Nebivolol Reverses Endothelial Dysfunction in Essential Hypertension

A Randomized, Double-Blind, Crossover Study

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Background—Vascular endothelial dysfunction may predict future atherosclerosis. Hence, an antihypertensive agent that reverses endothelial dysfunction and lowers blood pressure might improve the prognosis of patients with hypertension. We hypothesized that nebivolol, a vasodilating β-blocker, could improve endothelial dysfunction. We tested this hypothesis by comparing the effects of nebivolol and atenolol on endothelial function.

Methods and Results—Twelve hypertensive patients with a mean ambulatory blood pressure of 154±7/97±10 mm Hg were randomized after a 2-week placebo run-in period (baseline) in a double-blind, crossover fashion to 8-week treatment periods with either 5 mg of nebivolol with 2.5 mg of bendrofluazide or 50 mg of atenolol with 2.5 mg of bendrofluazide. Forearm venous occlusion plethysmography and intra-arterial infusions of acetylcholine and N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) were used to assess stimulated and basal endothelium-dependent nitric oxide release, respectively. Sodium nitroprusside was used as an endothelium-independent control. Nebivolol/bendrofluazide and atenolol/bendrofluazide each lowered the clinic blood pressure to the same extent (132±6/82±6 and 132±9/83±8 mm Hg, respectively; *P*<0.001 from baseline). The vasodilatory response to acetylcholine was significantly increased with nebivolol/bendrofluazide (maximum percentage change in forearm blood flow [mean±SEM], 435±27%; *P*<0.001) but not with atenolol/bendrofluazide. Similarly, the endothelium-dependent vasoconstrictive response to L-NMMA was significantly improved only with nebivolol treatment (percentage change in forearm blood flow, −54±5%; *P*<0.001). The response to sodium nitroprusside was not different between treatments, suggesting that the endothelium-independent pathway was unaffected.

Conclusions—Nebivolol/bendrofluazide increased both stimulated and basal endothelial nitric oxide release, whereas for the same degree of blood pressure control, atenolol/bendrofluazide had no effect on nitric oxide bioactivity. Thus, nebivolol may offer additional vascular protection in treating hypertension. *(Circulation. 2001;104:511-514.)*

Key Words: hypertension ■ endothelium ■ nitric oxide

An impairment in nitric oxide (NO) bioactivity or endothelial dysfunction involving resistance arteries may increase the systemic blood pressure in susceptible individuals, thus giving rise to hypertension.\textsuperscript{1} Interestingly but perhaps not unexpectedly, angiographically normal epicardial coronary arteries in hypertensives have been shown to have endothelial dysfunction, an abnormality that is now known to precede atherosclerosis.\textsuperscript{2} This is especially relevant because hypertension is a major risk factor for ischemic heart disease. The link between endothelial dysfunction and coronary artery disease is now well established.\textsuperscript{3} Hence, reversing endothelial dysfunction in hypertension is an attractive pharmacological aim if the natural history of the hypertension disease process is to be altered. One candidate drug is nebivolol, a selective β\textsubscript{1}-receptor blocker that has vasodilating properties (attributable to its ability to increase NO bioactivity, as demonstrated in animals and human volunteers).\textsuperscript{4,5} To discover whether nebivolol can reverse endothelial dysfunction, we conducted a double-blind, crossover trial comparing the effects of nebivolol and atenolol on vascular NO bioactivity in patients with essential hypertension.

Methods

Subjects

Study subjects were recruited from those attending the Tayside specialist hypertension clinic. Exclusion criteria included secondary hypertension, coronary artery disease, diabetes, hyperlipidemia (fasting cholesterol >5.8 mmol/L), renal impairment or other vascular...
diseases, and smoking. Written, informed consent was obtained from each study subject. The Tayside committee on research medical ethics approved this study.

**Experimental Design**

This was a randomized, double-blind, crossover study. Suitable study subjects had their antihypertensive drugs withdrawn at the beginning of a 2-week placebo run-in period, at the end of which each underwent 24-hour ambulatory blood pressure monitoring. Subjects with daytime ambulatory blood pressures >140 (systolic) and/or >90 (diastolic) mm Hg were then randomized to receive either 5 mg of nebivolol with 2.5 mg of bendrofluazide per day or 50 mg of atenolol with 2.5 mg of bendrofluazide per day for 8 weeks. Bendrofluazide (2.5 mg) was added at the beginning of each treatment period to allow consistent blood pressure control in all study subjects, rather than added later only for those in whom blood pressure control was not achieved with monotherapy. At the end of this first active treatment period, study subjects crossed over to the alternate treatment arm for another 8 weeks after a further 2-week placebo washout period.

Seated blood pressure was measured (mean of 3 measurements) at the beginning of each visit after a 10-minute rest using a semiautomatic oscillometric monitor (OMRON 705CP).

**Vascular Studies**

Vascular endothelial studies were undertaken at the end of the first 2-week placebo run-in period to provide baseline data and at the end of each 8-week active treatment period. Thus, each subject underwent 3 endothelial function assessments. All studies were conducted after an overnight fast and in a quiet, temperature-controlled laboratory (24±0.5°C) with dimmed lights. Alcoholic and caffeinated beverages were avoided for about 24 hours before the study day. After a supine rest of 30 minutes, the nondominant brachial artery was cannulated with a 27-gauge steel needle mounted onto a 16-gauge polyethylene epidural catheter under local anesthesia with 1% lidocaine. Forearm blood flow (FBF) was measured simultaneously in both arms by strain-gauge venous occlusion plethysmography, as previously described. Blood pressure and heart rate were noninvasively (OMRON, HEM-705CP) recorded in the noninfused (control) arm before each infusion.

**Hemodynamic Measurements and Drug Infusions**

FBF was measured during the last 2 minutes of each infusion period and was expressed as mL/100 mL forearm tissue/min, according to the Whitney method. Resting baseline FBF was obtained at least 30 minutes after needle placement to ensure that the blood flow in the cannulated arm had stabilized. After resting baseline FBF measurements, each study subject received intra-arterial infusions of incremental doses of acetylcholine (Miochol, CIBAVision), sodium nitroprusside (David Bull Laboratories), and N\(^\text{G}\)-monomethyl-L-arginine (L-NMMA; Clinalfa). The muscarinic agonist acetylcholine was used to assess endothelium-dependent vasodilatation, whereas sodium nitroprusside, an exogenous source of NO, was used to assess endothelium-independent vasodilatation.

Cumulative dose response curves were constructed after infusions of 2.5, 50, and 100 nmol/min acetylcholine and 4.2, 12.6, and 37.8 nmol/min sodium nitroprusside (each infusion lasted 5 minutes). Endothelial-dependent vasoconstriction was assessed by using the competitive NO synthase antagonist L-NMMA infused at 1, 2, and 4 μmol/min for 5 minutes. After each infusion, care was taken for FBF to reach baseline values; this generally took at least 30 minutes. The order of the vasoactive drugs infused was identical at all the study visits, and the study subjects were unaware of the substances infused. Drugs, saline, and 5% dextrose were infused at flow rates of 1 mL/min by means of a constant-rate infusion pump (Braun).

**Statistical Analysis**

The FBF ratio between the infused and control arms in response to drugs was expressed as a percentage of the ratio measured during the baseline control period (ΔFBF%). The coefficient of variation of FBF ranged from 7.8% to 16% (mean, 10.5%) when FBF was analyzed repeatedly in a steady state. From previous studies, the sample size was estimated with a power of 90% to detect a cumulative ΔFBF% difference between treatments of 100%. Clinical characteristics between clinic visits were compared by paired Student’s t test, and FBF measurements for individual treatments were compared between treatments using 2-way ANOVA with repeated measures with a correction for multiple comparisons for within-group effects. A 2-tailed P<0.05 was considered significant. FBF was expressed as mean±SEM; other values are mean±SD.

**Results**

**Patient Characteristics**

Twelve subjects (10 men and 2 postmenopausal women) with a mean age of 52±7 years and long-standing (>5 years) essential hypertension were studied. Daytime ambulatory blood pressure monitoring at the end of the 2-week placebo run-in period confirmed mild-to-moderate hypertension (Table). Both nebivolol/bendrofluazide and atenolol/bendrofluazide...
or fasting cholesterol levels between the 3 study periods.

ic treatments were well tolerated, and there were no signifi-

centage changes in FBF from baseline preceding each drug
infusion for 3 dose levels of acetylcholine, sodium nitroprusside,
and L-NMMA after placebo ( ● ), nebivolol ( ◦ ), and atenolol ( ○ )
treatment. Values are mean ± SEM. *P < 0.05 and **P < 0.001 for
differences between treatments.

zide treatments reduced systolic and diastolic blood pressures
to the same extent at the end of the each 8-week treatment
period compared with placebo (132 ± 7/82 ± 6 and 132 ± 9/
83 ± 8 versus 154 ± 8/98 ± 9 mm Hg, respectively; P < 0.001).
However, office blood pressure readings were always ob-
tained early in the morning; therefore, we cannot exclude
significant blood pressure discrepancies from daytime to
night-time between treatment periods. Both β-blocker/diuretic
treatments were well tolerated, and there were no significant
differences in baseline plasma electrolytes, urate levels,
or fasting cholesterol levels between the 3 study periods.

Blood pressure and absolute basal FBF (Table) were not
significantly different between the placebo lead-in period at
baseline and the washout period between the 2 active treat-
ments, and there were no significant carry-over effects.

Vascular Studies
Nebivolol/bendrofluazide treatment produced a significant
increase in forearm vasodilatation to acetylcholine compared
with baseline (maximum ΔFBF%, 435 ± 27% versus
185 ± 39%; P < 0.001 for the difference between the whole
dose-response curves; see Figure). In contrast, atenolol/
bendrofluazide had no effect on acetylcholine-mediated (NO-
dependent) vasodilation. Neither nebivolol- nor atenolol-
based treatments significantly affected sodium nitroprusside-
induced vasodilation, which suggests that the endothelium-
independent pathway was unaffected. Only nebivolol-based
treatment improved the vasoconstrictive response to
L-NMMA compared with baseline (ΔFBF%, −54 ± 5% ver-
sus −26 ± 4%, respectively; P < 0.001), which suggests an
additional and significant improvement of basal (tonic) NO
release.

Discussion
The present study demonstrated that nebivolol/bendrofluazide
treatment lowered blood pressure and reversed endothelial
dysfunction in patients with essential hypertension. Atenolol/bendrofluazide treatment similarly lowered blood
pressure but did not alter endothelial function.

Previous in vivo and in vitro studies have suggested that
nebivolol-mediated vasodilation is predominately endotheli-
um-dependent and favorably affects the L-arginine/NO path-
way.13,14 In humans, nebivolol evokes endothelial-dependent
vasodilation in healthy volunteers and in hypertensives. 5,8
However, these were short-term studies involving intrabra-
chial administration of the drug. Whether the same favorable
effects can be expected when given orally has not previously
been studied but would be of much greater relevance in
clinical practice.

The precise mechanism by which nebivolol enhances NO
bioavailability is unclear, but the drug was recently shown to
increase phospholipase C activity, which increases intracel-
lar free calcium concentrations.9 Because the activity of
the constitutive endothelial NO synthase is calcium/calmodulin-
dependent, an increase in the intracellular free calcium
concentration will activate this enzyme, with resultant in-
creases in NO release.10 However, the endothelial cell surface
receptor that nebivolol acts on to mediate phospholipase C
activation has not yet been identified, although it could be a
serotonin (5-HT1 ) receptor. Kakoki and colleagues11 showed
that 5-HT1 blockade with a specific antagonist almost abol-
ished the vasodilatory response to nebivolol in a rat kidney
preparation. This suggests that some degree of pharmacolog-
ic cross-reactivity between serotonin- and β-receptors does
occur, which may explain the vascular effects of nebivolol.12
Also, a possible antioxidant property of nebivolol has been
suggested as an additional factor in increasing NO bioactivity
or even reducing endothelin release.13,14 In the present study,
we could not tell if the improvement in NO bioavailability
was secondary to an increased synthesis and/or reduced
deactivation of NO. However, the improvement of NO
bioactivity seen in the study may well explain some of the
properties of nebivolol that are not shared by other
β-blockers.15

Epidemiological studies have consistently shown that the
dominant effect of blood pressure lowering is that of stroke
reduction; the effect on reducing coronary artery disease has
been disproportionately less in hypertension.16 This could be
because hypertension is only one of many cardiovascular risk
factors; thus, reducing blood pressure in itself may not be
sufficient to reduce mortality and morbidity. Pharmacological
treatment that reduces blood pressure and also reverses
endothelial dysfunction may have an additional impact on
reducing the incidence of cardiovascular events.
In conclusion, nebivolol/bendrofluazide but not atenolol/bendrofluazide–based treatment lowered office (daytime) blood pressure and reversed endothelial dysfunction in hypertensive patients. Its use as an antihypertensive agent might reduce the future atherosclerotic burden in hypertension and, hence, could reduce the risk for cardiac events over and above that expected by lowering blood pressure alone.

References
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Circulation. 2001;104:511-514
doi: 10.1161/hc3001.094207
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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