MYCOPHENOLATE MOFETIL: PHARMACOKINETIC STRATEGIES FOR OPTIMIZING IMMUNOSUPPRESSION

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Traditionally, the therapeutic response of patients to most new drugs has been evaluated by comparison of a single dose group with either a placebo group or one or more additional dose groups. The randomized dose-controlled clinical trial often leads to large variability in response within comparison groups, due to extensive interindividual **pharmacokinetic (PK) variability** and at the same time extensive interindividual **pharmacodynamic (therapeutic response) variability**. Thus understanding the factors which account for patient therapeutic response variability is hampered by the lack of control of drug concentration when both PK and PD variability are significant.

An approach to establishing a firmer scientific foundation for the relationship between active drug concentration and patient response is the **Randomized Concentration-Controlled Clinical Trial (RCCT)** /1-3/. In this experimental design for clinical trials the following are important features:

1. Study patients are randomly assigned to predetermined ranges of plasma or blood concentration.
2. Within each study patient dosage adjustments to achieve the assigned target concentration are based on a PK-controlled dosing scheme derived from prior experience.
3. An essential requirement for the success of this trial design is the use of validated analytical methodology and appropriate turn-around times to permit effective dosage adjustments.
4. If plasma (or blood) concentrations of active drug relate more strongly to response to the drug than dosage, the RCCT design can decrease response variability by reducing the variability in plasma concentrations within each study group.

I believe the RCCT experimental design is particularly appropriate in the evaluation of new immunosuppressive drugs for the following reasons:

1. The pharmacokinetic behavior of all immunosuppressive drugs studied to date is highly variable in transplant patients, particularly in the early post-transplant period.
2. In transplant patients the important overt clinical outcomes are discrete (e.g., acute rejection occurs or does not occur). The outcomes in these patients are not continuous like blood pressure is in evaluating antihypertensive drugs.
3. The concentration-effect study design is prospective and, by controlling PK variability, provides a stronger cause-effect foundation for the observed link between plasma (or blood) concentration and the outcome response.
4. In the past for immunosuppressive drugs such as cyclosporine A (CsA) as well as for other drugs for which therapeutic drug monitoring is essential most TDM strategies were based on retrospective data comparing concentrations with effects. The retrospective data are important and valuable for forming a hypothesis as to the most appropriate therapeutic range and dosing strategy.

The RCCT design goes one step further by formally testing the hypothesis thereby providing greater insight into the PK-outcome relationship, invaluable experience with a dose-adjustment strategy and a stronger scientific basis for deciding on a TDM strategy.

I consider next two recent studies which used the RCCT experimental design in renal transplant patients. The first is a study of tacrolimus concentration vs outcome. This is followed by a description of an RCCT study of mycophenolate mofetil (CellCept).

TACROLIMUS STUDY

120 renal transplant patients were enrolled in this multicenter (five centers), open label randomized clinical trial /4/. The patients were randomized to one of three target blood concentration ranges: low, middle and high. Each participating center used an ALG induction protocol with tacrolimus, azathioprine and prednisone as maintenance

immunosuppression. The incidence of acute rejection and of toxicity requiring tacrolimus dosage adjustment were the outcomes measured at 42 days following transplant surgery.

An ELISA immunoassay was the primary methodology used for measurement of tacrolimus concentration in blood. Each participating center measured the blood concentrations and dosage adjustments were made based on local experience with tacrolimus dosing. The analytical performance of the method for measuring tacrolimus is summarized in Table 1, showing the range of precision obtained by the participating centers for a common set of proficiency testing samples used throughout the study.

TABLE 1

Tacrolimus measurement

1. An enzyme linked immunosorbent immunoassay was used for analysis of tacrolimus in blood.

2. The interassay coefficients of variation for study centers enrolled in the concentration-ranging tacrolimus trial were:

   6.3 to 23.1% at 4 μg/l
   7.1 to 19% at 15 μg/l
   4.5 to 14.2% at 60 μg/l

In this study there was a general tendency to underdose patients such that the target ranges achieved were lower than originally intended. Nevertheless post-hoc analysis of the data using the lower ranges, summarized in Table 2, show that the relationship between increasing blood concentration of tacrolimus and (A) the decreasing rate of rejection, and (B) the increasing rate of toxicity were both statistically significant /4/.

TABLE 2

The incidence of rejection and of toxicity after post-hoc analysis of concentration-effect data in renal transplant patients receiving tacrolimus-based immunosuppression /4/

Tacrolimus blood concentration range	Rejection	Toxicity
0 - 5 µg/l	34%	0%
5-15 µg/l	17%	34%
> 15 µg/l	5%	54%

MYCOPHENOLATE MOFETIL STUDY

Mycophenolate mofetil, the pro-drug, is rapidly converted by esterases in blood and tissues to MPA, the pharmacologically active form of the drug. MPA is converted to the glucuronide metabolite primarily in the liver. The major features of MPA pharmacokinetics are summarized in Table 3. The primary driving processes believed responsible for the MPA area-under-the-plasma-concentration-time-curve (AUC) are clearance by the liver and an enterohepatic circulation involving: delivery of the glucuronide into the gut via bile flow, conversion of MPAG by the action of gut flora glucuronidase back to MPA, and subsequent gastro-intestinal absorption of MPA into the bloodstream.

TABLE 3

Major features of mycophenolic acid pharmacokinetics

1. Clearance of MPA by the liver and glucuronidation followed by enterohepatic circulation of the glucuronide metabolite MPAG are the primary processes responsible for the MPA area-under-the-concentration-time curve (AUC).
2. The average bioavailability of MPA is 94% in normal individuals and renal transplant patients.
3. For renal transplant patients, early after transplant surgery, on a fixed dose of CellCept, there is a more than 10-fold range in the 12 hour dose-interval MPA AUC.
4. MPAG is the sole metabolite of MPA and is pharmacologically inactive.
5. MPA binds avidly to HSA (avg 97%); the free fraction is the pharmacologically active fraction.

The average bioavailability of MPA is high - 94% - in normal and renal transplant patients /5/. However, the PK of MPA is significantly variable from patient to patient. For renal transplant patients early post-transplant on a fixed 2 g/day dose of CellCept there is a greater than 10-fold range in the 12-hour dose interval MPA AUC /5/. Correction of the AUC values for patient weight did not lessen the magnitude of the AUC range /6/. A possible reason for the latter observation could be a lack of correlation of the enterohepatic circulation pathway with subject weight /6/.

MPA binds avidly to human serum albumin and the free fraction has been shown to be the pharmacologically active fraction in *in vitro* tests of immunosuppressive activity /7/. Studies are in progress to assess the practical pharmacokinetic significance of the free fraction of MPA in several patient populations.

A multicenter, double blind, randomized concentration-controlled clinical trial was developed to test the hypothesis that increasing MPA AUC reduced the risk for rejection relative to what it would be in the absence of MPA exposure in renal transplant patients. As summarized in Table 4, the hypothesis was based on observations of a correlation between risk for rejection and the MPA dose-interval AUC in a Japanese clinical trial and later confirmed in other clinical trials /5,8/.

TABLE 4

The CellCept randomized concentration-controlled clinical trial

The following were the bases for this trial:

1. The strong correlation between risk for rejection and drug exposure (MPA dose-interval AUC) observed in a Japanese trial involving renal transplant patients (NSK24/100) receiving CsA-based immunosuppression and later confirmed in the US trial (MYCO 1866);
2. Development of a three parameter logistic model of risk for rejection (relative to immunosuppression without CellCept) vs natural log of MPA AUC;
3. Development of a dosage adjustment strategy for the trial.

Given the discrete nature of the measured clinical outcomes a logistical model for the risk for rejection as a function of the dose interval MPA AUC was judged to be the most appropriate model for

this study /5/. A dosing strategy for achieving target concentrations in the planned RCCT was also developed. The CellCept RCCT was a randomized, double-blind multicenter phase III study of 24 weeks duration and sponsored by Roche Bioscience /9/.

150 renal transplant patients were randomly assigned to one of three MPA AUC target range groups and studied concurrently. All patients received triple therapy including CellCept, cyclosporine and prednisone. CsA dosing was regulated according to the trough CsA blood concentration TDM strategy in use at each participating center. CellCept dosage adjustments were made based on linear pharmacokinetics: on post-op day 3 an initial 12 hour MPA AUC was determined and on day 5 dosage adjustments, if needed, were made based on the day 3 AUC. MPA AUCs were obtained at 1 week, 3 weeks and then at 4 week intervals thereafter for the duration of the study. MPA analysis in appropriately timed plasma samples were performed in a central reference laboratory by a validated HPLC method specific for MPA (Table 5) /10/. This method had been in use for the prior 3 years in at least five contract laboratories and two North American research laboratories with good analytical performance as reflected in proficiency test samples.

TABLE 5

Mycophenolic acid analysis

1. A validated HPLC method for the specific measurement of MPA and MPAG was developed.
2. Over a 3 year period this method was used in at least five contract laboratories and two North American research laboratories.
3. The overall results for control samples provided to each of these laboratories: MPA concentration in spiked plasma samples ranged from 0.2 to 36 mg/l; CV <5% up to 11%.

The results of this study were reported briefly /8/ and will be fully described at future scientific meetings. The outcome data verified the hypothesis that the risk for rejection, relative to what it would be using the immunosuppression regimen in the absence of CellCept, was significantly related to the 12 hour dose interval MPA AUC. The

incidence of acute rejection was below 10% in the middle target range group. The free fraction of MPA was measured in these study patients and this parameter is now under evaluation for its significance as a predictor of outcome.

At our institution we are using the results of the mycophenolate mofetil RCCT as the basis for a prospective study in renal transplant patients. Each patient enrolled is targeted to an MPA AUC range selected on the basis of earlier PK-PD studies and the RCCT study. The dosage of mycophenolate mofetil is guided by either MPA AUC or pre-dose concentration target ranges. The AUC variances for each sub-group will be compared to determine whether the pre-dose strategy will be effective compared to the AUC-based strategy for regulating mycophenolate mofetil dosage.

It is hoped that studies such as these will lead to a strengthening of the scientific basis for deciding on a therapeutic drug monitoring strategy for immunosuppressive drugs.

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