A Logistic-Regression Model Provides Novel Guidelines to Maximize the Anti-Acute Rejection Properties of Cyclosporine with a Minimum of Toxicity

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ABSTRACT
Although cyclosporine has become the mainstay of immunosuppression in organ transplantation, there is still no consensus on the criteria to optimize its antirejection activity with minimum toxicity. A clear and objective definition of target cyclosporine trough levels at different times from transplant have not been defined in sufficient detail, primarily because of the lack of a model correlating cyclosporine levels with probability of rejection or toxicity. In this study, logistic-regression model was developed that was applied to data collected retrospectively from two postoperative periods, i.e., Days 0 to 9 and 10 to 30, in 135 consecutive cadaveric renal transplant recipients, for a total of 1851 determinations. Only minimum and maximum trough levels were considered for each period. Concentration-response curves were estimated for Days 0 to 9 (P = 0.0001 for efficacy and P = 0.028 for toxicity) and for Days 10 to 30 (P = 0.015 for efficacy and P = 0.037 for toxicity). Therapeutic intervals of 330 to 430 ng/mL (parent compound in whole blood) for Days 0 to 9 and 260 to 390 ng/mL for Days 10 to 30 predicted an incidence of acute rejection of 22% and 12%, respectively, with a reasonably low toxicity that primarily consisted of elevation of serum aminotransferases.

Key Words: Cyclosporine dosage, cyclosporine blood concentration, acute graft rejection, nephrotoxicity, hepatotoxicity

Addition of cyclosporine (CsA) to conventional antirejection protocols has improved the short-term results of organ transplantation (1). However, acute rejection remains a major problem in transplantation and, concerning renal transplants, it is a recognized risk factor for subsequent chronic deterioration of renal function (2–4). On the other hand, CsA is a nephrotoxic agent (5), and doses high enough to avoid acute rejection may per se impair renal function. Thus studies have addressed the issue of the most appropriate strategy for desired immunosuppression with minimum toxicity. Complete pharmacokinetic profiles for area under the time-concentration curve determination are costly and time- and staff-consuming (6), and abbreviated profiles have been proved inaccurate (7). Thus, trough level monitoring was invariably preferred in clinical practice (8). Target values for CsA trough levels at different times from transplant have not been defined in sufficient detail, primarily because of the lack of a model correlating CsA blood levels with probability of rejection or toxicity. Burke and coworkers (4), in a recent retrospective analysis of data on 1663 renal transplant patients, have reported that average CsA trough levels considered to be “high” (e.g., >250 ng/mL by HPLC on whole blood) at 3 months post-transplant were associated with lower creatinine values in the first and third years, respectively. Lindholm (9) recommended a minimum trough level of 150 ng/mL (parent compound in whole blood) during the first postoperative month. However, he studied a very heterogeneous patient population, with both kidney and kidney-pancreas transplantations, and with approximately two-thirds of patients in triple therapy (CsA, azathioprine, steroids) and one-third in double therapy (CsA plus steroids). Other investigators suggested a therapeutic interval of 150 to 250 ng/mL (parent compound in whole blood) for the first few months after transplantation in patients on triple therapy (10–12), but they did not provide the scientific basis for such indication, or try to define recommended levels for the immediate post-transplantation period, when the frequency of rejection is maximal. Here we have developed a mathematical model on the basis of CsA trough levels measured during two different post-transplant periods—Days 0 to 9 and 10 to 30 after surgery—in 135 consecutive renal transplantations, all treated with CsA combined with steroids and azathioprine.

METHODS
Study Population and Immunosuppression Protocol

One hundred thirty-five consecutive patients (mean age, 45.1 yr) who received their first or second cadaveric renal transplant at the Ospedali Riuniti di Bergamo (Italy) from...
January 1990 to August 1994 were included in this study. All patients had undergone pretransplant evaluation. Cadaveric kidneys were allocated according to our regional agency, Nord Italia Transplant. On admission to the Hospital, all patients underwent routine history and physical examination and a battery of tests, including serum electrolytes, chest roentgenogram, and electrocardiogram. None of the 135 patients had liver dysfunction at the time of transplant or a record of antecedent liver disease, such as hepatitis C. If the patient had clinical evidence of fluid overload or was ≥5% above dry weight, or if indicated by pretransplant laboratory results, dialysis was done before the transplant. After a negative final crossmatch, the patients underwent kidney transplant. The Transplantation Unit of the Ospedali Riuniti di Bergamo adopts a standard triple immunosuppressive protocol, with a fixed CsA dosage, on the basis of the patient's body weight and the time elapsed since the intervention (CsA, 3 mg/kg per day iv for the first 3 days after surgery starting postoperatively as a continuous infusion through a central vein; 10 mg/kg per day orally, divided into two doses given at 12-h intervals until Day 30; then gradually tapered to 3 mg/kg per day divided into two doses during the following 6 months; methylprednisolone 500 mg iv, preoperatively, then given orally in a single morning dose of 16 mg until the end of the third month and progressively tapered to 8 mg/day; azathioprine given at the dose of 1 mg/kg per day). This immunosuppressive protocol is derived from the transplant literature (13). The CsA dose during the first 30 days post-transplant was not adjusted according to a target. Blood samples for CsA level determination during the first 3 days after transplantation are collected from a peripheral vein on the morning just before starting the new CsA infusion. Thereafter, CsA trough levels are evaluated daily during the first few days and then weekly, on blood samples collected before the morning CsA-dosing. For this reason, a wide range of CsA trough levels can be observed, which may be useful for the determination of concentration-response curves and therapeutic ranges. Whole-blood levels of CsA were determined by monoclonal RIA (Cyclo-Trac; Incstar, Stillwater, MN) (7). Concomitant medications administered to these patients did not include drugs, such as verapamil or diltiazem, as well as other medications that could interfere with CsA metabolism through the hepatic cytochrome P-450 enzyme system. None of the patients received antimicrobial prophylaxis; specifically, there was neither routine sulfamethoxazole-trimethoprim nor acyclovir prophylaxis.

To focus on the immediate post-transplant phase, the first month after surgery has been divided into two periods of increasing length: from Day 0 to 9 (first period) and from Day 10 to 30 (second period). The minimum number of documented daily CsA trough levels for admission to the study was three for the first period and six for the second period. This resulted in the selection of 115 and 90 cases, respectively, from a series of 135 consecutive renal transplantations. Only the minimum and maximum CsA trough levels for each period were considered. Trough levels evaluated during Orthocline OKT3 (muromonab-CD3; Ortho Pharmaceutical Corp., Raritan, NJ) cycles for steroid-resistant rejections were discarded, because in these cases, CsA doses are generally lowered to reduce the potential for opportunistic infections, whereas the anti-CD3 antibody affords the mainstay of immunosuppression.

Diagnosis of Acute Graft Rejection and Cyclosporine Toxicity

The diagnosis of acute rejection was made on the basis of clinical criteria in combination with positive findings on a core biopsy when available (23 out of 135 consecutive transplantations). They included the simultaneous occurrence of: (1) an increase in serum creatinine concentration >0.3 mg/dL for more than 2 days over the basal value associated with a stable trough blood CsA level; (2) a renal ultrasonogram that excludes any other cause of allograft dysfunction (including urinary tract obstruction); (3) a response to anti-rejection therapy (at least 3 days of iv methylprednisolone pulses, 0.5 g each, or OKT3 monoclonal antibody infusions in steroid-resistant cases) with improvement of renal function. Nephrotoxicity was defined as a serum creatinine concentration elevation greater than 0.3 mg/dL above baseline that responded to a reduced dose of CsA or persisted without evidence of graft rejection by clinical evaluation or of urinary tract obstruction by ultrasound examination. Hepatotoxicity was determined by liver function tests, if the total bilirubin was ≥1.5 mg/dL, or serum glutamic-pyruvic transaminase or serum glutamic-oxaloacetic transaminase was ≥100 U. No attempts were made at correlating the increase of transaminases with cytomegalovirus infection. When acute tubular necrosis, as defined by accepted clinical and histological features (14), occurred, CsA dose was reduced by 30%.

Statistical Analysis

The descriptive statistics for this study were presented as medians and interquartile ranges (25th and 75th percentiles) in the case of continuous variables and percentages in the case of discrete variables. Data analysis was based on logistic regression of rejection incidence versus logarithm of minimum CsA trough values, and toxicity incidence versus logarithm of maximum CsA trough values, for each post-transplant period. The left limit of efficacy and toxicity curves was constrained to 0, and the right limit of toxicity curves to 1. Logistic regressions, their likelihood ratio for overall significance, and 95% confidence intervals (CI) for regression parameters were evaluated by the LOGISTIC (15) and NONLIN (16) programs for the IBM PC (Vectra 386/20N; Hewlett Packard, Palo Alto, CA). Likelihood ratio statistics tests the significance of the independent variable (logarithm of the minimum or maximum CsA trough levels) on the outcome (graft rejection or toxicity, respectively). To assess the adequacy of the fitted model, a Hosmer-Lemeshow goodness-of-fit test, which compares the observed values with those predicted by the model, was carried out: a small P value was considered to indicate that predicted values did not fit the data. Statistical significance was set throughout at the 0.05 level.

RESULTS

First Period (Days 0 to 9 After Surgery)

During Days 0 to 9, at least one episode of acute rejection was observed in 54 of 115 patients (47%); all were treated with steroid pulses, whereas 13 steroid-resistant rejection patients needed OKT3 infusions. All patients with steroid-resistant rejections, except one, had minimum CsA trough levels <150 ng/mL. Toxicity was diagnosed in 18 of 115 patients (16%); 15 (13%) showed signs of hepatotoxicity (and among
them, five of the 54 with rejection), and three (3%) of nephrotoxicity. Likelihood ratio statistics were significant both for efficacy (P = 0.0001) and toxicity (P = 0.028). The distributions of minimum CsA trough levels versus rejection and maximum CsA trough levels versus toxicity are presented in Tables 1 and 2, respectively.

Second Period (Days 10 to 30)

During Days 10 to 30, the incidence of acute rejection was observed in 16 of 90 patients (18%); all were treated with steroid pulses, whereas six steroid-resistant rejection patients with minimum CsA trough levels ranging from 102 to 202 ng/mL needed OKT3 infusions. Toxicity was diagnosed in 21 of 90 patients (23%); 18 (20%) showed signs of hepatotoxicity (and among them, three of the 16 with rejection), and three (3%) of nephrotoxicity. Again, likelihood ratio statistics were significant (P = 0.015 for efficacy and P = 0.037 for toxicity). Data distributions are presented in Tables 1 and 2.

Concentration-Response Curves

Figure 1 shows the fitted logistic concentration-response curves for efficacy and toxicity, for Days 0 to 9 and 10 to 30 (Hosmer-Lemeshow goodness-of-fit P > 0.36). As the figure shows, the curves for efficacy are steeper than those for toxicity, evidencing a lower intrindividual variability for the antirejection response.

During the first 9 days, half-maximal efficacy (EC50) is reached at 102 ng/mL (95% CI, 59 to 177), maximum efficacy (EC95) at 330 ng/mL, with a predicted 22% incidence of acute rejection. For the rest of the first month, half-maximal efficacy is attained at 91 ng/mL (95% CI, 57 to 146), maximum efficacy at 260 ng/mL, with a predicted 12% incidence of acute rejection. The threshold values under which rejection is most probable (more than 90%) should be located around 50 ng/mL (EC10, 43 ng/mL for the first and 55 ng/mL for the second period). Half-maximal toxicity (TC50) is extrapolated at 2300 ng/mL for the first and 1500 ng/mL for the second period; 15% toxicity (TC15) is reached at 430 ng/mL until Day 9 and at 390 ng/mL thereafter.

DISCUSSION

CsA, by interfering with the calcium-dependent phosphatase calcineurin, blocks the interleukin-2 (IL-2) formation in T-lymphocytes, which promotes the proliferation of T cells in response to transplant antigens (17). This effect is reversible because the mitogen-induced proliferation of T cells from CsA-treated patients may be promptly restored by washing the cells with CsA-free medium (18). Hence, it is conceivable that even transient reductions in CsA blood levels under some threshold value may allow enough IL-2 to initiate the rejection response. For this reason, we used the lowest observed CsA trough value instead of the mean trough level, as was repeatedly done in previous studies (4,19,20). Likelihood ratio statistics were highly significant for both periods considered in the analysis presented here. The acute toxicity induced by CsA is similarly known to be dose-related (5) and probably dependent both on the release of vasoconstrictive factors (5,21,22) and the inhibition of the specific membrane translocase P-glycoprotein (23). Despite the maximum CsA trough values are only an indirect measure of the peak CsA levels that may have induced toxicity, likelihood ratio statistics were significant for the first 9 and 30 days.

Left and right limits of logistic regression curves were constrained to 0 and 1, respectively, assuming that complete CsA withdrawal from triple therapy during the early post-transplant phase would result in rejection and absence of CsA-induced toxicity, whereas CsA overload would very likely lead to toxicity. However, imposing or removing constraints did not substantially change the curves’ shape and location.

The therapeutic window for a given drug is generally calculated as the interval between the concentration effective in 99% of patients and that that causes toxicity in 1% of patients (EC99 to TC1); however, because these values overlap in the present situation, we suggest use of the EC95-TC5 interval, i.e., 330 to 430 ng/mL for the first 9 days, and 260 to 390 ng/mL for the rest of the month. No further improvement of the antirejection properties of CsA may be achieved by increasing blood levels beyond these values. In any case, CsA trough levels should never fall under the concentrations active in 90% of patients (EC90), i.e., 240 ng/mL for Days 0 to 9 and 150 ng/mL until the end of the first month. These levels are higher than those suggested elsewhere for the first few months after transplantation in patients on triple therapy (150 to 250 ng/mL) (10–12). Interestingly, a recent report

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TABLE 1. Distribution of minimum CsA trough levels in cases with or without rejection
TABLE 2. Distribution of maximum CsA trough levels in cases with or without toxicity

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Figure 1. Estimated concentration-response curves for efficacy versus minimum CsA trough levels and toxicity versus maximum CsA trough levels during Days 0 to 9 (N = 115) and Days 10 to 30 (N = 90). E9, efficacy curve for Days 0 to 9; E30, efficacy curve for Days 10 to 30; T9, toxicity curve for Days 0 to 9; T30, toxicity curve for Days 10 to 30.

has documented that at CsA trough levels of 150 to 190 ng/mL, the calcineurin phosphatase activity of peripheral blood leukocytes is still 45 to 55% of control subjects, indicating that higher levels may be required to achieve a more substantial inhibition of IL-2 production (24). The actual incidence of nephrotoxicity in our study was very low (2 to 3%), whereas elevations of aminotransferases were more common, reaching 20% during Days 10 to 30. Indeed, CsA-dependent hepatotoxicity generally appears to be mild and asymptomatic (25), reversible on dosage reduction (26), and not troublesome in patients with normal liver function at the time of transplantation (27). For these reasons, we feel confident that the chosen 15% maximal incidence of toxicity may be well tolerated in a clinical setting. All patients studied had no recorded antecedent liver disease before kidney transplant. One should be aware however of the possible confounding factor of previous liver diseases, such as hepatitis C, in evaluating the actual incidence of CsA-induced hepatotoxicity by this approach.

On the other hand, the concern that high early doses of CsA may be a risk factor for long-term renal dysfunction (5,28) has not been substantiated by a study specifically designed to address this point (4). In the latter study, patients with the highest whole-blood CsA trough levels (≥250 ng/mL by HPLC analysis) at 3 months post-transplant had better renal function at 1 and 3 yr as compared with those with lower CsA levels. Consistent with the above findings, in heart-transplanted patients given CsA as a chronic antirejection therapy after an initial decline at 2 to 3 yr from surgery (29), renal function did not worsen further at 5 to 6 yr (30), indicating that CsA renal toxicity is self-limited. This observation is supported by previous studies in 200 cardiac transplant recipients by Myers and Newton (31). They found that after 12 months, both low- and high-dose CsA treatment was associated with depression of GFR below the values recorded in another 100 recipients who had never been exposed to CsA. In contrast, there are reasons to believe that acute rejection reduces nephron units, which in the long-term may increase the risk of hyperfiltration damage (32,33). Thus the tendency to give low doses of CsA, under the assumption of avoiding early and late renal complications, is not substantiated by current findings. Instead, higher CsA levels during the first 30 post-transplant days, although remarkably reducing the incidence of acute rejection, may better preserve renal function over the long term (4,34). Our findings are in line with a recent observation by Nankivell et al. (35) in 92 consecutive renal allograft recipients receiving triple therapy and studied for episodes of renal dysfunction with respect to whole-blood CsA concentration within the first 100 days post-transplantation. They found a good specificity in the prediction of CsA nephrotoxicity by high (>400 ng/mL) levels and of acute rejection by low (<150 ng/mL) CsA levels, although the sensitivity was relatively low. However, in this study, 59% of rejection episodes occurred within the "therapeutic" (150 to 400 ng/mL) range for CsA, a value markedly higher with respect to what we have predicted by our present model. The apparent inconsistency between the two proposed regimens (that admittedly derives from different methodological approaches to the problem) is, however, easy to reconcile if one considers that the "therapeutic" range proposed by the above study (i.e., 150 to 400 ng/mL) is so wide that it includes a considerable number of patients who have trough levels well below what was shown by our mathemati-
nal model to be minimally protective. Difference in nephrotoxicity (63% versus 3%) has to take into account the fact that a single CsA-dosing regimen, as Nankivell and coworkers used, should not be assumed to be equivalent to a twice-daily regimen, as we used in terms of the negative impact on renal function of the very high blood CsA peak level reached in the former case.

CsA is metabolized in the liver by enzymes of the cytochrome P450 3A subfamily to more than 30 metabolites (36). They include first-generation metabolites, those generated by metabolism of the native compound CsA in one position (AM1, AM9, AM4N), and second-generation metabolites, those generated by further metabolism of other metabolites. Few and conflicting data are available on the potential immunosuppressive activity of these metabolites, and whether they contribute at least in part to the well-known toxic effect of the native compound is still a matter of debate (37-39). The present logistic-regression model is based on measurement of whole-blood CsA concentration by RIA using a specific monoclonal antibody that detects the circulating level only of the parent drug but not of its metabolites. Therefore, the results we obtained reflect the toxic/therapeutic profile of CsA "per se." On the basis of the results of the study presented here, we suggest that the maximal antirejection effect of CsA could be attained at concentrations of 330 to 430 ng/mL for the first 9 days after surgery, and 260 to 390 ng/mL for Days 10 to 30. The model predicts no further improvement of antirejection activity for trough levels of CsA higher than as indicated above. Within these therapeutic intervals, the model predicts a rather low frequency of toxic reactions. The incidence of acute rejection episodes predicted by the present model is considerably lower than that reported in most series, which, by utilizing more conservative protocols, have an incidence of acute rejection episodes ranging between 47% (4) and 60% (2) in the first 30 postoperative days. It goes without saying that the theoretical findings of this model should be validated by means of a prospective trial to verify whether the therapeutic intervals proposed here, if tested against more conventional protocols, did indeed offer the predicted degree of protection. The results of the study presented here, if confirmed prospectively, may have a major impact in the management of organ transplants other than those of the kidney.

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