Endogenous Endothelin in Human Coronary Vascular Function
Differential Contribution of Endothelin Receptor Types A and B

Julian P.J. Halcox, Khaled R.A. Nour, Gloria Zalos, Arshed A. Quyyumi

Abstract—Endothelin 1 mediates coronary vasoconstriction and endothelial dysfunction via endothelin receptor type A (ET_A) activation. However, the effects of selective endothelin receptor type B (ET_B) and combined ET_A+B Receptor blockade on coronary vasomotion are unknown. We measured coronary vascular tone and endothelium-dependent and -independent vasomotor function before and after selective infusion of BK-788 (an ET_B receptor antagonist) or combined infusion of BK-788+BQ-123 (an ET_A antagonist) into unobstructed coronary arteries of 39 patients with coronary atherosclerosis or risk factors undergoing cardiac catheterization. BK-788 did not affect epicardial diameter but constricted the microcirculation (P<0.0001), increased coronary sinus endothelin, and reduced nitrogen oxide levels. In contrast, BK-783+BK-788 dilated epicardial (P<0.0001) and resistance (P=0.022) arteries. Responses to acetylcholine and sodium nitroprusside were unaffected by BK-788 alone. Epicardial endothelial dysfunction improved after BK-123+BK-788 (P=0.007). Coronary microvascular responses to acetylcholine and sodium nitroprusside were unaffected by BK-123+BK-788. We conclude that selective ET_B receptor antagonism causes coronary microvascular constriction, without affecting epicardial tone or endothelial function, via reduced endothelin clearance and NO availability. Combined ET_A+B blockade dilates coronary conduit and resistance vessels and improves endothelial dysfunction of the epicardial coronary arteries. Thus, endogenous endothelin, predominantly via ET_A receptor stimulation, contributes to basal constrictor tone and endothelial dysfunction, whereas ET_B activation mediates vasodilation in human coronaries. Our data suggest that selective ET_A blockade may have greater therapeutic potential than nonselective agents, particularly for treatment of endothelial dysfunction in atherosclerosis. (Hypertension. 2007;49:1-8.)

Key Words: clinical science ■ blood flow regulation ■ endothelin ■ endothelium ■ atherosclerosis

The vascular effects of endothelin 1 (ET-1) are mediated via 2 distinct endothelin receptors, type A (ET_A) and type B (ET_B). ET_A receptors are selectively expressed on vascular smooth muscle cells, whereas ET_B receptors are present on both endothelial and vascular smooth muscle cells.1-5 Both subtypes mediate vascular smooth muscle constriction6-8; however, ET_B receptors also mediate the release of NO and prostacyclin from endothelial cells and increase pulmonary clearance and endothelial reuptake of ET-1.9-14 Thus, ET_B receptor stimulation has the potential to both vasodilate and vasoconstrict, and the balance between these opposing phenomena is critical in determining the physiological impact of ET_B receptor activity. Endogenous ET-1 is enhanced in hypertension, coronary artery disease (CAD), and heart failure,15-21 with ET_A receptor activation contributing to coronary constrictor tone and peripheral and coronary endothelial dysfunction via ET_A receptor activation.22-27 Differing effects of ET_B receptor activation are observed in health and disease and in different vascular beds.16,19,28-30 Combined ET_A+B antagonism causes coronary vasodilation,31 but the effects on endothelial function and the specific role of ET_B in coronary vasomotion remain unknown.

We hypothesized a differential contribution from endogenous ET_A, and ET_B receptor activation in human coronary vasomotor regulation. Herein we report the first clinical study investigating the effect of selective ET_B and combined ET_A+B receptor antagonism on coronary vascular function in subjects with CAD and its risk factors.

Methods

Patients
We studied 39 patients with either CAD (n=24) or normal coronary arteries and risk factors for CAD (n=15) undergoing diagnostic cardiac catheterization (details are available in a data supplement available online at http://hyper.ahajournals.org). The study was approved by the institutional review board of the National Heart,
Lung, and Blood Institute, and informed written consent was obtained from all of the patients.

**Study Protocols**

Study protocols were initiated after completion of diagnostic coronary angiography. A 7-French guide catheter was introduced into a coronary artery with insignificant stenosis (<20%). Blood flow velocity was measured using a 0.014-inch Doppler wire (FloWire, Volcano Corporation). Medications were infused via a 3-French infusion catheter, advanced over the FloWire. Infusion flow rates were similar in both protocols.

**Protocol 1**

In 25 patients, baseline coronary blood flow velocity and coronary angiography were performed after a 10-minute infusion of 5% dextrose at 2 mL/min. Endothelium-dependent coronary vasodilatation was estimated in 22 patients by administering incremental 2-minute infusions of acetylcholine (ACH) at 1.5, 15, and 50 μg/min (estimated intracoronary concentrations: 10⁻³, 10⁻², and 3.3×10⁻⁷ mol/L, respectively). Subsequently, a 3-minute intracoronary infusion of sodium nitroprusside (SNP) at a dose of 20 μg/min, was administered to assess endothelium-independent coronary vasodilation. BQ-788 (Basch, a selective ET₄ receptor antagonist, was then infused for 1 hour at a rate of 100 nmol/min. To record the maximal response, measurements were made over 1 hour, based on previous observations in the forearm circulation.³⁶ ACH and SNP infusions were then repeated during coadministration of BQ-788.

Systemic hemodynamics and coronary blood flow velocity were recorded, and coronary angiography was performed after each intervention and also after 30, 45, and 60 minutes of the BQ-788 infusion. In addition, pulmonary artery pressure, pulmonary capillary wedge pressure, and cardiac output were also measured using a Swan-Ganz catheter at baseline and during the 60-minute infusion of BQ-788.

In 12 patients in whom the native left anterior descending coronary artery was used as the study vessel, a 7-French gauge A2 catheter (Corid Inc) was introduced percutaneously via the coronary sinus into the great cardiac vein. Arterial and coronary venous blood was drawn at baseline and after 60 minutes of BQ-788 infusion.

**Protocol 2**

This protocol was conducted in a similar fashion to protocol 1 in 14 patients with measurement of systemic, pulmonary, and coronary hemodynamics and endothelium-dependent and -independent coronary vasodilation (with ACH at 1.5 and 15 μg/min for 2 minutes and SNP at 20 μg/min for 3 minutes, respectively) determined before and after a 1-hour combined intracoronary infusion of BQ-123 (Basch) and BQ-788 (at doses of 200 nmol/min and 100 nmol/min, respectively).

**Measurement of Coronary Blood Flow and Diameter**

Coronary blood flow, vascular resistance, and epicardial coronary diameter were assessed during each intervention using Doppler flow velocity and quantitative coronary angiography as described previously.²²,³²

**Measurement of Plasma Nitrogen Oxides, Big ET-1, and ET-1**

Blood was drawn in EDTA tubes and immediately chilled on ice, centrifuged at 4°C at 2500 rpm for 10 minutes, and plasma was frozen at −70°C. Coronal venous plasma nitrogen oxide (NOx) levels were measured with the use of the Sievers Nitric Oxide Analyzer (model 280).³³ Plasma levels of ET-1 and big ET-1 were measured using radioimmunoassay techniques.³⁴,³⁵

**Statistical Analysis**

Data are expressed as mean±SE. Differences between means were compared by paired or unpaired Student’s t-test as appropriate. Dose response curves with ACH were compared by ANOVA using the SAS software 6.12 (SAS Institute). If the F value was significant, a Bonferroni multiple comparison test was performed. Univariate correlations were performed using the Pearson’s correlation coefficient. Multiple stepwise regression analysis was performed to test whether the magnitude of change with BQ-788 or BQ-123+BQ-788 was related to the age, sex, presence of atherosclerosis, hypertension, diabetes, cigarette use, or the total, low-density lipoprotein, and high-density lipoprotein cholesterol levels (general linear models procedure). Subjects with a vasoconstrictor response in the epicardial vessels and/or in the lower 50% of the distribution for microvascular dilatation in response to ACH at a dose of 15 μg/min were considered as a subgroup with “worse endothelial function” for secondary analyses where appropriate (n=15 [63%] in protocol 1; n=8 [57%] in protocol 2). All of the P values are 2-tailed, and a value of <0.05 was considered of statistical significance.

**Results**

**Systemic and Pulmonary Hemodynamic Changes**

**Protocol 1: Effects of BQ-788**

Mild systemic vasoconstriction was observed with BQ-788; after 1 hour, mean arterial pressure rose by 4.9 mm Hg (P<0.001) and heart rate fell by 3.2 bpm (P=0.003). Cardiac index remained unchanged. Mean pulmonary artery pressure and pulmonary artery wedge pressure were unaffected by BQ-788 (Table).

**Protocol 2: Effects of Combined Administration of BQ-123+BQ-788**

Systemic blood pressure and cardiac index were unchanged and heart rate increased by 7 bpm (P=0.003). Mild pulmonary vasodilatation was observed with BQ-123+BQ-788; after 1 hour, mean pulmonary artery pressure and pulmonary artery wedge pressure fell by 3.0 mm Hg (P<0.01), and 2.6 mm Hg (P<0.01), respectively (Table).

**Resting Coronary Vascular Tone**

**Effect of BQ-788 on the Epicardial Arteries**

No change in epicardial vascular tone was observed after BQ-788 (diameter [D] change: 0.6±1%; P=0.4; Figure 1). This lack of effect was noted in both mid- and distal coronary arterial segments and in subgroups with and without CAD.

**Effect of BQ-788 or BQ-123+BQ-788 on Hemodynamic Parameters**

<table>
<thead>
<tr>
<th>Hemodynamic Parameter</th>
<th>Protocol 1</th>
<th>Protocol 2</th>
</tr>
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<tbody>
<tr>
<td>Heart rate, min⁻¹</td>
<td>77.0±2.5</td>
<td>73.8±2.6*</td>
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<tr>
<td>Mean blood pressure, mm Hg</td>
<td>107.8±3.1</td>
<td>112.7±2.6†</td>
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<tr>
<td>Mean pulmonary artery pressure, mm Hg</td>
<td>16.3±0.9</td>
<td>16.2±0.9</td>
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<tr>
<td>Mean pulmonary wedge pressure, mm Hg</td>
<td>8.8±0.9</td>
<td>8.9±0.7</td>
</tr>
<tr>
<td>Cardiac index, Lmin⁻¹ m⁻²</td>
<td>2.7±0.1</td>
<td>2.6±0.1</td>
</tr>
</tbody>
</table>

BQ-788 indicates after 60-minute intracoronary infusion of BQ-788 (100 nmol/min) or BQ-123+BQ788 (200 nmol/min+100 nmol/min). Data represent mean±SEM. *P<0.05; †P<0.001 versus baseline value.
Effect of BQ-788 on the Coronary Microcirculation

There was progressive coronary microvascular constriction with BQ-788; coronary blood flow fell by 13.1±3.8% (P=0.0003), and coronary vascular resistance (CVR) increased by 25.9±4.9% (P<0.0001) after 1 hour (Figure 1). We observed that the decrease in coronary blood flow (P=0.03) and increase in CVR (P=0.01) was apparent after 30 minutes of BQ-788 infusion, at which time blood pressure remained similar to baseline (P=0.2). There was no correlation between the presence of CAD or any of its risk factors with the magnitude of the microvascular response to BQ-788.

Effect of BQ-123+BQ-788 on the Epicardial Arteries

Significant vasodilatation of the epicardial coronary arteries was observed after 30 minutes and reached a maximum of 6.1±1.4% after 1 hour of BQ-123+BQ-788 (P<0.0001; Figure 2). There was no correlation between the magnitude of epicardial vasodilatation and the presence of atherosclerosis or its risk factors, and vasodilatation was similar in mid- and distal epicardial arteries.

Effect of BQ-123+BQ-788 on the Coronary Microcirculation

There was progressive coronary microvascular dilation with BQ-123+BQ-788; coronary blood flow increased by 24±8% (P=0.025), and CVR fell by −15±7% (P=0.022) after 1 hour (Figure 2). As with epicardial responses, the magnitude of microvascular vasodilatation did not correlate with the presence of atherosclerosis or its risk factors.
Endothelium-Dependent and -Independent Coronary Vascular Function

Effect of BQ-788 on the Epicardial Circulation

Epicardial coronary responses to ACH at baseline were heterogeneous with no net change in D (−0.7±1.4%; P = 0.36 at 15 μg/min of ACH). After BQ-788, a trend toward an improved epicardial vasodilator response to ACH (P = 0.06 ANOVA) was observed (Figure 3). There was no difference in the response to SNP (P = 0.19). To assess the effect of BQ-788 independent of both baseline changes in tone and any direct smooth muscle effects, we calculated the ratio of ACH:SNP responses in the epicardial coronary arteries before and after BQ-788. No significant difference in the epicardial ACH:SNP ratio was observed after BQ-788 in the whole group or in the subgroups defined by endothelial function.

Effect of BQ-123+BQ-788 on the Epicardial Circulation

Epicardial D was greater during ACH infusion after BQ-123+BQ-788 (1.83±0.09 versus 1.98±0.1 mm, with the 15-μg/min dose of ACH, pre- versus post-BQ-123+BQ-788; P < 0.01). When expressed as the percentage of change in D, ACH-induced epicardial vasoconstriction at baseline (−7.7±1.7%; P = 0.008 with 15 μg/min ACH) was significantly attenuated (P = 0.013 ANOVA) after BQ-123+BQ-788 (Figure 4). Epicardial responses to SNP were unchanged after BQ-123+BQ-788 (P = 0.18). Furthermore, a significant improvement in the ratio of epicardial ACH:SNP was noted after BQ-123+BQ-788 (P = 0.007 ANOVA), suggesting that combined ET_{A,B} receptor blockade selectively improves epicardial endothelial function. The subgroup with worse endothelial dysfunction at baseline had significant improvement after ET_{A,B} receptor blockade (−13% to −5%; P = 0.03), whereas in those with less severe dysfunction at baseline, the change did not reach significance (0.4% to −1%; P not significant).

Effect of BQ123+BQ-788 on Coronary Microvascular Function

After BQ-123+BQ-788, there was no difference in coronary blood flow with ACH (93±13 versus 102±11 mL/min pre-versus post-BQ-123+BQ-788 at the 15-μg/min dose of ACH; P = 0.1) or the percentage increase in flow with ACH (85±23 versus 76±22% pre-versus post-BQ-123+BQ-788 at the 15-μg/min dose of ACH; P = 0.7; please see the data supplement). Absolute flow and percentage increase in flow with SNP were also unchanged after BQ-123+BQ-788.
(85 ± 16 versus 88 ± 16 mL/min [P = 0.5] and 116 ± 33 versus 84 ± 23% [P = 0.2], pre-versus post-BQ-123 + BQ-788, respectively). Similarly, CVR, after both ACH and SNP, and the ACH:SNP resistance ratio remained unchanged after BQ-123 + BQ-788.

**Effect on Plasma Levels of Big ET-1, ET-1, and NOx in the Coronary Circulation**

No correlation was observed between baseline levels of ET-1 or big ET-1 and the change in coronary blood flow in response to BQ-788, BQ123/BQ-788, or ACH. Big ET-1 levels were unaltered in arterial and coronary venous blood after either BQ-788 or BQ-788. Arterial ET-1 levels were unchanged (9.3 ± 0.8 versus 9.1 ± 1.0 pg/mL), but coronary venous ET-1 levels increased after BQ-788 (10.1 ± 1.4 versus 12.4 ± 1.0 pg/mL), although there was absence of an arteriovenous difference in ET-1 (0.8 ± 1.9 pg/mL; P not significant) at baseline, after BQ-788, coronary venous ET-1 levels were higher (arteriovenous difference: 3.3 ± 1.6 pg/mL; P = 0.06). No changes in arterial or venous ET-1 levels were observed after BQ-123 + BQ-788. 

At baseline, coronary arterial and venous levels of NOx were similar (20.9 ± 2.4 versus 21.2 ± 2.4 μmol/L, arterial versus venous; P = 0.6). After BQ-788, a significant trans-coronary arterial and venous difference in plasma NOx was observed (20.0 ± 2.3 versus 18.8 ± 1.2 μmol/L, arterial versus venous; P = 0.01), predominantly because of the significant fall in coronary sinus NOx levels (P = 0.002).

**Discussion**

Selective ET<sub>B</sub> antagonism causes coronary microvascular vasoconstriction without affecting epicardial coronary vascular tone in patients with CAD and/or risk factors. In contrast, combined ET<sub>A+B</sub> receptor blockade results in both epicardial and microvascular coronary vasodilatation. Thus, endogenous ET-1 has a role in the maintenance of resting coronary vasomotor tone. This reflects a net balance of ET<sub>A</sub> receptor-mediated vasoconstriction and ET<sub>B</sub> receptor-mediated vasodilatation, the latter acting exclusively in resistance vessels. Furthermore, epicardial coronary endothelial dysfunction is improved by combined ET<sub>A+B</sub> receptor blockade, as observed with ET<sub>A</sub> antagonism, whereas selective ET<sub>B</sub> antagonism does not affect endothelial function. These effects were similar irrespective of angiographic CAD or specific risk factors.

**Effect of ET Receptor Blockade on Basal Coronary Vascular Tone**

**Epicardial Coronary Circulation**

Epicardial tone was unchanged after ET<sub>B</sub> receptor blockade, reflecting the sparse distribution of epicardial ET<sub>B</sub> receptors in conduit compared with resistance vessels. Notably, similar epicardial coronary dilation (6%) is seen with combined ET<sub>A+B</sub> and selective ET<sub>A</sub> receptor blockade using BQ-123 alone, indicating that basal epicardial coronary constriction is principally mediated via ET<sub>A</sub> receptors.

**Coronary Microcirculation**

Vasoconstriction is the predominant coronary microvascular effect of ET<sub>B</sub> receptor blockade. This response occurred before the increase in systemic blood pressure, implicating local rather than reflex systemic mechanisms. Possible explanations include reduced endothelial NO release and displacement of ET-1 from ET<sub>B</sub> receptors and/or ET<sub>B</sub>-mediated ET-1 clearance. The fall in coronary sinus NOx levels and the increase in coronary sinus levels of ET-1 after BQ-788 support both as potential mechanisms. In contrast, as with selective ET<sub>A</sub> receptor blockade, coronary microvas-
cular dilatation is the predominant local physiological effect of combined ET$_A$/ET$_B$ receptor blockade. In keeping with observations in the forearm microvessels of patients with CAD, the magnitude of coronary blood flow increase (mean 24%) with combined ET$_{A+B}$ receptor blockade in this study appears greater than that observed with selective ET$_A$ blockade (mean: 9% increase) in similar patients. This suggests a complementary effect of both ET$_B$ and ET$_A$ receptor blockades on microvascular smooth muscle. Although this suggests a lesser role for ET$_B$-mediated endothelial NO release than direct ET$_A$-mediated coronary microvascular constriction in our study, this may not be the case in healthy subjects with preserved endothelial NO bioavailability.

Effect of ET Receptor Blockade on Coronary Vascular Endothelial Function
Selective ET$_A$ antagonism did not affect epicardial vessel endothelium-dependent or -independent function. Combined
ET$_{A-B}$ receptor blockade improved epicardial endothelial dysfunction, consistent with our previous observations with selective ET$_{A}$ antagonism$^{22}$ and in keeping with the low to absent epicardial coronary ET$_{B}$ receptor expression.$^{2,37,39}$ These findings implicate ET$_{A}$ activation as an important mediator of epicardial coronary endothelial dysfunction. Reduced oxygen free-radical production with increased NO bioavailability may contribute to this effect.$^{40–42}$

In contrast, microvascular responses to ACH and SNP were unaffected by either BQ-788 alone or in combination with BQ-123. Because selective ET$_{B}$ receptor antagonism improves coronary and forearm microvascular endothelial dysfunction,$^{16,22,26}$ it is likely that attenuated NO release with ET$_{B}$ blockade, as seen in animal studies,$^{43}$ offsets the beneficial effect of ET$_{A}$ receptor antagonism on endothelial function. Alternatively, our cohort may be underpowered to demonstrate a smaller effect of dual antagonism on microvascular endothelial function.

**Limitations**

We cannot quantify the contribution of individual risk factors, and the impact of atherosclerosis burden (intravascular ultrasound) was not assessed. Although it is possible that the coronary vascular effects of ET receptor antagonists may differ somewhat according to these parameters, our cohort is representative of the general population with, or at increased risk, of developing CAD.

ET receptor antagonists may have different effects on coronary vascular function at different doses. Human coronary vascular physiology studies are technically challenging, time consuming, invasive, and, therefore, not without risk. Because a 60-minute infusion of each ET antagonist dose would be required, dose-ranging coronary studies cannot be justified. The concentrations of BQ-788 and BQ-123 were selected based on experience in the forearm circulation.$^{16,17,44}$ At these doses, systemic effects on blood pressure and autonomic tone may have modulated coronary physiology and accounted, in part, for our findings.$^{18–26}$ However, we observed an increase in coronary microvascular tone before the rise in systemic blood pressure during the BQ-788 infusion. Similarly, coronary vascular responses to ACH remained unchanged after adjustment for endothelium-independent vasomotor responses.

The fall in coronary NOx levels with associated increase in CVR after BQ-788 cannot be considered causal. Confirmation of this would require evaluation of responses to BQ-788 and decreased NO. Moreover, both selective ET$_{A}$ and combined ET$_{A-B}$ receptor blockade improve epicardial coronary endothelial dysfunction,$^{22}$ but dysfunction of the microcirculation was only improved by selective ET$_{A}$ blockade. Figure 5 summarizes our observations of the coronary vascular effects of endogenous ET-1.$^{22}$

Consistent with the conclusions of a recent comprehensive review of the literature, we found no incremental benefit with combined ET$_{A-B}$ antagonism in the coronary circulation, suggesting a potential therapeutic advantage for selective ET$_{A}$ receptor antagonism in atherosclerosis. In addition, selective ET$_{B}$ antagonism should be avoided.$^{49}$ The long-term value of appropriate doses of selective oral ET$_{A}$ receptor antagonists in CAD warrants further exploration.

**Source of Funding**

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**Disclosures**

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**References**

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ENDOGENOUS ENDOTHELIN IN HUMAN CORONARY VASCULAR
FUNCTION: DIFFERENTIAL CONTRIBUTION OF ET\textsubscript{A} AND ET\textsubscript{B} RECEPTORS

Endothelin and human coronary vascular function

(Manuscript# HYPERTENSION/2006/083303)

Julian P.J. Halcox M.A., M.D., M.R.C.P.\textsuperscript{1}
Khaled R.A. Nour M.D., F.A.C.C.\textsuperscript{2}
Gloria Zalos R.N.\textsuperscript{3}
Arshed A. Quyyumi M.D., F.R.C.P., F.A.C.C.\textsuperscript{4}

1- Institute of Child Health, University College London, UK,
2- Division of Cardiovascular Medicine, Henry Ford Hospital, Detroit, MI
3- Cardiology Branch, National Heart Lung and Blood Institute, Bethesda, MD,
4- Division of Cardiology, Emory University School of Medicine, Atlanta, GA

Address for Correspondence:
Julian P.J. Halcox
Senior Lecturer in Cardiology
Vascular Physiology Unit,
Institute of Child Health
30 Guildford St
London WC1N 1EH
UK
Tel +44 (0)20 7813 8223
Fax +44 (0)20 7813 8532
E mail j.halcox@ich.ucl.ac.uk
Supplemental Methods

Patients

CAD was defined as any angiographic evidence of plaque in any coronary artery. Risk factors assessed included age, gender, current or previous tobacco smoking, diabetes mellitus, dyslipidemia (elevated fasting cholesterol [>240 mg/dL], low density lipoprotein levels [>160mg/dL], reduced high density lipoprotein cholesterol levels [<40mg/dL]), and hypertension (blood pressure ≥140/≥90mmHg or receiving antihypertensive agents). Patients with unstable coronary syndromes within the previous month, significant valvular heart disease, congestive heart failure, and women of child-bearing potential were excluded. Angiotensin converting enzyme therapy and aspirin were withheld for at least 1 week, and all other cardiac medications were discontinued for at least 5 half-lives prior to the study. Patients consumed a low-nitrate diet for at least 24 hours before the study. The study was approved by the Institutional Review Board of the National Heart, Lung and Blood Institute and informed, written consent was obtained from all patients.
Supplemental Figure Numbers, Titles and Legends

**Figure I: Effect of BQ-788 on coronary microvascular function**

Effect of BQ-788 on coronary vascular resistance (CVR) during (A) acetylcholine (1.5 - 50 µg.min⁻¹), and (B) sodium nitroprusside (SNP, 20µg.min⁻¹) administration. Ratio of acetylcholine/sodium nitroprusside CVR responses before and after BQ-788 (C). Data represent mean ± SEM; p-values represent results of two-way ANOVA for panels A and C, and results of paired t-test in panel B.

**Figure II: Effect of BQ-123 + BQ-788 on coronary microvascular function**

Effect of combined administration of BQ-123+BQ-788 on CVR during (A) acetylcholine (1.5 - 50 µg.min⁻¹), and (B) sodium nitroprusside (SNP, 20µg.min⁻¹) administration. Ratio of acetylcholine/sodium nitroprusside CVR responses before and after BQ-123+BQ-788 (C). Data represent mean ± SEM; p-values represent results of two-way ANOVA for panels A and C, and results of paired t-test in panel B.
Supplemental Table

Table I. Patient characteristics

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<th>Patient Characteristic</th>
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<th>Protocol 2</th>
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<td>Patients Studied (n [%])</td>
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<tr>
<td>Mean Age (years)</td>
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<td>Subjects with 2 risk factors*</td>
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<tr>
<td>Subjects with ≥3 risk factors*</td>
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<td>3</td>
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</tbody>
</table>

Legend:

* modifiable risk factors considered include dyslipidemia, smoking, diabetes mellitus and hypertension (as defined in methods).