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Aldosterone inhibition and coronary endothelial function in women without obstructive coronary artery disease: An ancillary study of the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE)

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Abstract

Background—Endothelial dysfunction is highly prevalent and associated with adverse outcomes among patients without obstructive coronary artery disease (CAD). Angiotensin II inhibition may improve endothelial function, but with continued treatment “aldosterone escape” may occur. Thus it is unknown if adding aldosterone blockade further improves endothelial function.

Methods—In a double-blind, parallel-group, repeated measures study, women with symptoms and signs of ischemia, no significant CAD, and coronary endothelial dysfunction receiving an angiotensin converting enzyme-inhibitor (ACE-I) or receptor blocker were randomized to aldosterone blockade or placebo. The primary outcome at 16 weeks was percent change in coronary diameter to ACh and secondary outcomes coronary flow reserve to adenosine, both adjusted for baseline reactivity.

Results—Forty-one women completed the treatment period with repeat coronary reactivity testing. Their mean age was 54±10 years, body mass index 30±7.4 kg/m², 12% had diabetes, and 15% had metabolic syndrome. There were no significant differences between treatment groups. At baseline, the percent change in reference vessel coronary diameter to ACh was −5.0% in the

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aldosterone blockade group and -3.4% in the placebo group, and at 16 weeks, -7.2% in the aldosterone blockade group versus -14.3% in the placebo group ($p=0.15$). At 16 weeks, the change in coronary flow reserve to intracoronary adenosine was -0.13 in the aldosterone blockade group versus -0.25 in the placebo group ($p=0.66$).

Conclusion—Adding aldosterone receptor blockade to angiotensin II inhibition did not improve coronary endothelial or microvascular function among women with signs and symptoms of ischemia in the setting of non-obstructive CAD.

Keywords

Women; Microcirculation; Ischemia; Adenosine; Acetylcholine; Endothelial dysfunction

Introduction

Women with symptoms and/or signs of myocardial ischemia referred for angiography have a high frequency of non-obstructive coronary artery disease.¹⁻⁴ This sub-population is highly prevalent,⁴ costly,⁵ and at higher risk for adverse outcomes including mortality.^{4,6} Although the precise mechanisms are unclear, coronary vascular dysregulation (e.g. endothelial and/or smooth muscle dysfunction at the macrovascular and/or microvascular level) is highly prevalent and linked with adverse outcomes.⁷⁻⁹ This association appears independent of underlying angiographically evident obstructive coronary artery disease.

Multiple randomized trials as summarized in a recent meta analysis have shown that angiotensin-converting-enzyme inhibitors (ACE-I) improve macrovascular endothelial dysfunction.¹⁰ We have recently shown this class of medications improves coronary microvascular function and reduces angina frequency in symptomatic women with signs of ischemia without obstructive CAD.¹¹

Aldosterone may act synergistically with angiotensin II and other factors (e.g., dysglycemia) in development of vascular inflammation, fibrosis, and remodeling. Both preclinical and clinical trials indicate that aldosterone inhibition may have microvascular protective effects in a variety of models¹²⁻¹⁴ as well as in patients.¹⁵⁻¹⁷ These findings suggest that aldosterone per se, beyond the influence of angiotensin II, is an important mediator of microvascular damage, at least in other in other organs.^{18,19} Thus dual blockade of both RAS and aldosterone may result in a more complete inhibition of RAAS than blockade of either system alone, providing increased beneficial effects on microvascular disease. Accordingly we hypothesized that adding an aldosterone antagonist to ACE-I will improve coronary vasomotor function.²⁰⁻²²

Methods

Study Funding

Eplerenone and matching placebo were provided by Pfizer. The Women's Ischemia Syndrome Evaluation (WISE) study was supported by contracts from the National Heart, Lung and Blood Institutes, nos. N01-HV-68161, N01-HV-68162, N01-HV-68163, N01-HV-68164, grants U0164829, U01 HL649141, U01 HL649241, T32HL69751,

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The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.

Study Design

The Eplerenone Women's Ischemia Syndrome Evaluation (EWISE) was a multi-center, double-blind, randomized trial designed to investigate effects of adding aldosterone blockade to ACE-I in women with signs and symptoms of ischemia, coronary endothelial dysfunction, and no angiographically evident obstructive coronary disease. This was an ancillary study of the National Heart Lung and Blood Institute sponsored WISE and was conducted according to the Declaration of Helsinki (NCT 00187889). The WISE protocol and patient population have been previously published.²³ Institutional Review Boards, which are responsible for ethical review, at the University of Florida (Gainesville, FL), Cedars-Sinai Heart Institute (Los Angeles, CA), Emory University (Atlanta, GA), Rhode Island Hospital (Providence, RI), and Mayo Clinic (Rochester, MD) each reviewed and approved the protocol and informed consent document. Written informed consent was obtained from each participant, and data were monitored by an independent data and safety monitoring committee.

Selection Criteria

Women 21 to 75 years of age with angina, other signs of ischemia, and the following findings were eligible to participate: 1) no obstructive coronary artery disease (<50% luminal diameter stenosis) and 2) endothelial dysfunction defined as no dilation (<5% increase in mean luminal diameter) to intracoronary acetylcholine (ACh). The following conditions were excluded: breast-feeding or pregnancy, acute myocardial infarction or unstable angina within the prior month, uncontrolled hypertension (>160/95 mm Hg), left ventricular dysfunction (ejection fraction <35%) with New York Heart Association class III or IV symptoms on treatment, hypertrophic obstructive cardiomyopathy, prior coronary artery bypass grafting or percutaneous coronary intervention, significant lung or liver disease, serum creatinine >1.8 mg/dL or creatinine clearance <50 mL/min, surgically uncorrected significant congenital heart disease, medical noncompliance or substance abuse, hypersensitivity to study medication, significant aortic stenosis (valve area <1.5 cm²), serum potassium >5.0 meq/L, use of potassium supplements, potassium sparing diuretics, or CYP3A4 inhibitors.

Study Protocol

Eligible women were randomized in a double-blind fashion stratified by center to aldosterone blockade (eplerenone; 25 mg daily for 4 weeks, then up-titrated to 50 mg daily for 12 weeks) or matching placebo (1 tablet daily for 4 weeks, then uptitrated daily for 12 weeks). The drugs and randomization code were provided by the University of Florida/Shands Hospital Investigational Pharmacy.

Patients were using an ACE-I at a maximum tolerated dose before the coronary study was initiated and continued throughout the study period: an angiotensin receptor blocker (ARB) could be substituted for ACE-I intolerant patients. Stable doses of calcium antagonists and nitrates were acceptable as long as sublingual nitrates were withheld for 2 hours prior to endothelial function testing. Short-acting calcium antagonists and long-acting nitrates were withheld for 24 hours prior to testing, and long-acting calcium antagonists were withheld 48 hours prior to endothelial function testing. Study medication could be down-titrated or stopped if the patient developed hyperkalemia or an adverse event.

Catheterization Procedure

Left heart catheterization and quantitative coronary angiography were performed according to standard technique with a 6 French guiding catheter. Unfractionated heparin was administered to achieve an activated clotting time of at least 250 to 300 seconds. A 0.014" Doppler-tipped guide wire (FloWire; JOMED/Cardiometrics; Mountain View, CA) was advanced into the proximal or mid segment of the left anterior descending coronary artery (preferred) or left circumflex artery branch if dictated by coronary anatomy. Baseline coronary flow velocity and coronary angiogram were obtained.

Protocol stipulated that vasoactive substances were sequentially administered through the guiding catheter and coronary flow velocity recorded after each. This consisted of 3 doses (to ensure a stable average coronary velocity) of adenosine 18 µg (Adenocard; Fujisawa USA; Deerfield, IL) followed by a 5 mL normal saline bolus, ACh 10^{-6} mol/L at 0.8 mL/min for 3 minutes (coronary concentration 0.18 µg/mL) (Miochol-E; Novartis Pharmaceuticals; Stein, Switzerland), ACh 10^{-4} mol/L at 0.8 mL/min for 3 minutes (coronary concentration 18 µg/mL), and then nitroglycerin 150 µg. Coronary angiograms were performed after the third dose of adenosine and each subsequently administered vasoactive substance.

Quantitative Coronary Angiography

Angiograms were analyzed by an established WISE angiographic core laboratory with a computer-based edge-detection method (Rhode Island Hospital, Providence, RI) blinded to patient treatment assignment.²⁴ Luminal diameters were determined before and after ACh. During catheterization, measurements were made at the same vessel location before and after ACh and with the same camera angulation.

Study Outcomes

The primary measure of interest was the relative change in coronary diameter to ACh at 16 weeks, adjusted for baseline reactivity to ACh.⁸ The secondary measure of interest was

coronary flow reserve (CFR) at 16 weeks adjusted for baseline CFR. This was defined as the ratio of average peak coronary velocity after administration of adenosine to the average peak coronary velocity immediately prior to adenosine.²⁵ Angina was assessed in the following domains from the validated Seattle Angina Questionnaire: angina frequency, angina stability, disease perception, physical limitation, and treatment satisfaction.²⁶ Exercise duration was compared at baseline and 16 weeks using the modified Asymptomatic Cardiac Ischemia Pilot protocol.

Statistical Analysis

Baseline characteristics were presented according to treatment group. Categorical variables were summarized as percentages and compared with the Fisher's exact test. Continuous variables were summarized as mean $\pm$ standard deviation and compared with the two-tailed, two-sample t-test.

We tested the null hypothesis of no difference in coronary diameter response to ACh between the aldosterone blockade and placebo groups over the follow-up period. Within each treatment group, coronary diameter before and after ACh was evaluated with the two-tailed, two-sample t-test after checking the distribution of residuals. Change in coronary diameter before and after ACh was also analyzed using a matched segment linear regression model (SAS PROC GLM) and an all-segment mixed model with repeated measures (SAS PROC MIXED), assuming the covariance model is compound symmetry. Matched-segment analysis used the primary outcome of one matched segment at baseline versus 16 weeks for each patient. All-segment analysis used the primary outcome of all segments that were available for each patient. Multiple segments in the same patient were considered as repeated measures.

Sample size considerations

Prior studies in WISE patients with endothelial dysfunction revealed that coronary vessel diameter response to ACh 10^{-4} mol/L was -14.4% with standard deviation 13.8% . If the mean change of diameter response to ACh for the placebo group remains at -14.4% after 16 weeks and the treatment group improves to a mean -2.4% change, it would require a total of 44 women to detect a significant difference with 80% power and a two-sided p-value of 0.05. A -2.4% mean change (SD 13.8%) would be clinically important as it reflects approximately 20% of women to have changed from abnormal to normal endothelial function (5% change). Thus, a sample size of 22 per group (total 44) would be adequate to detect a 12% absolute difference in diameter response to ACh. Assuming a worse case 20% dropout rate, we planned to recruit 50 patients (25 per group, 5 per clinical center) for this study to obtain 44 completed patients. A p-value <0.05 was considered statistically significant. All analyses were performed with SAS software version 9.3 (SAS Institute, Inc., Cary, North Carolina).

Results

Baseline characteristics

From 70 screened women, 19 patients in the aldosterone blockade group and 22 patients in the placebo group completed the study drug and had repeat cardiac catheterization at 16 weeks (Figure 1). The principal reason for participant withdrawal after randomization was unwillingness to complete the 16-week angiogram. The treatment was well tolerated with no episodes of hyperkalemia or hypotension requiring discontinuation of study drugs. Baseline characteristics were similar between the groups (Table I). One subject, who signed the informed consent, developed coronary spasm with ACh during the baseline screening evaluation which, after NTG, was associated with a filling defect believed to be thrombus. Percutaneous coronary intervention resulted in complete resolution of the defect. This patient was not randomized to study drug and was withdrawn from the study. A second patient, randomized to placebo, developed deep vein thrombophlebitis approximately 2 weeks after coronary angiography and was treated with anticoagulation. This patient was also withdrawn at the request of her primary physician. Systolic blood pressure at 16 weeks was 120 mm Hg in the aldosterone blockade group versus 117 mm Hg in the placebo group ($p = 0.72$).

All patients had endothelial dysfunction at baseline with a percent change in coronary diameter with ACh 10^{-4} mol/L of -5.0% in the aldosterone blockade group and -3.4% in the placebo group. At 16 weeks, the percent change in reference vessel diameter with ACh was -7.2% in the aldosterone blockade group versus -14.3% in the placebo group ($p = 0.15$ between groups; Figure 2). Among 9 patients with $\text{CFR} < 2.4$, the percent change in reference vessel diameter with ACh was -2.6% in the aldosterone blockade group versus -16.9% in the placebo group ($p = 0.43$). In a matched segment linear regression model that controlled for baseline vessel reactivity, age, hypertension, diabetes/metabolic syndrome, and lipid lowering medications, aldosterone blockade did not improve ACh-mediated vasomotion ($p = 0.22$). Also, an all-segment linear regression model yielded the same conclusion ($p = 0.24$). Detailed data on coronary diameter and coronary flow reserve in response to adenosine is presented in Table II. Coronary volumetric flow and coronary flow velocity data are provide in Table III.

Over 16 weeks, angina frequency score by Seattle Angina Questionnaire increased in both groups: from 67 to 73 in the aldosterone blockade group ($p = 0.24$) and from 51 to 65 in the placebo group ($p = 0.0057$) ($p = 0.27$ between groups; Figure 3). There was no change between treatment groups in the other angina domains of the Seattle Angina Questionnaire. Serum potassium essentially remained the same over follow-up: 4.2 mEq/L at baseline to 4.3 mEq/L at 16 weeks in the aldosterone blockade group and 4.2 mEq/L at baseline and 16 weeks in the placebo group.

Discussion

Among women with coronary endothelial dysfunction treated with angiotensin inhibition, addition of aldosterone blockade did not improve epicardial coronary endothelial dysfunction compared with placebo. Aldosterone blockade also failed to improve coronary

flow reserve, angina frequency, or exercise duration. Aldosterone blockade with eplerenone was well-tolerated when added to angiotensin inhibition. At 16 weeks, systolic blood pressure was similar between groups.

To our knowledge, this is the first report of aldosterone blockade added to angiotensin inhibition among patients with endothelial dysfunction, but without heart failure. Mineralocorticoid receptor blockade in addition to optimal medical therapy has been extensively studied in heart failure patients. Spironolactone (non-selective blockade) was tested and proved to be beneficial in patients with severe heart failure mostly due to ischemic heart disease.²⁷ Spironolactone was also evaluated among patients with heart failure with preserved ejection fraction. While spironolactone did not reduce adverse cardiovascular events, it did prevent hospitalizations due to heart failure.²⁸ Eplerenone (selective blockade) was also demonstrated to be beneficial in mild heart failure and in heart failure due to myocardial infarction.^{29–31}

The reason for the lack of apparent benefit from aldosterone blockade on improving endothelial function may be due, in part, to the fact that all participants were already treated with an angiotensin inhibitor (ACE-I or ARB). Our group previously documented improvement in microvascular function and angina frequency with 16 weeks of ACE-I therapy (quinapril).¹¹ Several other studies have also documented benefit of ACE-I in similar patients.^{32–35}

Intracoronary ACh is not just a marker for endothelial function within the coronary arteries, but may also reflect smooth muscle cell sensitivity to vasoconstrictor responses (e.g. intracoronary ACh causes release of endothelin into the coronary circulation).³⁶ A potential limitation of this study was insufficient power to detect a difference between treatment groups. Based on data from prior WISE studies, EWISE was powered for 44 total patients; however, there were only 41 patients with analyzable data. However, this is likely not of major significance. A more relevant issue is the fact that the actual mean diameter response was smaller than anticipated. The potent effect of background therapy with an ACE-I/ARB was not fully appreciated at the design phase of the study. The improvement in angina score among ACE-I/ARB plus placebo patients, which was not different from ACE-I/ARB plus aldosterone patients, is supportive of the beneficial effects of these agents. It is possible that benefit from additional aldosterone blockade could be limited to patients with the most impaired microvascular dysfunction. In the small sample of 9 patients with CFR < 2.4 at baseline, there was a suggestion of possible benefit from aldosterone blockade; however, this subgroup would need to be prospectively tested. Another limitation is that study design required patients to return for a repeat coronary angiogram which some chose not to have. A future study utilizing a surrogate outcome, such as brachial flow mediated dilatation could help to enhance patient enrollment; however, this might be tempered by less reliable results. Lastly, these results only apply to women.

Conclusions

Among women with chest pain, endothelial dysfunction, and no obstructive coronary artery disease, the addition of aldosterone blockade to already optimal ACE-I or ARB therapy did

not significantly improve endothelial function. This group of symptomatic women with microvascular/endothelial dysfunction continues to pose a challenge in providing a long term effective therapy and deserves further investigation.

References

1. Lagerqvist B, Safstrom K, Stahle E, et al. Is early invasive treatment of unstable coronary artery disease equally effective for both women and men? FRISC II Study Group Investigators. *J Am Coll Cardiol*. 2001; 38:41–48. [PubMed: 11451294]
2. Glaser R, Herrmann HC, Murphy SA, et al. Benefit of an early invasive management strategy in women with acute coronary syndromes. *JAMA*. 2002; 288:3124–3129. [PubMed: 12495392]
3. Clayton TC, Pocock SJ, Henderson RA, et al. Do men benefit more than women from an interventional strategy in patients with unstable angina or non-ST-elevation myocardial infarction? The impact of gender in the RITA 3 trial. *Eur Heart J*. 2004; 25:1641–1650. [PubMed: 15351164]
4. Jespersen L, Hvelplund A, Abildstrom SZ, et al. Stable angina pectoris with no obstructive coronary artery disease is associated with increased risks of major adverse cardiovascular events. *Eur Heart J*. 2012; 33:734–744. [PubMed: 21911339]
5. Shaw LJ, Merz CN, Pepine CJ, et al. The economic burden of angina in women with suspected ischemic heart disease: results from the National Institutes of Health--National Heart, Lung, and Blood Institute--sponsored Women's Ischemia Syndrome Evaluation. *Circulation*. 2006; 114:894–904. [PubMed: 16923752]
6. Gulati M, Cooper-DeHoff RM, McClure C, et al. Adverse cardiovascular outcomes in women with nonobstructive coronary artery disease: a report from the Women's Ischemia Syndrome Evaluation Study and the St James Women Take Heart Project. *Arch Intern Med*. 2009; 169:843–850. [PubMed: 19433695]
7. Pepine CJ, Anderson RD, Sharaf BL, et al. Coronary microvascular reactivity to adenosine predicts adverse outcome in women evaluated for suspected ischemia results from the National Heart, Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) study. *J Am Coll Cardiol*. 2010; 55:2825–2832. [PubMed: 20579539]
8. von Mering GO, Arant CB, Wessel TR, et al. Abnormal coronary vasomotion as a prognostic indicator of cardiovascular events in women: results from the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation*. 2004; 109:722–725. [PubMed: 14970106]
9. Halcox JP, Schenke WH, Zalos G, et al. Prognostic value of coronary vascular endothelial dysfunction. *Circulation*. 2002; 106:653–658. [PubMed: 12163423]
10. Shahin Y, Khan JA, Samuel N, et al. Angiotensin converting enzyme inhibitors effect on endothelial dysfunction: a meta-analysis of randomised controlled trials. *Atherosclerosis*. 2011; 216:7–16. [PubMed: 21411098]
11. Pauly DF, Johnson BD, Anderson RD, et al. In women with symptoms of cardiac ischemia, nonobstructive coronary arteries, and microvascular dysfunction, angiotensin-converting enzyme inhibition is associated with improved microvascular function: A double-blind randomized study from the National Heart, Lung and Blood Institute Women's Ischemia Syndrome Evaluation (WISE). *Am Heart J*. 2011; 162:678–684. [PubMed: 21982660]
12. Fujisawa G, Okada K, Muto S, et al. Spironolactone prevents early renal injury in streptozotocin-induced diabetic rats. *Kidney Int*. 2004; 66:1493–1502. [PubMed: 15458443]
13. Kang YS, Ko GJ, Lee MH, et al. Effect of eplerenone, enalapril and their combination treatment on diabetic nephropathy in type II diabetic rats. *Nephrol Dial Transplant*. 2009; 24:73–84. [PubMed: 18682491]
14. Trachtman H, Weiser AC, Valderrama E, et al. Prevention of renal fibrosis by spironolactone in mice with complete unilateral ureteral obstruction. *J Urol*. 2004; 172:1590–1594. [PubMed: 15371767]
15. Campese VM, Park J. Use of antagonists of aldosterone in patients with chronic kidney disease: Potential advantages and risks. *J Hypertens*. 2006; 24:2157–2159. [PubMed: 17053534]

16. Epstein M, Williams GH, Weinberger M, et al. Selective aldosterone blockade with eplerenone reduces albuminuria in patients with type 2 diabetes. *Clin J Am Soc Nephrol*. 2006; 1:940–951. [PubMed: 17699311]
17. Sato A, Hayashi K, Naruse M, et al. Effectiveness of aldosterone blockade in patients with diabetic nephropathy. *Hypertension*. 2003; 41:64–68. [PubMed: 12511531]
18. Epstein M. Aldosterone as a mediator of progressive renal disease: pathogenetic and clinical implications. *Am J Kidney Dis*. 2001; 37:677–688. [PubMed: 11273866]
19. Schjoedt KJ. The renin-angiotensin-aldosterone system and its blockade in diabetic nephropathy: main focus on the role of aldosterone. *Dan Med Bull*. 2011; 58:B4265. [PubMed: 21466768]
20. Brown NJ. Aldosterone and vascular inflammation. *Hypertension*. 2008; 51:161–167. [PubMed: 18172061]
21. Min LJ, Mogi M, Iwanami J, et al. Cross-talk between aldosterone and angiotensin II in vascular smooth muscle cell senescence. *Cardiovasc Res*. 2007; 76:506–516. [PubMed: 17706954]
22. Montezano AC, Callera GE, Yogi A, et al. Aldosterone and angiotensin II synergistically stimulate migration in vascular smooth muscle cells through c-Src-regulated redox-sensitive RhoA pathways. *Arterioscler Thromb Vasc Biol*. 2008; 28:1511–1518. [PubMed: 18467645]
23. Merz CN, Kelsey SF, Pepine CJ, et al. The Women's Ischemia Syndrome Evaluation (WISE) study: protocol design, methodology and feasibility report. *J Am Coll Cardiol*. 1999; 33:1453–1461. [PubMed: 10334408]
24. Sharaf BL, Pepine CJ, Kerensky RA, et al. Detailed angiographic analysis of women with suspected ischemic chest pain (pilot phase data from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation [WISE] Study Angiographic Core Laboratory). *Am J Cardiol*. 2001; 87:937–941. A3. [PubMed: 11305981]
25. Reis SE, Holubkov R, Lee JS, et al. Coronary flow velocity response to adenosine characterizes coronary microvascular function in women with chest pain and no obstructive coronary disease. Results from the pilot phase of the Women's Ischemia Syndrome Evaluation (WISE) study. *J Am Coll Cardiol*. 1999; 33:1469–1475. [PubMed: 10334410]
26. Spertus JA, Winder JA, Dewhurst TA, et al. Development and evaluation of the Seattle Angina Questionnaire: a new functional status measure for coronary artery disease. *J Am Coll Cardiol*. 1995; 25:333–341. [PubMed: 7829785]
27. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med*. 1999; 341:709–717. [PubMed: 10471456]
28. Shah SJ, Heitner JF, Sweitzer NK, et al. Baseline characteristics of patients in the treatment of preserved cardiac function heart failure with an aldosterone antagonist trial. *Circ Heart Fail*. 2013; 6:184–192. [PubMed: 23258572]
29. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med*. 2003; 348:1309–1321. [PubMed: 12668699]
30. Zannad F, McMurray JJ, Krum H, et al. Eplerenone in patients with systolic heart failure and mild symptoms. *N Engl J Med*. 2011; 364:11–21. [PubMed: 21073363]
31. Rogers JK, McMurray JJ, Pocock SJ, et al. Eplerenone in patients with systolic heart failure and mild symptoms: analysis of repeat hospitalizations. *Circulation*. 2012; 126:2317–2323. [PubMed: 23042980]
32. Motz W, Strauer BE. Improvement of coronary flow reserve after long-term therapy with enalapril. *Hypertension*. 1996; 27:1031–1038. [PubMed: 8621193]
33. Rizzoni D, Muiesan ML, Porteri E, et al. Effects of long-term antihypertensive treatment with lisinopril on resistance arteries in hypertensive patients with left ventricular hypertrophy. *J Hypertens*. 1997; 15:197–204. [PubMed: 9469796]
34. Giugliano D, Marfella R, Acampora R, et al. Effects of perindopril and carvedilol on endothelium-dependent vascular functions in patients with diabetes and hypertension. *Diabetes Care*. 1998; 21:631–636. [PubMed: 9571355]

35. Higuchi T, Abletshauser C, Nekolla SG, et al. Effect of the angiotensin receptor blocker Valsartan on coronary microvascular flow reserve in moderately hypertensive patients with stable coronary artery disease. *Microcirculation*. 2007; 14:805–812. [PubMed: 17907015]
36. Rubanyi GM. The discovery of endothelin: the power of bioassay and the role of serendipity in the discovery of endothelium-derived vasocative substances. *Pharmacol Res*. 2011; 63:448–454. [PubMed: 20813186]

Figure 1.
Patient flow diagram.

Figure 2.
Assessment of endothelial function: percent change in coronary diameter to acetylcholine.

Figure 3.
Change in angina frequency score, assessed by Seattle Angina Questionnaire.

Table I

Baseline characteristics of study participants*

Baseline characteristic	Aldosterone Blockade (n = 19)	Placebo (n = 22)
Age, years	54 ± 10	54 ± 11
Caucasian, %	89	91
Waist circumference, inches	35.1 ± 5.8	37.4 ± 8.2
BMI, kg/m ²	29.6 ± 6.5	30.0 ± 8.2
Ejection fraction, %	71.2 ± 8.6	71.9 ± 8.7
History of, (%)		
Hypertension	42	57
Diabetes [†]	11	14
Metabolic syndrome	7	21
Dyslipidemia [‡]	68	64
Smoking within last 30 days	11	14
Cerebrovascular disease [§]	12	5
SBP, mm Hg	118 ± 18	120 ± 19
DBP, mm Hg	71 ± 10	74 ± 11
Heart rate, beats per minute	74 ± 11	72 ± 10
Pulse pressure, beats per minute	46 ± 15	48 ± 16
Laboratory data, mean ± SD		
Total cholesterol, mg/dL	187 ± 36	177 ± 38
Triglycerides, mg/dL	125 ± 94	141 ± 72
HDL cholesterol, mg/dL	60 ± 22	57 ± 18
LDL cholesterol, mg/dL	102 ± 38	92 ± 41
Serum creatinine, mg/dL	0.75 ± 0.10	0.79 ± 0.14
Hemoglobin, g/dL	16 ± 7.3	14 ± 1.4
Medications, (%)		
Aspirin	63	59
Beta-blocker	33	45
Nitrate	26	50
Statin	39	59
Diuretics	14	42
Antidepressant	26	50

BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; SD, standard deviation; SBP, systolic blood pressure.

* Values are reported as mean ± standard deviation unless reported otherwise. There was no difference in baseline characteristics between groups. Fisher exact test was used to compare frequencies and 2-sided t-test used for continuous values.

[†] Defined as diagnosis of diabetes or use of oral hypoglycemic medications and/or insulin.

[‡]Defined as diagnosis of hypercholesterolemia or use of lipid lowering medications;

[§]Defined as stroke, transient ischemic attack, or carotid revascularization.

Table II

Secondary outcomes

	Aldosterone Blockade	Placebo	p value*
Coronary diameter (mm) after intracoronary nitroglycerin			
Coronary diameter at baseline	2.35 ± 0.57	2.65 ± 0.55	0.21
Coronary diameter at 16 weeks	2.42 ± 0.57	2.60 ± 0.57	0.46
Coronary diameter change (16 weeks – baseline)	0.07 ± 0.53	– 0.05 ± 0.33	0.51
Coronary flow reserve (CFR) response to intracoronary adenosine			
CFR at baseline	2.71 ± 0.62	2.93 ± 0.80	0.34
CFR at 16 weeks	2.64 ± 0.62	2.79 ± 0.52	0.46
CFR change (16 weeks – baseline)	–0.13 ± 0.83	–0.25 ± 0.80	0.66
Angina frequency score; Seattle Angina Questionnaire			
Angina frequency at baseline	67 ± 19	51 ± 29	0.09
Angina frequency at 16 week	73 ± 25	65 ± 26	0.29
Angina frequency change (16 weeks – baseline)	6 ± 22	14 ± 20	0.27
Exercise duration by modified Asymptomatic Cardiac Ischemia Pilot protocol (minutes)			
Baseline	7.18 ± 1.25	7.68 ± 3.30	0.76
16 weeks	7.67 ± 2.34	8.20 ± 3.37	0.76
Change (16 weeks – baseline)	0.48 ± 1.38	0.53 ± 1.81	0.96

Data presented as mean ± standard error

* Two-tailed t-test used to compare the aldosterone blockade and placebo groups

Table III

Coronary volumetric flow, coronary flow velocity and coronary cross-sectional area

Aldosterone Blockade (n = 19)			Placebo (n = 22)		p value*
Week 0	Week 16	Week 0	Week 16		
Coronary volumetric flow / (heart rate × systolic blood pressure) (mL/ (beats per minute × mm Hg))					
Baseline	5.4 ± 4.3	5.0 ± 3.6	3.2 ± 1.8	3.6 ± 1.6	
Adenosine	14.3 ± 9.5	13.2 ± 8.7	9.8 ± 6.0	10.9 ± 5.7	
Change (%)	199.9 ± 92.9	182.4 ± 82.7	203.0 ± 97.3	205.3 ± 104.1	0.56
Coronary flow velocity (cm/second)					
Baseline	24.9 ± 10.0	23.7 ± 6.6	18.8 ± 6.8	23.3 ± 6.7	
Adenosine	62.3 ± 15.6	63.2 ± 18.9	53.9 ± 16.9	59.2 ± 13.7	
Change (%)	166.5 ± 59.0	172.7 ± 73.9	199.1 ± 82.3	165.8 ± 61.0	0.33
Coronary cross-sectional area (cm ²)					
Baseline	0.053 ± 0.024	0.049 ± 0.018	0.049 ± 0.026	0.043 ± 0.016	
Adenosine	0.057 ± 0.026	0.052 ± 0.018	0.05 ± 0.026	0.048 ± 0.021	
Change (%)	10.7 ± 22.7	6.6 ± 11.5	1.7 ± 14.3	10.9 ± 22.1	0.13

Data presented as mean ± standard error

* Two-sided t-test was used to compare the difference of the % change (week 16 – week 0) between aldosterone blockade and placebo groups.