Nebivolol Reverses Endothelial Dysfunction in Essential Hypertension
A Randomized, Double-Blind, Crossover Study

Nikolaos Tzemos, MRCP; Pitt O. Lim, MRCP; Thomas M. MacDonald, MD, FRCP, FESC

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 L-NAME (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 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.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.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.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 β1-receptor blocker that has vasodilating properties (attributable to its ability to increase NO bioactivity, as demonstrated in animals and human volunteers).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 at least 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.6 Blood pressure and heart rate were noninvasively (OMRON, HEM-705CP) recorded in the noninfused (control) arm before each infusion.

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.7 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, CIBA Vision), sodium nitroprusside (David Bull Laboratories), and NG-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 25, 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 nmol/min sodium nitroprusside, an exogenous source of NO, was used to assess endothelium-independent vasodilatation. 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 (AFBF%). The coefficient of variation of drug response in each subject was 7.8% to 16% (mean, 10.5%) when FBF was expressed as mean±SEM. A 2-tailed P<0.05 was considered significant. 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...
Vascular Studies

Nebivolol/bendroflumethiazide 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/bendroflumethiazide 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% versus −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/bendroflumethiazide treatment lowered blood pressure and reversed endothelial dysfunction in patients with essential hypertension. Atenolol/bendroflumethiazide 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 endothelium-dependent and favorably affects the L-arginine/NO pathway.4,5 In humans, nebivolol evokes endothelial-dependent vasodilation in healthy volunteers and in hypertensives.5,8 However, these were short-term studies involving intrabrachial 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 intracellular 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 increases 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 abolished the vasodilatory response to nebivolol in a rat kidney preparation. This suggests that some degree of pharmacological 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
Nebivolol Reverses Endothelial Dysfunction in Essential Hypertension: A Randomized, Double-Blind, Crossover Study
Nikolaos Tzemos, Pitt O. Lim and Thomas M. MacDonald

Circulation. 2001;104:511-514
doi: 10.1161/hc3001.094207

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/5/511