Sirolimus affects cardiomyocytes to reduce left ventricular mass in heart transplant recipients

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Aims
The cellular mechanisms underlying cardiac hypertrophy may result from changes in cardiac myocyte growth and differentiation. We tested whether sirolimus, an immunosuppressive agent that inhibits mTOR, a protein that regulates cell division and differentiation, might modify cardiac hypertrophy after cardiac transplantation.

Methods and results
Fifty-eight cardiac transplant recipients were withdrawn from treatment with calcineurin inhibitors (CNIs) and treated with sirolimus. Eighty-three control subjects were maintained on CNIs. After 12 months, left ventricular (LV) mass decreased from 196.15 ± 48.28 to 182.21 ± 43.56 g (P = 0.05) and LV mass index from 99.25 ± 20.08 to 93.82 ± 20.22 g/m² (P = 0.031) in sirolimus-treated subjects but did not change in controls. The left atrial volume index of sirolimus-treated subjects decreased from 52.44 ± 17.22 to 48.40 ± 15.14 cm³/m² (P = 0.008) and increased from 52.07 ± 19.45 to 57.03 ± 19.93 cm³/m² (P = 0.0012) in controls. The difference between the groups was independent of blood pressure. The number of cells in myocardial biopsies positive for p27Kip1, a protein induced by mTOR inhibition, increased in sirolimus-treated subjects (P = 0.0005) and did not change in controls (P = 0.54) suggesting sirolimus acted directly on myocardium.

Conclusion
Sirolimus may inhibit adverse ventricular remodelling resulting in cardiac hypertrophy and have potential in the treatment of conditions in which severe hypertrophy compromises cardiac function.

Keywords
Cardiac transplantation • Remodeling • Sirolimus • Left ventricular hypertrophy

Introduction
Left ventricular hypertrophy (LVH) and increased left ventricular (LV) mass occur frequently in those with hypertension, coronary artery disease, and other conditions.1,2 While these changes ascribed to ventricular remodelling might initially enable the heart to adapt to haemodynamic challenges, they ultimately compromise LV structure and diastolic function and adversely affect survival.3,4 Therefore, hypertrophy in response to pathological stress is not truly adaptive.5,6

LVH occurs frequently in cardiac transplants and adversely affects survival.7 In this setting cardiac hypertrophy contributes to diastolic abnormalities, elevation of filling pressures, and atrial remodelling.8 LVH may be stimulated by increased afterload due to hypertension, increased wall stress secondary to high intra-ventricular pressure or volume, and compensatory cellular responses to myocyte injury such as ischaemia, organ preservation injury, and allograft rejection.9,10 Excessive neurohormone production and hypertension caused by immunosuppressive drugs may also contribute.9,11

Cardiac hypertrophy generally involves an increase in cardiac mass secondary to growth of cardiomyocytes, which increase in size.12 Increased protein synthesis is the main feature of cardiac hypertrophy and most likely underlies the increased cell and organ size observed under this condition.12 Several cellular signaling pathways regulate the rate of protein synthesis and are thus implicated in the cardiac hypertrophic response.13,14 Among these pathways, the mTOR pathway plays a central role in regulating mRNA translation and cell growth.15

We explored the involvement of the mTOR in the cardiac hypertrophic process by studying whether sirolimus, a direct inhibitor of mTOR, modifies ventricular hypertrophy in cardiac transplant recipients. Sirolimus (Rapamune, rapamycin) is a...
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macrolide antibiotic with potent immunosuppressive, antiproliferative, and anti-migratory properties. The immunosuppressive properties of sirolimus are owed to stabilization of p27Kip1, a cell-cycle inhibitor protein. Since the protein targeted by sirolimus (target of rapamycin, mTOR) is expressed in myocardium, treatment with sirolimus might directly modify myocardial remodelling. Consistent with this possibility, sirolimus reverses pressure overload-induced increase in heart weight without deleterious effects on cardiac function or mortality in mice.

The aim of this study was to examine if treatment with sirolimus reverses LVH and improves diastolic function of the transplanted heart through a direct action on the myocardium.

Methods

This study was a retrospective, non-randomized, single-centre study, approved by the Mayo Clinic institutional review board.

Subject characteristics

We identified cardiac transplant recipients who were maintained on standard immunosuppression with calcineurin inhibitors (CNIs) (cyclosporine A or tacrolimus) and subjects in whom CNIs were withdrawn and replaced with sirolimus. All surviving subjects who underwent at least two echocardiograms 1 year apart over a 3 year period (between the beginning of 2004 and the end of 2006) were included in the study and comprise 141 cardiac transplant recipients. 6.11 ± 4.56 years after transplantation. This number represents 81% of the total number of living cardiac transplant recipients, followed-up over that 3 year period at Mayo Clinic. Subjects with severe mitral regurgitation, severe tricuspid regurgitation, and less than 6 months after transplantation were excluded. At the time of assessment all subjects were in sinus rhythm without cardiac pacing. All included subjects survived for the duration of the study period and there were no subjects who refused analysis of their echocardiographic data.

In 58 subjects with impaired renal function secondary to CNI or/and allograft vasculopathy, detected on annual coronary angiography, immunosuppression was changed from CNI to sirolimus as previously described. 4.96 ± 3.89 (mean ± standard deviation) years following cardiac transplantation. Echocardiographic measurements were performed at baseline prior to conversion and after 12 months of sirolimus therapy as well as when these patients were on ciclosporine therapy 12 month before conversion to sirolimus.

The control group comprised 83 subjects 6.89 ± 4.83 years from transplant maintained on standard primary immunosuppression with ciclosporine A (n = 69) or tacrolimus (n = 14). Two echocardiographic studies, 1 year apart were analysed in these patients. Secondary immunosuppressant (mycophenolate mofetil or azathioprine) and the existing dose of prednisone were not changed (see Table 1).

At these time points, subjects underwent the standard post-transplant follow-up, including endomyocardial biopsy, to determine rejection status and angiographic evaluation. All cardiac transplant recipients undergo routine echocardiography during scheduled follow-up. The echocardiographic parameters obtained allow us to assess changes in LVH and mass.

Echocardiographic measurements

Clinical echocardiographic interpretations were used for this study. All echocardiograms were performed at Mayo Clinic using a standardized protocol by experienced physicians, who were unaware of treatment assignment. Left ventricular end diastole (LVED); left ventricular end systole (LVES); left ventricular posterior wall thickness (EDPWT); interventricular septum thickness (EDST) were measured by two-dimensional echocardiography using American Society of Echocardiography recommendations. Left ventricular mass (LVM) was calculated using the formula 0.8 × (LVED+LVPW+IVS)² − (LVED)² and was adjusted for differences in body size (left ventricular mass index, LVMi) by use of body surface area (BSA). A cut-off of 116 g/m² for men and 96 g/m² for women was used for the upper limit of normal for LVMi based on evaluations performed in a normal population and was defined as LVH.

Stroke volume was determined by an invasively validated Doppler method. The ejection fraction was calculated using the formula: (LV diastolic diameter − LV systolic diameter)/LV diastolic diameter. The left atrial (LA) dimension was measured by the area-length method and calculated as (0.85 × A1 × A2)/L. (A1=LA area, 4-chamber view, A2=LA area, 2-chamber view, L=LA length). BSA (m²) was used for body size indexing.

Paraffin-embedded heart biopsy specimens were stained using pre-diluted polyclonal rabbit anti-human p27Kip1 antibodies (Novus Biologicals) as directed by the manufacturer. Antibody binding to p27Kip1 was revealed using EnVision + System-HRP polymer (Dako-Cytomation Inc.). A pathologist (Y.S.), who was blinded to treatment group, estimated the level of p27Kip1 nuclear expression by counting the number of p27Kip1-positive cardiomyocyte nuclei in each of five representative high-powered fields. Expression of p27Kip1 was summarized as the number of p27-positive cardiomyocytes per mm².

Statistical analysis

Data are displayed as mean ± standard deviation or count and percent, where appropriate. Variables with heavily skewed distribution (time post transplantation) are reported as medians with first and third quartiles in parenthesis. Univariable analysis was performed using the two-tailed t-test for numerical data and the χ² test for categorical data. Subsequently, the ANCOVA accounting for baseline measurement was used to test for differences between the treatment groups in echocardiographic and haemodynamic characteristics. Multivariable linear regression analysis, included variables with significance level P ≤ 0.1 in univariable analysis. In addition time after transplantation (to account for the differences between the groups), SBP and DBP were included in the multivariable model to assess independent predictors of changes in left ventricle mass index and left atrial volume index (LAVI). A P-value < 0.05 was considered to be statistically significant.

Results

Baseline characteristics of study subjects

The potential impact of sirolimus on ventricular hypertrophy was investigated in 58 cardiac transplant recipients treated with...
sirolimus in lieu of a CNI and 83 cardiac transplant recipients treated with a CNI. Pertinent characteristics of the subjects are presented in Table 1. The groups did not differ significantly in number of episodes of rejection, degree of immunosuppression, medical therapy, donor characteristics, and prevalence of hypertension or diabetes at the initiation of the study. The incidence of angiographically assessed coronary allograft vasculopathy was higher in the subjects treated with sirolimus.

**Degree of immunosuppression and incidence of rejection**

No subject had clinical or histological evidence of acute rejection during the study period. In subjects treated with sirolimus, the sirolimus level remained stable (11.57 ± 2.59 at entry, 11.94 ± 3.25 ng/mL at follow-up, \( P = 0.19 \)). In subjects treated with CNIs, the cyclosporine level decreased from 146.24 ± 75.73 to 129.96 ± 45.45 ng/mL (\( P = 0.003 \)) during the study period. The steroid dose (\( P = 0.9 \)) and use of secondary immunosuppressive agents did not differ between the two groups over the course of the study (Table 1). Azathioprine (\( P = 0.16 \)) or mycophenolate mofetil (\( P = 0.66 \)) dose did not differ between the two groups. Similarity in use of immunosuppression and number of rejection episodes suggest that both groups had similar levels of alloimmunity.

**Changes in blood pressure**

There was no significant difference between the two groups in antihypertensive treatment (Table 1). The groups did not differ in initial blood pressures (\( P = 0.05 \) for systolic and \( P = 0.1 \) for diastolic) (Table 2). The systolic blood pressure (\( P = 0.05 \)) and diastolic blood pressure (\( P = 0.0004 \)) decreased in those treated with sirolimus, and antihypertensive medications were discontinued in eight patients (clonidine in three patients and calcium-channel antagonists in five patients 3.2 ± 1.5 months after initiation of sirolimus). Subsequently the blood pressure did not significantly change in these patients. The blood pressure did not change in those treated with CNIs (Table 2). Despite the decrease in

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**Table 1** Demographic and clinical characteristics of the patients prior to study

<table>
<thead>
<tr>
<th></th>
<th>Sirolimus group, ( n = 58 )</th>
<th>Calcineurin inhibitor group, ( n = 83 )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at study entrance (years)</td>
<td>50.21 ± 14.35</td>
<td>56.38 ± 13.44</td>
<td>0.013</td>
</tr>
<tr>
<td>Male gender, ( n ) (%)</td>
<td>47 (81)</td>
<td>64 (77)</td>
<td>0.50</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>32.67 ± 13.43</td>
<td>31.77 ± 13.10</td>
<td>0.73</td>
</tr>
<tr>
<td>Time post HTx (years)</td>
<td>median 4.03, IQR 1.23–7.91</td>
<td>median 6.98, IQR 2.90–11.10</td>
<td>0.011</td>
</tr>
</tbody>
</table>

**Reason for HTx, \( n \) (%)**

- ICMP: 17 (29) vs. 27 (33)
- DCMP: 25 (43) vs. 33 (40)
- Other: 16 (28) vs. 22 (27)

**Angiographic CAV present, \( n \) (%)**

- Sirolimus: 34 (60) vs. 32 (41)
- Calcineurin: 15 (26) vs. 15 (18)

**GFR**

- Sirolimus: 50.86 ± 18.95 vs. 57.44 ± 20.39
- Calcineurin: 50.86 ± 18.95 vs. 57.44 ± 20.39

**Patients with rejection before study entry**, \( n \) (%)

- Sirolimus: 15 (26) vs. 15 (18)
- Calcineurin: 26 (46) vs. 27 (33)

**ACE-inhibitor, \( n \) (%)**

- Sirolimus: 26 (46) vs. 27 (33)
- Calcineurin: 26 (46) vs. 27 (33)

**Beta blocker, \( n \) (%)**

- Sirolimus: 4 (7) vs. 11 (13)
- Calcineurin: 4 (7) vs. 11 (13)

**Calcium-channel blocker, \( n \) (%)**

- Sirolimus: 18 (31) vs. 30 (36)
- Calcineurin: 18 (31) vs. 30 (36)

**Clonidine, \( n \) (%)**

- Sirolimus: 8 (14) vs. 10 (12)
- Calcineurin: 8 (14) vs. 10 (12)

**Statin, \( n \) (%)**

- Sirolimus: 52 (91) vs. 70 (84)
- Calcineurin: 52 (91) vs. 70 (84)

**Cyclosporin, \( n \) (%)**

- Sirolimus: 51 (88) vs. 69 (83)
- Calcineurin: 51 (88) vs. 69 (83)

**Tacrolimus, \( n \) (%)**

- Sirolimus: 7 (12) vs. 14 (17)
- Calcineurin: 7 (12) vs. 14 (17)

**Azathioprine, \( n \) (%)**

- Sirolimus: 33 (57) vs. 38 (47)
- Calcineurin: 33 (57) vs. 38 (47)

**MMF, \( n \) (%)**

- Sirolimus: 25 (43) vs. 41 (51)
- Calcineurin: 25 (43) vs. 41 (51)

**Prednisone, \( n \) (%)**

- Sirolimus: 34 (59) vs. 44 (52)
- Calcineurin: 34 (59) vs. 44 (52)

**Prednisone dose (mg)**

- Sirolimus: 3.80 ± 4.23 vs. 3.70 ± 4.74
- Calcineurin: 3.80 ± 4.23 vs. 3.70 ± 4.74

**Cyclosporin, \( n \) (%)**

- Sirolimus: 69 (83) vs. 69 (83)
- Calcineurin: 69 (83) vs. 69 (83)

**Tacrolimus, \( n \) (%)**

- Sirolimus: 14 (17) vs. 14 (17)
- Calcineurin: 14 (17) vs. 14 (17)

**Sirolimus, \( n \) (%)**

- Sirolimus: 58 (100) vs. 58 (100)
- Calcineurin: 33 (57) vs. 33 (57)

**Azathioprine, \( n \) (%)**

- Sirolimus: 33 (57) vs. 33 (57)
- Calcineurin: 33 (57) vs. 33 (57)

**MMF, \( n \) (%)**

- Sirolimus: 25 (43) vs. 25 (43)
- Calcineurin: 25 (43) vs. 25 (43)

**Azathioprine dose (mg)**

- Sirolimus: 100.78 ± 58.02 vs. 122.30 ± 66.66
- Calcineurin: 100.78 ± 58.02 vs. 122.30 ± 66.66

**MMF dose (mg)**

- Sirolimus: 1465.22 ± 812.33 vs. 1553.85 ± 657.66
- Calcineurin: 1465.22 ± 812.33 vs. 1553.85 ± 657.66

HTx, heart transplantation; IQR, inter-quartile range; ICMP, ischaemic cardiomyopathy; DCMP, dilated cardiomyopathy; MMF, mycophenolate mofetil; ACE, angiotensin-converting enzyme; GFR, glomerular filtration rate.

\( ^{*} \) ISHLT 2004 ± grade 2R.
blood pressure in those treated with sirolimus, the changes in systolic (P = 0.18) or diastolic (P = 0.15) blood pressure did not correlate with LV mass index. Hence, changes in blood pressure did not affect ventricular structure and function in the two groups.

**Left ventricular structure: changes in left ventricular mass and mass index**

To determine if treatment with sirolimus might modify ventricular hypertrophy, we examined changes in LV mass and LV mass index over a period of 12 months. LV mass decreased from 196.15 ± 48.28 to 185.13 ± 49.48 (P = 0.05) and LV mass index decreased from 99.25 ± 20.08 to 93.10 ± 20.23 (P = 0.031) in those treated with sirolimus. LV mass (P = 0.09) and LV mass index (P = 0.09) did not change in those treated with CNIs. The groups differed significantly in the change in LV mass (P = 0.024) and LV mass index (P = 0.02) (Figure 1A and B) (Table 2). One hundred and twenty-four records of echocardiograms from the year prior to the initiation of the observation period were available (49 for sirolimus group and 75 for CNI group). All subjects in the sirolimus group were on CNI in the year prior to the observation period. LV mass increased non-significantly from 186.80 ± 45.38 to 195.63 ± 47.44 (P = 0.09) in the sirolimus group and from 175.15 ± 49.48 to 182.04 ± 42.79 (P = 0.11) in the CNI group.

**Table 2  Echocardiographic and haemodynamic assessment of study (sirolimus) and control (calcineurin inhibitor) subjects**

<table>
<thead>
<tr>
<th></th>
<th>Sirolimus group, n = 58</th>
<th>Calcineurin-inhibitor group, n = 83</th>
<th>P-value (sirolimus vs. calcineurin inhibitor group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time FU (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>12.92 ± 5.01</td>
<td>12.85 ± 5.20</td>
<td>0.93</td>
</tr>
<tr>
<td>Baseline</td>
<td>124.80 ± 15.36</td>
<td>122.23 ± 16.31</td>
<td>0.36</td>
</tr>
<tr>
<td>Follow-up</td>
<td>120.09 ± 12.23</td>
<td>123.20 ± 17.52</td>
<td>0.07</td>
</tr>
<tr>
<td>ΔSBP</td>
<td>−4.71 ± 18.71</td>
<td>0.97 ± 15.33</td>
<td>0.04</td>
</tr>
<tr>
<td>P-value(^{b})</td>
<td>0.054</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>78.02 ± 10.30</td>
<td>75.70 ± 11.16</td>
<td>0.21</td>
</tr>
<tr>
<td>Follow-up</td>
<td>72.05 ± 9.97</td>
<td>77.36 ± 12.03</td>
<td>0.0005(^{a})</td>
</tr>
<tr>
<td>ΔDBP</td>
<td>−5.97 ± 11.87</td>
<td>1.66 ± 13.53</td>
<td>0.0018</td>
</tr>
<tr>
<td>P-value(^{b})</td>
<td>0.0004</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>LVM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>196.15 ± 48.28</td>
<td>182.21 ± 43.56</td>
<td>0.08</td>
</tr>
<tr>
<td>Follow-up</td>
<td>185.13 ± 49.48(^{b})</td>
<td>189.21 ± 54.31</td>
<td>0.024(^{a})</td>
</tr>
<tr>
<td>ΔLVM</td>
<td>−11.03 ± 42.09</td>
<td>7.00 ± 36.76</td>
<td>0.01</td>
</tr>
<tr>
<td>P-value(^{b})</td>
<td>0.05</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>LVMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>99.25 ± 20.08</td>
<td>93.45 ± 19.34</td>
<td>0.09</td>
</tr>
<tr>
<td>Follow-up</td>
<td>93.10 ± 20.23(^{b})</td>
<td>96.65 ± 23.83</td>
<td>0.020(^{a})</td>
</tr>
<tr>
<td>ΔLVMI</td>
<td>−6.15 ± 21.02</td>
<td>3.32 ± 17.67</td>
<td>0.007</td>
</tr>
<tr>
<td>P-value(^{b})</td>
<td>0.031</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>LVEF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>64.04 ± 7.23</td>
<td>64.05 ± 5.91</td>
<td>0.88</td>
</tr>
<tr>
<td>Follow-up</td>
<td>61.73 ± 7.37</td>
<td>63.70 ± 6.29</td>
<td>0.38(^{a})</td>
</tr>
<tr>
<td>ΔLVEF</td>
<td>−2.31 ± 7.00</td>
<td>−0.35 ± 5.65</td>
<td>0.36</td>
</tr>
<tr>
<td>P-value(^{b})</td>
<td>0.011</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>LAVI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>52.45 ± 17.22</td>
<td>52.07 ± 19.45</td>
<td>0.91</td>
</tr>
<tr>
<td>Follow-up</td>
<td>48.45 ± 15.14</td>
<td>57.34 ± 20.58</td>
<td>0.0001(^{a})</td>
</tr>
<tr>
<td>ΔLAV index</td>
<td>−4.00 ± 10.56</td>
<td>5.27 ± 12.99</td>
<td>0.0001</td>
</tr>
<tr>
<td>P-value(^{b})</td>
<td>0.008</td>
<td>0.0012</td>
<td></td>
</tr>
</tbody>
</table>

**ANCOVA using baseline characteristics as co-variables.**

\(^{a}\)Paired test.
For all panels, the data are expressed as box-and-whiskers plots. The centreline depicts the median. The box depicts the interquartile range and whiskers extend from the box to the outer-most data point that falls within one and a half times the interquartile range of the box. Points beyond that are displayed individually. Mean ± SD is also displayed below the figure. (A and B) Changes in left ventricular mass and left ventricular mass index, respectively, for all subjects in the study. (C and D) Changes in left ventricular mass and left ventricular mass index in those subjects with left ventricular hypertrophy. (E) Changes in left atrial volume index in the sirolimus and calcineurin inhibitor cohorts.

Figure 1
Sirolimus and LV mass

Table 3 Predictors of changes in LVMI

<table>
<thead>
<tr>
<th>Reason for HTx</th>
<th>Univariable analysis</th>
<th>Multivariable analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>CNI vs. sirolimus</td>
<td>4.74 (1.46–8.02)</td>
<td>0.06</td>
</tr>
<tr>
<td>Age at study entrance (years)</td>
<td>0.22 (–0.01–0.45)</td>
<td>0.65</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>1.91 (–2.15–5.97)</td>
<td>0.35</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>0.49 (–0.037–1.022)</td>
<td>0.07</td>
</tr>
<tr>
<td>Time post HTx (years)</td>
<td>0.33 (–0.40–1.06)</td>
<td>0.37</td>
</tr>
<tr>
<td>CIT</td>
<td>0.09 (–0.16–0.014)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

CIT: cold ischaemic time; HTx, heart transplantation; ICMP, ischaemic cardiomyopathy; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GFR, glomerular filtration rate; ACE, angiotensin-converting enzyme.

We next considered the change in LV structure in subjects with LVM at baseline. The LV mass and LVM index decreased in subjects with LV hypertrophy at baseline who were treated with sirolimus (n = 12). The LV mass and LVM index did not change in subjects with LV hypertrophy at baseline who were treated with CNI (n = 15) (ΔLVM: –42.89 ± 32.65 in the sirolimus group compared with 2.27 ± 53.95 in CNI, P = 0.013; ΔLVMI: –23.97 ± 19.92 in the sirolimus group compared with –0.65 ± 25.85 in CNI, P = 0.017) (Figure 1C and D). In subjects without LVH the changes in LV mass and LVM index did not differ significantly between the groups (ΔLVM: –2.39 ± 40.55 in the sirolimus group compared with 8.07 ± 32.10 in CNI, P = 0.14; ΔLVMI: –1.40 ± 18.80 in the sirolimus group compared with –4.24 ± 15.33 in CNI, P = 0.10).

In the multivariable analysis the LVM (P = 0.037) and LVMi (P = 0.04) decreased significantly in those treated with sirolimus when compared with CNI-treated subjects (Table 3).

Left ventricular function: changes in left atrial volume index

We next asked whether treatment with sirolimus modifies ventricular function by examining the LAVI, an indirect measure of diastolic function and assessing LV wall stiffness. LAVI decreased (P = 0.008) in those treated with sirolimus and increased (P = 0.0012) in those treated with CNI (Table 2). The groups differed significantly in the change in LAVI from baseline (ΔLAVI: –4.00 ± 10.57 in sirolimus group compared with 5.28 ± 12.99 in CNI group, P < 0.0001) (Figure 1E).

After multivariable regression analysis, changes in LAVI remained significantly lower in the sirolimus group (P = 0.004, Table 4).

Although pulse wave Doppler assessment of mitral and pulmonary venous inflow was performed in each subject, E, and A velocity waves were uninterpretable in the majority of patients because of E and A velocity wave fusion due to resting tachycardia and competition between native and donor atria. Although LAVI is not a true representation of diastolic function, changes in LAVI would be correlated with changes in LV filling pressure. However, E/e' from baseline to post-sirolimus conversion showed no significant difference (10.2 ± 4.4 compared with 9.7 ± 4.0, P = 0.76). Therefore, in most subjects diastolic function could not be graded using this method. LAVI, however, would not be affected because it is a morphological marker.

Although not clinically significant, there was a slight but statistically significant decrease in LVEF in the sirolimus group (P = 0.011), but no significant difference in LVEF changes between the groups was demonstrated (P = 0.38, Table 2).

Changes in p27 Kip-1 expression

Since the changes in LV mass, LV mass index, and LAVI could not be explained by differences in rejection or in blood pressure, we asked whether sirolimus might act directly on the heart. Toward this end, we evaluated expression of p27Kip-1 in endomyocardial biopsies. Sirolimus acts on mTOR and slows cell-cycle progression by stabilizing cellular expression of the cell-cycle inhibitor protein p27Kip-1, which can therefore be a surrogate marker of sirolimus activity, whereas CNIs have no impact on p27Kip-1.29,30 As shown in Table 5, the number of p27- positive nuclei increased (P = 0.0005) in those treated with sirolimus but did not change in those treated with CNIs (P = 0.54) (Figure 2).

Discussion

Adverse cardiac function caused by ventricular hypertrophy remains a major therapeutic challenge in cardiovascular medicine.
sirolimus, an inhibitor of mTOR, usually used as an immunosuppressive agent, reverses ventricular hypertrophy and improves ventricular diastolic dysfunction. These structural and functional changes occur independently of any impact sirolimus might have on blood pressure or immunosuppression and may reflect the direct action of sirolimus on myocardium.

In the general population, diastolic dysfunction as evidenced by LA size post-transplantation also correlates inversely with survival and may reflect the cumulative effect of increased filling pressures over time. Therefore, LA size has been suggested as a marker of the severity and duration of diastolic dysfunction. In the present study, sirolimus treatment improved diastolic function and was the only independent predictor of reduction in LV mass.

In animals, sirolimus attenuates cardiac hypertrophy caused by pressure overload and reverses cardiac hypertrophy. Cardiomyocyte proliferation is thought to be completed by birth and whether cardiac hyperplasia is solely due to increased mass of cardiac muscle fibres, or whether proliferation of cardiomyocytes may contribute to increased cardiac muscle mass is a matter of controversy. Following myocardial infarction, cell proliferation occurs in viable tissue bordering the infarct, and in more distant tissue where oxygenation is maintained. That myocardium expresses proteins associated with cell cycle progression, for example cyclins, cyclin-dependent kinases, and PCNA, suggests that cardiomyocytes maintain the ability to proliferate, and cellular proliferation may therefore contribute to myocardial hypertrophy.

We postulate that sirolimus acts directly on cardiac tissue. Sirolimus suppresses the function of mTOR, which is a key regulator of both cell cycle progression and also cellular energy utilization and protein metabolism. Progression through the cell cycle and ultimately cellular division depends upon production of cyclin-cdk complexes, the levels of which build to surmount the block imposed by cyclin-dependent kinase (Cdk) inhibitors, or the threshold may be reduced by degradation of the inhibitor. p27Kip1 has an important role in the inhibition of cell growth and division. Antiproliferative signals lead to accumulation and stabilization of p27Kip1 resulting in cell-cycle arrest through inhibition of Cdk2. Mice with genetic deletion of p27Kip1 develop multiorgan hyperplasia and tumor development. A recent study in p27Kip1 knockout mice demonstrated that p27Kip1 suppressed physiological hypertrophy in the adult heart and pathological cardiac growth in response to pressure overload. The same study also suggested that inactivation of p27Kip1 may be a key

<table>
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<th>Table 4 Predictors of changes in LAVI</th>
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<td><strong>Univariable analysis</strong></td>
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<td><strong>Estimate (95% CI)</strong></td>
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<td>CNI vs. sirolimus</td>
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<tr>
<td>Age at study entrance (years)</td>
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<tr>
<td>Male gender, n (%)</td>
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<tr>
<td>Donor age (years)</td>
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<tr>
<td>Time post HTx (years)</td>
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**Reason for HTx**
- ICMP
- BMI
- SBP
- DBP
- GFR
- ACE-inhibitor
- Calcium-channel blocker

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<th>Table 5 p27Kip1 expression</th>
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<td><strong>Sirolimus group, n = 22</strong></td>
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<td>p27Kip1 expression</td>
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*p27Kip1 expression, summarized as the number of p27-positive cardiomyocytes per mm2 in sirolimus and calcineurin inhibitor groups.*
We have observed that sirolimus appears to increase expression of p27Kip1, prevents cell-cycle progression, and this may be the mechanism by which sirolimus reduces cardiomyocyte proliferation and myocardial hypertrophy.

mTOR also regulates cellular energy utilization and protein metabolism. Blockade of mTOR by sirolimus activates a complex cellular program of autophagy, by which proteins and organelles are catabolized in lysosomes to provide amino acids and energy. Autophagy is stimulated by cellular stress and helps cells survive starvation and stress, but also is a normal mechanism by which cells deal with damaged organelles and proteins and maintain proper protein and organelle turnover. Indeed autophagic vacuoles are found in normal cardiomyocytes and are increased in ischaemic hearts and in human and animal models of failing hearts. Mice lacking Atg5 gene function, critical for initiation of autophagy rapidly develop heart failure characterized by LV enlargement. Thus, autophagy is required for normal cardiac myocyte function and sirolimus may help preserve this critical function in transplanted hearts.

**Study limitations**

The main limitation to this study is that it is a retrospective analysis and the two patient groups were not prospectively matched and there is therefore potential for selection bias. Despite that limitation, LVH was diminished following treatment with sirolimus, a change that was independent of changes in blood pressure. However, we acknowledge that despite the analysis, it is possible that differences in blood pressure may be a confounding influence. Although we speculate that diastolic function improves and this is suggested by changes in LAVI, we acknowledge that no data exists in the cardiac transplant population to verify the validity of LAVI as a measure diastolic function in this population.

**Conclusions**

Adverse cardiac remodelling resulting in cardiac hypertrophy and diastolic dysfunction remains widely prevalent, is difficult to manage, and has deleterious consequences. In principal there are two approaches to management: the first is to correct the underlying pathophysiological abnormalities, the second is to improve the function of the heart by inducing regression of established hypertrophy. The first approach forms the basis of existing pharmacotherapy and generally produces only limited benefits in regression of cardiac hypertrophy. The findings of the present study suggest that sirolimus, or other anti-proliferative agents, have potential as a possible therapeutic approach for the treatment of adverse cardiac remodelling characterized by ventricular hypertrophy. In particular, earlier use of sirolimus following cardiac transplantation may prevent the development of LVH.

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**References**

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