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Use of Rapamycin Slows Progression of Cardiac Transplantation Vasculopathy

Donna Mancini, MD; Sean Pinney, MD; Daniel Burkhoff, MD, PhD; John LaManca, PhD; Silviu Itescu, MD; Elizabeth Burke, RN; Niloo Edwards, MD; Mehmet Oz, MD; Andrew R. Marks, MD

Background — Cardiac transplantation vasculopathy is the leading cause of late death in heart transplantation recipients. Rapamycin is an immunosuppressant drug with potent antiproliferative and antimigratory effects. We investigated whether rapamycin could prevent progression of graft vasculopathy in 46 patients (age, 54±10 years; 4.3±2.3 years after transplantation) with severe disease.

Methods and Results — At annual cardiac catheterization, patients were randomly assigned to treatment with rapamycin (n=22) versus continued current immunosuppression (n=24). Clinical characteristics including recipient age and sex, underlying cause of congestive heart failure, donor age and sex, and ischemic time were recorded. Cardiac catheterization was graded with the use of a semiquantitative scale and repeated annually. Clinically significant adverse events were defined as death, need for angioplasty or bypass surgery, myocardial infarction, and a >25% worsening of the catheterization score. These events were monitored as primary study end points. Anti-HLA class I and II antibody production and lymphocyte growth assays were measured with each biopsy. Patients selected for rapamycin had azathioprine or mycophenolate mofetil discontinued and were given rapamycin. Outcomes were compared by means of log-rank analysis. There were no significant differences in baseline characteristics. Duration of follow-up was comparable (rapamycin, 689±261; control, 630±207 days; NS). In the rapamycin group, 3 patients reached primary end points versus 14 patients in the control group (P<0.001). There was no difference in baseline or subsequent anti-HLA class I or II antibody production.

Conclusions — In this patient cohort with cardiac vasculopathy, treatment with rapamycin slowed disease progression probably by its antiproliferative and antimigratory effects. (Circulation. 2003;108:48-53.)

Key Words: transplantation ■ vasculature ■ atherosclerosis ■ drugs
the time of their annual angiogram, patients with severe transplantation coronary artery disease defined as epicardial stenosis >50%, intravascular ultrasound intimal thickening >0.5 mm, and/or severe diffuse vessel tapering were recruited for participation into the trial. Demographic data including age, sex, date of transplantation, donor characteristics, previous biopsy results, and background immunosuppressive and medical therapy was recorded. Patients were randomly assigned to treatment with rapamycin or continued standard care. For patients randomly assigned to rapamycin, mycophenolate mofetil or azathioprine was discontinued. A loading dose of rapamycin (6 mg) was administered followed by 2 mg daily. Patients in the rapamycin group were seen within 2 weeks of initiating therapy and again at 4 weeks. An endomyocardial biopsy specimen was taken within 1 month of starting rapamycin. After this initial period, the patients returned to their usual clinic visits. Rapamycin levels were determined by liquid chromatography on whole blood obtained at each visit, and the dose was titrated to a level between 4 to 15 ng/mL. Serum chemistries, complete blood counts, lipid panel, and cyclosporine or tacrolimus levels were obtained at each clinic visit. Left ventricular ejection fraction by echocardiography or nuclear techniques was obtained annually.

The Columbia University College of Physicians and Surgeons Institutional Review Board approved the study. All patients gave written informed consent.

Catheterization Analysis
Semi-quantified scoring of catheterization films was performed by 2 independent observers blinded to treatment assignments. When feasible, intravascular ultrasound (IVUS) was obtained with automatic pullback at a rate of 0.5 mm/s, with a transducer at 30 MHz. A minimum of 1 vessel (left anterior descending artery) was examined, and maximum intimal thickness was measured.

Histological Grading of Biopsy Specimens
Each biopsy specimen was graded according to the International Society of Heart Lung Transplant System. The pathologists were blinded to the treatment assignment. For each patient, a baseline biopsy score was derived, with each biopsy grade converted to a numeric value and averaged.

Immunologic Assays
Serological typing of HLA-A and HLA-B loci was done by standard microcytotoxicity techniques. HLA-DR typing was performed by analysis of serology and DNA techniques with sequence-specific oligonucleotide primers and PCR. At each biopsy, serum was screened for the presence of anti-HLA class I and II antibodies as previously described.

Lymphocyte growth assay was also performed at each biopsy. A biopsy fragment was placed in medium supplemented with recombinant interleukin-2 and examined at 48 hours with a phase-inverted microscope. Circumferential T-cell aggregation denoted a positive test.

Study End Points
The primary end point was a composite of clinically significant events including death, acute myocardial infarction, need for angioplasty or bypass surgery, and/or a >25% increase in the catheterization score. Secondary end points included cardiac hospitalization and relisting for transplantation. Adverse events including infections, and physical findings were monitored.

Statistical Analysis
All data are expressed as mean±SD. Variables were compared by means of a nonpaired t test or χ² analysis as appropriate. Kaplan-Meier outcomes in each group were compared by using the log-rank test to analyze time to events. A probability value <0.05 was considered statistically significant.

Results

Patient Characteristics
The clinical characteristics of the patients are shown in Table 1. No significant differences were observed in recipient or donor age, sex, underlying cause of CHF, ischemic time, or left ventricular ejection fraction. Patients in the rapamycin group had a shorter time after transplantation than the control subjects. Background immunosuppressive therapy was comparable (Table 1). Baseline biopsy score was not different between the groups. Indication for enrollment into the study was similar. In the rapamycin group, 9 patients had IVUS intimal thickening >0.5 mm or diffuse vessel tapering and 13 patients had significant epicardial disease. In the control group, 6 patients had IVUS >0.5 mm or diffuse vessel tapering and 13 patients had epicardial disease.

Clinical Follow-Up
Therapeutic rapamycin levels were achieved by 4 weeks in all patients and were maintained at 4 to 15 ng/mL (Figure 1). Serum creatinine, cholesterol, and hematocrits were similar throughout the year in both groups. Though cholesterol levels were not different between the two groups, cholesterol levels tended to be higher in the rapamycin group at all time points. The average cyclosporine level was significantly lower in the rapamycin group by the end of the first year (P<0.04) (Figure 2).
Incidence of acute allograft rejection was comparable between the two groups. Endomyocardial biopsy specimens were taken every 6 months as per our institutional protocol. Five grade 2 rejections occurred in 3 patients in the control group versus 4 grade 2 rejections in 4 patients in the rapamycin group. Two grade 3A rejections occurred in the control group and none occurred in the rapamycin group. Left ventricular ejection fractions were similar at 1 year of follow-up (control, 59±10; rapamycin, 58±8%; \( P=\text{NS} \)).

**Catheterization Scores**

The baseline catheterization scores for the control and rapamycin-treated groups were comparable (control, 19.0±10.3; rapamycin, 16.5±7.3; NS): 18 of the 22 patients in the rapamycin-treated group had at least 2 catheterizations. One patient underwent repeat transplantation before repeat catheterization, 2 patients withdrew from the study, and 1 patient had severe renal insufficiency precluding repeat catheterization. The catheterization scores in the rapamycin group demonstrated no significant change (baseline, 16.5±7.3; 1 year, 16.6±8.3; \( P=0.41 \)). Three patients (17%) had a >25% decrease in the catheterization score, whereas 2 patients (11%) had a >25% increase in the score.

In the control group, 19 patients had serial catheterizations. Two patients died, 1 patient underwent repeat transplantation before repeat testing, 1 patient did not undergo repeat catheterization because of renal insufficiency, and 1 patient

![Figure 1. Rapamycin levels at 1, 6, and 12 months of therapy.](image1)

![Figure 2. Cyclosporine A, creatinine, hematocrit, and cholesterol levels at baseline and 6 and 12 months.](image2)

\* \( p <0.05 \) rapamycin 12 mo vs baseline
did not reach 1 year follow-up. In contrast to the rapamycin group, the control group demonstrated a significant increase in catheterization score (baseline, 19.0±10.3; 1 year: 23.4±10.9; P < 0.01). Moreover, none of the patients in the control group showed a >25% decrease in catheterization score and 8 patients (33%) demonstrated a >25% increase in catheterization score.

Ten patients underwent baseline intracoronary ultrasound (IVUS; 5 patients in the rapamycin group and 5 patients in the control group). In 5 patients, introduction of the IVUS catheter resulted in significant hypotension, resulting in termination of the study. Intimal thickening of the left anterior descending artery was comparable in both groups (control, 0.6±0.24; rapamycin, 0.6±0.12 mm; NS).

Immunologic Assays
The presence of anti-HLA IgG class I or II antibodies at baseline was comparable for the control and rapamycin-treated groups. There was no difference in the incidence of new anti-HLA antibody production, persistent production, or resolution of antibody production between the two groups (P = 0.43). Moreover, immune activation reflected by the presence of any positive lymphocyte growth tests were similar between the two groups (Table 2).

Study End Points
Clinically significant adverse outcomes occurred more frequently in the control versus rapamycin group (Table 3). Figure 3 shows the Kaplan-Meier curves of time to clinically significant event of each group compared by log-rank analysis. A highly statistically significant difference was observed between the two groups (P < 0.01). Analysis of the subgroup of control patients with comparable time after transplantation (n = 16) to the rapamycin group demonstrated an even higher statistical decrease in primary events (P < 0.001).

The odds ratio of achieving a primary end point was 9:1 for the control group versus the rapamycin group. In the control group, 14 patients achieved a primary end point. Two patients died suddenly, 2 patients had an myocardial infarction and died, 4 patients underwent angioplasty, 1 patient had PTCA with stent placement followed by myocardial infarction, in-stent stenosis, and bypass grafting, 2 patients had isolated myocardial infarction, and 3 patients had an isolated worsening of their catheterization score.

In the rapamycin group, only 3 patients achieved primary end points. One patient who had a >25% increase in the semiquantitative catheterization score went on to have a myocardial infarction and die. Another patient required angioplasty just 2 months after enrollment, and a third patient had >25% increase in catheterization score.

Secondary end points were also significantly more common in the control than in the rapamycin group (Table 3). Cardiac hospitalizations occurred in 15 patients in the control group versus 5 patients in the rapamycin group (P < 0.01). Time to the first cardiac hospitalization was statistically significant by log-rank analysis (Figure 4).

Adverse Effects
The frequency of admission to the hospital for noncardiac reasons was similar between the two groups (Table 4). Infections constituted the most common indication for readmission in both groups. In the control group, infections occurred from pneumocystis pneumonia,1 bacterial pneumonia,2 bronchitis,1 acute gastroenteritis,1 and viral syndrome. In the rapamycin group, causes of infections were bacterial pneumonia,4 bronchitis,1 cellulitis,1 diverticulitis1 and urosepsis.2 Two patients in each group were hospitalized with new onset diabetes. Two patients in the rapamycin group were hospitalized for kidney failure. In one patient, the kidney failure was irreversible. With regard to malignancy, 2 patients in the rapamycin group were diagnosed with solid organ tumors versus none in the control group. None of the patients had posttransplantation lymphoproliferative disease.

Figure 3. Time to primary end point (death, angioplasty, myocardial infarction, or >25% increase in catheterization score) in the control and rapamycin groups.

### Table 2. IgG Class I and II Anti-HLA Antibody Production in Control and Rapamycin Groups

<table>
<thead>
<tr>
<th>IgG Class I or II HLA ab</th>
<th>Control</th>
<th>Rapamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab at baseline, n</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Ab production, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>New producers</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Resolution</td>
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<td>4</td>
</tr>
<tr>
<td>Lymphocyte growth assay, n</td>
<td>9</td>
<td>8</td>
</tr>
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</table>

### Table 3. Study End Points

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Rapamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary end points</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>PTCA</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>CABG</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>&gt;25% increase in catheterization score</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Secondary end points</td>
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<td></td>
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<tr>
<td>Cardiac hospitalizations</td>
<td>20</td>
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</tr>
<tr>
<td>Congestive heart failure</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Chest pain</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Relist for transplantation</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>7</td>
</tr>
</tbody>
</table>
to limit disease progression and improve outcome.21 Oxidants, and intensified immunosuppression have been suggested with a localized sustained inflammatory response characterized by mediated and humoral response to vascular endothelial injury occur over time,26 results in expansion of T-cell clones with specificity of CD4 T-cell activation assumes an increasingly important role rejection early after organ transplantation. The indirect pathway primary immunologic pathway responsible for acute cellular rejection.27–29 Since the indirect T-cell activation pathway gives rise to B-cell activation, development of anti-HLA IgG antibodies to the graft has been shown to be predictive of the development of graft atherosclerosis.30 Moreover, antigen-specific B cells may drive the indirect T-cell activation pathway by acting as potent antigen-presenting cells of soluble allo-HLA-DR peptides to self-CD4 T cells.31,32 Therefore, prominent anti-HLA antibody production in a given transplantation recipient may correlate with a high risk for transplantation-related vasculopathy by both reflecting an activated indirect T-cell recognition pathway and an efficient alloantigen-presentation arm of the immune response.

In this study, the rapamycin group did not show reduced levels of anti-HLA antibodies in comparison to the control group nor reduced outgrowth from the graft of IL2-receptor positive T cells, therefore rapamycin did not effectively suppress intragraft T-cell activation or the indirect T-cell recognition pathway associated with systemic B-cell activation. Consequently, our results suggest that the primary mechanism by which rapamycin reduced transplantation-related vasculopathy in this patient cohort was derived from its effects on vascular smooth muscle.

Transplantation vasculopathy is characterized by intense intimal proliferation in large- and small-caliber vessels. Severe intimal thickening is associated with an increased rate of cardiac events and decreased survival.33 In animal models of transplantation coronary artery disease and angioplasty, an endovascular proliferative response is prevented by treatment with rapamycin.10 As rapamycin combines antiproliferative as well as antimigratory properties with potent immunosuppressant activity, it is an ideal drug to both prevent and treat graft vasculopathy. Moreover, since rapamycin targets central regulators of cell cycle progression in vascular smooth muscle cells, including the cyclin-dependent kinase inhibitor p27,193 it is likely to be efficacious in a wide range of patients, even if the stimuli for intimal proliferation in the transplanted coronaries varies among patients.

Unlike the recent renal transplantation trial where the incidence of allograft rejection was lower in those receiving rapamycin,14,17 in our study, no significant reduction in allograft rejection was observed in patients treated with rapamycin at later stages after transplantation. The frequency of allograft rejection may not have been high enough in our cohort to discern a significant difference between the groups. Alternatively, in this study, mycophenolate mofetil was primarily used as the other antiproliferative agent.

The rate of progression of transplantation vasculopathy is variable. Patients who have the disease earlier after transplantation may have more aggressive disease, whereas those presenting later after transplantation may have a greater disease burden. As the time after transplantation was shorter in the rapamycin group, one could infer that their disease was more aggressive. Moreover, in one study examining transplantation recipients with a discrete epicardial stenosis >40%, the mortality rate was >50% at 2 years.34 By comparison, the survival of our rapamycin group was extraordinarily good, with only 1 death in 2 years of follow-up.

Table 4. Noncardiac Hospitalizations

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Rapamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Kidney failure</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
Study Limitations
There are several limitations to this study. First, although patients were randomly assigned, it was an open-labeled study. In patients with severe epicardial disease, insertion of the IVUS catheter was not feasible because of the development of hypotension at the time of catheter insertion. However, in the patients who underwent this procedure, significant intimal hyperplasia was visualized, confirming the presence of active transplantation vasculopathy. Our semi-quantitative catheterization scoring can be criticized as being subjective; however, the physicians reading the angiograms were blinded to treatment assignment.

Patients assigned to the rapamycin group had closer follow-up in the initial month of therapy because of the need to achieve therapeutic levels and to ensure that acute allograft rejection did not occur with the alteration in therapy. After therapeutic levels were documented, the patients returned to their usual clinic schedule.

Side Effects
Hospitalizations for infection were common in both groups. Non-cardiac peripheral edema with or without polyarthralgia occurred more frequently in the rapamycin group. There was a trend to worsening renal function in the rapamycin-treated patients despite statistically significant lower cyclosporine levels. Though rapamycin has no intrinsic nephrotoxicity, it may potentiate the nephrotoxic effects of the calcineurin inhibitors.

Conclusions
Rapamycin appears to be an effective therapy to slow transplantation arteriopathy. The mechanism of action is probably due to its antiproliferative and antimigratory effects and not related to B-cell suppression or lipid-lowering actions. The promising findings of this single center, open-labeled, randomized study need confirmation in a multicenter trial.

Acknowledgments
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