has allowed organ transplant recipients in developing countries and the underinsured patient in the USA to receive CsA when they would not have been able to afford it if prescribed alone. It is unlikely that organ transplant recipients in these countries will be able to benefit from tacrolimus unless a similar cost reduction strategy is employed since this new immunosuppressive drug is more expensive than CsA. When used for kidney transplantation, tacrolimus is NOT currently funded via the 'section 100' scheme in Australia with the result that this hospital pays for the costs (approximately $13,000 per patient per annum). Assuming that DTZ spares tacrolimus to the same extent that it spares cyclosporin, anticipated savings that might result from the coprescription of DTZ will be approximately $4000 per annum per patient.

Study participants will be offered $100 per study day to recompense them for inconvenience, taxi fares etc. This sum is not considered to be large enough to induce people to enrol in the study and is in keeping with similar remuneration offered to participants in similar trials in Adelaide. Some participants may be bored by the need to stay in hospital but because participants will serve as their own controls, we are keen that participants should not withdraw for non medical reason. To reduce the likelihood that participants will withdraw, we will offer a $200 completion bonus. Participants will be withdrawn from the trial if it is considered by their doctor to be injurious to their health.

**Drugs:**

Tacrolimus is a powerful immunosuppressive drug that has been used both to prevent and to treat rejection for a variety of organ transplant recipients. It is currently approved for liver transplantation only in Australia but indications for kidney transplantation have been granted in USA, UK and have been applied for in Australia. Patients invited to enrol in the study will already be receiving tacrolimus for immunosuppression to prevent kidney transplant rejection.

DTZ is a calcium channel blocking agent that has been widely used throughout Australia for the treatment of hypertension and angina pectoris. It is considered by most authorities to have a safe adverse reaction profile, especially in the doses that are being used in this trial. Adverse effects that have been reported include:
- Headache, dizziness, bradycardia, first degree heart block, oedema, asthenia, rash and dyspepsia.
Because DTZ is known to interact with the CYP3A4 enzyme system and because tacrolimus' metabolism is known to be affected by the use of drugs which interact with this system, no other drug which is known to affect tacrolimus (or cyclosporin) metabolism will be permitted, nor will any drug whose metabolism is affected by DTZ be permitted during the course of the trial. These drugs include erythromycin, cisapride, terfenadine, ketoconazole, itraconazole, verapamil and grapefruit juice. In addition, the use of beta blockers will be restricted since the combined use of 'normal doses' (viz ≥180mg per day) of DTZ can result in additive cardiac effects.

**Funding:** Funding sources have yet to be finalised but we anticipate that these might include TQEH since the savings that will result from the coprescription of DTZ may well exceed the costs of performing the trial.

**Housing:** Patients will be housed in a suitable area (perhaps a vacant ward) for the 24h period. Meals will be provided by the hospital's catering dept and a television will be provided.

**CTN/CTX:** A CTN application will be made because the capsule of DTZ that are made by the hospital pharmacy are not a marketed formulation. The same processes that were used in 1994/5 will be used in this trial. The CTN application is also required because tacrolimus is not yet approved for kidney transplantation in Australia.

**Refs:**
APPENDICES

Patient Information Sheet
Tacrolimus-Diltiazem Interaction Study

You are invited to participate in this study which aims to define the size and frequency of the dose of Diltiazem which will affect the blood concentration of Tacrolimus. The study will also address the safety of prescribing these two drugs together.

Tacrolimus is an immunosuppressive drug (suppresses the immune system) which prevents your transplanted kidney from being rejected. Diltiazem is used for the treatment of high blood pressure and angina (chest pain) and the recommended doses in Australia are between 180-360mg per day. In this trial, you will be given increasing doses of Diltiazem each fortnight, starting at nil and increasing up to a maximum dose of 180mg per day.

Diltiazem is considered to have a safe side effect profile but you may suffer one or more of the following side effects:

- low blood pressure, headache, nausea, rash, water retention and abnormal heart conduction.

Most of these side effects are related to the dose used and since most of the doses used in this trial are very low, it is unlikely that you will suffer any side effects. Your blood pressure will be carefully monitored during the course of the study and if you experience this or any other side effect, you may be withdrawn from the study. You may also withdraw from the study at anytime if you wish and your treatment at this hospital will not be affected by this decision. If you do decide to withdraw from the study, we may need to take a few additional blood samples to ensure the tacrolimus concentration in your blood is at the correct level to prevent your kidney from rejecting.

Diltiazem has been prescribed routinely with the other major immunosuppressive drug used in Australia (cyclosporin) for many years because there is evidence that diltiazem protects the kidney from some of the side effects of cyclosporin. Since tacrolimus and cyclosporin cause the same type of kidney damage, we anticipate that diltiazem may reduce the kidney damage that can sometimes be seen in patients treated with tacrolimus. Diltiazem also reduces the rate of metabolism of cyclosporin and thereby allows the dose of cyclosporin to be reduced by approximately 35% without impairing its efficacy. The savings to the taxpayer from this lower dose of cyclosporin are significant (approximately $7million per annum). Tacrolimus is more expensive than cyclosporin and if diltiazem reduces tacrolimus' metabolism by a similar amount, we anticipate that approximately $4,000 will be saved for every patient each year.

You will be required to come into hospital for a full day (a little over 24 hours) each fortnight for a total of 8 visits spanning 4 months. In the morning of each study day, an indwelling catheter will be placed in a convenient vein in the forearm and blood samples will be drawn from this tube over the next 24 hour period. Sample times will be 0, 1, 2, 3, 4, 6 and 12 hours after both morning and evening doses of tacrolimus. The tube will be removed the next morning when you will be free to return home. Each blood sample will be 8mL (approximately 2 teaspoonsful) so that a total volume
of 104mL (approximately half a cupful) of blood will be taken each fortnight. The tube will be injected with a low dose of heparin to help keep it working - this drug has been used for decades to prevent blood clotting. One of the side effects which may be caused by heparin is reduced numbers of platelets and we will monitor your blood for this problem as well as for anaemia which may occur due to the blood we take. You may suffer some pain and bruising as a result of the indwelling catheter.

While in hospital, you will be free to move around and a television will be provided for your entertainment. Meals will be provided by the hospital. To compensate you for out of pocket expenses and taxi fares, you will be given $100 each study day and upon completion of the study, you will be given an additional bonus of $200.

Because diltiazem and tacrolimus are affected by (and can affect) other drugs, you will not be allowed to take any other drug which can interfere with the metabolism of tacrolimus or whose metabolism could be affected by diltiazem. These drugs include terfenadine ('Teldane'), erythromycin ('Eryc'), cisapride ('Prepulsid'), ketoconazole ('Nizoral'), verapamil ('Isoptin', 'Anpec', 'Cordilox'). You will not be allowed to take grapefruit juice either and, as a matter of principle, before any new drug is taken, one of the researchers should be contacted so that the prescriber can be advised of the potential for interaction and alternatives sought.

If diltiazem proves to reduce the side effects of tacrolimus (especially any damage to your kidney), then this may prolong the life of your kidney. You may also be able to reduce the number of blood pressure medications that you take. We cannot guarantee that you will derive any benefit from participation in this trial however. If you would like to speak to either of the researchers about the project, they can be contacted thus: Terry Jones 82226000 (ask switchboard to page) Ray Morris 82226753 Dr Matthew 82226665

If you wish to speak to someone who is not involved with the trial, please contact Paul Miller, Executive Officer, The Ethics Of Human Research Committee on 82226000 (ask switchboard to page)
APPENDICES

Appendix 4. Log-trapezoid rule used to calculate AUC

\[
AUC = \frac{(c_1-c_2) \times (t_2-t_1)}{\ln c_1 - \ln c_2}
\]

Where \(c_1\) = concentration in blood at time 1

\(c_2\) = concentration in blood at time 2

Appendix 5. Sigmoid Emax equation used to derive population estimates from individual renal transplant data (Chapter 4)

\[
E = E_0 + \frac{Emax \cdot CE^\gamma}{EC_{50}^\gamma + CE^\gamma}
\]

Where \(E\) = Effect intensity at any concentration,

\(E_0\) = effect intensity at concentration 0,

\(Emax\) = maximal effect intensity,

\(EC_{50}\) = concentration required to produce an effect intensity midway between \(E_0\) and \(Emax\)

\(CE\) = concentration required to produce the intensity of effect, \(E\)

\(\gamma\) = sigmoidicity parameter