

Individual Differences in Cyclosporine A Pharmacokinetics and Its Association with Acute Renal Function Following Heart Transplantation

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Abstract: *Background:* The secondary metabolites of cyclosporine A (CsA), AM19, AM1c and AM1c9, have been indicated to be nephrotoxic. The aim of the present pilot study was to investigate the relationship between acute renal failure and CsA metabolite levels, including relevant pharmacokinetic genotypes, following heart transplantation.

Methods: Whole-blood samples were drawn the first posttransplant week in 22 patients (median 54 years, range from 27 to 65). Whole blood concentrations of CsA and its six main metabolites were analyzed with a validated HPLC-MS/MS method, and relevant *CYP3A5* and *ABCB1* genotypes determined. Renal function was monitored daily during the first posttransplant month and also at months 3, 6 and 12.

Results: One patient died early posttransplant. Six patients were in need of dialysis directly after transplantation. Nine patients developed sustained impaired renal function, while six had stable renal function. Sustained renal impairment tended to be associated with high levels of toxic metabolites ($P=0.08$). Six of the patients with possible *ABCB1* TTT-haplotype developed renal impairment ($P=0.12$).

Conclusion: The present study indicates that toxic CsA metabolites seems to be associated with development of impaired renal function and together with *ABCB1* genotyping they might be promising biomarkers for optimization of immunosuppressive drug treatment in future studies.

Key Words: Cyclosporine A, heart transplantation, renal impairment, metabolites, nephrotoxicity.

INTRODUCTION

Renal failure following heart transplantation is a frequent complication and associated with impaired survival [1]. There are several risk factors but most of them are non-modifiable [2-6]. Cyclosporine A (CsA) has a narrow therapeutic window and induce reduced renal function following each dose [7, 8]. The mechanisms of renal impairment following heart transplantation are not fully elucidated, but CsA is often mentioned as a risk factor [9-11]. Several clinical trials have shown encouraging results by lowering CsA concentrations [12].

CsA undergoes extensive metabolism, primarily *via* CYP3A, to over 30 metabolites [13-15] and shows high variation [15, 16]. The general understanding is that CsA metabolites are less toxic than the parent drug [17-19]. However, secondary metabolites, AM19, AM1c and AM1c9, may be associated with nephrotoxicity [17, 20-24]. *In vitro* studies indicate that CYP3A5 expressers produce more AM19 and AM1c9 metabolites [15], and they directly affect renal haemodynamics. In addition is AM1 and AM9 cytotoxic [17, 25].

P-glycoprotein (P-gp) is an efflux transporter coded by the *ABCB1* gene that affects CsA pharmacokinetics [26, 27]. The TTT-haplotype of C1236T, G2677T and C3435T SNP's has been suggested to have impaired activity [28], which may attenuate the efflux of compounds out of kidney cells and therefore be a risk factor for nephrotoxicity.

In the present pilot study in heart transplant recipients the association between CsA metabolite levels, and acute renal impairment were studied. Renal function was followed for one year and the effects of different genotypes were in addition examined.

MATERIAL AND METHODOLOGY

Patients

Twenty-two heart transplant recipients (11 men), median age of 54 years (range from 27 to 65 years), were enrolled consecutively in this single centre, pilot study. The patients received CsA, mycophenolate mofetil (MMF) and steroids as immunosuppressive therapy. Treatment was initiated with 100 mg CsA intravenously at transplantation, followed by oral twice daily dosing. C0 first month; 250 to 300 µg/L and then tapered to between 60 to 120 µg/L during the one year follow-up. All patients received 1.5 g twice daily MMF and steroids in accordance to the following protocol; intravenous methylprednisolone (500 mg) at the time of transplantation

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and three more doses (125 mg) followed by oral prednisolone (0.2 mg/kg) twice daily from the second day with tapering after two weeks. Basiliximab induction (first dose of 20 mg intravenous within 2 hours pretransplant and a second dose of 20 mg intravenous on the fourth posttransplant day) was used in two patients. Demographic baseline data are shown in Table 1. The study was performed in accordance with the Declaration of Helsinki and all patients signed a written informed consent. The study was evaluated by the Regional Ethics Committee of Health Region South in Norway.

Study Design

Renal function was monitored daily (plasma creatinine) during the first posttransplant month and also at months 3, 6 and 12. Patients were divided in three groups based on renal function pattern during the first posttransplant month. Group A: patients with no, or only modest increase in plasma creatinine (i.e. < 30% increase from pretransplant level), group B: patients with sustained increase in plasma creatinine of > 30% from pretransplant levels but not in need of dialysis and group C: patients in need of dialysis after transplantation.

A blood sample was drawn during the first posttransplant week (median 4 days, range from 1 to 5) for the analysis of CsA and its six main metabolites as well as *CYP3A5* (*3) and *ABCBI* (G1199A, C1236T, G2677A/T, C3435T) genotypes. Blood sampling was performed at a median 2.5 hours (range from 1 to 25) following CsA administration.

Cyclosporine Analyses

Whole blood CsA and metabolite concentrations were analyzed with a validated HPLC-MS/MS method [29]. In brief, after protein precipitation with methanol and centrifugation, the supernatants were subjected to solid phase extraction using Oasis[®] HLB cartridges. CsA and the six metabolites AM19, AM1c9, AM1, AM9, AM1c, AM4N were all separated chromatographically before MS/MS detection. The method has a linear range of 2.5 - 3000 ng/mL for all analytes. Metabolite concentrations were expressed as a percent of CsA levels.

Genotyping

Genotyping was performed with previously reported polymerase chain reaction-restriction fragment length poly-

Table 1. Demographic Data at Time of Sampling (Mean ±SD)

	Group A N=6	Group B N=9	Group C N=6	All N=21	P-Value [#]
Age (years)	49 ± 13	51 ± 16	51 ± 13	50 ± 14	0.73
Gender (M/F)	5/1	2/7	4/2	11/10	0.048
Body weight (kg)	90 ± 24	72 ± 15	70 ± 16	77 ± 19	0.11
Diuresis (mL/24 hours)	2083 ± 1388	2316 ± 951	493 ± 563	1670 ± 1219	0.018
P-creatinine preTx. (mmol/L)	114 ± 29	82 ± 28	107 ± 34	97 ± 30	0.13
ASAT (U/L)	74 ± 53	71 ± 53	631 ± 1360	232 ± 729	0.66
ALAT (U/L)	41 ± 19	41 ± 24	631 ± 1451	209 ± 776	0.98
GGT (U/L)	80 ± 55	77 ± 44	131 ± 197	93 ± 109	0.83
SBP (mmHg)	125 ± 19	126 ± 24	126 ± 22	126 ± 21	1.00
DBP (mmHg)	81 ± 13	66 ± 13	67 ± 15	71 ± 14	0.090
CsA dose (mg/day)*	425 ± 95	303 ± 63	321 ± 99	343 ± 96	0.054
CsA dose (mg/kg/day)	4.9 ± 1.0	4.3 ± 0.9	4.6 ± 1.0	4.5 ± 0.9	0.73
CsA C2 conc (µg/L)***	1061 ± 593	1058 ± 656	1352 ± 875	1143 ± 686	0.72
Days since Tx.	3.7 ± 1.4	3.0 ± 1.3	3.7 ± 0.8	3.4 ± 1.2	0.50
Sampling time (hour)	2.5 ± 0.8	3.0 ± 0.7	7.9 ± 9.8	4.3 ± 5.6	0.38
<i>CYP3A5</i> *1 genotype	1/6	2/9	2/6**	5/21	0.56
Possible <i>ABCBI</i> TTT-haplotype	3/6	6/9	1/6	10/21	0.16

Abbreviations: P-creatinine: plasma creatinine, ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase, GGT: γ -glutamyl transferase, Tx: transplantation, SBP: systolic blood pressure, DBP: diastolic blood pressure. Group A: unchanged renal function; Group B: sustained renal dysfunction; Group C: patients in need of dialysis from the day of transplantation.

*Five patients received their dose intravenously. The value given is the normalized oral dose (IV dose * 3).

One of these patients were homozygote *CYP3A51/*1.

***C2: estimated concentration 2 hours postdose.

[#]Statistics performed with Chi² and Kruskal-Wallis Tests, respectively.

morphism assays on deoxyribonucleic acid (DNA), extracted from EDTA blood by QIAamp (Qiagen, Valencia, Calif), using specific primers and separation on 3% agarose gels [30-32]. All patients were screened for relevant polymorphisms in *CYP3A5* (*2 [C27289A, Thr398Asn] and *3 [A6986G, splicing defect]) and *ABCB1* (G1199A, C1236T, G2677A/T, and C3435T). Positive controls were kindly supplied by Dr D. Katz, Abbott Laboratories, Abbott Park, IL (*ABCB1*), and Dr R. van Schaik, Department of Clinical Chemistry, Erasmus MC, The Netherlands (*CYP3A5*).

Statistical Analyses

The statistical analyses were performed using Chi²/Mann-Whitney U Test/unpaired student *t*-test and Kruskal-Wallis Test with P-values < 0.05 considered as significant. All statistical analyses were performed using SPSS version 14.0.

RESULTS

Patients

The patients included in this study were representative of the over all heart transplant population at our centre. One patient died six days following transplantation due to severe acute rejection, and was excluded from further analyses.

Baseline demographic parameters and relevant *CYP3A5* and *ABCB1* genotypes were not relevantly different between groups, with the exception of gender (Table 1), with more females in group B developing renal impairment (P = 0.048). Four patients in group C and one patient in group A were treated with intravenous CsA at the time of sampling.

Renal Function

Six patients needed dialysis from the day of transplantation (group C). Six patients had stable renal function throughout the follow-up period (group A) while nine patients developed sustained renal impairment (group B). One of the patients in group B needed dialysis 15 days following transplantation and one died after 33 days. One patient in group C died after month 3, resulting in an overall patient survival of 86.4%. Six patients had mechanical circulatory support at transplantation. Seven patients experienced CMV

infection and four patients received treatment for acute rejection episodes (three steroid resistant). One of the six patients in group A developed impaired renal function by one year. The renal function improved over the year to near pretransplant levels in four of the nine patients in group B and patients on dialysis were later able to stop renal replacement therapy.

Cyclosporine A

Weight adjusted CsA doses and whole blood concentrations were not significantly different between group A and B (P = 0.28, and P = 0.99, Table 1), neither were any of the six metabolites measured (Table 2, and Fig. 1). Patients with impaired renal function (group B) showed a tendency of

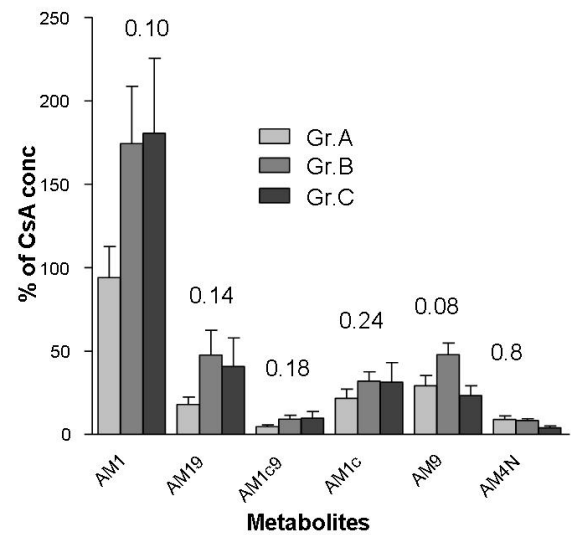


Fig. (1). The level of metabolites expressed as percent of cyclosporine A for the three study groups: Group A: Stable renal function, Group B: impaired renal function and group C: patients requiring dialysis. P-value: unpaired t-test between group A and group B.

Table 2. Mean (\pm SD) CsA and Metabolite Levels (μ g/L) in the Different Groups

	Group A N=6	Group B N=9	Group C N=6	P-Value
CsA	620 \pm 261	471 \pm 290	474 \pm 341	0.80
AM19	102.6 \pm 75.0	180.0 \pm 151.1	113 \pm 50.5	0.27
AM1c	116.6 \pm 68.8	136.3 \pm 71.2	153 \pm 219.6	0.61
AM1c9	25.2 \pm 15.2	33.1 \pm 17.2	26 \pm 11.6	0.38
AM1	517.8 \pm 265.7	753 \pm 495	640 \pm 578.4	0.31
AM9	153.8 \pm 78.8	206.8 \pm 124.8	113 \pm 113.1	0.38
AM4N	49.6 \pm 32.7	34.2 \pm 14.3	20 \pm 20.5	0.23

P-value: unpaired t-test between group A and group B.

Table 3. CYP3A5 and ABCB1 Genotype Allelic Frequencies in the Different Groups

SNP	Group A N=6	Group B N=9	Group C N=6	All N=21
<i>CYP3A5</i> *3	0.75	0.89	0.92	0.86
<i>ABCB1</i> 1199A	0.00	0.00	0.00	0.00
<i>ABCB1</i> 1236T	0.33	0.67	0.33	0.48
<i>ABCB1</i> 2677T	0.17	0.39	0.17	0.26
<i>ABCB1</i> 3435T	0.50	0.78	0.50	0.62

higher metabolite formation ($P > 0.08$). Metabolite AM19 showed the greatest difference and were 164% higher than in group A ($P = 0.14$). The three patients with highest relative concentrations of secondary metabolites were all in group B. The patients in need of dialysis (group C) tended to have higher metabolite concentrations compared to group A ($P > 0.16$).

CYP3A5 and ABCB1 Genotypes

No significant differences in *CYP3A5* or *ABCB1* genotype frequencies were present in this material (Table 3). Two of the five patients expressing functional *CYP3A5* enzymes (*CYP3A5**1) developed sustained renal impairment while one of the five patients showed stable renal function during the study period. With regards to P-gp functionality; six of the ten patients that expressed genotypes previously linked with impaired function (*ABCB1* TTT-haplotypes) developed renal impairment, while three of the ten patients showed stable renal function. Patients with TTT-haplotype and renal impairment had lower AM19 and AM1c9 levels compared to non-TTT-haplotype patients with renal impairment, $37.7 \pm 38.0\%$ vs $81.4 \pm 46.7\%$ ($P < 0.16$) and $6.7 \pm 3.9\%$ vs $14.9 \pm 9.5\%$ ($P < 0.08$), respectively. No significant difference in metabolite formation was observed with regards to functional expression of *CYP3A5*, with exception of AM9 formation.

DISCUSSION

Recent trends in immunosuppressive treatment have been either to reduce or replace CsA. Previous studies have shown that secondary CsA metabolites (AM19, AM1c9 and AM1c) may be associated with renal impairment following transplantation [24]. An inverse correlation between renal function and AM19 levels has also been presented [33]. The present study also indicates an association between acute renal impairment following transplantation and CsA metabolic pattern. The question whether the increased formation of nephrotoxic metabolites is a result of impaired renal function or the reason for the nephrotoxicity can not be elucidated by the present study. Increased level of metabolites in dialysis patients indicates that these metabolites at least partly are affected by renal impairment. However, *in vitro* studies report that the metabolite AM19 and AM1c9 also may be responsible for nephrotoxicity [17] and that *CYP3A5* produce more AM19 and AM1c9 [15]. The present study could however neither confirm nor reject this hypothesis, as only three

of the fifteen non-dialysis patients expressed *CYP3A5*. The patient material was also too small to draw any conclusions regarding *ABCB1* genotypes. However, six of the nine patients experiencing renal failure were TTT-haplotypes ($P = 0.12$), as compared to the previously reported frequency of approximately 0.4 [34]. In addition, the six patients with TTT-haplotype in group B all showed lower exposure of metabolites AM1 and AM1c9 (46.3% and 45.0%) compared to non-TTT-haplotypes. This indicates that impaired P-gp activity may induce higher sensitivity to CsA toxicity since intrarenal exposure may be higher. However, all four of the patients with acute rejections had also TTT-haplotype opposing the hypothesis of increased intracellular concentrations in these patients [35].

The major limitation of the study is the small sample-size, but also the facts that blood samples were drawn at different times following last dose of CsA and that some patients received intravenous CsA introduces additional limitation.

CONCLUSION

The present pilot study indicates that heart transplant recipients who experience reduced renal function early post-transplant have an altered metabolic pattern of CsA. The primary metabolites AM1 and AM9 and their secondary metabolites AM19, AM1c9 and AM1c tended to be higher in patients with impaired renal function compared to patients with stable renal function. This study also indicates that reduced P-gp activity may augment CsA nephrotoxicity. Both CsA metabolic pattern and *ABCB1* genotyping deserve further investigations to explore their potential as biomarkers for CsA induced nephrotoxicity.

TRIAL REGISTRATION

The trial is registered on ClinicalTrials.gov (NCT00264-355).

ACKNOWLEDGEMENTS

Beata Mohebi is acknowledged for the analytical work performed, and we would also like to thank all participating patients for giving their consent and donating blood samples.

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Received: February 2, 2009

Revised: March 18, 2009

Accepted: March 20, 2009

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