Chronic Nebivolol Treatment Suppresses Endothelin-1–Mediated Vasoconstrictor Tone in Adults With Elevated Blood Pressure

Kyle J. Diehl, Brian L. Stauffer, Caitlin A. Dow, Tyler D. Bammert, Danielle L. Brunjes, Jared J. Greiner, Christopher A. DeSouza

Abstract—Endothelin-1 (ET-1) plays a major role in the pathophysiology of hypertension and its associated cardiovascular risk. We tested the hypothesis that chronic nebivolol treatment reduces ET-1–mediated vasoconstrictor tone in adult humans with elevated blood pressure (BP). Furthermore, reducing ET-1 vasoconstrictor activity contributes to the improvement in endothelial vasodilator function associated with nebivolol treatment. Forty-two middle-aged adults with elevated BP (systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg) completed a 3-month, double-blind, randomized, placebo controlled trial: 14 received nebivolol (8 men/6 women; 5 mg per day); 14 received metoprolol succinate (9 men/5 women; 100 mg per day); and 14 received placebo (9 men/5 women). Forearm blood flow (plethysmography) responses to selective (BQ-123: 100 nmol/min; 60 minutes) and nonselective (BQ-123+BQ-788 [50 nmol/min]; 60 minutes) ET-1 receptor blockade, as well as acetylcholine (4.0, 8.0, and 16.0 μg per 100 mL of tissue per minute) in the absence and presence of nonselective ET-1 receptor blockade were determined before and after each treatment intervention. Forearm blood flow responses to BQ-123 and BQ-123+BQ-788 were similarly and significantly elevated (≈30% and 60%, respectively) from baseline in all 3 groups. Nebivolol, but not metoprolol or placebo, therapy resulted in a marked (≈25% and 45%; P<0.05) reduction in forearm blood flow response to BQ-123 and BQ-123+BQ-788. Moreover, after nebivolol therapy only, vasodilator response to acetylcholine was not significantly increased by ET-1 receptor blockade. These results demonstrate that nebivolol, but not metoprolol, treatment reduces ET-1–mediated vasoconstrictor tone in adult humans with elevated BP. In addition, nebivolol-induced reduction in ET-1–mediated vasoconstrictor tone underlies the favorable effects of this β-blocker on endothelial vasodilation.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01395329.

Key Words: blood pressure ■ endothelin-1 ■ metoprolol ■ nebivolol ■ vasoconstriction
Nebivolol, a third generation β-blocker with high selectivity for β₁-adrenergic receptors, has proven to be effective in treating elevated BP. A distinguishing feature of nebivolol from other β-blockers is its hemodynamic profile, specifically the unique ability to enhance both basal and stimulated nitric oxide release resulting in peripheral vasodilation, improved endothelial function and increased myocardial compliance. Cockcroft et al demonstrated that the vasodilatory effects of nebivolol were attenuated by the infusion of the nitric oxide synthase inhibitor NG-monomethyl L-arginine, indicating that nebivolol-induced improvement in vasodilator function is mediated, in part, by increased nitric oxide bioavailability. However, the favorable vascular effects of nebivolol that contribute to its BP-lowering action may not be limited to nitric oxide. Indeed, there are in vitro data to suggest that nebivolol suppresses endothelial ET-1 production, but there is currently no in vivo clinical evidence that treatment with nebivolol reduces ET-1–mediated vasoconstrictor tone.

Accordingly, we tested the hypothesis that chronic nebivolol treatment will reduce ET-1–mediated vasoconstrictor tone in adult humans with elevated BP. Moreover, reducing ET-1 vasoconstrictor activity contributes to the improvement in endothelial vasodilator function associated with nebivolol treatment. To address this hypothesis, we used a 3-month randomized, double-blind, placebo controlled study to determine the effects of nebivolol, compared with metoprolol and placebo, on ET-1 vasoconstrictor tone in adults with suboptimal BP.

### Methods

#### Subjects

Forty-two middle-aged adults with elevated BP (systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg) participated in a 3-month, double-blind, randomized, placebo controlled trial: 14 received nebivolol (8 men/6 women; 5 mg per day; Forest Laboratories, Inc, Jersey City, NJ); 14 received metoprolol succinate (9 men/5 women; 100 mg per day; AstraZeneca LP, Wilmington, DE); and 14 received placebo (9 men/5 women; 1 gelatin capsule per day; Forest Laboratories, Inc). The doses of nebivolol and metoprolol were chosen to elicit similar reductions in BP. Resting BP was determined by the average of ≥2 seated BP readings from 2 separate visits per American Heart Association guidelines. All subjects were free of overt coronary and metabolic disease as assessed by medical history, physical examination, fasting blood chemistries, and electrocardiograms and BP at rest and during incremental exercise performed to exhaustion. In addition, all subjects presented with a resting heart rate more than 50 bpm. None of the subjects smoked, were taking medications (including vitamins), or performed regular physical exercise for at least 1 year before the start of the study. All of the women were at least 1-year postmenopausal and had never taken or had discontinued the use of hormone replacement therapy at least 1 year before the start of the study. After baseline testing, subjects were randomly assigned to 1 of the 3 experimental groups. Before participation, all of the subjects had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder. The study was approved by the Institutional Review Board of University of Colorado, Boulder.

#### Measurements

**Blood Pressure**

Resting BP measurements were performed in the sitting position on at least 2 separate days at least 1 week apart. Subjects were instructed not to...
ingest caffeine-containing beverages before all BP measurements. The recordings were made under quiet, comfortable ambient (≈24°C) laboratory conditions. To avoid the possibility of investigator bias, measurements were made with a semiautomated device (Dinamap, Critikon, FL) that uses an oscillometric technique over the brachial artery. Recordings were made in triplicate in the upright sitting position. All measurements conformed to American Heart Association guidelines as established by the Council for High Blood Pressure Research.

**Body Composition**

Body mass was measured to the nearest 0.1 kg using a medical beam balance (Detecto, Webb City, MO). Percent body fat was determined by dual energy x-ray absorptiometry (Lunar Radiation Corporation, Madison, WI). Body mass index was calculated as weight (kg) divided by height (m) squared. Minimal waist circumference was measured according to previously published guidelines.

**Metabolic Measurements**

Fasting plasma lipid, lipoprotein, glucose, and insulin concentrations were determined using standard techniques by the clinical laboratory affiliated with the Clinical Translational Research Center at the University of Colorado at Boulder.

**Intra-arterial Infusion Studies**

All studies were performed between 7:00 AM and 10:00 AM after a 10-hour overnight fast in a temperature-controlled room. Under strict aseptic conditions a 5-cm, 20-gauge catheter was inserted into the brachial artery of the nondominant arm under local anesthesia (1% lidocaine). Heart rate and arterial BP were continuously measured throughout the infusion protocol. FBF at rest and in response to each pharmacological agent was measured in both the experimental (non-dominant) and the contralateral (dominant) forearm using strain-gauge venous occlusion plethysmography (D.E. Hokanson, Bellevue, WA) as previously described by our laboratory. Baseline FBF was measured for 5 minutes and for 5 minutes before each drug infusion thereafter. After the measurement of resting blood flow, FBF was assessed in response to infusions of acetylcholine (IOLAB Pharmaceuticals, Duluth, GA) at 4.0, 8.0, and 16.0 μg per 100 mL of tissue per minute and sodium nitroprusside (SNP; Nitroprusside, Abbott Laboratories, Abbott Park, IL) at 1.0, 2.0, and 4.0 μg per 100 mL of tissue per minute. Each dose of acetylcholine and SNP was infused for ≈5 minutes, and sufficient time (≈20 minutes) was allowed for FBF to return to resting levels between each vasoactive agent. To avoid an order effect, the sequence of acetylcholine and SNP administration was randomized. After the initial infusion of acetylcholine and SNP and allowing FBF to return to baseline (≈20 minutes), BQ-123 (Clinalfa, AG, Bubendorf, Switzerland), a selective ETα receptor antagonist, was infused at a rate of 100 nmol/min for 60 minutes. FBF was measured every 10 minutes throughout the infusion period. The selected dose of BQ-123 has been shown to completely inhibit the vasoconstrictor effect of ET-1 in the human forearm of healthy adults. After 60 minutes of BQ-123 infusion, the FBF response to nonselective ET-1 receptor blockade was assessed by the coadministration of BQ-123 and BQ-788 (Clinalfa, AG) for an additional 60 minutes. BQ-788, a specific antagonist of ETβ receptors, was infused at a rate of 50 nmol/min for 60 minutes. FBF was measured every 10 minutes throughout the infusion period. The selected dose of BQ-123 has been shown to completely inhibit the vasoconstrictor effect of ET-1 in the human forearm of healthy adults.

**Statistical Analysis**

Differences in subject baseline characteristics were determined by between-groups ANOVA. Differences in FBF responses to acetylcholine, SNP, BQ-123, BQ-123+BQ-788, and BQ-123/BQ-788+acetylcholine involving both main effects and interactions (group×intervention) were determined by repeated-measures ANOVA. Post hoc comparisons were performed using the Tukey procedure. There were no significant sex interactions; therefore, the data were pooled and presented together. All data are expressed as mean±SEM. Statistical significance was set a priori at P<0.05.

**Results**

Selected subject characteristics are presented in Table 1. There were no differences in age and anthropometric, metabolic, or

<table>
<thead>
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<th>Variable</th>
<th>Nebivolol Before</th>
<th>Nebivolol After</th>
<th>Metoprolol Before</th>
<th>Metoprolol After</th>
<th>Placebo Before</th>
<th>Placebo After</th>
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<td>Heart rate, bpm</td>
<td>66±1</td>
<td>64±2*</td>
<td>70±2</td>
<td>73±2</td>
<td>71±2</td>
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<tr>
<td>Systolic BP, mmHg</td>
<td>144±2*</td>
<td>125±3*</td>
<td>139±1</td>
<td>134±2</td>
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<td></td>
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<td>Diastolic BP, mmHg</td>
<td>89±1</td>
<td>77±1*</td>
<td>90±2</td>
<td>77±1*</td>
<td>86±2</td>
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<td>MAP, mmHg</td>
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<td>94±2*</td>
<td>105±2</td>
<td>93±2*</td>
<td>104±2</td>
<td>100±2</td>
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</table>

Values are presented as mean±SEM. BP indicates blood pressure; and MAP, mean arterial pressure. *P<0.05 vs before intervention.
hemodynamic variables between the groups. Table 2 shows the BP responses among the groups. There were no differences in resting BP between the nebivolol, metoprolol, and placebo groups. Both nebivolol and metoprolol treatment resulted in similar and significant reductions in systolic (≈10%), diastolic (≈15%), and mean arterial (≈15%) BPs. There were no significant changes in BP in the placebo group.

Before randomization to nebivolol/metoprolol/placebo, FBF responses to selective ET<sub>A</sub> receptor blockade with BQ-123 were similarly and significantly elevated (≈30%) from baseline in all 3 groups. Nebivolol, but not metoprolol or placebo, therapy resulted in a marked (≈25%; P<0.05) reduction in FBF response to BQ-123 (Figure 1). The vasodilator response to BQ-123 was almost identical before and after...
either metoprolol or placebo treatment. The FBF responses to nonselective ET\_A receptor blockade with BQ-123 and BQ-788 were similar among the groups before treatment. There was a significant increase (≈35%) in FBF beyond that of ET\_A receptor blockade in each group (Figure 2). However, after 3 months of treatment, only nebivolol therapy significantly reduced (≈40%) the FBF response to nonselective ET\_A receptor blockade (Figure 2). Neither metoprolol therapy nor placebo significantly altered the FBF responses to BQ-123/BQ-788 infusion (Figure 2). Across the groups, the FBF response to BQ-123/BQ-788 after each intervention was significantly greater in the nebivolol treatment group.

FBF responses to the endothelium-dependent vasodilator acetylcholine were not significantly different between the 3 groups before intervention (nebivolol: from 5.1±0.3 to 13.3±0.8 mL per 100 mL of tissue per min; metoprolol: from 5.3±0.4 to 13.9±1.1 mL of 100 mL of tissue per min; and placebo: from 4.9±0.2 to 13.0±0.2 mL per 100 mL of tissue per min). Nebivolol treatment resulted in a significant increase (≈20%) in the vasodilator response to acetylcholine (from 4.9±0.4 to 16.4±0.6 mL per 100 mL of tissue per min; Figure 3). In stark contrast, there was no change in the FBF responses to acetylcholine in either the metoprolol (from 5.7±0.5 to 14.1±1.1 mL per 100 mL of tissue per min) or placebo (from 5.1±0.3 to 13.8±0.7 mL per 100 mL of tissue per min) groups after each respective intervention. Across the groups, the FBF response to acetylcholine after each intervention was significantly greater in the nebivolol treatment group. The FBF responses to SNP were not affected by each intervention (Figure 3). The coinfusion of acetylcholine with nonselective ET\_A receptor blockade (BQ-123+BQ-788) resulted in significantly greater (≈30%) vasodilator responses in all 3 groups before each intervention (Figure 4). However, after nebivolol therapy (Figure 4), but not metoprolol (Figure 5) or placebo (Figure 6), FBF response to acetylcholine was not significantly increased by the coinfusion of BQ-123+BQ-788 (Figure 4). In both the metoprolol and placebo groups, nonselective ET\_A receptor blockade augmented acetylcholine-mediated vasodilation to similar extent when compared with before each intervention.

Figure 4. Forearm blood flow (FBF) responses (top) and total FBF (bottom) to acetylcholine in the absence or presence of nonselective endothelin A/B blockade (BQ-123+BQ-788) before and after nebivolol intervention. Values are presented as mean±SEM. *P<0.05 vs saline.
Discussion

The BP-lowering effects of nebivolol are well established. The seminal and novel finding of the present study, however, is that in addition to, and independent of, lowering BP, nebivolol markedly and favorably affects ET-1 system activity. Indeed, the results reported herein demonstrate that (1) chronic nebivolol, but not metoprolol, therapy reduces ET-1–mediated vasoconstrictor tone in adults with elevated BP and (2) reductions in ET-1 vasoconstriction underlie nebivolol-induced improvements in endothelium-dependent vasodilation. Diminished ET-1–mediated vasoconstrictor tone may represent an important endovascular pleiotropic effect of nebivolol, contributing to its favorable effect on overall cardiovascular risk.

In vitro, nebivolol has been shown to blunt endothelial production, and in turn release, of ET-1. The results of this study complement and significantly extend these findings by demonstrating that nebivolol reduces ET-1–mediated vasoconstrictor tone in vivo in adults with elevated BP. After 3 months of nebivolol therapy, there was a marked reduction in the vasodilator response to both selective ETA and nonselective ETA/B receptor blockade. Of note, the nebivolol-induced reduction in ET-1 vasoconstrictor tone was independent of concomitant reductions in BP. Indeed, BP was equally and significantly reduced in adults randomized to either the nebivolol or metoprolol treatment groups. However, metoprolol therapy had no effect on the vascular responses to...

Figure 5. Forearm blood flow (FBF) responses to acetylcholine in the absence or presence of nonselective endothelin A/B blockade (BQ-123+BQ-788) before and after metoprolol intervention. Values are presented as mean±SEM. *P<0.05 vs saline.
either selective or nonselective ET-1 receptor blockade despite its BP-lowering effect. Moreover, there were no significant changes in body composition or cardiometabolic risk factors in response to nebivolol (or metoprolol) treatment. Collectively, this provides further evidence for a direct clinical effect of nebivolol on the ET-1 system. Several mechanisms may underlie this unique feature of nebivolol. Most notably, nebivolol has been shown to ameliorate prepro–ET-1 mRNA production in human coronary endothelial cells. Prepro–ET-1 is the peptide transcribed from prepro–ET-1 mRNA that is post-translationally modified to ET-1. Reduction in prepro–ET-1 would ultimately lead to less ET-1 formation. Other contributing factors may include greater nitric oxide bioavailability and reduced oxidative mediators. Nebivolol increases nitric oxide bioavailability by enhancing endothelial nitric oxide synthase activity through calcium and noncalcium-dependent pathways. Nitric oxide, in turn, has a potent inhibitory influence on ET-1 at the level of transcription and endothelin-converting enzyme activity. About oxidative stress, nebivolol has been shown to block nicotinamide adenine dinucleotide phosphate oxidase, a known activator of the ET-1 system.

Concurrent with the nebivolol-induced reduction in ET-1–mediated vasoconstrictor tone, we also demonstrate that the nebivolol-induced improvement in endothelium-dependent vasodilation is due, at least in part, to the reduction in ET-1 vasoconstriction. It is important to note that before intervention, the FBF responses to acetylcholine in all 3 groups were similar to that previously reported in prehypertensive and hypertensive adults, supporting diminished endothelium-dependent vasodilation in our study population. Consistent with previous studies, we demonstrate that chronic nebivolol therapy significantly improves acetylcholine-mediated endothelium-dependent vasodilation in adults with elevated BP. In stark contrast, there was no effect of metoprolol therapy (or placebo) on endothelial vasodilator function. It has been suggested that the nebivolol-induced improvement in endothelium-dependent vasodilation is largely because of an increase in nitric oxide bioavailability. A seminal finding of this study is that reduced ET-1 vasoconstrictor tone seems to be a primary contributor to improved endothelial vasodilator function. Indeed, before intervention, the coinfusion of nonselective ET receptor blockade resulted in a significant increase (=35%) in

![Figure 6. Forearm blood flow (FBF) responses (top) and total FBF (bottom) to acetycholine in the absence or presence of nonselective endothelin A/B blockade (BQ-123+BQ-788) before and after placebo intervention. Values are presented as mean±SEM. *P<0.05 vs saline.](image-url)
acetylcholine-stimulated endothelial vasodilation in all 3 treatment groups. After the 3-month intervention period, this effect was unchanged in the metoprolol and placebo groups; however, in the nebivolol group, nonselective ET_{A/B} receptor antagonism no longer enhanced the FBF responses to acetylcholine. Although we did not assess whether the nebivolol-induced improvement in acetylcholine-mediated vasodilation was nitric oxide dependent, it is plausible that the previously reported increase in nitric oxide bioavailability with nebivolol is due, in part, to an uncoupling of ET-1–mediated nitric oxide inhibition. Moreover, relieving ET-1–mediated vasoconstriction would allow nitric oxide, and other endothelium-derived relaxing factors, to act without opposition and dilate the vessel appropriately in response to stimulation. Thus, the unique vasomotor properties of nebivolol seem to involve both vasodilator and vasoconstrictor factors. To the best of our knowledge, this is the first study to assess the involvement of the ET-1 system in nebivolol-induced improvements in endothelial vasodilator function.

There are a few experimental considerations about the present study that deserve to be mentioned. First, given the extended half-life of ET receptor antagonists, our study design did not involve the singular administration of the selective ET_{B} receptor antagonist BQ-788, and therefore, we cannot comment on the effects of nebivolol (or metoprolol) on the independent vascular actions of the ET_{B} receptor. Second, we did not measure circulating plasma levels of ET-1 in the present study. ET-1 produced by the endothelium is predominantly (>80%) released abuminally toward the vascular smooth muscle; thus, the pathophysiological significance of circulating ET-1 levels can be variable. Circulating plasma concentrations of the peptide may not necessarily reflect local vascular production but rather spillover into, and clearance from, the bloodstream. However, elevations in plasma ET-1 concentrations have been linked with ET receptor activity. Third, consistent with previous studies, we infused BQ-123 for 60 minutes before the coinfusion with BQ-788; the time course for the slow-onset vasodilation with BQ-123 has been shown to maximize by 60 minutes in some studies and 90 minutes in another study. As a result, we cannot rule out the possibility that further increase in FBF noted in the groups in response to the addition of BQ-788 to BQ-123 may involve some residual effects of BQ-123.

Perspectives

In conclusion, the results of this study indicate that nebivolol, but not metoprolol, treatment reduces ET-1–mediated vasoconstrictor tone in adult humans with elevated BP. Moreover, nebivolol-induced reduction in ET-1–mediated vasoconstrictor tone seems to be an important factor underlying the favorable effects of this β-blocker on endothelial vasodilation. Importantly, the direct effect of nebivolol on ET-1 system activity is in addition to, and independent of, its established BP-lowering effects and may be a key factor contributing to the improvement in endovascular health and reduction in cardiovascular morbidity and mortality associated with chronic nebivolol treatment.

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Disclosures

None.

References


Novelty and Significance

What Is New?

- The novel and seminal finding of this study is that nebivolol, independent of lowering blood pressure, reduces endothelin-1-mediated vasoconstrictor tone in adults with elevated blood pressure. In addition, reduction in endothelin-1 vasoconstriction underlies the nebivolol-induced improvement in endothelin-dependent vasodilation.

What Is Relevant?

- Endothelin-1 plays a pivotal role in the regulation of vascular tone and the cause of hypertension and atherosclerotic vascular disease. Although both nebivolol and metoprolol are highly effective in lowering blood pres-
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