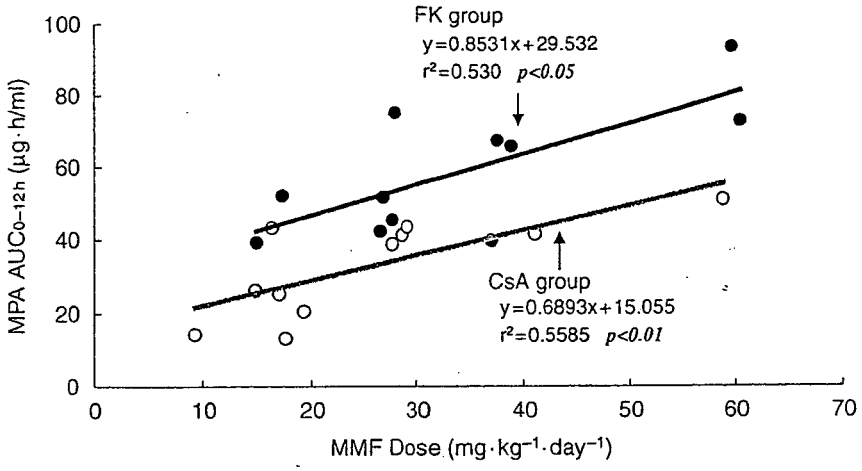

Fig 2. Relationship between mycophenolate mofetil (MMF) dose·kg^{-1}·day^{-1} and mycophenolic acid (MPA) AUC_{0-12h} for tacrolimus (FK) group (closed circle) and cyclosporine (CsA) group (open circle).

Table 3 Multiple Regression Analysis to Correlate Abbreviated MPA AUC Values With AUC Values Calculated Using the Full Set of 12 Timed MPA Concentrations (CsA Group)

Model	Sampling times (h)	Model equation	r^2	Prediction error (%)				VIF					
				Mean±SD	Within±15%	<−15%	>15%	C_{0h}	C_{1h}	C_{2h}	C_{4h}	C_{6h}	C_{12h}
1	0	$8.73C_{0h}+25.11$	0.29	15.77±48.21	1 (9.1)	5 (45.5)	5 (45.5)						
2	1	$1.40C_{1h}+23.14$	0.36	14.27±44.60	5 (45.5)	2 (18.2)	4 (36.3)						
3	2	$2.67C_{2h}+13.38$	0.46	11.30±35.83	10 (90.9)	0 (0)	1 (9.1)						
4	4	$4.00C_{4h}+21.39$	0.28	31.57±6.97	9 (81.8)	1 (9.1)	1 (9.1)						
5	6	$8.18C_{6h}+20.24$	0.43	12.94±39.31	3 (27.3)	3 (27.3)	5 (45.5)						
6	12	$13.64C_{12h}+18.93$	0.54	11.90±42.13	4 (36.3)	3 (27.3)	4 (36.3)						
7	0,1	$15.34+8.92C_{0h}+1.43C_{1h}$	0.66	7.53±27.91	5 (45.5)	2 (18.1)	4 (36.3)	1.00	1.00				
8	0,2	$-0.51+11.47C_{0h}+3.24C_{2h}$	0.94	0.49±10.35	10 (90.9)	0 (0)	1 (9.1)	1.04		1.04			
9	0,4	$21.31+5.36C_{0h}+2.39C_{4h}$	0.35	14.42±42.57	10 (90.9)	0 (0)	1 (9.1)	1.74			1.74		
10	0,6	$19.27-4.90C_{0h}+11.60C_{6h}$	0.44	12.68±40.79	3 (27.3)	3 (27.3)	5 (45.5)	5.55				5.55	
11	0,12	$18.80-2.38C_{0h}+15.81C_{12h}$	0.55	12.02±43.02	5 (45.5)	3 (27.3)	3 (27.3)	2.78					2.78
12	0,1,2	$0.10+11.15C_{0h}+0.42C_{1h}+2.80C_{2h}$	0.96	0.15±7.85	11 (100)	0 (0)	0 (0)	1.68	1.06	1.61			
13	0,2,4	$-0.23+12.70C_{0h}+3.36C_{2h}-0.80_{4h}$	0.94	0.36±9.80	10 (90.9)	0 (0)	1 (9.1)	1.23		2.04	2.08		
14	1,2,4	$1.28+1.91C_{1h}+0.26C_{2h}+5.91C_{4h}$	0.95	0.77±7.94	10 (90.9)	0 (0)	1 (9.1)		2.21	2.02	1.43		

CsA, cyclosporine; VIF, variance inflation factor. Other abbreviations see in Table 2.

Table 4 Multiple Regression Analysis to Correlate Abbreviated MPA AUC Values With AUC Values Calculated Using the Full Set of 12 Timed MPA Concentrations (FK Group)

Model	Sampling times (h)	Model equation	r^2	Prediction error (%)				VIF						
				Mean±SD	Within±15%	<−15%	>15%	C_{0h}	C_{1h}	C_{2h}	C_{4h}	C_{6h}	C_{8h}	C_{12h}
1	0	$1.53C_{0h}+55.48$	0.01	7.96±30.17	6 (54.5)	3 (27.3)	2 (18.2)							
2	1	$-0.23C_{1h}+60.75$	0.01	8.03±30.64	3 (27.3)	3 (27.3)	5 (45.5)							
3	2	$1.66C_{2h}+41.16$	0.65	3.50±20.06	6 (54.5)	2 (18.2)	3 (27.3)							
4	4	$2.97C_{4h}+44.54$	0.32	5.84±26.97	5 (45.5)	2 (18.2)	4 (36.3)							
5	6	$3.03C_{6h}+49.93$	0.10	7.03±27.03	4 (36.3)	3 (27.3)	4 (36.3)							
6	8	$5.90C_{8h}+36.99$	0.22	5.71±22.48	6 (54.5)	1 (9.1)	4 (36.3)							
7	12	$1.57C_{12h}+53.61$	0.04	7.57±28.68	3 (27.3)	3 (27.3)	5 (45.5)							
8	0,1	$57.83+1.14C_{0h}-0.17C_{1h}$	0.01	7.97±30.42	3 (27.3)	3 (27.3)	5 (45.5)	1.16	1.16					
9	0,2	$34.87+2.95C_{0h}+1.69C_{2h}$	0.68	3.26±19.57	6 (54.5)	2 (18.2)	3 (27.3)	1.01		1.01				
10	0,4	$47.63-2.01C_{0h}+3.17C_{4h}$	0.33	5.80±26.71	4 (36.3)	3 (27.3)	4 (36.3)	1.13			1.13			
11	0,6	$48.52+0.79C_{0h}+2.97C_{6h}$	0.10	7.01±26.99	4 (36.3)	3 (27.3)	4 (36.3)	1.02				1.02		
12	0,8	$39.36-2.93C_{0h}+6.86C_{8h}$	0.24	5.48±21.92	6 (54.5)	1 (9.1)	4 (36.3)	1.27					1.27	
13	0,12	$50.41+1.58C_{0h}+1.581C_{12h}$	0.05	7.49±28.42	3 (27.3)	3 (27.3)	5 (45.5)	1.00						1.00
14	0,1,2	$26.93+4.17C_{0h}+0.49C_{1h}+1.77C_{2h}$	0.70	2.97±18.28	7 (63.6)	1 (9.1)	3 (27.3)	1.08	1.20	1.24				
15	0,2,4	$35.03+2.39C_{0h}+1.59C_{2h}+0.43C_{4h}$	0.68	3.24±19.48	6 (54.5)	2 (18.2)	3 (27.3)	1.688		1.882	1.3			
16	1,2,4	$23.56+1.05C_{1h}+1.25C_{2h}+2.53C_{4h}$	0.73	2.73±17.09	6 (54.5)	1 (9.1)	4 (36.3)		2.21	1.645	3.105			

FK, tacrolimus. Other abbreviations see in Tables 2,3.

AUC_{0-12h} >60 μg·h/ml, and in the CsA group, there was no recipient who had an MPA AUC_{0-12h} >60 μg·h/ml. No recipient had severe side effects in either group.

Limited Sampling Strategy

The correlations between MPA concentrations at various time points and the full MPA AUC_{0-12h} values and prediction errors for the abbreviated AUC_{0-12h} profiles in the CsA and FK groups are summarized in Tables 3 and 4. Fourteen

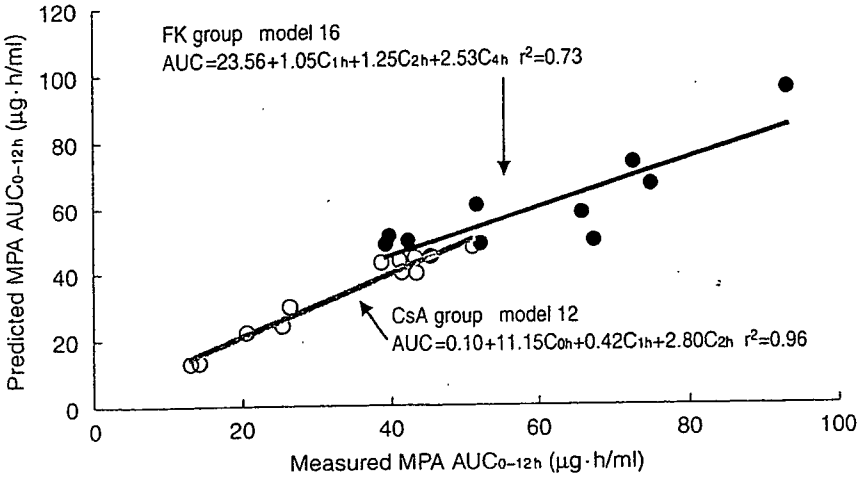

Fig 3. Linear regression plots of mycophenolic acid (MPA) area under the plasma concentration curve (AUC) values predicted by regression model 12 in cyclosporine (CsA) group (open circle; 3 samples is 0-h, 1-h and 2-h MPA concentration), 16 in tacrolimus (FK) group (closed circle; 3 samples; 1-h, 2-h and 4-h MPA concentrations) vs the corresponding each 11 MPA AUC values calculated from the full sets of samples by the linear trapezoidal rule.

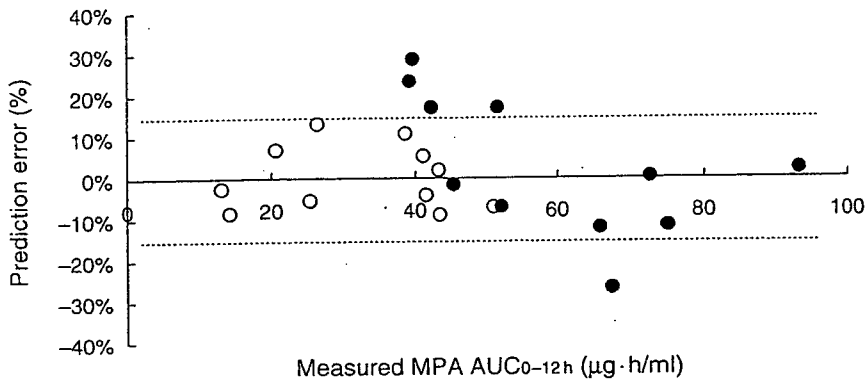

Fig 4. Prediction errors for the abbreviated mycophenolic acid (MPA) area under the plasma concentration curve (AUC) profiles. model 12 in cyclosporine (CsA) group (open circle; 3 samples is 0-h, 1-h and 2-h MPA concentration), 16 in tacrolimus (FK) group (closed circle; 3 samples; 1-h, 2-h and 4-h MPA concentrations) vs the corresponding each 11 MPA AUC values calculated from the full sets of samples by the linear trapezoidal rule.

models were developed and analyzed for their ability to estimate the MPA AUC_{0-12h} based on a limited number of samples in the CsA group. Sixteen models were developed in the FK group. The collinearity check for these models was not violated if the VIF of each model was smaller than 10. Fig 3 shows the MPA AUC values predicted using regression model 12 in the CsA group (model 16 in the FK group) plotted against the corresponding 11 MPA AUC values calculated from the full sets of 12 timed samples by the linear trapezoidal rule. Fig 4 shows prediction errors for the abbreviated MPA AUC profiles (model 12 in the CsA group and 16 in the FK group) plotted against the corresponding 11 MPA AUC values calculated from the full set of 12 timed samples by the linear trapezoidal rule. The best model for predicting the full MPA AUC_{0-12h} in the CsA group was a 3-time-point model (model 12; C_{0h}, C_{1h}, C_{2h}; r^2=0.96) with a mean prediction error of 0.15±7.85% (Table 3, Figs 3 and 4). The estimated prediction errors fell within ±15% in 100% of the profiles (11/11) with this model. The 2-sample model that gave the best r^2 value (0.94) was model 8 (C_{0h}, C_{2h}) with a mean prediction error of 0.49±10.35%. At this model, 90.9% of profiles (10/11) had an estimated prediction error within ±15%. The highest coefficient of determination between the MPA AUC_{0-12h} and a single concentration was observed with C_{12h} (r^2=0.54). The mean prediction error was 11.90±42.13% and the estimated prediction error fell within ±15% in only 36.3% of the profiles (4/11). The best model for predicting the full MPA AUC_{0-12h} in the FK group was a 3-time-point model (model 16; C_{1h}, C_{2h}, C_{4h}; r^2=0.73) with a mean prediction error of 2.73±17.09% (Table 4, Figs 3 and 4). The estimated prediction errors fell within ±15% in 54.5% of the profiles (6/11) with this model. The 2-sample model that gave the best r^2 value (0.68) was model 9 (C_{0h}, C_{2h}) with a mean prediction error of 3.26±19.57%. At this model, 54.5% profiles (6/11) had an estimated prediction error within ±15%. The highest coefficient of determination between the MPA AUC_{0-12h} and a single concentration was observed with C_{2h} (r^2=0.65). The mean prediction error was 3.50±20.06% and the estimated prediction error fell within ±15% in 54.5% of the profiles (6/11).

Discussion

The present study showed that the best model for predicting the full MPA AUC_{0-12h} was a 3-time-point model that included C_{0h}, C_{1h} and C_{2h} in the CsA group. The 2-time-point model that included C_{0h} and C_{2h} was also useful for predicting the full MPA AUC_{0-12h}. However, reliable models for a limited sampling strategy could not be obtained in the FK group. The measurement of the MPA AUC using a full set of samples requires considerable personnel time, laboratory resources and large quantities of blood. To support therapeutic drug monitoring of MPA in clinical practice, limited-sampling strategies should be developed for estimation of the MPA AUC. The r^2 values of other 3-time-point models (C_{0h}, C_{2h}, C_{4h}; C_{1h}, C_{2h} C_{4h}) in the CsA group were 0.94 and 0.95, respectively, The mean estimated prediction errors for these models were 0.36±9.80 and 0.77±7.94, respectively. These models could also be used for predicting the full MPA AUC_{0-12h}. In contrast, the r^2 values of 3-time-point models (C_{0h}, C_{1h}, C_{2h}; C_{0h}, C_{2h},

C_{4h}; C_{1h}, C_{2h}, C_{4h}) in the FK group were 0.70, 0.68 and 0.73, respectively. The mean estimated prediction errors for these models were 2.97±18.28, 3.24±19.48 and 2.73±17.09, respectively. Therefore, these models should not be used for predicting the full MPA AUC_{0-12h}. In the 2-sample model (C_{0h}, C_{2h}) with the best r^2 value (0.94) in the CsA group, the estimated prediction errors fell within ±15% in 90.9% of the profiles (10/11). This model is also suitable for predicting the full MPA AUC_{0-12h}. On the contrary, in the 2-sample model (C_{0h}, C_{2h}) with the best r^2 value (0.68) in the FK group, the estimated prediction errors fell within ±15% in 54.5% of the profiles (6/11). Therefore, this model is not suitable for predicting the full MPA AUC_{0-12h}. A single-point method would be useful to support therapeutic drug monitoring of MPA in the clinical management of our recipients; however, a reliable single-point method for predicting the full MPA AUC_{0-12h} was not obtained in either the CsA or FK groups. Moreover, reliable models for a limited-sampling strategy were not obtained in the FK group.

Cho et al reported that MPA concentrations before and at 1 and 8 h after dosing were positively correlated with the AUC in kidney transplant recipients treated with CsA.[19] Pawinski et al reported that a 3-sample model with sampling before and at 0.5 and 2 h after dosing was the best model for predicting the full MPA AUC in kidney transplant recipients treated with FK (r^2 value of 0.862 and a prediction error of 6.1±19.0%).[20] In another report, an r^2 value of 0.946 was obtained by a 3-sample model with sampling at 20 min, 1 and 3 h after dosing in kidney transplant recipients treated with CsA.[21] The discrepancy among the results of these previous studies might partly be explained by variation of the transplant organ (kidney or heart), concomitant drug therapy and the sampling times used to determine the full MPA AUC. It has been reported that a significant decrease of the MPA AUC and an increase of the oral apparent clearance are observed in renal-impaired recipients.[8] The suggested mechanism for these phenomena is a uremia-induced increase of the MPA-free fraction, leading to a temporary increase in the clearance of this restrictively-cleared drug. Thus, the MPA absorption profile might be different between kidney and heart transplant recipients. Cho et al collected blood samples before dosing and at 0.5, 1, 2, 4, 6 and 8 h after dosing.[19] Pawinski et al collected blood samples before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 9, 10, 11, and 12 h after dosing.[20] Le Guellec et al collected blood samples before dosing and at 20 min, 40 min, 1, 1.5, 2, 3, 4, 6 and 9 h after dosing.[21] In our study, blood samples were collected before dosing and at 1, 2, 4, 6, and 12 h after dosing in the CsA group and at 1, 2, 4, 6, 8 and 12 h after dosing in the FK group. Differences in the limited sampling strategies recommended in these studies could be attributable to sampling time differences.

In our study, reliable models for prediction of the MPA AUC were obtained in the CsA group, but not in the FK group. This suggests that concomitant drug therapy might be an important factor for predicting the MPA AUC. The difference in the results between the CsA and FK groups was attributed to distinct MPA pharmacokinetics. It has been reported that a secondary plasma peak of MPA attributed to enterohepatic circulation occurs 6 to 12 h after administration in recipients treated with FK.[22] In contrast, CsA might inhibit the transport of 7-O-MPA glucuronide, an inactive metabolite of MPA, into the bile and reduce the enterohepatic recirculation of MPA; therefore, a secondary plasma peak of MPA does not occur when MMF is used concomitantly with CsA.[22]

There was no recipient who experienced ISHLT Grade III rejection in the present study. No serious adverse effects were observed in 22 recipients enrolled in the study, including the recipients with an MPA AUC_{0-12h} >60 μg·h/ml. Consequently, the present study could not determine whether the target range of 30–60 μg·h/ml for the MPA AUC is suitable for reducing the risk of acute rejection and adverse effects. Further studies should be conducted on the relationship between the MPA AUC and the risk of rejection and adverse effects in Japanese heart transplant recipients. For this purpose, the development of a limited sampling strategy for estimation of the MPA AUC in Japanese heart transplant recipients is desirable. The 3-time-point model that included C_{0h}, C_{1h} and C_{2h} in the CsA group was useful for predicting the full MPA AUC_{0-12h}. In addition, the 2-time-point model that included C_{0h}, and C_{2h} in the CsA group was also reliable for predicting the full MPA AUC_{0-12h}. Although our study is limited in that a small number of recipients were evaluated, it is the first study to characterize the pharmacokinetic parameters of MPA in Japanese heart transplant recipients. A more detailed study is necessary to verify the assumption that the 2- and 3-time-point models for predicting the MPA AUC are valuable in Japanese heart transplant recipients treated with CsA. Furthermore, limited sampling strategies for predicting the MPA AUC need to be developed in FK-treated transplant recipients.

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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) AUC_{0-12h} <30 $\mu g \cdot h \cdot ml^{-1}$ and low CsA AUC (AUC_{0-4h}; 2,408 $ng \cdot h \cdot ml^{-1}$, 1,735 $ng \cdot h \cdot ml^{-1}$). However, 1 patient (man in his 30s) with a high CsA AUC_{0-4h} (4,019 $ng \cdot h \cdot ml^{-1}$) 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 C_0, C_2, AUC_{0-4h} and MPA AUC_{0-12h}.

Conclusions The findings suggest that a high CsA AUC_{0-4h} 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

A 3-drug combination therapy consisting of cyclosporine (CsA) or tacrolimus (FK) plus mycophenolate mofetil (MMF) and prednisolone (PSL) is commonly used for basic immunotherapy in heart transplant patients. At the National Cardiovascular Center (NCVC), approximately 30 heart transplant patients, including several from overseas, have received the 3-drug combination therapy, and its usefulness has been recognized[1,2] However, despite this treatment, some patients develop acute rejection. It is well known that, in order to obtain good clinical effects and to prevent acute rejection, it is important to monitor the blood levels of immunosuppressive agents. Generally, the trough level (C_0) has been used in such monitoring; however, in recent times, analysis of the full area under the curve (AUC) of CsA was demonstrated to be a precise predictor of acute rejection and graft survival[3] In addition, it was reported that in renal transplant patients, the AUC during the absorption phase (AUC_{0-4h}) was highly correlated with the full AUC and was a better marker for rejection and nephrotoxicity than the blood trough level[4] It has therefore come to be recognized that absorption profiling is needed in order to monitor the CsA microemulsion (Neoral) more effectively[4–11]

After oral administration, MMF is rapidly and extensively absorbed and hydrolyzed to mycophenolic acid (MPA), the active immunosuppressive agent. Several studies have demonstrated a significant relationship between the MPA AUC and acute rejection[12–19] A low AUC in the first 6 months is associated with a high incidence of rejection[13] and recent reports suggest that a target of 30–60 $ng \cdot h \cdot ml^{-1}$ may be suitable during both the early post transplant period and later for maintenance therapy in heart transplant patients[13,14,18,19]

We present 3 Japanese heart transplant recipients who showed a correlation between the development of acute rejection and the relevant pharmacokinetic parameters, including the CsA AUC_{0-4h}, the 2h post-dose concentration (C_2) and the MPA AUC_{0-12h}.

Methods

Of 14 patients who had received the 3-drug combination therapy between May 1999 and November 2005, 3 had acute rejection episodes (International Society for Heart & Lung Transplantation (ISHLT) grade 3a). In 2 of them, blood levels of CsA and MMF were measured before and after the acute rejection episode. Among the remaining 11 patients who did not have acute rejection episodes, 1 patient stopped the MMF for a long period and only received a 2-drug therapy (CsA and PSL). The 2 patients who had acute rejection episodes and the 1 who did not have an acute rejection episode during withdrawal of MMF were enrolled.

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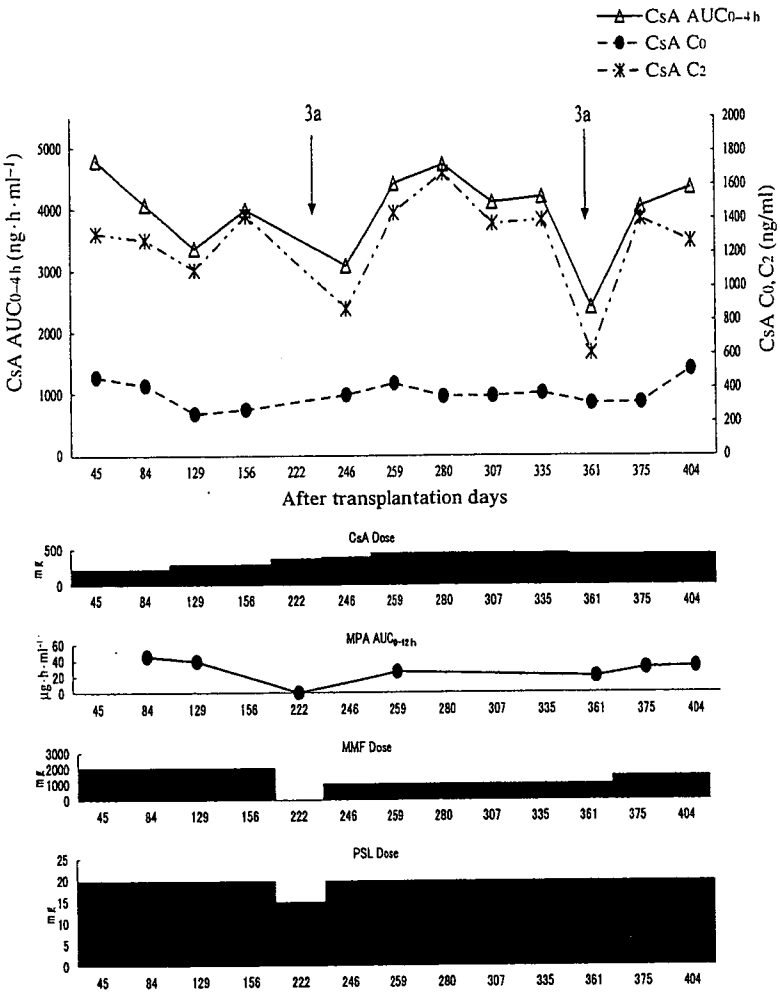

Fig 1. The blood concentration profiles of CsA and MPA, and the doses of CsA, MMF and PSL in patient 1. CsA, cyclosporine; AUC_{0-4}, area under the curve during the absorption phase; C_0, measurement of whole blood trough levels; C_2, 2 h post-dose concentration; MPA, mycophenolic acid; MMF, mycophenolate mofetil; PSL, prednisolone.

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-perfomance liquid chromatography[20] respectively. AUC was calculated using the trapezoidal method. The AUC_{0-4h} was calculated as:

$$AUC_{0-4h}=1/2\times\{(C_0+C_1)\times1\}+1/2\times\{(C_1+C_2)\times1\}+1/2\times\{(C_2+C_4)\times2\}$$

where C_1 is the 1 h post-dose concentration and C_4 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 30 s 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-administrated with the addition of MMF and PSL.

The blood concentration profiles of CsA and MPA, and the doses of CsA, MMF and PSL are showed 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 ($10\,mg\cdot kg^{-1}\cdot day^{-1}$) 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 360 mg/day, MMF administration had ceased, and the dose of PSL was 15 mg/day. At this time, blood levels of CsA and MMF were not measured. Three-day pulse therapy with methyl prednisolone (MP, 1 g/day) was instituted, followed by an increase in the doses of CsA and PSL to 380 and 20 mg/day, respectively. MMF treatment was reinstated at 1 g/day. After 2 weeks, a myocardial biopsy showed improvement in the acute rejection, which was ISHLT grade 2. The target C_0 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

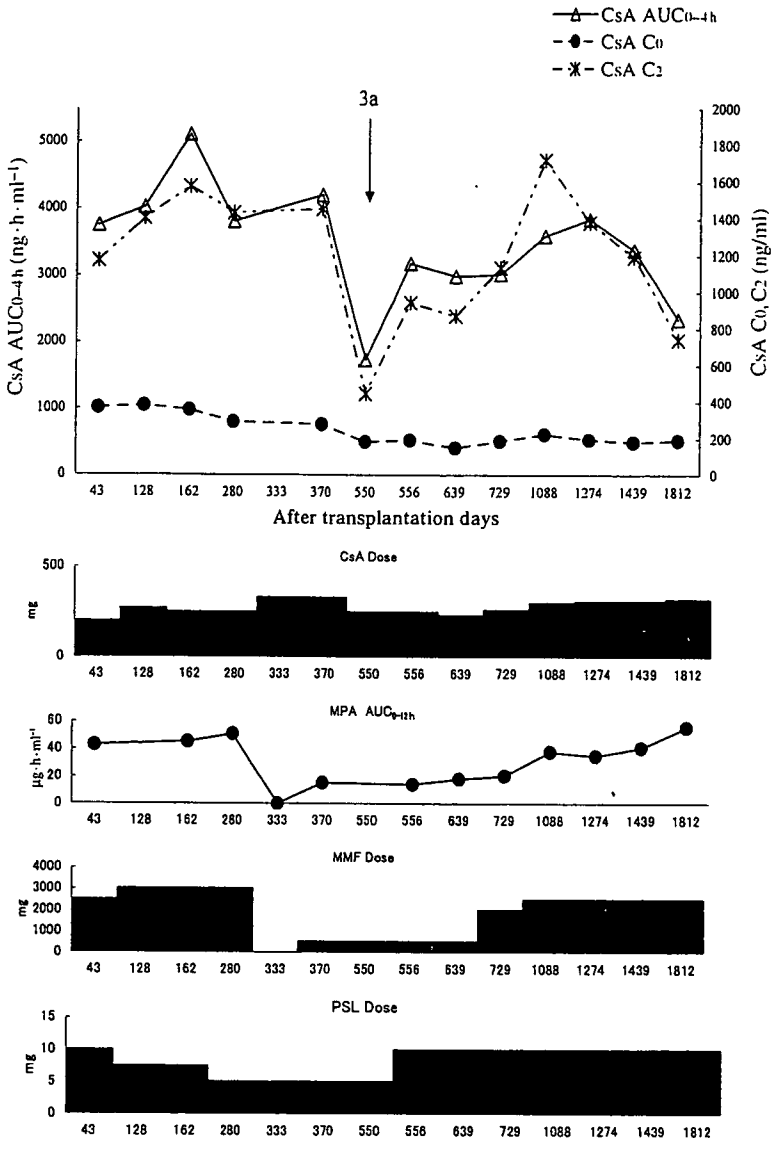

Fig 2. The blood concentration profiles of CsA and MPA, and the doses of CsA, MMF and PSL in patient 2.

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 40s with DCM as the underlying disease underwent cardiac transplantation after being on a NCVC extracorporeal left ventricular assist system (LVAS) (Toyobo, Tokyo, Japan)[21] 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-

day 550 in patient 2. Although the MPA AUC_{0-12h} decreased to 15.3 $\mu g \cdot h \cdot ml^{-1}$ on day 370, the high CsA AUC_{0-4h} (4,204 $ng \cdot h \cdot ml^{-1}$) and C_2 (1,452 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 be attributed to the high AUC_{0-4h} (4,019 $ng \cdot h \cdot ml^{-1}$) or C_2 (1,249 ng/ml). These findings suggest that the high CsA AUC_{0-4h} (>4,000 $ng \cdot h \cdot ml^{-1}$) or C_2 (>1,200 ng/ml) might prevent acute rejection, even if the MPA AUC_{0-12h} is 30 $\mu g \cdot h \cdot ml^{-1}$ 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.[4-8] The AUC_{0-4h} is the important parameter of the absorption profile; however, a 1-point monitoring strategy needs to be developed for predicting the AUC_{0-4h} in clinical practice, in particular for outpatients.[22] It has been reported that C_2 is the most accurate surrogate marker for AUC_{0-4h},[4-6] and has been found to be a better marker for rejection and nephrotoxicity than C_0.[4,5] Our experience also suggests that the CsA C_2 values changed in relation to the AUC_{0-4h}. Cantarovich et al[6,9,23] 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.[6,11] At present, at the NCVC, the AUC_{0-4h} 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_{0-4h} or C_2 of CsA in heart transplant recipients is not fixed.

On the other hand, the target AUC_{0-12h} value for MPA after heart transplantation has been reported to be 30–60 $\mu g \cdot h \cdot ml^{-1}$.[13] In addition, the 3-point monitoring of C_0, $C_{0.5}$, and C_2 has been reported to be highly correlated with the AUC_{0-12h}.[24]

We demonstrated that a high CsA AUC_{0-4h} 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_{0-4h} (>4,000 $ng \cdot h \cdot ml^{-1}$). 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_{0-4h} or MPA AUC_{0-12h} and the risk of rejection, and the effectiveness of CsA C_2 monitoring in heart transplant patients should be confirmed.

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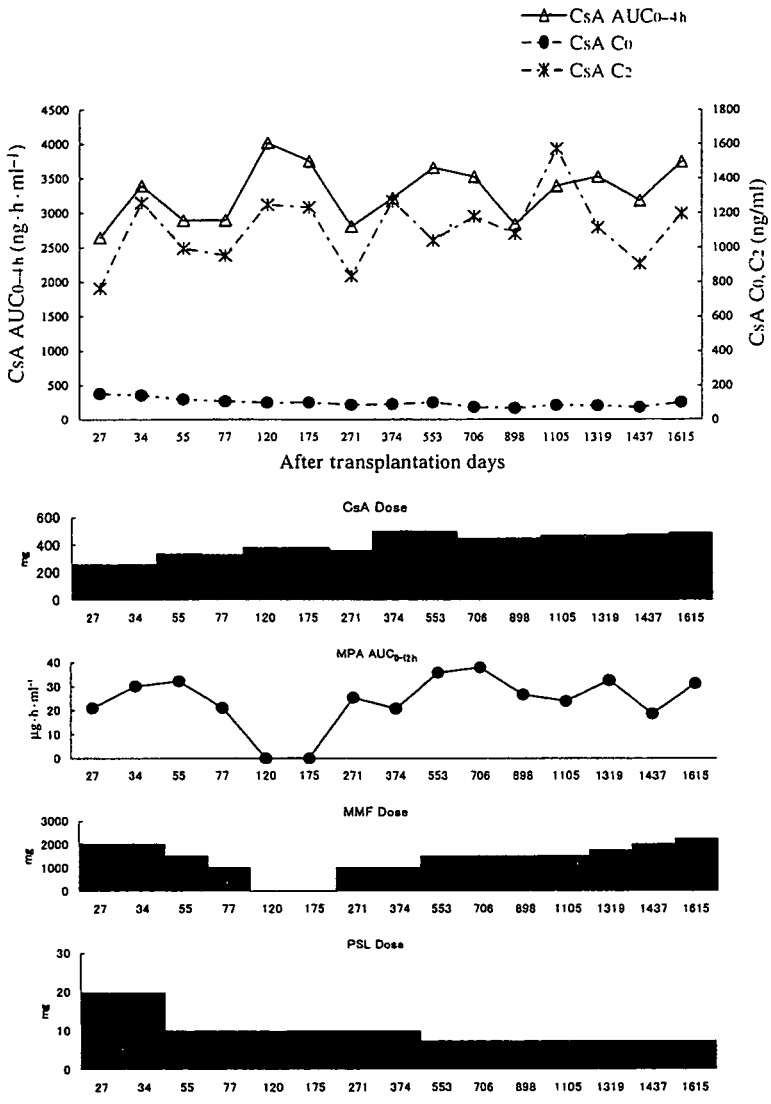

Fig 3. The blood concentration profiles of CsA and MPA, and the doses of CsA, MMF and PSL in patient 3.

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 C_2 and AUC_{0-4h}, 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 Fig 3. 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, C_0 was at 267 ng/ml, C_2 at 954 ng/ml, and AUC_{0-4h} at 2,897 ng·h·ml^{-1}. When the CsA dose was at 380 mg/day, C_0 was at 247 ng/ml, C_2 at 1,249 ng/ml, and AUC_{0-4h} 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 AUC_{0-4h} or C_2 may be useful in preventing acute rejection, as may a high AUC_{0-4h} or C_2, even if MMF is stopped or drastically decreased.

In patient 1, the CsA AUC_{0-4h} and C_2 were greatly decreased, with a low MPA AUC_{0-12h} (20.8 ng·h·ml^{-1}) on day 361 post-transplant (ISHLT grade 3a). In patient 2, the CsA AUC_{0-4h} and C_2 greatly decreased on day 550 post-transplant (ISHLT grade 3a). Although the MPA AUC_{0-12h} value on day 550 was not calculated, approximately 15 ng·h·ml^{-1} could be predicted because the MPA AUC_{0-12h} values on days 370 and 556 were 15.3 μg·h·ml^{-1} and 14.3 μg·h·ml^{-1}, respectively. The MPA dose remained unchanged from day 370 to day 556. Low CsA AUC_{0-4h} and MPA AUC_{0-12h} might have been the cause of acute rejection on

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Case Report

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Drug interactions between tacrolimus and phenytoin in Japanese heart transplant recipients: 2 case reports

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Key words
phenytoin – tacrolimus – drug interactions – Japanese heart transplant recipients

Abstract. Objective: The purpose of the study was to demonstrate how the interaction between phenytoin and tacrolimus (FK 506) can be managed clinically and to characterize the change in FK 506 levels after discontinuation of phenytoin in two Japanese heart transplant recipients with different dosing periods of phenytoin. Methods: A drug interaction between phenytoin and FK 506 was investigated in 2 patients. The concentration-dose ratios (CDR: trough blood FK 506 level (ng/ml)/FK 506 dose (mg/day) on the previous day) were calculated as an index of the induction of the CYP3A4 enzyme during and after phenytoin therapy. Results: About 2- to 3-fold dosages of FK 506 were required to maintain the required blood level when phenytoin was used concomitantly in the two cases examined. The FK 506 dose was constant within 21 days after discontinuing phenytoin in Patient 1 who had 36 days of phenytoin therapy. In Patient 2 with 21-day phenytoin therapy, the FK 506 doses and CDR varied for 10 days after discontinuing phenytoin, and expected FK 506 C_0 levels were achieved within 11 days. Conclusions: The persistence of CYP induction after discontinuing phenytoin is dependent on the history of administration and, perhaps, on the dosing period in particular.

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Introduction

Tacrolimus (FK 506), a neutral macrolide antibiotic isolated from Streptomyces tsukubaensis, is widely used in solid organ transplantation [Kino et al. 1987]. It is a potent immunosuppressive agent that inhibits calcineurin to prevent the production of interleukin-2 by T cells, inhibiting their maturation and proliferation [Ochiai et al. 1987]. Therapeutic drug monitoring (TDM) is required for FK 506 because of a narrow therapeutic window. Metabolism of the drug largely occurs through the cytochrome P450 (CYP) 3A4 enzyme system, which is present in the liver and gut [Lamba et al. 2002]. Agents that affect the metabolism of FK 506 by inducing or inhibiting CYP3A4 enzymes may change blood levels of FK 506, resulting in transplant rejection or side effects. Inhibitors of CYP3A4, such as clarithromycin, diltiazem, fluconazole, indinavir and grapefruit juice, have been reported to increase FK 506 levels [Fireman et al. 2004]. In contrast, inducers of CYP3A4, such as rifampicin, phenobarbital and St. John's wort have been shown to decrease FK 506 levels [Fireman et al. 2004].

Phenytoin is a hydantoin anticonvulsant for the control of generalized tonic-clonic and complex partial seizures. Phenytoin accelerates CYP1A-, CYP2B-, CYP2C- and CYP-3A-mediated oxidative metabolism of antiepileptic drugs (e.g. zonisamide, carbamazepin, valproate), cardioactive drugs (e.g. nifedipine, disopyramide, procainamide), oral anticoagulants (e.g. warfarin) and immunosuppressants (e.g. cyclosporine (CsA), FK 506) [Anderson 1998]. A drug interaction between phenytoin and FK 506 has been previously reported [Formea et al. 2005, Karasu et al. 2001, Moreno et al. 1999]. However, changes in FK 506 blood levels after discontinuation of phenytoin have not been characterized in previous reports. In this report, we present two cases of a drug interaction between phenytoin and FK 506 in Japanese heart

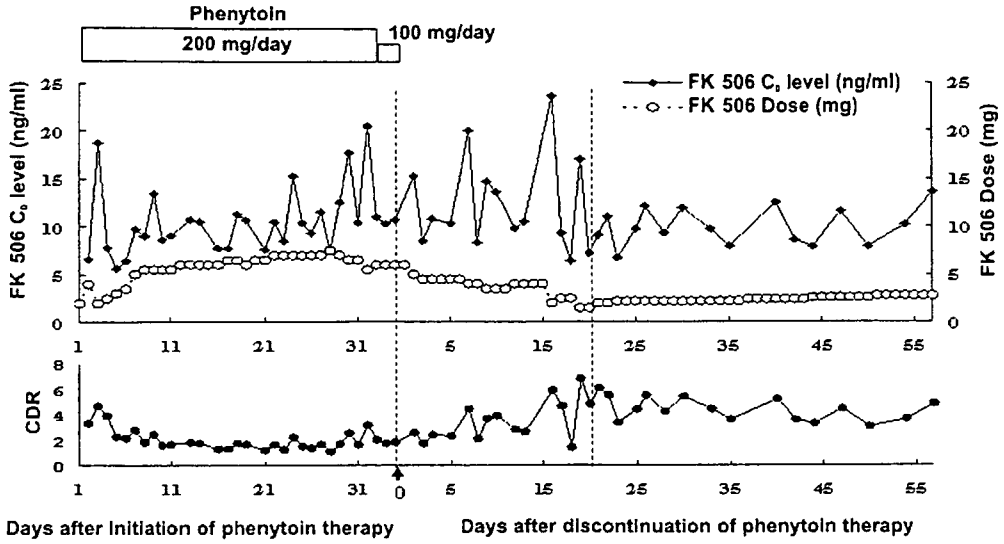

Figure 1. The effect of phenytoin treatment on trough serum FK 506 levels in Case 1 patient.

transplant recipients. The purpose of the study was to show how the drug interaction between phenytoin and FK 506 can be managed clinically and to characterize the changes in FK 506 levels after discontinuation of phenytoin in Japanese heart transplant recipients.

Methods

Among the 38 patients who had received immunosuppression therapy with CsA or FK 506, mycophenolate mofetil (MMF) and prednisone (PSL) between May 1999 and November 2006, there were 2 patients who received FK 506 and phenytoin concomitantly. One patient was a woman in her 40's who underwent heart transplantation at the National Cardiovascular Center (NCVC). The primary disease of the patient was dilated cardiomyopathy. The other patient was a woman in her 20's with dilated cardiomyopathy as a primary disease, who underwent heart transplantation at an overseas hospital. These 2 patients were investigated to evaluate a drug interaction between phenytoin and FK 506. Blood levels of FK 506 and CsA were measured by fluorescent polarization immunoassay (TDx, Abbott Japan CO., Tokyo, LTDA). The concentration-dose ratios (CDR: trough blood FK 506 level) (ng/ml)/FK 506 dose (mg/day) on the previous day were calculated as an index for the induction of the CYP3A4 enzyme during and after phenytoin therapy [Keown et al. 1984]. The changes in CDR for FK 506 between combination periods and FK 506-only periods were investigated. All research procedures were conducted according to the clinical research guidelines in our institute. All patients gave their written informed consent concerning the disclosure of their clinical data.

Results

Patient 1

This patient received triple-drug immunosuppression with CsA, MMF and PSL after surgery. The patient developed an impaired level of consciousness and orientation on the 7th day after surgery. For the next 7 days, she had narrowed visual fields, and on the 16th day after surgery she developed a convulsive seizure. The CsA trough (C_0) levels were measured routinely. The maximum CsA C_0 level throughout the neurological events was 439 ng/ml, which was within the target range. The magnesium level was 0.44 mmol/l. Magnetic resonance imaging (MRI) of the head demonstrated multifocal areas of increased signal in the white matter of the right mater lobus occipitalis. Oral phenytoin was started for treatment of a convulsive seizure at a dose of 200 mg/day, and CsA was changed to FK 506 on the next day because of potential CsA neurotoxicity. Abnormal MRI signals signifi-

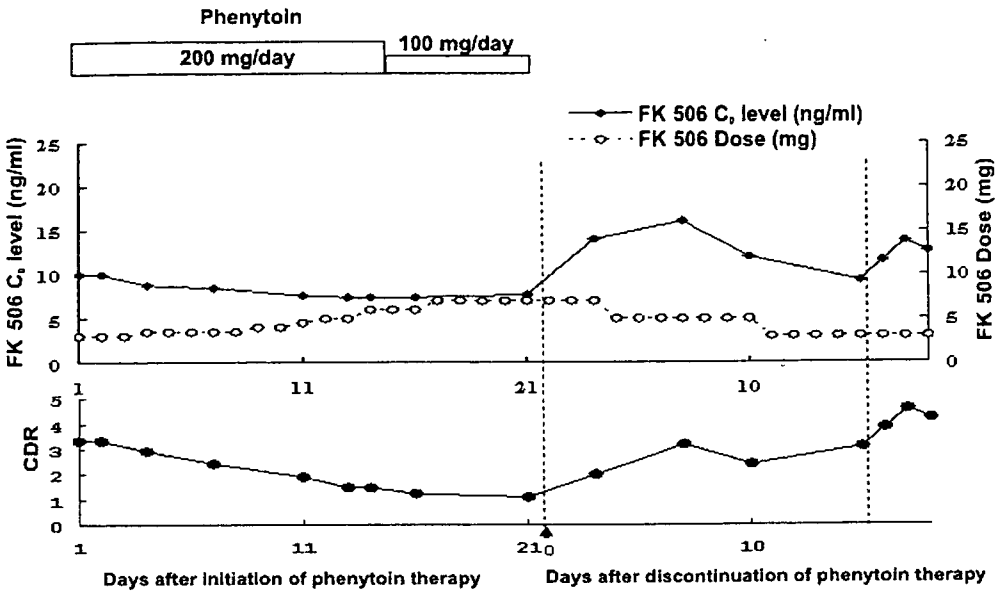

Figure 2. The effect of phenytoin treatment on trough serum FK 506 levels in Case 2 patient.

cantly diminished, and narrowed visual fields disappeared on the 6th day after discontinuing CsA. The abnormal MRI signals disappeared on the 28th day after CsA was stopped. Phenytoin was decreased to 100 mg/day on the 34th day after the initiation of therapy and was stopped within 2 days.

Changes of phenytoin dose, FK 506 dose, blood FK 506 C_0 levels and the CDR are shown in Figure 1.

FK 506 was started at an initial daily dose of 2 mg. FK 506 C_0 levels were measured routinely. The dose of FK 506 was gradually increased and a C_0 level of 10.5 ng/ml was obtained with a daily dose of 6.0 mg on the 14th day after initiation of phenytoin therapy. Thereafter, 5.5 – 7.5 mg/day of FK 506 gave C_0 levels of 7.7 – 20.5 ng/ml. The CDR was 4.7 on the 3rd day after initiation of FK 506 therapy and gradually decreased.

On the 7th day after phenytoin was stopped, 4 mg/day of FK 506 resulted in a C_0 level of 20 ng/ml. Although the FK 506 dose was finely adjusted thereafter, the C_0 levels varied widely. After discontinuing phenytoin therapy, the FK 506 dose was gradually reduced. Consequently, C_0 levels of 10 – 13 ng/ml were maintained with daily doses of 2.4 – 2.8 mg for 3 weeks after stopping phenytoin. The mean value of CDR for 3 weeks after stopping phenytoin was 3.63 ± 1.67. The mean value of CDR for the next 21 days (22nd – 42nd day after stopping phenytoin) was 4.52 ± 0.84. The coefficient of variation of the CDR for 3 weeks after discontinuing phenytoin was 46%. The coefficient of variation of the CDR for the next 21 days was 18.7%. These observations suggest that enzyme induction by phenytoin persisted for 21 days. Concomitant drugs were not changed during phenytoin therapy, and there was no hepatic disease.

Patient 2

This patient received triple-drug immunosuppression with FK 506, MMF and PSL. She developed a convulsive seizure on the 16th and 20th day after surgery and was treated with oral levetiracetam (unapproved in Japan). The convulsive seizure immediately disappeared. On the 70th day after surgery, the patient returned to Japan to enter the NCVC. On the day after admission, oral levetiracetam was changed to 200 mg/day of oral phenytoin. The phenytoin daily dose was decreased to 100 mg on the 15th day and was stopped on the 21st day after the initiation of therapy. Changes of phenytoin dose, FK 506 dose, blood FK 506 C_0 levels and CDR are shown in Figure 2. The FK 506 C_0 levels were measured routinely. Before the start of phenytoin dosing, the FK 506 C_0 level was 10 ng/ml with a daily dose of 3 mg. Thereafter, there was a decrease in the FK 506 C_0 level

(8.8 ng/ml) on the 4th day after the start of phenytoin. Despite increasing the daily dose of FK 506 to 6 mg, a C_0 level of 7.4 ng/ml and a CDR value of 1.5 were obtained on the 14th day after the start of phenytoin. Phenytoin was discontinued on the 21st day after it was initiated, and the FK 506 C_0 level reached 14 ng/ml within 3 days of a daily dose of 7 mg. For the next 7 days, the FK 506 dose was reduced to 5 mg/day, however, the C_0 level did not decrease. Thereafter, the FK 506 dose was reduced to 3 mg/day and C_0 levels of 9 – 14 ng/ml were obtained. Fixed dosing of FK 506 was achieved within 11 days after discontinuing phenytoin.

The mean CDR was 2.53 ± 0.61 for 10 days after discontinuing phenytoin. The mean CDR for the next 8 days (11th – 18th day after discontinuing phenytoin) was 3.95 ± 0.64. A CDR value of 3.9 on the 16th day after discontinuing phenytoin was about the same as obtained at the onset of phenytoin dosing. The coefficient of variation of the CDR for 10 days after stopping phenytoin was 24.1%. The coefficient of variation of the CDR for the next 8 days was 16.2%. This suggests that the effect of enzyme induction by phenytoin disappeared in 11 days. Other concomitant drugs were not changed during phenytoin therapy, and there was no hepatic dysfunction.

Discussion

The present study suggested that the difference of the persistence of CYP induction after discontinuing phenytoin may partly be explained by variation of the dosing period. Drug interactions associated with CYP induction are expected to occur gradually because CYP induction requires increased production of metabolic protein. In general, the effects of CYP induction occur and dissipate slowly in a few weeks after the start and discontinuation of inducers. In the case of phenytoin, it has been reported that induction of the CYP3A4 enzyme occurs within 2 – 5 days, reaching a maximum 10 – 14 days after the initiation of therapy [Karasu et al. 2001]. In our patients, the CDR was stable within 14 – 21 days after initiation of phenytoin. About a 2- to 3-fold higher dose of FK 506 is required to maintain expected blood levels when phenytoin is given concomitantly.

The impact of CYP3A4 enzyme induction on blood levels of FK 506 disappeared slowly within 21 and 11 days after stopping phenytoin in Patients 1 and 2, respectively. There were differences in the dosing period and total dosage of phenytoin between these 2 patients. The dosing period of phenytoin in Patient 2 was shorter than in Patient 1. The half-life of CYP enzyme turnover ranges from 1 – 6 days. In addition, the time course of induction is dependent on the time period required for enzyme degradation and new enzyme production [Michalets 1998]. The persistence of CYP induction after discontinuing inducers may change depending on the history of administration. It was reported that the time period of CYP induction depended on the half-life of inducers, the half-life of the CYP enzyme, the patient's hepatic function and the patient's age [Michalets 1998]. The effect of CYP induction by phenytoin could depend on these factors, and the persistence of CYP induction after discontinuing phenytoin disappears slowly and is not constant. In our 2 patients, the persistence of CYP induction varied with the dosing period of phenytoin.

In conclusion, the 2 cases in the present study suggest that the persistence of CYP induction after discontinuing phenytoin depends on the history of administration and, perhaps, on the dosing period in particular. When FK 506 is used concomitantly with phenytoin, the dose of FK 506 after discontinuing phenytoin should be carefully adjusted taking into account the dosing period of phenytoin, and FK 506 blood levels should be closely monitored during and after phenytoin therapy. Further study is required to clarify the relationship between the dosing period of phenytoin and the persistence of CYP induction.

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Reduction of Antigenicity and Risk of Infection in Regenerative Tissue Transplantation by Cold Isostatic Pressing

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Abstract
Tissue engineered heart valves based on acellular tissue have been studied to have more durability and bio-functionality with growth potential and less immunogenicity. Whereas they have still several problems to be solved such as complete cell removal and transfer of unknown animal related infectious diseases. In this paper, our novel tissue processing for decellularization using ultrahigh pressure for the safe tissue transplantation was reported. Porcine cardiac tissues were isolated and treated by a cold isostatic pressing for a disruption of donor cells. The cell debris was then washed out by washing solution at 4°C. The tissues treated were completely cell free when they were applied to 980 MPa for 10 min. There was no porcine endogeneous retrovirus detected. There were no significant changes in biomechanical properties of the breaking strength and elastic modulus. The acellular grafts of pulmonary valve were transplanted to allogeneic miniature pigs. The explanted grafts showed remarkable cell infiltration and endothelialization. This processing may provide more durable and safe scaffold for the regenerative tissue transplantation.

Keywords: tissue engineering, tissue transplantation, acellular, scaffold

1. Introduction

The implantable cardiovascular medical devices have been clinically used for more than 30 years as substitution for the patient's deficient tissues. The artificial heart valve is one of the most clinically used medical devices applied to about 300,000 patients per year worldwide. There are two kinds of artificial heart valves currently used. A xenograft heart valve is made of the chemically crosslinked porcine valve or bovine pericardium to reduce antigenicity of the xenogeneic tissue. A mechanical heart valve is made of pyrolytic carbon or titanium. The former has good biocompatibility, hemodynamics, and resistant to infections compared with the latter. However, the durability of the xenograft valve is relatively short especially in pediatric patients for about 5-10 years by the calcification of the gltaraldehyde-fixed animal tissue. Recent establishment of the human tissue bank has made it easy to use allogeneic tissues for the transplantation that are superior to the current artificial devices. However, since they are donated from the cadavers, the supply is very limited and some donated tissues may not be applicable due to infection. In addition to the above issues, all the devices and tissues lack the growth potential and they may be replaced repeatedly through the patients' growth process.

All of the current medical devices remain as foreign bodies even after the implantation. If a device accepts host cell impregnation and is replaced by the host tissue after the implantation,

it may acquire perfect biocompatibility and growth ability. An ideal candidate for such a regenerative scaffold is a decellularized allogeneic or xenogeneic tissue since it does not require tissue fixation for removal of antigenicity. Detergents and/or enzymes such as Triton® X-100, sodium dodecyl sulphate, deoxycholate, trypsine, DNase, and RNase have been commonly used for the cell removal media from the tissue [1-4]. However, the decellularization depends on their permeation in the tissue and may not be achieved completely in large or hard tissues. And furthermore, since the detergents are generally cytotoxic and it takes time for their removal, it may lead denature of biological properties and contamination in the process. Recent BSE (Bovine Spongiform Encephalopathy) and vCJD (variant Creutzfeldt-Jakob disease) issues have been affecting to the tissue transplantation from the point of view of safety. In this paper, a cold isostatic pressing (CIP) was applied for removal of the cells and inactivation of viruses in the cardiovascular tissues to have scaffold for the safe regenerative tissue transplantation.

2. Material and methods

The porcine heart valves were isolated from 4 month-old Clawn miniature pigs (Japan Farm Co. Ltd, Kagoshima, Japan) weighing about 10 kg under the sterile condition. The harvested tissues were packed immediately in sterile bags filled with phosphate buffered saline (PBS) and treated by ultrahigh pressure of 980 MPa for 10 min using a CIP apparatus (Dr. Chef, Kobe Steel Ltd, Kobe, Japan) for cell demolition (Fig. 1). The range of temperature in the process is about 5 to 30°C. They were then rinsed by PBS for 2 weeks under gentle stirring at 4°C for removal of the residues of the broken cells. They were subjected to the histological observation by the light and electron microscopy, DNA and phospholipids assay, detection of porcine endogeneous retrovirus (PERV) by the PCR, and biomechanical study by the tensile strength measurement.

Fig. 1 Packed porcine heart valves for CIP treatment.

The acellualr tissues were transplanted orthotopically into nine allogeneic miniature pigs. The pulmonary valves were transplanted at right ventricular outflow tract through a median sternotomy with extracorporeal circulation without blood oxygenation [5]. The postoperative anticoagulation or anti-platelet therapy was not instigated. They were explanted 4, 12, and 24 weeks (n=3) after the transplantation and examined histologically and immunohistologically. All animals were carefully reared in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH publication No.85-23, revised in 1985).

3. Results and discussion

The tissues were completely cell free when they were treated by the CIP for 10 min followed by washing for 2 weeks from the H-E staining (Fig. 2). The amount of DNA and phospholipids were lower than 1 μg/ml and 0.5 mg/wet g, respectively and those were less than 10% in the native tissue (Fig. 3).

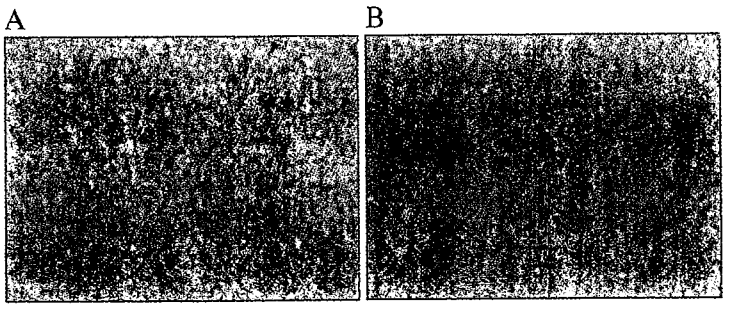

Fig. 2 Cross sections of (A) native and (B) treated tissues (H-E staining).

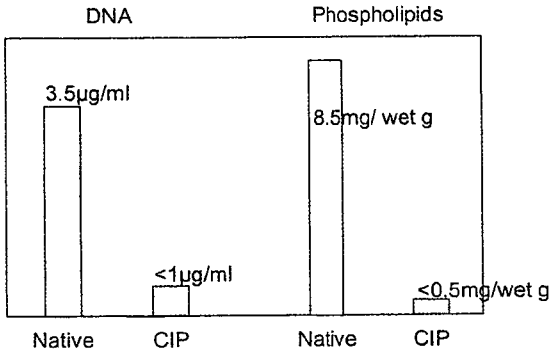

Fig. 3 Residual amounts of DNA and phospholipids in native and treated tissues.

The collagen and elastin fibers were well maintained in the acellular tissue and there were no significant changes in biomechanical properties of the breaking strength and elastic modulus. We have already found that this process could be successfully applied to cartilage tissues for decellularization (not shown). More effectively, it has been reported that the most of viruses including HIV are inactivated by the CIP only of more than 600 MPa without washing [6]. This means the treatment is able to sterilize the tissue in addition to the decellularization. The Clawn miniature pig was chosen as a donor animal since its size adapts human tissues well and its genome has been well studied in order to develop a human gene induced transgenic animal for the organ transplantation. There was no PERV detected in PCR assay from the tissue treated (Fig. 4).

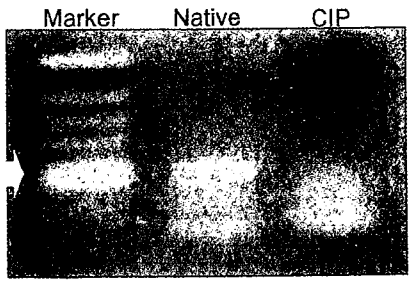

Fig. 4 PCR products of PERV (arrow) in native and treated tissues.

The animals survived after the transplantation in the all cases. The explanted grafts showed no macroscopical abnormality and no dilatation and aneurysmal changes including their anastomosis. The inner surface was completely covered with endothelial cells and the inside was infiltrated by cells from both sides of endothelium and outer tissue after 12 weeks. It was dominant in the latter. Almost of the tissue including cusps were filled by the cells at 24 weeks, mainly by smooth muscle cells (Fig. 5). There was no inflammation and calcification observed in the tissue.

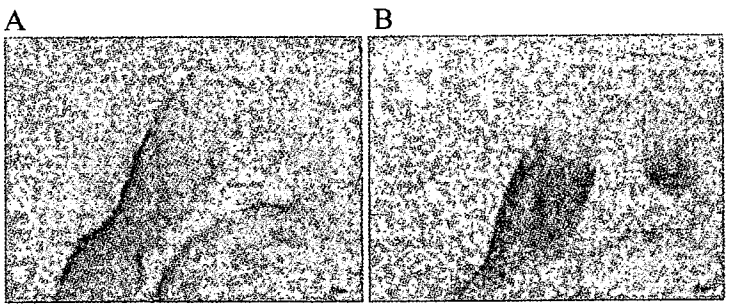

Fig. 5 Cross sections of (A) anti-vWF (endothelial cells) and (B) anti-αSMA (smooth muscle cells) immunostained treated tissues 24 weeks after the transplantation.

Recently, some groups have reported excellent clinical results of acellular pulmpnary heart valve transplantation [7-9]. We are planning a clinical application of the acellular grafts made by this process in the near future.

4. Conclusion

Porcine cells and PERV were removed completely by the CIP treatment without using any detergents. The acellular grafts showed remarkable ability in repopulation after the transplantation. This CIP treatment may have more secure acellular graft for the regenerative tissue transplantation.

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