Relationship Between Acute Rejection and Cyclosporine or Mycophenolic Acid Levels in Japanese Heart Transplantation

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Background  Cyclosporine (CsA), Mycophenolate mofetil (MMF) and prednisolone (PSL) are widely used for the prevention of acute rejection after heart transplantation. Recently, the serum concentration–time curves (AUC) of CsA and MMF have been demonstrated to be precise predictors of acute rejection.

Methods and Results  Fourteen heart transplant patients were treated concomitantly with CsA, MMF, and PSL between May 1999 and November 2005 at the National Cardiovascular Center and of them 3 had acute rejection episodes [International Society for Heart & Lung Transplantation grade 3a]. Two patients (man in his 30s; woman in her 40s) had acute rejection with a mycophenolic acid (MPA) AUC0–12h <30 ng·h·ml$^{-1}$ and low CsA AUC (AUC0–4h: 2,408 ng·h·ml$^{-1}$, 1,735 ng·h·ml$^{-1}$). However, 1 patient (man in his 30s) with a high CsA AUC0–4h (4,019 ng·h·ml$^{-1}$) did not develop cardiac allograft rejection even if the MMF was temporarily stopped. These 3 patients were investigated to evaluate the relationship between acute rejection and pharmacokinetic parameters, including the CsA C0, C2, AUC0–4h and MPA AUC0–12h.

Conclusions  The findings suggest that a high CsA AUC0–4h may prevent rejection of a cardiac allograft, even if MMF is stopped or drastically reduced. (Circ J 2007; 71: 289–293)

Key Words: Cyclosporine; Japanese heart transplantation; Mycophenolate mofetil; Serum concentration–time curve
Blood for calculating the AUC of CsA and MPA was sampled at 6 time points: before dosing and at 1, 2, 4, 6, and 12 h after dosing. Blood levels of CsA and MPA were measured by fluorescent polarization immunoassay (TDx, Abbott Japan Co, Ltd) and reverse-phase high-performance liquid chromatography, respectively. AUC was calculated using the trapezoidal method. The AUC0–4 h was calculated as:

$$AUC0–4 h = \frac{1}{2} \times \{ (C0 + C1) \times 1 \} + \frac{1}{2} \times \{ (C1 + C2) \times 1 \} + \frac{1}{2} \times \{ (C2 + C4) \times 2 \}$$

where $C1$ is the 1 h post-dose concentration and $C4$ is the 4 h post-dose concentration. All research procedures were conducted according to the institutional clinical research guidelines and all patients gave written informed consent concerning the disclosure of their clinical data.

**Results**

**Patient 1 (Acute Rejection)**

A man in his 30s man with dilated cardiomyopathy (DCM) as the underlying disease received a heart transplant under catecholamine treatment. At the time of transplantation, Human Leukocyte Antigen (HLA) (A, B, DR) compatibility was 0/6; cytomegalovirus (CMV) was (+) for the donor and (−) for the recipient. Initial immunosuppressive therapy was CsA, but the serum creatinine increased to 2.2 mg/dl, so CsA was discontinued from day 3 post-transplant and replaced with orthoclone-OKT3. After renal function improved, CsA was re-administered with the addition of MMF and PSL.

The blood concentration profiles of CsA and MPA, and the doses of CsA, MMF and PSL are shown in Fig 1. The patient took oral ganciclovir (1,500 mg/day) to prevent CMV infection; however, on day 169 post-transplant, the CMV-polymerase chain reaction test showed a copy number of 1,900 and the antigenemia assay was positive. The patient was therefore hospitalized and ganciclovir injection therapy (10 mg·kg⁻¹·day⁻¹) was started.

The patient’s leukocyte count decreased to 1,900/μl, which was an adverse reaction caused by ganciclovir and MMF. Therefore, both the ganciclovir injection and MMF (2 g/day) were stopped. The dose of CsA was increased from 300 to 360 mg. After that, on day 222 post-transplant, ISHLT grade 3a acute rejection was confirmed by myocardial biopsy. The CsA dose was increased to 380 mg/day and the dose of PSL was increased to 20 mg/day. After 2 weeks, a myocardial biopsy showed improvement in the acute rejection, which was ISHLT grade 2. The target $C0$ value of CsA was set at approximately 300 ng/ml.

On day 361 post-transplant, myocardial biopsy again revealed acute rejection of ISHLT grade 3a. The dose of CsA...
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was at 420 mg/day, C0 at 308 ng/ml, C2 at 607 ng/ml, and the AUC0-4h at 2,408 ng·h·ml⁻¹; the dose of MMF was 1 g/day, AUC0-12h of MPA was 20.8 µg·h·ml⁻¹, and the dose of PSL was at 20 mg/day. The grade of acute rejection improved following a 3-day course of pulse therapy with MP at 1 g/day. The C0 of CsA was at the target level with few variations, but despite that, acute rejection of ISHLT grade 3a occurred twice, and the patient sustained a pressure fracture of a vertebra because of PSL. In view of these findings, CsA was changed to FK.

Patient 2 (Acute Rejection)

A woman in her 40 s with DCM as the underlying disease underwent cardiac transplantation after being on a NCVC extracorporeal left ventricular assist system (LVAS) (Toyobo, Tokyo, Japan). HLA (A, B, DR) compatibility was 2/6, and CMV antibody was (+) for the donor and (+) for the recipient. At the time of the transplant, Panel Reactive Antibody was (+), as the cross-match test. Specific anti-HLA antibodies against the donor were found in the recipient. In addition, owing to concern about the possibility of a renal function disorder because of long-term use of the LVAS, immunosuppressive therapy was begun with OKT-3, then switched to the 3-drug combination therapy.

The blood concentration profiles of CsA and MPA, and the doses of CsA, MMF and PSL are shown in Fig 2. On day 333 post-transplant, the patient’s leukocyte count had decreased to 3,400/µl, so MMF treatment (3 g/day) was stopped and the dose of CsA was increased from 280 to 330 mg/day. However, the serum creatinine level increased mildly to 1.3 mg/dl. On day 370 post-transplant, MMF treatment was reinstated at 0.5 g/day. CsA was maintained at 330 mg/day. The C0 of CsA was at 275 ng/ml, C2 at 1,452 ng/ml, and AUC0-4h at 4,204 ng·h·ml⁻¹. In addition, the AUC0-12h of MPA was 15.3 µg·h·ml⁻¹. subsequently, the serum creatinine level increased to 1.1 mg/dl and the CsA dose was decreased from 330 to 250 mg/day in order to obtain a target C0 of CsA of ≈200 ng/ml. On day 550 post-transplant, a myocardial biopsy was performed and acute rejection of ISHLT grade 3a was identified. At this point C0 was at 182 ng/ml, C2 at 445 ng/ml, AUC0-4h at 1,735 ng·h·ml⁻¹, the dose of MMF was 0.5 g/day, and that of PSL was 5 mg/day. Blood MPA level was not measured. After a 3-day course of pulse therapy with MP 1 g/day, the PSL dose was increased to 10 mg/day and acute rejection improved on day 556 post-transplant. The patient’s leuko-
Cyte count recovered to the normal value, so MMF was gradually increased to 2.5 g/day. Thereafter, the dose of CsA was set according to the monitoring of C2 and AUC0-4h, and no further episodes of acute rejection occurred.

**Patient 3 (Without Acute Rejection)**

A man in his 30s with DCM as the underlying disease, underwent cardiac transplantation under the support of LVAS. HLA (A, B, DR) compatibility was 0/6, CMV antibody was donor (+) and recipient (+). After the transplant, the patient’s serum creatinine level increased to 2.2 mg/dl so immunosuppressive therapy was initiated with OKT-3, followed by the 3-drug combination therapy.

The blood concentration profiles of CsA and MPA, and the doses of CsA, MMF and PSL are showed in Fig3. Up to day 75 post-transplant, the MMF dose was at 1.5 g/day, but the leukocyte count decreased to 3,770/μl, so the MMF dose was decreased from 1.5 to 1 g/day. On day 99 post-transplant, the leukocyte count decreased further to 3,270/μl, MMF was stopped and the CsA dose was increased from 320 to 380 mg/day. When the CsA dose was at 320 mg/day, C0 was at 267 ng/ml, C2 at 954 ng/ml, and AUC0–4 h at 2,897 ng·h·ml⁻¹. When the CsA dose was at 380 mg/day, C0 was at 247 ng/ml, C2 at 1,249 ng/ml, and AUC0–4 h at 4,019 ng·h·ml⁻¹. On day 262 post-transplant, the leukocyte count recovered to 8,000/μl, which is within the normal range, so MMF treatment was reinstated at 0.5 g/day. During the washout of MMF, myocardial biopsy was performed twice, but acute rejection was not seen.

**Discussion**

Our experience with the 3 heart transplant patients presented here suggests that monitoring of the CsA AUC0-4h or C2 may be useful in preventing acute rejection, as may a high AUC0-4h or C2, even if MMF is stopped or drastically decreased.

In patient 1, the CsA AUC0-4h and C2 were greatly decreased, with a low MPA AUC0-12h (20.8 ng·h·ml⁻¹) on day 361 post-transplant (ISHLT grade 3a). In patient 2, the CsA AUC0-4h and C2 greatly decreased on day 550 post-transplant (ISHLT grade 3a). Although the MPA AUC0-12h value on day 550 was not calculated, approximately 15 ng·h·ml⁻¹ could be predicted because the MPA AUC0-12h values on days 370 and 556 were 15.3 ng·h·ml⁻¹ and 14.3 ng·h·ml⁻¹, respectively. The MPA dose remained unchanged from day 370 to day 556. Low CsA AUC0-4h and MPA AUC0-12h might have been the cause of acute rejection on Fig 3. The blood concentration profiles of CsA and MPA, and the doses of CsA, MMF and PSL in patient 3.
day 550 in patient 2. Although the MPA AUC_{0-12 h} decreased to 15.3 \text{g} \cdot \text{h} \cdot \text{ml}^{-1} on day 370, the high CsA AUC_{0-4 h} (4,204 \text{ng} \cdot \text{h} \cdot \text{ml}^{-1}) and C_2 (1,452 \text{ng/ml}) were maintained, which may have prevented acute rejection in patient 2. Patient 3 did not experience acute rejection during the MMF washout period from day 99 to day 262, which may have prevented acute rejection, even if the MPA AUC_{0-12 h} is 30 \text{g} \cdot \text{h} \cdot \text{ml}^{-1} or less.

We calculate the AUC of CsA in heart transplant patients who are admitted for myocardial biopsy and monitor the C_0 and C_2 levels (the reference levels) of outpatients to determine the dose of CsA. However, there was no clear link between the risk of acute rejection and CsA C_0 levels in these 3 patients. It has been reported that, in determining the appropriate dose, monitoring of the absorption profile is more important than conventional C_0 monitoring of CsA.\(^4\)

The AUC_{0-4 h} is the important parameter of the absorption profile; however, a 1-point monitoring strategy needs to be developed for predicting the AUC_{0-4 h} in clinical practice, in particular for outpatients.\(^5\) It has been reported that C_2 is the most accurate surrogate marker for AUC_{0-4 h} and has been found to be a better marker for rejection and nephrotoxicity than C_0.\(^5\) Our experience also suggests that the CsA C_2 values changed in relation to the AUC_{0-4 h}. Cantarovich et al.\(^6\) report a clinical benefit of CsA C_2 monitoring (as opposed to C_0 monitoring) in long-term heart transplant patients. The C_2 target levels of their study were as follows: 0–3 months, 600–800 ng/ml; 4–6 months, 500–700 ng/ml; >6 months, 400–600 ng/ml. Other groups report that high C_2 values (1,015±422 ng/ml) are associated with fewer episodes of acute cellular rejection in patients who have undergone heart transplantation,\(^10\) and that acute cellular rejection should be suspected when the C_2 level is below 600 ng/ml.\(^11\) At present, at the NCVC, the AUC_{0-4 h} is predicted from C_0 and C_2, which are monitored in outpatients to determine the dose of CsA. However, the appropriate target value for either the AUC_{0-4 h} or C_2 of CsA in heart transplant recipients is not fixed.

On the other hand, the target AUC_{0-12 h} value for MPA after heart transplantation has been reported to be 30–60 \text{g} \cdot \text{h} \cdot \text{ml}^{-1}.\(^11\) In addition, the 3-point monitoring of C_0, C_2, and CsA has been reported to be highly correlated with the AUC_{0-12 h}.\(^2\)

We demonstrated that a high CsA AUC_{0-4 h} may help prevent cardiac allograft rejection in patients who temporarily stop MMF treatment. When MMF is stopped or drastically reduced, the dose of CsA should be increased to maintain the high CsA AUC_{0-4 h} (>4,000 \text{ng} \cdot \text{h} \cdot \text{ml}^{-1}). Although our study had a limited number of patients, it is the first to characterize the relationship between acute rejection and either the CsA or MPA level in heart transplant recipients. Further studies should be conducted to investigate the relationship between the CsA AUC_{0-4 h} or MPA AUC_{0-12 h} and the risk of rejection, and the effectiveness of CsA C_2 monitoring in heart transplant patients should be confirmed.

References