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TARGET RANGE MAXIMUM OF CYCLOSPORINE BLOOD CONCENTRATION TWO HOURS POST DOSE IN STABLE LIVER TRANSPLANT PATIENTS*

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Abstract
Recently, single blood level measurement 2 hours after cyclosporine administration (C2) is taken as a more sensitive indicator of drug exposure in de novo transplant recipients than trough levels (C0). However, few studies focused on the determination of the C2 target range maximum and its associated adverse events in stable liver recipients. This prospective study was designed to assess the relative risk of developing CsA related side effects in patients with high C2-levels. Adverse effects were determined clinically, and by using a specially designed questionnaire. Eventual adverse events as well as C2 levels were determined repeatedly up to 4 times in 3-months intervals (observation period 9 ± 3 months) in 36 long-term liver recipients (1-13.5 years post-transplant), in addition to conventional C0 levels. Cyclosporine dose was adjusted according to a predefined C0 target level range and clinical status. Totally 103 questionnaires and the corresponding paired CsA blood level records were obtained. C0 levels and C2 levels ranged from 90 to 287 (143 ± 31) ng/ml and from 212 to 1358 (672 ± 203) ng/ml respectively. No patient experienced a rejection episode during the observation period, demonstrating the efficiency of the immunosuppressive therapy. However, 33/36 patients (91%) showed symptoms attributable to CsA therapy. C2 levels above 750 ng/ml, determined at least twice in an interval of 3 months, were identified as a relevant risk factor for the presence of multiple adverse effects, which were defined as the combination of hypertension, renal insufficiency and more than two neurological complaints (RR = 3.11, $p<0.01$). This risk population was not completely identified by determination of C0 level.

Key words: Cyclosporine, C2, liver transplantation, adverse events

Abbreviations: CCr: creatinine clearance; CsA: cyclosporine A; C0: predose blood concentration of cyclosporine; C2 : 2 hours post dose blood concentration of cyclosporine; EMIT: enzyme multiplied immunologic technique; FPIA : fluorescence polarisation immuno assay; LTx: liver transplantation; MMF: mycophenolate mofetil; RR: relative risk

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INTRODUCTION

Cyclosporine A (CsA) has been a mainstay of immunosuppressive treatment following liver transplantation since its introduction in the 1980s [1]. The absorption phase for the microemulsion form Neoral (Novartis Pharmaceutical Corp., Switzerland) occurs during the first 4 hours after administration and is characterized by rapid changes in blood CsA concentrations and a high degree of variability [2]. It has been shown repeatedly that blood concentration measurement 2 hours after Neoral administration (C2) had a higher correlation with the absorption during the first 4 hours postdose (correlation coefficients ranging from 0.81 to 0.93) than trough levels (correlation coefficients ranging from 0.03 to 0.41) [3], leading to the introduction of C2-monitoring in the early posttransplant period [4, 5, 6]. Incidence of rejection in the first three postoperative months was reduced in patients reaching the recommended C2 target level minimum within a few days [7, 8].

However, the superiority of C2 monitoring for long-term stable liver graft recipient remains controversial, especially in respect to the prevention of drug associated side effects [9, 10, 11]. Moreover, suggested target ranges of C2-level for maintenance therapy vary from 300 up to 750 ng/ml [3, 12, 13, 14]. This study was designed to analyse CsA associated adverse events in respect to their corresponding C2- and C0-levels in stable liver graft recipients.

METHODS

Stable liver graft recipients transplanted at least one year prior to study entry and subjected to a CsA based immunosuppressive regimen were selected as study population. All participating patients provided written informed consent prior to study entry. During the routine follow-up visits with monitoring of the trough levels, patients were asked to stay additional two hours in the outpatient department after being sampled for C0-levels and subsequent intake of their medication.

During the course of the study, the blood sample for C0 measurement was first collected. The second blood sample was taken within 15 minutes of the 2 hours post-dose time for C2 measurement as recommended [3]. The CsA blood concentrations were measured with a monoclonal antibody-based fluorescence

polarization immunoassay system on a TDx analyser (Cyclosporine Monoclonal Whole Blood, Abbott Laboratories, Abbott Park, IL, USA). Patients on standard triple immunosuppressive regimen received CsA in microemulsion form (Neoral) in combination with mycophenolate mofetil (MMF) (CellCept, F.Hoffmann-La Roche AG, Germany) and prednisone (Decortin, Merck KGaA, Germany). Dual therapy consisted of CsA and MMF. CsA monotherapy consisted of 2 daily oral doses of 50-150 mg. The CsA dose was adjusted according to predetermined C0 target range (100-200 ng/ml for CsA monotherapy) [12, 13, 15] and clinical status by two hepatologists (S.B. and V.C.) blinded to the C2 values. In patients with clinically suspected CsA associated adverse events, reduction of the CsA dosage with co-immunosuppression with MMF or steroids was carried out [16, 17]. Percutaneous liver biopsy was performed in case of clinically suspected rejection or recurrence of viral hepatitis.

In addition to the regular physical examination and routine laboratory measurements, patients were interviewed specifically regarding the adverse effects of CsA on the cardiovascular, renal and neurological system by another physician (J.L), see Appendix 1. Hypertension was defined as diastolic blood pressure > 90 mmHg, systolic blood pressure > 160 mmHg or the initiation of new antihypertensive agents post-transplant [18]. Creatinine clearance (CCr) was estimated using serum creatinine and body weight according to the Cockcroft-Gault formula [19]. An arbitrary classification was employed to categorize renal insufficiency. Renal insufficiency was defined as mild (CCr>70 ml/min), moderate (CCr 40-70 ml/min) or severe (CCr 20-40 ml/min) [20]. Common neurological symptoms in stable liver transplant patients such as tremor, motorial weakness and paresthesia, were documented according to the patient's complaints [21]. Multiple adverse effects were defined as the combination of hypertension and of moderate to severe renal insufficiency together with more than two neurological complaints.

Results were reported as means ± standard deviation. Means of variables were compared with a Student's t-test for unpaired data. For assessment of correlations, a bivariate correlation using the Pearson correlations was performed. The chi-square test was used to compare the incidence of side effects between the groups. P-values of <0.05 were considered statistically significant.

Results

Thirty-six patients (10 women and 26 men), with a mean age of 55 ± 8 (39-70) years, were enrolled in the study (Table 1). Cirrhosis due to viral hepatitis (n = 14) and alcoholic liver disease (n = 13) were the major primary diagnoses of the recipients. Thirty-one patients (86%) received CsA-monotherapy. Three patients were on dual regimen (CsA plus MMF). Two patients were on a triple immunosuppressive regimen (CsA plus MMF and prednisone).

The mean observation time for each patients was 9 ± 3 (7-18) months. Each patient had at least 2 interviews (2-4 interviews) with an interval of 3 months (2-4 months). Totally 103 clinical records together with the corresponding paired CsA blood level records were obtained from the 36 patients.

Table 1. Characteristics of the study population (n = 36).

Gender (female: male)	10:26
Age (yr ± SD)	39-70 (55 ± 8)
Primary diagnosis in recipients (n)	
Viral hepatitis	14
Alcoholic liver disease	13
Hepatocellular carcinoma	4
Acute liver failure due to intoxication	1
Primary biliary cirrhosis	1
Cyst liver disease	1
Malignant haemogioma	1
Metabolic liver disease	1
Years post LTx	1-13.5 (4.4 ± 3.5)
Immunosuppression regimen (n)	
CsA	31
CsA+MMF	3
CsA+Prednisone+MMF	2

LTx: liver transplantation
CsA: cyclosporine
MMF: mycophenolate mofetil

The C0 levels ranged from 90 to 287 ng/ml (143 ± 31 ng/ml) with 92% (95/103) of the results within the therapeutic range and 4% (4/103) above range. The corresponding C2 value ranged from 212 to 1358 ng/ml (672 ± 203 ng/ml). The target range 450-750 ng/ml as suggested by Barakat [12] was used as basis for the analysis. Following this recommendation 14 results (14%) were below the range, 60 results (58%) within the range and 29 results (28%) above the range. In total 37/103 results (36%) were discrepant in respect to both, C0 and C2 target range. A poor correlation between C0 and C2 values was found (correlation coefficient = 0.54) (Fig. 1).

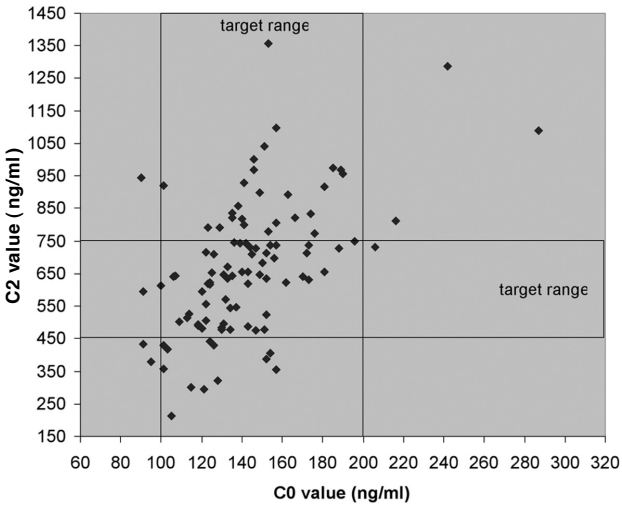

Fig. 1. C0 values and corresponding C2 values in 103 CsA-profiles from 36 stable adult patients more than 1 year after liver transplantation. Target ranges of C0 (100-200 ng/ml) and C2 (450-750 ng/ml) were marked as box. A poor correlation between C0 and C2 value was revealed (correlation coefficient = 0.54).

Table 2. Possible CsA-associated adverse events observed in 103 interviews of 36 LTx patients.

	Before LTx	At the end of the study
Hypertension	14% (5/36)	75% (27/36)
Moderate to severe renal insufficiency	19% (7 moderate)	53% (3 severe; 16 moderate)
Neurotoxicity		
Tremor	No*	34% (35/103)
Paresthesia	No*	39% (40/103)
Motorial weakness	No*	41% (42/103)

LTx: liver transplantation
* Absence of symptoms during the evaluation for LTx

Table 3. Distribution of the 17 patients (numbered from 1 to 17) with CsA associated multiple adverse events (MAE) according to the predefined C0 and C2 target ranges*.

	C0	C2
Below the target range	None	None
Within the target range	1,2,3,4,5,6,9,10, 11,13,15,16,17 (n = 13)	2,7,9,10,11,15,17 (n = 7)
Above the target range		
determined once	7,8,12,14 (n = 4)	4,13 (n = 2)
determined more than once	None	1,3,5,6,8,12,14,16 (n = 8)

*Target range of C0: 100-200 ng/ml; Target range of C2: 450-750 ng/ml

Immunosuppressive therapy was effective as no patient experienced rejection. Clinically indicated liver biopsies remained negative for acute cellular rejection in all 8 cases performed during the study period. In contrast, CsA associated adverse events, such as hypertension, elevation of serum creatinine or neurological symptoms, were observed in 33/36 patients during the study period. The incidence of hypertension was 75% (27/36) compared with 14% (5/36) before transplantation (Table 2). Elevated serum creatinine levels were found in 75% patients (27/36) leading to the diagnosis of moderate (16 patients) to severe (3 patients) renal insufficiency according to estimated creatinine clearance. Three out of seven patients who had moderate renal insufficiency before transplantation, showed further deterioration of kidney function. Neurological complaints, such as tremor, paresthesia or motorial weakness, were recorded in 34% (35/103), 39% (40/103) and 41% (42/103) of all interviews respectively. Eighteen patients (50%) experienced more than two neurological complaints at the end of the study period. Multiple adverse effects as defined above were observed in 17 patients (47%).

It was found that repeated C2 levels above 750 ng/ml, which were determined consecutively at least twice in an interval of 3 months, were always associated with multiple adverse effects (n = 8). From all 17 patients with multiple adverse effects, only 4 presented with C0 values above the target range (>200 ng/ml) (Table 3). Statistical analysis using the chi-square test confirmed that repeated C2 levels above 750 ng/ml represented a relevant risk factor for developing CsA associated multiple adverse effects (relative risk = 3.11, $p<0.01$) whereas a single C2 value exceeding the target range was not a statistically significant risk factor (relative risk = 1.6, $p>0.05$).

Discussion

Due to the narrow therapeutic window, monitoring of drug levels was used since the introduction of CsA as a key immunosuppressive drug in transplantation. In the past individualization of therapy was based exclusively on trough levels until late 1990's, when the microemulsion form of CsA and C2-monitoring were introduced into clinical practice. Clinical trials were performed based on a well-documented pharmacokinetic rationale and disclosed a reduced incidence and severity of rejection in de novo patients, suggesting an optimised use of Neoral [3, 4, 7, 22], when subjecting the patients to C2-monitoring. The advantage of C2 monitoring compared to C0 monitoring in reducing rejection in the early postoperative period is now well accepted [23]. However, most centres still adjust the CsA dose especially in long-term patients guided by predose blood concentration, not only because of simplicity of sample collection and cost, but also because of the wide acceptance of the therapeutic blood levels.

Target levels were mainly evaluated in respect to the reduction of rejection in the early posttransplant period [3, 4]. With the increasing number of liver transplant patients surviving 5 years and more, the long-term effects of calcineurin inhibitor-associated side effects, such as hypertension, renal failure and neuropathy are becoming a clinically more and more relevant problem [24]. C2 monitoring and its relevance to the drug toxicity profile in stable liver recipients has not yet been fully evaluated [3, 11, 13]. The target range maximum of CsA blood concentration and its relationship to the incidence and severity of the adverse events are not clear [3,4,11], leading to at least 4 different suggestions reported in the literature (Table 4) [12, 13, 14, 25].

We designed this prospective study to evaluate the upper C2-target level in respect to the occurrence of long-term multiple adverse effects. Drug associated side effects and corresponding C2-levels were determined repeatedly in 3 months intervals and the relative risk was calculated accordingly.

A literature survey on C0 target level showed a tendency towards reduction of target maximum along with increasing co-immunosuppression (Table 5). Eighty-nine percent patients in this study were receiving CsA-monotherapy. The target range of C0 was relatively higher in comparison of the target range in co-immunosuppressive regimens [10, 14]. Ninety-two percent C0 profiles were within the target range of 100-200 ng/ml. More than 80% (33/36) of the pa-

Table 4. C2 target range recommended in the literature.

Authors	Year of publication	C2 target range (method)	Related study group and patient number	CsA monotherapy
Cantarovich [13]	1998	300-600 ng/ml (EMIT)	11 liver recipients more than 12 months post-LTx	8/11 patients
Levy [25]	2001	600 ng/ml (not mentioned)	110 liver recipients more than 3 months post-LTx	Not mentioned
Barakat [12]	2002	450-750 ng/ml (EMIT)	10 liver recipients more than 12 months post-LTx	1/10 patients
Langers [14]	2004	510-690 ng/ml (FPIA)	31 liver recipients more than 6 months post-LTx	6/31 patients

EMIT: enzyme multiplied immunologic technique
FPIA : fluorescence polarisation immunoassay
LTx : liver transplantation

Table 5. C0 target range mentioned in the literature.

Authors	Year of publication	Time after LTx	Target range of C0	Immunosuppression regimen: Nr. of patients
Cantarovich [13]	1998	Over 1 year	100-200ng/ml	CsA:28 (80%) CsA+Pred.: 6 CsA+Pred+AZA:1
Cohen [15]	2002	Over 4 months	100-200ng/ml	CsA+Pred.+MMF/AZA
Barakat [12]	2002	Over 1 year	100-200ng/ml	CsA:1 (10%) CsA+Pred.: 1 CsA+AZA:6 CsA+MMF: 2
Sterneck [11]	2002	Over 6 months	100-150ng/ml	CsA:11 (18%) CsA+Pred.: 21 CsA+Pred+AZA:8 CsA+Pred+MMF:1 CsA+AZA:7 CsA+MMF: 14
Teisseyre [10]	2003	Over 1 year	100-150ng/ml	CsA:9 (20%) CsA+Pred.: 22 CsA+Pred+AZA:8 CsA+Pred+MMF:3 CsA+AZA:2
Langers [14]	2004	Over 6 months	90-150ng/ml	CsA:6 (19%) CsA+Pred.: 8 CsA+Pred+AZA:4 CsA+Pred+MMF:4 CsA+AZA:4 CsA+MMF: 5

AZA: azathioprine; CsA: cyclosporine; MMF: mycophenolate mofetil; LTx : liver transplantation; Pred: prednisone

tients presented at the respective visit with at least one symptom leading to the suspicion of CsA related side effects. The high rate of CsA related side effects is very similar to the results from other authors, observed in regimen based on C0 monitoring: the development of hypertension occurred in 62-82% of the patients, the occurrence of abnormal creatinine value in 43%-73% of the patients and presence of neurotoxicity in 25%-47% of liver recipients receiving CsA-based immunosuppression [9,18,21,26,27]. In other words, the potential risk of side effects is not identified utilizing exclusively C0 levels.

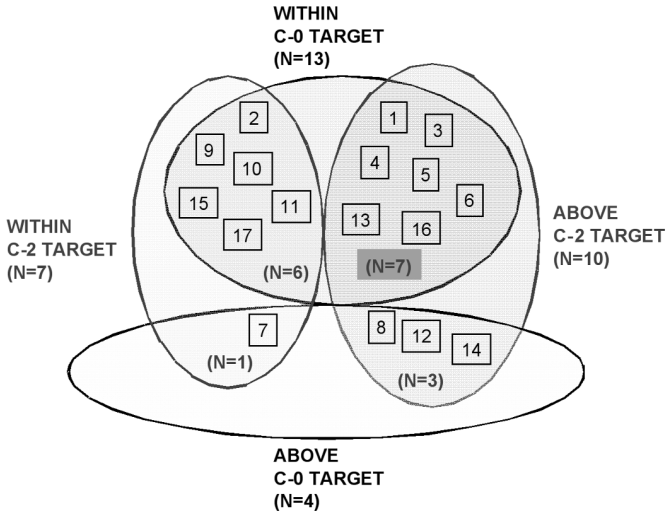

Fig. 2. CsA overdosing, identified by repeated C2-levels but not always by C0-monitoring in patients with multiple adverse effects.

Following the target range of Barakat (450-750 ng/ml) who suggested the highest target range maximum reported in long-term liver recipients, 47% (17/36) of our patients had at least one C2 value exceeding the target range. All of them presented with at least one adverse event.

We evaluated C2 levels repeatedly in 3-months intervals and analysed the relationship between side effects and at least 2 repeated C2 levels exceeding the upper range level of 750 ng/ml. Statistical analysis revealed a relative risk of 3.11 to develop multiple adverse effects in this population. Repeated C2 levels above range were always associated with the presence of multiple adverse effects. The data presented here support that the target level above 750 ng/ml should not be exceeded.

In our study, 10 patients with multiple adverse effects had C2 values above the predefined maximum. However, C0 values in seven of them (patient number 1, 3, 4, 5, 6, 13, 16) were always within the target range (Table 3 and Fig. 2). In other words, CsA overdosing was not identified as possible cause of adverse events when the CsA monitoring was only based on C0 value, but was only visible upon repeated C2-measurements. Those patients may potentially benefit from a dose reduction based on C2-monitoring without putting them at risk for rejection due to under-immunosuppression. As C2 is regarded as a surrogate of maximal concentration of CsA, the repeated exposure to high CsA concentration might be the cause of the adverse events. Keeping the C2 levels below the target range maximum may help to reduce the risk for developing multiple adverse effects associated with CsA.

Conclusion

In this study we prospectively analysed the role of C2 monitoring in stable liver transplant recipients on maintenance immunosuppression. C2 levels exceeding 750 ng/ml at 2 repeated time points in 3 months intervals was identified as a risk factor for development of multiple adverse effects related to CsA.

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Appendix 1. Questionnaire for liver transplanted patients regarding C0/C2 monitoring and general adverse effects

Date _____

1. General data of the patient:
   - Name _____
   - Birthday _____
   - Sex _____
   - Date of liver transplantation _____
   - Indication of liver transplantation _____
   - Current body weight: _____ kg
   - Current blood pressure _____ mmHg

2. Document of CsA:

   First blood sample at _____clock

   ↓

   Actual dose of CsA (Sandimmun® Optoral) _____ mg

   ↓

   Second blood sample at _____ clock

CsA level
C0_____ng/ml
C2_____ng/ml
(only for medical staff)

3. Did you have any of the following disease before LTx?
   - o Hypertension .....yes / no
   - o Diabetes mellitus .....yes / no

4. Do you have any of the following symptoms/complaints since last visit?
   - o Fever or chills .....yes / no
   - o Increased body hair growth .....yes / no
   - o Gingival hyperplasia .....yes / no
   - o Gastrointestinal complains
   - o Upper abdominal pain .....yes / no
   - o Reduced appetite .....yes / no
   - o Nausea/vomiting .....yes / no
   - o Diarrhea .....yes / no
   - o Neurological complaints
   - o Tremor .....yes / no
   - o Unusual tiredness or weakness .....yes / no
   - o Persisting headache .....yes / no
   - o Cribbed finger/numbness or tingling .....yes / no
   - o "Burning" hands or feet .....yes / no
   - o Weakness of legs .....yes / no
   - o Renal symptoms
   - o Obvious less urine output than before (i.e.: less than 2 time a day) .....yes / no
   - o Unusual urine output (i.e.: painful or difficult urination) .....yes / no
   - o Elevated blood pressure with/without medications .....yes / no
   - o Uncontrolled blood sugar with/without medications .....yes / no

5. Other complaints

_____

_____

_____