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Circulation 2003;108;48-53; originally published online May 12, 2003;
DOI: 10.1161/01.CIR.0000070421.38604.2B
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

Use of Rapamycin Slows Progression of Cardiac Transplantation Vasculopathy

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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

Cardiac allograft vasculopathy is the leading cause of late death after heart transplantation.[1] In a multicenter study of 2609 heart transplantation recipients, 42% had evidence of graft vasculopathy on angiography by 5 years after transplantation.[2] Both immunologic and nonimmunologic endothelium damage may initiate pathological remodeling of the transplanted coronary arteries resulting in graft vasculopathy.[3–5] Currently there is no known therapy to prevent the development or progression of this disease.

See p 6

Rapamycin is a new immunosuppressant with potent antiproliferative[6] and antimigratory[7] activity in vascular smooth muscle that has been shown in animal models to prevent cardiac transplantation vasculopathy.[8] In both animal models and clinical trials, use of rapamycin-coated stents has been shown to prevent in-stent restenosis.[9–11] Unlike calcineurin inhibitors, rapamycin does not inhibit interleukin production from antigen-induced T-cell activation but inhibits cellular proliferation and migration in response to alloantigens. Rapamycin binds to FK506-binding protein 12 (FKBP12). The rapamycin-FKBP12 dimeric molecule inhibits the mammalian target of rapamycin (mTOR) and upregulates the cyclin-dependent kinase inhibitor $p27^{kip1}$,[12] leading to inhibition of cell cycle progression at the G1 to S phase.[13] Use of rapamycin in de novo kidney allograft recipients has been shown to be effective in reducing allograft rejection.[14,15] Use of rapamycin in heart transplantation recipients is more limited.

The primary aim of this prospective, randomized trial was to investigate the efficacy of rapamycin in preventing disease progression in a cohort of patients with angiographically documented transplantation vasculopathy. Clinically significant end points including death, myocardial infarction, need for surgical or percutaneous interventions, and semiquantitative catheterization scores were the primary study end points.

Methods

Study Design

This was an open-label, prospective, randomized study of patients with graft atherosclerosis, using rapamycin versus standard care. At

Received December 16, 2002; revision received March 4, 2003; accepted March 5, 2003.
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Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000070421.38604.2B**

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

Semiquantified scoring of catheterization films was performed[17] 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.

Histiological Grading of Biopsy Specimens

Each biopsy specimen was graded according to the International Society of Heart Lung Transplant System.[18] 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.[19]

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.[20]

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, laboratory abnormalities, 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 χ^2 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 18 patients had epicardial disease.

TABLE 1. Clinical Characteristics of Patients Studied

	Control (n=24)	Rapamycin (n=22)
Age, y	53±10	54±11
Sex, n		
Male	18	20
Female	6	2
Reason for transplantation, n		
Coronary artery disease	11	12
Dilated cardiomyopathy	13	12
Donor age, y	39±9	41±11
HLA match, n*		
A match	9	10
B match	7	3
DR match	5	9
Ischemic time, min	176±40	176±53
Time after transplantation, y	5.1±3.1	3.3±1.5*
Left ventricular ejection fraction, %	54±10	56±7
Biopsy score	0.68±0.28	0.77±0.33
Rejection episodes (>3A)	11	9
Follow-up, d	630±207	689±261
Prednisone dose, mg/d	5.4±2.0	6.2±2.8
Mycophenolate mofetil, n	16	20
Azathioprine, n	5	2
Cyclosporine, n	20	17
Tacrolimus, n	4	5
HMG-CoA reductase inhibitors, n	22	20
Diabetic medications, n	9	5
Antihypertensive medications, n	21	17
ACE or ARB	13	10
Ca channel blocker	8	10

*No. of patients with at least one HLA-A, B, or DR match.

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).

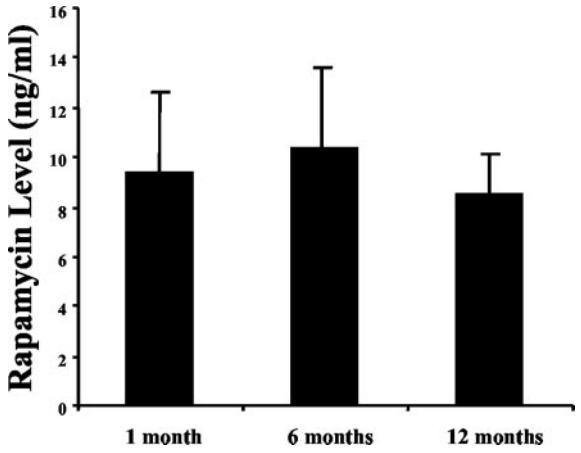

Figure 1. Rapamycin levels at 1, 6, and 12 months of therapy.

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=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

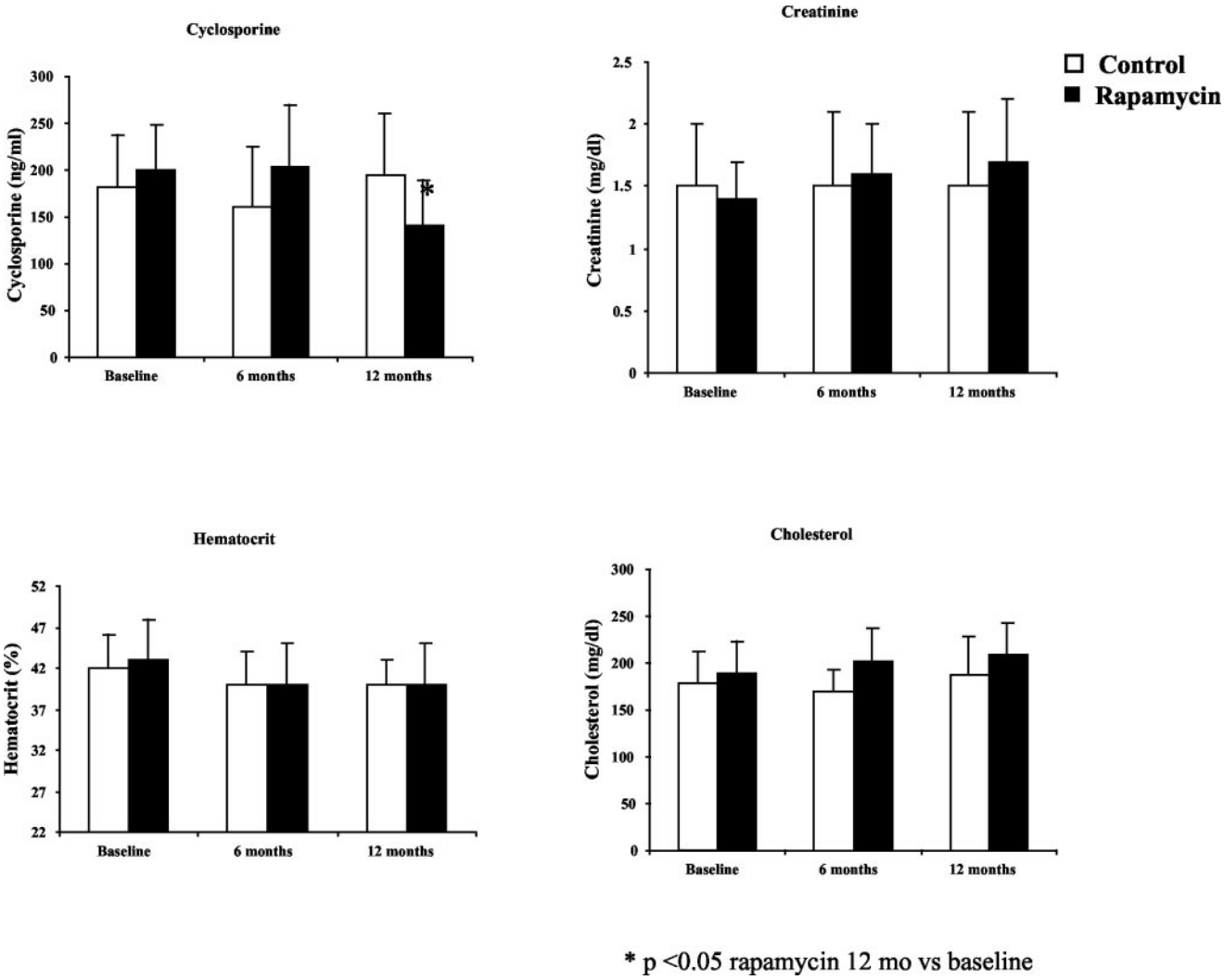

Figure 2. Cyclosporine A, creatinine, hematocrit, and cholesterol levels at baseline and 6 and 12 months.

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

IgG Class I or II HLA ab	Control	Rapamycin
Ab at baseline, n	6	7
Ab production, n		
Persistent	4	3
New producers	3	5
Resolution	2	4
Lymphocyte growth assay, n	9	8

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.

TABLE 3. Study End Points

	Control	Rapamycin
Primary end points		
Death	4	1
PTCA	5	1
CABG	1	0
Myocardial infarction	7	1
>25% increase in catheterization score	8	2
Total	25	5
Secondary end points		
Cardiac hospitalizations	20	5
Congestive heart failure	14	5
Chest pain	6	
Relist for transplantation	5	2
Total	25	7

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,[3] bronchitis,[1] acute gastroenteritis,[1] and viral syndrome. In the rapamycin group, causes of infections were bacterial pneumonia,[4] bronchitis,[1] cellulitis,[1] diverticulitis[1] 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.

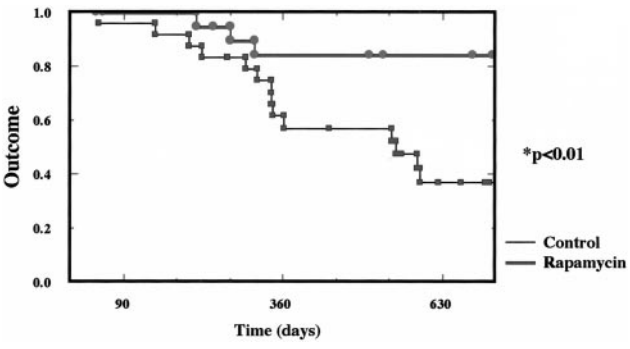

Figure 3. Time to primary end point (death, angioplasty, myocardial infarction, or >25% increase in catheterization score) in the control and rapamycin groups.

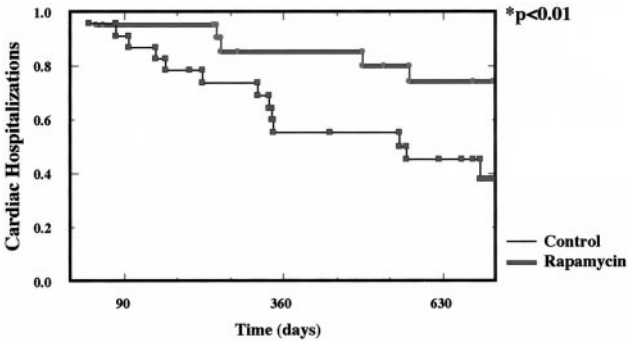

Figure 4. Time to cardiac hospitalization in the control and rapamycin groups.

Four patients discontinued rapamycin therapy. Two patients were withdrawn <1 month after beginning therapy because of renal insufficiency. The other 2 patients were discontinued after 1 year of treatment because of gastrointestinal intolerance and refractory leg edema. Polyarthralgias and peripheral edema were observed more frequently in the rapamycin-treated patients.

Discussion

This is the first study to demonstrate effective therapy for patients with documented severe transplantation vasculopathy. The use of rapamycin effectively slowed the progression of graft vasculopathy and reduced the incidence of clinical significant cardiac events. Production of anti-HLA class I and II antibodies was not reduced with rapamycin, suggesting that its mechanism of action is not mediated by B-cell suppression.

Allograft vasculopathy remains the most important cause of late graft deterioration and death. Vascular remodeling results in decreased caliber of the arterial lumen, with resultant ischemia and graft failure. Vasculopathy is associated with immunologic mechanisms, such as alloreactive T lymphocytes and antibodies and nonimmunologic factors such as hyperlipidemia, obesity, ischemia/reperfusion injury, donor age, and CMV infection.[3–5] Both cell-mediated and humoral response to vascular endothelial injury occur with a localized sustained inflammatory response characterized by myofibroblast proliferation and fibrosis. There is no known treatment for graft vasculopathy. The use of calcium antagonists, ACE inhibitors, hydroxymethylglutaryl Co-A reductase inhibitors, antioxidants, and intensified immunosuppression have been suggested to limit disease progression and improve outcome.[21–25]

Chronic allograft rejection is associated with the development of transplantation vasculopathy. Direct allorecognition is the primary immunologic pathway responsible for acute cellular rejection early after organ transplantation. The indirect pathway of CD4 T-cell activation assumes an increasingly important role over time,[26] results in expansion of T-cell clones with specificity for multiple HLA-DR allopeptides presented by self-antigen presenting cells, and correlates closely with onset of chronic allograft 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.

TABLE 4. Noncardiac Hospitalizations

	Control	Rapamycin
Infection	7	9
Diabetes	2	2
Kidney failure	0	2
Other	2	0

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 $p27,^{kip1}$ 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.

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 semiquantitative 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. Noncardiac 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

This study was supported by the Foundation for the Advancement of Cardiac Therapies (FACT) and the Doris Duke Charitable Foundation.

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