PHARMACOKINETICS AND DRUG DISPOSITION

P450 3A activity and cyclosporine dosing in kidney and heart transplant recipients

Interpatient differences in the kinetics of cyclosporine appear to result in part from interindividual differences in the catalytic activity of an enzyme termed P450 3A. We investigated the relationship between P450 3A activity, as measured by the erythromycin breath test (ERMBT), and the appropriate stable daily dose of cyclosporine as currently determined by physicians at our institution. The ERMBT was administered to kidney and heart allograft recipients who had attended at least two monthly clinic visits without having their daily cyclosporine dose changed. There was a significant positive correlation between the ERMBT result and the daily cyclosporine doses (in milligrams per kilogram) in both the heart ($r = 0.68$; $p = 0.04$; $n = 9$) and kidney ($r = 0.68$; $p = 0.03$; $n = 10$) recipients. To confirm our findings, we prospectively administered the ERMBT on multiple occasions to 20 patients who were undergoing kidney transplantation. Although the transplant physicians were blinded to the ERMBT results, the test predicted the stable daily doses of cyclosporine that they ultimately prescribed to the patients ($r = 0.54$; $p = 0.015$). When data from all 39 patients were pooled and subjected to multiple regression analysis, the ERMBT was the only variable examined that significantly correlated with the stable daily cyclosporine dose ($r = 0.63$; $p < 0.001$; $n = 39$). In the 20 patients prospectively studied, the prescribed daily dose of cyclosporine generally decreased during the months after surgery and the percentage changes in cyclosporine daily dose correlated with changes in P450 3A activity during this period ($r = 0.47$; $p = 0.03$). We conclude that interpatient and intrapatient differences in P450 3A activity in part account for the cyclosporine dosing practices of transplant physicians. (CLIN PHARMACOL THER 1994;56:253-60.)

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The daily dose of cyclosporine that suppresses organ rejection while minimizing toxicity varies at least tenfold among organ transplant recipients.[1] The major pathway for elimination of cyclosporine involves hydroxylation and demethylation by a cytochrome P450, termed P450 3A[1-4] (in this article, the term P450 3A is used to denote all the possible gene products of the *CYP3A* gene subfamily[5]), which is present in the liver[6,7] and in intestinal mucosa.[8,9] We have previously used the [^{14}C *N*-methyl]erythromycin breath test (ERMBT) to show that interpatient differences in P450 3A activity[10,11] account in part for interpatient heterogeneity in the pharmacokinetics of cyclosporine. The ERMBT is based on the observations that P450 3A exclusively catalyzes the *N*-demethylation of erythromycin in the liver. The fate of the cleaved

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Table I. Stable organ recipients

Sex	Age (yr)	Months since transplant	ERMBT (% ^{14}C/1 hr)	Cyclosporine level (ng/ml)*	Cyclosporine dose (mg/kg)	Dosing	Months receiving this dose	Azathioprine dose (mg/kg)	Prednisone dose (mg/kg)
Kidney transplant									
Male	33	12	3.36	71.25	3.43	qd	2	0.86	0.11
Male	30	11	2.96	58.8	4.71	qd	9	1.47	0.12
Female	49	49	3.70	83.25	5.89	qd	18	0.00	0.13
Male	57	30	1.17	165.0	1.97	qd	5	0.87	0.12
Male	40	11	2.79	52.25	3.75	qd	9	1.56	0.13
Male	32	21	1.43	74.5	4.72	qd	6	1.97	0.16
Male	59	26	3.42	67.5	4.76	qd	19	0.95	0.05
Male	30	39	4.72	86.3	4.54	qd	1.5	1.77	0.14
Male	52	9	1.32	107.25	2.82	qd	4	0.56	0.11
Female	54	33	1.22	100.3	1.90	qd	12	0.00	0.09
Mean	43.6	24.1	2.61	86.64	3.85		9	1.00	0.12
Heart transplant									
Male†	55	36	8.24	209.3	15.25	tid	26	1.63	0.20
Female	60	18	2.04	242.7	4.05	bid	7	1.73	0.23
Male	52	19	0.88	108.3	1.23	bid	5	1.76	0.18
Male	32	22	2.21	100.2	2.97	bid	6	1.98	0.20
Male	42	13	1.1	267.0	3.34	bid	5	1.48	0.15
Male	54	35	3.27	189.7	3.48	bid	8	0.70	0.07
Male	44	52	2.06	271.8	3.22	bid	7	0.24	0.12
Female	54	7	1.22	322.3	4.10	bid	3	1.51	0.18
Female	51	25	4.68	88.2	6.00	bid	8	2.00	0.15
Male	49	9	3.07	191.0	7.13	bid	2.5	1.53	0.13
Mean	49.3	23.6	2.88	199.07	5.087		8	1.46	0.16

ERMBT, [^{14}C *N*-methyl]Erythromycin breath test; qd, every day; tid, three times a day; bid, twice a day.
*Mean of two to three trough blood levels obtained while receiving the indicated dose.
†Patient A.

methyl carbon is to promptly appear in the breath as carbon dioxide.[12] Hence, the rate at which $^{14}CO_2$ is exhaled after an intravenous injection of [^{14}C *N*-methyl]erythromycin reflects the liver activity of P450 3A. We have previously reported that the ERMBT results correlated with mean trough blood levels of cyclosporine in 32 patients with psoriasis receiving fixed doses of cyclosporine for 16 weeks.[13] In a second study of 16 kidney transplant recipients, we showed that the ERMBT results correlated with the apparent oral clearance of cyclosporine at steady state.[14]

Because there does not always appear to be a good correlation between the pharmacokinetics and pharmacodynamics of cyclosporine, it cannot necessarily be concluded from our previous studies that measurements of P450 3A activity will provide useful information to clinicians administering cyclosporine. Some patients maintained with relatively low trough blood levels of cyclosporine will show significant drug toxicity, whereas others with relatively high cyclosporine blood levels will experience organ rejection.[15-17] Thus, although the physician may use target cyclosporine trough level ranges as a general guide to medication administration, the ultimate maintenance dose for a given patient is frequently determined after many small dosage adjustments based largely on clinical suspicion of organ rejection or drug toxicity.[1] Cyclosporine dosing is particularly difficult in kidney transplant recipients because organ dysfunction from nephrotoxicity can be difficult to distinguish from rejection.

In this study, we investigated the relationship between the ERMBT result and appropriate cyclosporine dosing as it is currently determined for solid organ transplant recipients by their physicians. Our data support the hypothesis that interpatient and intrapatient variation in P450 3A activity largely accounts for the cyclosporine dosing requirements of transplant recipients.

METHODS

Patients studied. In the first phase of the study, we administered the ERMBT to 10 kidney and 10 heart transplant recipients. Patient characteristics are dis-

played in Table I. Women with reproductive potential and children under 18 years of age were excluded. Each subject had to have been prescribed the identical daily dose of cyclosporine for at least 6 weeks (mean, 8 weeks) and must have attended at least two consecutive monthly clinic visits without changes in their cyclosporine dosing. All patients were considered by their primary physician to be medically stable and to be without evidence of organ rejection or significant cyclosporine toxicity.

Dosing of cyclosporine was determined for each patient by his or her own physician (five kidney transplant physicians and a single heart transplant physician). Kidney recipients received cyclosporine as a single daily dose, whereas nine of the 10 heart transplant recipients received cyclosporine in two equal daily doses. The remaining heart recipient received three daily doses of cyclosporine. All patients were tested at least 6 months after transplant surgery (mean, 22½ months). The patients took their usual cyclosporine morning doses immediately after the breath test.

In the prospective phase of the study, patients were enrolled before undergoing kidney transplant surgery. Women with reproductive potential and children under 18 years of age were excluded. Patients received the ERMBT on four occasions: before surgery (ERMBT 1), in the immediate postoperative period just before cyclosporine therapy was started (ERMBT 2), at an early outpatient clinic visit 1 to 4 months (mean, 2½ months) after transplantation (ERMBT 3), and after the patient had attended two consecutive monthly clinic visits without a change in his or her prescribed daily dose of drug (ERMBT 4). At the time of the fourth breath test, these patients satisfied the entrance criteria for our initial (retrospective) study. Physicians adjusting the cyclosporine dosing were blinded to the ERMBT results throughout the study.

A sample size of 20 was chosen to allow an 80% likelihood of rejecting the null hypothesis (no correlation between the ERMBT results and the stable cyclosporine dose) if the true correlation between the parameters was 0.6. Therefore the data was analyzed after the first 20 consecutively enrolled patients completed the final breath test (ERMBT 4). Five additional patients were enrolled but never completed the study. Four of these patients died of causes unrelated to cyclosporine (or the ERMBT) and one patient rejected his transplanted kidney.

It was our intent to enroll every eligible patient undergoing kidney transplantation surgery. Twenty-three women with reproductive potential, four children, two patients with possible erythromycin allergy, and two patients undergoing combined kidney and pancreas transplantation were excluded. One patient refused to participate in the study.

Investigational administration of the ERMBT to these patients was approved by the Institutional Review Board of the University of Michigan Medical Center. Participants provided separate written consent for the ERMBT portion of the study.

[^{14}C N-methyl]Erythromycin breath test. The erythromycin breath test was administered as described previously,[12] except that a single breath collection was made. Three microcuries of [^{14}C *N*-methyl]erythromycin (0.074 μmol) (DuPont NEN Research Products, Boston, Mass.) were dissolved in 2.5 ml of 5% dextrose in water immediately before intravenous administration. Twenty minutes after the injection, patients were instructed to exhale through a tube, creating bubbles in 4 ml of hyamine hydroxide and ethanol (1:1) to which a trace amount of thymolphthalein had been added. When 2 mmol carbon dioxide had been trapped, which took approximately 40 seconds of exhalation, the blue color vanished. The vials were then capped and transported to the laboratory. Twelve milliliters of Aquasol (DuPont NEN Research Products) were added to each vial and the specific activity of carbon 14 determined by scintillation counting. The percentage of administered carbon 14 exhaled per minute was calculated assuming an endogenous carbon dioxide production equal to 5 mmol CO_2/m^2 body surface area/min.[18] We have found that the rate of carbon 14 exhalation calculated from a single breath collection obtained at 20 minutes correlated well with the percentage of administered label exhaled in 1 hour calculated from breath samples obtained every 10 minutes (r = 0.95; Watkins PB, unpublished results, May 1990). Breath test results are expressed as the percentage of administered carbon 14 exhaled during the first hour after injection of the erythromycin[12] as estimated by the following equation:

$$(\%\ ^{14}\text{C administered exhaled/min at 20 minutes} \times 43.917) + 0.38338$$

We have shown that the results of the ERMBT remain valid when patients are receiving treatment with cyclosporine.[13]

Measurement of cyclosporine blood levels. Parent cyclosporine was measured in whole blood according to an established HPLC technique.[19]

Statistical analysis. Multiple regressions were performed with SAS (Statistical Analysis System) GLM procedure (SAS Institute Inc., Cary, N.C.). Hematocrit and serum lipid fractions were not uniformly obtained in patients and were therefore not considered to be independent variables in the regressions. However,

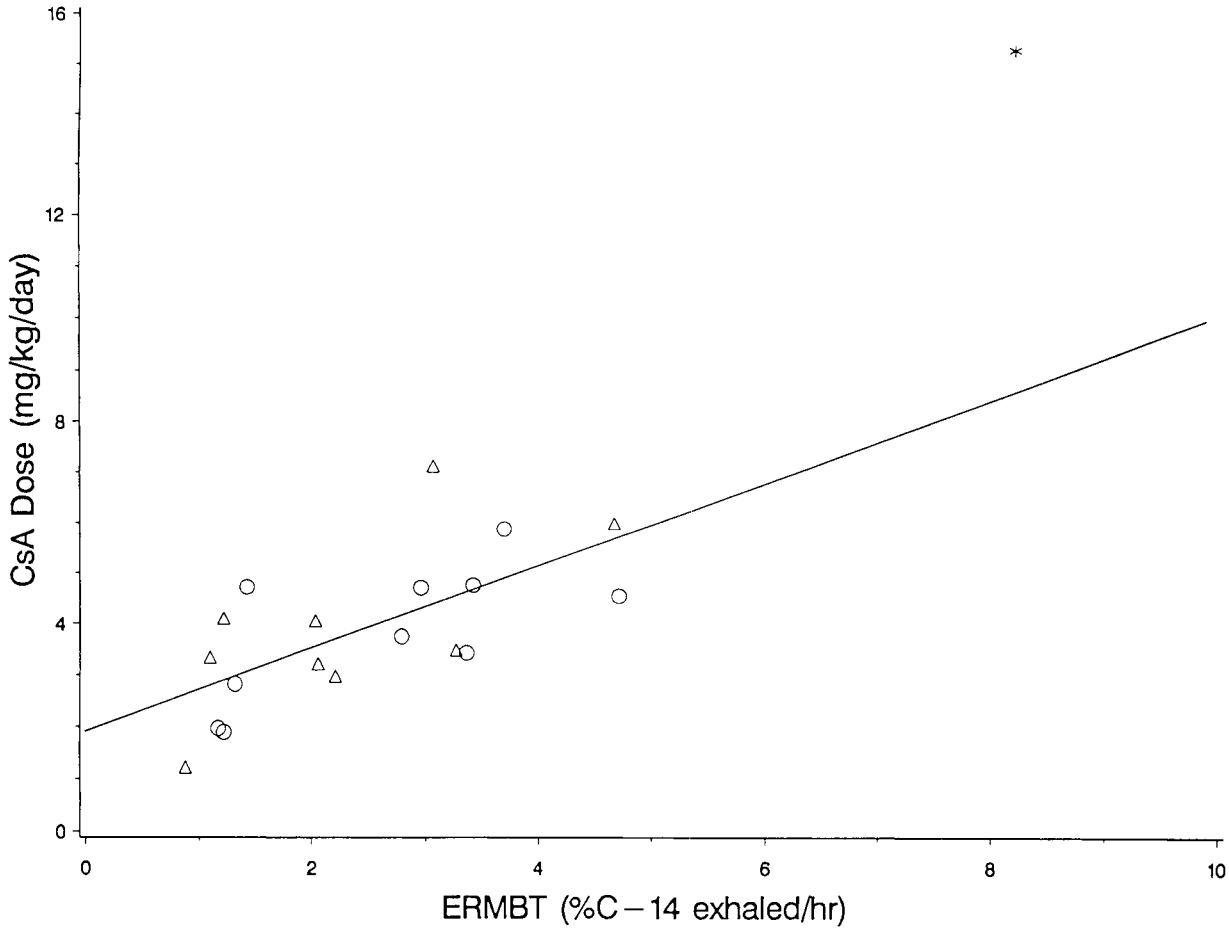

Fig. 1. The correlation between the erythromycin breath test (ERMBT) result and the daily dose of cyclosporine (CsA) prescribed to 10 stable heart *(triangles)* and kidney *(circles)* transplant recipients. There was a significant correlation between the ERMBT result and the daily dose of cyclosporine prescribed to each patient when all patients were considered ($r = 0.75$; $p < 0.001$; $n = 20$). The correlation remained significant when the patient receiving 15 mg/kg/day *(asterisk,* patient A in Table I) was excluded ($r = 0.67$; $p = 0.002$; $n = 19$). When analyzed separately, significant correlations were also observed in the kidney recipients ($r = 0.68$; $p = 0.03$; $n = 10$) and in the heart recipients ($r = 0.68$; $p = 0.04$; $n = 9$; excluding patient A).

we have not detected significant correlations between these variables and cyclosporine pharmacokinetics in two previous studies.[13,14] One heart transplant patient (patient A in Table I) received 15.25 mg/kg/day cyclosporine and had a breath test result of 8.24 (percentage of administered carbon 14 exhaled in breath in 1 hour); each of these values is almost twice that of any other patient. Patient A had a seizure disorder and was receiving phenytoin, a known P450 3A inducer.[20] All analyses were performed with and without data from this patient. When the results differed, the results of both analyses are given.

RESULTS

Correlation between the ERMBT result and stable cyclosporine dose. The ERMBT was administered to 10 kidney and 10 heart transplant recipients who were receiving cyclosporine therapy (see Table I for patient characteristics). Each patient had attended at least two consecutive monthly clinic sessions since the last change in his or her daily cyclosporine dose. We therefore assumed that each patient was receiving an "appropriate" dose of cyclosporine in the opinion of his or her physician. The stable daily dose of cyclosporine (in milligrams per kilogram) received by the

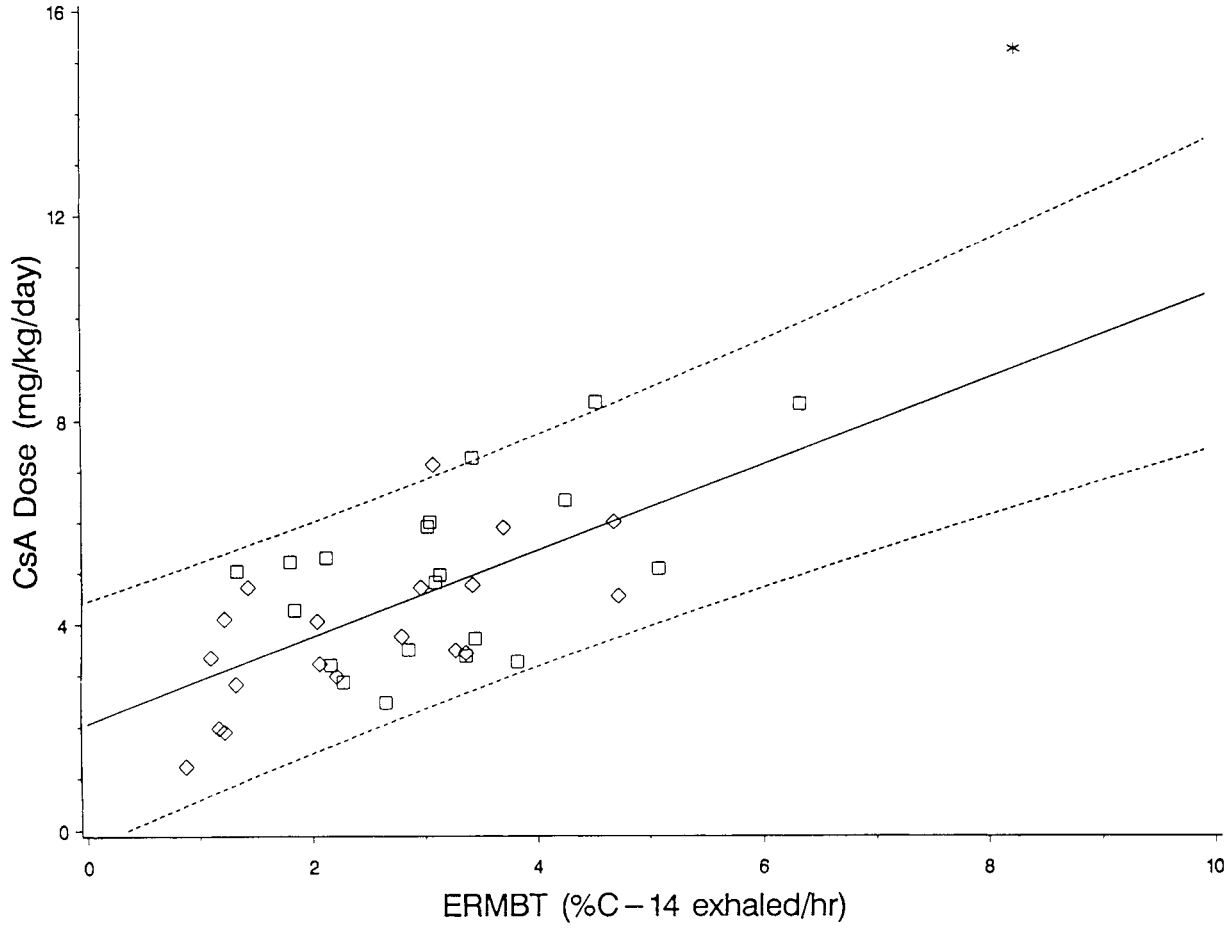

Fig. 2. The correlation between the ERMBT result and the stable dose of cyclosporine prescribed to the 20 prospectively followed kidney transplant recipients *(squares)* and to the 10 heart and 10 kidney recipients initially studied *(diamonds,* patient A shown by *asterisk).* Among the prospectively followed patients, there was a significant correlation between the ERMBT result (ERMBT 4) and the stable cyclosporine dose the patients were taking at the time of the test ($r = 0.54$; $p = 0.015$; $n = 20$). The 90% prediction limits *(broken lines)* are shown for all 39 patients (excluding patient A *[asterisk]*).

patients varied approximately sixfold (Table I). Whole blood parent cyclosporine levels varied approximately threefold in each transplant group (Table I). This is consistent with the concept that physicians' decisions regarding administration of cyclosporine are not guided solely by blood level monitoring. Among these 20 patients, the correlation between the ERMBT results and the ratios of observed cyclosporine blood level to daily cyclosporine dose (in milligrams per kilogram per day) was 0.63 ($p = 0.003$; data not shown).

As shown in Fig. 1, there was a positive correlation between the ERMBT result and the cyclosporine dose ($r = 0.75$; $p < 0.001$; $n = 20$). When the single patient with both the highest ERMBT result and largest daily cyclosporine dose (patient A, Fig. 1) was excluded, the correlation remained significant ($r = 0.67$; $p = 0.002$; $n = 19$).

To determine if other patient characteristics or laboratory parameters might also predict dosing of cyclosporine, a stepwise regression was performed. The predictor variables ERMBT result, cyclosporine blood concentration, transplant type (kidney or heart), months after transplant, gender, age, weight (in kilograms), azathioprine dose (in milligrams per kilogram), prednisone dose (in milligrams per kilogram), and traditional liver chemistries (alanine aminotransferase, aspartate aminotransferase, alkaline phos-

phatase, and bilirubin) were entered into a regression equation with the dependent variable cyclosporine dose (in milligrams per kilogram per day). Predictor variables were then deleted until only those significant at a $p < 0.05$ level remained ("backward" selection). When all 20 patients were included, the resulting model (not shown) included only the ERMBT ($p < 0.001$) and prednisone dose ($p = 0.03$). When patient A was deleted, the final model contained only the ERMBT ($p < 0.01$).

Within the heart transplant group, the coefficient of correlation *(r)* between the ERMBT and the cyclosporine dose was 0.92 ($p < 0.001$) when patient A was included and 0.68 ($p = 0.04$; $n = 9$) when patient A was excluded (Fig. 1). In the kidney recipients, the correlation coefficient was also 0.68 ($p = 0.03$; $n = 10$).

Prospective study of kidney transplant recipients. We next prospectively administered the ERMBT on four occasions to patients undergoing kidney transplantation. The ERMBT results rose in most patients between the first and second breath test (corresponding to immediately before surgery and after surgery just before cyclosporine was started, respectively); however, this increase did not reach significance (not shown). There was also no significant difference between the mean ERMBT result at the time of the second and third tests (not shown). However, there was a significant decrease in the mean ERMBT result between the third ERMBT (mean, 4.2%; SD, 1.8%), performed at an early outpatient clinic visit, and the fourth ERMBT (mean, 3.2%; SD, 1.2%), performed a mean of 8.6 months after surgery ($p = 0.002$, paired *t* test).

At an early outpatient clinic visit (the third ERMBT, a mean of 2½ months after surgery), there was a significant correlation between the patients' ERMBT values and the doses of cyclosporine prescribed ($r = 0.55$; $p = 0.01$). There was also a significant correlation between the final stable dose of cyclosporine prescribed and ERMBT results obtained at the time of the fourth ERMBT, a mean of 8.6 months after surgery ($r = 0.54$; $p = 0.015$; Fig. 2). At this time, the correlation between the ERMBT results and the ratios of cyclosporine blood level to daily dose was 0.60 ($p = 0.005$).

There was a significant correlation between the percentage of change in cyclosporine dose and the percentage of change in ERMBT result between the time of the third and fourth ERMBT ($r = 0.472$; $p = 0.037$). Hence, patients who had decreases in ERMBT results generally had a reduction in their prescribed doses of cyclosporine.

Table II. Correlations between cyclosporine dose (in milligrams per kilogram per day) and various patient characteristics or laboratory parameters in the 39 patients shown in Fig. 2*

Variable	*r*	*p*
ERMBT (% ^{14}C exhaled/hr)	0.63	0.0001
Gender	0.089	0.59
Age (yr)	−0.12	0.62
Weight (kg)	−0.30	0.061
Azathioprine dose (mg/kg)	0.22	0.90
Prednisone dose (mg/kg)	−0.080	0.63
AST (IU/L)	−0.16	0.33
ALT (IU/L)	−0.011	0.95
Alkaline phosphatase (IU/L)	0.051	0.76
Total bilirubin (mg/dl)	−0.007	0.96

AST, Aspartate aminotransferase; ALT, alanine aminotransferase.
*Patient A not included.

At the time of the fourth breath test, the prospectively recruited kidney transplant recipients satisfied the entrance criteria used in the original retrospective study (i.e., attending two consecutive clinic visits without a change in the daily cyclosporine dose). We therefore pooled the prospective data with those obtained from the kidney and heart transplant patients originally studied (Fig. 1). As shown in Table II, the ERMBT was the only variable examined that significantly correlated with daily cyclosporine dose. A multiple regression was then performed with the stable cyclosporine dose as the dependent variable and ERMBT result, gender, age, weight (in kilograms), azathioprine dose (in milligrams per kilogram), prednisone dose (in milligrams per kilogram), and traditional liver chemistries (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin) as independent variables. After stepwise deletion of variables (see Methods section), the resulting model included only the ERMBT ($p < 0.0001$). When patient A (Table I) was deleted, the final model also contained only the ERMBT ($r = 0.63$; $p < 0.001$). Thus interpatient differences in the ERMBT results accounted for 40% of the variability in prescribed cyclosporine dosing in the 39 patients ($r^2 = 0.40$). The correlation between the ERMBT results and the cyclosporine blood level/dose ratios was essentially identical ($r = 0.63$; $r^2 = 0.40$).

DISCUSSION

Interindividual differences in P450 3A activity account in part for interpatient differences in the pharmacokinetics of cyclosporine.[13,14] The ERMBT provides a convenient means of measuring P450 3A

activity[12] and we have shown that the ERMBT predicts the pharmacokinetics of cyclosporine at steady state. The significant correlations we found between the ERMBT results and the cyclosporine blood level/daily dose ratios in the current studies further confirm our previous observations. However, the relationship between an individual's P450 3A catalytic activity and the actual dose of cyclosporine administered in clinical practice had not been investigated previously.

In the first phase of this study, we demonstrated that the ERMBT result correlated with the stable doses prescribed to 20 kidney and heart transplant recipients who were apparently at steady state for cyclosporine (Fig. 1). This correlation was then confirmed in a prospective analysis of patients undergoing kidney transplantation. We found that the patients' P450 3A activities, as measured by the ERMBT, correlated with the daily cyclosporine doses prescribed by physicians both at an early outpatient clinic visit and when patients achieved stable cyclosporine dosing at a mean of 8.6 months after surgery (Fig. 2). When the data from the initial and prospective studies were pooled, the ERMBT result was the only variable that significantly predicted the stable dose of the drug (Table II). Even when the data from patient A was excluded, the ERMBT result accounted for 40% of the variability in cyclosporine dosing observed in this population (r^2 = 0.40). Other patient characteristics or laboratory parameters (including traditional liver chemistries) appeared to have no correlation with the cyclosporine dose in these multiple regression analyses. We conclude that interpatient differences in P450 3A activity may largely account for the heterogeneity in cyclosporine dosing requirements.

A reduction in the cyclosporine dosing requirement is generally observed in kidney transplant recipients during the first year after surgery, although there is no accepted explanation for this phenomenon.[20,21] There was also a reduction in the mean daily dose of cyclosporine prescribed to our patients during the study (not shown). This was associated with a decrease in the mean ERMBT result and there was a correlation between the percentage of change in the ERMBT result and the percentage of change in the daily prescribed dose of cyclosporine between the third and fourth tests. It therefore seems likely that the reduction in required dose of cyclosporine that occurs during the first year after transplant surgery in part results from a tendency for P450 3A activity to decrease during this period.

The reasons for the decrease in P450 3A activity in the months after transplant surgery are unclear. In this regard, it may be important that all of the patients received prednisone, a known inducer of P450 3A in cultured human hepatocytes.[22] The mean daily dose of prednisone received by patients was reduced by approximately 50% between the third and fourth breath tests (data not shown). A plausible hypothesis is that withdrawal of this inducer partially accounted for the reduction in P450 3A activity.

In a previous study involving patients treated with cyclosporine for psoriasis, we derived a mathematical model that could account for the majority of variation in observed cyclosporine blood levels using the variables of cyclosporine daily dose, ERMBT result, and patient age.[13] When this model was applied to the data in this study, a statistically significant correlation between the observed blood levels and those predicted from the model was found in each subset of patients (data not shown). However, the actual predictive capacity of the model was far lower in the transplant recipients than in the patients with psoriasis. For example, the correlation was only 0.5 when all 40 patients were examined (p = 0.001), whereas a correlation of 0.83 was obtained in the 32 patients with psoriasis.[13] The poorer correlation in the transplant recipients probably reflects the numerous differences between our psoriasis study and the current study. For example, the dosing regimens differed among our transplant recipients (Table I). In addition, the transplant recipients were receiving multiple medications and had concomitant diseases that would be likely to influence the predictive model.

An important question not addressed by our studies is whether the ERMBT would be useful in prescribing cyclosporine. Based on our data (Fig. 2), it seems likely that the test could potentially identify some individuals who may require unusually high or unusually low doses of the drug. Because of the long blood half-life of cyclosporine, it can take many days or weeks to arrive at the appropriate steady-state dose of cyclosporine for these individuals when guided solely by blood levels. The ERMBT may also be useful in evaluating patients with unexpected changes in their apparent dosing requirements. The discovery that such a patient's prescribed daily cyclosporine dose and ERMBT result lie outside the 90% prediction limits (Fig. 2) could support suspicions of noncompliance. Alternatively, if the patient's values lie within the prediction limits, concomitant treatment with P450 3A inducers or inhibitors[10,11] would be suggested.

In summary, our data supports the hypothesis that interpatient and intrapatient differences in P450 3A activity largely account for the heterogeneity in

physician-derived dosing regimens for cyclosporine. Prospective clinical trials should now be undertaken to evaluate the usefulness of the ERMBT as a guide to monitoring therapy with cyclosporine.

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