Pretransplant assessment of cyclosporine level as a predictor of cyclosporine dose requirements after kidney transplantation

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Introduction and aims
Pretransplant administration of cyclosporine (CsA) may reduce post-transplant maintenance dose and consequently CsA inhibitor nephrotoxicity and helps in achieving the desired target C2 levels earlier. The optimum dose or timing of administration of CsA induction dose is still debatable.
Patients and methods
We compared three different protocols for pretransplant administration of CsA aiming to reach a target C2 therapeutic level of greater than 800 ng/ml on the third day post-transplant. Sixty kidney transplant recipients from Cairo University hospitals were divided into: group 1 (n=20) who received a single CsA induction dose of 2 mg/kg 12 h pretransplant; group 2 (n=20) who received four CsA consecutive doses of 4 mg/kg 48 h pre-transplant; and group 3 (n=20) who received four CsA consecutive doses of 2 mg/kg 48 h pretransplant.
Results
The desired therapeutic level in the earlier post-transplantation period was achieved in 65% in group 1, 100% in groups 2 and 3). In group 2 a lower dose was needed to maintain C2 within the therapeutic range during the first year post-transplantation ($P<0.01$). Furthermore, a lower number of cases were complicated by CsA nephrotoxicity in group 2 in comparison to groups 1 and 3 (25, 0, 5% in group 1, 2, 3, respectively, $P<0.039$). A higher longer dose of CsA pretransplant associated with early withdrawal of CsA had a better effect on graft function than lower or shorter induction doses with late withdrawal as evidenced by lower serum creatinine levels all through the follow-up period in group 2 compared with group 3.
Conclusion
Forty-eight-hour pretransplant induction with CsA at a dose of 4 mg/kg with early dose reduction post-transplant was associated with lower CsA maintenance and a better 1-year graft function.

Keywords:
cyclosporine, early, graft function, renal transplantation

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Introduction

The introduction of cyclosporine (CsA) in the 1980s was associated with statistically significant improvement in graft survival and a decrease in mortality rates [1]. Different immunosuppression regimens are used since then as maintenance therapy in kidney transplantation (KTX). Although new drugs allowed the exponential growth of organ and tissue transplantation in medicine over the last three decades, the standard immunosuppressive regimen remained consisting of CsA and prednisone [1]. Calcineurin inhibitor nephrotoxicity (CIN), however, is a well-known complication in all types of transplantations with an even higher risk of chronic kidney disease in nonrenal transplantations [2]. In this line of concept, reducing calcineurin inhibitor (CNI) and simultaneously avoiding graft rejection has been the main concern of many trials [3]. CsA-free immunosuppressive drug protocols were hampered by a high frequency of acute graft rejection episodes or treatment failure especially in immunologically high risk KTX [4].

Some studies demonstrated a large within-day variation in CsA absorption [5,6]. Other studies have shown that food could alter CsA absorption [7]. This high variation of pharmacokinetic profile and short limited time during the early post-transplantation period make it difficult to modify the CsA dose that can achieve the target level on time early after transplantation [8].

Pretransplant administration of CsA, as a part of induction therapy, was highlighted recently as a new tool in order to avoid minimization strategies that may lead to underimmunosuppression with increased risk of rejection. Furthermore, achieving lower CsA target blood concentrations at 2 h after the dose (C2) early after KTX resulted in a more favorable renal graft

function [8,9]. Unfortunately, the optimum pretransplant dose and the time to start CsA are still debatable.

The aim of our study was to compare three different protocols for pretransplant administration of CsA aiming to reach the target therapeutic (C2>800 ng/ml) level on the third day posttransplant and to assess its relation to the effect on graft function as regards rejection episodes and CIN.

Patients and methods

Study population

This study is a prospective multicenter study aiming to compare three different protocols conducted in the Nephrology Department of King Fahd Unit, Manial Hospital and French Hospital at Cairo University Hospitals. The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and was approved by the ethics committee of Internal Medicine, Faculty of Medicine, Cairo University, Egypt.

Sixty kidney transplant recipients (KTRs), minimum 18 years old, were selected randomly from the nephrology unit and were included in this study after obtaining a written consent. All participants had living related well-matched KTX.

The patients were divided to three groups:

(1) Group 1 (n=20) received a single dose of CsA (2 mg/kg) 12 h pretransplant.
(2) Group 2 (n=20) received four consecutive doses of CsA (4 mg/kg each) 48 h pretransplant.
(3) Group 3 (n=20) received four consecutive doses of CsA (2 mg/kg each) 48 h pretransplant.

All groups received a daily dose of CsA (6 mg/kg/d) after transplantation as initial dose and then the dose was adjusted to achieve a C2 greater than 800 ng/ml on third-day post-transplant, 1000–1200 ng/ml over the first month, 600–800 ng/ml at 6 months, and 400–500 ng/ml at 12 months after KTX.

All participants received 250 mg of methylprednisolone before transplantation and 500 mg intraoperatively, followed by 250 mg postoperatively and then 225 mg on day 1. The steroids were tapered by 25 mg each day until 20 mg oral drug at day 10, 10–15 mg after 3 months and 5–10 mg/day maintenance dose and 5 mg/day at 6 months was reached. MMF was administered 48 h preoperatively as 1 g every 12 h to all KTRs.

Standard prophylactic treatment for pneumocystis carinii consisted of oral daily sulfamethoxazole-trimethoprim (800/160 mg daily) for 3 months which was taken by all groups.

All patients were subjected to full history taking and medical exanimation. Demographic data collected included age at transplantation, gender, body weight, duration of hemodialysis before transplantation, and history of graft rejection or CsA nephrotoxicity.

The CsA level at 2 h after administration of the dose (C2) in all the patients were recorded pretransplant.

C2 levels during the 1-year follow-up period post-transplant and the levels of creatinine at 1, 6 months, and 12 months were also registered for all participants. Creatinine was measured in mg/dl. The patients were followed up at least for 1 year.

Exclusion criteria

Patients with pretransplant positive crossmatch, gastrointestinal malabsorption diseases, and medications that have a significant drug interaction with CsA (e.g. diltiazem, verapamil, macrolide antibiotics, and other antifungal agents) during the 7 days prior to and the 6 months trial period were excluded from the study. Patients who previously received an organ transplant other than a kidney or if the kidney's warm ischemia time was more than 60 min, or HIV or hepatitis B positive have a positive pregnancy test or those who have significant liver diseases or a history of malignancy were also excluded.

Determination of C2

The C2 levels were assessed by radioimmunoassay using commercially available kits (Immunotech, Beckman-Coulter, France) according to the manufacturer procedures. C2 was measured in ng/ml.

Determination of CNI nephrotoxicity

CNI was determined by a high C2 level associated with elevated serum creatinine (Scr) after administration of CsA and responding to lowering of its dose.

Immunosuppression protocol for rejection

Clinical suspicion of rejection was confirmed by renal biopsy. First line antirejection treatment was prescribed for all patients consisting of intravenous methylprednisolone for 5 consecutive days followed by 40 mg oral prednisolone, tapered daily by 10 mg until achieving the baseline steroid maintenance dose. Steroid-resistant acute rejection episodes were treated with the polyclonal anti-T-cell antibody

anti-thymocyte globulin (3 mg/kg/day) for a minimum of 7 days and up to 10 days.

Statistical analysis

Data were coded and entered using the SPSS (statistical package for the social sciences; SPSS Inc., Chicago, Illinois, USA) version 23. Data were summarized using mean and standard deviation in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between groups were done using analysis of variance with multiple comparisons post-hoc test in normally distributed quantitative variables while non-parametrical Kruskal–Wallis test and Mann–Whitney test were used for non-normally distributed quantitative variables. For comparing categorical data, χ^2-test was performed. An exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman's correlation coefficient. Logistic regression was done to detect independent predictors of regression and CsA toxicity. Survival curves were plotted by the Kaplan–Meier method and were compared using the log-rank test. *P* values of less than 0.05 were considered as statistically significant.

Results

Study population

Sixty KTRs with living related kidney transplant who met the inclusion criteria were included in the study. The baseline characteristics were compared between the groups studied. There was no statistically significant difference between the three groups as regards age, sex, and body weight; 100% of the patients in groups 1 and 3 had hemodialysis before transplantation in contrast to 85% in group 2. Patient survival was 100% in all groups (Table 1).

Rejection and cyclosporine nephrotoxicity in the study groups

Rejection occurred in nine patients out of 60 (15%) proven by renal biopsy. They were five men and four women. Rejection was least common in group 2 in comparison to groups 1 and 3 (20, 10, and 15% in-group 1, 2, and 3, respectively). However, this was statistically insignificant (Table 1).

Cyclosporine nephrotoxicity (CIN) occurred in six patients out of the studied population (10% of the whole sample). CIN did not occur in group 2 patients (0%) in comparison to 25% and 5% in groups 1 and 3, respectively and this was statistically significant (P=0.039) (Table 1).

Mean C2 pretransplant level

Mean C2 levels were comparable in groups 2 and 3 with no statistically significant difference between them.

Mean C2 level and doses post-transplant

The mean C2 post-transplant levels and doses needed to maintain the C2 level within the therapeutic range were lowest in group 2 and highest in group 1. This was statistically significant (P<0.005, <0.001, respectively) at 1, 6, and 12 months of the follow-up (Table 2).

When comparing between all groups regarding the proportion of the patients that achieved C2 greater than 800 ng/ml, 100% of patients in groups 2 and 3 achieved that level on the third day posttransplant in comparison to 65% in group 1. It was also noticed that while groups 2 and 3 took only 3 days to achieve a C2 level greater than 800 ng/ml, group 1 achieved that level by day 180. This was statistically significant (P<0.001). The number of patients with C2 above the recommended therapeutic range (>1200 ng/ml) was highest in group 1. This was a statistically significant difference (P<0.001) at day 3, 6 months, and 12 months after transplantation (Table 3).

Serum creatinine in the three groups

Better serum creatinine levels were noticed among patients of group 2 especially at 12 months post-transplant, but with no statistically significant difference between the three groups (Table 4).

There was no difference between patients experiencing rejections and patients without rejection regarding age, C2 levels pretransplant, and C2 levels after 3 days, and 6 and 12 months post-transplant. Patients who experienced rejections had higher levels of serum creatinine early post-transplant and at 6 and 12 months after KTX. This was statistically significant (P<0.001) (Table 5).

Discussion

CIN is one of the most challenging complications after KTX and is frequently related to longer treatment duration, larger cumulative doses, and higher daily dose of CsA [10]. There is increasing interest in CIN avoidance, withdrawal, and minimization. Maintaining CsA concentrations within target ranges was proven to be difficult due to its high inter- and intraindividual pharmacokinetic variability [11].

Acute CIN is primarily due to acute arteriolopathy resulting from a combination of an increase in

Table 1 Demographic data of the studied population

	Group 1 [*n* (%)]	Group 2 [*n* (%)]	Group 3 [*n* (%)]	*P* value
Age	34.55±10.40	38.30±8.20	33.50±10.39	0.247
Body weight	61.55±10	63±12	62.3±12.8	0.732
Gender				
Female	10 (50.0)	6 (30.0)	7 (35.0)	0.4
Male	10 (50.0)	14 (70.0)	13 (65.0)	
HD before TX				
Yes	20 (100.0)	17 (85.0)	20 (100.0)	0.1
No	0 (0.0)	3 (15.0)	0 (0.0)	
Rejection				
Yes	4 (20.0)	2 (10.0)	3 (15.0)	0.9
No	16 (80.0)	18 (90.0)	17 (85.0)	
Cyclosporine toxicity				
Yes	5 (25.0)	0 (0.0)	1 (5.0)	0.039
No	15 (75.0)	20 (100.0)	19 (95.0)	

This table compares the baseline clinical data of the three groups included in the study. HD, hemodialysis; TX, transplantation.

Table 2 Mean cyclosporine levels and dosage changes over 1 year in the three groups

Day	Mean cyclosporine level (C2) (ng/ml) 1 year follow-up				Mean CsA dose (mg/kg/day) 1 year follow-up			
	Group 1	Group 2	Group 3	*P* value	Group 1	Group 2	Group 3	*P* value
C2 pre-Tx		300.35±62	308.2±80	0.685				
0		300±65	308±82	0.734				
3	1051±664	854±35	974±138.5	0.285	5.9±1.36	3.8±0.9	4.67±0.4	<**0.001**
30	1049±516	721±258	905±105	**0.013**	5.1±0.85	3.7±0.7	4.29±0.5	<**0.001**
45	1051±377	690±211	837±157	<**0.001**	5±1.35	3.6±0.8	4.24±0.7	<**0.001**
90	1035±257	682±190	767±245	<**0.001**	4.8±0.67	3.4±0.6	3.77±0.8	<**0.001**
180	1050±202	674±184	701±342	<**0.001**	4.2±0.68	3.1±0.7	3.86±0.7	<**0.001**
360	991±386	451±83	680±335	<**0.001**	3.9±0.96	3±0.5	3±0.8	<**0.001**

This table show cyclosporine levels (C2) and maintenance dosage during the follow-up period. CsA, cyclosporine; pre-Tx, pretransplant.

vasoconstrictive factors, activation of the renin–angiotensin–aldosterone system, reduction of vasodilator factors, and formation of free radicals. Secondary release of aldosterone is thought to play a significant role in chronic CIN nephropathy with upregulation of the transforming growth factor-β [12].

Earlier studies have found that early achievement of C2 concentration was associated with decreased CIN and improved graft function. Furthermore, early achievement of C2 concentration is important for early inhibition of reactive immune competent cells, which in turn reduces the rejection episodes [8,9].

In this study, we aimed at comparing the effect of pretransplant administration of different CsA doses on graft function as regards rejection and CIN.

The most important findings in our study was that CIN did not occur (0%) in patients receiving four doses of CsA of 4 mg/kg 48 h pretransplant. This was in contrast to a higher percentage of CIN in patients receiving a single dose of CsA of 2 mg/kg 12 h pretransplant (25%) and in patients receiving four doses of CsA of 2 mg/kg 48 h pretransplant (5%).

This may be explained by the fact that in our study we found that the pretransplant administration of CsA 48 h in either doses of 4 mg/kg or 2 mg/kg (groups 2 and 3) helped to achieve the targeted therapeutic level of CsA on day 3 post-transplant. In addition, the lowest levels and doses needed to maintain the C2 level within the therapeutic range were noticed in group 2, which was not the situation in group 1, who received a single low CsA dose (2 mg/kg) only 12 h pretransplant. Those receiving a single dose of CsA pretransplant achieved C2-targeted therapeutic level 6 months after KTX. Furthermore, the number of patients with C2 above the recommended therapeutic range (>1200 ng/ml) was highest in this group. The higher level of C2 level in group 1, despite the fact that they received a lower dose of pretransplant CsA induction, might be explained by the fact that those patients received higher CsA doses of 6 mg/kg after transplant for longer duration before starting withdrawal.

A concentration toxicity relationship has been established with CsA and therefore its concentration must be monitored. CsA with its narrow therapeutic index can result in acute and chronic CIN. Therefore, current practice and research target lower normal plasma concentrations of CsA, which appears to be safe [12].

Table 3 Comparison of C2 time by time in the three groups

	Group 1		Group 2		Group 3		*P* value
	Count	%	Count	%	Count	%	
C2 on third day >1200							
Yes	9	45	0	0.0	1	5.0	<0.001
No	11	55	20	100	19	95	
C2 on 6 months >1200							
Yes	7	35	1	5.0	1	5.0	0.013
No	13	65	19	95.0	19	95	
C2 on 12 months >1200							
Yes	9	45	0	0.0	2	10	0.001
No	11	55	20	100	18	90	
C2 3 days after TX							
<800	7	35.0	0	0.0	0	0.0	
>800	13	65.0	20	100	20	100	
Time to achieve C2 >800 in 100% of the group's patients							
Day 3	0	0.0	20	100.0	20	100.0	<0.001
Day 180	20	100.0	0	0.0	0	0.0%	

This table shows the C2 level at the third day, and 6 and 12 months post-transplant and show the percentage of patients reach C2 greater than 800 at the third day and at 6 months of follow-up. TX, transplantation.

Table 4 Serum creatinine level on the third day post-transplantation and after 6 and12 months in the three groups

	Group 1		Group 2		Group 3		*P* value
	Mean	SD	Mean	SD	Mean	SD	
Creatinine on third day	1.41	1.04	1.42	0.27	1.44	1.05	0.056
Creatinine after 6 months	1.52	0.98	1.40	0.25	1.56	0.76	0.223
Creatinine after 12 months	1.57	0.94	1.28	0.27	1.63	0.83	0.473

This table shows the mean creatinine levels during different times of follow-up in the studied groups.

Table 5 Comparison between rejections versus whole sample

	Rejection				*P* value
	Yes		No		
	Mean	SD	Mean	SD	
Age	34.56	7.67	35.61	10.16	0.876
C2 before TX	304.00	57.98	304.31	66.69	0.713
C2 after 3 days of TX	936.83	485.92	963.84	381.49	0.462
C2 after 6 months	880.44	377.91	796.31	286.70	0.521
C2 after 12 months	728.61	396.10	704.55	366.69	0.975
creatinine post TX	2.87	1.37	1.17	0.34	<0.001
creatinine after 6 months	2.81	1.05	1.26	0.27	<0.001
creatinine after 12 months	2.72	1.16	1.27	0.34	<0.001

This table shows data on those experienced rejection versus those did not in the studied groups. TX, transplantation.

In the current study, better serum creatinine levels were noticed among patients of group 2 especially at 12 months post-transplant. It is not clear whether the improved 1-year graft function was the result of reduced chronic CsA exposure (and therefore potentially less CIN) or of less cumulative allograft damage conferred by fewer acute rejection episodes as the least rejection rate (10%); although insignificant, it also occurred in the same group compared with 20% and 15% in groups 1 and 3. Unfortunately, we did not perform protocol biopsies to distinguish the two possibilities.

This result is in agreement with earlier studies that demonstrated that with low CsA dose regimens (<5 mg/kgday), stable serum creatinine levels have been observed for up to 15–20 years after KTX [13,14].

Many different studies also have shown that achievement of target C2 concentration within 3 days post-transplantation minimizes the risk of graft rejection and CIN since the intra-patient PK variation causes unpredictable CsA levels [11,12,15,16].

Our results come in agreement with an earlier study by Maamoun *et al.* [9] who proved that pretransplant administration of CsA helps to achieve low and safe target C2 concentrations with better 1-year graft function compared with the standard CsA drug regimen starting CsA in dose of 6 mg/kg post-transplant. However, in their study they found that the incidence of delayed graft function (DGF) was higher among the group who received pretransplant CsA (20%) compared with the standard regimen (17.5%). The difference was not significant though. This finding may be due to a CsA effect when given 48 h pre-transplant, compromising renal hemodynamics in the presence of ischemia followed by reperfusion related to the operative procedure itself. The strategy of postponing or reducing the initial dose of CsA during induction therapy seeking to gain a favorable influence on the incidence and course of DGF remains a topic of debate.A study done by Sukhavasharin *et al.* also found that the early postoperative optimal CsA dose could be effectively predicted by pretransplant C2 measurement [8].

The difference between groups 2 and 3 who both received CsA 48 h pretransplant might be explained by the fact that group 3 who received a lower dose of pretransplant CsA needed long-term higher doses to reach the therapeutic target. Furthermore not all KTRs on CsA develop CIN, because added to the degree of renal CsA exposure, there is also evidence that the susceptibility to CIN is determined by local renal factors, independent of the CsA levels [17]. Other factors determining CIN susceptibility include the age of the recipient and the age of the transplanted kidney. In addition, genetic polymorphisms are also involved in the pathogenesis of CIN [18].

Finally the strategy of induction with CsA, its timing and dosage, delaying CsA dosage, and time of achieving optimum C2 level in different KTRs in order to maintain a balance between obtaining a longer graft survival and avoiding DGF, CIN, infection, and acute or chronic rejection is still debatable.

Study limitations

It has to be taken into account that this trial was done on low immunological risk cases without the use of induction therapy. Furthermore, the study was conducted on a small number of patients. A longer follow-up period may be needed to estimate the risks associated with low-dose CsA. Further studies with a larger number of KTRs are needed for the validation of the results of this study.

Conclusion

Induction by CsA (4 mg/kg) given 48 h before RTX has a beneficial effect on 1-year graft survival. This was evidenced by lower rates of rejection, lower CIN incidence, ability to achieve target C2 on third day post-KTX and lower serum creatinine all through the 1-year follow-up in the group who received 4 mg/kg 48 h before RTX in comparison to KTRs who receive 4 mg/kg 12 h or those who received 2 m/kg/48 h pre-transplant.

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Conflicts of interest

There are no conflicts of interest.

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