SMGroup

Therapeutic Drug Monitoring of Immunosuppressive Agents

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ABSTRACT

The pharmacokinetics of the immunosuppressive drugs is complex and unpredictable. A narrow therapeutic index unique to each patient, as well as variable absorption, distribution, and elimination, are characteristics of these drugs. Therapeutic drug monitoring plays a key role in helping clinicians maintain blood and plasma levels of immunosuppressive drugs within their respective therapeutic ranges. Variation in concentrations outside the narrow therapeutic ranges can result in adverse clinical outcomes. Therapeutic drug monitoring ensures that concentrations are not too high or too low, thereby reducing the risks of toxicity or rejection, respectively. This chapter reviews the general principles of therapeutic drug monitoring of the immunosuppressive drugs: cyclosporine, tacrolimus, mycophenolic acid, sirolimus and everolimus.

INTRODUCTION

The first successful kidney transplantation was performed in the mid-1950s and marked the advent of a new effective therapy for end-stage renal disease. Since then, the field of kidney transplantation has witnessed improved graft outcomes, with reduced rates of early acute rejection and increased patient survival [1]. The principal reason for this success can

be attributed to the use of drugs that inhibit the immune response and thus prevent rejection. Optimal immunosuppressant drug therapy is essential for maintaining a viable organ allograft. Nowadays, most patients are treated with combined immunosuppressive therapy consisting of a calcineurin inhibitor [CNI; either ciclosporin A **(CsA)** or Tacrolimus **(TAC)**] in association with an anti-proliferative agent (most oftenmycophenolate) and glucocorticoids [2].

Individualizing a patient's drug therapy to obtain the optimum balance between therapeutic efficacy and the occurrence of adverse events is the physician's goal. However achieving this goal is not always straight forward, being complicated by within and between patient variability in both pharmacokinetics and pharmacodynamics. In the early 1960s new analytical techniques became available allowing the measurement of the low drug concentrations seen in biological fluids during drug treatment. This offered the opportunity to reduce the pharmacokinetic component of variability by controlling drug therapy using concentrations in the body rather than by dose alone. This process became known as therapeutic drug monitoring [3].

THERAPEUTIC DRUG MONITORING (TDM)

The aim of TDM is to optimize pharmacotherapy by maximizing therapeutic efficacy, while minimizing adverse events, in those instances where the blood concentration of the drug is a better predictor of the desired effect (s) than the dose. The reasons why these principles have gained wide acceptance include the following: (i) although imperfect, a better relationship often exists between the effect of a given drug and its concentration in the blood than between the dose of the drug and the effect; (ii) a thorough understanding of pharmacokinetics, i.e., the processes of drug absorption, distribution, metabolism, and drug excretion in individual patients and in patient populations is available; and (iii) the development of reliable and relatively easy to use drug-monitoring assays. In addition, TDM can also be useful in cases in which compliance is in question, where it is not clear if the right drug is being taken, where dosage adjustment is required as a result of drug-drug or drug-food interactions, and where intoxication is suspected.

TDM is more than simply the analysis of a single drug concentration in the blood of a patient and a report of this number. It also comprises interpretation of the value measured using the mathematical (pharmacokinetic) principles mentioned above, drawing the appropriate conclusions about the result, and advising the physician who ordered the test how to optimize treatment. It is important to apply a uniform definition of TDM here, because different definitions have previously been used in cost-effectiveness studies and reviews of TDM.

Consequently, comparisons have been made based on different approaches, which may influence the results. The International Association for Therapeutic Drug Monitoring and Clinical Toxicology has adopted the following definition:

Therapeutic drug monitoring is defined as the measurement made in the laboratory of a parameter that, with appropriate interpretation, will directly influence prescribing procedures.

Commonly the measurement is in a biologic matrix of a prescribed xenobiotic, but it may also be of an endogenous compound prescribed as replacement therapy in an individual who is physiologically or pathologically deficient in that compound.

This definition places TDM within the total therapeutic approach and should give not only more emphasis to the medication and patient-safety aspects of pharmacotherapy, but also more insight into why there are differences in efficacy among different patients. This definition implies that clinical pharmacologists have an active involvement in drug therapy, something that is not yet realized in many countries [4].

WHY TDM FOR IMMUNOSUPPRESSANTS?

For a drug to be a suitable candidate for therapeutic drug monitoring it must satisfy the following criteria [3]:

1. There should be a clear relationship between drug concentration and effect.
2. The drug should have a narrow therapeutic index; that is, the difference in the concentrations exerting therapeutic benefit and dose causing adverse events should be small.
3. There should be considerable between-subject pharmacokinetic variability and, therefore, a poor relationship between dose and drug concentration/response.
4. The pharmacological response of the drug should be difficult to assess or to distinguish from adverse events.

The most commonly used immune suppressants require TDM because of their narrow therapeutic index and significant variability in blood concentrations between individuals. In transplant recipients, both supra therapeutics and sub therapeutics drug concentrations can have devastating results. At sub therapeutics drug concentrations, the transplant recipient is at risk for allograft rejection. At supratherapeutics drug concentrations, the patient is at risk for over-immunosuppression which can potentially lead to infection or drug specific side effects. It is it known that neurological and gastrointestinal side effects occur more frequently at higher concentrations of TAC [5]. Immuno suppressants displays significant inter individual variability in plasma drug concentrations, which creates the demand for TDM when such drugs are used.

FACTORS CONTRIBUTING TO THE VARIABILITY

Immunosuppressant's display significant inter individual variability in plasma drug concentrations, which creates the demand for TDM when such drugs are used. It is appropriate to look into the multitude of factors that contribute to the inter individual variability. Some of the factors include drug-nutrients interactions, drug-disease interactions, renal insufficiency, inflammation and infection, gender, age, polymorphism and liver mass. Drug nutrient interactions are becoming very widely appreciated. The metabolism of drugs sometimes also depends on the type of diet taken by the patients. Renal transplant patients may have reduced oral bioavailability

for TAC. When given with meals, especially with high fat content food, oral bioavailability of TAC decreases [6].

To avoid the possible effect of food on TAC bioavailability, the drug should be given at a constant time in relation to meals. Several studies have demonstrated that grapefruit juice can increase plasma concentrations of CsA by inhibiting CYP3A-mediated metabolism and by increasing drug absorption via inhibition of P-glycoprotein **(P-gp)** efflux transporters. Also, oral TAC should not be taken with grapefruit juice since this vehicle inhibits CYP3A4 and/or P-gp contained in the gastrointestinal tract and markedly increases bioavailability. Similarly, drug disease interactions can also contribute to interindividual variability in plasma concentration of immune suppressants. Renal insufficiency can result in an altered free fraction of MPA due to the reduction in protein binding. MMF is rapidly converted to its active form, MPA, upon reaching the systemic circulation. MPA is metabolized to its glucuronide metabolite, MPA glucuronide **(MPAG)**, by glucoronyltransferases in the liver and possibly elsewhere.

MPAG is then excreted by the kidney. MPA is extensively and avidly bound to serum albumin. Previous studies have demonstrated that it is only the free (non-protein-bound) fraction of MPA that is available to exert its action. *In vivo* and *in vitro* studies demonstrate that renal insufficiency decreases the protein binding of MPA and increases free drug concentrations. This decrease in protein binding seems to be caused both by the uremic state itself and by competition with the retained metabolite MPAG. The disposition of MPA in patients with severe renal impairment may be significantly affected by this change in protein binding [7].

The concomitant administration of TAC and non steroidal anti-inflammatory drugs has been described as a possible cause of increased TAC nephrotoxicity because of the reduction of vasodilator prostaglandin synthesis through a blockade of the enzyme cyclo-oxygenase. Coadministration of ibuprofen and TAC has resulted in acute renal failure. Drugs such as amino glycosides, cotrimoxazole (trimethoprim/sulfamethoxazole), amphotericin B and aciclovir, which cause significant renal dysfunction on their own, may also enhance TAC nephrotoxicity in the absence of careful monitoring of both renal function and drug concentrations [8].

It has been demonstrated that the *in vitro* metabolism of CsA in human liver microsomeswas significantly reduced by TAC [9].

Interaction between MMF and TAC or CsA is probably related to a possible inhibitory effect of TAC on MPA metabolism and an inhibition of the enterohepatic recirculation of MPA by CsA, resulting in a substantial reduction in the MMF dosage when associated with TAC as compared with CsA. This has been reported in pediatric renal allograft patients and animal models [10,11].

Gender also influences drug concentration. Biologic differences exist between men and women that can result in differences in responses to drugs. Both pharmacokinetic and pharmacodynamic differences between the sexes exist, with more data on pharmacokinetic differences. Bioavailability

after oral drug dosing, for CYP3A substrates in particular, may be somewhat higher in women compared to men [12].

It is known that MPA is primarily metabolized in the liver to its MPAG derivative. Morissette et al., found that men treated with MMF and TAC showed a lower ratio than patients treated with this couple of drugs, confirming that TAC inhibits glucuronidation of MPA. Because MPAG can favor the elimination of MPA, they concluded that gender differences and co treatment with TAC must be taken into consideration when MMF is being administered [13].

Velickovic et al., investigated the gender differences in pharmacokinetics of TAC, their result show remarkable gender-related differences between women and men after the first oral dose among kidney transplant recipients on quaternary immunosuppressive therapy, including TAC, MMF, methylprednisolone and basiliximab [14].

Likewise, age can also contribute to inter individual difference in immunosuppressant plasma concentration. Pharmacokinetic parameters observed in adults may not be applicable to children, especially to the younger age groups. In general, patients younger than 5 years of age show higher clearance rates regardless of the organ transplanted or the immunosuppressive drug used [15].

Young children (1–6 years of age) appear to need higher doses per kilogram body weight of TAC than older children and adults to maintain similar trough concentrations. The reason for this age-related faster clearance rate is unknown [16]. Pediatric transplant recipients require higher doses of CsA to maintain blood concentrations equal to those found in adults [17]. Studies using intravenous CsA demonstrate that this is not because of any metabolic differences, as CsA clearance is not related to age [18].

Polymorphism has demonstrated functional consequences of many drug metabolizing enzymes. For example, CsA is known substrate for CYP3A4/5 and P-gp. CYP3A5 is one of the main CYP3A enzymes and its expression is clearly polymorphic and shows ethnic dependence. TAC is primarily metabolized by cytochrome P450 (CYP) 3A enzymes in the gut wall and liver. It is also a substrate for P-gp, which counter-transports diffused TAC out of intestinal cells and back into the gut lumen. Age-associated alterations in CYP3A and P-gp expression and/or activity, along with liver mass and body composition changes would be expected to affect the pharmacokinetics of TAC in the elderly [19].

The importance of interethnic differences in the pharmacokinetics of immunosuppressant's has been recognized as having a significant impact on the outcome of transplantation. In a retrospective analysis Fitzimmons et al., found that the oral bioavailability of TAC in African American healthy volunteers and kidney transplant patients was significantly lower than in non-African Americans, but there was no statistically significant difference in clearance [20].

These results were confirmed in a healthy volunteer study. The absolute oral bioavailability of TAC in African American and Latin American subjects was significantly lower than in Caucasians.

The results suggested that the observed ethnic differences in TAC pharmaco kinetics were, instead, related to differences in intestinal P-glycoprotein-mediated efflux and CYP3A-mediated metabolism rather than differences in hepatic elimination [21].

Other ethnic groups such as the Japanese populations are not different from the Caucasian population because their transplant outcomes were comparable under usual TAC dosages [22].

All this factors contribute to the variability of immunosuppressant concentrations which has to be maintained within therapeutic range in order to achieve the optimal benefit of drug therapy, rendering TDM necessary for these drugs.

MONITORING OF INDIVIDUAL IMMUNOSUPPRESSIVE AGENTS

Cyclosporine (CSA)

The introduction of CsA in the early 1980s was immediately associated with an enhanced one year renal allograft survival; however, the argument for the therapeutic monitoring to optimize efficacy and safety, has been discussed in the last 25 years and it is still debated [23].

Therapeutic Monitoring

Trough concentration (c_0) monitoring

Over the past two decades, there have been changes to recommended CsA dosing, changes in concomitant medications, and one major change to the oral drug formulation. Lately, there has also been the introduction of generic formulations of CsA [24].

In 1988, in a prospective study showed that although C_0 (trough concentration) levels of CsA correlated poorly with dose, Cmax was significantly correlated with dose, area under the curve **(AUC)** and elimination half-life (t1/2). Those who suffered acute rejection had a significantly lower Cmax by 15–31% [25]. The problem with this method for adjusting the dosage of CsA is that it relies on only one aspect of CsA pharmacokinetics, the predose or trough concentration. With the original formulation of CsA, Sand immune®, this was the best practice, but during the conversion of patients from that formulation to the improved formulation, Neoral® the 2 h post-dose concentration has been advocated as a single concentration monitoring alternative to C_0 [26].

The micro emulsion formulation of CsA, Neoral®, makes CsA pharmacokinetics more predictable and reduces the effects of bile and food on absorption [27].

Nevertheless the predose concentration is still widely used in clinical practice. Currently, most transplant centers measure a single steady-state CsA concentration as either a C_0 predose trough or 2 hours postdose, while some conduct multiple measurements to determine CsA AUC estimates [28].

The target predose concentrations varied not only with transplanted organ and time after transplant but also with the analytical method used. The therapeutic range of CsA used by clinicians varies greatly according to the type of assay used to measure CsA and whether blood or serum concentrations are determined by the clinical laboratory. Thus, it has reported by high pressure liquid chromatography, monoclonal fluorescence polarization immunoassay (monoclonal TDx assay, Abbott Diagnostics®), or monoclonal radioimmunoassay (various manufacturers), the level of therapeutic concentrations in blood are 10-400 ng/mL. By high pressure liquid chromatography, monoclonal fluorescence polarization immunoassay (monoclonal TDx assay, Abbott Diagnostics®), or monoclonal radioimmunoassay (various manufacturers), the level of therapeutic concentrations in serum are 50-150 ng/mL. By polyclonal fluorescence polarization immunoassay (monoclonal TDx assay, Abbott Diagnostics®), or polyclonal radioimmunoassay (various manufacturers) the level of 324 Current Issues and Future Direction in Kidney Transplantation therapeutic concentrations in blood are 200-800 ng/mL, and by polyclonal fluorescence polarization immunoassay (monoclonal TDx assay, Abbott Diagnostics®), or polyclonal radioimmunoassay (various manufacturers), the level of therapeutic concentrations in plasma are 100-400 ng/mL [28].

AREA UNDER THE BLOOD CONCENTRATION-TIME CURVE (AUC)

The first steps towards the development of a more precise monitoring strategy for CsA resulted from the landmark studies by Lindholm and Kahan and Kahan et al., which identified a link between the pharmacokinetics of CsA and clinical outcomes in the individual transplant recipient [29,30].

The area under the concentration-time curve for CsA over a 12-hour drug administration interval (AUC0-12h) was a more precise predictor of graft loss and incidence of acute rejection than other parameters, including the C_0. Since then, subsequent studies on the pharmacokinetics of CsA in renal transplant patients have identified that intra patient variability in AUC values over time was directly correlated with the risk of chronic rejection [28,31].

Proper calculation of AUC requires administration of a dose, followed by blood collection according to an intensive sampling strategy. Concentration values obtained are used to calculate AUC, usually by the trapezoidal method [28].

Some advantages of AUC monitoring are that it is the most precise indicator of drug exposure, can characterize abnormal absorption patterns, appears to be a predictor of clinical outcomes, generates a concentration-time profile, allows calculation of oral pharmacokinetic parameters, and reduces the problems associated with laboratory errors and single concentrations [23,32,33].

Despite its appealing potential advantages, the major disadvantage of AUC monitoring is its inherent need for multiple blood samples. The increased number of samples required, makes AUC monitoring impractical for routine clinical use, more expensive in the short term because of

increased sample collection, analysis and interpretation of results, and inconvenient for patients, especially those in an outpatient setting [26,34].

AUC has been advocated as a better parameter to monitor than trough concentrations, because trough concentrations give no indication of exposure to CsA. For example, 2 patients could have the same trough concentration, but one could have a much lower AUC and, therefore, exposure to CsA. Unfortunately, AUC monitoring is not clinically feasible because of the added time, expense and inconvenience required to collect a sufficient number of samples to properly calculate AUC. Although the full AUC for CsA has been demonstrated as being a sensitive monitoring tool, there may be an alternative approach to the determination of the degree and variability of CsA exposure in the individual patient [26,32].

TWO HOURS POST DOSE CONCENTRATION MONITORING (C2)

This approach, which is termed 'absorption profiling', has the underlying rationale that the 4-hour absorption phase following administration provides measurements that are more informative than C_0 monitoring in the assessment of likely CsA exposure and subsequent clinical response [35,36].

AUC0-4h monitoring is a sensitive tool used to optimize CsA immunosuppression in renal transplant recipients. However, the tool is not practical in the clinical pharmacology and therapeutic drug monitoring of immunosuppressive agents setting because of 3 drawbacks: (1) it requires multiple sampling of blood for determination of the AUC0-4h, (2) the actual value requires a mathematical calculation step, and (3) the test may be too expensive for many clinical hospitals or institutions because of the use of added costly laboratory tests for CsA concentrations and the subsequent increase in workload. Therefore, the search for a single blood-sampling point that best reflects the sensitivity of AUC0-4h was the focus of several research initiatives that resulted in a broad approval for C2 monitoring [82,85]. This method is done by measuring either the area under the blood CsA concentration-time curve in the first hours after dose, AUC0-4 or, more simply, by measuring the blood CsA concentration at 2 hours after dose, C2 [31].

In the novo patients this monitoring method has led to result in the following clinical benefits compared with trough concentration monitoring [37]: (1) reduced incidence of acute rejection, (2) reduced severity of rejection episodes and (3) reduced incidence of nephrotoxicity.

BAYESIAN FORECASTING

The initial pharmacokinetic models for CsA were complicated by the nonlinear, segmented, zero order absorption of the drug from the gut [26,38].

Bayesian forecasting is a TDM tool that has been successfully used clinically in the monitoring of drugs that have a narrow therapeutic index, including antiepileptic drugs, theophylline and amino glycosides; however, although Bayesian forecasting has proven useful clinically with other drugs, this is not the case with CsA [27]. Bayesian forecasting, in its modern form, was first

proposed in 1979 by Sheiner et al. [39].Since that time, user-friendly computer programs that perform this technique have become widely available. These programs are capable of calculating dosage regimens and pharmacokinetic parameters, as well as predicting drug concentrations by blending population values with patient-specific values [27].

However, these methods were technically complex and were not practical or successful for individualizing CsA therapy in a routine clinical setting and therefore did not gain widespread use. The introduction of Neoral, with its less variable and more predictable blood concentration profile, has rekindled interest in the pharmacokinetic modeling of CsA and in the use of Bayesian forecasting to predict CsA blood concentrations [26].

TACROLIMUS (TAC)

The therapeutic range for TAC used by most transplantation centers is 5-20 ng/mL in blood. Although, plasma TAC concentrations have been measured and an equivalent therapeutic range in this matrix suggested (0.5-2 ng/mL), the two most widely used assays for the drug use blood samples. Because this drug is extensively bound to erythrocytes, blood concentrations average about 15 times greater than concurrently measured serum or plasma concentrations [6,28,40].

As a result, whole blood has become the principal sample used for TAC concentration monitoring, with extraction accomplished through cell lysis and protein denaturation steps that are similar or identical to those used for CsA analysis [41].

The pharmacokinetics of TAC is highly variable. Since TAC shares many of the pharmacokinetic and pharmacodynamic problems associated with CsA the rationale for TDM is similar. Although the feasibility of a limited sampling scheme to predict AUC 326 Current Issues and Future Direction in Kidney Transplantation has been demonstrated, as yet, through or predose whole blood concentration monitoring is still the method of choice [3].

THERAPEUTIC MONITORING

TAC whole-blood through concentrations have been found to correlate well with the area under the concentration-time curve measurements in liver, kidney and bone marrow transplant recipients (r= 0.91-0.99). Thus, through concentrations are good index of overall drug exposure, and are currently used for routine monitoring as part of patient care post transplantation [40,42].

This approach offers the opportunity to reduce the pharmacokinetic variability by implementing drug dose adjustments based on plasma/blood concentrations. Drug levels are obtained as predose (12 hours after previous dose) trough concentrations in whole blood [37]. These trough levels correlate reasonably well with area under the curve, with total area under the curve being an accurate measure of drug exposure [43].

Therapeutic ranges of TAC after kidney transplantation are reported as a range for various times after transplant: 0-1 month, 15-20 μg/L; 1-3 months, 10-15 μg/L; and more than 3 months,

5-12 μg/L [44]. TAC blood concentrations are monitored 3 to 7 days a week for the first 2 weeks, at least three times for the following 2 weeks, and whenever the patient comes for an outpatient visit thereafter [45].

On the basis of the terminal half-life of TAC, it was suggested to start monitoring blood concentrations 2 to 3 days after initiation of TAC treatment after the drug has reached steady state. However it is important to reach effective drug concentrations early after transplantation to decrease the risk of acute rejection and to avoid excessive early calcineurin inhibitors concentrations that may be severely damaging after reperfusion of the transplanted organ [46].

The frequency of TDM of TAC should be increased in the case of suspected adverse events or rejection, when liver function is deteriorating, after dose adjustments of the immune suppressants, change of route of administration, or change of drug formulations, when drugs that are known to interact with CYP3A or P-gP are added or discontinued, or when their doses are changed, in case of severe illness that may affect drug absorption or elimination such as severe immune reactions and sepsis, or if noncompliance is suspected [47].

MYCOPHENOLIC ACID (MPA)

In 1995, for preventing rejection in renal transplant patients, MMF, the morpholinoethyl ester prod rug from MPA was approved for clinical use. This drug has since become the predominant anti-metabolite immunosuppressive used in the transplant setting. Although the current labeling information for MMF does not indicate any need for therapeutic monitoring of plasma MPA concentrations, there were a number of studies showing a relationship between MPA pharmacokinetics and clinical outcome [48].

Definitive determination of the pharmacokinetics of the drug in renal allograft recipients after transplantation is not without difficulty. In principle, substantial changes in pharmacokinetics could be produced by changes following transplantation, both in the immediate post-transplant period (reflecting rapid alterations in drug therapy, renal function, hemodynamics and gastrointestinal motility) as well as more gradual changes (reflecting change in bodyweight, plasma proteins and organ function) [49].

The greatest variability in MPA pharmacokinetic is noted in the initial 2 months following transplantation, when adequate immunosuppression is critical to graft function and survival. It has also become apparent from longer term pharmacokinetic studies that exposure to MPA increases over time due to reduced clearance of the drug. A possible additional factor that could contribute to the higher oral clearance of MPA early after transplantation is corticosteroid therapy, which is significantly higher in that period but then is tapered to low dose levels or completely withdrawn. Based upon the marked pharmacokinetic variability observed with MPA and the pharmacodynamic relationship of pharmacokinetic parameters to rejection outcome, several scientific societies and consensus conferences have advocated the use of concentration monitoring for patients undergoing treatment with MMF or enteric-coated MPA [50].

THERAPEUTIC MONITORING

The incorporation of MMF into immunosuppressive regimens has been associated with decrease rates of acute rejection and decreased chronic allograft loss. Indications for TDM of mycophenolates were reviewed in a consensus meeting [50].

They included high-risk patients, patients with delayed graft function, or patients with immunosuppressive protocols excluding induction therapy or steroids or calcineurin inhibitor or patients with calcineurinminimization. Most of these patients (especially high-risk patients) are often excluded from the clinical trials. In fact, MPA TDM is currently only used in a few transplant centers on a routine basis, whereas a few others only checked MPA exposure in case of unexpected acute rejection or adverse event or drug interaction. Most of the centers never measure MPA. It is clear that the use of MPA TDM is conditioned by the faith of the physicians in its use, local availability of MPA measurements, and organization of the nursing staff [51].

TROUGH CONCENTRATION (C_0) MONITORING

Although a relationship between AUC and outcome exists, the clinical utility of concentration monitoring, particularly C_0 monitoring for MMF, has been questioned. Over the past decade, several studies were conducted to evaluate the clinical utility of prospective concentration controlled MMF therapy. While these studies were anticipated to fully clarify the utility of monitored MMF therapy, the outcomes from these studies are conflicting and have done little to settle the controversies surrounding this area of therapeutic drug monitoring [49].

With trough concentration, plasma concentration of MPA is measured immediately before a dose, it is easy to measure because only -ask patient to return to give sample, it is immediately before a dose, and only requires single simple possible association between C_0 and decreased rejection noted in transplant recipients. However this method represents some disadvantages. Timing may not be accurate (depends on remembering time of last dose). Timing may vary from the “ideal” (12 h after last dose) by several hours. There is no high-level evidence of a strong association between C_0 and outcome, or between C_0 and AUC0-12, C_0 is not a very informative time point for estimation of individual pharmacokinetic parameters. Single time-point samples such as the trough concentration or others do not correlate well with the MPA AUC, especially in the early post transplantation period [52].

LIMITED SAMPLING STRATEGIES FOR ESTIMATION OF MPA AUC

The dose interval MPA AUC0-12 h is generally regarded as the most reliable pharmacokinetic parameter index of risk for acute rejection but is impractical to measure in routine clinical practice. Single time-point samples such as the trough concentration or others, do not correlate well with the MPA AUC, especially in the early post transplantation period renal transplant patients and for regimens that include MMF plus CsA, TAC, or SRL [53].

Therefore, assessment of whether C_0 concentrations or other single time points correlate well with the AUC is important for establishing routine monitoring of the drug. Apart of the C_0 level other single time points after MMF dosing are examined for their ability to predict full AUC values. A full MPA AUC typically requires at least eight blood samples during 12 hour dose interval. In clinical practice this is impractical; therefore, abbreviated sampling schemes involving the collection of there to five plasma samples have been investigated. The abbreviated sampling approach has provided estimations of MPA AUC with high correlations (r2>0.8). Several models have been developed all of them in renal transplant patients [54-57].

SIROLIMUS (SRL)

SRL (formerly known as rapamycin) is a macrolide antibiotic with immune suppressive properties that was introduced relatively recently (September 1999) into clinical practice for maintenance therapy in organ transplantation [58].

Pharmacokinetics studies of SRL in renal transplant patients have been shown great variability between patients. Several features contribute to the inter patient pharmacokinetic variability observed with SRL and can include any combination of the following: absorption, distribution, metabolism and/or excretion [59].

This drug presents a rapid gastrointestinal absorption (tmax from 0.33 to 5 hours) as well as alow (mean value 14%) and variable bioavailability. It has been reported that SRL is a substrate for the multidrug P-glycoprotein transporter and that the biotransformation of SRL is mediated by CYP3A enzymes. Accordingly, considerable variability in its pharmacokinetic parameters may be expected (apparent blood clearance rates after oral administration from 87to 416 mL/h/kg). In addition, the disposition of SRL in humans includes a large volume of distribution, a long half-life (35 to 95 hours) and dose proportionality for Cmax and AUC. Also, some interracial variability and an influence of hepatic dysfunction have been noted with SRL [60].

Although structurally similar to TAC, SRL has a novel mechanism of action, which leads to synergy with CsA. The long half-life of the drug necessitates a loading dose to achieve therapeutic concentrations quickly, and also allows for once daily administration. Highly variable absorption and metabolism of the drug result in large differences in blood concentrations among patients receiving the same dose. Efficacy for the prevention of acute rejection episodes, and the rate of common adverse effects (thrombocytopenia, leucopenia and hypertriglyceridemia),are concentration-dependent [61].

THERAPEUTIC MONITORING

Clinical data suggest that the immunosuppressive efficacy and the occurrence and severity of adverse effects of SRL correlate with blood concentrations [61].

Drug interactions with concomitant immunosuppressant medications will alter SRL whole blood concentrations. The appropriate SRL through concentration at steady state (Cmin,ss) for acute rejection episode prophylaxis is a function of the concomitant immunosuppressive regimen.

When it is used asbase therapy with azathioprine and prednisone, a regimen stipulating initial Cmin,ss values equal to 30 μg/L during the first 2 months, and 15 μg/L(LC/UV assay) thereafter, led to a 41%rate of acute rejection episodes among 41 cadaveric kidney transplant recipients [62].

When combined with MMF and prednisone, this SRL regimen was associated with a 27.5%rate of acute rejection episodes among 40 cadaveric renal transplant recipients. Indeed, the combination of SRL (Cmin,ss of 10 to 20 μg/L; LC/UV assay) and basiliximab with late introduction of low dosage CsA has provided excellent prophylaxis of acute rejection episodes and renal function for primary, non-African-American recipients of cadaveric kidney transplants that displayed delayed graft function [61,63,64].

In purely Caucasian low-risk liver and kidney-pancreas transplant recipients, Cmin,ss of 6 to12 μg/L (IMx® assay) in combination with low dosage TAC has been reported to yield low rates of acute rejection episodes and toxicity [65].

Because of the long half-life and extensive tissue distribution of the drug, steady-state concentrations are not reached before day 6 after initiation of therapy or after a dosage change. Thus, daily concentration monitoring is not necessary; the first SRL measurements should not be obtained before day 4 after inception of,or change in therapy. Thereafter, recommend monitoring Cmin,ss weekly for the first month and bi-weekly for the next month, targeting a 5 to 15 μg/L range if CsA is being used concomitantly at Cmin,ss concentrations of 75 to 150 μg/L. If the patient fails to attain these values despite a dosage of 20mg/day, a full pharmacokinetic study should be performed to assess whether the defect is due to limited absorption or rapid clearance rates [61]. Modest correlation($r = 0.59$) exists between SRL dose and peak plasma concentration (Cmax) or AUC, but a good correlation ($r = 0.85$) exists between trough concentration prior to the dose (minimum Cmin,ss and AUC. For this reason, Cmin,ss is a simple and useful index for therapeutic monitoring of SRL [61,66,67].

EVEROLIMUS (EVL)

In April 2010, EVL, a more water-soluble analog of SRL was approved for use in CsA-sparingregimens, including the requirement for adjusting EVL doses using target trough blood concentrations in renal transplant patients [51]. EVL, which has greater polarity than SRL, was developed in an attempt to improve the pharmacokinetic characteristics of SRL, particularly to increase its oral bioavailability. After a single oral dose of EVL 4mg in 12 healthy volunteers, it was absorbed rapidly (within 30 minutes after drug intake). The Cmax of EVL amounted to 44.2 ± 13.3 μg/L and was reached (t_{max}) after 30 minutes (range 0.5–1 hour). The AUC was 219± 69 μg*h/L. The overall absorption of EVL, like that of SRL, is probably affected by the activity of P-gp. It is recommended that patients should take the drug consistently with or without food to reduce fluctuations in drug exposure [68].

In an international study, the pharmacokinetics of EVL, were characterized over the first 6 months post-transplant in 731 patients receiving either 0.75 or 1.5mg bid EVL in addition to CsA and corticosteroids. The within- and between-patient variability of dose interval AUC was 27% and 31% respectively. There was no detectable influence of sex, age (16–66 years), or weight (42-132 kg) on AUC, but EVL exposure was significantly lower by an average of 20 % in blacks. In a study of 659 AUC profiles the correlation between trough concentration and overall exposure (AUC) there was a significant linear correlation with a regression coefficient of 0.89 and corresponding coefficient of determination of 0.79 [69].

For example, Buddeet. al., reported that multiple daily dosing of EVL, in doses up to 5 mg/day, isadequately well tolerated as add-on therapy in stable renal transplant patients receiving maintenance Neoral® immune suppression. Similar degrees of correlation between EVL trough concentration and thrombocytopenia, leukopenia, hypertriglyceridemia, or hyper cholesterolemiain 54 stable renal transplant patients (18-68 years) were found [70].

THERAPEUTIC DRUG MONITORING

EVL is a drug with a narrow therapeutic index. The limited and variable bioavailability, intrinsic inter individual pharmacokinetic variability, the number of factors affecting the pharmacokinetics, and the number of drug interactions limits the use of fixed doses of this drug. The EVL Cmin is a good surrogate marker of EVL exposure (AUC), and correlates with pharmacological response and clinical outcomes. Therefore, prospective dose adjustments to obtain and maintain a therapeutic EVL Cmin have the potential to improve efficacy and reduce toxicity [71].

A role for EVL drug monitoring has been suggested because of the potential for improving efficacy and reducing adverse effects, the EVL Cmin is a good surrogate marker of EVLexposure (AUC), and correlates with pharmacological response and clinical outcomes. Therefore, prospective dose adjustments to obtain and maintain a therapeutic EVL Cmin have the potential to improve efficacy and reduce toxicity [72].

Mere clinical monitoring of efficacy is insufficient because clinical presentations of graft rejection vary for each patient and are nonspecific. Thus, some authors have used a previously published 9-step decision-making algorithm to evaluate the utility of TDM for EVL. The recommended therapeutic range for EVL is a trough concentration of 3 to 8 ng/mL, as concentrations over 3 ng/mL have been associated with a decreased incidence of rejection, and concentrations >8 ng/mL with increased toxicity. Patients on EVL who have problems with absorption, who take concurrent cytochrome P450 inhibitors or inducers, or are noncompliant will attain the greatest benefit from therapeutic drug monitoring [73].

PERSPECTIVES OF TDM AND PHARMACOGENETICS

Utilization of pharmacokinetic principles and dose-concentration relationships are central to TDM in optimizing drug dosing to target concentrations; at the present time we are entering

an era in which combination therapy will be the norm and clinicians will tailor the immune suppression to the characteristics of the individual patient, changing dose and drugs as time progresses and conditions change. Individualizing a patient's drug therapy to balance therapeutic efficacy and adverse events has become an important goal for transplant physicians. TDM with subsequent dose adaptation is an indispensable tool to target CNIs to their therapeutic window and is now universally applied. However, although the use of TDM limits the time a patient is exposed to supra- or sub-therapeutic concentrations, it essentially remains a reactive, trial-and-error approach and has no predictive value. Pharmaco genetics, on the other hand, may be of help in predicting an individual's response to a given drug and can be applied before the start of pharmacotherapy [74,75].

The major promise of pharmacogenetics lies in its potential to identify high risk patients who would benefit from an alternative dosage and/or drug regimen prior to the start of therapy. Given its pro-active nature, pharmacogenetics may thus be a potential complementary tool to TDM for optimizing immunosuppressive treatment thereby improving effectiveness and reducing adverse drug reactions [74].

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