

## Cyclosporine Plasma Unbound Fraction in Heart and Lung Transplantation Recipients

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### Summary:

To investigate the variability in the unbound fraction ( $f_U$ ) of cyclosporine in recipients of heart, heart-lung, and lung transplantation, cyclosporine  $f_U$  was determined *ex vivo* in plasma by equilibrium dialysis. In a retrospective study, 260 samples of plasma (one to seven per patient) were obtained from 89 heart (86%), lung (9%), and heart-lung (5%) transplant patients. The unbound fraction ( $\times 100$ ) of cyclosporine ranged from 0.52% to 3.94%, with an overall mean of  $1.53\% \pm 0.375\%$  (SD). The mean percentage unbound for individual patients ranged from 0.71% to 1.98%, giving a 2.8-fold interpatient variation. In heart transplant recipients (66 patients), the values of  $f_U$  were significantly lower ( $p < 0.01$ ) during more severe rejection episodes, which required antirejection treatment (endomyocardial biopsy result of grade 3a and higher) than in the absence of rejection (grade 0) or during grade 1a rejections. The value of  $f_U$  did not vary with organ transplanted ( $p = 0.35$ ) or etiology of organ failure ( $p = 0.32$ ). Cyclosporine  $f_U$  was negatively correlated with the age of the patient ( $r = -0.18$ ,  $p < 0.05$ ). Correlations were not observed between  $f_U$  and blood biochemical and cytologic indices. However,  $f_U$  was significantly lower ( $p < 0.01$ ) in hypercholesterolemic transplant recipients ( $1.37 \pm 0.52\%$ ) than in normocholesterolemic patients ( $1.60 \pm 0.63\%$ ). Administration of simvastatin resulted in a significant increase in the mean  $f_U$  from  $1.40 \pm 0.09\%$  to  $1.82 \pm 0.13\%$  (paired *t* test,  $n = 13$ ;  $p < 0.01$ ). In patients who received ketoconazole,  $f_U$  was not different from controls. These findings suggest that the level of cyclosporine  $f_U$  may be an important determinant immunosuppressive activity of cyclosporine. Moreover, the variation in  $f_U$  could be strongly related to the concentration of serum lipoproteins; interpretation of the results of cyclosporine monitoring thus requires consideration of the lipidemic status of the patient.

The immunosuppressive agent, cyclosporine, is highly lipophilic and extensively bound to blood and plasma components (1). In plasma, it binds mainly to lipoproteins, including very low density lipoprotein (VLDL) (10%), low density lipoprotein (LDL) (35%), and high density lipoprotein (HDL) (33% [2]), leaving approximately 2% unbound (3,4).

Unbound drug concentration is an important determinant of the pharmacokinetic and pharmacodynamic characteristics of pharmacologic agents (5). Variation in the fraction unbound ( $f_U$ ) may alter pharmacokinetic characteristics, including clearance and volume of distribution (6). Pharmacologic effect of pharmaceutical agents is usually thought to depend on their concentration at the receptor site, which is directly related to the concentration of unbound drug in plasma. Therefore, changes in the concentration of unbound drug in plasma may have a significant impact on the clinical effect of a drug; that is, an increase in the plasma protein binding may decrease the clinical efficacy of the medication (7). Levy and Moreland (8) suggested that monitoring of unbound concentration should be considered for highly protein bound drugs with a narrow therapeutic index that require routine therapeutic monitoring of total concentration.

The measurement of cyclosporine  $f_U$  is inherently difficult and laborious because of cyclosporine's extensive nonspecific binding to apparatus. To our knowledge, the only comprehensive investigation of cyclosporine  $f_U$  was performed by Lindholm and colleagues in 66 renal transplant recipients (3,9,10).

The objectives of this study were to investigate the relationship between cyclosporine  $f_U$  and the incidence of rejection episodes in heart transplantation recipients. Moreover, this study's aim was to examine the variability in cyclosporine  $f_U$  among cardiopulmonary transplant recipients. The cardiopulmonary transplant recipients exhibit different pathologic conditions and receive different medications from those of kidney transplant recipients. These differences may be manifested in the biochemical characteristics of blood and plasma that, in turn, may affect cyclosporine  $f_U$ .

## METHODS

### Patients

Blood samples were obtained from transplant recipients admitted to the Heart and Lung Transplant Unit of St. Vincent's Hospital, Sydney, Australia. Blood was obtained during a randomized clinical trial of ketoconazole as an inhibitor of cyclosporine metabolism (11). The Research and Ethics Committee of St. Vincent's Hospital approved the study and written consent was obtained from each patient participating in the trial. A total of 260 plasma samples (separated from blood at 37°C) was collected from 89 patients, including heart (66), heart-lung (8), heart-kidney (2), and lung (13) transplantation recipients. From one to seven samples were collected from each patient, with a median of three samples per patient. The majority of samples (n = 172) was obtained consecutively from 43 heart transplantation recipients during outpatient visits at 1, 3, 6, and 12 months after transplantation. An additional 88 blood samples were obtained from another 46 recipients of heart, lung, and heart-lung transplants. Blood samples were collected during outpatient visits between 7 a.m. and 9 a.m., coincident with the collection of blood for routine biochemical, cytologic, cyclosporine trough total blood concentration measurements, or all three. Patients were asked to take their cyclosporine dose at 9 p.m. on the day before sampling and to fast overnight. Characteristics of the patient group are shown in Table 1.

| Variable  | Mean $\pm$ SD   | Range      |
|---|-----------------|------------|
| Sex (% male)  | 66              |            |
| Age (yr)  | 47.6 $\pm$ 12.2 | 14–68      |
| Body mass index (kg $\cdot$ m <sup>-2</sup> )                   | 24.2 $\pm$ 3.2  | 15.8–33.6  |
| Time after transplantation (days)                               | 175 $\pm$ 259   | 6–1893     |
| Hematocrit (%)  | 36 $\pm$ 5      | 23–48      |
| Estimated creatinine clearance (mL $\cdot$ min <sup>-1</sup> )* | 65.7 $\pm$ 21.7 | 23.6–144.2 |

\* Calculated from serum creatinine concentration using the Cockcroft and Gault equation (see reference 16).

TABLE 1. Patient characteristics

### Immunosuppression and Other Maintenance Therapy

Routine maintenance immunosuppressive therapy involved cyclosporine, azathioprine, and prednisolone. Before transplantation surgery, cyclosporine (2–3 mg  $\cdot$  kg<sup>-1</sup>) was given (unless there was moderate hepatic or renal impairment), together with 1 g of intravenous (IV) methylprednisolone. An additional 1 g of methylprednisolone was given at the completion of surgery, followed by 125 mg IV every 8 hours for three doses. Oral cyclosporine (8 mg/kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup>) was initiated 24 hours after surgery, together with azathioprine (1.5–2.0 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup>) and prednisolone (1 mg kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup>), reducing dosage to 0.18 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup> by day 14 and 0.10 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup> by 6 months. Cyclosporine dosage was adjusted to maintain whole blood concentrations within the following ranges: 350 to 450  $\mu$ g  $\cdot$  L<sup>-1</sup> in the first 2 months after transplantation, 300 to 400  $\mu$ g  $\cdot$  L<sup>-1</sup> at 2 to 3 months; 250 to 300  $\mu$ g  $\cdot$  L<sup>-1</sup> at 3 to 6 months; 200 to 300  $\mu$ g  $\cdot$  L<sup>-1</sup> at 6 to 12 months, and 150 to 200  $\mu$ g  $\cdot$  L<sup>-1</sup> after 12 months (12).

Patients in this study variously received ketoconazole or diltiazem as cyclosporine-sparing agents, or itraconazole or fluconazole as antimicrobial agents. All heart transplantation patients received trimethoprim (160 mg) with sulphamethoxazole (800 mg) daily for 2 days per week during the first year after transplantation as prophylaxis against *Pneumocystis carinii* pneumonia and toxoplasmosis. Patients with elevated levels of serum cholesterol (> 6.5 mmol  $\cdot$  L<sup>-1</sup>) were treated with lipid-lowering agents, especially HMG-CoA reductase inhibitors, such as simvastatin or pravastatin.

### Clinical Evaluation

Endomyocardial biopsies were performed in heart transplantation patients to diagnose rejection at the following intervals: weekly to 1 month, at the second week to 3 months, thereafter at 4, 5, 6, 9, and 12 months, and at other times on clinical indication. Histologic rejection was graded according to the International Society of Heart and Lung Transplantation classification for cardiac rejection (13). Patients with a grade of 0, 1a, 1b, or 2 rejection did not receive augmented immunosuppression, but patients with higher grades were treated with oral or IV steroids. Resistant or recurrent rejections were treated with antithymocyte globulin or total lymphoid irradiation. The results of endomyocardial biopsies performed within 3 days of sampling for cyclosporine  $f_u$  were used to determine rejection status at the time of sampling.

### Determination of Cyclosporine Unbound Fraction

Cyclosporine  $f_u$  was measured using a previously described equilibrium dialysis method (14) using stainless steel cells to minimize nonspecific binding and high-performance liquid chromatography-purified [<sup>3</sup>H]cyclosporine. The method had an intraday coefficient of variation (CV) of 15% (n = 16).

### Statistical Analysis

Statistical analyses were performed using SPSS for Windows (Release 6.1, SPSS Inc., Chicago, IL, U.S.A.). Unless otherwise stated, all *p* values are based on two-tailed tests and *p* values less than 0.05 are considered significant. The normal distribution of data was verified using the Kolmogorov-Smirnov test for goodness-of-fit. Pearson's bivariate correlation coefficient was used for normally distributed data and Spearman's coefficient for non-normally distributed data. Partial correlation analyses were performed to examine the relationship between a pair of variables while adjusting for the linear effects of one or more additional variables. Significance of a difference between the means of two independent samples was examined using an independent sample *t* test or the Mann-Whitney *U* test. For paired samples, a paired-sample *t* test or the Wilcoxon signed-ranks test was used. Differences between two

or more independent samples were assessed using one-way or two-way analysis of variance. The Bonferroni post hoc test was used for multiple comparisons among individual groups. For non-normally distributed data, Kruskal-Wallis one-way test and Friedman's two-way tests were used.

#### Biochemical and Cytologic Measurements

Measurement of biochemical and hematologic indices was performed by the Department of Chemical Pathology, St. Vincent's Hospital. Hematocrit, hemoglobin concentration, platelet, red blood cell (RBC), and total and differential white blood cell (WBC) counts were measured. Differential WBC counts included neutrophil, lymphocyte, monocyte, basophil, and eosinophil counts. Concentrations of total plasma proteins, albumin, creatinine, glucose, urea, bilirubin, alkaline phosphatase, [gamma]-glutamyl transpeptidase (GGT) and alanine transaminase (ALT) were also measured. Concentrations of serum lipids, including serum total cholesterol, HDL cholesterol, and total triglyceride were measured using standard enzymatic techniques. The LDL cholesterol concentration was estimated using the formula of Friedewald and colleagues (15).

Total cyclosporine levels in whole blood were measured by the Department of Clinical Pharmacology, St. Vincent's Hospital, by the monoclonal TDx method (Abbott Laboratories; Abbott Park, IL, U.S.A.) using a fluorescence polarization immunoassay.

#### RESULTS

The median cyclosporine  $f_U$  over all observations was 1.40%. Values ranged from 0.52% to 3.94%, indicating wide variability (7.5-fold). Mean values for individuals ranged from 0.71% to 1.98%, giving a mean 2.8-fold interpatient variability. The inpatient variability among patients with two or more estimates of  $f_U$  was  $1.96 \pm 0.73$ -fold (mean  $\pm$  SD), ranging from 1.00- to 4.28-fold. Values of cyclosporine  $f_U$  were not normally distributed ( $p < 0.05$ ; Fig. 1).

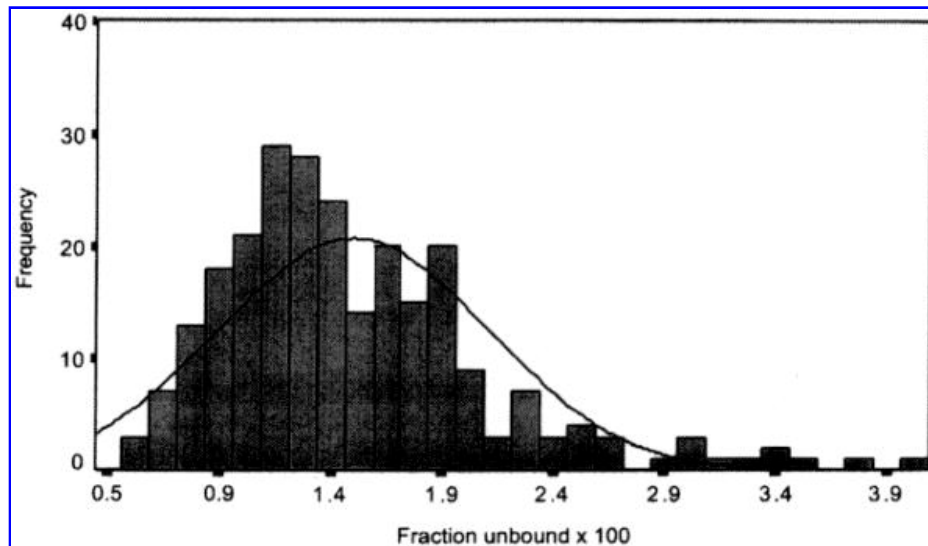


FIG. 1. Distribution histogram of cyclosporine fraction unbound and fitted normal curve.

#### Cyclosporine Fraction Unbound and Cardiac Rejection

Cyclosporine  $f_U$ , total concentration in whole blood, and time after transplantation were examined for a relationship with the grade of cardiac rejection (Table 2). Significant differences in mean  $f_U$  were observed among the three rejection groups ( $p < 0.01$ ). Mean  $f_U$  was significantly lower in patients with Grade 3a and higher rejections (grade 3b and 4), than in those with either Grade 0 or Ia (Bonferroni multiple comparison,  $p < 0.05$ ). On the other hand, the total blood concentration of cyclosporine did not vary significantly with rejection status ( $p = 0.1$ ).

| Grade of endomyocardial biopsy* | Number of occurrences† | Time after transplantation (days) | Total concentration‡ (µg L <sup>-1</sup> ) | Fraction unbound × 100§ (mean ± SD) |
|---------------------------------|------------------------|-----------------------------------|--|-------------------------------------|
| 0 <sup>  </sup>                 | 83                     | 183 ± 123                         | 273 ± 104                                  | 1.64 ± 0.64                         |
| 1a <sup>  </sup>                | 42                     | 168 ± 330                         | 340 ± 167                                  | 1.65 ± 0.60                         |
| 3a and above <sup>#</sup>       | 44                     | 100 ± 93                          | 312 ± 153                                  | 1.34 ± 0.52**                       |

\* International Society of Heart and Lung Transplantation grades.  
† Includes only occurrences when cyclosporine fraction unbound was determined on the same day as or within one day of endomyocardial biopsy.  
‡ Measured in whole blood using monoclonal fluorescence polarization immunoassay.  
§ Three groups are significantly different (Kruskal-Wallis one-way,  $p < 0.01$ ).  
<sup>||</sup> No rejection.  
<sup>||</sup> Focal, perivascular, or interstitial infiltrate without necrosis.  
<sup>#</sup> Multifocal aggressive infiltrates, myocyte damage, or both.  
\*\* Significantly different from grades 0 and 1a (Bonferroni test, log transform,  $p < 0.05$ ).

TABLE 2. Cyclosporine fraction unbound in plasma at the time of cardiac rejection

#### Cyclosporine Unbound Fraction in Different Groups of Transplant Recipients

The mean value of cyclosporine  $f_U$  was not significantly different ( $p = 0.62$ ) between men and women transplantation recipients (mean ± SD was  $1.55 \pm 0.60\%$  for men,  $1.50 \pm 0.57\%$  for women). A negative correlation was observed between age and mean  $f_U$  for individuals ( $r = 0.18$ ,  $p = 0.008$ ), indicating a slight but significant decline in cyclosporine  $f_U$  with age. Significant positive correlations between age and concentrations of serum total ( $r = 0.18$ ) and LDL cholesterol ( $r = 0.17$ ) pointed to an age-dependent increase in serum lipids that may account for the lower  $f_U$  in older patients. No significant correlations were observed between cyclosporine  $f_U$  and patient's body weight, body mass index, or estimated ideal body weight.

Cyclosporine  $f_U$  was not significantly different ( $p = 0.35$ ) among patients receiving different types of transplantation, including heart ( $n = 64$ ,  $f_U = 1.51 \pm 0.38$ ), heart and kidney ( $n = 2$ ,  $f_U = 1.09$ ), heart-lung ( $n = 8$ ,  $f_U = 1.47 \pm 0.38$ ), and lung (single or double) transplants ( $n = 13$ ,  $f_U = 1.38 \pm 0.44$ ). Cyclosporine  $f_U$  was not significantly different ( $p = 0.32$ ) among groups with various etiologies of heart failure, including ischemic heart disease ( $n = 22$ ,  $f_U = 1.43 \pm 0.32$ ), cardiomyopathy ( $n = 39$ ,  $f_U = 1.56 \pm 0.43$ ) or congenital heart disease ( $n = 7$ ,  $f_U = 1.39 \pm 0.21$ ) or lung failure ( $p = 0.86$ ) including emphysema ( $n = 10$ ,  $f_U = 1.33 \pm 0.41$ ), cystic fibrosis ( $n = 6$ ,  $f_U = 1.44 \pm 0.33$ ) and Eisenmenger's syndrome ( $n = 2$ ,  $f_U = 1.42$ ).

One of the patient subjects included in this study became pregnant 9 months after transplantation. Estimated values of cyclosporine  $f_U$  for this patient were 1.55%, 1.26%, and 1.68% before her pregnancy, and 1.31% and 1.90% at the beginning of her pregnancy. The apparent increase in  $f_U$  at the last sampling date was associated with elevated concentrations of serum creatinine and bilirubin ( $0.13 \text{ mmol} \cdot \text{L}^{-1}$  and  $146 \text{ mmol} \cdot \text{L}^{-1}$  respectively), attributable to cholestasis at 5 months of pregnancy. However, little can be concluded from findings in a single patient.

In patients with diabetes mellitus (seven patients), values of  $f_U$  were significantly higher than in nondiabetic patients. The mean  $f_U$  (SD) in seven diabetic patients was  $1.80 \pm 0.51\%$  as compared with  $1.45\% \pm 0.36$  for the remainder of the study group ( $p = 0.05$ ). Furthermore, concentrations of serum bilirubin, ALT, and GGT was significantly higher in these patients (bilirubin,  $19.7 \pm 8.3$  versus  $6.1$ ]  $7.1 \text{ mmol/L}^{-1}$   $p < 0.04$ ; ALT,  $154 \pm 112$  versus  $88 \pm 47 \text{ U} \cdot \text{L}^{-1}$   $p < 0.01$ ; GGT,  $256 \pm 292$  versus  $117 \pm 283 \text{ U} \cdot \text{L}^{-1}$   $p < 0.01$ ). Moreover, in the diabetics patients included in this study, the concentrations of serum total and LDL cholesterol and total triglyceride values were significantly lower than those of the rest of the patients (total cholesterol,  $5.2 \pm 1.16$  versus  $6.5 \pm 1.5 \text{ mmol/L}^{-1}$   $p < 0.01$ ; LDL cholesterol,  $2.63 \pm 0.94$  versus  $3.97 \pm 1.37 \text{ mmol} \cdot \text{L}^{-1}$ ,  $p < 0.01$ ; triglyceride,  $1.81 \pm 0.88$  versus  $2.37 \pm 1.10$ ,  $\text{mmol} \cdot \text{L}^{-1}$   $p = 0.02$ ) despite the fact that these patients were not treated with lipid-lowering agents.

#### Time Posttransplantation

In the first year after transplantation, cyclosporine  $f_U$  increased, showing a positive correlation with time ( $r = 0.29$ ,  $p < 0.005$ ). In the 43 heart transplantation recipients who were sampled successively,  $f_U$  at 12 months after transplantation ( $1.95\% \pm 0.75\%$ ) was significantly higher ( $p < 0.01$ ) from  $f_U$  at 1, 3, and 6 months ( $1.54\% \pm 0.65\%$  at 1 month,  $1.47\% \pm 0.49\%$  at 3 months, and  $1.61\% \pm 0.56\%$  at 6 months after transplantation).

#### Cyclosporine Total Blood Concentration, Dose, and Time After Last Dose

There was no significant correlation between  $f_U$  and either cyclosporine dose, or time since the last dose, although there was a weak but significant and inverse correlation between  $f_U$  and total blood concentration (Table 3). The partial correlation coefficient between  $f_U$  and total concentration adjusted for time since transplantation ( $-0.05$ ) was not significant ( $p = 0.5$ ). It can be concluded that cyclosporine  $f_U$  is independent of cyclosporine total concentration.

|   | Mean $\pm$ SD   | Range     | $r^\dagger$ |
|---|-----------------|-----------|-------------|
| Dose ( $\text{mg} \cdot \text{day}^{-1}$ )                                    | 242 $\pm$ 169   | 30–900    | –0.101      |
| Dose ( $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ )               | 3.55 $\pm$ 2.70 | 0.36–15.2 | –0.102      |
| Total blood concentration<br>( $\mu\text{g} \cdot \text{L}^{-1}$ ) $\ddagger$ | 291 $\pm$ 135   | 74–976    | –0.19*      |
| Time after previous dose (hours)  | 12.2 $\pm$ 1.33 | 8.2–20    | –0.04       |

\*  $p < 0.05$ .  
 $\dagger$  Spearman correlation coefficient.  
 $\ddagger$  Monoclonal fluorescence polarization immunoassay method.

TABLE 3. Correlation between cyclosporine fraction unbound and cyclosporine dose, total blood concentration, and time after the previous dose

#### Biochemistry and Hematology

The relationship between cyclosporine  $f_U$  and serum biochemical and hematologic indicators was analyzed. In general,  $f_U$  did not show significant covariation (based on correlation analysis) with albumin, total plasma protein, alkaline phosphatase, alanine transaminase, creatinine, glucose, or urea concentrations in serum ( $p > 0.5$ ) or with creatinine clearance ( $p = 0.8$ ) estimated by the Cockcroft and Gault method (16). At times early after transplantation (less than 3 months),  $f_U$  exhibited a significant correlation with the concentration of serum bilirubin and serum GGT (Table 4).

|                               | Time after transplantation |                              |                              |                               |
|-------------------------------|----------------------------|------------------------------|------------------------------|-------------------------------|
|                               | $\geq 1$ month<br>(n = 47) | $\geq 3$ months<br>(n = 110) | $\geq 6$ months<br>(n = 154) | $\geq 12$ months<br>(n = 189) |
| Serum bilirubin concentration | 0.35*                      | 0.15                         | 0.11                         | 0.05                          |
| Serum GGT concentration       | 0.37 $\dagger$             | 0.23*                        | 0.14                         | 0.08                          |

\*  $p < 0.05$ .  
 $\dagger$   $p < 0.01$ .  
 Data are Spearman correlation coefficients. GGT,  $\gamma$ -glutamyl transferase.

TABLE 4. Correlation between cyclosporine fraction unbound and serum bilirubin and [gamma]-glutamyl transferase concentrations at various times after transplantation

Correlations could not be demonstrated between cyclosporine  $f_U$  and hematocrit, hemoglobin concentration, or platelet, red blood cell, and total and differential white blood cell counts ( $p > 0.1$ ).

#### Serum Lipid Concentrations

Cyclosporine  $f_U$  was negatively correlated with concentration of total cholesterol ( $r = -0.22$ ,  $p = 0.004$ ) and triglyceride ( $r = -0.18$ ,  $p = 0.017$ ). However, the correlations with the concentrations of HDL-cholesterol ( $r = -0.08$ ,  $p = 0.35$ ) and LDL cholesterol ( $r = -0.13$ ,  $p = 0.106$ ) could not be demonstrated. Mean  $f_U$  was lower in hypercholesterolemia ( $>6.5 \text{ mmol} \cdot \text{L}^{-1}$ ) and hypertriglyceridemia ( $>2.0 \text{ mmol} \cdot \text{L}^{-1}$ ), but the difference was significant only in hypercholesterolemia (Table 5).

| Plasma lipid concentration       | Sample number | Fraction unbound $\times 100$ (mean $\pm$ SD) |
|----------------------------------|---------------|---|
| Normal cholesterol*              | 90            | 1.60 $\pm$ 0.63                               |
| High cholesterol*                | 70            | 1.37 $\pm$ 0.52 <sup>††</sup>                 |
| Normal triglyceride <sup>†</sup> | 69            | 1.54 $\pm$ 0.54                               |
| High triglyceride <sup>†</sup>   | 91            | 1.47 $\pm$ 0.63                               |

\* Mean  $\pm$  SD cholesterol plasma concentration in normocholesterolaemic group is 5.44  $\pm$  0.71 mmol  $\cdot$  L<sup>-1</sup> and in hypercholesterolaemic group is 7.78  $\pm$  1.19 mmol  $\cdot$  L<sup>-1</sup>.

<sup>†</sup> Mean  $\pm$  SD triglyceride plasma concentration in normotriglyceridemic group is 1.48  $\pm$  0.33 mmol  $\cdot$  L<sup>-1</sup> and in hypertriglyceridemic group is 3.07  $\pm$  1.00 mmol  $\cdot$  L<sup>-1</sup>.

<sup>‡</sup> Significantly different than normal (Mann-Whitney *U* test,  $p < 0.01$ ).

TABLE 5. Cyclosporine fraction unbound in plasma from patients with normal and elevated plasma lipid concentrations

#### Concomitant Medication

Fifty medications other than cyclosporine were administered, including immunosuppressive agents, cyclosporine-sparing agents, lipid-lowering agents, and a number of miscellaneous medications (i.e., co-trimoxazole [to 76% of patients], captopril [to 62% of patients], ranitidine [to 34% of patients], aspirin [to 36% of patients], furosemide [to 19% of patients], hydralazine [to 16.6% of patients], nifedipine [to 12.6% of patients], calcium and calciferol [to 11.6% of patients], and prazosin [to 17.7% of patients]). Differences in  $f_U$  were not observed between patients who received these medications and those who did not ( $p > 0.1$ ).

All patients received immunosuppressive therapy with azathioprine, prednisolone, and cyclosporine. There were significant negative bivariate correlations between  $f_U$  and the daily dose of azathioprine and prednisolone that may possibly reflect the routine adjustment of azathioprine and prednisolone doses after transplantation (Table 6).

|              | Mean dose (SD)<br>(mg $\cdot$ kg <sup>-1</sup> $\cdot$ day <sup>-1</sup> ) | Correlation coefficient |                      |
|--------------|--|-------------------------|----------------------|
|              |  | Bivariate*              | Partial <sup>†</sup> |
| Azathioprine | 1.73 (0.48)  | -0.22 <sup>‡</sup>      | -0.17 <sup>‡</sup>   |
| Prednisolone | 0.22 (0.16)  | -0.13 <sup>§</sup>      | -0.06                |

\* Spearman correlation coefficient.

<sup>†</sup> Partial correlation coefficient, controlling for time after transplantation.

<sup>‡</sup>  $p < 0.01$ .

<sup>§</sup>  $p < 0.05$ .

TABLE 6. Correlation between cyclosporine fraction unbound and dosage of azathioprine and prednisolone

A group of 55 patients received inhibitors of cyclosporine metabolism as sparing agents with the intention of reducing the cost of cyclosporine therapy (11,17). The mean cyclosporine  $f_U$  was not statistically different in patients who received ketoconazole or itraconazole relative to others (Table 7) and was not significantly different between the ketoconazole group and the untreated group at 1, 3, 6, and 12 months after transplantation ( $p = 0.2$ ; Fig. 2).

| Sparing agent | Fraction unbound $\times 100^*$ |                      | $p^\dagger$ |
|---------------|---------------------------------|----------------------|-------------|
|               | Sparing agent                   | No sparing agent     |             |
| Diltiazem     | 1.34 $\pm$ 0.35 (30)            | 1.54 $\pm$ 0.39 (59) | 0.02        |
| Ketoconazole  | 1.56 $\pm$ 0.33 (29)            | 1.43 $\pm$ 0.41 (60) | 0.15        |
| Itraconazole  | 1.32 $\pm$ 0.36 (5)             | 1.48 $\pm$ 0.39 (84) | 0.35        |

\* Unweighted mean  $\pm$  SD of individual patient means; values in parentheses are the numbers of patients in each group.  
 $\dagger p$  value for two-sample  $t$  test.

TABLE 7. Cyclosporine fraction unbound in patients receiving cyclosporine-sparing agents

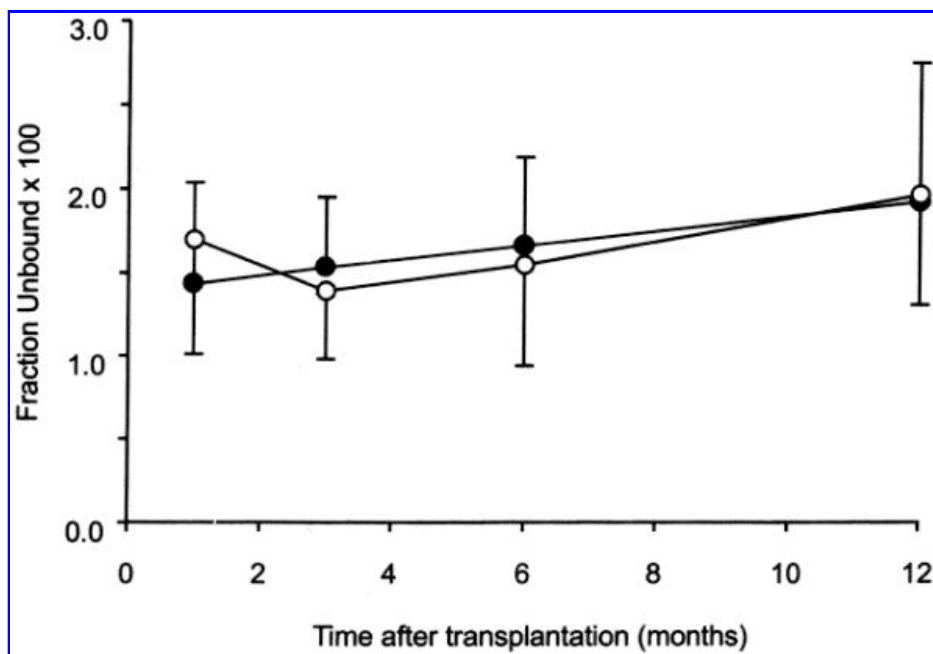


FIG. 2. Mean cyclosporine fraction unbound in ketoconazole ([black circle]) and control (o) groups; error bars are standard deviation (SD).

Mean  $f_u$  was lower in the patients receiving diltiazem (see Table 7). This decrease was associated with a significantly greater concentration of serum HDL-cholesterol ( $p < 0.01$ ) in patients receiving diltiazem ( $1.67 \pm 0.52$  mmol/L<sup>-1</sup>) than others ( $1.42 \pm 0.42$  mmol/L<sup>-1</sup>).

Simvastatin was the major lipid-lowering agent administered to patients included in this study (14). The mean  $f_u$  increased by 29%, from  $1.40 \pm 0.1\%$  to  $1.82 \pm 0.22\%$  after simvastatin administration ( $p < 0.01$ ).

## DISCUSSION

In heart transplantation, the failure of the transplanted organ results in the death of the patient; optimal patient management is thus essential to prevent graft rejection. Routine monitoring of cyclosporine total concentration in whole blood is commonly practiced after transplantation to account for inter- and inpatient variability in cyclosporine pharmacokinetics. The usefulness of monitoring in controlling the extent of immunosuppression is the subject of debate (18). For highly protein bound drugs, such as cyclosporine, unbound fraction is important both from pharmacokinetic and pharmacodynamic points of view. Cyclosporine is eliminated predominantly by hepatic metabolism (19). Because the hepatic extraction ratio of cyclosporine is low to intermediate (20), its total, but not unbound, clearance depends on the unbound fraction in plasma (21). It is also usually accepted that pharmacologic response correlates most closely with the concentration of unbound drug at the receptor site (7). Therefore, unbound cyclosporine blood and plasma concentration may be a better indicator of cyclosporine pharmacologic outcomes than total concentrations. It has also been speculated that cyclosporine may enter blood lymphocytes throughout the LDL receptors on the surface of these cells (22); however, this appears to be an unlikely mechanism for transport of cyclosporine through the cell membrane, considering that in the presence of physiologic concentrations of plasma lipoproteins, these receptors are down regulated (23).

The unbound fraction of cyclosporine in cardiopulmonary transplantation recipients has not previously been measured. The main aims of this study were to investigate the variability of cyclosporine  $f_u$  in this group of patients, to evaluate the impact of concomitant medications used after cardiopulmonary transplant, and to evaluate, if possible, the association between cyclosporine  $f_u$  and organ rejection.

The most important finding of this study was that  $f_U$  was significantly lower during cardiac rejection episodes. In a study performed in renal transplantation recipients, Lindholm (10) showed that cyclosporine  $f_U$  and total and unbound plasma concentrations were lower during graft rejection episodes than before. In this study, total plasma concentrations of cyclosporine were not available, but total whole blood concentrations were not significantly different during cardiac rejection episodes.

The inter- and intra patient variability in cyclosporine  $f_U$  observed in this study is comparable with the 2.3-fold interpatient and 2.8-fold inpatient variability observed by Lindholm and Henricsson (9) in renal transplant recipients. The median  $f_U$  in renal transplant recipients was 1.30%, compared with 1.40% in this study.

We found a significant negative correlation between patient age and cyclosporine  $f_U$  that was attributable to higher levels of serum cholesterol in the elderly. Yee and coworkers (24) observed significant, negative correlations between patient age and cyclosporine clearance and volume of distribution at steady state in 69 nonobese bone marrow transplantation recipients. Their speculation that increased cyclosporine binding resulting from elevated levels of serum lipoproteins in the elderly might be responsible for this relationship is confirmed by the finding of the present study.

No difference was found in  $f_U$  among the recipients of heart, heart-kidney, heart-lung, or single- or double-lung transplantation. There was also no significant difference among patients with various disease etiologies. The failure to find differences in cyclosporine  $f_U$ , particularly between groups with known variations in lipoprotein levels, such as patients with a history of ischemic heart disease or cystic fibrosis (25,26), might be related to the small sample size.

In this study, as in a study by Lindholm and colleagues (3), cyclosporine  $f_U$  was higher in diabetic than in nondiabetic transplantation recipients. In diabetes, the protein binding of drugs may be influenced by glycosylation of serum proteins, including albumin; by increases in the concentrations of free fatty acids; by the displacing effects of oral hypoglycemic agents; and by a decrease in the concentration of serum albumin (27). Unlike Lindholm and colleagues (3), we did not find a significant difference in the concentration of serum albumin, but lower concentrations of serum total and LDL cholesterol and total triglyceride were observed in diabetic patients.

The increase in cyclosporine  $f_U$  with respect to time posttransplantation was an interesting finding of this study. Lindholm and coworkers (3,10) did not observe time-dependency in  $f_U$  over 6 months of follow-up of renal transplantation recipients and concluded that cyclosporine  $f_U$  does not change with time after transplantation. The difference between the results of the two studies might be explained by the difference in the type of transplantation and the longer period of follow-up in the present study.

In the present study, serum GGT and bilirubin were significantly correlated with  $f_U$  for as long as 3 months after transplantation (see Table 4). The increased concentration of serum GGT has been attributed to cyclosporine hepatotoxicity (28). Gulbis and coworkers (29) also speculated that the increase in the concentrations of serum bile acids and GGT after heart transplantation in patients who had impaired liver function before transplantation was an indication of cyclosporine hepatotoxicity. In liver transplant patients, Wisecarver and colleagues (30) showed that the elevated levels of GGT correlated with the blood concentration of cyclosporine. The concentrations of GGT and serum bilirubin may be indicators of hepatotoxicity and thus the possibility of an association between  $f_U$  and hepatotoxicity may need further investigation in a larger number of patients. Another explanation for the positive correlations observed may be the reduced capacity of the damaged liver to produce albumin and lipoproteins after transplantation.

One of the major objectives of this study was to evaluate the effects of concomitant medications on cyclosporine  $f_U$ . Administration of the antifungal agent ketoconazole is commonly practiced after transplantation to reduce the cost of cyclosporine treatment (11,31). Ketoconazole has been shown to reduce the concentration of serum cholesterol (32) in heart transplant recipients, so it might also increase cyclosporine  $f_U$ . In this study, we did not find a significant difference in the values of cyclosporine  $f_U$  in patients receiving ketoconazole as compared with others. We also did not observe any differences in serum lipid concentrations in the patients receiving ketoconazole.

Cyclosporine  $f_U$  was lower in patients who were receiving diltiazem than in those who were not. Administration of diltiazem is thought to increase the concentration of serum HDL cholesterol (33) and to prevent accelerated coronary artery disease (34). In this study, the higher HDL cholesterol concentrations were found in the patients receiving diltiazem and presumably, resulted in the observed decreases in  $f_U$ .

In light of this study it can be concluded that cyclosporine  $f_U$  is an important indicator of cyclosporine immunosuppressive activity and that lower levels of cyclosporine  $f_U$  may be associated with cardiac rejection episodes. Variability in cyclosporine  $f_U$  can be related to a number of factors including age, diabetes, hyperlipidemia, and concomitantly administered medications, such as simvastatin and diltiazem. We observed that most of these factors affect cyclosporine  $f_U$  through alterations in lipoprotein levels. To account for the relationship between plasma lipoproteins and cyclosporine  $f_U$ , we developed a mathematic model to predict  $f_U$  from the concentrations of serum total and HDL cholesterol, serum albumin, and time after transplantation (35). Routine monitoring of cyclosporine  $f_U$ , although important, is inherently difficult and costly. In view of the inverse association between serum lipid concentrations and cyclosporine  $f_U$ , routine monitoring of serum lipids may be useful after transplantation to detect hyperlipidemia, to initiate appropriate lipid-lowering treatment, and as an indirect method of detection for potentially low cyclosporine fraction unbound and organ rejection.

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## REFERENCES

1. Niederberger W, Lemaire M, Maurer G, Nussbauer K, Wagner O. Distribution and binding of cyclosporine in blood and tissues. *Transplant Proc* 1983;15:2419-21. [[Context Link](#)]

2. Gurecki J, Warty V, Sanghvi A. The transport of cyclosporine in association with plasma lipoproteins in heart and liver transplant patients. *Transplant Proc* 1985;22:1997-2002. [\[Context Link\]](#)
3. Lindholm A, Henricsson S, Gang P. The free fraction of cyclosporine in plasma: Clinical findings with a new method. *Transplant Proc* 1988;20:377-81. [Bibliographic Links](#) | [\[Context Link\]](#)
4. Akhlaghi F. *Distribution of cyclosporine in plasma from heart transplant recipients*. [doctoral thesis], University of Sydney, 1996. [\[Context Link\]](#)
5. Rowland M, Tozer TN. In: *Clinical pharmacokinetics: Concepts and applications*. 3rd ed. Philadelphia: Williams & Wilkins, 1995. [\[Context Link\]](#)
6. Rowland M. Protein binding and drug clearance. *Clin Pharmacokinet* 1984;9:10-17. [Buy Now](#) | [Bibliographic Links](#) | [\[Context Link\]](#)
7. du Souich P, Verges J, Erill S. Plasma protein binding and pharmacological response. *Clin Pharmacokinet* 1993;24:435-40. [Buy Now](#) | [Bibliographic Links](#) | [\[Context Link\]](#)
8. Levy RH, Moreland TA. Rationale for monitoring free drug levels. *Clin Pharmacokinet* 1984;9:1-9. [Buy Now](#) | [Bibliographic Links](#) | [\[Context Link\]](#)
9. Lindholm A, Henricsson S. Intra- and interindividual variability in the free fraction of cyclosporine in plasma in recipients of renal transplant. *Ther Drug Monit* 1989;11:623-30. [Buy Now](#) | [Bibliographic Links](#) | [\[Context Link\]](#)
10. Lindholm A. Monitoring of the free concentration of cyclosporine in plasma in man. *Eur J Clin Pharmacol* 1991;40:571-5. [Bibliographic Links](#) | [\[Context Link\]](#)
11. Keogh A, Spratt P, McGosker C, Macdonald P, Mundy J, Kaan A. Ketoconazole to reduce the need for cyclosporine after cardiac transplantation. *N Engl J Med* 1995;333:628-33. [Bibliographic Links](#) | [\[Context Link\]](#)
12. Morris RG, Tett SE, Ray JE. Cyclosporine A monitoring in Australia: Consensus recommendations. *Ther Drug Monit* 1994;16:570-6. [\[Context Link\]](#)
13. Billingham ME, Cary NR, Hammond ME, Kemnitz J, Marhoe C, McCallister HA. A working formulation for the standardisation of nomenclature in the diagnosis of heart and lung rejection: Heart rejection study group. *J Heart Transplant* 1990;9:587-95. [\[Context Link\]](#)
14. Akhlaghi F, McLachlan AJ, Keogh AM, Brown KF. Effect of simvastatin on cyclosporine unbound fraction and apparent blood clearance in heart transplant recipients, *Br J Clin Pharmacol*. 1997;44:537-42. [\[Context Link\]](#)
15. Converse CA, Skinner ER, eds. *Lipoprotein analysis: A practical approach*. Oxford: Oxford University Press, 1992. [\[Context Link\]](#)
16. Walker R, Edward C, eds. *Clinical pharmacy and therapeutics*. New York: Churchill Livingstone, 1994. [\[Context Link\]](#)
17. Macdonald P, Keogh A, Connell J, Harvison A, Richens D, Spratt P. Diltiazem co-administration reduces cyclosporine toxicity after heart transplantation: A prospective randomised study. *Transplant Proc* 1992;24:2259-62. [Bibliographic Links](#) | [\[Context Link\]](#)
18. Lindholm A. Cyclosporine A: Clinical experience and therapeutic drug monitoring. *Ther Drug Monit* 1995;17:631-7. [Buy Now](#) | [Bibliographic Links](#) | [\[Context Link\]](#)
19. Fahr A. Cyclosporine clinical pharmacokinetics. *Clin Pharmacokinet* 1993;2:472-95. [\[Context Link\]](#)
20. Venkataramanan R, Ptachinski RJ, Burckart GJ, Yang S, Starzl TE. Extraction ratio of cyclosporine in a liver transplant patient with organ rejection. *J Pharm Sci* 1985;74:901-2. [Bibliographic Links](#) | [\[Context Link\]](#)
21. Rowland M. Protein binding and drug clearance. *Clin Pharmacokinet* 1984;9:10-7. [Buy Now](#) | [Bibliographic Links](#) | [\[Context Link\]](#)
22. deGroen PC. Cyclosporine, low-density lipoprotein, and cholesterol. *Mayo Clin Proc* 1988;63:1012-21. [\[Context Link\]](#)
23. Stulnig TM, Klocker H, Harwood HJ, et al. In vivo LDL receptor and HMG-CoA reductase regulation in human lymphocytes and its alterations during aging. *Arterioscler Thromb Vasc Biol* 1995;15:872-8. [Ovid Full Text](#) | [Bibliographic Links](#) | [\[Context Link\]](#)
24. Yee GC, Lennon TP, Gmur DJ, Kennedy MS, Deeg HJ. Age-dependent cyclosporine pharmacokinetics in marrow transplant recipients. *Clin Pharmacol Ther* 1986;40:438-43. [Bibliographic Links](#) | [\[Context Link\]](#)
25. Vaughan WJ, Lindgren FT, Whalen JB, Abraham S. Serum lipoprotein concentrations in cystic fibrosis. *Science* 1978;199:783-6. [Bibliographic Links](#) | [\[Context Link\]](#)
26. Levey E, Roy C, Lacaille F, et al. Lipoprotein abnormalities associated with cholesteryl ester transfer in cystic fibrosis patients: The role of essential

fatty acid deficiency. *Am J Clin Nutr* 1993;57:573-9. [Bibliographic Links](#) | [\[Context Link\]](#)

27. Zini R, Riant P, Barre J, Tillemont JP. Disease induced variations in plasma protein levels: Implications for drug and protein regimens. *Clin Pharmacokinet* 1990;19:147-229. [Buy Now](#) | [Bibliographic Links](#) | [\[Context Link\]](#)

28. McKenzie FN, Moses GC, Henderson AR. Routine 'cardiac' and 'hepatic' serum enzyme profiles in cardiac transplant patients treated with cyclosporine A: Operative and post operative findings. *Clin Chem* 1985;31:822-5. [Bibliographic Links](#) | [\[Context Link\]](#)

29. Gulbis B, Adler M, Ooms HA, Desmet JM, Jeclerc JL, Primo G. Liver-function studies in heart transplant recipients treated with cyclosporine A. *Clin Chem* 1988;34:1772-4. [\[Context Link\]](#)

30. Wisecarver JL, Earl RA, Haven MC, Timmins PW, Shaw BW, Stratta RJ. Histologic changes in liver allograft biopsies associated with elevated whole blood and tissue cyclosporine concentrations. *Mod Pathol* 1992;5:611-6. [Bibliographic Links](#) | [\[Context Link\]](#)

31. First MR, Schroeder TJ, Alexander JW, et al. Cyclosporine dose reduction by ketoconazole administration in renal transplant recipients. *Transplantation* 1991;2:365-70. [\[Context Link\]](#)

32. Butman SM, Wild JC, Nolan PE, et al. Prospective study of the safety and financial benefit of ketoconazole as adjunctive therapy to cyclosporine after heart transplantation. *J Heart Lung Transplant* 1991;10:351-8. [Bibliographic Links](#) | [\[Context Link\]](#)

33. Pool PE, Seagren SC, Salel AF. Effects of diltiazem on serum lipids, exercise performance and blood pressure: Randomized, double-blind, placebo-controlled evaluation for systemic hypertension. *Am J Cardiol* 1985;56:86-91. [\[Context Link\]](#)

34. Gheissari A, Yokoyama T, Hendel J, Capouya E, Fuentes J, Jimenez P. Diltiazem prevents accelerated graft coronary artery disease in heart transplant recipients. *Transplant Proc* 1995;27:2625-7. [Bibliographic Links](#) | [\[Context Link\]](#)

35. Akhlaghi F, Ashley JJ, Keogh AM, Brown KF. Indirect estimation of the unbound fraction of cyclosporine in plasma. *Ther Drug Monit* 1998;20:301-8. [Ovid Full Text](#) | [Bibliographic Links](#) | [\[Context Link\]](#)

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