Exploring Vascular Benefits of Endothelium-Derived Nitric Oxide

John R. Cockcroft

Although the regulation of arterial blood flow has been a subject of intensive medical research, the precise circulatory mechanisms involved are still not fully understood. It has been increasingly recognized that the endothelium plays a vital role in regulating vascular tone, structure, and function. A seminal discovery was made with the identification of endothelium-derived relaxing factor, a key mediator of vasodilation, which was later identified as nitric oxide (NO). Nitric oxide is synthesized from the amino acid L-arginine in the endothelium. Decreased bioavailability of NO is associated with arterial stiffness, hypertension, atherosclerosis, and cardiovascular disease (CVD).

Nebivolol is a novel β-blocker that is highly selective for β1-adrenergic receptors. Nebivolol also causes vasodilation through a mechanism involving endothelium-derived NO. In clinical studies in hypertensive subjects, nebivolol significantly improves vasodilator responses to endothelium-dependent agonists such as acetylcholine. In addition, nebivolol significantly reduces pulse wave velocity (PWV), a measure of arterial stiffness, whereas the β-blocker atenolol has no effect on PWV. Because endothelial dysfunction and arterial stiffness play an integral part in the early atherosclerotic process and are associated with poor outcomes and increased mortality, independent of blood pressure, the ability of nebivolol to enhance release of endothelium-derived NO may have significant clinical implications for the use of this agent in the treatment of hypertension and CVD. Am J Hypertens 2005; 18:177S-183S © 2005 American Journal of Hypertension, Ltd.

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Modern understanding of the circulation of blood and the cardiovascular (CV) system may be traced to the publication in 1628 of Dr. William Harvey’s famous treatise “On the Motion of the Heart and Blood in Animals.”1 Harvey, an eminent English physician, showed that blood is passed through the lungs, propelled through the arteries by the pulsations caused by the contractions of the left ventricle of the heart, and returned to the heart through the veins. He also hypothesized that the arteries close to the heart are larger and thicker than veins, and are distensible because they “sustain the shock of the impelling heart and streaming blood.” Almost 400 years later, however, the precise mechanisms of the arteries in regulating the flow of blood in health and in disease states remain elusive. A major research focus remains on the functions and effects of the endothelium, the inner lining of the epithelial cells of the heart and blood vessels, and its release of the vasodilator nitric oxide (NO).

The endothelium is a dynamic organ that regulates vascular tone, structure, and function by sensing various physiologic stimuli and triggering release of multiple vasodilator substances, including NO.2,3 Endothelial dysfunction, characterized by decreased bioavailability of NO, contributes to hypertension, atherogenesis, and the progression of CV disease (CVD).2-4 Therefore, the effects of an antihypertensive agent on endothelial dysfunction may be important in terms of that drug’s ability to provide end-organ protection, independent of blood pressure (BP) lowering, and to reduce the risks of CV morbidity and mortality.5-7 This article reviews the known functions of endothelium-derived NO and the effects of the novel β-blocker nebivolol on the NO pathway.

Benefits of Endothelium-Derived Nitric Oxide

The endothelium plays an essential role in vasodilation. In an early, seminal in vitro experiment, Furchgott and Zawadzki8 found that unintentional rubbing of the intimal surface of a helical strip of a rabbit descending aorta decreased the vasodilating effect of acetylcholine (ACh) on the strip. The investigators then compared the effects of

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ACh on rubbed and unrubbed strips and found that the unrubbed strips were markedly more sensitive to the vasodilating effect of ACh. However, rubbing had no effect on response to vasconstrictive agents, and rubbing of the adventitial surface of the strip, in contrast to the intimal surface, had no effect on the response to ACh, suggesting that the response observed was selectively endothelium-derived and involved vasodilation of vascular smooth muscle cells. Furchgott and Zawadzki were unable, however, to identify the vasodilating substance or substances released by the endothelium, which became known as endothelium-derived relaxing factor.

Endothelium-derived relaxing factor has subsequently been identified as NO. A number of other vasodilators have since been shown to depend on the integrity of the vascular endothelium for their activity, including bradykinin and substance P. An important component of such vascular endothelium for their activity, including bradykinin and substance P. An important component of such endothelium is associated with selectively impaired endothelium-derived relaxing factor. 

Because of these apparent benefits of endothelium-derived NO, techniques have been developed and clinical studies performed to assess endothelium-dependent vasodilation by comparing it to endothelial-independent vasodilation. In one such study, the effects of ACh, an endothelium-dependent vasodilator, and sodium nitroprusside, a direct endothelium-independent vasodilator, on forearm blood flow (FBF) in the brachial artery were compared in 18 men and women with hypertension and 18 normotensive controls. As measured by strain-gauge plethysmography, FBF, and vascular resistance to ACh were significantly reduced in the hypertensive patients compared to the control patients (P < 0.0002), but no significant differences in FBF and vascular resistance were observed between hypertensive and control patients in those infused with sodium nitroprusside.

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**FIG. 1.** Infusion of 3 mg/kg N-monomethyl-L-arginine, an inhibitor of nitric oxide synthase, significantly increased mean arterial pressure (MAP) by 10% (P < .05) and increased total peripheral resistance by 46%, compared with saline placebo, in eight healthy subjects. TPRI – total peripheral resistance index. *P < .05.
Endothelial Dysfunction and Clinical Outcomes

Endothelial dysfunction has been considered an early phase of the atherosclerotic process. One study followed 157 patients with mild atherosclerosis (without evidence of coronary spasm) to determine whether the presence or degree of endothelial dysfunction, measured at baseline by coronary blood flow response to ACh, was associated with cardiac outcomes. After an average of 28 months of follow-up, no cardiac events were reported in group 1 (patients with baseline normal endothelial function, n = 83) or in group 2 (patients with mild endothelial dysfunction, n = 32), whereas patients with severe endothelial dysfunction at baseline (group 3, n = 42) experienced 10 cardiac events, occurring in six (14%) patients (P < .05 group 3 v groups 1 and 2) (Fig. 3). These findings suggested not only that severe endothelial dysfunction is associated with an increased risk of cardiac events, but that it may also play a pathogenic role in the progression of atherosclerosis.

A study with similar objectives was conducted in 225 patients with never-treated hypertension whose endothelial function was assessed at baseline by measurement of the FBF response to infusion of increasing doses of ACh and sodium nitroprusside. The patients were divided into three groups on the basis of their increase from basal FBF following ACh infusion: tertile 1, severe endothelial dysfunction; tertile 2, mild endothelial dysfunction; and tertile 3, normal endothelial function. After a mean follow-up of 31.5 months, 29 major cardiac or cerebrovascular events occurred, including myocardial infarction (MI), coronary revascularization procedures, stroke, transient ischemic attack, and aortoiliac occlusive disease. Patients with severe endothelial dysfunction experienced a higher rate of CV events. The event rate per 100 patient-years was 8.17 in tertile 1, 4.34 in tertile 2, and 2.02 in tertile 3. The cumulative CV event rate in tertile 1 was 57.2% at 7 years, compared with a rate of 14.4% in tertile 3 (P = .0012) (Fig. 4). There were no differences in event rates between patients in the sodium nitroprusside response groups.

These findings raise the question of whether there is a pathogenic mechanism through which endothelial dysfunction increases the risk of CV events in high-risk patients. Arterial stiffness is a major risk factor for CVD. Brachial artery pulse pressure (the difference between systolic and diastolic BP) is a reliable surrogate measure of increased arterial stiffness and a strong predictor of MI. However, pulse pressure measured peripherally in the brachial artery may not always be in close agreement with central aortic pulse pressure. This is because, in addition to their role as conduits, the large arteries, such as the aorta, are elastic structures with elastin and smooth muscle in the arterial wall. They therefore have an important role in buffering the changes in pressure resulting from intermittent ventricular ejection and smoothing peripheral blood flow. The rate at which pressure waves travel along the arterial tree—the pulse wave velocity (PWV)—is, in part, determined by the stiffness of the arterial wall. The forward-going pressure wave is reflected back from sites of impedance mismatch and normally returns to the aorta during diastole, where it serves to maintain coronary artery blood flow. With age and conditions associated with premature vascular aging, such as diabetes and hypercholesterolemia, arteries stiffen, increasing both the PWV and the amplitude of the reflected wave. Consequently, a larger reflected wave returns to the aorta earlier and augments the late systolic central pressure. Although valuable data may be obtained on arterial stiffness from both central and peripheral pressure waveforms, absolute measures of wave reflection amplitude and wasted left ventricular (LV) pressure energy can only be derived from the central aortic pressure waveform. Beneficial changes on central arterial pressure induced with antihypertensive therapies may not be reflected in brachial artery cuff BP readings. This is important as it is central pressure that the heart and coronary arteries are exposed to.
and it is the central pressure that determines LV workload. Analysis of the central arterial pressure wave provides more information regarding arterial stiffness and central aortic BP than peripheral measurements using conventional sphygmomanometry. It would therefore be of considerable interest to examine the relationship between parameters of arterial stiffness, derived noninvasively from the central aortic waveform, and coronary artery disease. Until recently, such studies were impractical as assessment of the aortic pressure waveform could only be performed invasively. Fortunately, noninvasive assessment of the aortic waveform is now possible using a validated transfer function with the SphygmoCor system (AtCor Medical, Sydney, Australia).

Pulse Wave Analysis

Noninvasive analysis of central arterial waveforms has been made possible with the development of the SphygmoCor system by O’Rourke and Gallagher. Using the SphygmoCor system, peripheral pressure waveforms are recorded noninvasively with a high-fidelity applanation tonometer. The tonometer, with a Millar micromanometer at its tip, is used to flatten but not occlude a peripheral artery (radial or carotid). Circumferential pressures are thus equalized and accurate recording of the pressure waveform is obtained. The peripheral waveform is then transformed into the corresponding central arterial waveform, using Fourier analysis, and a validated generalized transfer function based on data obtained from invasive measurements. Augmentation index, a measure of systemic arterial stiffness, can then be calculated as the difference between the first and second systolic peaks expressed as a percentage of the pulse pressure, and central pressure can then be derived. In addition, by gating the measurements to the R wave of an electrocardiogram and recording at two sites, aortic (carotid/femoral) PWV can also be measured.

The hemodynamic and structural vascular changes associated with age-related arterial stiffening are significant predictors of CV morbidity and mortality risk. A study in 1924 men and women without hypertension or CVD, participating in the Framingham Heart Study, found that elevated pulse pressure was at least as strong an independent predictor of coronary heart disease (CHD) risk as systolic and diastolic BP. Increased PWV has also been shown to be a significant, independent risk factor for CV morbidity and mortality. A study in 710 hypertensive Framingham Heart Study participants demonstrated that all CV risks increased continuously with increasing PWV, and that PWV was the strongest predictor of CV mortality at a given age, compared with other common risk factors such as hypertension, smoking, and LV hypertrophy. Other studies have found that PWV is a strong, independent predictor of all-cause and CV mortality in patients with end-stage renal disease (n = 241), and is a more significant, independent predictor of mortality than systolic BP in patients with type 2 diabetes.

Because hypertension is a major cause of increased PWV, a study was conducted in 150 patients with end-stage renal failure (ESRF), of whom 106 were taking one or more antihypertensive medications, to determine whether sustained BP reduction would significantly lower PWV and reduce mortality risk. After a mean follow-up of 51 months, changes in PWV were significantly correlated with changes in systolic BP (P < .0001). However, neither systolic BP nor diastolic BP had a significant, independent effect on the risk for all-cause or CV mortality. In contrast, each decrease of 1 m/sec in PWV was independently associated with an adjusted relative risk reduction of 29% for all-cause mortality and 21% for CV mortality. These findings suggest that loss of aortic PWV sensitivity to BP lowering could be an indicator of more advanced arterial stiffness, atherosclerosis, and CVD, thus explaining the higher death rate in patients without decreased PWV. Another important implication from the study is that reduction of BP alone may not be sufficient to reduce mortality risk in high-risk patients with CVD. Evaluation of the effects of antihypertensive agents on ancillary parameters such as endothelial function, NO release, and arterial stiffness may be critical to understanding their potential effectiveness in providing end-organ protection and in reducing morbidity and mortality risk.

The Role of Nitric Oxide in Large-Artery Stiffness

Studies have shown that substances associated with increased NO production, including glyceryl trinitrate (GTN), reduce arterial stiffness and wave augmentation, whereas inhibitors of NO, such as L-NMMA, increase measures of arterial stiffness. In addition, increased endothelial dysfunction and arterial stiffness occur in populations at high risk for CVD. Patients with hypercholesterolemia, for example, have been shown separately to have significant endothelial dysfunction in forearm vasculature, and to have increased arterial stiffness and central pulse pressure. Impairment of endothelium-derived NO function and arterial stiffness has also been observed separately in patients with type 1 diabetes. In addition, coexisting arterial stiffness or reduced vascular compliance and endothelial dysfunction have been observed in patients with heart failure and in patients with β-thalassemia major, a risk factor for LV dysfunction.

A study in never-treated hypertensive patients (n = 262) also found that increased pulse pressure, a strong correlate of arterial stiffness as previously noted, was associated with endothelial dysfunction as measured by ACh-stimulated FBF. After adjustment for other covariates, ACh-stimulated FBF decreased by 8.7% for each 1 mm Hg in pulse pressure. The study investigators hypothesized that increased pulse pressure reduces FBF by increasing oxidative stress and reducing the production of nitric oxide and hemodynamics.
NO as a result of the reduced diastolic shear stress occurring with decreased diastolic BP (diastolic shear stress is an important physiologic stimulant of peripheral NO activity). Conversely, NO may play a role in regulating arterial stiffness. An experimental study found that 24-h BP and pulse pressure were significantly increased in mice that lacked the gene for endothelial NO synthase, compared with the corresponding levels in control mice. Another animal study, in anesthetized sheep, showed that infusion of ACh and GTN to stimulate production of NO resulted in significant reductions of PWV (P = .03 with ACh; P < .01 with GTN), as measured by a high-fidelity dual-pressure sensing catheter placed in the common iliac artery. Furthermore, only the effect of ACh was inhibited by co-infusion of L-NMMA (P = .03), and neither ACh nor L-NMMA affected PWV when infused distal to the common iliac artery (through a sheath in the femoral artery). These results demonstrated for the first time that NO affects large-artery distensibility in vivo.

These findings were reproduced in a similarly designed clinical study that evaluated the effects of NO on human iliac artery distensibility in 18 patients found to be free of significant CVD or iliac artery disease after diagnostic cardiac catheterization for atypical chest pain. Catheter infusion of 8 µmol/min and 16 µmol/min L-NMMA significantly increased PWV, compared with a control saline infusion, in a dose-dependent manner (P = .001 for both doses). Moreover, iliac pulse pressure, which is also inversely related to arterial distensibility, increased significantly with 16 µmol/min L-NMMA compared with saline infusion (P < .05).

**Effects of Nebivolol on Endothelium-Derived Nitric Oxide**

Nebivolol is a novel β-blocker that is a racemic mixture of l- and d-enantiomers, and consists of four asymmetric carbon atoms. Nebivolol has a β1 selectivity higher than that of other available agents in the class, and has also demonstrated vasodilatory activity independent of β1 inhibition. To determine whether these vasodilatory mechanisms are associated with endothelial NO synthesis, the effects of brachial infusion of nebivolol and of the nonvasodilating β1-selective adrenergic blocker atenolol on FBF in 40 healthy men were measured using venous occlusion plethysmography. This study found that, compared with baseline saline infusion, 354 µg/min nebivolol significantly increased FBF by approximately 80% (P < .01), whereas atenolol had no significant effect on FBF (Fig. 5). Furthermore, inhibition of NO synthesis with infusion of L-NMMA reduced the vasodilatory response to nebivolol by 65.1%, compared with nitroprusside, the endothelium-independent mediator of NO synthesis and vasodilation (P < .01). Therefore, the vasodilatory effects of nebivolol were shown to be mediated through endothelium-derived NO.

Another clinical study was conducted to evaluate the effects of nebivolol on endothelial function, as measured by FBF with venous occlusion plethysmography, in 12 hypertensive patients (mean ambulatory BP of 154/97 mm Hg). After a 2-week placebo run-in period, patients were randomized in a double-blind, crossover fashion to 8-week treatment periods with once-daily doses of 5 mg of nebivolol plus 2.5 mg of bendrofluazide or 50 mg of atenolol plus 2.5 mg of bendrofluazide, with the treatment periods separated by a second 2-week placebo washout period. Infusions of ACh, L-NMMA, and nitroprusside were used for confirmation of endothelium-dependent versus endothelium-independent vasodilation. Effects on BP were similar between the nebivolol/bendrofluazide and atenolol/bendrofluazide treatment groups. After the first treatment period, the endothelium-dependent vasodilatory response to ACh was significantly increased with nebivolol/bendrofluazide, compared with atenolol/bendrofluazide (P < .001), which had no effect on ACh-mediated vasodilation (Fig. 6). Neither nebivolol/bendrofluazide nor atenolol/bendrofluazide affected FBF response to endothelium-independent sodium nitroprusside. Only nebivolol/bendrofluazide significantly improved FBF response at baseline despite the vasoconstrictive effect of L-NMMA...
PWV as a measure of arterial stiffness were studied in an ovine model.\(^3\)\(^4\) Arterial stiffness occurs not only because of age-related replacement of elastin with stiffer collagen tissue, but is also regulated by changes in smooth muscle tone.\(^3\)\(^4\) Endothelial substances regulate vascular smooth tissue, but is also regulated by changes in smooth muscle of age-related replacement of elastin with stiffer collagen significantly reduced PWV (\(-26\% \pm -54\%\) change in FBF; \(P < .001\)), suggesting a significant increase of NO release with nebivolol/bendrofluazide.

In related investigations, the effects of nebivolol on PWV as a measure of arterial stiffness were studied in an ovine model.\(^4\) \(^9\) Arterial stiffness occurs not only because of age-related replacement of elastin with stiffer collagen tissue, but is also regulated by changes in smooth muscle tone.\(^3\)\(^4\) Endothelial substances regulate vascular smooth muscle tone and function, and in vivo studies suggest that endothelium-derived NO plays a role in regulating progression of large-artery stiffness.\(^3\)\(^4\)\(^5\)\(^0\) The effects of nebivolol on arterial stiffness have been evaluated in recent animal studies. In these studies, the effects on PWV of infusion of nebivolol and atenolol at equimolar concentrations of 250 and 500 nmoL/min for 5 min each were compared using a dual-pressure sensing catheter in the common iliac artery of six anesthetized sheep for each drug.\(^4\)\(^9\) Compared with baseline, both doses of nebivolol significantly reduced PWV (\(P < .05\) and \(P < .01\) with 250 and 500 nmoL/min, respectively), whereas atenolol had no significant effect on PWV (Fig. 7). Infusion of 2, 4, and 8 nmoL/min GTN caused significant, dose-dependent reductions in PWV (\(P < .001\)). The reduction with GTN at the 2 nmoL/min dose was similar to that observed with 500 nmoL/min nebivolol (\(-6\%; P < .001\)). Co-infusion of L-NMMA with nebivolol greatly attenuated the significant reduction in PWV seen with nebivolol alone, thereby confirming that the direct effects of nebivolol on arterial stiffness are mediated through endothelium-derived NO (Fig. 8).

**Conclusion**

Endothelium-derived NO plays a major role in the regulation of vascular tone, structure, and function, and endothelial dysfunction plays an important role in the pathogenesis of hypertension and CVD. Endothelial dysfunction contributes to the progression of arterial stiffness, an age-related process that is a significant, independent risk factor for hypertension, CVD, and mortality. Research also indicates that increased PWV, a robust measure of arterial stiffness, is a more important risk factor for mortality than BP in high-risk patients with ESRF. The novel β-blocker nebivolol has been shown to increase synthesis and release of endothelium-dependent NO, thus stimulating vasodilation and decreasing PWV. The effects of nebivolol on endothelial function suggest that nebivolol may provide clinical benefits independent of BP lowering, particularly in high-risk populations. Further study of the potential clinical impact of the effects of nebivolol on the l-arginine/NO pathway is warranted.

**References**