Cumulative Episodes of Rejection Altered Myocardial Sarcoplasmic Reticulum Ca²⁺-ATPase and Ryanodine Receptor-2 mRNA Expression in Heart Transplant Recipients

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SUMMARY

The aim of the present study was to investigate the relationship between rejections and gene expression of Ca²⁺-handling proteins in heart transplant recipients. Thirty-seven heart transplant recipients underwent routine endomyocardial biopsy. Levels of sarcoplasmic reticulum Ca²⁺-ATPase (SERCA2) and ryanodine receptor-2 mRNAs in endomyocardial tissue were quantified by a real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) method. Rejections were diagnosed according to the conventional International Society for Heart and Lung Transplantation criteria. Patients were classified as follows; group AR⁺ (*n* = 9) with rejection grade of 2 or higher versus group AR⁻ (*n* = 28) with rejection grade of 0, 1a or 1b at the time of biopsy, and group Rec-AR⁺ (*n* = 6) with a history of more than 4 episodes of treatment required rejection versus group Rec-AR⁻ (*n* = 31) without history of recurrent rejection. The mRNA levels of the SERCA2/GAPDH ratio and ryanodine receptor-2/GAPDH ratio were not different between group AR⁺ and group AR⁻; however, they were reduced in group Rec-AR⁺ more than in group Rec-AR⁻ (0.83 ± 0.07 versus 0.90 ± 0.07, *P* = 0.034, 0.74 ± 0.06 versus 0.84 ± 0.10, *P* = 0.027, respectively). A single episode of on-going rejection would not affect myocardial Ca²⁺-handling proteins; however, cumulative rejection episodes might alter the gene expression of myocardial Ca²⁺-handling proteins in heart transplant recipients. (Int Heart J 2010; 51: 259-263)

Key words: Transplantation, Gene expression, Biopsy, Sarcoplasmic reticulum

Acute rejection of heart transplants is characterized histologically by infiltration of inflammatory cells and interstitial edema, which ultimately result in increases in myocardial stiffness and abnormal relaxation.^{1,2)} Several studies demonstrated the associations between rejected hearts and abnormal diastolic function in heart transplant recipients.¹⁻⁴⁾

The alterations in gene expression of Ca²⁺-handling proteins have been demonstrated in failing human myocardium.^{5,6)} however, the gene expression of Ca²⁺-handling proteins in rejected heart remains to be clarified.

In the present study, we investigated the relationship between rejection profile and the mRNA levels of sarcoplasmic reticulum Ca²⁺-ATPase (SERCA2) and ryanodine receptor-2 in endomyocardial tissue obtained from transplant recipients.

METHODS

Study design: Thirty-seven consecutive adult heart transplant recipients (26 men, 11 women, transplanted at 36.5 ± 13.2 years old, transplanted between July 1993 and December

2006) who underwent routine endomyocardial biopsy between October 2006 and September 2007 were enrolled in this study. The patients underwent scheduled cardiac catheterization and endomyocardial biopsy at 1 week, 2 weeks, 7 weeks, 3 months, 4.5 months, 6 months, 9 months, 1 year, 1.5 years, and then every 6 months or 1 year after transplantation surgery, or when rejection was suspected on the basis of clinical symptoms. For patients who underwent multiple catheterizations during the study period, the results of first catheterization and the endomyocardial tissue were used for the analysis.

The diagnosis of cellular acute rejection was based on the conventional International Society for Heart and Lung Transplantation (ISHLT) criteria.⁷⁾ Given that none of the study subjects showed antibody-mediated rejection alone (without associated cellular rejection), only the grade of cellular rejection was used for classification of acute rejection in this study. The biopsy specimens showing an ISHLT grade of 0, 1a or 1b were classified as the on-going acute rejection-negative group (group AR⁻), and those of grade 2 or higher were classified as the on-going rejection positive group (group AR⁺). The biopsy specimens obtained from patients who had a history of less than 4 episodes of treatment required rejection were classified as the

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recurrent-rejection negative group (group Rec-AR⁻), and those who had a history of more than or equal to 4 episodes of rejection were classified as the recurrent-rejection positive group (group Rec-AR⁺).

Endomyocardial biopsy and hemodynamic testing are accepted and financially covered by the National Health Insurance System of Japan for the diagnosis of rejection and for obtaining information on the clinical condition of heart transplant recipients. The present study, including the biopsy procedure, was approved by the Institutional Review Board and Institutional Ethical Committee for Human Research of the National Cardiovascular Center, and was executed in accordance with the Declaration of Helsinki. All study subjects provided written informed consent with regard to the study procedures.

Cardiac catheterization: All patients underwent right heart catheterization for hemodynamic evaluation and endomyocardial biopsy. Several (≥ 5) biopsy specimens were obtained from the right side of the interventricular septum at each examination, some of which were used for mRNA analysis. Transplant vasculopathy was evaluated by coronary angiography with intracoronary ultrasound.⁸⁾

Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis: Quantitative PT-PCR analysis of the amounts of the mRNAs for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), SERCA2, and ryanodine receptor-2 was

performed with a Prism 7700 Sequence Detector (Perkin-Elmer, Foster City, CA) as previously described.⁹⁾ Variability in the efficiency of cDNA synthesis was corrected by dividing the levels of SERCA2 and ryanodine receptor-2 mRNAs by that of GAPDH mRNA.

Echocardiography: Conventional echocardiography accompanied with tissue Doppler imaging was performed within 12 hours after right heart catheterization. Echocardiographic images were obtained in the parasternal long- and short-axis views and apical two- and four-chamber views with standard transducer positions and with the use of a Vivid Seven digital ultrasound system (GE VingMed Ultrasound, Horten, Norway). The LV ejection fraction was calculated by the Teichholz method. Continuous-wave Doppler echocardiography was performed, and peak early (*E*) and late (*A*) transmitral filling velocities and their ratio (*E/A*), deceleration time of *E*, and isovolumic relaxation time were measured from mitral inflow velocities. On completion of the standard echocardiographic measurements, tissue Doppler imaging was performed as described previously.¹⁰⁾ Early diastolic mitral annular velocity (*E_m*) was obtained by placing a tissue Doppler sample volume at the septal mitral annulus in the apical four-chamber view, and the *E/E_m* ratio was calculated.

Statistical analysis: Data are presented as the mean $\pm$ SD. Normality was evaluated for each variable on the basis of normal

Table I. Clinical Characteristics

Parameter	Group AR ⁺ (n = 9)	Group AR ⁻ (n = 28)	P [*]	Group Rec-AR ⁺ (n = 6)	Group Rec-AR ⁻ (n = 31)	P [†]
Age at transplant (years)	35.6 $\pm$ 12.7	36.7 $\pm$ 13.6	0.82	34.5 $\pm$ 13.9	36.9 $\pm$ 13.2	0.68
Age at examination (years)	42.9 $\pm$ 10.4	41.2 $\pm$ 12.9	0.70	43.9 $\pm$ 10.6	41.2 $\pm$ 12.6	0.62
Posttransplant duration (years)	7.3 $\pm$ 4.5	4.3 $\pm$ 3.0	0.29	9.4 $\pm$ 4.1	4.3 $\pm$ 2.8	0.0006
Male (%)	100%	60.1%	0.067	100%	64.5%	0.21
Immunosuppression (%)						
CSA-based regimen	55.6%	64.2%	0.81	50.0%	61.2%	0.46
Tac-based regimen	44.4%	35.8%	0.81	50.0%	38.8%	0.46
MMF administration	100%	100%	-	100%	100%	-
Steroid withdrawal	33.3%	35.7%	0.61	50.0%	38.7%	0.46

CSA indicates cyclosporine A; Tac, tacrolimus; MMF, mycophenolate mofetil; *, P values obtained from the comparison between group AR⁺ and group AR⁻; and †, P values obtained from the comparison between group Rec-AR⁺ and group Rec-AR⁻.

Table II. Echocardiographic Parameters

Parameter	Group AR ⁺ (n = 9)	Group AR ⁻ (n = 28)	P [*]	Group Rec-AR ⁺ (n = 6)	Group Rec-AR ⁻ (n = 31)	P [†]
LVDd (mm)	41.3 $\pm$ 7.0	41.0 $\pm$ 4.1	0.88	43.7 $\pm$ 7.1	40.6 $\pm$ 4.3	0.16
LVDs (mm)	26.1 $\pm$ 4.5	25.7 $\pm$ 4.2	0.81	25.3 $\pm$ 3.8	25.9 $\pm$ 4.3	0.77
IVST (mm)	9.9 $\pm$ 1.1	9.0 $\pm$ 1.4	0.74	9.3 $\pm$ 0.8	9.2 $\pm$ 1.4	0.78
PWT (mm)	9.7 $\pm$ 1.4	8.9 $\pm$ 1.1	0.10	8.8 $\pm$ 0.8	9.2 $\pm$ 1.3	0.55
LVEF (%)	58.9 $\pm$ 15.0	61.7 $\pm$ 9.0	0.50	66.0 $\pm$ 11.3	60.1 $\pm$ 10.4	0.21
TMF <i>E</i> (cm/s)	77.0 $\pm$ 16.3	79.5 $\pm$ 19.5	0.72	72.8 $\pm$ 17.3	80.1 $\pm$ 18.9	0.39
TMF <i>E/A</i>	2.3 $\pm$ 0.9	2.4 $\pm$ 0.9	0.89	2.3 $\pm$ 1.0	2.4 $\pm$ 0.9	0.93
TMF DcT (ms)	169.7 $\pm$ 30.2	162.2 $\pm$ 26.2	0.50	165.3 $\pm$ 33.9	164.1 $\pm$ 26.1	0.92
IRT (ms)	67.4 $\pm$ 16.0	62.3 $\pm$ 17.3	0.43	66.1 $\pm$ 13.4	63.3 $\pm$ 17.0	0.71
<i>E_m</i> (cm/s)	9.2 $\pm$ 1.9	9.3 $\pm$ 2.8	0.96	9.0 $\pm$ 2.5	9.3 $\pm$ 2.7	0.82
<i>E/E_m</i>	8.4 $\pm$ 1.5	9.3 $\pm$ 3.7	0.53	8.0 $\pm$ 1.3	9.2 $\pm$ 3.6	0.46

LVDd indicates LV end-diastolic internal dimension; LVDs, LV end-systolic internal dimension; IVST, thickness of the interventricular septum; PWT, thickness of the LV posterior wall; LVEF, LV ejection fraction; FS, fraction shortening; TMF, transmitral flow; DcT, deceleration time of *E*; IRT, isovolumic relaxation time; *, P values obtained from the comparison between group AR⁺ and group AR⁻; and †, P values obtained from the comparison between group Rec-AR⁺ and group Rec-AR⁻.

Table III. Hemodynamic Parameters and Transplant Vasculopathy

Parameter	Group AR ⁺ (n = 9)	Group AR ⁻ (n = 28)	P*	Group Rec-AR ⁺ (n = 6)	Group Rec-AR ⁻ (n = 31)	P†
CI (L min ⁻¹ m ⁻²)	3.7 ± 0.2	3.8 ± 0.1	0.67	3.7 ± 0.7	3.8 ± 0.8	0.56
PAWP (mmHg)	9.4 ± 3.9	6.4 ± 0.5	0.076	10.3 ± 4.7	6.5 ± 2.3	0.0039
Mean PA (mmHg)	14.2 ± 3.0	13.6 ± 3.0	0.60	14.5 ± 3.5	13.6 ± 2.8	0.50
RA (mmHg)	3.8 ± 3.3	3.0 ± 1.8	0.39	4.5 ± 3.9	3.0 ± 1.8	0.13
Transplant vasculopathy Stanford class-IV IMT	44.4%	50%	0.77	50%	48.4%	0.94

CI indicates cardiac index; PAWP, pulmonary arterial wedge pressure; PA, pulmonary artery pressure; RA, right atrial pressure; IMT, intimal thickness detected by intracoronary ultrasound; *, P values obtained from the comparison between group AR⁺ and group AR⁻; and †, P values obtained from the comparison between group Rec-AR⁺ and group Rec-AR⁻.

Table IV. Relative Expression Levels of Ca^{2+} -Handling Protein mRNAs in Ventricular Tissue

Parameter	Group AR ⁺ (n = 9)	Group AR ⁻ (n = 28)	P*	Group Rec-AR ⁺ (n = 6)	Group Rec-AR ⁻ (n = 31)	P†
SERCA2/GAPDH ratio	0.86 ± 0.10	0.90 ± 0.07	0.17	0.83 ± 0.07	0.90 ± 0.07	0.034
Ryanodine receptor-2 /GAPDH ratio	0.76 ± 0.08	0.82 ± 0.10	0.06	0.74 ± 0.06	0.84 ± 0.10	0.027

*indicates P values obtained from the comparison between group AR⁺ and group AR⁻ and †, P values obtained from the comparison between group Rec-AR⁺ and group Rec-AR⁻.

distribution plots and histograms and by the Kolmogorov-Smirnov test. Clinical characteristics, echocardiographic indexes, hemodynamic variables, and mRNA levels were compared between groups by Student's unpaired two-tailed *t* test or chi-square analysis. A *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed with JMP7.0 software (SAS Institute, Cary, NC, USA).

RESULTS

The demographic and clinical data of the patients are summarized in Table I. The reason for heart transplantation was nonischemic cardiomyopathy for all patients. All patients were in NYHA class I at the time of examination. The ages at the time of transplantation and examination were not significantly different between group AR⁻ and group AR⁺, or between group Rec-AR⁻ and group Rec-AR⁺. Posttransplant duration, defined as years between the date of transplant surgery and the date of biopsy, were longer in group Rec-AR⁺ than that in group Rec-AR⁻. Gender distribution and distribution of immunosuppressive regimen were not significantly different between group AR⁺ and group AR⁻, or between group Rec-AR⁺ and group Rec-AR⁻. Mycophenolate mofetil was administered to all patients as an inhibitor of nucleic acid synthesis. Neither azathioprine nor mammalian targets of rapamycin inhibitors were prescribed to any patients at our institution during the study period.

Neither conventional echocardiographic indexes nor E_m or the E/E_m ratio differed significantly between group AR⁻ and group AR⁺, or between group Rec-AR⁻ and group Rec-AR⁺ (Table II).

Hemodynamic variables obtained by right heart catheterization were not significantly different between the groups, except for pulmonary arterial wedge pressure (PAWP). The PAWP was significantly higher in group AR⁺ than in group AR⁻, and in group Rec-AR⁺ than in Rec-AR⁻, albeit within the

Figure. Comparison of the results of RT-PCR analysis of SERCA2 (a, c) and ryanodine receptor-2 (b, d) mRNA levels in endomyocardial biopsy samples between group AR⁺ and group AR⁻ (a, b) and between group Rec-AR⁺ and group Rec-AR⁻ (c, d). The open circles represent group AR⁻ and group Rec-AR⁻, the gray closed circles represent group AR⁺, and the black closed circles represent group Rec-AR⁺.

normal limit (Table III). None of the patients showed angiographically apparent coronary stenosis. The proportion of those showing an intimal thickness of Stanford Class IV on intracoronary ultrasound⁸⁾ did not differ significantly between the groups (Table III).

The quantitative data for each target Ca^{2+} -handling protein, normalized to the GAPDH transcript, are shown in Table IV and the Figure. No significant difference in the mRNA expression of either SERCA2 or ryanodine receptor-2 mRNA was found between group AR⁺ and group AR⁻; however, there was a significant decrease in the level of SERCA2 mRNA expression and ryanodine receptor-2 mRNA in the ventricular myocardium obtained from group Rec-AR⁺ compared with group Rec-AR⁻.

DISCUSSION

The present study has demonstrated for the first time that (i) mRNA levels of SERCA2 and the ryanodine receptor-2 in the ventricular myocardium of transplanted hearts were not significantly different between those with on-going rejection and those free from rejection, and (ii) in contrast, mRNA levels of both SERCA2 and the ryanodine receptor-2 in the ventricular myocardium of transplanted hearts were significantly reduced in those with recurrent episodes of the rejection compared to those without a history of rejection, regardless of the hemodynamic changes.

It has been reported that transient diastolic dysfunction may be associated with rejection in heart transplant recipients.¹⁻⁴⁾ The Ca^{2+} -handling proteins were recognized as a major determinant of myocardial contractile and lucitrophic property.^{5,11)} Several studies of myocardial samples from patients with hypertrophic cardiomyopathy after myectomy have suggested that functional abnormalities in Ca^{2+} -handling proteins, including SERCA2, might be involved in abnormal intracellular Ca^{2+} handling and thus, in impaired myocardial performance in HCM patients.^{12,13)} Alteration in gene expression of Ca^{2+} -handling proteins has been demonstrated in failing human myocardium;^{5,6)} however, the gene expressions of Ca^{2+} -handling proteins in transplanted heart and their associations with rejection remain to be clarified.

In the present study, none of the studied patients demonstrated hemodynamic abnormalities, although pulmonary artery wedge pressure was higher within normal limits in patients with on-going rejection (group AR⁺) compared with that in patients without rejection (group AR⁻), as well as in patients with a history of recurrent rejections (group Rec-AR⁺) compared with that in patients without such a history (Rec-AR⁻).

Stüdeli, *et al* demonstrated that prolongation of isovolumic relaxation time determined by echocardiography was related with reduced SERCA2 gene expression.¹⁴⁾ In contrast, we did not observe any relationships between mRNA expression of Ca^{2+} -handling proteins and hemodynamic or/and echocardiographic parameters in the present study. However, regardless of hemodynamic or echocardiographic abnormalities, the mRNA levels of SERCA2 and ryanodine receptor-2 were reduced in transplanted hearts with recurrent episodes of rejection compared with hearts without a history of rejection.

Acute rejection of heart transplants is characterized histologically by infiltration of inflammatory cells and interstitial edema, which ultimately result in structural and functional abnormalities of the allograft.¹⁾ In addition to such interstitial alteration during acute rejection, we speculate that the interstitial microenvironment would have an influence on cardiac sarcoplasmic reticulum. Cumulative episodes of these changes during acute rejection affect both myocardial contractile function and left ventricular filling due to an increase in myocardial stiffness and abnormal relaxation, which would cause alteration of mRNA levels of Ca^{2+} -handling proteins in transplanted hearts with recurrent rejections.

The major limitation of this study is the small number of samples studied. In the present study, we did not experience severe rejection with hemodynamic compromise, accompanied by greater than conventional ISHLT grade 3a or antibody mediated rejection. It is conceivable that even a single episode of such a grade of severe rejection would alter myocardial Ca^{2+} -

handling proteins. Another limitation is the possibility of coexistence of transplant coronary artery disease, which was not taken into consideration. Myocardial ischemia can also result in the alteration of myocardial Ca^{2+} -handling proteins. In addition, long-term exposure to cyclosporine A may cause the downregulation of mRNA expression of SERCA2 as a result of an interaction between P-glycoprotein and calcineurin inhibitors.^{15,16)} In the present study, the distribution of the immunosuppressive regimens was not significantly different between the groups; therefore, we believe that the immunosuppressants did not influence the results. Further studies will be necessary to clarify the precise mechanism involved in this issue.

In conclusion, we found that a single episode of rejection did not have an effect on Ca^{2+} -handling proteins in transplanted heart. However, cumulative rejection episodes alter myocardial Ca^{2+} -handling, regardless of the existence of diastolic dysfunction.

REFERENCES

1. Puleo JA, Aranda JM, Weston MW, *et al*. Noninvasive detection of allograft rejection in heart transplant recipients by use of tissue Doppler tissue imaging. *J Heart Lung Transplant* 1998; 17: 176-84.
2. Nagueh SF, Kopelen HA, Lim DS, *et al*. Tissue Doppler imaging consistently detects myocardial contraction and relaxation abnormalities, irrespective of cardiac hypertrophy, in a transgenic rabbit model of human hypertrophic cardiomyopathy. *Circulation* 2000; 102: 1346-50.
3. Mouly-Bandini A, Vion-Dury J, Viout P, Mesana T, Cozzone PJ, Montiès JR. Value of Doppler echocardiography in the detection of low-grade rejections after cardiac transplantation. *Transpl Int* 1996; 9: 131-6.
4. Valantine HA, Yeoh TK, Gibbons R, *et al*. Sensitivity and specificity of diastolic indexes for rejection surveillance: temporal correlation with endomyocardial biopsy. *J Heart Lung Transplant* 1991; 10: 757-65.
5. Pieske B, Maier LS, Bers DM, Hasenfuss G. Ca^{2+} handling and sarcoplasmic reticulum Ca^{2+} content in isolated failing and non-failing human myocardium. *Circ Res* 1999; 85: 38-46.
6. Vanderheyden M, Mullens W, Delrue L, *et al*. Myocardial gene expression in heart failure patients treated with cardiac resynchronization therapy responders versus nonresponders. *J Am Coll Cardiol* 2008; 51: 129-36.
7. Billingham ME, Cary NR, Hammond ME, *et al*. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. The International Society for Heart Transplantation. *J Heart Transplant* 1990; 9: 587-93.
8. St Goar FG, Pinto FJ, Alderman EL, *et al*. Intracoronary ultrasound in cardiac transplant recipients. In vivo evidence of "angiographically silent" intimal thickening. *Circulation* 1992; 85: 979-87.
9. Somura F, Izawa H, Iwase M, *et al*. Reduced myocardial sarcoplasmic reticulum Ca^{2+} -ATPase mRNA expression and biphasic force-frequency relations in patients with hypertrophic cardiomyopathy. *Circulation* 2001; 104: 658-63.
10. Kato TS, Noda A, Izawa H, *et al*. Discrimination of nonobstructive hypertrophic cardiomyopathy from hypertensive left ventricular hypertrophy on the basis of strain rate imaging by tissue Doppler ultrasonography. *Circulation* 2004; 110: 3808-14.
11. Lompré AM, Anger M, Levitsky D. Sarcoplasmic reticulum calcium pumps in the cardiovascular system: function and gene expression. *J Mol Cell Cardiol* 1994; 26: 1109-21. (Review)
12. Gwathmey JK, Warren SE, Briggs GM, *et al*. Diastolic dysfunction in hypertrophic cardiomyopathy. Effect on active force gener-

- ation during systole. *J Clin Invest* 1991; 87: 1023-31.
13. Schotten U, Koenigs B, Rueppel M, *et al.* Reduced myocardial sarcoplasmic reticulum $Ca(2+)$ -ATPase protein expression in compensated primary and secondary human cardiac hypertrophy. *J Mol Cell Cardiol* 1999; 31: 1483-94.
 14. Stüdeli R, Jung S, Mohacsi P, *et al.* Diastolic dysfunction in human cardiac allografts is related with reduced SERCA2a gene expression. *Am J Transplant* 2006; 6: 775-82.
 15. Mistry P, Stewart AJ, Dangerfield W, *et al.* In vitro and in vivo reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res* 2001; 61: 749-58.
 16. Seres M, Poláková E, Krizanová O, *et al.* Overexpression of P-glycoprotein in L1210/VCR cells is associated with changes in several endoplasmic reticulum proteins that may be partially responsible for the lack of thapsigargin sensitivity. *Gen Physiol Biophys* 2008; 27: 211-21.