

ENDOTHELIAL PATHOPHYSIOLOGY

Endothelial Dysfunction and Essential Hypertension

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ABSTRACT

Systemic hypertension is a chronic disorder of cardiovascular system characterized by an increase in systemic vascular resistance (SVR). Although the level of blood pressure is a product of SVR and cardiac output, it is the former which is responsible for chronic blood pressure elevation. A number of biochemical, biophysical, and neuro-humoral factors participate in the maintenance of SVR. Whatever the underlying molecular mechanism may be for elevated SVR, the end consequence is endothelial dysfunction. Normal endothelium promotes vasodilation and prevention of local thrombotic phenomena whereas abnormal endothelium promotes vasoconstriction and thrombotic processes. One of the basic pathophysiological aberrations in hypertension is abnormal endothelial function. A number of blood pressure lowering strategies (life-style modification and or anti-hypertensive drugs) result in reversing endothelial dysfunction in hypertension. Thus, endothelial function is considered both as a mechanism and a therapeutic target in hypertension. This review summarizes the physiology and pathophysiology of endothelium in hypertension.

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INTRODUCTION

Endothelial cells form the innermost monolayer of the vascular wall in arteries, veins, and capillaries. The endothelium functions both as an endocrine organ, expressing receptors for cellular and hormonal communication, and as a paracrine organ, producing vasoactive, inflammatory, vasculoprotective, angiogenic, thrombotic,

and antithrombotic molecules.^{1,2} It exists in persistent homeostasis, balancing blood fluidity and thrombosis, vascular inflammation and immunologic processes, and importantly, regulating vascular tone. We will focus on endothelial regulation of vascular tone in hypertension in this review.

Vascular tone is critically regulated by the endothelium via the synthesis and release of a variety of endothelium-derived factors that exist in a delicate balance with each other. Endothelin-1 (ET-1), angiotensin II, thromboxane A₂, and reactive oxygen species mediate vasoconstriction, whereas nitric oxide (NO), prostacyclin, carbon monoxide, and other endothelium-derived hyperpolarizing factors mediate vasodilation.^{3,4} Among these, NO appears to be a critical regulator of vascular homeostasis.⁵

PATHOPHYSIOLOGY OF ENDOTHELIAL DYSFUNCTION AND HYPERTENSION

Endothelial dysfunction (ED) occurs early after the diagnosis of essential hypertension and may even precede it.⁶ A major characteristic of ED is decreased NO bioavailability. Endothelial cells synthesize NO through the constitutive expression of nitric oxide synthase-3 (NOS-3), also known as endothelial nitric oxide synthase (eNOS),⁶ which facilitates both coupled and uncoupled reactions. In the healthy endothelium, a coupled eNOS leads to a Ca²⁺/calmodulin (CaM)-dependent phosphorylation that converts L-arginine to L-citrulline, resulting in generation of NO.¹ The release of NO leads to smooth muscle vasodilation via a cyclic guanylate monophosphate (cGMP)-mediated activation of guanylate cyclase, altering resting vasomotor tone.

In conditions of increased oxidative stress (OS), an uncoupled Ca²⁺/CaM-independent reaction generates superoxide anions rather than NO.¹ In this state, L-arginine and tetrahydrobiopterin (BH₄) are depleted and peroxynitrite and asymmetric dimethylarginine (ADMA) levels are increased. Excess superoxide typically leads to further depletion of NO by formation of peroxynitrite, destabilization of eNOS, and an overall reduction in NO bioavailability. Overall, a reduction in substrate (L-arginine) levels, presence of eNOS antagonists, elevated breakdown of NO due to OS, and decreased cofactors for eNOS, such as tetrahydrobiopterin lead to decreased NO bioavailability.² Decreased NO availability leads to excess vasomotor tone, which in turn leads to hypertension, spasm, triggers of ischemic events, and other deleterious

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effects on vasculature. Depletion of NO also results in nuclear factor-kappa β (NF- κ B)-dependent activation of adhesion molecules, such as selectins that promote vascular inflammation and increase the thrombotic potential of platelets and blood coagulability, ultimately increasing the risk of atherosclerosis and thrombosis.⁷ Inflammation and OS are associated with traditional and nontraditional cardiovascular risk factors, including essential hypertension.² Furthermore, reduced NO is associated with decreased endothelial progenitor cell (EPC) activity and function, thus impairing vascular regenerative potential.⁸ Thus, decreased NO bioavailability leads to an alteration in endothelial homeostasis, creating a vasoconstrictive, proinflammatory, proatherosclerotic, prothrombotic, and antiregenerative milieu.¹

INVASIVE EVALUATION OF ENDOTHELIAL FUNCTION

Endothelial function can be estimated with both invasive testing of the coronary or peripheral vasculature and by noninvasive techniques. Assessment of coronary endothelial function is performed by direct infusion of endothelium-dependent vasodilators, such as acetylcholine, bradykinin, substance P, and others followed by measurement of changes in epicardial diameter and coronary blood flow. In the setting of normal endothelial function, acetylcholine causes epicardial dilation and increase in coronary blood flow. With ED, epicardial arteries constrict and flow increases to a lesser extent than in the normal setting.^{1,9,10}

Measurements of blood flow changes during intrabrachial arterial infusion of these endothelium-dependent vasodilators can be used to assess endothelial function in the forearm circulation.⁴ During forearm plethysmography, venous drainage is briefly interrupted by inflating a blood pressure cuff, while arterial inflow is maintained. Flow measurements are recorded as endothelial vasodilators are infused. In the setting on normal endothelial function, acetylcholine, a commonly used endothelium-dependent agonist, stimulates release of NO and other endothelium-dependent vasodilators, resulting in vasodilation of the forearm circulation.¹¹ To study the contribution of NO to the observed vasodilation, inhibitors of NO and other endothelium-derived relaxing factors can be employed.¹¹⁻²⁰ Finally, to study endothelium-independent function, NO donors, such as nitroprusside or nitroglycerine are given and blood flow responses measured.

NONINVASIVE EVALUATION OF ENDOTHELIAL FUNCTION

Flow-mediated dilation (FMD) uses high-resolution ultrasound to assess endothelium-dependent brachial

reactivity. The FMD of the brachial artery is almost entirely due to shear-mediated release of NO from the intact brachial artery endothelium and correlates with coronary artery endothelium-dependent responses to acetylcholine, effectively serving as a surrogate marker for coronary endothelial function.²¹ After transiently inducing ischemia by cuff inflation for 5 minutes and subsequent deflation, the resulting hyperemia increases brachial artery shear stress that releases NO from the healthy endothelium and causes the brachial artery to dilate. The magnitude of this FMD is proportional to the NO release from the endothelium.⁶

ENDOTHELIAL FUNCTION AND ADVERSE CARDIOVASCULAR OUTCOMES

Endothelial dysfunction, regardless of the underlying cause, is an independent predictor of future adverse cardiovascular events.²²⁻²⁴ In 3,026 subjects free of cardiovascular disease from the Multi-Ethnic Study of Atherosclerosis (MESA), followed for 5 years, each SD increase in FMD conferred a hazard ratio of 0.84 for incident cardiovascular events. Importantly, FMD also improved net reclassification of risk when compared with the Framingham risk score.²⁴ Two further studies in more selected populations, including the Cardiovascular Health Study of elderly subjects and a study by Rossi et al²⁵ in over 2,000 postmenopausal women, support these findings by demonstrating significant association between impaired FMD and cardiovascular outcomes.²⁴⁻²⁶

ENDOTHELIAL DYSFUNCTION IN HYPERTENSION

A traditional and widely accepted viewpoint is that hypertension is a *cause* rather than a consequence of ED. Acute and chronic hypertension precipitates ED.^{27,28} The Cardiovascular Risk in Young Finns study found that abnormal blood pressure in youth tended to predict future impaired endothelial function.⁴ In a cross-sectional analysis of 3,500 middle-aged participants in MESA, hypertension was associated with a lower FMD in all ethnicities.²⁹ Finally, the degree of ED is related to the magnitude of blood pressure elevation.^{30,31}

Multiple mechanistic studies have shown that the ED observed in hypertension is associated with reduced NO bioavailability in both conductance vessels and the microvasculature.³² Acetylcholine-mediated coronary and forearm vasodilation is blunted in hypertension compared with normotensive controls.¹¹ However, a study in a younger hypertensive population did not confirm this finding.³³ To determine whether the reduced vasodilation with acetylcholine is due to reduced NO bioavailability, acetylcholine infusion was repeated after administration

of N^G-monomethyl-L-arginine, a competitive inhibitor of eNOS synthesis. Inhibition of acetylcholine response was greater in normotensive than in hypertensive subjects, indicating reduced NO bioavailability in hypertension.³⁴ Further studies demonstrated that, at least partly, this reduction in NO activity is compensated by release of endothelium-derived hyperpolarizing factors.³⁴ Infusion of vitamin C, that quenches free radicals when infused intrarterially, restored the reduced endothelium-dependent vasodilation in hypertensives, indicating the contribution of OS to the reduced NO bioavailability in hypertension.³⁵

Endothelin-1, a powerful endogenous, endothelium-derived vasoconstrictor peptide, is continuously released from the vascular endothelium and contributes to tonic vasomotor constrictor tone. The NO may inhibit the synthesis and hemodynamic effects of ET-1; ET-1 can stimulate NO production by stimulating the endothelial ET(B) receptors.³⁶ Blockade of ET-1 receptors, either ET(A/B) or ETA, resulted in a significant increase in forearm blood flow in hypertensive, but not in normotensive controls. Moreover, ET blockade also improved acetylcholine-mediated responses in hypertensive patients, indicating that increased ET-1 activity may play a role in the pathophysiology of hypertension.³⁷ This increased ET-1 activity was shown to be particularly higher in hypertensive black participants compared with white subjects.³⁸

ENDOTHELIAL DYSFUNCTION AS A PRECURSOR TO HYPERTENSION

There is increasing evidence that ED, when present among normotensive subjects, may lead to future development of hypertension.⁶ For example, eNOS knockout mice typically develop hypertension,⁶ infusion of NO synthase antagonists leads to elevation of blood pressure,³⁹ hypertensive subjects appear to have more NOS-3 gene mutations,⁴⁰ and normotensive offspring of hypertensive patients demonstrate impaired endothelial function.⁴¹ In 952 postmenopausal women, free of risk factors including hypertension, Rossi et al²⁵ found that the incidence of hypertension over a 3.6-year follow-up period was 5.77-fold higher in those in the lowest FMD quartile compared with the highest, indicating a role for ED as a precursor in the development of hypertension.⁴² In MESA, 1869 patients without hypertension were followed over a median of 4.8 years for incident hypertension. While the association between low FMD and incident hypertension was significant, this did not withstand multivariate adjustment for important confounders.⁴³

BIOMARKERS OF ENDOTHELIAL DYSFUNCTION

Endothelial function can be estimated indirectly with certain circulating biomarkers, including ADMA, oxidized

low-density lipoprotein, aminothiols including glutathione and cystine, certain adhesion molecules, such as intercellular adhesion molecule-1, endothelial microparticles (EMPs), EPCs, endothelial glycocalyx, monocyte-platelet aggregates, and others.

Asymmetric Dimethylarginine

Asymmetric dimethylarginine is an endogenous competitive antagonist of NO synthase.⁴⁴ Hypertensive patients have higher ADMA levels compared with normotensive, healthy controls⁴⁵ and higher ADMA levels have been associated with both ED and increased intima-media thickness (IMT).⁴⁶ The ADMA levels correlate with pulse wave velocity, further indicating its contribution to hypertension and increased arterial stiffness.⁴⁷ The ADMA levels are also elevated in those with a high-risk factor burden, chronic kidney disease,⁴⁸ coronary heart disease, and stroke. Importantly, higher ADMA levels are associated with adverse long-term outcomes.^{49,50}

Aminothiols

Oxidative stress is implicated in the pathophysiology of ED as described earlier and in multiple conditions including CVD.⁵⁰ Recent studies have shown the importance of nonfree radical species as indicators and mediators of OS.⁵¹ Proteins are susceptible to oxidation through alterations of reactive aminothiol residues and such covalent modifications serve to alter their cellular signaling activity, thereby coupling redox modifications of aminothiols to functional activity.⁵² Importantly, these aminothiols can be quantified in plasma to assess the oxidant burden *in vivo*.⁵³ Of these, cysteine constitutes the major aminothiol pool extracellularly that reacts readily with oxidants to form its oxidized disulfide cystine. Intracellularly, glutathione is a major antioxidant that helps eliminate peroxides and maintain cellular redox, and its oxidized form is glutathione disulfide.⁵⁴ We have shown that increased OS, measured as higher levels of cystine, lower levels of glutathione, or altered ratios of oxidized to reduced aminothiols, is associated with cellular dysfunction, aging, risk factors for CVD including hypertension and subclinical vascular disease including ED, microvascular dysfunction, arterial stiffness, increased carotid IMT, and pulmonary hypertension.^{52,55-62}

Endothelial Progenitor Cells

Endothelial progenitor cells are bone marrow-derived stem cells with the potential to differentiate into mature vascular endothelium. Endothelial dysfunction may be considered to be a result of a balance between the magnitude of injury due to exposure to risk factors and the capacity for endothelial repair.⁶³ Although risk factor-mediated injury to the

vascular endothelium is well understood, the mechanisms underlying regeneration and the pivotal role of progenitor cells (PCs) in vascular repair and hence, to cardiovascular health have only recently been appreciated.⁶³⁻⁶⁵ The EPCs are mononuclear cells that originate primarily (but not exclusively) from the bone marrow and differentiate into endothelial cells both *in vitro* and *in vivo*.^{66,67} The PCs reside primarily in bone marrow, circulate, and contribute to blood vessel formation during tissue repair.⁶⁷⁻⁷⁹ Endogenous PCs contribute to reendothelialization of tissues after endothelial injury, attenuating progression to frank atherosclerosis.⁸⁰⁻⁸⁵ Circulating PCs are multilineage, but the most common circulating PCs are of hematopoietic and EPCs that are capable of vascular repair, largely by their paracrine activities.^{86,87} Our recent studies have shown reduction in the number and migratory activity of PCs in patients with coronary artery disease (CAD) compared with healthy subjects.⁸⁸⁻⁹³ Endothelial dysfunction correlates with PC number and function.^{89,94} Importantly, a low PC count appears to be an independent predictor of poor outcome in patients with CAD,^{89,95} stroke, or acute lung injury.⁹⁴⁻⁹⁷

Endothelial Microparticles

Endothelial microparticles are composed of endothelial cellular debris that breaks off into small membrane vesicles comprised of their native cell membrane and cytoplasm. Using flow cytometry, these microparticles are classified into EMPs, leukocyte microparticles, and platelet microparticles (PMPs).⁹⁸ The EMPs have been used as surrogates for ED⁹⁹ and been associated with decreased NO bioavailability and with the severity of hypertension.¹⁰⁰⁻¹⁰⁴ In 844 participants enrolled in the Framingham offspring cohort,⁹⁹ circulating EMP levels were associated with the development of traditional cardiovascular risk factors, including hypertension.⁹⁹ Patients with severe hypertension, compared with those with mild hypertension or normal blood pressure, had significantly elevated EMPs and PMPs.¹⁰³

Therapeutic Targets

Both classic antihypertensive therapies, therapy targeted toward cardiovascular risk factors and therapy targeting the NO pathway, have been studied in patients with hypertension.¹⁰⁵ Antihypertensive agents, including angiotensin-converting enzyme inhibitors (ACEi), angiotensin-II type I receptor blockers (ARBs), nebivolol, a third-generation beta receptor antagonist, and amlodipine, appear to improve ED in patients with hypertension.¹⁰⁶⁻¹⁰⁸ In a meta-analysis involving 1,129 heterogeneous patients at increased cardiovascular risk, ACEi and ARBs significantly improved brachial

FMD compared with beta-blockers and calcium channel blockers.¹⁰⁸ Both irbesartan and nebivolol in combination with hydrochlorothiazide improved vascular function in hypertension.¹⁰⁷ Nebivolol, in particular, has demonstrated increased NO availability, enhanced antithrombotic activity, and improvement in markers of ED.¹⁰⁷ Both in patients with essential hypertension and in murine models, the combination of amlodipine and atorvastatin improved vascular function.¹⁰⁹ The BH4 acts as a cofactor of NO synthase and a scavenger for free radicals.¹¹⁰ In patients with hypertension receiving BH4 supplementation, investigators have demonstrated improvement in endothelial function.¹¹⁰

SUMMARY

Hypertension is characterized by ED, reduced NO bioavailability, and increased OS, and ED may even precede the development of hypertension. The magnitude of ED is predictive of adverse cardiovascular outcomes, and improvement in ED by medications and other means may reflect reduced risk. Markers that reflect ED are being studied for utility, validity, and clinical application.

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