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—We 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 ramipril or placebo. They underwent coronary angiography, endothelial function testing, measurement of fractional flow reserve (FFR), coronary flow reserve (CFR) and the index of microcirculatory resistance (IMR), and intravascular ultrasound (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.

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RESULTS—Plaque volume at 1 year was similar between the ramipril and placebo groups (162.1 ± 70.5 vs. 177.3 ± 94.3 mm^3, p =0.73). Patients receiving ramipril had improvement in microvascular function as evidenced 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.

CONCLUSION—Ramipril does not slow development of epicardial plaque volume but does stabilize EPC levels and improve microvascular function, which have been associated with improved long-term survival after HT.

Keywords

Cardiac allograft vasculopathy; Microcirculation; Coronary physiology

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 non-transplant patients independent of antihypertensive effects, presumably by improving endothelial function, decreasing inflammation, and reducing oxidative stress, all of which stabilize and reduce plaque development (2). Previous single center and retrospective studies have suggested that ACEIs decrease coronary plaque development after cardiac transplantation, however, 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.

METHODS

STUDY DESIGN

Consecutive HT recipients at Stanford University, the Palo Alto Veterans Affairs Health Care System, and Cedars Sinai Medical Center who were 12 years old and had a serum creatinine <2.0 mg/dL were included. Patients were excluded if they were undergoing more than 1 solid organ transplant or repeat HT. Within 8 weeks of HT, patients were randomized in an equal and double-blind fashion to either ramipril or to matching placebo. These 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 ultrasound (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 endpoint of the study was the difference in plaque volume as assessed by intravascular ultrasound (IVUS) examination of the LAD at 1 year between those the ramipril and placebo groups. Key secondary endpoints included the safety and efficacy of

ramipril administration early after HT, the 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 (age 18 years old) received induction therapy with methylprednisolone 500 mg IV when coming off bypass and rATG at 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 hours. 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 (1000 mg twice daily) in both adults and pediatrics 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 HMG-CoA reductase inhibitor (pravastatin) was initiated in all patients at 20 mg per day within 1 week of transplant 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 mmHg. 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 remained on valganciclovir 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 per day 1 week after baseline coronary angiography. Two weeks later, if the serum creatinine was 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 the

adult patients. The dose was titrated to a maximum of 20 mg per day in adult patients if a serum creatinine 2 weeks later was stable and there were no adverse sequelae. If a subject developed a side effect felt 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 upwards as necessary. A beta-blocker or dihydropyridine calcium antagonist was added if the blood pressure was not controlled with the above medications. Patients had routine blood pressure evaluation 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 mmHg 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 the Palo Alto Veterans Affairs Health Care System. Using a 6 French guiding catheter, baseline coronary angiography of the LAD without panning the table was performed in a cranial projection. Intracoronary acetylcholine (20 mcg) was administered over 30 seconds. An angiogram of the LAD was recorded. A second bolus of intracoronary acetylcholine (50 mcg) was administered over 30 seconds. Another angiogram of the LAD was recorded. Intracoronary nitroglycerin (100 to 200 mcg) was administered and a final angiogram of the LAD 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 at baseline and after injection were done with an automatic edge detection system (QAngio XA 7.3®; MEDIS, Leiden, Netherlands). Endothelial dysfunction was defined as 20% decrease in the LAD diameter after acetylcholine infusion compared with baseline.

CORONARY PHYSIOLOGY ASSESSMENT

Coronary physiology assessment was performed in the adult patients enrolled from Stanford University and the Palo Alto Veterans Affairs Health Care System. After endothelial function assessment and administration of intracoronary nitroglycerin, intravenous heparin (60 units/kg) was administered. A coronary pressure wire (Certus, St. Jude Medical) 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. 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 by the 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 mcg/kg/min) was then administered. Once maximal hyperemia was achieved, 3 injections of approximately 3 ml of room temperature saline were performed and the hyperemic mean transit time was calculated as described above. Coronary flow reserve (CFR) was calculated as the resting mean transit time divided by the hyperemic mean transit time; the index of microcirculatory resistance (IMR) was calculated as the hyperemic mean transit time multiplied by the hyperemic distal coronary pressure; and fractional flow reserve (FFR) was calculated as the mean distal pressure divided by the mean proximal pressure during maximal hyperemia (7).

INTRAVASCULAR ULTRASOUND

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. A recording was performed 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 Laboratory, Stanford, California) blinded to the patient's clinical and randomization information, using commercially available 3-dimensional 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. For volumetric analyses, lumen and vessel volumes were calculated using Simpson's rule and divided by the axial analyzed length (mm^3) to adjust for any differences of analyzed longitudinal lengths among the cases. Plaque volume was defined as vessel volume – lumen volume; percent plaque volume (%) was defined as 100 x plaque volume/vessel volume. Maximal intimal thickness (MIT) was also obtained from the interpolated data set as a 2-dimensional 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 via centrifugation over Ficoll-Paque Plus (GE Healthcare) following the manufacturer's directions and preserved in 10% DMSO/90% fetal calf serum (FCS) in liquid nitrogen vapor. To perform flow cytometry, samples were thawed and rested for 2 hours in complete media (RPMI 1640 with10%FCS) at 37°C and 5% CO_2 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) (Online Table 1). Samples were then stained with LiveDead Blue (ThermoFisher), fixed with 2% paraformaaldehyde in phosphate-buffered saline (Electron Microscopy Sciences) and stored at 4°C until acquisition. Data was acquired on LSR II instrument (BD Biosciences) running FACSDIVA software in the Shared FACS Facility obtained. Post-acquisition analysis was done using FlowJo (Treestar). PBMCs, initially gated 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 was then further assessed on those events that were CD45(−) and lineage(−) (Supplemental Figure 1). Events that were CD34(++) and CD31(+) were recorded as EPCs and reported as a percent of total live cells, which were transformed to $\log_{10}$ 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 with the Shapiro-Wilk test. Depending on the result of the Levene test for homoscedasticity, variables with normal distribution were compared with Student t-test or Welch t-test. If the normality test failed, variables were compared with Mann-Whitney U test. For paired comparisons, variables with normal distribution were compared with paired t-test. If the normality test 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 mm^3 in the control arm and a plaque volume of 133 mm^3 in the ramipril treated patients with a standard deviation of 60 mm^3, 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.

RESULTS

Ninety-six patients, including 7 pediatric patients (12 to 19 years old) were enrolled into the study and randomized to ramipril or matching placebo. As outlined in Figure 1, 39 patients in the ramipril arm 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 dosage of ramipril was 13.8 ± 7.7 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 as compared to the placebo group (40 vs. 64%, p =0.026). This was primarily because a significantly lower proportion of patients who received amlodipine in the ramipril compared to the placebo group (22.2% vs. 45.5%, respectively; p =0.02), as well as a significantly lower dose of amlodipine in the ramipril versus the placebo group (1.3 ± 2.9 vs. 5.9 ± 18.1 mg, respectively; p =0.012). The blood urea nitrogen, 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 was no significant difference 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 was no difference in acute cellular rejection (Grade 2R diagnosed by right ventricular biopsy) between the ramipril and placebo groups (14.9 vs. 10.2%, p =0.49).

Serial IVUS examination demonstrated significant increases in maximum intimal thickness and plaque volume and significant decreases in lumen and vessel volume in both cohorts of patients during the first year (Online Table 2). At baseline there were no significant

differences in IVUS parameters between the 2 groups; at 1 year there were also no significant differences (Table 3). Plaque volume at 1 year was similar between the patients treated with ramipril versus placebo (162.1 ± 70.5 vs. 177.3 ± 94.3 mm^3 respectively; p =0.73) (Online Figure 2). The mean change in plaque volume also was similar between the ramipril and placebo groups (26.7 ± 37.5 vs. 29.7 ± 56.9 mm^3 respectively; p =0.68). Ramipril did not have a differential effect based on whether the MIT 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, while 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). CFR 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 was no difference in endothelial function at baseline or at 1 year between the 2 groups; nor was there any difference in change of endothelial function from baseline to 1 year in the 2 groups. The $\log_{10}$ percentage of circulating EPCs was 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 towards lower circulating EPCs in the control group compared to 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 that 1) the use of ramipril appears safe and effective at lowering blood pressure; 2) the use of ramipril did not significantly affect plaque progression based on IVUS examination; and 3) the use of ramipril improved microvascular function based on IMR and CFR assessment during the first year after HT (Central Illustration).

Ramipril was well tolerated by transplant patients, even when 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 lowering blood pressure, as the ramipril-treated patients required significantly less antihypertensive medication compared to the placebo group. These findings have clinical relevance because of previous concern regarding the tolerability and effectiveness of ACEIs after HT (10). They 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 non-transplant populations.

The renin angiotensin system has been implicated in the development of native coronary atherosclerosis, as well as 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 contribute 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 compared to similar controls (3). In a retrospective comparison of transplant recipients treated with ACE I, 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 compared to 17 patients without plaque regression (5). However, these studies were not prospective, randomized or blinded, and therefore may have been subject to bias.

In this prospective trial, a significant increase in plaque volume, % 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. IMR (an index specific for the 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, while there was no significant change in the placebo-treated patients (Figure 2). FFR (an index that independently assesses the functional significance of epicardial CAV) decreased significantly from baseline to 1 year in the ramipril treated patients, while 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 retransplantation (12). In addition, the IMR measurement at 1 year was an independent predictor of death or retransplantation, while 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 non-transplant recipients IMR has also been found to be useful in identifying the cause of chest pain in patients with non-obstructive 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 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. found significantly decreased numbers of circulating EPCs in transplant recipients with angiographic evidence of CAV compared to matched recipients without evidence of CAV (17). In the non-transplant population, ACEI therapy may increase the number and the 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 greater than 2-fold increase in circulating EPC number, 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 transplant in the placebo group, whereas the levels at baseline and at 1 year were similar 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.

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 physiologic effect. Additionally, the dosage achieved was similar to 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 appears to be a safe and effective antihypertensive 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 are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

ACEI	Angiotensin Converting Enzyme Inhibitor
CAV	Cardiac Allograft Vasculopathy
CFR	Coronary Flow Reserve
EPC	Endothelial Progenitor Cell
FFR	Fractional Flow Reserve
IMR	Index of Microcirculatory Resistance
IVUS	Intravascular Ultrasound
LAD	Left Anterior Descending
HT	Heart Transplantation

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CONDENSED ABSTRACT

In this prospective, multicenter, randomized placebo controlled trial we tested the safety and efficacy of the angiotensin converting enzyme inhibitor, ramipril in 96 patients early after heart transplantation (HT). We found that ramipril was safe and effective, but did not slow coronary artery plaque progression during the first year after HT. However, ramipril did stabilize circulating endothelial progenitor cell number and did lead to significant improvement in microvascular function, which has been associated with improved long-term survival after HT.

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.
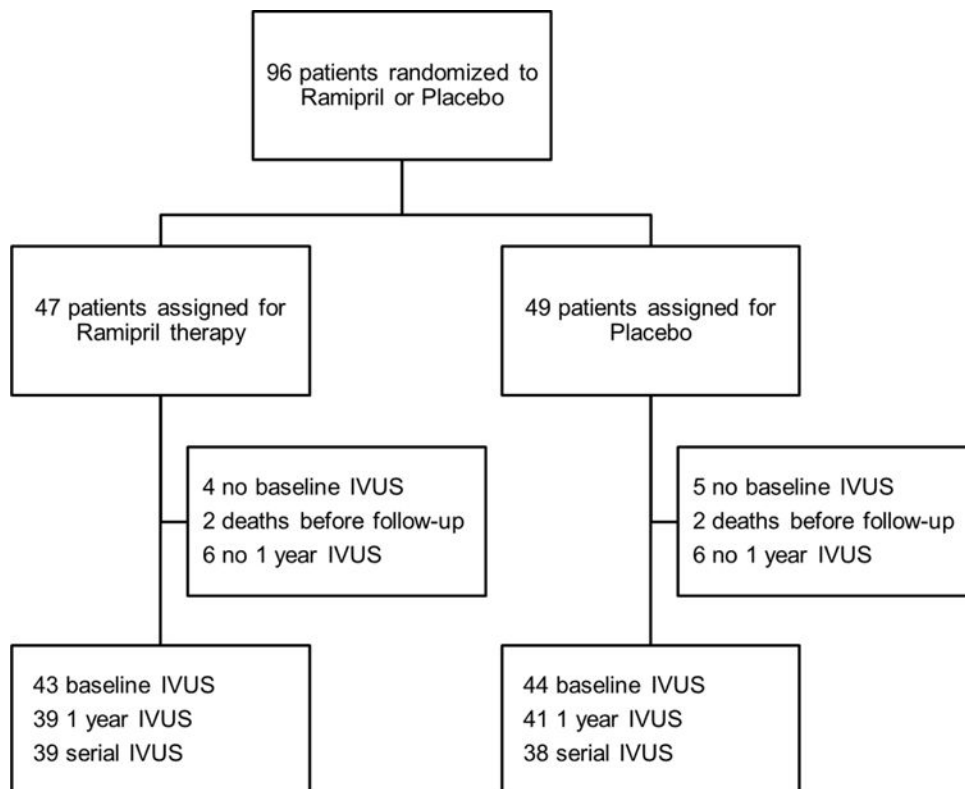
Figure 1. Enrollment flowchart

A total of 96 patients were randomized to ramipril (n = 47) or placebo (n = 49).
Comparisons of baseline IVUS data were performed between 43 patients in the ramipril
group and 44 patients in the placebo group where IVUS images were analyzable.
Comparisons of 1 year IVUS data were performed between 39 patients in the ramipril group
and 41 patients in the placebo group where IVUS images were available (survived at 1 year)
and analyzable. In patients both baseline and 1 year IVUS were analyzed, we performed
paired comparisons within the group (39 serial IVUS cases in the ramipril group and 38
serial IVUS cases in the placebo group). IVUS = Intravascular Ultrasound.
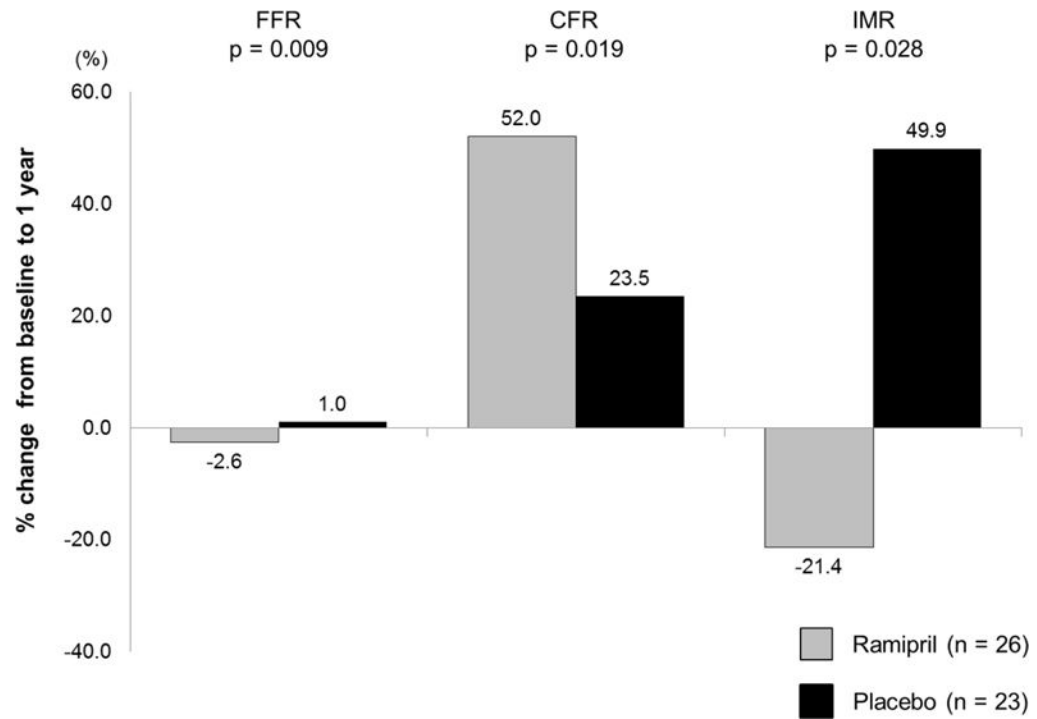
Figure 2. Percent change in FFR, CFR and IMR from baseline to 1 year
In patients assigned to the ramipril therapy, improvement of microvascular function as assessed by CFR and IMR were observed. CFR = Coronary Flow Reserve; FFR = Fractional Flow Reserve; IMR = Index of Microcirculatory Resistance.
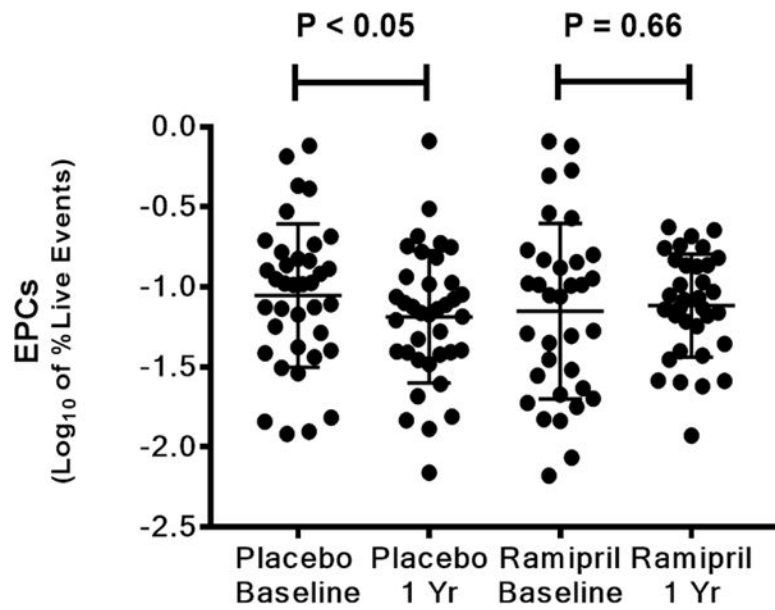
Figure 3. Log$_{10}$ of the percentage of circulating EPCs at baseline and at 1 year after transplant for the placebo group or the ramipril-treated group

Percentage of circulating EPCs significantly decreased in the placebo group from baseline to 1 year whereas it was similar in the ramipril group. EPC = Endothelial Progenitor Cell.
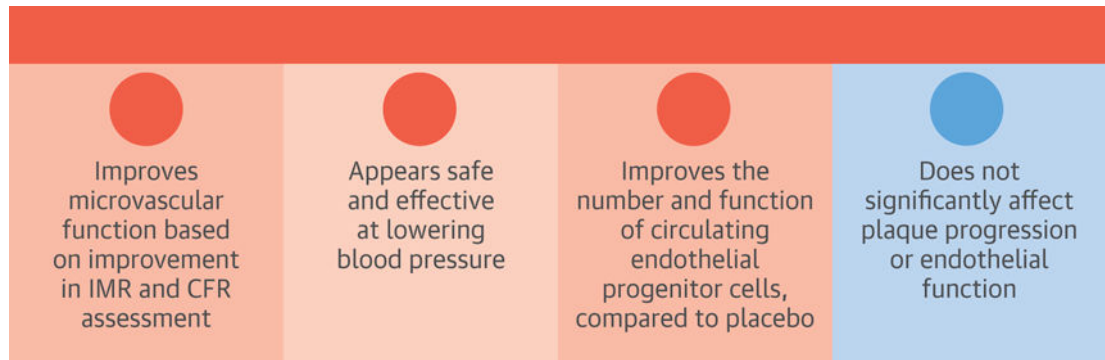
Central Illustration. Effects of ACE Inhibition Early After Heart Transplantation
Key positive and negative effects of the ACEI ramipril when administered early after heart transplantation.

Table 1

Baseline Recipient and Donor Clinical Characteristics

Variable	Ramipril n = 47	Placebo n = 49	p value
Recipient characteristics:			
Age, years	55 ± 15	52 ± 17	0.57
Male, n (%)	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, n (%)	33 (70.2)	34 (70.8)	0.95
Diabetes, n (%)	13 (27.7)	14 (28.6)	0.92
Hypertension, n (%)	27 (57.4)	28 (57.1)	0.98
Systolic blood pressure (mmHg)	134 ± 16	132 ± 12	0.68
Diastolic blood pressure (mmHg)	82 ± 12	84 ± 12	0.40
*Hypercholesterolemia, n (%)	24 (51.1)	16 (32.7)	0.07
Ischemic cardiomyopathy, n (%)	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, n (%)	26 (55.3)	27 (55.1)	0.98
β-blocker, n (%)	1 (2.1)	3 (6.1)	0.32
Diuretics, n (%)	32 (68.1)	28 (57.1)	0.27
Statin, n (%)	43 (91.5)	46 (93.9)	0.48
Induction therapy			0.35
None	14 (14.7)	8 (8.4)	
γ-antithymocyte globulin, n (%)	26 (27.4)	32 (33.7)	
Daclizumab, n (%)	5 (5.3)	6 (6.3)	
Baciliximab, n (%)	1 (1.1)	3 (3.2)	
Tacrolimus, n (%)	42 (89.4)	43 (87.8)	0.81
Tacrolimus level, ng/mL	10.8 ± 3.1	12.1 ± 3.9	0.12
Cyclosporine, n (%)	4 (8.5)	5 (10.2)	0.53
Cyclosporine level, ng/mL	365.6 ± 57.9	401.3 ± 69.6	0.39
Mycophenolate mofetil, n (%)	47 (100)	49 (100)	NA
Corticosteroid dose, mg	21.1 ± 9.3	25.6 ± 21.8	0.67
Donor characteristics:			
Age, years	33 ± 14	33 ± 12	0.90
Male, n (%)	28 (59.6)	32 (66.7)	0.47
Sex mismatch, n (%)	9 (19.1)	11 (22.9)	0.65
Blood type mismatch, n (%)	6 (12.8)	6 (12.5)	0.97

Variable	Ramipril n = 47	Placebo n = 49	p value
CMV IgG mismatch, n (%)	18 (38.3)	15 (31.3)	0.47
Cold ischemic time, minutes	219 ± 39	208 ± 50	0.24

Values are mean and standard deviation or n (%).

*
Hypercholesterolemia was defined as patient either taking statin or diagnosed as hypercholesterolemia by a treating physician before heart transplantation. BUN = blood urea nitrogen; CMV = cytomegalovirus; IgG = immunoglobulin G.

Table 2

One-Year Recipient Clinical Characteristics

Variable	Ramipril n = 45	Placebo n = 47	p value
Comorbidities:			
Diabetes, n (%)	16 (34.0)	18 (36.7)	0.24
Hypertension, n (%)	32 (68.1)	36 (73.5)	0.64
Systolic blood pressure, mmHg	125 ± 16	127 ± 15	0.45
Diastolic blood pressure, mmHg	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, n (%)	18 (40)	28 (64)	0.026
β-blocker, n (%)	3 (6.4)	2 (4.1)	0.76
Diuretics, n (%)	12 (25.5)	10 (20.4)	0.74
Statin, n (%)	41 (87.2)	39 (79.6)	0.45
Tacrolimus, n (%)	40 (85.1)	39 (79.6)	0.74
Tacrolimus level, ng/mL	8.1 ± 3.6	8.0 ± 3.2	0.86
Cyclosporine, n (%)	4 (8.5)	3 (6.1)	0.79
Cyclosporine level, ng/mL	155.8 ± 68.0	154.3 ± 109.1	0.98
Mycophenolate mofetil, n (%)	36 (76.6)	37 (75.5)	0.86
Steroid dose, mg	2.7 ± 3.6	3.4 ± 7.0	0.74

Values are mean and standard deviation or n (%). BUN = blood urea nitrogen.

Table 3

Baseline and 1-year IVUS indices.

Variable	Ramipril	Placebo	p value
Baseline	(n = 43)	(n = 44)	
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	031
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
One year	(n = 39)	(n = 41)	
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 and standard deviation.

[*] Plaque, lumen, and vessel volumes were standardized for analyzed longitudinal length (mm^3/mm). IVUS = intravascular ultrasound. Includes patients without serial IVUS assessment.

Table 4

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

Variable	Baseline	One year	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 and standard deviation.

[*] Serial IMR assessment was possible in 22 patients. Numbers are smaller because only Stanford patients underwent physiology and endothelial function assessment. FFR = fractional flow reserve, CFR = coronary flow reserve, IMR = index of microcirculatory resistance, EPC = Endothelial Progenitor Cell.