Cross-Correlation of Cyclosporine Concentrations and Biochemical Measures of Kidney and Liver Function in Heart and Heart–Lung Transplant Recipients

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Cross-correlation of cyclosporine concentrations with results of biochemical tests of renal and liver function, measured during the first three months post-operatively, was carried out retrospectively in 24 heart and eight heart–lung transplant recipients to assess the temporal relationship between cyclosporine treatment and the development of possible toxic side-effects. We found a statistically significant negative correlation (95% confidence interval of the mean correlation coefficient did not overlap zero) between the five-day mean concentration of cyclosporine in whole blood (but not plasma) as measured with nonselective (NSRIA) and selective radioimmunoassays (SRIA) and the mean reciprocal creatinine concentration measured in the subsequent five days. In 15 of 32 (47%) patients the negative correlation coefficient exceeded 0.7 (high susceptibility), whereas in 11 of 32 (34%) it was between 0.5 and 0.7 (medium susceptibility), and in only six of 32 (19%) was it less than 0.3 (low susceptibility). We found no consistent correlations between cyclosporine measurements and results of other renal-function tests or liver-function tests. This suggests that therapeutic doses of the drug are not hepatotoxic in most patients. There was, however, a significantly correlated decrease in the NSRIA/SRIA ratio and in serum bilirubin concentration with time postoperatively, reflecting improvement in hepatic function and clearance of the cyclosporine metabolites that are detected by NSRIA. Assays of cyclosporine in whole blood, but not in plasma, are of value in anticipating changes in renal function after heart and heart–lung transplantation.

Additional Keyphrases: radioimmunoassay • monoclonal vs polyclonal antibodies • monitoring therapy • nephrotoxicity

Despite the introduction of triple therapy (cyclosporine, azathioprine, and prednisone) in 1984, nephrotoxicity remains a very common and serious side effect of immunosuppression with cyclosporine. At Papworth Hospital the most significant complication associated with long-term use of cyclosporine in heart-transplant patients is chronic nephrotoxicity, which can be irreversible and lead to end-stage renal failure (1). After heart transplantation, isolated or combined increases in results of liver-function tests have also been attributed to cyclosporine toxicity (2–6). The persistence of these complications of cyclosporine therapy in heart transplantation may be partly associated with a perceived or actual requirement for heart-transplant recipients to be maintained on relatively high concentrations of cyclosporine in blood, compared with other transplant groups (7).

After heart transplantation, the overriding clinical priority in adjusting cyclosporine dosage is the prevention of life-threatening transplant rejection (8). In some circumstances this may be at the expense of deteriorating renal function due to cyclosporine nephrotoxicity. By contrast, in kidney transplant patients, the minimization of cyclosporine nephrotoxicity is a prerequisite for the maintenance of good allograft function. Measurements of cyclosporine concentrations in blood are used in conjunction with clinical and other laboratory data to assess each heart-transplant patient’s current tolerable dosage requirements for cyclosporine. Some patients are clearly more prone to develop the severe side effects associated with cyclosporine therapy than others. The objective stratification of individual patients according to their susceptibility to these complications may provide a more rational basis for deciding the appropriate cyclosporine dosage adjustment at any given time.

We have carried out time-series analysis of cyclosporine concentrations in blood and biochemical tests of renal and liver function in a group of heart and heart–lung transplant recipients to define any putative temporal cause-and-effect relationships between changes in these measurements in the first three months after transplantation.

Patients and Methods

Samples

Included in this pilot study were 24 consecutive heart transplant recipients and eight heart–lung transplant recipients, all on quadruple therapy (cyclosporine, azathioprine, and steroids, plus, on days 1 to 3, anti-thymocyte globulin), who received transplants between November 1987 and March 1988. Cyclosporine was generally introduced more slowly in the heart transplant group because of poor pre-operative renal function. Heart–lung recipients did not receive oral prednisolone until two weeks after transplantation or after treatment for acute rejection with intravenous methylprednisolone. The mean (and SD) age of heart-transplant patients was 44.9 (10.9) years; 22 were men. Fifteen patients received heart allografts for ischemic heart disease and nine for chronic cardiomyopathy. The mean age of the heart–lung group was 27.6 (12.2) years; five were men. Four patients received heart–lung allografts for cystic fibrosis, two for primary pulmonary hypertension, one for Eisenmenger’s syndrome, and one for pulmonary tumor.

In all, 624 paired EDTA-anticoagulated whole-blood and plasma samples, sent for routine measurement of morning trough cyclosporine concentrations, were collected from the 32 patients over the three months after the operation; 452 (72%) of the 624 specimens were received within the first month after transplantation. Plasma was separated after...
equilibration of blood samples for 2 h at room temperature. A mean of 18.4 (SD 5.6) paired samples (range 12 to 37) were obtained from each patient.

Biochemical Analyses

Whole-blood and plasma concentrations of cyclosporine were measured by polyclonal antibody-based, nonselective radioimmunoassay (NSRIA; Cyclo-Trak®; Incstar, Stillwater, MN 55082); whole-blood specimens were also tested by monoclonal antibody-based selective radioimmunoassay (SRIA; Cyclo-Trak® SP). The between-assay coefficients of variation for both NSRIA and SRIA were <10% at cyclosporine concentrations of 150, 600, and 1000 µg/L. We measured potassium, creatinine, and urea in plasma (renal-function tests; RFTs) and bilirubin, alkaline phosphatase, and alanine aminotransferase in serum (liver-function tests; LFTs) routinely with an Hitachi 717 discrete analyzer (Boehringer Mannheim, Mannheim, F.R.G.), according to the manufacturer's instructions, at Papworth Hospital.

Time Series Analyses

Biochemical data that did not follow gaussian distribution were normalized by appropriate transformations before statistical analysis (9). Data from each patient were divided into five-day periods from the date of transplantation, and the mean result for each period was calculated. We chose a five-day mean period to overcome the problem of missing daily data in time series analyses; this served to spread data points evenly over the three months during which each patient was studied. The use of five-day means may also have the advantage of diluting the effects of analytical error in daily biochemical measurements as well as errors in the collection time of blood samples for measuring "trough" concentrations of cyclosporine, which may obscure underlying correlations.

We determined for each patient the correlation between five-day mean cyclosporine concentrations and five-day mean RFT or LFT data, when RFT and LFT data were either in phase with cyclosporine measurements or lagged out of phase by as many as three five-day periods. For example, with one five-day lag, the mean cyclosporine concentrations for days 1 to 5 and 6 to 10 were correlated with mean RFT or LFT data for days 6 to 10 and 11 to 15, respectively, and so on. The correlation coefficients (r) for all 32 patients were then transformed (Fisher transformation) to the quantity z (10), which has an approximately gaussian distribution, by the following formula:

\[ z = 0.5 \times \log_e[(1 + r)/(1 - r)] \]

The mean (\( \bar{z} \)) and standard error (SE) were then calculated for the 32 z values. The 95% confidence interval for the mean was calculated as

\[ z_1 = \bar{z} + t \ SE \]
\[ z_2 = \bar{z} - t \ SE \]

where \( t \) is the 97.5th percentile from the Student's t-distribution with \( n-1 \) (i.e., 31) degrees of freedom. \( \bar{z}, z_1, \) and \( z_2 \) were then back-transformed to the original scale by using

\[ r = \frac{(e^{2\bar{z}} - 1)}{(e^{2\bar{z}} + 1)}, \quad r_1 = \frac{(e^{2z_1} - 1)}{(e^{2z_1} + 1)}, \quad r_2 = \frac{(e^{2z_2} - 1)}{(e^{2z_2} + 1)} \]

to give the upper (\( r_1 \)) and lower (\( r_2 \)) limits of the 95% confidence interval for the population correlation coefficient.

Results

The mean (and SD) concentration of cyclosporine measured by SRIA in whole blood in the 32 heart and heart–lung transplant recipients increased from 146 (102) µg/L in the first week to 282 (101) µg/L by the third week. This equated with mean blood cyclosporine concentrations measured by NSRIA of 504 (304) and 836 (212) µg/L, respectively. The mean whole-blood concentration of cyclosporine in the heart and heart–lung transplant patients was 50% greater than the mean blood cyclosporine concentrations measured in 30 liver- and 30 kidney-transplant recipients studied during the same period in Cambridge.

Cyclosporine and Renal Function

There was a statistically significant negative correlation (i.e., the 95% confidence interval for the true correlation coefficient did not overlap zero) between both five-day mean whole-blood cyclosporine concentrations as measured by NSRIA and SRIA and the subsequent five-day mean reciprocal creatinine concentration (Figure 1). The reciprocal creatinine was the statistically appropriate transformation for stabilizing the distribution of variance (normalization) of these data. It is also physiologically more relevant than the concentration in plasma of creatinine itself because the glomerular filtration rate, the underlying indicator of renal function, is inversely proportional to the concentration of creatinine in plasma (11).

The overall correlation between five-day mean whole-

![Fig. 1. Mean correlation (with 95% confidence interval) between five-day mean cyclosporine and reciprocal creatinine concentrations in 32 heart and heart–lung transplant recipients](image-url)

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blood cyclosporine and reciprocal creatinine concentrations was not found for NSRIA-measured cyclosporine in plasma. Furthermore, no consistent correlations were found (i.e., the 95% confidence interval for the correlation coefficient overlapped zero) between five-day mean whole-blood or plasma measurements of cyclosporine and either five-day mean concentrations of urea or potassium in plasma, whether in phase or out of phase by as many as three five-day periods.

Patients were arbitrarily divided into "susceptible" and "nonsusceptible" groups, according to the magnitude of the correlation between five-day mean cyclosporine and reciprocal creatinine concentrations measured in phase and lagged out of phase by as many as three one- to five-day periods. This descriptive exercise helped us to visualize the entire time series for patients with extremes of observed correlation. In 15 of 32 (47%) patients, the negative correlation coefficient exceeded 0.7 (high susceptibility), whereas in 11 of 32 (34%) it was between 0.5 and 0.7 (medium susceptibility), and in only six of 32 (19%) was it <0.3 (low susceptibility). There was no significant difference between the mean cyclosporine concentration, measured by SRIA or NSRIA, in high- and low-susceptibility groups (Student's t-test). Longitudinal cyclosporine and creatinine data profiles from selected high and low susceptibility individuals are illustrated in Figure 2.

Heart-transplant patients had more early post-operative renal dysfunction than did the heart–lung group, probably associated with pre-operative cardiac failure. In the first three days after transplantation, the mean (SD) creatinine concentration in the heart group was 164 (89) μmol/L, whereas in the heart–lung group it was 91 (49) μmol/L. Cyclosporine concentrations in both groups were always <200 μg/L (SRIA) in the first three days after transplantation, and usually <100 μg/L during treatment with antithymocyte globulin. The post-operative renal dysfunction in most heart recipients improved within the first week and by days 7 to 9 the mean creatinine concentration had decreased to 124 (SD 50) μmol/L.

Although post-operative renal function was better in the heart–lung group, there was no evidence that they were less susceptible to cyclosporine nephrotoxicity. Among the heart–lung recipients, three of eight patients were in the high-susceptibility group, four in the medium group, and one in the low group.

Cyclosporine and Liver Function

No consistent correlations were found between either whole-blood or plasma cyclosporine concentrations (by NSRIA) or whole-blood SRIA measurements and LFT data. There was, however, an overall correlation between the five-day mean whole blood NSRIA/SRIA ratio and the five-day mean serum bilirubin concentration [when in phase, but not out of phase (r = 0.47; 95% confidence interval, 0.31 to 0.59)]. This correlation reflects the concurrent decrease in concentrations of both bilirubin and cyclosporine metabolites in blood with time after transplantation (Figure 3).

Discussion

In Cambridge, heart and heart–lung transplant patients are, on average, maintained on 50% higher whole-blood cyclosporine concentrations (SRIA) than are liver and kidney recipients. The requirement for effective immunosuppression in the prevention of acute rejection is the major clinical priority in the management of heart and heart–lung recipients, the consequence of early graft failure being death. Heart and heart–lung transplant patients at Papworth were, until recently, also routinely monitored for cyclosporine in both whole blood and plasma. They were, therefore, an ideal group in which to carry out time-series analysis of the relative predictive values of different cyclosporine assays and sample matrices, with respect to their temporal association with biochemical measures of renal and hepatic toxicity.

Fig. 2. Whole-blood SRIA cyclosporine and plasma creatinine concentration profiles (untransformed) in three “susceptible” heart-transplant recipients (A–C; negative correlation coefficient >0.7 with a one- to five-day lag in creatinine) and one “nonsusceptible” heart-transplant recipient (D; r<0.3, with no lag or up to three one- to five-day lags in creatinine)

Fig. 3. Changes in mean (± SE) serum bilirubin (—–) and the NSRIA/SRIA ratio (——) with time after heart and heart–lung transplantation (HTx)
In this retrospective analysis, 32 consecutive heart and heart–lung recipients were studied during the first three months post-transplantation. A common association between changes in cyclosporine concentrations and renal function emerged when five-day mean concentrations of cyclosporine in whole blood were correlated with mean reciprocal creatinine concentrations measured in the subsequent five-day period. This correlation was found for both whole-blood NSRIA and SRIA cyclosporine assays; NSRIA measurements of cyclosporine in plasma were less sensitive in the prediction of changes in renal function.

The lack of sensitivity of the plasma assay may be in part due to the lower precision of assays for measuring the relatively low concentrations of cyclosporine found in plasma separated at room temperature. Kennedy et al. (12) did find an association between the incidence and rate of development of renal dysfunction and trough concentrations of cyclosporine measured by polyclonal antibody-based radioimmunoassay in serum separated at 20 °C, but the bone-marrow transplant patients in their study were maintained on high doses of cyclosporine (standard 12.5 mg/kg per day orally) and no adjustment was made to maintain cyclosporine concentrations within a specific range.

The separation of plasma or serum equilibrated at 37 °C yields higher cyclosporine concentrations but does not improve the poor correlation found between plasma and whole-blood cyclosporine measured by NSRIA (13). Sommer et al. (14) analyzed >300 episodes of cyclosporine nephrotoxicity in 85 kidney recipients within six months of transplantation. Pharmacokinetic indices for cyclosporine, estimated from cyclosporine concentration-time measurements (nonselective assay) in serum separated at 37 °C, did not correlate with or predict the subsequent incidence of renal dysfunction. However, comparison of the relative pharmacodynamic merits of whole blood and plasma for measuring cyclosporine concentration with nonselective assays is complicated by the variable partitioning of different cyclosporine metabolites between plasma and blood cell compartments (15). Further studies are warranted to make this comparison by selective cyclosporine assay.

Kasiakse et al. (16) suggested that their failure to find a correlation between cyclosporine pharmacokinetic indices and renal function in a prospective study of 45 kidney recipients was attributable to the relatively low doses of cyclosporine given their patients, compared with studies in which a correlation had been found. The success or failure to find an association between cyclosporine concentrations measured in a given matrix and changes in renal function may, therefore, depend on the dosage regime used. However, the variable susceptibility to nephrotoxicity of transplant recipients maintained on similar concentrations of cyclosporine in blood suggests that it may not be appropriate to analyze the temporal relationships between the drug concentration and renal function by grouping data from all patients together as in classical studies. The understanding of this relationship in each individual will probably be more informative biologically and more helpful in clinical practice for individualizing dosage requirements. For example, the judicious reduction of cyclosporine dosage in patients who are identified as susceptible to cyclosporine nephrotoxicity may decrease the incidence of chronic, irreversible renal damage and, as a consequence, the requirement for cyclosporine withdrawal, which can precipitate acute rejection (1).

The failure to observe a correlation between concurrent five-day mean measurements of cyclosporine and creatinine concentrations may have many explanations. First, there will probably be a temporal delay between an initial nephrotoxic insult from cyclosporine and the onset of biochemically overt renal impairment. Second, heart-transplant recipients in particular often have significant renal dysfunction in the immediate post-operative period, probably attributable to pre-operative cardiac failure (7) rather than cyclosporine nephrotoxicity. This is borne out by the observed decrease in mean creatinine concentrations in heart-transplant patients over the first week, at a time when cyclosporine concentrations were universally rising.

Although any post-operative renal dysfunction in heart recipients usually improved rapidly, it created statistical "noise" in the cross-correlation of cyclosporine and creatinine concentrations. Lagging five-day mean creatinine concentration measurements by one one- to five-day period out of phase with cyclosporine measurements effectively removed this early "noise" from the analysis.

Some liver dysfunction is common after heart transplantation but we found no evidence to connect changes in blood or plasma cyclosporine concentrations with changes in biochemical tests of liver function, whether correlated concurrently or out of phase. It is conceivable that differences in the kinetics of changes in cyclosporine and LFTs may make them unamenable to this type of time-series analysis. The "4-State" Kalman filter may be more appropriate statistical tool under such circumstances (11). However, a more likely explanation is that increases in liver-function tests post-transplantation are again attributable to cardiac failure (17), not to cyclosporine toxicity.

The simultaneous decrease in both serum bilirubin and the NSRIA/SRIA cyclosporine assay ratio with time after transplantation is consistent with previous observations in both heart- and liver-transplant recipients (18, 19). This probably reflects the overall improvement in biliary clearance of cyclosporine metabolites (which are significantly detected only in the NSRIA) and of bilirubin, both of which appear to follow similar elimination kinetics. The clinical significance of this observation is that nonselective cyclosporine assays may provide a particularly poor measure of immunosuppression in the early weeks after heart transplantation if detected metabolites actually do have low activity (20). It is perhaps more surprising that both NSRIA and SRIA measurements of cyclosporine concentrations in blood correlated in a similar way with renal function, despite the changes in the NSRIA/SRIA ratio, although the mean ratio did stabilize within a month of transplantation.

The interpretation of sequences of biochemical data in relation to clinical events can be difficult even if the experienced physician is able to anticipate the forms of the pattern that might arise and the changes in pattern that are most likely. Important changes in observed sequences can be masked by the "noise" resulting from biological variability and from errors in collection, measurement, and processing of the data. Cross-correlating the five-day mean periods of data, as applied in this study, effectively obviated these problems.

Biostatistic time-series analysis proved useful in revealing the temporal relationship between cyclosporine therapy and the development of renal toxicity. Further studies are required to assess the clinical value of the technique in objectively distinguishing between those heart or heart–
lung transplant patients who are susceptible to severe cyclosporine-induced nephrotoxicity and those who are better able to tolerate the high concentrations of this drug that might be necessary to prevent life-threatening rejection. The temporal relationship between cyclosporine treatment and other associated risk factors such as infection and rejection may also be amenable to this type of analysis. Prospective evaluation of such analyses in heart-, liver-, and kidney-transplant recipients is in progress.

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References