Iranian Red Crescent Medical Journal

ORIGINAL ARTICLE

A comparison between the levels of cyclosporine C0 and C2 in children undergoing renal transplantation

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Abstract

Background: Cyclosporine A (CSA) with intra- and inter-individual variability in absorptive property needs individualized dose adjustment in patients receiving the drug. This study was performed to compare cyclosporine C0 (before morning dose) and C2 (two hours after morning dose) levels in order to adjust the maintenance dose of CSA in stable renal transplant patients in Shiraz, southern Iran.

Methods: From October 2004 to June 2005, 64 kidney transplants of Nemazee Hospital entered our study. All patients underwent renal transplantation for the first time except one subject who received the second transplant. All patients received three immunosuppressive drugs of which CSA was administered in two divided doses in the form of microemulsion. The height, weight, blood pressure, periodical tests and C0 and C2 levels were determined at the time of referral, as well as one and 5 months later. The amount of CSA was adjusted based on C0 levels of 100-250 ng/mL. The patients were divided into two C0 subgroups with C0 levels of <100 and ≥100 ng/mL. In regard to C2, the two subgroups were <800 and ≥800 ng/mL. In addition to CSA, cellcept and prednisolone were administered to 47, immuran with prednisolone to 15, and only prednisolone to 2 patients.

Results: Comparing the two subgroups of C0 and C2, no differences were observed between serum creatinine level, CSA doage and the drug complications. A significant correlation was found between C0 and C2 levels, and also between C2 level and CSA dosage. A negative correlation was seen between C0 level and serum creatinine. The coefficient of variation of the three samples of each patient was 10.89% for C0, and 8.94% for C2 with constant drug regimen.

Conclusion: As there was no significant difference between mean C0 and C2 levels, and renal function at the start and the end of study, there seemed to be no need to recommend C2 level for follow up of renal transplantation.

Keywords: Cyclosporine; children; renal transplantation

Introduction

The introduction of cyclosporine (CSA) in early 1980's improved up to 15% the annual graft survival curve in patients undergoing transplantation.[1] However, it did not improve the graft survival for prolonged period, because of its inability to suppress the destruc-

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tion of renal tissue, due to antigen dependent and independent immunologic factors.[2-4]

CSA binds to cyclophilins, a family of cytoplasmic proteins, which are constituents of many cells. The complex of drug and receptor binds to and suppress calcineurin competitively, which is a calcium and calmodulin dependent phosphatase.[5-7] This process prevents the translocation of a series of transcription factors, and leads to reduction of primary cytokines of gene transcription such as GM-CSF, gamma-INF, IL-2, TNF-α, IL-3, IL-4 and CD40L.[5-6,8] CSA has narrow therapeutic index with variations in its absorptive properties even with new formulation of the drug.[9] The dose should therefore be adjusted for each patient by drug monitoring. The most accurate way of dose adjustment is serial measuring of serum level of Cmax and area under the curve (AUC).[10,11] This is a time- consuming process and is not practical. Several regimens were therefore searched in order to decrease sampling frequency and facilitate AUC detection.[10,11] AUC had maximum sensitivity in the first 4 hour after drug consumption and also the Cmax exhibited highest inhibition of calcineurin. C2 was therefore considered to be more suitable for drug monitoring.[9,10] This was confirmed by several clinical trials in kidney liver and heart recipients.[10-18]

Unfortunately, few prospective studies on pediatric age group were undertaken to determine the risks and benefits of C2 monitoring.[19-22] This study was undertaken to compare CSA C0 (before morning dose) and C2 (two hours after morning dose) levels in order to adjust the maintenance dose of CSA in stable renal transplant patients in Shiraz, southern Iran.

Materials and Methods

In a prospective study conducted in Shiraz, southern Iran, from October 2004 to June 2005, 64 renal transplanted patients, with at least three months post-renal transplantation period, of Nemazee Hospital affiliated to Shiraz University of Medical Sciences, were followed and investigated in the outpatient clinics of the University during a six-month period. The method and goal of investigation were explained to each patient and written consent was obtained from all cases entering the study. All patients received microimmulation form of CSA (Neoral), according to the protocol of the drug and were treated with CSA , prednisolone and, cellcept (MMF) or azathioprine. All patients were initially monitored to adjust the dose of CSA at C0 level. Other drugs were administered for treatment of hypertension, hypercholesterolemia or other electrolyte-associated problems. Patients receiving drugs that interacted with CSA were excluded. During a six-months period, the blood level of CSA was checked for C0 (before morning dose) and C2 (two hours±10 min after morning dose) upon referral, one month after the first and 5 months after the second referral, two times on each occasion. Blood samples were collected in tubes containing EDTA, transferred to the laboratory and kept at -70 °C for the tests. In order to control the accuracy of the test result and determine the mean, two blood samples were taken from each patient (12 samples in all, namely 6 for C0 and 6 for C2). Pharmacokinetic evaluation of C0 and C2 blood samples was performed by radioimmunoassay (RIA) using Immunotech CSA kits. The height and weight of patients as well as BUN, creatinine, electrolyte, blood sugar, calcium, phosphorus, and uric acid were all checked on the first referred and the creatinine, medications as well as important clinical aspects including rejection, CSA toxicity, infection and hospital admission

were determined on the following period. The dose of CSA was also adjusted according to C0 level of 100–250 ng/mL. The drug dose was reduced in patients with high CSA level, based on C0 level. However, the dose was not raised in stable patients due to concern about increasing the dose of CSA and its related complications. The results of the study were compared with the initial baseline measurements determined for each patient on first visit.

Acute clinical rejection was suggested by increasing creatinine or reduced renal output, confirmed by renal biopsy. Decreasing renal function established clinically, suggested CSA toxicity that was reversed by improving renal function through adjustment of the drug dose and confirmed by renal biopsy. Infections were monitored by clinical and laboratory tests and presence of fever, high CRP, WBC changes, positive urine culture and chest X-Ray. GFR for each patient was determined by Schwartz formula using height of the patient and serum creatinine and a constant k coefficient.[23-25] The mean and standard deviation of C0 and C2 levels, creatinine, GFR and serum cholesterol at the beginning and at the end of monitoring were measured for all patients. In order to evaluate the CSA level and the clinical events, the patients were divided into two subgroups, based on the level of C0. The patients were also divided into two groups based on the level of C2. Statistical analysis was conducted using SPSS software, version 11.0 and $P<0.05$ was considered significant. *Student's t*, χ^2, Pearson correlation and Mann-Whiteny U tests were used for statistical analysis.

Results

The male/female, live donor/cadaver, and multiple/single transplantation for 64 patients of outpatient clinics of the university were 41:23, 31:33 and 1:63, respectively. The mean±SD age, transplantation age, weight and height of patients and post-transplantation period were 16.18±3.63 years, 13.85±3.56 years, 45.47±12.24 kg, 147.08±13.77 cm, and 29.9±26 months, respectively. The mean of C0, C2 and creatinine levels and GFR at the beginning of the study were 128.89±75.38, 529.22±276.71 ng/mL, 1.24±0.63 mg% and 78.22±27 mL/min/1.73 m^2, respectively and the creatinine mean level at the end of study was 1.27±1.08 mg/dL. Thus, 26 (40.6%) patients had a level of C0<100 ng/mL and 38 (54.4%) had a level of C0≥100 ng/mL. In this regard, 10 patients (15.6%) had C2≥800 ng/mL and 54 (84.4%) had C2<800 ng/mL (Table 1).

A total of 800 tests, 400 for C0 and 400 for C2, of CSA level were carried out one month from the first and 5 months after the second visit. Except the two subjects who only received CSA and prednisolone, the other patients were treated with the three drugs. One patient expired despite normal renal function before sample collection, and was excluded from the study. All patients were previously

Table 1: Mean±SD creatinine, GFR, C0 and C2 levels at the start and completion of the study

Time	Creatinine (mg/dL)	GFR (mL/min)	C0 level (ng/mL)	C2 level (ng/mL)
Start of study	1.24±0.63	78.22±27.08	128.89±75.38	529.22±276.71
Completion of study	1.27±1.08	84.91±27.03	127.56±51.15	569.96±195.82

monitored to determine CSA dose at C0 level and none received any drug having serious interaction with CSA. All patients underwent treatment by maintaining CSA at the mean dose of 4.18±1.43 mg/kg/day and as supplementary drugs, 47 patients (73.4%) received prednisolone and cellcept, 15 cases (23.4%) were treated with prednisolone and azathioprine and 2 individuals (3.11%) received prednisolone alone. The drug combination remained unchanged for all patients during monitoring period. The mean of C0 level was 128.89±75.38 ng/mL and that of C2 being 529.22±276.7 ng/mL in all cases. On the other hand, 26 (40.6%) patients had a level of C0<100 ng/mL and 38 (54.4%) had a level of C0≥100 ng/mL. No significant difference was found between creatinine levels (1.23±0.72 *vs* 1.25±0.57 mg/dL), and CSA dose (3.49±1.08 *vs* 4.62±1.46 mg/kg/day)) and the incidence of complications, such as hypercholesterolemia, hyperuricemia and hypertension in both groups. In group C0<100 ng/mL, the level of C2 was significantly lower (347.17±153.6) than group C0≥100 ng/mL (653.78±274.14 ng/mL) (P=0.003).

As to the grouping of patients based on C2 level, no significant difference was observed in regard to serum creatinine (1.29±0.75 *vs* 1.24±0.61 mg/dL), CSA dose (5.3±1.58 *vs* 4.02±1.35 mg/kg) and incidence of complications, such as hypercholestrolemia, hyperuricemia and hypertension between two groups. In the group C2<800 ng/mL, the level of C0 was also lower (116.41±73.5 ng/mL) than the group C2≥800 ng/mL (196.25±44.38) (P=0.001). In respect of immunosuppressive therapy, no significant difference was observed between subgroups C0<100 and C0≥100 ng/mL. In this study, the correlation between C0 and C2 was highly significant (P<0.001) and this correlation between the C2 level and CSA dose was also significant (P<0.001). A negative correlation was noticed between the C0 level and serum creatinine (r=-0.07). The patients with constant drug dose who were in stable condition, the study showed a favorable C0 correlation (P=0.05) with conformity for C2 levels (P=0.03). The coefficient of variation of three samples without any change in CSA dose was 10.89% for C0 and 8.94% for C2 levels.

All patients except one (1.5%), survived during the six-month follow-up. As some cases did not appear for the third series of tests, only 50 patients were evaluated. During the follow-up period, 7 patients had elevated serum creatinine of more than 20%, of whom 2 were due to infection—one because of urinary tract and the other because of oral herpes infection—while their serum creatinine decreased to the pre-infection level following treatment. In one of these two patients, with recently increased drug dose, the C0 level was >100 ng/mL but the amount of C2 was <800 ng/mL. The corresponding values were >100ng/mL and ≥800 ng/mL. Of the other 5 cases with C2<800 ng/mL, one developed an acute rejection and in another case, an acute rejection was superimposed on a chronic rejection. Both of these patients had poor compliance. None of the 7 patients showed any evidence of CSA toxicity based on clinical and kidney biopsy findings.

Discussion

The optimization of immunosuppression seems to be necessary for long-term improvement of transplantation outcome. However, the target level of CSA C2 remains to be determined. The children in present study were followed according to conventional C0 level, but checked for both C0 and C2 levels. The patients were also monitored in regard to

clinical ground, drug complications and renal functions. The mean of C0 and C2 levels were consistent with those of previous studies performed on children and adults.[26-28] The mean of CSA dose was also in agreement with the study conducted in children.[26] Previous studies showed a poor correlation between C0 and C2 levels[26,28-30], but favorable results were obtained in the present study (P=0.001). This might be due to drug absorption in different populations and pharmacokinetic in various individuals. The results of present study were in agreement with those of previous reports in that a significant correlation was found between C2 level and CSA dose (P=0.001) with no correlation between C0 level and CSA dose.[26-28,31] There was a negative correlation between C0 and creatinine levels which was similar to those reported on adults,[28] indicating a negative correlation between CSA level and the reduction in renal function. On the other hand, no difference in renal function was found between C0 (C0≥100, C0<100 ng/mL) and C2 (C2<800, C2≥800 ng/mL) subgroups determined by serum creatinine level and GFR. The results of present investigation were in contrast to those reported previously.[28] Similar results were reported in another study.[30] This may be due to the dependence of renal function on various factors in addition to CSA level and drug doses, and perhaps related to the drug dose before the beginning of the study. Interestingly, and similar to the findings of another study on adults,[28] fewer patients had C2≥800 ng/mL in our study. This was contrary to the findings of other studies in which 40% to 69% of patients were overexposed to the drug.[29-30,32-33] This problem could be due to economic reasons (high drug cost), or prescribing a more precise dose or a more careful monitoring of the patients.

Similar to some previous studies, coefficient of variation for C2 level, based on three samplings, was less than C0.[27,31-32] Therefore, C2 level seemed to be more accurate in relation to the drug dose, although different results were obtained by other studies with C0, lower than C2 level.[28] No significant difference was found between evaluating the mean of C0, C2, renal function and drug complications at the beginning and completion of the study. Regardless of C2 level, in the presents study transplanted kidneys in most patients had stable function. Therefore, determination of C2 level during patients' follow-up was not recommended in our study. Finally, we concluded that the target level of C2 should be determined, but due to the presence of low rate of clinical problems, it was not possible to obtain the favorable cut off point anticipating the foregoing, which hampered the determination of target C2 level. Further studies are thus needed in relation to follow up C0 and C2 levels in children in order to correctly judge the C2 value. Also, a larger sample size with a longer follow up seems to be more beneficial.

Acknowledgement

The authors would like to thank the Office of Vice Chancellor for Research of Shiraz University of Medical Sciences for financial support and Dr. D. Mehrabani for editorial assistance.

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