

UNBOUND CYCLOSPORINE AND ALLOGRAFT REJECTION AFTER HEART TRANSPLANTATION

FATEMEH AKHLAGHI,^{1,2} ANNE M. KEOGH,³ AND KENNETH F. BROWN¹

Department of Pharmacy, University of Sydney and Heart and Lung Transplant Unit, St. Vincent's Hospital, Sydney, New South Wales, Australia

Background. To determine the impact of cyclosporine plasma protein binding on organ rejection after cardiac transplantation, the incidence of cardiac rejection episodes was compared among patients who had differing levels of cyclosporine plasma fraction unbound (f_U).

Methods. Forty-six consecutive cardiac transplant recipients were sampled at 1, 3, 6, and 12 months after transplantation, and cyclosporine plasma f_U was determined, using a specially developed equilibrium dialysis method. At the completion of the study, incidences of cardiac rejection episodes were compared among patients having mean cyclosporine f_U (Csfu) that were low (L_{Csfu} ; mean \pm SD, $1.33 \pm 0.10\%$, $n=15$), intermediate (I_{Csfu} ; $1.60 \pm 0.07\%$, $n=16$), and high (H_{Csfu} ; $1.99 \pm 0.30\%$, $n=15$).

Results. Percentage of endomyocardial biopsies (grade 3a, 3b, and 4) with respect to the total number of biopsies performed in the first 3 months after transplant was significantly higher in the L_{Csfu} group than the other groups (40.9% in L_{Csfu} vs. 28.5% for I_{Csfu} and 32.1% for H_{Csfu} groups, $P=0.02$). The linearized rate of rejection (episodes of rejection/100 patient-days) in the first month after transplant was 6.5 ± 1.7 for L_{Csfu} , 3.5 ± 0.8 for I_{Csfu} and 4.3 ± 0.9 for the H_{Csfu} group ($P<0.05$, low vs. intermediate-high). The mean (95% confidence interval) of time interval between the first and second episodes of rejections was 10.7 (5.6–16.0) days for L_{Csfu} , 18.0 (8.6–29.0) days for the I_{Csfu} , and 26.0 (15.1–36.9) days for the H_{Csfu} group ($P<0.01$). The total number of rejections requiring treatment per patient in the first 3 months after transplant was higher in the L_{Csfu} group compared with the others (4.0 ± 1.7 episodes for L_{Csfu} vs. 2.9 ± 1.1 for I_{Csfu} and 3.2 ± 1.2 episodes for H_{Csfu} ; $P<0.05$). Four patients in the low group, one patient in the intermediate group, and no patients in the high group required treatment with total lymphoid irradiation ($P<0.02$).

Conclusions. This finding suggests that patients with lower levels of cyclosporine f_U are more prone to cardiac rejection and that the level of cyclosporine fraction unbound may be clinically important for determination of response to cyclosporine therapy.

Allograft rejection remains a critically important problem after organ transplant. In heart transplantation, the danger of organ rejection is exacerbated by the fact that graft failure often results in death of the patient (1). Therapeutic moni-

toring of total blood concentration of cyclosporine is routinely practiced after transplantation. The usefulness of the monitoring of total cyclosporine concentrations as an indicator of organ rejection is subject to debate (2). Cyclosporine is a hydrophobic molecule, which is highly bound to blood cells and plasma components. In plasma, it is bound predominantly to cholesterol-rich lipoproteins including low-density lipoprotein (LDL*) and high-density lipoprotein (3). The unbound or free fraction (f_U) of cyclosporine in plasma, as determined by equilibrium dialysis, is approximately 1–2% (4, 5).

In a retrospective study of heart transplant recipients, we have observed that the level of cyclosporine f_U was significantly lower at the time of an endomyocardial biopsy result of grade 3a or above (rejection requiring treatment) than at the time of no rejection (grade 0) or grade 1a rejection episodes (6). This finding is consistent with the finding of Lindholm and colleagues (7), in renal transplant recipients, who observed that both cyclosporine unbound fraction and unbound concentration were significantly lower at the time of rejection.

The aim of the present study was to determine whether the overall level of cyclosporine f_U is an important indicator of organ rejection after cardiac transplantation.

PATIENTS AND METHODS

Patients. Forty-six consecutive heart transplant recipients were included in the study and followed for 1 year after transplant. The patients included in this study were part of a randomized clinical trial in which ketoconazole was administered as a cyclosporine metabolic inhibitor (8). 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. Blood samples were collected during outpatient visits, coincident with the collection of blood for routine biochemical, cytological, and cyclosporine total concentration measurements.

Determination of cyclosporine f_U . Samples of blood were obtained at outpatient visits at 1, 3, 6, and 12 months after transplantation. Cyclosporine f_U was measured in plasma by the previously described equilibrium dialysis method (9) employing stainless steel cells in order to minimize extensive nonspecific binding of cyclosporine to apparatus. Samples of [³H]cyclosporine were purified using high-performance liquid chromatography. The method had an intra-day coefficient of variation of less than 15% ($n=16$). At the completion of the study (end of first year after transplant) mean values of cyclosporine f_U were calculated for individual patients. Mean number of observations per patient was 4.16 (median: 4), ranging from 2 to 7. Mean values were ranked from lowest to highest using the SPSS™ computer package, and three arbitrarily defined groups of patients

* Abbreviations: AUC, area under the concentration-time curve; f_U , fraction unbound; H_{Csfu} , high cyclosporine f_U ; I_{Csfu} , intermediate cyclosporine f_U ; L_{Csfu} , low cyclosporine f_U ; LDL, low-density lipoprotein; TLI, total lymphoid irradiation.

¹ Department of Pharmacy, University of Sydney.

² Address correspondence to: Dr. Fatemeh Akhlaghi. Present affiliation: Pathology Clinical Research Unit, Papworth Hospital, Papworth Everard, Cambridge CB3 8RE, United Kingdom. E-mail: fa213@cam.ac.uk.

³ Heart and Lung Transplant Unit, St. Vincent's Hospital.

having low ($L_{C_{sfu}}$), intermediate ($I_{C_{sfu}}$), and high ($H_{C_{sfu}}$) values of cyclosporine f_U were identified. The means \pm SD of cyclosporine plasma f_U expressed as a percentage were $1.33 \pm 0.10\%$, $1.60 \pm 0.07\%$, and $1.99 \pm 0.30\%$ for $L_{C_{sfu}}$, $I_{C_{sfu}}$, and $H_{C_{sfu}}$ groups, respectively.

Immunosuppression and other maintenance therapy. Routine maintenance immunosuppression consisted of cyclosporine, azathioprine, and corticosteroids. Before transplantation surgery, cyclosporine ($2\text{--}3 \text{ mg kg}^{-1}$) was given as a single oral dose (unless moderate hepatic or renal impairment was present), together with 1 g of intravenous methylprednisolone. At the completion of surgery, an additional 1 g of methylprednisolone was given, followed by 125 mg intravenously every 8 hr for 3 doses. Oral cyclosporine ($8 \text{ mg kg}^{-1} \text{ day}^{-1}$) was begun 24 h after surgery, together with azathioprine ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$) and prednisolone ($1 \text{ mg kg}^{-1} \text{ day}^{-1}$, reducing to $0.18 \text{ mg kg}^{-1} \text{ day}^{-1}$ by day 14 and $0.10 \text{ mg kg}^{-1} \text{ day}^{-1}$ by 6 months). Cyclosporine dosage was adjusted to maintain whole blood concentrations within the following ranges: $350\text{--}450 \mu\text{g L}^{-1}$ in the first 2 months after transplantation, $300\text{--}400 \mu\text{g L}^{-1}$ at $2\text{--}3$ months, $250\text{--}300 \mu\text{g L}^{-1}$ at 3 to 6 months, $200\text{--}300 \mu\text{g L}^{-1}$ at 6 to 12 months, and $150\text{--}200 \mu\text{g L}^{-1}$ after 12 months (10). Patients in this study variously received ketoconazole or diltiazem as cyclosporine-sparing agents, or itraconazole or fluconazole as needed for antimicrobial therapy. All heart transplant patients received trimethoprim (160 mg) with sulphamethoxazole (800 mg) daily for 2 days per week for the first posttransplant year as prophylaxis against *Pneumocystis carinii* pneumonia and toxoplasmosis.

Definition of heart rejection. Endomyocardial biopsies were performed routinely at the following intervals: weekly to 1 month, every other week to 3 months, and thereafter at $4, 5, 6, 9,$ and 12 months and at other times on clinical indication. Histological rejection was graded according to the International Society of Heart and Lung Transplantation classification for cardiac rejection (11). Patients with grade $0, 1a, 1b,$ or 2 rejection did not receive augmented immunosuppression, but patients with higher grades (grade 3 and above) were treated with oral or intravenous steroids. Resistant or recurrent rejection was treated with antithymocyte globulin (OKT3) or total lymphoid irradiation (TLI).

Statistical analysis. Statistical analyses were performed using SPSS for Windows (release 6.1, SPSS Inc). Unless otherwise stated, all P -values were based on two-tailed tests and P -values less than 0.05 were considered significant. Differences between two or more independent samples were assessed using the one-way test, followed by the Bonferroni *post hoc* test to identify differences among individual groups. The absolute number of events or patients in different groups were compared by the chi-square test. Preliminary statistical analysis of data revealed that the values in the low group were almost consistently different from the other two groups, whereas the differences between intermediate and high were not significant. To simplify expressions of statistical differences, on a number of occasions, the low group was compared against combined results from the intermediate and high group (intermediate-high) using an independent sample t test.

RESULTS

Demographic characteristics of patient population including number of female patients, age, weight, etiology of organ failure, number of patients who were receiving ketoconazole or diltiazem, number of patients with diabetes mellitus, and mean values of HLA mismatches were comparable among the three study groups (Table 1). In addition, the dosages of cyclosporine, azathioprine, and prednisolone and the total concentration of cyclosporine measured using monoclonal fluorescence polarization immunoassay were not significantly different at $1, 3, 6,$ and 12 months after transplantation, with the exception of higher azathioprine dosage in the

TABLE 1. Patient demographic characteristics

Variable	Group ^a		
	Low	Intermediate	High
No. of patients	15	16	15
Female recipients (%)	33	37	20
Mean age	52	48	46
Weight kg (mean \pm SD)	71 ± 16	71 ± 12	76 ± 12
Ketoconazole	8	11	8
Diltiazem	1	3	0
Diabetes mellitus	1	1	4
Diagnosis			
Cardiomyopathy	8	8	9
Ischaemic Heart Disease	4	6	4
Others	3	2	2
No. of HLA mismatches			
HLA A	1.42 ± 0.51	1.25 ± 0.68	1.46 ± 0.64
HLA B	1.85 ± 0.36	1.50 ± 0.51	1.73 ± 0.45
HLA DR	1.71 ± 0.46	1.60 ± 0.50	1.40 ± 0.50

^a $P = \text{NS}$; plus-minus values are mean \pm SD.

low group at the first month after transplant ($P < 0.05$) (Table 2).

Endomyocardial biopsy results. The percentage of biopsy-proven rejection episode of grade $3a, 3b,$ or 4 relative to the total number of biopsies performed in the first 3 months after transplant was significantly higher ($P = 0.02$) in the $L_{C_{sfu}}$ group than either of the other groups (40.9% in $L_{C_{sfu}}$ vs.

TABLE 2. Cyclosporine unbound fraction, whole blood monoclonal trough levels, and daily dosage of cyclosporine, azathioprine, and prednisolone for each group of patients at monthly intervals after transplant^a

Variable	Group		
	Low	Intermediate	High
CsA unbound fraction (%)			
Mo 1	1.23 ± 0.35	1.47 ± 0.35	1.94 ± 0.86
Mo 3	1.23 ± 0.26	1.50 ± 0.39	1.67 ± 0.42
Mo 6	1.23 ± 0.28	1.66 ± 0.19	1.95 ± 0.67
Mo 12	1.65 ± 0.38	1.80 ± 0.50	2.41 ± 0.78
CsA total concentration ($\mu\text{g L}^{-1}$) ^b			
Mo 1	437 ± 172	465 ± 218	401 ± 128
Mo 3	350 ± 156	303 ± 123	268 ± 82
Mo 6	253 ± 111	252 ± 34	259 ± 81
Mo 12	248 ± 78	218 ± 73	249 ± 190
CsA dose (mg/kg/day)			
Mo 1	4.07 ± 3.29	4.09 ± 3.03	4.70 ± 4.60
Mo 3	2.77 ± 1.98	2.93 ± 2.28	3.13 ± 3.16
Mo 6	2.48 ± 1.91	2.75 ± 1.98	2.82 ± 2.42
Mo 12	2.13 ± 1.59	2.13 ± 1.45	2.51 ± 2.42
Azathioprine (mg/kg/day)			
Mo 1	1.95 ± 0.21^c	1.75 ± 0.23	1.71 ± 0.24
Mo 3	1.71 ± 0.19	1.58 ± 0.38	1.57 ± 0.44
Mo 6	1.58 ± 0.30	1.38 ± 0.42	1.50 ± 0.39
Mo 12	1.61 ± 0.30	1.49 ± 0.36	1.60 ± 0.26
Prednisolone (mg/kg/day)			
Mo 1	0.23 ± 0.03	0.22 ± 0.03	0.26 ± 0.11
Mo 3	0.20 ± 0.02	0.20 ± 0.02	0.19 ± 0.02
Mo 6	0.19 ± 0.02	0.18 ± 0.04	0.18 ± 0.02
Mo 12	0.17 ± 0.02	0.16 ± 0.03	0.16 ± 0.03

^a Plus-minus values are mean \pm SD; CsA, cyclosporine.

^b Measured in whole blood using monoclonal fluorescence polarization immunoassay.

^c $P < 0.05$, significantly higher than intermediate and high groups.

TABLE 3. Percentages of a particular endomyocardial biopsy result with respect to the total number of biopsies performed in the first 3 months after transplant^a

EMB grade (percentage) ^b	Group		
	Low	Intermediate	High
Grade 0	31.2±15.1	33.4±17.6	32.8±12.2
Grade 1a, 1b, and 2	27.9±11.8 ^c	38.0±13.6	35.1±15.6
Grade 3a, 3b, and 4	40.9±13.8 ^d	28.5±12.6	32.1±11.4

^a Data are expressed as mean±SD; EMB, endomyocardial biopsy; ISHLT, International Society of Heart and Lung Transplantation.

^b Definition of different grades of endomyocardial biopsy determinations according to ISHLT (11); 0=no rejection; 1a=focal (perivascular or interstitial) infiltrate without necrosis; 1b=diffuse but sparse infiltrate without necrosis; 2=one focus only with aggressive infiltration and/or focal myocyte damage; 3a=multifocal aggressive infiltrates and/or myocyte damage; 3b=diffuse inflammatory process with necrosis; 4=diffuse aggressive polymorphous infiltrate±edema, ±hemorrhage, ±vasculitis, with myocyte necrosis.

^c $P=0.03$, low vs. intermediate-high.

^d $P=0.01$, low vs. intermediate-high.

28.5% for $I_{C_{sfu}}$ and 32.1% for $H_{C_{sfu}}$ group) (Table 3). Furthermore the percentage of less severe rejection episodes (grades 1a, 1b, or 2, which are not treated with augmented immunosuppression) was lower in the $L_{C_{sfu}}$ group than in the others (28% for $L_{C_{sfu}}$ vs. 38% for $I_{C_{sfu}}$ and 35% for $H_{C_{sfu}}$ group, $P=0.03$). There was no significant difference in the percentage of grade 0 (no rejection) endomyocardial biopsies among the three groups of patients.

Time of cardiac rejection episodes. The period of freedom from rejection was longer in the patient $I_{C_{sfu}}$ group than in either the $L_{C_{sfu}}$ or $H_{C_{sfu}}$ groups ($P<0.05$ $I_{C_{sfu}}$ vs. $L_{C_{sfu}}$ or $H_{C_{sfu}}$) (Table 4). Every patient in the $L_{C_{sfu}}$ group had experienced the first episode of rejection by 24 days after transplant as compared with 110 days for the patients with an intermediate level and 52 days for the patients with a high level of cyclosporine f_U ($P<0.05$, low vs. intermediate) (Fig. 1). No significant difference was observed between the low and high group with respect to time of first cardiac rejection ($P=0.8$). The time to the second episode of cardiac rejection was shorter for the patients in $L_{C_{sfu}}$ group than the $I_{C_{sfu}}$ or the $H_{C_{sfu}}$ group ($P=0.02$) (Table 4). Occurrence of the third episode of cardiac rejection was not significantly different between the three group of patients; however, it should be noted that a smaller number of patients ($n=11$) in the high group experienced a third episode of graft rejection as com-

TABLE 4. Time of the first, second, and third episodes of cardiac rejection and the linearized rejection rate

	Group			P values ^a
	Low	Intermediate	High	
First episode (days)	16±5 (n=15)	35±33 (n=16)	17±13 (n=15)	0.053
Second episode (days)	26±10 (n=14)	50±39 (n=14)	41±21 (n=14)	0.065
Third episode (days)	84±85 (n=14)	109±102 (n=14)	75±39 (n=11)	0.77

^a Level of significance among the three groups (one-way analysis of variance); values in parentheses indicate number of patients in each group who experienced rejection.

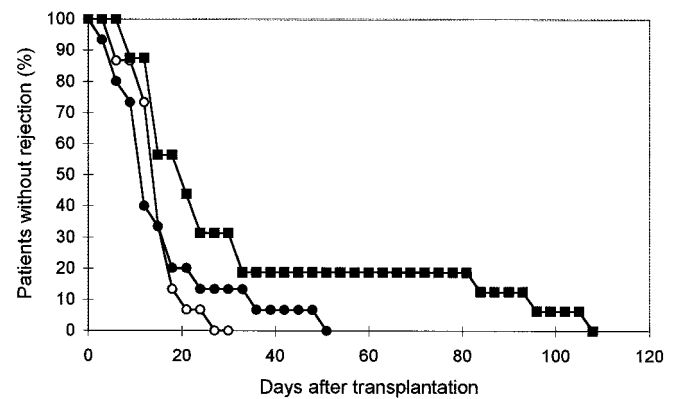


FIGURE 1. Life-table analysis showing the occurrence of first episodes of cardiac rejection. Low (○), intermediate (■), and high (●) (first episode of cardiac rejection occurred in significantly later times ($P<0.05$) in the group with intermediate level of cyclosporine f_U compared with the other two groups.

pared with 14 patients in either low or intermediate groups (Table 4).

Rate of rejection and intervals between rejection episodes. The linearized heart rejection rate (rejections per 100 patient-days) in the first month after transplant was significantly higher ($P<0.05$) in the $L_{C_{sfu}}$ than in the $I_{C_{sfu}}$ group of patients (Table 5). The linearized rate of rejection was not significantly different in the subsequent months after transplantation. The time intervals (days) between first and second episodes of rejection were calculated as an indicator of how rapidly rejection episodes occurred. The mean and 95% confidence interval between the first and second episodes of rejections was 10.7 (5.6–16.0) days for the low group, 18.0 (8.6–29.0) days for the intermediate group, and 26.0 (15.1–36.9) days for the high group ($P<0.01$, low vs. intermediate-high), indicating that patients with lower level of cyclosporine f_U had a shorter interval between their first and second episodes of rejection than the intermediate and the high groups (Fig. 2).

Treatment of rejections. Total number of treatment episodes per patient was higher for the patients with a low level of cyclosporine f_U as compared with the other two groups ($4.0±1.7$ episodes for $L_{C_{sfu}}$ vs. $2.9±1.1$ for $I_{C_{sfu}}$ and $3.2±1.2$ episodes for $H_{C_{sfu}}$; $P<0.05$, low vs. intermediate-high) (Table 6). Moreover, the number of patients who required treatment of recurrent rejection episodes using TLI was greater in the low group that in the intermediate and high groups (four patients in the low group, one patient in the intermediate group, and no patient in the high group required treatment with TLI; $P<0.02$, low vs. intermediate-high) (Fig. 3). The

TABLE 5. Linearized rejection rate in the first 3 months after transplantation^a

	Group		
	Low	Intermediate	High
Month 1	6.5±1.7 ^b	3.5±0.8	4.3±0.9
Month 2	2.1±0.7	3.1±0.8	3.3±0.8
Month 3	2.9±0.8	1.9±0.6	2.3±0.7

^a Data are expressed as mean±SD; rejection rate is episodes of rejection/100 patient-days.

^b $P<0.05$ low vs. intermediate-high group.

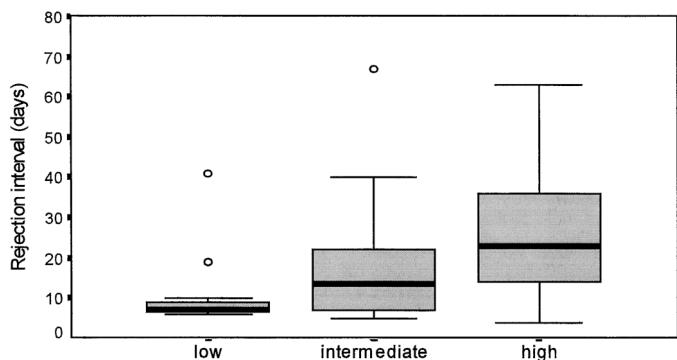


FIGURE 2. Time interval between the first and second episodes of cardiac rejection in three groups of patients grouped according to their cyclosporine f_U . Lower and upper levels of the box plot indicate 25th and 75th percentiles, lines in the middle of boxes indicate median, whiskers indicate lowest and highest values, and open circles indicate extreme values. The low group was significantly different from high ($P < 0.05$).

TABLE 6. Episodes of treatment

Treatment episodes	Low	Intermediate	High
Total episodes of treatment/patient	4.0 ± 1.7 ^a	2.9 ± 1.1	3.2 ± 1.2
Intravenous methylprednisolone/patient	1.93 ± 0.59 ^b	1.37 ± 0.80	1.66 ± 0.72
Oral prednisolone/patient	1.40 ± 0.98	1.12 ± 0.61	1.20 ± 0.67

^a $P = 0.03$ low vs. intermediate-high.

^b $P = 0.05$ low vs. intermediate-high.

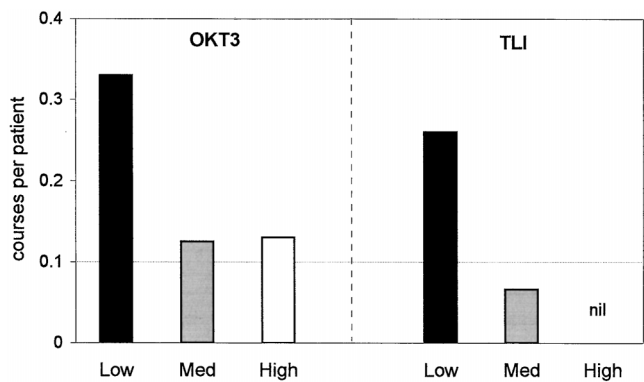


FIGURE 3. Immunosuppressive treatment using OKT3 and TLI to overcome recurrent or resistance episodes of rejections. OKT3 and intravenous methylprednisolone treatment, $P = 0.12$, low vs. intermediate-high; TLI treatment, $P = 0.03$, low vs. intermediate-high.

number of episodes of pulse therapy per patient, using intravenous methylprednisolone or oral prednisolone, or the number of patients that require treatment using OKT3 and intravenous methylprednisolone was greater in L_{Csfu} , although the difference among the three groups was not significant (Table 6, Fig. 3).

Infection. The number of episodes of infection including bacterial, viral, and fungal infections and the linearized rate of infection in the first 4 months after transplantation was not significantly different at any time point among the three groups.

Biochemical and cytological indices. Concentrations of serum creatinine, urea, bilirubin, total protein, albumin, alkaline phosphatase, alanine transaminase, γ -glutamyl transpeptidase, and electrolytes were not significantly different among the three groups. In addition, hematocrit and white cell counts were not different. At 6 months after transplant, the absolute number of lymphocytes was significantly lower ($0.97 \pm 0.52 \times 10^9/L$) in the patients with a low level of cyclosporine f_U than for the intermediate and high groups ($1.26 \pm 0.46 \times 10^9/L$ and $1.75 \pm 0.83 \times 10^9/L$, respectively) ($P = 0.02$). The lower lymphocyte count in the patients with low levels of cyclosporine f_U may be explained by more frequent episodes of treatment using OKT3 and TLI in this group of patients.

Total serum cholesterol was significantly higher at 3 ($P < 0.001$) and 6 ($P < 0.01$) months after transplant, and the concentration of LDL cholesterol was significantly higher at 3 months after transplant in patients having low fraction unbound than for the other two groups (Fig. 4).

DISCUSSION

The main objective of the present study was to consider the overall impact of the extent of protein binding of cyclosporine in plasma with clinical outcomes, in particular rejection. This study showed that increased protein binding of cyclosporine, which results in the lower levels of cyclosporine f_U , is associated with higher frequency in cardiac rejection and an increase in the requirement for more oppressive immunosuppressive treatments including use of TLI or cytolytic therapy. Patient demographic characteristics that can influence organ rejection, including proportion of female transplant recipients and number of HLA mismatches, were not significantly

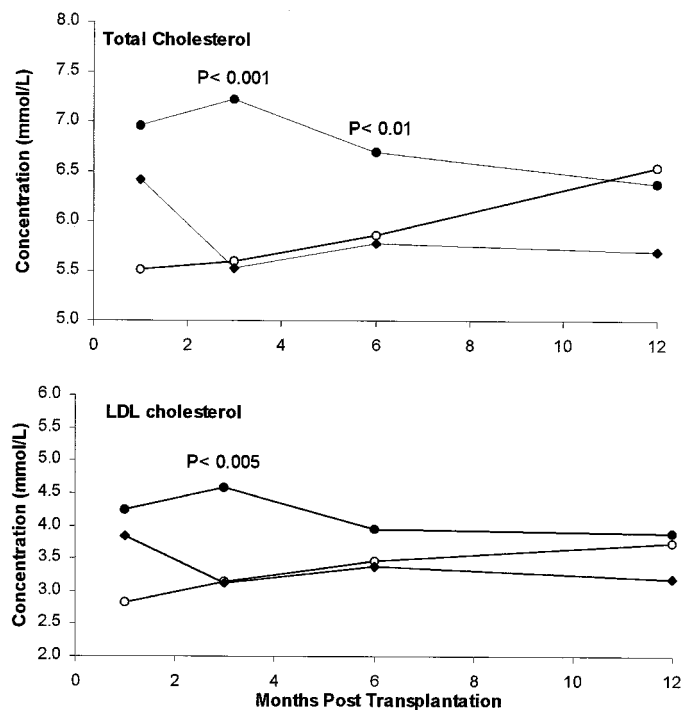


FIGURE 4. Concentrations of total and LDL cholesterol at various time points after transplantation for patients in low (●), intermediate (○), and high (◆) groups. P -values represent the level of significance between the low group and intermediate-high group.

different among the three groups, excluding the possible effects of these parameters on rejection outcomes.

In this study, we followed 46 consecutive heart transplant recipients for a period of 1 year after transplant and measured plasma fraction unbound of cyclosporine on four occasions. At the completion of the study period, patients were divided into three arbitrary groups according to tertile having low, intermediate, and high levels of cyclosporine f_U . It is important to note that no cutoff value was defined to indicate the optimum level of cyclosporine f_U ; therefore, the above mentioned classification appears credible. Measurement of cyclosporine fraction unbound requires a complex method not easily applied in the clinical setting. To our knowledge, the association between cyclosporine fraction unbound and rejection has not been characterized previously in heart transplant recipients.

In heart transplantation, failure of the transplanted organ may result in the loss of the patient. Intensive patient management is therefore essential to prevent graft rejection. Total trough concentration of cyclosporine in whole blood is routinely measured to provide information on bioavailability and metabolism, effect of concomitant medications, and patient compliance. Moreover, cyclosporine dosage is usually adjusted to maintain desired total concentration levels for the respective time after transplant (10). For a highly protein-bound drug such as cyclosporine, it is generally accepted that unbound drug, which is available for membrane transport and active receptor binding, should correlate more closely with clinical outcomes. On the other hand, an increase in the extent of plasma protein binding, which results in a lower fraction unbound, increases the steady-state total concentrations required to produce drug response (12). Protein binding of cyclosporine in plasma is a linear and nonsaturable process; therefore, f_U is essentially independent of total concentration and depends only on the concentration of binding proteins. The unbound concentration, however, is directly related to fraction unbound in plasma (13); at any time point during the dosing interval, unbound concentration in plasma can be calculated by multiplying the total plasma concentration by fraction unbound. In this study, we did not have the plasma concentration data or any other pharmacokinetic information such as area under the concentration-time curve (AUC), which indicates total exposure to cyclosporine. However, if one assumes a constant value for cyclosporine plasma AUC, the mean unbound AUC values for a patient in the low group at the first month after transplant will be approximately 20% lower than the intermediate and 57% lower than the high group.

In this study, we did not observe a significant difference in the rejection characteristics between the group with intermediate levels of cyclosporine fraction unbound and the group with a high level. This finding may be explained by considering the sigmoidal association between cyclosporine concentration and its immunosuppressive effects. Using a mitogenic lymphocyte proliferative assay, it has been shown that the total concentration of cyclosporine exhibits a sigmoidal relationship with its inhibitory effects (14). In cardiac transplant recipients, Best and colleagues (15) demonstrated an inverse relationship between the relative risk of rejection and cyclosporine concentration and showed that the rejection risk is linearly associated with total concentration of cyclosporine at

lower concentrations. However, this association reaches a plateau at the concentrations of 375 ng ml⁻¹ and above.

The patients in the group with a low level of cyclosporine f_U had higher total and LDL cholesterol concentrations at the third and sixth months after transplant. It has been speculated that hypercholesterolemia may reduce the biological activity of cyclosporine and subsequently produce cyclosporine nonresponsiveness (16–19). In long-term survivors of renal transplantation, hyperlipidemia and pretransplant lipid abnormalities were found to be important risk factors for chronic graft rejection (16). In pediatric patients with nephrotic syndrome, nonresponsiveness to cyclosporine also correlated with hypercholesterolemia. Higher doses of cyclosporine were required to achieve immunosuppressive treatment in this group of patients (17). Dimeny and colleagues (18) showed that the concentration of serum lipids, including serum total cholesterol and triglyceride, as well as the cholesterol and triglyceride content of very-low-density lipoprotein and LDL fractions, were significantly higher in renal transplant recipients with greater incidence of acute rejections and higher serum creatinine as a result of frequent rejections.

In summary, this preliminary study shows that the unbound fraction of cyclosporine in plasma may be important with respect to allograft rejection. Lower levels of cyclosporine f_U are associated with greater incidence of cardiac rejection episodes requiring treatment. A more detailed clinical study in de novo heart transplant recipients in the first few months after transplantation, together with both pharmacokinetic and immunopharmacodynamic characterization, is warranted to further elucidate the association between cyclosporine fraction unbound, hypercholesterolemia, and organ rejection.

REFERENCES

- White D. Immunosuppression in heart transplantation. *Br J Biomed Sci* 1993; 50: 277.
- Lindholm A. Cyclosporine A: clinical experience and therapeutic drug monitoring. *Ther Drug Monit* 1995; 17: 631.
- Niederberger W, Lemaire M, Maurer G, Nussbaumer K, Wagner O. Distribution and binding of cyclosporine in blood and tissues. *Transplant Proc* 1983; 15: 2419.
- Lindholm A, Henricsson S, Lind M, Dahlqvist R. Intraindividual variability in the relative systemic availability of cyclosporine after oral dosing. *Eur J Clin Pharmacol* 1988; 34: 461.
- Akhlaghi F. Distribution of cyclosporine in plasma from heart transplant recipients [Ph.D. dissertation]. Sydney: University of Sydney, 1996.
- Akhlaghi F, Ashley JJ, Keogh AM, Brown KF. Cyclosporine plasma unbound fraction in heart and lung transplant recipients. *Ther Drug Monit*; in press.
- Lindholm A. Monitoring of the free concentration of cyclosporine in plasma in man. *Eur J Clin Pharmacol* 1991; 40: 571.
- Keogh A, Spratt P, McCosker C, Macdonald P, Mundy J, Kaan A. Ketoconazole to reduce the need for cyclosporine after cardiac transplantation. *N Engl J Med* 1995; 333: 628.
- Akhlaghi F, McLachlan AJ, Keogh A, Brown KF. Effect of simvastatin on cyclosporine unbound fraction and apparent blood clearance in heart transplant recipients. *Br J Clin Pharmacol* 1997; 44: 537.
- Morris RG, Tett SE, Ray JE. Cyclosporine A monitoring in Australia: consensus recommendations. *Ther Drug Monit* 1994; 16: 570.
- Billingham ME, Cary NR, Hammond ME, et al. 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.
12. Hladky SB. Pharmacokinetics. Manchester, UK: Manchester University Press, 1990.
 13. Rowland M, Tozer TN. Clinical pharmacokinetics: concepts and applications. 3rd Ed. Baltimore: Williams & Wilkins, 1995.
 14. d'Uscio CH, Aweeka FT, Prueksaritanont T, et al. Immunopharmacodynamic studies of cyclosporine in patient awaiting renal transplantation. *J Clin Pharmacol* 1995; 35: 967.
 15. Best NG, Tan KKC, Trull AK, Spiegelhalter DJ, Stewart S, Wallwork J. Pharmacodynamics of cyclosporine in heart and heart-lung transplant recipients. I. Blood cyclosporine concentrations and other risk factors for cardiac allograft rejection. *Transplantation* 1996; 62: 1429.
 16. Dimeny E, Wahlberg J, Lithell H, Fellstrom B. Hyperlipidaemia in renal transplantation: risk factor for long-term graft outcome. *Eur J Clin Invest* 1995; 25: 574.
 17. Ingulli E, Tejani A. Severe hypercholesterolemia inhibits cyclosporine A efficacy in a dose-dependent manner in children with nephrotic syndrome. *J Am Soc Nephrol* 1992; 3: 254.
 18. Dimeny E, Tufveson G, Lithell H, Larsson E, Siegbahn A, Fellstrom B. The influence of pre-transplant lipoprotein abnormalities on the early results on renal transplantation. *Eur J Clin Invest* 1993; 23: 572.
 19. Guijarro C, Massy ZA, Kasiske BL. Clinical correlation between renal allograft failure and hyperlipidaemia. *Kidney Int* 1995; 48: S56.

Received 18 March 1998.

Accepted 26 August 1998.

0041-1337/99/6701-59\$03.00/0

TRANSPLANTATION

Copyright © 1999 by Lippincott Williams & Wilkins

Vol. 67, 59–65, No. 1, January 15, 1999

Printed in U.S.A.

SERUM TRANSFORMING GROWTH FACTOR- β_1 LEVELS IN BONE MARROW TRANSPLANT RECIPIENTS CORRELATE WITH BLOOD CELL COUNTS AND CHRONIC GRAFT-VERSUS-HOST DISEASE¹

LINDA M. LIEM,² WIM E. FIBBE,³ HANS C. VAN HOUWELINGEN,⁴ AND ELS GOULMY^{2,5}

Departments of Immunohematology and Blood Bank, Hematology, and Medical Statistics, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

Objective. Profound immunosuppression and extensive fibrotic changes in the skin are characteristic for graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation (BMT). Transforming growth factor (TGF)- β is a potent immunosuppressive cytokine that plays an important regulatory role in the immune response. In addition, TGF- β promotes wound repair but has also been implicated in tissue fibrosis. These characteristics prompted us to question whether serum TGF- β levels would be associated with GVHD after BMT.

Methods. In this study, total TGF- β_1 levels in serum from HLA-identical BMT recipients before and at several time intervals after transplantation were quantified and correlated with platelet and white blood cell (WBC) counts and with the presence of acute and chronic GVHD in a multivariate analysis.

Results. TGF- β_1 levels were readily detectable in healthy controls and in BMT recipients before BMT. In all patients, a rapid drop in TGF- β_1 levels was seen

during the BMT conditioning regimen. After 20–50 days postBMT, TGF- β_1 levels started to increase to normal levels. Platelet and WBC counts were strongly correlated with TGF- β_1 levels ($r=0.810$, $P<0.001$, and $r=0.733$, $P<0.001$, respectively). Multivariate analysis also revealed that TGF- β_1 levels were significantly increased during chronic GVHD and that the increase during acute GVHD reached levels of significance ($P=0.009$ and $P=0.053$, respectively).

Conclusions. These results show that total TGF- β_1 levels correlate significantly with platelet and WBC counts and that chronic GVHD is associated with an increase in serum TGF- β_1 , independent of platelet or WBC counts.

Graft-versus-host disease (GVHD*) is an important cause of morbidity and mortality after allogeneic bone marrow transplantation (BMT). GVHD occurs in approximately 35% of recipients of an HLA genotypically identical bone marrow graft, depending on the age of the recipients and the amount of T-cell depletion (1). GVHD is mediated by donor T cells recognizing antigenic differences in the recipient and is primarily aimed at tissue of the skin, gastrointestinal tract, and

¹ This work was supported by the J.A. Cohen Institute for Radiopathology and Radiation Protection (IRS), by European Community grant BIO2 CT92 0300, and by a grant from the MACROPA Foundation.

² Department of Immunohematology and Blood Bank.

³ Department of Hematology.

⁴ Department of Medical Statistics.

⁵ Address correspondence to: E. Goulmy, PhD, Department of Immunohematology and Blood Bank, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

* Abbreviations: aGVHD, acute graft-versus-host disease; APC, antigen-presenting cells; BMT, bone marrow transplantation; cGVHD, chronic graft-versus-host disease; GVHD, graft-versus-host disease; MANOVA, multivariate analysis of variance; TGF, transforming growth factor; WBC, white blood cell.