

Angiotensin-Converting Enzyme Inhibition Early After Heart Transplantation



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ABSTRACT

BACKGROUND Cardiac allograft vasculopathy (CAV) remains a leading cause of mortality after heart transplantation (HT). Angiotensin-converting enzyme inhibitors (ACEIs) may retard the development of CAV but have not been well studied after HT.

OBJECTIVES This study tested the safety and efficacy of the ACEI ramipril on the development of CAV early after HT.

METHODS In this prospective, multicenter, randomized, double-blind, placebo-controlled trial, 96 HT recipients were randomized to undergo ramipril or placebo therapy. They underwent coronary angiography, endothelial function testing; measurements of fractional flow reserve (FFR) and coronary flow reserve (CFR) and the index of microcirculatory resistance (IMR); and intravascular ultrasonography (IVUS) of the left anterior descending coronary artery, within 8 weeks of HT. At 1 year, the invasive assessment was repeated. Circulating endothelial progenitor cells (EPCs) were quantified at baseline and 1 year.

RESULTS Plaque volumes at 1 year were similar between the ramipril and placebo groups ($162.1 \pm 70.5 \text{ mm}^3$ vs. $177.3 \pm 94.3 \text{ mm}^3$, respectively; $p = 0.73$). Patients receiving ramipril had improvement in microvascular function as shown by a significant decrease in IMR (21.4 ± 14.7 to 14.4 ± 6.3 ; $p = 0.001$) and increase in CFR (3.8 ± 1.7 to 4.8 ± 1.5 ; $p = 0.017$), from baseline to 1 year. This did not occur with IMR (17.4 ± 8.4 to 21.5 ± 20.0 ; $p = 0.72$) or CFR (4.1 ± 1.8 to 4.1 ± 2.2 ; $p = 0.60$) in the placebo-treated patients. EPCs decreased significantly at 1 year in the placebo group but not in the ramipril group.

CONCLUSIONS Ramipril does not slow development of epicardial plaque volume but does stabilize levels of endothelial progenitor cells and improve microvascular function, which have been associated with improved long-term survival after HT. (Angiotensin Converting Enzyme [ACE] Inhibition and Cardiac Allograft Vasculopathy; [NCT01078363](https://clinicaltrials.gov/ct2/show/study/NCT01078363)) (J Am Coll Cardiol 2017;69:2832-41) © 2017 by the American College of Cardiology Foundation.

Cardiac allograft vasculopathy (CAV) manifesting as progressive coronary intimal thickening remains a leading cause of morbidity and mortality after heart transplantation (HT) (1). Angiotensin-converting enzyme inhibitors

(ACEIs) attenuate coronary atherosclerotic events in nontransplant patients independent of antihypertensive effects, presumably by improving endothelial function, decreasing inflammation, and reducing oxidative stress, all of which stabilize and reduce



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Manuscript received January 30, 2017; revised manuscript received March 27, 2017, accepted March 31, 2017.

plaque development (2). Previous single-center and retrospective studies have suggested that ACEIs decrease coronary plaque development after cardiac transplantation, but ACEIs have not been well studied in this setting (3-5). The goal of this prospective, multicenter, randomized, double-blind, placebo-controlled trial was to test the safety and efficacy of the ACEI ramipril on the development of CAV early after HT. The primary endpoint was the difference in plaque volume at 1 year, with key secondary endpoints including the effect of ramipril on coronary physiology, endothelial function, and endothelial progenitor cell levels.

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METHODS

STUDY DESIGN. Consecutive HT recipients at Stanford University, Palo Alto Veterans Affairs Health Care System, and Cedars Sinai Medical Center who were ≥ 12 years of age and had a serum creatinine concentration of < 2.0 mg/dl were included. Patients were excluded if they were undergoing more than 1 solid organ transplantation or repeated HT. Within 8 weeks of HT, patients were randomized in an equal and double-blind fashion to receive either ramipril or a matching placebo. These protocols were initiated 1 week after coronary angiography, coronary endothelial function testing, fractional flow reserve (FFR), coronary flow reserve (CFR), the index of microcirculatory resistance (IMR) and intravascular ultrasonography (IVUS) examination of the left anterior descending (LAD) coronary artery were performed. Blood was collected to quantify circulating endothelial progenitor cells. One year after HT, the invasive and serologic tests were repeated. The primary endpoints of the study were differences in plaque volume as assessed by IVUS examination of the LAD artery at 1 year between the ramipril and the placebo groups. Key secondary endpoints included the safety and efficacy of ramipril administration early after HT, effect of ramipril on coronary endothelial function, coronary physiology, and circulating endothelial progenitor cell number. This study was approved by the institutional review committee at each participating site. Informed written consent was obtained from all patients.

IMMUNOSUPPRESSIVE AND OTHER MEDICAL THERAPY. Adult patients (≥ 18 years of age) received induction therapy with intravenous methylprednisolone, 500 mg, when coming off bypass and rATG (rabbit antithymocyte globulin) therapy, 1 mg/kg (maximum dose: 125 mg) given on post-operative days 1, 2, and 3. Pediatric patients received daclizumab, 1 mg/kg. Two additional doses of methylprednisolone were given

over the next 24 h. Pediatric patients received daclizumab every other week for a total of 5 doses after transplantation. The daily maintenance immunosuppressive regimen included cyclosporine (2 to 4 mg/kg/day) in children, tacrolimus (0.5 mg twice daily) in adults, and mycophenolate mofetil (1,000 mg twice daily) in both adults and pediatric patients. Dose adjustments were made as indicated by adverse effects and/or acute rejection. Prednisone was initiated at 1 mg/kg/day and tapered to 0.5 mg/kg/day by month 6 and to 0.1 mg/kg/day by month 12. Concomitant medications were initiated as soon as the patient was able to tolerate oral intake. All patients received co-trimoxazole (1 double-strength tablet daily) for pneumocystis pneumonia prophylaxis, except for patients with sulfa allergy who received atovaquone instead of co-trimoxazole.

An hydroxymethylglutaryl-coenzyme A reductase inhibitor (pravastatin) therapy was initiated in all patients at 20 mg/day within 1 week of transplantation regardless of plasma cholesterol or triglyceride concentration. The dose of pravastatin was increased to 40 mg as tolerated. Unless contraindicated by hypotension or allergic reaction, patients were placed on a calcium channel-blocking drug. The first-line agent was diltiazem, commencing at a dose of 120 mg daily. Dose adjustments of the calcium channel blocker were based on routine blood pressure measurements, targeting a goal of $< 130/80$ mm Hg. Aspirin was routinely given unless contraindicated.

All patients in whom either the donor or recipient was cytomegalovirus-positive by serologic testing received standard ganciclovir prophylaxis, consisting of valganciclovir (900 mg twice daily) for 1 month, initiated as soon as the patient could tolerate oral medications, followed by 900 mg daily until the end of the third post-operative month. Thereafter, all patients continued valganciclovir therapy, 450 mg daily for the remainder of the first year post HT. Patients presenting with signs or symptoms of cytomegalovirus infection underwent testing for confirmation and treatment. Patients were monitored for acute rejection by routine surveillance endomyocardial biopsy and echocardiography to evaluate allograft function at 2 weeks and 1, 2, 3, 4, 6, 9, and 12 months after HT.

TITRATION OF STUDY DRUG AND HYPERTENSION MANAGEMENT. Ramipril or placebo was initiated at 2.5 mg/day 1 week after baseline coronary angiography. Two weeks later, if serum creatinine was

ABBREVIATIONS AND ACRONYMS

ACEI = angiotensin converting enzyme inhibitor
CAV = cardiac allograft vasculopathy
CFR = coronary flow reserve
EPC = endothelial progenitor cell
FFR = fractional flow reserve
HT = heart transplantation
IMR = index of microcirculatory resistance
IVUS = intravascular ultrasonography
LAD = left anterior descending artery

stable and the patient had not developed any adverse sequelae, the dose was increased to 5 mg per day, and serum creatinine was checked again 2 weeks later. If the creatinine was stable and the patient had not developed any adverse sequelae, the dose was further increased to 10 mg per day in adult patients. The dose was titrated to a maximum of 20 mg/day in adult patients if serum creatinine 2 weeks later was stable and there were no adverse sequelae. If a subject developed a side effect felt to be secondary to study drug, the dose was first decreased and then discontinued if the side effect persisted.

If a subject developed hypertension, diltiazem was increased. If a patient remained hypertensive despite a maximal dose of diltiazem, hydrochlorothiazide was started at 12.5 mg daily and increased to 25 mg as necessary. If a patient continued to be hypertensive despite a maximal dose of hydrochlorothiazide, then clonidine, 0.1 mg twice daily, was added and titrated upward as necessary. A beta-blocker or dihydropyridine calcium antagonist was added if the blood pressure was not controlled with the above medications. Patients underwent routine blood pressure evaluations on a weekly basis during the first month, on a monthly basis during the first 4 months, and then at months 6, 9, and 12 after HT. The blood pressure goal was <130/80 mm Hg in all patients. Patients were not allowed to take an angiotensin receptor blocker or direct renin inhibitor during the study period. The invasive coronary artery assessments are described in the order in which they were performed in each patient.

CORONARY ENDOTHELIAL FUNCTION ASSESSMENT.

Endothelial function assessment was performed in the adult patients enrolled at Stanford University and Palo Alto Veterans Affairs Health Care System. Using a 6-F guide catheter, baseline coronary angiography of the LAD artery without panning the table was performed in a cranial projection. Intracoronary acetylcholine (20 μ g) was administered over 30 s. An angiogram of the LAD artery was recorded. A second bolus of intracoronary acetylcholine (50 μ g) was administered over 30 s. Another angiogram of the LAD artery was recorded. Intracoronary nitroglycerin (100 to 200 μ g) was administered, and a final angiogram of the LAD artery was recorded. All coronary angiograms were analyzed by an independent core laboratory (Cardiovascular Core Analysis Laboratory, Stanford, California) blinded to the patient's clinical and randomization information. Quantitative and qualitative analyses of the LAD artery at baseline and after injection were carried out using an automatic edge detection system (QAngio XA 7.3, Medis, Leiden,

the Netherlands). Endothelial dysfunction was defined as $\geq 20\%$ decrease in LAD artery diameter after acetylcholine infusion compared with that at baseline.

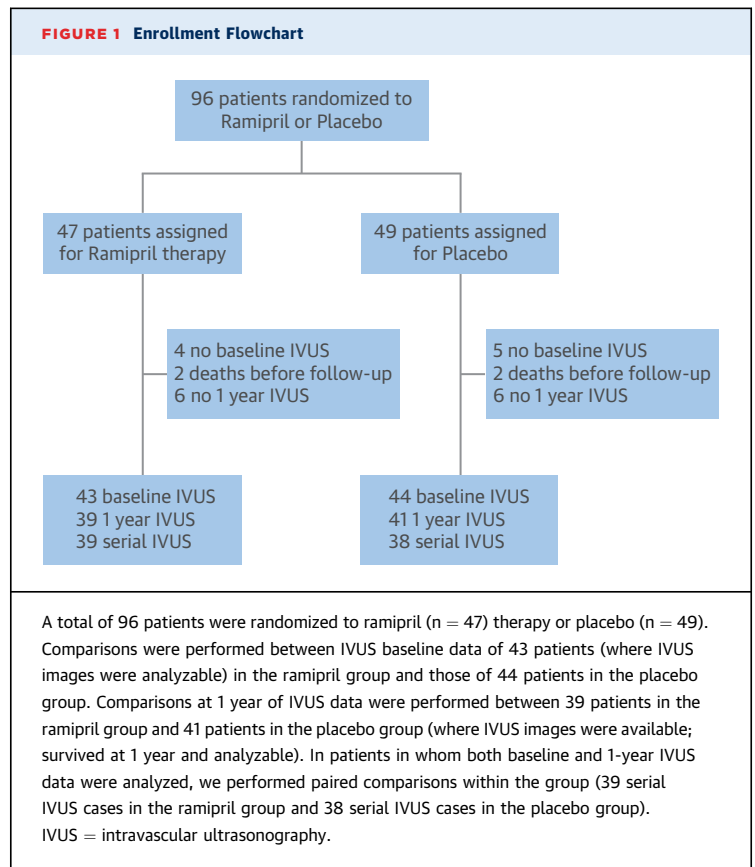
CORONARY PHYSIOLOGY ASSESSMENT. Coronary physiology assessment was performed in the adult patients enrolled from Stanford University and Palo Alto Veterans Affairs Health Care System. After endothelial function assessment and administration of intracoronary nitroglycerin, intravenous heparin (60 U/kg) was administered. A coronary pressure wire (Certus, St. Jude Medical, Luxembourg, Germany) was calibrated outside of the body and advanced so that the sensor was positioned at the tip of the guide catheter and pressures were equalized. The wire was then advanced to the distal two-thirds of the LAD artery. Care was taken to position the wire in the same location at baseline and at the 1-year follow-up. Three intracoronary injections of approximately 3 ml of room temperature saline were performed using a 3-cc syringe connected to a stopcock at the back end of the manifold. The resting mean transit time was calculated automatically (Radi Analyzer, St. Jude Medical) as previously described (6). The 3 resting transit times were automatically averaged and displayed as a resting mean transit time. Intravenous adenosine (140 μ g/kg/min) was then administered. Once maximal hyperemia was achieved, 3 injections of approximately 3 ml of room temperature saline each were performed, and the hyperemic mean transit time was calculated as described above. Coronary flow reserve was calculated as the resting mean transit time divided by the hyperemic mean transit time; the IMR was calculated as the hyperemic mean transit time multiplied by the hyperemic distal coronary pressure; and FFR was calculated as the mean distal pressure divided by the mean proximal pressure during maximal hyperemia (7).

INTRAVASCULAR ULTRASONOGRAPHY. In all patients, a commercially available 40-MHz IVUS catheter (Atlantis SR Pro 2 or OptiCross, Boston Scientific, Natick, Massachusetts) was advanced over the coronary guidewire to the mid to distal LAD artery. A recording was performed by using an automated motorized pullback (0.5 mm/s) of the IVUS catheter. IVUS imaging was analyzed offline by an independent core laboratory (Cardiovascular Core Analysis) blinded to the patient's clinical and randomization information, using commercially available 3D reconstruction software (echoPlaque 4.0, Indec Medical Systems, Santa Clara, California). Lumen and vessel contours were traced with 1-mm axial intervals for the first 50 mm from the ostium

of the LAD artery. For volumetric analyses, lumen and vessel volumes were calculated using Simpson's rule and divided by the axial analyzed length (cubic millimeters) to adjust for any differences of analyzed longitudinal lengths among the cases. Plaque volume was defined as vessel volume minus lumen volume; percentage of plaque volume was defined as: $[100 \times \text{plaque volume}/\text{vessel volume}]$. Maximal intimal thickness was also obtained from the interpolated data set as a 2D IVUS index.

QUANTIFICATION OF ENDOTHELIAL PROGENITOR CELL NUMBER. Whole blood was collected in heparinized collection tubes. Most samples were processed the same day. Other samples were shipped overnight to Stanford University from Cedars Sinai, Los Angeles, at ambient temperature and then processed immediately. Peripheral blood mononuclear cells were separated by centrifugation (Ficoll-Paque Plus, GE Healthcare, Little Chalfont, United Kingdom) following the manufacturer's directions and preserved in a solution of 10% dimethyl sulfoxide-90% fetal calf serum (FCS) in liquid nitrogen vapor. To perform flow cytometry, samples were thawed and rested for 2 h in complete medium (RPMI 1640 medium with 10% FCS) at 37°C in 5% CO₂ prior to surface staining with a cocktail of fluorochrome-conjugated monoclonal antibodies reactive with human CD3, CD14, CD16, CD19, CD20, CD56, CD31, CD34, and CD45 (BD Biosciences, San Jose, California) (Online Table 1). Samples were then stained (Live-Dead Blue; ThermoFisher, Halethorpe, Maryland), fixed with 2% paraformaldehyde in phosphate-buffered saline (Electron Microscopy Sciences, Hatfield, Pennsylvania) and stored at 4°C until acquisition. Data were acquired using an LSR II instrument (BD Biosciences) running FACSDIVA software in the Shared FACS Facility obtained. Post-acquisition analysis was done using FlowJo (Trestar). Peripheral blood mononuclear cells, initially gated based on light scatter properties and viability staining, were tested for surface expression of CD45 and multiple cell lineage markers (CD3, CD14, CD16, CD19, CD20, CD56). CD31 and CD34 surface expression levels were then further assessed based on those events that were CD45(-) and lineage(-) (Online Figure 1). Events that were CD34(++) and CD31(+) were recorded as endothelial progenitor cells (EPCs) and reported as the percentage of total live cells, which was transformed to log₁₀ values (8,9).

STATISTICAL ANALYSIS. Categorical variables are presented as counts and percentages. Pearson's chi-square test or Fisher's exact test was used for comparisons of categorical variables, as appropriate.



Normality of the continuous variables was confirmed using the Shapiro-Wilk test. Depending on the result of the Levene test for homoscedasticity, variables with normal distribution were compared using Student or Welch *t* test. If the normality test results failed, variables were compared with Mann-Whitney *U* test. For paired comparisons, variables with normal distribution were compared with paired Student *t* test. If the normality test results failed, variables were compared with Wilcoxon signed-rank test. A *p* value of <0.05 was considered statistically significant. Assuming a plaque volume at 1 year of 170 mm³ in the control arm and a plaque volume of 133 mm³ in the ramipril-treated patients with a SD of 60 mm³, a sample size of 42 was required in each arm to have 80% power to detect a significant difference. All analyses were performed using SPSS version 21 software (IBM, Armonk, New York).

RESULTS

Ninety-six patients, including 7 pediatric patients (12 to 19 years of age) were enrolled in the study and randomized to receive ramipril or matching placebo. As outlined in Figure 1, 39 patients in the ramipril arm

TABLE 1 Baseline Recipient and Donor Clinical Characteristics			
	Ramipril (n = 47)	Placebo (n = 49)	p Value
Recipient characteristics			
Age, yrs	55 ± 15	52 ± 17	0.57
Male	35 (74.5)	32 (65.3)	0.33
Body mass index, kg/m ²	25.4 ± 4.2	25.4 ± 6.0	0.57
Comorbidities			
CMV IgG-positive	33 (70.2)	34 (70.8)	0.95
Diabetes	13 (27.7)	14 (28.6)	0.92
Hypertension	27 (57.4)	28 (57.1)	0.98
Systolic blood pressure, mm Hg	134 ± 16	132 ± 12	0.68
Diastolic blood pressure, mm Hg	82 ± 12	84 ± 12	0.40
Hypercholesterolemia*	24 (51.1)	16 (32.7)	0.07
Ischemic cardiomyopathy	10 (21.3)	5 (10.2)	0.14
Laboratory data			
Creatinine, mg/dl	1.14 ± 0.35	1.03 ± 0.36	0.16
BUN, mg/dl	29.0 ± 8.9	30.7 ± 11.8	0.81
Sodium, mmol/l	139 ± 3	138 ± 3	0.29
Potassium, mmol/l	4.3 ± 0.4	4.2 ± 0.5	0.61
Medications			
Calcium-channel blocker	26 (55.3)	27 (55.1)	0.98
Beta-blocker	1 (2.1)	3 (6.1)	0.32
Diuretics	32 (68.1)	28 (57.1)	0.27
Statin	43 (91.5)	46 (93.9)	0.48
Induction therapy			
None	14 (14.7)	8 (8.4)	0.35
γ-antithymocyte globulin	26 (27.4)	32 (33.7)	
Daclizumab	5 (5.3)	6 (6.3)	
Baciliximab	1 (1.1)	3 (3.2)	
Tacrolimus	42 (89.4)	43 (87.8)	0.81
Tacrolimus level, ng/ml	10.8 ± 3.1	12.1 ± 3.9	0.12
Cyclosporine	4 (8.5)	5 (10.2)	0.53
Cyclosporine level, ng/ml	365.6 ± 57.9	401.3 ± 69.6	0.39
Mycophenolate mofetil	47 (100)	49 (100)	NA
Corticosteroid dose, mg	21.1 ± 9.3	25.6 ± 21.8	0.67
Donor characteristics			
Age, yrs	33 ± 14	33 ± 12	0.90
Male	28 (59.6)	32 (66.7)	0.47
Sex mismatch	9 (19.1)	11 (22.9)	0.65
Blood type mismatch	6 (12.8)	6 (12.5)	0.97
CMV IgG mismatch	18 (38.3)	15 (31.3)	0.47
Cold ischemic time, min	219 ± 39	208 ± 50	0.24

Values are mean ± SD or n (%). *Hypercholesterolemia was defined as a patient who was either taking statin or had a diagnosis of hypercholesterolemia by a treating physician before heart transplantation.
BUN = blood urea nitrogen; CMV = cytomegalovirus; IgG = immunoglobulin G.

and 38 patients in the placebo arm completed both baseline and 1-year IVUS examinations. Baseline characteristics of the 2 groups were well matched and are described in **Table 1**. After 1 year, there were no significant adverse effects related to treatment with ramipril or to the matching placebo. Of this group, 1 patient stopped ramipril because of low blood pressure. The mean dose of ramipril was 13.8 ± 7.7

TABLE 2 1-Yr Recipient Clinical Characteristics			
	Ramipril (n = 45)	Placebo (n = 47)	p Value
Comorbidities			
Diabetes	16 (34.0)	18 (36.7)	0.24
Hypertension	32 (68.1)	36 (73.5)	0.64
Systolic blood pressure, mm Hg	125 ± 16	127 ± 15	0.45
Diastolic blood pressure, mm Hg	78 ± 12	80 ± 11	0.31
Laboratory data			
Creatinine, mg/dl	1.29 ± 0.33	1.11 ± 0.30	0.01
BUN, mg/dl	24.5 ± 7.1	20.9 ± 7.5	0.01
Sodium, mmol/l	139 ± 3	140 ± 2	0.21
Potassium, mmol/l	4.3 ± 0.4	4.0 ± 0.3	0.003
Medications			
Calcium-channel blocker	18 (40)	28 (64)	0.026
Beta-blocker	3 (6.4)	2 (4.1)	0.76
Diuretics	12 (25.5)	10 (20.4)	0.74
Statin	41 (87.2)	39 (79.6)	0.45
Tacrolimus	40 (85.1)	39 (79.6)	0.74
Tacrolimus level, ng/ml	8.1 ± 3.6	8.0 ± 3.2	0.86
Cyclosporine	4 (8.5)	3 (6.1)	0.79
Cyclosporine level, ng/ml	155.8 ± 68.0	154.3 ± 109.1	0.98
Mycophenolate mofetil	36 (76.6)	37 (75.5)	0.86
Steroid dose, mg	2.7 ± 3.6	3.4 ± 7.0	0.74

Values are n (%) or mean ± SD.
BUN = blood urea nitrogen.

mg. As outlined in **Table 2**, at 1 year, the systolic and diastolic blood pressures were similar in both groups. There was a significantly lower rate of calcium channel blocker use in the ramipril-treated patients than in the placebo group (40% vs. 64%, respectively; $p = 0.026$). This was primarily because a significantly lower proportion of patients received amlodipine in the ramipril arm than in the placebo group (22.2% vs. 45.5%, respectively; $p = 0.02$) and a significantly lower dose of amlodipine in the ramipril than in the placebo group (1.3 ± 2.9 mg vs. 5.9 ± 18.1 mg, respectively; $p = 0.012$). The blood urea nitrogen and creatinine and potassium levels were all significantly higher in the ramipril group, but no patient had to stop ramipril because of renal dysfunction or hyperkalemia. There were no significant differences between the ramipril and placebo groups with regard to the change in creatinine (0.14 ± 0.31 vs. 0.10 ± 0.29 , respectively; $p = 0.56$) or potassium (0.0 ± 0.6 vs. -0.2 ± 0.5 , respectively; $p = 0.12$). At 1 year, there were no differences in acute cellular rejection (grade $\geq 2R$, diagnosed by right ventricular biopsy) between the ramipril and placebo groups (14.9% vs. 10.2%, respectively; $p = 0.49$).

Serial IVUS examinations demonstrated significant increases in maximum intimal thickness and plaque

volumes and significant decreases in lumen and vessel volumes in both cohorts of patients during the first year (Online Table 2). At baseline and at 1 year, there were no significant differences in IVUS parameters between the 2 groups (Table 3). Plaque volume at 1 year was similar between the patients treated with ramipril and those treated with placebo (162.1 ± 70.5 mm³ vs. 177.3 ± 94.3 mm³, respectively; p = 0.73) (Online Figure 2). The mean changes in plaque volume also were similar between the ramipril and placebo groups (26.7 ± 37.5 mm³ vs. 29.7 ± 56.9 mm³, respectively; p = 0.68). Ramipril did not have a differential effect based on whether the maximal intimal thickness at baseline was greater or less than 0.5 mm. The above results were similar when the 7 pediatric patients were excluded from the analyses.

Serial coronary physiology analyses showed that FFR did not change significantly, whereas CFR increased and IMR tended to decrease for the entire cohort during the first year. Compared to baseline, IMR decreased significantly at 1 year in the ramipril group (21.4 ± 14.7 to 14.4 ± 6.3; p = 0.001) but did not change significantly in the placebo arm (17.4 ± 8.4 to 21.5 ± 20.0; p = 0.72). Coronary flow reserve increased significantly in the ramipril group (3.8 ± 1.7 to 4.8 ± 1.5; p = 0.017) but did not change in the placebo arm (4.1 ± 1.8 to 4.1 ± 2.2; p = 0.60). Finally, FFR decreased significantly in the ramipril group (0.90 ± 0.06 to 0.88 ± 0.04; p = 0.007) but did not change in the placebo arm (0.89 ± 0.04 to 0.90 ± 0.04; p = 0.39) (Figure 2, Table 4). There were no differences in endothelial function at baseline or at 1 year between the 2 groups; nor were there any changes in endothelial function from baseline to 1 year in the 2 groups. The log₁₀ percentages of circulating EPCs were similar at baseline between the 2 groups. In the ramipril group, this value did not change significantly at 1 year, whereas in the control group, there was a significant decline in circulating EPCs at 1 year compared to the baseline value (Figure 3, Table 4). At 1 year, there was a trend toward lower circulating EPCs in the control group than in the ramipril group (-1.19 ± 0.41 vs. -1.12 ± 0.32, respectively; p = 0.17).

DISCUSSION

Our main findings regarding the use of the ACEI ramipril early after HT are ramipril seems to be safe and effective at lowering blood pressure; use of ramipril did not significantly affect plaque progression based on IVUS examination; and use of ramipril improved microvascular function based on IMR and CFR assessment during the first year after HT (Central Illustration).

TABLE 3 Baseline and 1-Yr IVUS Indices

	Ramipril (n = 43)	Placebo (n = 44)	p Value
Baseline			
Maximal intimal thickness, mm	0.74 ± 0.44	0.72 ± 0.45	0.60
Plaque volume, mm ³	135.4 ± 58.2	147.5 ± 70.4	0.42
Plaque volume, mm ³ /mm*	2.8 ± 1.2	3.2 ± 1.6	0.35
% Plaque volume	19.9 ± 7.8	20.3 ± 9.2	0.95
Lumen volume, mm ³	558.7 ± 164.5	596.8 ± 183.0	0.31
Lumen volume, mm ³ /mm*	11.5 ± 3.0	12.5 ± 3.2	0.15
Vessel volume, mm ³	694.0 ± 185.8	744.3 ± 210.7	0.29
Vessel volume, mm ³ /mm*	14.3 ± 3.3	15.6 ± 3.7	0.14
1 yr			
Maximal intimal thickness, mm	0.89 ± 0.49	0.91 ± 0.52	0.90
Plaque volume, mm ³	162.1 ± 70.5	177.3 ± 94.3	0.73
Plaque volume, mm ³ /mm*	3.4 ± 1.7	3.7 ± 1.9	0.55
% Plaque volume	25.2 ± 12.0	24.3 ± 9.9	0.87
Lumen volume, mm ³	503.3 ± 158.4	542.4 ± 176.3	0.46
Lumen volume, mm ³ /mm*	10.2 ± 3.1	11.2 ± 3.4	0.21
Vessel volume, mm ³	665.4 ± 162.3	719.7 ± 218.2	0.21
Vessel volume, mm ³ /mm*	13.6 ± 3.2	14.8 ± 4.2	0.22

Values are mean ± SD. *Plaque, lumen, and vessel volumes were standardized for analyzed longitudinal length (mm³/mm).
 IVUS = intravascular ultrasonography; includes patients without serial IVUS assessment.

Ramipril was well tolerated by transplant patients, even when therapy started within the first few weeks after transplantation. There were no cases of acute renal failure or hyperkalemia requiring discontinuation of the ACEI. Ramipril was also effective at

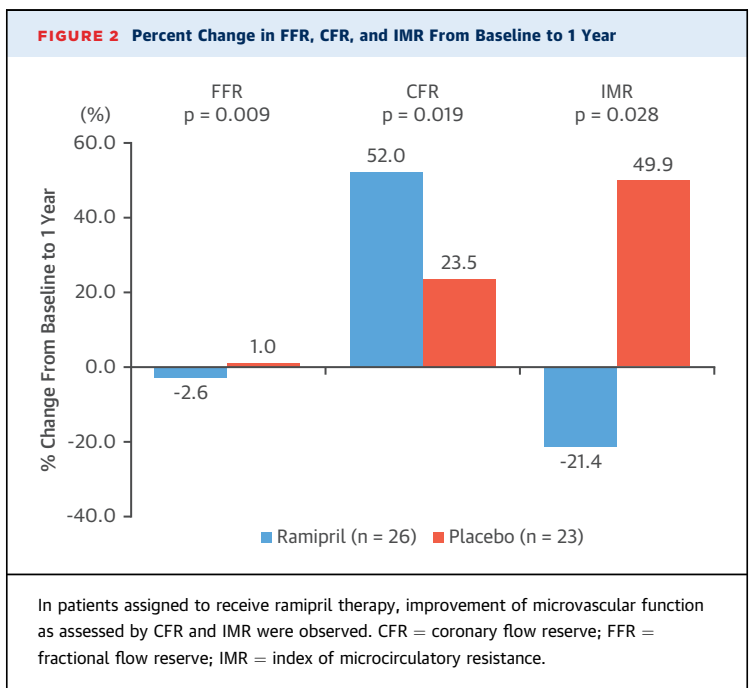


TABLE 4 Serial Coronary Physiology, Endothelial Function, and Endothelial Progenitor Cell Data

	Baseline	1 Yr	p Value
Ramipril (n = 26)			
FFR	0.90 ± 0.06	0.88 ± 0.04	0.007
CFR	3.8 ± 1.7	4.8 ± 1.5	0.017
IMR	21.4 ± 14.7	14.4 ± 6.3	0.001
Placebo (n = 23)			
FFR	0.89 ± 0.04	0.90 ± 0.04	0.39
CFR	4.1 ± 1.8	4.1 ± 2.2	0.60
IMR*	17.4 ± 8.4	21.5 ± 20.0	0.72
Ramipril (n = 25)			
Endothelial dysfunction, %	6.7 ± 20.5	9.4 ± 25.6	0.63
Placebo (n = 21)			
Endothelial dysfunction, %	9.3 ± 21.1	5.6 ± 27.8	0.39
Ramipril (n = 34)			
Log ₁₀ % EPC	-1.15 ± 0.55	-1.12 ± 0.32	0.66
Placebo (n = 38)			
Log ₁₀ % EPC	-1.05 ± 0.45	-1.19 ± 0.41	0.035

Values are mean ± SD. *Serial IMR assessment was possible in 22 patients. Values are smaller because only Stanford patients underwent physiological and endothelial function assessments.
CFR = coronary flow reserve; EPC = endothelial progenitor cell; FFR = fractional flow reserve; IMR = index of microcirculatory resistance.

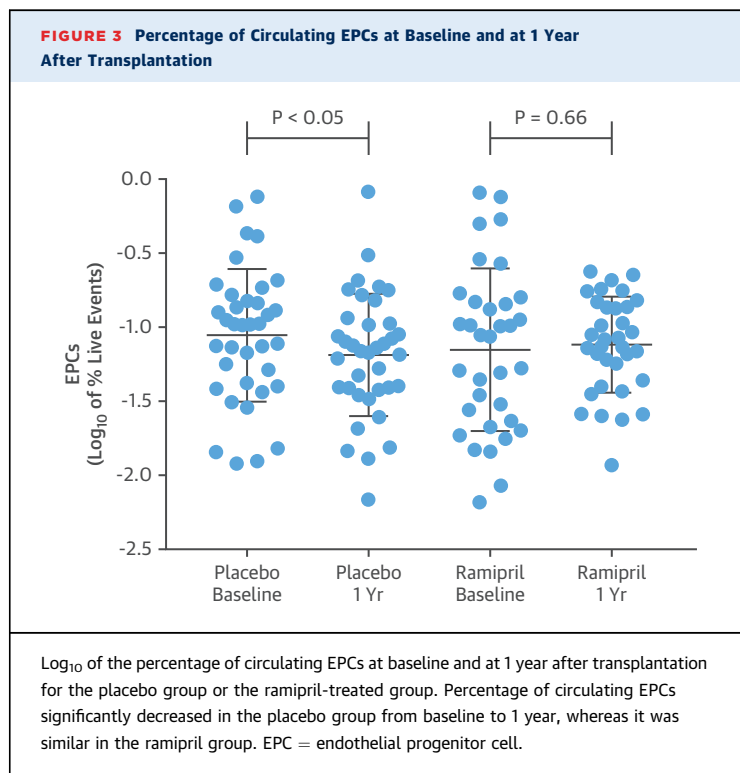
lowering blood pressure, as the ramipril-treated patients required significantly less antihypertensive medication than the placebo group. These findings have clinical relevance because of previous concern regarding the tolerability and effectiveness of ACEIs

after HT (10). Findings suggest that ACEIs can be considered a reasonable antihypertensive agent in this population and may have ancillary beneficial effects on the cardiovascular system and renal system with longer-term use, as demonstrated in nontransplant populations.

The renin angiotensin system has been implicated in the development of native coronary atherosclerosis, as was CAV (11). ACEIs inhibit the renin angiotensin system and decrease angiotensin II, reduce oxidative stress, inhibit smooth muscle cell proliferation, and improve fibrinolysis, all of which likely contributes to their antiatherogenic properties. Previous single-center and retrospective studies suggested that ACEIs reduce coronary plaque development after cardiac transplantation (3-5). For example, a small, nonrandomized study comparing the degree of CAV at 1 year after HT based on IVUS measurement of intimal thickness showed that patients receiving an ACEI had less CAV than similar controls (3). In a retrospective comparison of transplant recipients treated with ACEI, calcium channel blocker or both, an ACEI alone appeared to be better than a calcium channel blocker with respect to development of CAV, and the combination of ACEI and calcium channel blocker appeared to be best (4). In another retrospective evaluation, 22 transplant recipients with plaque progression based on serial IVUS evaluation were less likely to have been treated with an ACEI than 17 patients without plaque regression (5). However, those studies were not prospective, randomized or blinded and therefore might have been subject to bias.

In the present prospective trial, a significant increase in plaque volume, percentage of plaque volume, and maximal intimal thickness occurred from baseline to 1 year in both arms, with no apparent protective effect conferred by ramipril. There was concomitant loss of lumen due to a combination of plaque progression and negative vessel remodeling, which occurred to a similar degree in both arms.

Interestingly, although there was no effect on the epicardial anatomy based on IVUS examination in this study, treatment with ramipril did significantly improve microvascular function. Index of microcirculatory resistance (an index specific for coronary microvasculature) and CFR (an index that interrogates the entire coronary circulation, both the microcirculation and the epicardial vessel) improved significantly in the ramipril-treated patients from baseline to 1 year, whereas there were no significant changes in the placebo-treated patients (Figure 2). fractional flow reserve (an index that independently assesses the functional significance of epicardial CAV) decreased



CENTRAL ILLUSTRATION Effects of ACE Inhibition Early After Heart Transplantation

Effects of ACEI Ramipril Early After Heart Transplantation



Improves microvascular function based on improvement in IMR and CFR assessment



Appears safe and effective at lowering blood pressure



Improves the number and function of circulating endothelial progenitor cells, compared to placebo



Does not significantly affect plaque progression or endothelial function

Fearon, W.F. et al. *J Am Coll Cardiol.* 2017;69(23):2832-41.

Key positive and negative effects of the angiotensin-converting enzyme inhibitor (ACEI) ramipril are shown when administered early after heart transplantation. CFR = coronary flow reserve; IMR = index of microcirculatory resistance.

significantly from baseline to 1 year in the ramipril-treated patients, whereas it did not change in the placebo group. Because plaque progression was similar in the 2 treatment arms, the numerically small but statistically significant decrease in FFR in the ramipril group is likely a reflection of improved microvascular function in this group at 1 year, which allowed greater hyperemic flow, a larger pressure gradient, and a lower FFR, despite the similar plaque volume.

The improved microvascular function at 1 year in the ramipril-treated patients is clinically relevant because we demonstrated in a prior study that transplant recipients with low IMR (<20) measured at 1 year and those with a decrease or no change in IMR from baseline to 1 year after transplantation had significantly lower rates of long-term mortality or the need for re-transplantation (12). In addition, the IMR measurement at 1 year was an independent predictor of death or re-transplantation, whereas IVUS parameters were not, suggesting that microvascular function may be a more important indicator of long-term outcome as compared to epicardial plaque. Moreover, in another study IMR was found to predict development of CAV and graft dysfunction (13). In nontransplant recipients, IMR has also been found to be useful in identifying the cause of chest pain in patients with nonobstructive epicardial coronary disease and in predicting poor recovery of left ventricular function and mortality when measured after primary percutaneous coronary intervention in patients with ST-segment elevation myocardial infarction (14,15).

Despite having this positive effect on microvascular function, ramipril did not appear to improve endothelial function, as the percentage of patients with endothelial dysfunction was similar between the 2 groups. These findings contradict a previous report which found that short-term intracoronary infusion of the ACEI quinaprilat to HT recipients resulted in improved endothelial function based on improved epicardial artery diameter and flow (16). The discordant finding may be explained by the delivery method, the duration of exposure, and/or the ACEI used in the 2 studies.

The role of circulating EPCs in the development of CAV remains unclear. In a clinical study evaluating this topic, Simper et al. (17) found significantly decreased numbers of circulating EPCs in transplant recipients with angiographic evidence of CAV compared to those in matched recipients without evidence of CAV. In the nontransplant population, ACEI therapy may increase the number and function of circulating EPCs. For example, after 4 weeks of ramipril therapy in patients with stable coronary artery disease, the ACEI-treated patients demonstrated a >2-fold increase in circulating numbers of EPCs, with improved proliferative and migratory capacity (18). In the present study, we observed a significant decline in the level of EPCs at 1 year after transplantation in the placebo group, whereas the levels at baseline were similar to those at 1 year in the ramipril group. This raises the possibility that ramipril treatment may be beneficial for microvascular function following cardiac allograft

transplantation, at least in part, by stabilizing circulating EPC number and function.

STUDY LIMITATIONS. Limitations of this study include the sample size; however, our study was powered to detect a 20% difference in plaque volume at 1 year, and we were able to detect significant differences in microvascular function. Another limitation of this study is the relatively short duration of therapy with ramipril, although other therapies such as calcium channel blockade and statin administration showed benefit during the first year of treatment (19,20). It is unlikely that the dose of ramipril was too low, particularly given the adequate blood pressure control, the changes in potassium and creatinine seen, and the beneficial effects on the microvasculature suggesting a physiological effect. Additionally, the dosage achieved was similar to that in previous trials showing a benefit of ramipril (21). Why ramipril improved microvascular function without decreasing epicardial plaque remains unclear. It may be that the positive effects of ACEIs on oxidative stress, endothelial function, smooth muscle cell proliferation, and fibrinolysis selectively benefit the microvasculature. Based on this study, we cannot conclude whether this is a class effect associated with all ACEIs or unique to ramipril.

CONCLUSIONS

Ramipril seems to be a safe and effective antihypertensive agent early after HT. It does not

significantly affect plaque progression during the first year, but it does stabilize circulating endothelial progenitor cell levels and improve microvascular function, which has been associated with improved long-term survival after HT. Further study of the longer term clinical effects of ACEIs in this population is warranted.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The angiotensin-converting enzyme inhibitor ramipril is a safe and effective antihypertensive agent that stabilizes circulating endothelial progenitor cell levels and improves coronary microvascular function early after heart transplantation but does not prevent thickening of the epicardial coronary artery intima.

TRANSLATIONAL OUTLOOK: Further research is needed to understand the interactions between endothelial progenitor cell levels and microvascular function after cardiac transplantation.

REFERENCES

- Lund LH, Edwards LB, Kucheryavaya AY, et al. The registry of the International Society for Heart and Lung Transplantation: thirty-second official adult heart transplantation report—2015: focus theme: early graft failure. *J Heart Lung Transplant* 2015;34:1244-54.
- Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin-converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006;368:581-8.
- Mehra MR, Ventura HO, Smart FW, Collins TJ, Ramee SR, Stapleton DD. An intravascular ultrasound study of the influence of angiotensin-converting enzyme inhibitors and calcium entry blockers on the development of cardiac allograft vasculopathy. *Am J Cardiol* 1995;75:853-4.
- Erinc K, Yamani MH, Starling RC, et al. The effect of combined angiotensin-converting enzyme inhibition and calcium antagonism on allograft coronary vasculopathy validated by intravascular ultrasound. *J Heart Lung Transplant* 2005;24:1033-8.
- Bae JH, Rihal CS, Edwards BS, et al. Association of angiotensin-converting enzyme inhibitors and serum lipids with plaque regression in cardiac allograft vasculopathy. *Transplantation* 2006;82:1108-11.
- De Bruyne B, Pijls NH, Smith L, Wievegg M, Heyndrickx GR. Coronary thermodilution to assess flow reserve: experimental validation. *Circulation* 2001;104:2003-6.
- Ng MK, Yeung AC, Fearon WF. Invasive assessment of the coronary microcirculation: superior reproducibility and less hemodynamic dependence of index of microcirculatory resistance compared with coronary flow reserve. *Circulation* 2006;113:2054-61.
- Shim Y, Nam MH, Hyuk SW, Yoon SY, Song JM. Concurrent hypermulticolor monitoring of CD31, CD34, CD45 and CD146 endothelial progenitor cell markers for acute myocardial infarction. *Anal Chim Acta* 2015;853:501-7.
- Lachmann R, Lanuti P, Miscia S. OMIP-011: characterization of circulating endothelial cells (CECs) in peripheral blood. *Cytometry A* 2012;81:549-51.
- Brozena SC, Johnson MR, Ventura H, et al. Effectiveness and safety of diltiazem or lisinopril in treatment of hypertension after heart transplantation. Results of a prospective, randomized multicenter trial. *J Am Coll Cardiol* 1996;27:1707-12.
- Yousufuddin M, Yamani MH. The renin-angiotensin hypothesis for the pathogenesis of cardiac allograft vasculopathy. *Int J Cardiol* 2004;95:123-7.
- Yang HM, Khush K, Luikart H, et al. Invasive assessment of coronary physiology predicts late mortality after heart transplantation. *Circulation* 2016;133:1945-50.
- Haddad F, Khazanie P, Deuse T, et al. Clinical and functional correlates of early microvascular dysfunction following heart transplantation. *Circ Heart Fail* 2012;5:759-68.
- Lee BK, Lim HS, Fearon WF, et al. Invasive evaluation of patients with angina in the absence of obstructive coronary artery disease. *Circulation* 2015;131:1054-60.
- Fearon WF, Low AF, Yong AS, et al. Prognostic value of the index of microcirculatory resistance measured after primary percutaneous coronary intervention. *Circulation* 2013;127:2436-41.
- Steinhauff S, Pehlivanli S, Bakovic-Alt R, et al. Beneficial effects of quinaprilat on coronary vasomotor function, endothelial oxidative stress, and endothelin activation after human heart transplantation. *Transplantation* 2004;77:1859-65.

17. Simper D, Wang S, Deb A, et al. Endothelial progenitor cells are decreased in blood of cardiac allograft patients with vasculopathy and endothelial cells of noncardiac origin are enriched in transplant atherosclerosis. *Circulation* 2003;108:143-9.

18. Min TQ, Zhu CJ, Xiang WX, Hui ZJ, Peng SY. Improvement in endothelial progenitor cells from peripheral blood by ramipril therapy in patients with stable coronary artery disease. *Cardiovasc Drugs Ther* 2004;18:203-9.

19. Schroeder JS, Gao SZ, Alderman EL, et al. A preliminary study of diltiazem in the prevention of coronary artery disease in heart-transplant recipients. *N Engl J Med* 1993;328:164-70.

20. Kobashigawa JA, Katznelson S, Laks H, et al. Effect of pravastatin on outcomes after cardiac transplantation. *N Engl J Med* 1995;333:621-7.

21. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular

events in high-risk patients. *N Engl J Med* 2000;342:145-53.

KEY WORDS cardiac allograft vasculopathy, coronary physiology, microcirculation

APPENDIX For supplemental tables and figures, please see the online version of this article.