

# Chapter IV

## Materials and Methods

### Materials

#### 1. Apparatus

- 1.1) Automated Fluorescence Polarization Analyzer  
(Diagnostic Division, Abbott Laboratories, Inc., Irving, TX, USA.)
- 1.2) Vortex
- 1.3) TDx<sup>®</sup> centrifuge

#### 2. TDx<sup>®</sup> Cyclosporin monoclonal whole blood kit

- 2.1) Cyclosporin monoclonal whole blood calibrators
- 2.2) Cyclosporin monoclonal whole blood controls
- 2.3) Cyclosporin monoclonal reagent pack
- 2.4) Solubilizing reagent
- 2.5) Precipitation reagent
- 2.6) Dilution buffer
- 2.7) Sample cups
- 2.8) Cuvettes
- 2.9) Centrifuge tubes

#### 3. Medical equipment

- 3.1) 5 ml Syringes
- 3.2) 23 G Needles
- 3.3) 22 G Cathelons
- 3.4) Injection plugs
- 3.5) Normal Saline
- 3.6) Heparin 5,000 IU
- 3.7) 3 ml EDTA containing tubes
- 3.8) Others: Gauze, Micropore, Alcohol, etc.

## **Patients and Method**

### **1. Patient selection**

Renal transplant patients who consented and fulfilled the following entry criteria were studied: patients with more than 6 months of follow-up at Chulalongkorn hospital, 20-65 years of age, receive CsA microemulsion formulation (Sandimmun Neoral<sup>®</sup>) twice daily, and no evidence of significantly abnormal liver function.

### **2. Methods**

The pharmacokinetic profiles were determined on patients in steady state, which is normally reached after the third day of the same CsA oral dose administration. Blood samples (3 ml) were obtained before their morning dose of CsA and then 1, 2, 4, 6, 8, and 12 hours after dosing. The samples were collected in tubes containing EDTA as the anticoagulant. All whole blood samples were stored at room temperature not more than 24 hours before they were assayed by specific-monoclonal antibody Fluorescence Polarization Immunoassay (FPIA, TDx<sup>®</sup>, Abbott Diagnostics).

The routine biochemical measurements collected for patient monitoring and the available patient data were recorded.

### **CsA Assay**

The FPIA assay was performed in a manner designed by the manufacturer (Abbott Diagnostics, TDx<sup>®</sup> Fluorescence polarization analyzer, Irving, TX, USA) as follows.

- Step 1. Let sample, control and reagent come to room temperature
- Step 2. Mix the above components by gentle inversion.
- Step 3. Perform the following for each sample and control.
  - 3a. Accurately pipette 150  $\mu$ l of sample or control into centrifuge tube
  - 3b. Accurately pipette 50  $\mu$ l of solubilizing reagent into centrifuge tube
  - 3c. Accurately pipette 300  $\mu$ l of precipitation reagent into centrifuge tube
- Step 4. Cap and vortex each centrifuge tube for 10 seconds. Then place centrifuge tubes evenly balanced into TDx<sup>®</sup> centrifuge
- Step 5. Centrifuge all centrifuge tubes for at least 5 minutes.
- Step 6. Verify the presence of a protein pellet in each centrifuge tube.

Step 7. Decant each centrifuge tube in the corresponding sample cup on the TDx<sup>®</sup> carousel

Step 8. Place carousel with sample cups and cuvettes into TDx<sup>®</sup> analyzer along with TDx<sup>®</sup> Cyclosporin monoclonal reagent pack. Close door, then press the run button

## Pharmacokinetic and Statistical Analysis

The area under the blood concentration-time curve (AUC) for each patient was calculated by linear trapezoidal rule from the seven concentrations in the full profile (0, 1, 2, 4, 6, 8, and 12 hours). The average steady-state concentration ( $C_{ssav}$ ) was calculated as  $AUC/\tau$ , where  $\tau$  is the dosing interval. The highest measured blood concentration and the corresponding sampling time were defined as  $C_{max}$  and  $t_{max}$  respectively. Two trough levels were measured, before drug administration ( $C_{min, 0 \text{ hr}}$ ) and 12-hour after drug dosing ( $C_{min, 12 \text{ hr}}$ ). To detect any significant difference between  $C_{min, 0 \text{ hr}}$  and  $C_{min, 12 \text{ hr}}$ , paired-samples T Test was done. Half-life ( $t_{1/2}$ ) was determined by the equation:  $t_{1/2} = 0.693/\beta$ , where  $\beta$  is the terminal slope of the linear least-squares regression line of a semilogarithmic plot of blood concentration VS time. Non-compartmental analysis was used to compute clearance (Cl/F) and apparent volume of distribution (Vd/F), according to the following equations:  $Cl/F = \text{Dose}/AUC$ ,  $Vd/F = Cl/\beta$ , where Cl is clearance and F is a bioavailability factor.

Use two methods to select the optimum sampling times. (1) Perform multiple linear regression analysis to create a formula for the AUC prediction. (2) Select time point(s) that yield a good predictive AUC when calculate by linear trapezoidal rule (Appendix A). Multiple linear regression was done by the AUC was used as the dependent variable and the blood concentrations grouped by time as the independent variables.

To select 2 sampling time points to calculate AUC by trapezoidal rule,  $t_{max}$  was usually chosen first as the first time point to be included since the peak level is theoretically known to be correlated well with AUC. Another time point was determined according to the pair of samples which gave the best prediction of AUC, that is, the two sampling time points which could predict AUC with least prediction error.

To select 3 sampling time points to calculate AUC by trapezoidal rule, again  $t_{max}$  was the first sampling time point to be chosen. Then, another two time points were determined by compared the prediction error of all

combination and selected those sampling time points which yielded the best prediction of AUC in term of least prediction error.

Pearson product-moment correlation coefficients were calculated to evaluate the linear relations between CsA dose, AUC and blood concentration at a given time including average concentration at steady state ( $C_{ssav}$ ). The correlation between the predicted and measured AUC was evaluated by the prediction error calculated as follows:

$$\text{Prediction error} = \frac{\text{Predicted AUC} - \text{Measured AUC}}{\text{Measured AUC}} \times 100\%$$



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