Supplement to
Clinical Cardiology

Volume 20

11'97

Clinical Cardiology™

A Journal for Advances in Cardiovascular Disease

Endothelial Dysfunction, the Renin-Angiotensin System, and Nitric Oxide: Impact on Coronary Artery Disease and Therapeutic Interventions

Carl J. Pepine, M.D.
Guest Editor

November 1997
Foundation for Advances in Medicine and Science, Inc.

Clinical Cardiology™

Supplement II

Endothelial Dysfunction, the Renin-Angiotensin System, and Nitric Oxide: Impact on Coronary Artery Disease and Therapeutic Interventions

Guest Editor: C.J. PEPINE, M.D.

This supplement is based on the Vascular Biology Working Group meetings held in the United States and Europe in late 1996 and early 1997. The meetings and publication of the proceedings were developed under the auspices of the University of Florida College of Medicine and supported by unrestricted grants from Parke-Davis, Division of Warner-Lambert Company.

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The editorial content of supplements is reviewed by the guest editor and approved by C. Richard Conti, M.D., Editor-in-Chief of the Journal. The findings presented in this supplement are those of the contributors and not necessarily those of the sponsor, the publisher, or the editors of Clinical Cardiology.

The Publisher

CLINICAL CARDIOLOGY: A Journal for Advances in Cardiovascular Disease (ISSN 0160-9289) is published monthly and copyrighted © 1997 by the Foundation for Advances in Medicine and Science, Inc., 111 Oweno Road, Mahwah, N.J. 07430. Second class postage paid at Mahwah, New Jersey, and additional mailing offices. POSTMASTER: Send address changes to Clinical Cardiology, Box 832, Mahwah, NJ 07430-0832.

Subscription per annum (12 issues plus supplements): Institutional and personal—$80 U.S. and $126.50 foreign; single copy $15.50. Subscriptions calendar year only. Payment for subscriptions should be in U.S. funds drawn on U.S. bank to Clinical Cardiology Publishing Co., Inc., Box 832, Mahwah, N.J. 07430-0832.

This journal is fully refereed and is included in Index Medicus, Current Contents/Clinical Practice, ISI BioMed, Science Citation Index and Index Internacional de Cardiologia.

Manuscript submission: Refer to last page of Table of Contents for Instructions to Authors page listing.

Printed in USA by Mack Printing Co., Easton, PA

November 1997

Publisher: Tony Bourgholtzer
Managing Editor: Joey Marie Bourgholtzer, Ph.D.
Art Director: John Romano
Circulation Manager: Lillian Conly
Telecommunications Editor: Lynn M. Savino
Copyreader: Helga Politzer

To implement a change of address, please remove the mailing label affixed to your copy and send it together with your new address to Clinical Cardiology, Box 832, Mahwah, NJ 07430-0832.

Clin. Cardiol. Vol. 20 (Suppl. II), II-1 (1997)

Introduction

Endothelial Dysfunction, the Renin-Angiotensin System, and Nitric Oxide: Impact on Coronary Artery Disease and Therapeutic Interventions

CARL J. PEPINE, M.D., Guest Editor

The Vascular Biology Working Group was formed under the auspices of the University of Florida College of Medicine to bring together researchers from various fields to explore the clinical implications of recent basic research related to the endothelium. A central focus was work dealing with the pathogenesis of cardiovascular disease. The initial meetings of the Working Group were held in March 1994 (North America) and March 1995 (Europe), and annual meetings have refined our understanding of the complex processes that influence the development of atherosclerosis, hypertension, and other vascular diseases. The primary goal of these meetings is to translate basic and clinical research into a message useful to the practicing physician, with the hope that it could impact on adverse outcomes of patients with cardiovascular disease.

This supplement to *Clinical Cardiology* is based on the proceedings of Working Group meetings held in the United States and Europe in late 1996 and early 1997. The nine articles by prominent cardiologists review the current understanding of the endothelium as a mediator of cardiovascular tone and structure. They also discuss interventions that are proposed (e.g., estrogen in postmenopausal women, L-arginine supplementation) and proven [e.g., HMG-CoA reductase inhibitors, angiotensin-converting enzyme (ACE) inhibitors, and others] to improve endothelial function in the coronary circulation of patients with atherosclerosis or hypertension.

To begin, Thomas F. Lüscher, M.D., from University Hospital Zürich and the University of Zürich, Switzerland, establishes the basis for the ensuing discussions with an overview of the biology of the endothelium. As he explains, the endothelium produces substances that regulate both relaxation and contraction of blood vessels, and it also contributes to the maintenance of vascular structure.

Next, David G. Harrison, M.D., of Emory University School of Medicine, Atlanta, examines oxidant stress and endothelial function. We now know that oxidation inactivates nitric oxide and likely contributes to many abnormalities of endothelium that characterize atherosclerosis, hypertension, and other disease processes.

The article on the homeostatic balance between angiotensin II and nitric oxide by Gary H. Gibbons, M.D., from Brigham and Women's Hospital in Boston, provides an excellent overview of the balance between vasoconstrictors and vasodilators as well as between growth promoters and growth inhibitors. In this regard, angiotensin II in particular mediates vascular remodeling. Dr. Gibbons indicates that blocking angiotensin II by ACE inhibition may have profound effects on vascular function and structure.

Although recent research has established that endothelial dysfunction of both large and small blood vessels contributes to hypertension, the exact cause of the pathologic disturbance that causes endothelial dysfunction in patients with hypertension has not been defined. Julio A. Panza, M.D, and colleagues at the National Institutes of Health, Bethesda, have conducted a series of investigations designed to clarify the potential contributions of various endothelium-dependent and -independent factors to abnormal endothelial function. He provides an outstanding overview of the relation between hypertension and endothelial dysfunction as well as an update on the results of experiments to identify potential mechanisms.

A primary focus of the Vascular Biology Working Group is the role of the renin-angiotensin system and ACE in endothelial function. Douglas E. Vaughan, M.D., of Vanderbilt University Medical Center, Nashville, explores this relationship as it pertains to local fibrinolysis, one of the primary endogenous mechanisms for preventing intravascular thrombosis. He provides results of the newest studies showing that ACE inhibition can interrupt the occurrence of acute ischemic events in some populations.

As the inner lining of blood vessels, the endothelium is involved early in the development of atherosclerotic plaque. Plaque disruption causes coronary thrombosis, which is, in turn, the primary mechanism responsible for acute coronary syndromes such as unstable angina, acute myocardial infarction, and sudden cardiac death. The article by Prediman K. Shah, M.D., of Cedars-Sinai Medical Center, Los Angeles, provides an update on the pathogenesis and prevention of plaque disruption and coronary thrombosis, including the recent concept of plaque stabilization as a potential clinical intervention.

In his discussion of the endothelium as a target organ, John P. Cooke, M.D., Ph.D., from Stanford University School of Medicine, Stanford, reviews risk factors known to lead to endothelial dysfunction. These range from well-recognized risk factors for atherosclerosis such as hypertension and increased levels of low-density lipoprotein cholesterol to a recently identified factor, asymmetric dimethylarginine (ADMA), an endogenous antagonist of nitric oxide synthase. A number of potential therapeutic interventions, both pharmacologic (ACE inhibitors, lipid-lowering agents) and nonpharmacologic (e.g., antioxidants), have been shown to improve endothelial function. They accomplish this by modifying or reducing the effects of these factors or by decreasing the vulnerability of the endothelium to damage.

Christophe Bauters, M.D., of the University and Cardiology Hospital of Lille, France, presents results of experimental studies performed at his laboratory on the beneficial effects exerted by several vascular growth factors, including basic fibroblast growth factor and vascular endothelial growth factor, on endothelial function. These growth factors offer a new avenue for therapy of ischemia in either the limbs or the heart.

The final article by myself examines the potential role of ACE inhibition in clinical myocardial ischemia. A number of recently completed or ongoing randomized, clinical trials are reviewed. Results of these trials will provide critical information on the potential benefits of ACE-inhibitor therapy in improving endothelial function.

It is hoped that the publication of this supplement, made possible through an unrestricted grant from Parke-Davis, will further our goal of making the latest research in vascular biology accessible to the practicing physician and provide insights into the basis for clinical interventions to improve outcomes in coronary artery disease.

Clin. Cardiol. Vol. 20 (Suppl. II), II-3–II-10 (1997)

Biology of the Endothelium

THOMAS F. LÜSCHER, M.D., AND MATTHIAS BARTON, M.D.

Department of Cardiology, University Hospital Zürich, and Cardiovascular Research, Institute of Physiology, University of Zürich, Zürich, Switzerland

Summary: The endothelium releases factors that control vascular relaxation and contraction, thrombogenesis and fibrinolysis, and platelet activation and inhibition. Maintaining the functional integrity of the endothelium, therefore, is critical for the preservation of blood flow and the prevention of thrombosis. This article reviews the primary endothelium-dependent substances that promote either relaxation (e.g., nitric oxide, prostacyclin) or contraction (e.g., endothelin) of blood vessels, including their physiology, mechanism of effect, and role in endothelial dysfunction. Risk factors for cardiovascular disease, such as hypertension, hypercholesterolemia, diabetes, vascular aging, and estrogen deficiency, are discussed in terms of their contributions to endothelial dysfunction, which may be the initial step in atherogenesis.

Key words: atherosclerosis, endothelial dysfunction, endothelin, hypercholesterolemia, hypertension, nitric oxide, risk factors

Introduction

Since the pioneering work of Furchgott and Zawadzki,[1] the endothelium has been recognized as a major regulator of vascular hemostasis. Endothelial cells, as the inner lining of blood vessels, are strategically located between circulating blood and blood cells and the vascular smooth muscle. In a person with a body weight of 70 kg, the endothelium covers an area of approximately 700 m^2 and weighs about 1 to 1.5 kg.[2] Functional integrity of the endothelium is crucial for the maintenance of blood flow and antithrombotic capacity, because the endothelium releases humoral factors that control relaxation and contraction, thrombogenesis and fibrinolysis, and platelet activation and inhibition. Thus, the endothelium contributes to blood pressure control, blood flow, and vessel patency. It is now clear that impaired endothelial function contributes substantially to cardiovascular disorders such as atherosclerosis, hypertension, and heart failure, which lead to hypoperfusion, vascular occlusion, and end-organ damage.

Supported by the Swiss National Foundation (grants no. 32-32541.91/2 and no. 32-45878.95), and the Deutsche Forschungsgemeinschaft (Ba 1541/1-1).

Address for reprints:

Thomas F. Lüscher, M.D., F.A.C.C., F.E.S.C.
Professor and Head of Cardiology
University Hospital
CH-8091 Zürich, Switzerland

Physiology of the Endothelium

Endothelium-Derived Relaxing Factors

Stimulation of intact endothelial cells by neurotransmitters, hormones, and substances derived from platelets and the coagulation system causes release of a substance that, in turn, induces relaxation of the underlying vascular smooth muscle (Fig. 1).[1, 3] Furthermore, shear forces generated by circulating blood induce endothelium-dependent vasodilation, which is an important adaptive response of the vasculature during exercise. This endothelium-derived relaxing factor, a diffusible substance with a half-life of a few seconds,[1] has been identified as the free radical, nitric oxide (NO). Nitric oxide is formed from L-arginine by oxidation of the guanidine-nitrogen terminal.[4] The NO-synthesizing enzyme exists in several isoforms in endothelial cells, platelets, macrophages, vascular smooth muscle cells, nerves, and the brain.[5] In endothelial cells, gene expression of NO synthase, although constitutively activated, can be upregulated by shear stress and estrogens. The activity of NO synthase can be inhibited by the circulating amino acid, asymmetrical dimethylarginine (ADMA), which accumulates in patients with renal failure.[6] This observation has been further extended to hypercholesterolemia; increased levels of ADMA were seen in hypercholesterolemic rabbits despite normal renal function,[7] and elevated circulating ADMA was subsequently observed in patients with occlusive peripheral atherosclerotic disease.[8] An inducible isoform of NO synthase exists in vascular smooth muscle and macrophages. When activated by cytokines such as endotoxin, interleukin-1β, and tumor necrosis factor α, this calcium-independent enzyme produces

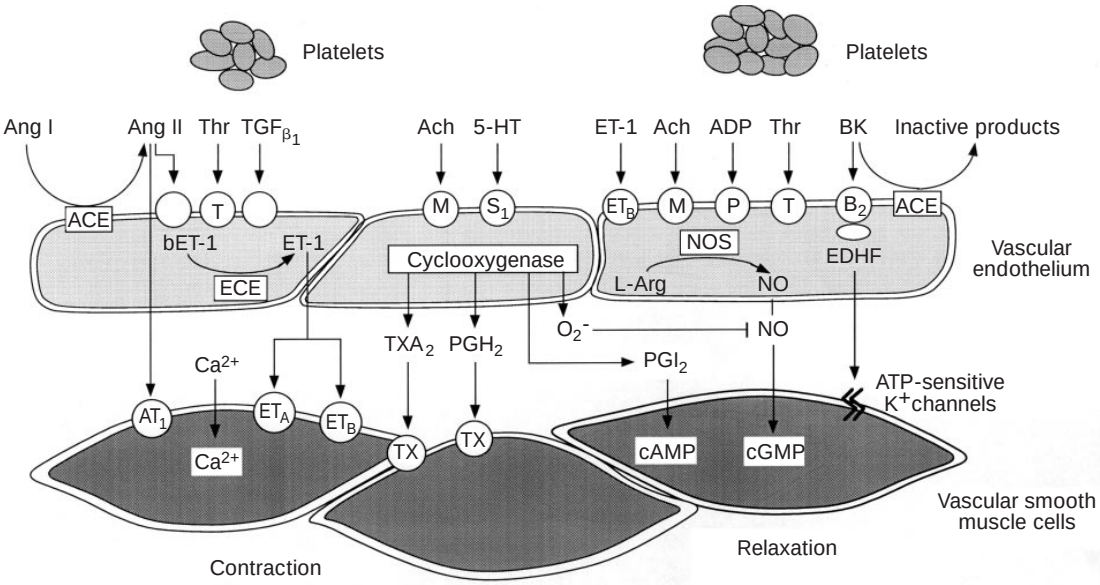

FIG. 1 Vasoactive mediators released by the endothelium. The endothelium produces factors that promote both relaxation (right) and contraction (left). Ang = angiotensin, ACE = angiotensin-converting enzyme, Ach = acetylcholine, ADP = adenosine diphosphate, ATP = adenosine triphosphate, Bk = bradykinin, cAMP/cGMP = cyclic adenosine/guanosine monophosphate, ECE = endothelin-converting enzyme, EDHF = endothelium-derived hyperpolarizing factor, ET = endothelin-1, 5HT = 5-hydroxytryptamine (serotonin), L-Arg = L-arginine, NO = nitric oxide, NOS = nitric oxide synthase, O_2^- = superoxide, PGH_2 = prostaglandin H_2, PGI_2 = prostacyclin, TGFβ_1 = transforming growth factor β_1, Thr = thrombin, TXA_2 = thromboxane A_2. Circles represent receptors (AT = angiotensinergic, B = bradykinergic, ET = endothelin receptor, M = muscarinic, P = purinergic, S = serotonergic, T = thrombin receptor, TX = thromboxane receptor).

large amounts of NO, and hence is activated in inflammatory processes and endotoxic shock.

Endothelium-dependent relaxations due to NO involve formation of cyclic 3',5'-guanosine monophosphate (cGMP) via the soluble enzyme guanylyl cyclase[9] (Fig. 1). Nitric oxide-induced endothelium-dependent relaxation can be pharmacologically inhibited by analogues of L-arginine such as L-N^G-monomethyl arginine (L-NMMA) or L-nitroarginine methylester (L-NAME), which compete with the natural precursor L-arginine at the catalytic site of the enzyme.[5] In isolated arteries, these inhibitors cause endothelium-dependent contractions, whereas in perfused hearts, inhibition of NO formation markedly decreases coronary flow. Local infusion of L-NMMA into the human forearm circulation induces an increase in peripheral vascular resistance. When infused intravenously, L-NMMA induces long-lasting increases in blood pressure. This indicates that the vasculature is in a constant state of vasodilation due to continuous basal release of NO by the endothelium.

In addition to NO, endothelial cells release prostacyclin in response to shear stress, hypoxia, and several substances (see above) that also release NO (Fig. 1). Prostacyclin increases cyclic 3',5'-adenosine monophosphate (cAMP) in smooth muscle and platelets. Its platelet-inhibitory effects play a greater physiologic role than its contribution to endothelium-dependent relaxation. Nitric oxide and prostacyclin synergistically inhibit platelet aggregation, suggesting that the presence of both mediators is required for maximal inhibition of platelet activation.

In the epicardial coronary circulation, inhibitors of the L-arginine pathway do not prevent all endothelium-dependent relaxations, particularly in intramyocardial vessels.[10] Because vascular smooth muscle cells become hyperpolarized during NO-independent relaxations, the existence of endothelium-dependent hyperpolarizing factors has been proposed.[11, 12] However, C-type natriuretic peptide, previously proposed as an endothelium-derived hyperpolarizing factor, does not cause endothelium-dependent hyperpolarization.[13]

Endothelium-Derived Contracting Factors

Soon after endothelium-derived relaxing factor/NO was discovered, it became clear that endothelial cells also can mediate contraction[3] (Fig. 1). Endothelium-derived contracting factors include the 21-amino acid peptide endothelin-1 (ET-1), vasoconstrictor prostanoids such as thromboxane A_2 and prostaglandin H_2, and components of the renin-angiotensin system such as angiotensin II. Three isoforms of the endothelin peptide family exist: endothelin-1, endothelin-2, and endothelin-3. Endothelial cells produce ET-1 exclusively.[14] Translation of messenger RNA generates preproendothelin, which is converted to big endothelin (bET-1) that is further converted by endothelin-converting enzyme (ECE) to the mature peptide ET-1. Four isoforms of this enzyme—ECE-1a, ECE-1b, ECE-1c, and ECE-2—have been cloned.[15, 16] Expression of messenger RNA and release of ET-1 are stimulated by thrombin, transforming growth factor β, interleukin-1, epinephrine, angiotensin II, arginine vaso-

pressin, calcium ionophore, and phorbol ester[14, 17] (Fig. 1).

Endothelin-1 causes vasodilation at lower concentrations but marked and sustained contractions at higher concentrations;[14, 18] in the heart, the latter eventually leads to ischemia, arrhythmias, and death. Intramyocardial vessels are more sensitive to the vasoconstrictor effects of ET-1 than are epicardial coronary arteries, suggesting that endothelin has particular importance in the regulation of flow. Very low circulating levels of ET-1 indicate that most of the peptide is formed locally in the vascular wall. This may be due to the absence of stimuli for endothelin production, the presence of potent inhibitory mechanisms, or the preferential release of endothelin abluminally toward smooth muscle cells.[19] Four inhibitory mechanisms regulating ET-1 production have been delineated: (1) cGMP-dependent inhibition,[17] (2) cAMP-dependent inhibition,[20] (3) an inhibitory factor produced by vascular smooth muscle cells,[21] and (4) inhibition by estrogens via an estrogen-receptor-dependent mechanism.[22] Inhibition of the endothelial L-arginine pathway augments thrombin-induced or angiotensin-induced production of ET-1; conversely, nitrates and atrial natriuretic peptide (which activate particulate guanylyl cyclase) prevent thrombin-induced ET-1 release via a cGMP-dependent mechanism. Endothelin-1 may also promote release of NO and prostacyclin from endothelial cells through ET_B receptors; as a negative feedback mechanism, this process reduces ET-1 production in the endothelium[17] and its vasoconstrictor action in smooth muscle. It is interesting that endothelin inhibits the expression and function of inducible NO synthase.[23]

Two distinct endothelin receptors have been identified: the ET_A- and ET_B-receptors (Fig. 1).[24] Both are G protein-coupled receptors with seven transmembrane domains and are linked to phospholipase C and protein kinase C. Endothelial cells express ET_B-receptors involved in the formation of NO and prostacyclin, which explains the transient vasodilator effects of endothelin when infused into intact organs or organisms. ET_A-receptors and, to some extent, ET_B-receptors mediate contraction and proliferation in vascular smooth muscle. Several endothelin-receptor antagonists have been developed and are currently being clinically evaluated in normal subjects and patients.

The cyclooxygenase pathway also produces endothelium-derived vasoconstrictors. Particularly in veins, but also in the cerebral and ophthalmic circulation, agonists such as arachidonic acid, acetylcholine, histamine, and serotonin can evoke endothelium-dependent contractions that are mediated by thromboxane A_2 or prostaglandin H_2 (Fig. 1).[3] Thromboxane A_2 and prostaglandin H_2 activate the thromboxane receptors in vascular smooth muscle and platelets, thereby counteracting the effects of NO and prostacyclin in both types of cell. In addition, the cyclooxygenase pathway is a source of superoxide anions, which rapidly inactivate NO to form the potent cytotoxic oxidant peroxynitrite.

The endothelium also regulates the activity of the renin-angiotensin system. Angiotensin-converting enzyme (ACE), which converts angiotensin I to angiotensin II, is expressed on the endothelial cell membrane. Angiotensin-converting enzyme is identical to kinase II, which inactivates bradykinin. Angiotensin II can activate endothelial angiotensin receptors; these receptors stimulate the production of ET-1 and other mediators such as plasminogen activator inhibitor.[25] Furthermore, superoxide anion production due to the activation of NADH/NADPH oxidase has recently been linked to angiotensin II-induced hypertension.[26]

Endothelium and Vascular Structure

Removal of endothelial cells by balloon injury invariably leads immediately to deposition of platelets and white blood cells at the site of injury; intimal hyperplasia occurs within days to weeks. This observation suggests that the endothelium regulates vascular structure and that it protects the vessel wall from activation of vascular smooth muscle cells (Fig. 2). Endothelial dysfunction is therefore an important factor in atherosclerosis, restenosis, and hypertensive vascular disease. Vascular structure is determined mainly by vascular smooth muscle cell growth. Endothelial cells may affect vascular structure directly and indirectly. Nitric oxide and prostacyclin inhibit platelet adhesion.[27] Endothelial dysfunction and/or denudation, however, allow platelets to adhere to the vessel wall, where they may cause contraction through the release of thromboxane A_2 and serotonin and may stimulate proliferation and migration of vascular smooth muscle cells via release of platelet-derived growth factor.[28]

Endothelial cells produce growth promoters and growth inhibitors. Under physiologic conditions, the effects of growth inhibitors appear to outweigh those of growth promoters, which may explain why the blood vessel wall is normally quiescent with no proliferation of smooth muscle cells. Heparan sulfates, NO, and transforming growth factor β_1 are potent inhibitors of vascular smooth muscle cell migration and proliferation.[29–31] In contrast, endothelial cells under certain conditions may produce various growth factors, particularly platelet-derived growth factor, epidermal growth factor, and angiotensin II (Fig. 2). These factors may become important in disease states in which the endothelium remains morphologically intact but dysfunctional and may thereby contribute to smooth muscle cell proliferation.

Pathophysiology of the Endothelium

Endothelial Dysfunction: Marker or Mediator?

Endothelial dysfunction is characterized by an imbalance of endothelium-derived relaxing and contracting factors. It may be the cause or consequence of vascular disease and is a hallmark of known cardiovascular risk factors (see below). It is interesting that endothelial dysfunction precedes structural vascular alterations, indicating a protective role of the functionally intact endothelium. While some vessels are particularly prone to developing endothelial dysfunction and atherosclerosis (epicardial coronary arteries, large arteries such as the aorta or iliac artery), others appear to be protected (internal

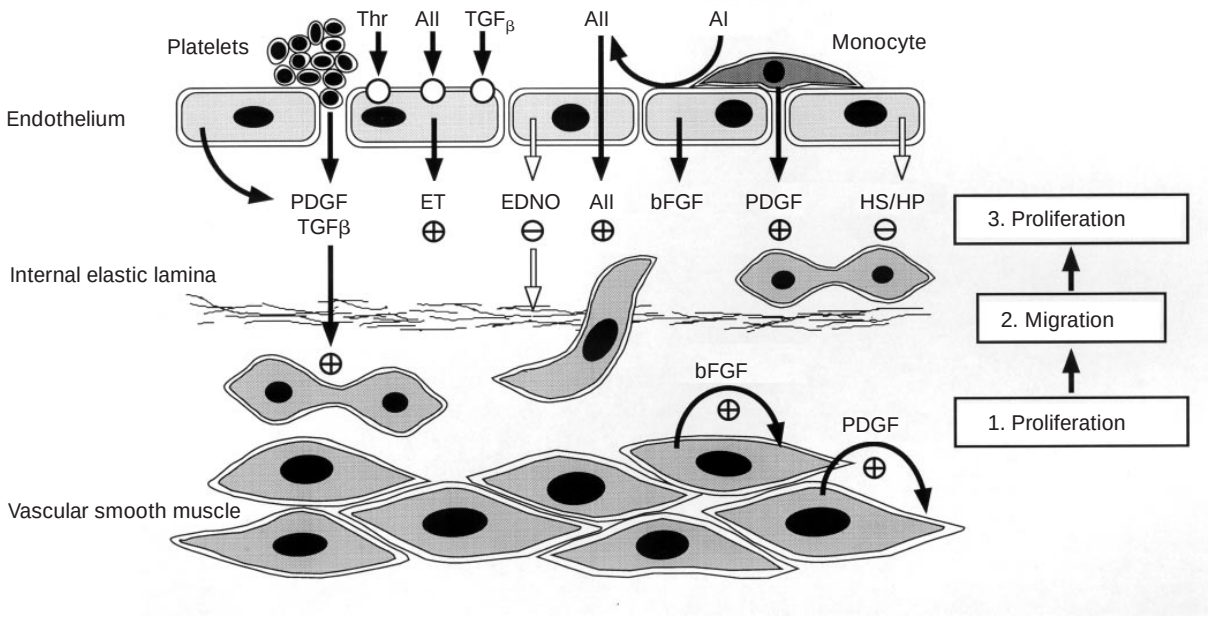

FIG. 2 The endothelium and control of vascular structure. Under normal conditions, the endothelium does not stimulate migration and proliferation of vascular smooth muscle cells. With onset of endothelial dysfunction, platelets and monocytes adhere to the vessel wall, and growth factors are released from these cells as well as from the endothelium. AII = angiotensin II, bFGF = basic fibroblast growth factor, EDNO = endothelium-derived nitric oxide, HP/HS = heparan sulfates, PDGF = platelet-derived growth factor. Other abbreviations as in Figure 1.

mammary artery, brachial artery). This difference may relate to selective alterations due to pulse pressure and/or alterations in endothelial cell function in different areas of the vascular tree. Endothelial cell denudation, however, occurs only in very late stages of atherosclerosis and plaque rupture. These changes in endothelial cell morphology are almost invariably associated with functional alterations and intimal thickening, with accumulation of white blood cells, vascular smooth muscle cells, and fibroblasts and matrix deposition.

Cardiovascular Risk Factors and Endothelial Dysfunction

Hypercholesterolemia: Hypercholesterolemia per se, without atherosclerotic vascular changes, inhibits endothelium-dependent relaxations, which are further reduced in atherosclerosis.[32] It appears that low-density lipoprotein (LDL) is a major determinant of this phenomenon (Fig. 3). Indeed, incubation of isolated coronary arteries with oxidized but not native LDL selectively inhibits endothelium-dependent relaxations to serotonin, aggregating platelets, and thrombin, whereas the response to bradykinin is not affected.[33] A similar diminution of the response can be achieved by pertussis toxin or an inhibitor of NO formation, suggesting defective activation of the L-arginine pathway by G_i protein-coupled receptors.[33, 34] Exogenous L-arginine improves or restores reduced endothelium-dependent relaxation in the presence of oxidized LDL, which suggests that oxidized LDL impairs the activity of NO synthase. The active component of LDL appears to be lysolecithine, which mimics most of the effects of LDL. In vitro experiments in the coronary arteries of hypercholesterolemic pigs have demonstrated selective dysfunction of endothelium-dependent relaxation in response to serotonin and to aggregating platelets and thrombin. Endothelial dysfunction is more extensive in more advanced stages of atherosclerosis. Experiments in the aorta of hypercholesterolemic rabbits suggest that the overall production of NO is not reduced but rather augmented; however, increased production of NO is inactivated by superoxide radicals produced within the endothelium[35] (Fig. 3). Similar observations have been made in rabbits with fully developed atherosclerosis. Under the conditions of both hypercholesterolemia and atherosclerosis, biologically active NO is markedly reduced, a fact also supported by bioassay experiments with coronary arteries of hypercholesterolemic pigs.[36]

Endothelin is activated in atherosclerotic vascular disease. In hyperlipidemia and atherosclerosis, endothelial cell production of endothelin is increased[37] (Fig. 3), while the expression of endothelin receptors is downregulated.[38] A most likely stimulus for the increased endothelin production is LDL, which increases endothelin gene expression and endothelin release from porcine and human aortic endothelial cells[39] (Fig. 3). Vascular smooth muscle cells, particularly those that migrate into the intima during the atherosclerotic process, also produce endothelin. In cultured vascular smooth muscle cells, endothelin can be released by growth factors such as platelet-derived growth factor and transforming growth factor β_1 and by vasoconstrictors such as arginine vasopressin.[40] Hence, several mediators involved in atherosclerosis stimulate vascular endothelin production, perhaps explaining why plasma endothelin levels are increased and correlate positively with

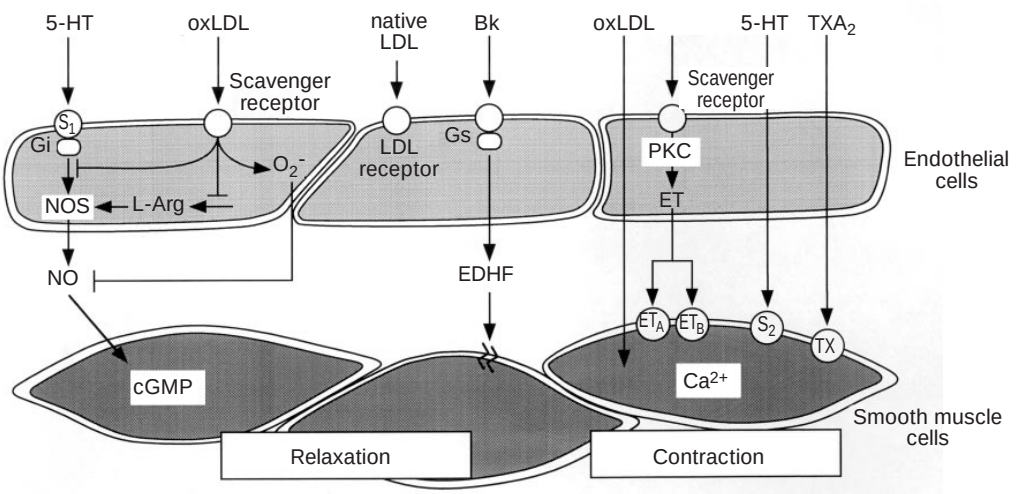

FIG. 3 Endothelial dysfunction in hyperlipidemia and atherosclerosis. The major contributor is oxidized low-density lipoprotein (oxLDL), which, by activating scavenger receptors, impairs the activity of the L-arginine-NO pathway. The mechanism may involve inactivation of G_i proteins (G_i), decreased intracellular availability of L-arginine (L-Arg), and increased breakdown of NO by superoxide (O_2^-). oxLDL further activates endothelin (ET) gene expression and production via protein kinase C (PKC). Other abbreviations as in Figure 1.

the extent of atherosclerotic lesion formation.[37] Furthermore, unstable lesions removed from coronary arteries by atherectomy exhibit marked staining for ET-1.[41] Thus, local vascular endothelin may contribute to both abnormal coronary vasomotion in patients with unstable angina, which may be stimulated by ischemia or thrombin, and to vasoconstriction and the proliferation of vascular smooth muscle cells observed in atherosclerosis.

Hypertension: Endothelial dysfunction in hypertension may contribute to an increase in peripheral vascular resistance (in small arteries) or to vascular complications of the disease (in large and medium-sized conduit arteries). In most models of hypertension, high blood pressure is associated with reduced endothelium-dependent relaxation.[3] Endothelial dysfunction is more prominent in some blood vessels than in others and appears to occur as blood pressure rises; thus, endothelial dysfunction is a consequence rather than a cause of hypertension. In hypertensive subjects, acetylcholine causes paradoxical vasoconstriction of epicardial coronary arteries. The mechanism of endothelial dysfunction differs in various models of hypertension. In the spontaneously hypertensive rat model of genetic hypertension, the activity of the enzyme NO synthase is markedly increased but inefficacious, probably due to increased inactivation of NO by superoxide anion[42] (Fig. 4). In addition, the endothelium of spontaneously hypertensive rats and ren-2 transgenic rats produces increased amounts of prostaglandin H_2, which offsets the effects of NO in vascular smooth muscle and platelets. Whether or not this occurs in humans is uncertain; however, in the forearm circulation of patients with essential hypertension, infusion of a cyclooxygenase inhibitor such as indomethacin enhances vasodilation to acetylcholine.[43] In contrast, salt-induced hypertension is associated with a marked impairment of endothelial NO synthase activity[44] (Fig. 4). Plasma levels of endothelin remain normal in most patients with hypertension except in the presence of renal failure or atherosclerosis. Increased local vascular production of endothelin, however, is likely; because most of the peptide is released abluminally,[19] plasma levels of endothelin do not necessarily reflect local tissue levels. Vascular endothelin production is reduced in the spontaneously hypertensive rat but increased in angiotensin II-induced hypertension in Wistar-Kyoto rats. In the latter model, functional ECE activity is also increased.[45] However, endothelin by itself does not appear to cause hypertension.[46]

Vascular aging: Aging is a physiologic process associated with an increase in cardiovascular morbidity and mortality even in the absence of known cardiovascular risk factors. This may be related to cellular changes in response to increased oxidative stress[47] or to other factors such as impaired release of vasoactive mediators. In most studies, endothelium-dependent relaxations decrease with aging. In humans, the increase in coronary flow induced by acetylcholine infusion lessens with age.[48] Recent studies have demonstrated that the decline in endothelium-dependent relaxation may be related to a decrease in basal[49] and stimulated[50] release of NO and to reduced expression of the endothelial NO synthase gene. Vascular function is preserved with aging, however, in some arteries such as the femoral artery (Fig. 5).[49] Although plasma levels of endothelin increase with age, the response to endothelin decreases, presumably due to downregulation of receptors in most vessels. Similarly, aging heterogeneously affects functional ECE activity, which may increase in some but not all arteries.[49]

Diabetes: Elevated glucose levels in patients with diabetes cause endothelial dysfunction. The underlying mechanism may involve increased synthesis of endothelin[51] and/or impairment of the L-arginine-NO pathway. Recent studies have

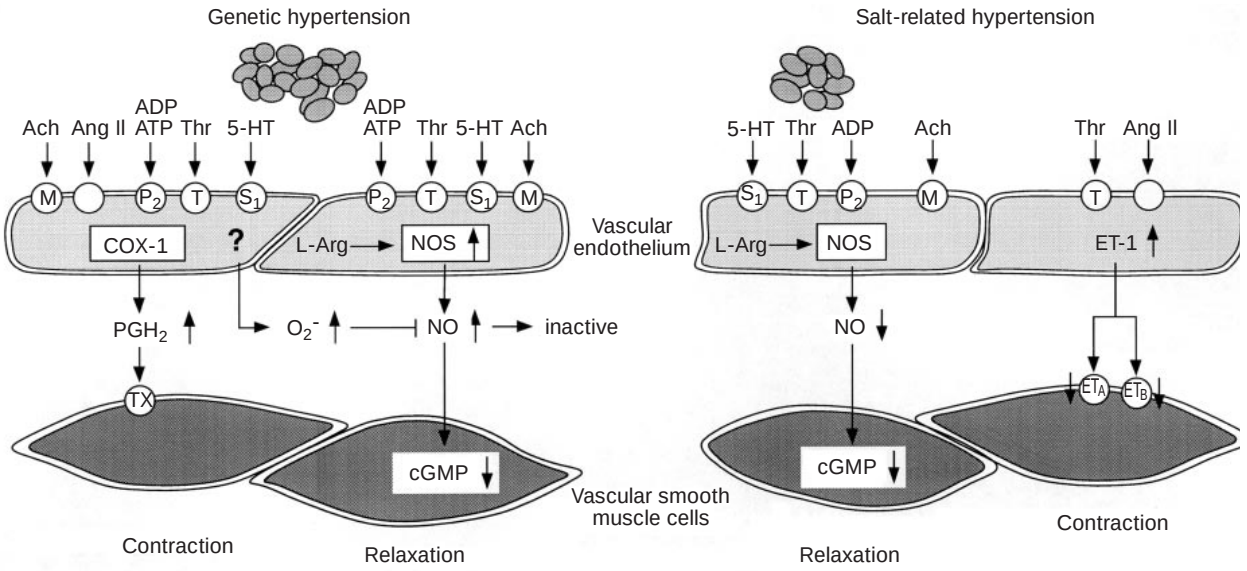

FIG. 4 Endothelial function and hypertension. In spontaneously hypertensive rats (SHR; left), nitric oxide synthase (NOS) activity is increased, but the biological activity of nitric oxide (NO) is reduced, possibly due to inactivation by superoxide (O_2^-). In addition, the production of thromboxane A_2 (TXA_2) and prostaglandin H_2 (PGH_2) via cyclooxygenase (COX-1) is increased. In contrast, in salt-related hypertension (Dahl rats, Sabra rats, Doca® salt hypertension), NO production is reduced. Production of endothelin (ET-1) is increased in Dahl or Doca® salt hypertension but reduced in SHR. Doca® = desoxycorticosterone acetate. Other abbreviations as in Figure 1.

shown that elevated glucose concentrations increase expression of NO synthase and production of superoxide anion in vitro.[52] Vascular dysfunction due to high glucose levels appears to be mediated in vivo by vascular endothelial growth factor via an NO synthase-linked pathway.[53]

Estrogen deficiency: Estrogen is an important modulator of vascular function. Estrogen replacement therapy is associated with a decreased risk of cardiovascular morbidity and mortality in postmenopausal women.[54] Accordingly, male gender is considered an independent risk factor for coronary artery disease. Estrogen modulates NO synthase activity and the formation of NO in vitro and in vivo. Estrogen deficiency is associated with endothelial dysfunction[55] and increased circulating levels of endothelin.[56] Endothelin can be inhibited by estrogen in vitro[22] and in vivo.[56]

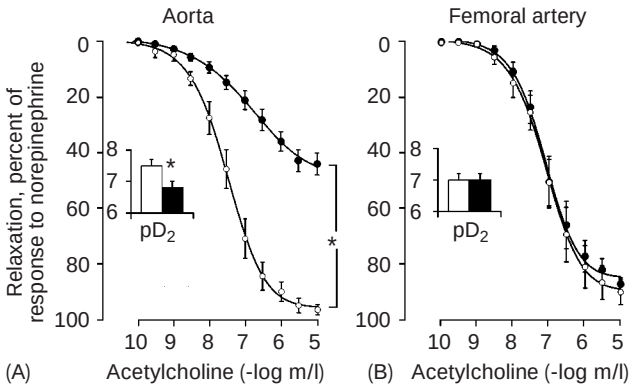

FIG. 5. Endothelial function and vascular aging. Aging impairs NO-mediated endothelium-dependent relaxations to acetylcholine in the aorta of Wistar rats (A), whereas endothelial function in the femoral artery is maintained (B). The different responses indicate an anatomical heterogeneity in the aging process of the endothelium. • Old (n = 6), ◦ young (n = 8). Reprinted with permission from Ref. No. 49.

Clinical Implications

Experimental and clinical evidence suggests that endothelial dysfunction is a major determinant for the development and progression of cardiovascular and renovascular diseases. A major goal of therapy in patients with these diseases should be to improve or preserve endothelial function. Furthermore, since endothelial dysfunction occurs prior to structural vascular changes, therapy should be initiated early in patients at risk (e.g., familial hypercholesterolemia, hypertension). Prevention or correction of endothelial dysfunction in cardiovascular disease with agents targeting the endothelium, such as ACE inhibitors, HMG-CoA reductase inhibitors, and estrogen, is likely to improve the clinical outcome in these patients.

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Clin. Cardiol. Vol. 20 (Suppl. II), II-11–II-17 (1997)

Endothelial Function and Oxidant Stress

DAVID G. HARRISON, M.D.

Cardiology Division and Department of Medicine, Emory University School of Medicine, and the Atlanta Veterans Administration Medical Center, Atlanta, Georgia, USA

Summary: Both endothelial cells and vascular smooth muscle cells are capable of producing reactive oxygen species from a variety of enzymatic sources. In disease states such as atherosclerosis and hypertension, vascular production of these reactive oxygen metabolites can increase substantially. Increases in the production of superoxide anion can lead to decreases in ambient levels of nitric oxide via a facile radical/radical reaction that occurs more rapidly than the reaction of superoxide anion with superoxide dismutase. This phenomenon alters endothelial regulation of vasomotion in a variety of disease conditions. Recent evidence suggests that the major source of vascular superoxide ion and hydrogen peroxide is a membrane-bound, reduced nicotinamide-adenine dinucleotide (NADH)-dependent oxidase. The activity of this enzyme system is regulated by angiotensin II and is elevated following prolonged exposure to nitroglycerin. Alterations of vascular oxidant state caused by angiotensin II may contribute substantially to vascular pathology and may also provide a link between hypertension and atherosclerosis.

Key words: atherosclerosis, angiotensin II, endothelial function, hypertension, nitric oxide, superoxide anion, superoxide dismutase

Introduction

During the past decade, it has become apparent that numerous disease states are associated with abnormalities of endothelium-dependent vascular relaxation in both large and arteriolar vessels. While the mechanisms whereby these various disease processes alter endothelium-dependent vascular relaxation are likely multifactorial, several studies from our laboratory and others have suggested that oxidative inactivation of nitric oxide (NO) may be important in some circumstances. These studies indicate that a tenuous balance exists in the vessel wall between the steady-state levels of NO and superoxide anion ($\bullet O_2^-$). This review will discuss factors that may modulate vascular levels of $\bullet O_2^-$ and NO and the evidence that imbalances between these two can predispose to alterations of vascular regulation in several important disease conditions.

Interactions between Nitric Oxide and Superoxide: A Potential Mechanism for Modulating Vasomotor Tone?

Even before the endothelium-derived relaxing factor (EDRF) was shown to be NO or a closely related compound, it was known that its half-life could be shortened by exposure to artificially generated superoxide and prolonged by superoxide dismutase (SOD).[1, 2] Shortly after the identification of EDRF with NO, Eric Feigl, in a commentary to the journal *Science*, suggested that an important role of the EDRF might be to scavenge $\bullet O_2^-$.[3] This suggestion was based on knowledge of chemical reactions in which superoxide and NO reacted with one another in a facile radical/radical reaction. Since NO could be inactivated by superoxide, it was reasoned that superoxide itself might be inactivated by NO.

At first glance, a role for NO in scavenging superoxide seemed unlikely. Both prokaryotic and eukaryotic cells contain large amounts of various SOD enzymes with a high affinity for superoxide. One might suspect that these SODs would scavenge all of the superoxide made in the vessel wall, preventing the radical from reacting with NO. Recent evidence suggests, however, that in the vessel wall there exists a tenuous balance between superoxide, NO, and cellular antioxidant defense mechanisms (particularly SODs) and that reactions between superoxide and NO may be quite important. For example, it has been shown that the reaction rate between superoxide and NO is extremely rapid ($6.7 \times 10^9\ M^{-1}/s^{-1}$).[4] This rate is approximately three times faster than the reaction rate of superoxide with either the manganese or the copper-zinc form of SOD. In a compartment containing both NO and SOD, there

Address for reprints:

Dr. David G. Harrison
1639 Pierce Drive
319 Woodruff Memorial Research Building
Cardiology Division
Emory University School of Medicine
Atlanta, GA 30329, USA

may be a propensity for superoxide to react preferentially with NO rather than with SOD, depending on the relative concentrations of each.

In addition, there may exist compartments in the cellular and extracellular space in which the scavenging of superoxide by SOD is limited. For example, both SOD and vascular cell membranes carry a negative charge, leading to an electrostatic repulsion between the two.[5] Therefore, interactions between NO and superoxide may occur preferentially over reactions between superoxide and SODs in or near cellular membranes. Such reactions might also occur in interstitial spaces.

Regulation of Vascular Superoxide Production

Studies in Hypercholesterolemia: Possible Role of Xanthine Oxidase

Some of the earliest evidence that vascular levels of superoxide could modulate NO bioactivity came from studies of vessels from cholesterol-fed rabbits. Several conditions, including hypercholesterolemia and atherosclerosis, are associated with altered bioactivity of NO as reflected by abnormal endothelium-dependent vascular relaxation. The responsible mechanisms may vary depending on the stage of the disease, the vascular bed examined, and the animal model studied. Nevertheless, we found that aortas from cholesterol-fed rabbits produce even larger amounts of NO than aortas from control rabbits,[6] but that the NO seemed to be oxidatively degraded. Treatment of the rabbits with polyethylene-glycolated SOD dramatically increased endothelium-dependent vascular relaxation, further suggesting a role for superoxide.[7] Finally, direct measurements of superoxide production using lucigenin chemiluminescence have shown that aortic segments from cholesterol-fed rabbits (4 weeks on diet) produce approximately threefold more superoxide than the aortas of control rabbits.[8] The superoxide production seemed to come from xanthine oxidase, as it was inhibited by oxypurinol. In more recent studies, White *et al.* have shown that levels of xanthine oxidase are increased in the plasma of cholesterol-fed rabbits and that this circulating xanthine oxidase binds to heparin-binding sites on the vessel wall, where it acts to produce excess superoxide.[9] Inhibition of xanthine oxidase with oxypurinol improves endothelium-dependent vascular relaxation, as does treatment of vessels from these animals with heparin, which displaces the xanthine oxidase.

The role of xanthine oxidase may be limited to the early stages of hypercholesterolemia. As atherosclerosis develops and more complicated plaques form, other oxidases become predominant, and oxypurinol or similar agents become less effective in either decreasing superoxide or improving endothelium-dependent vascular relaxation. Preliminary studies in apolipoprotein E-deficient mice indicate a substantial increase in reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase in atherosclerotic vessels, perhaps due to accumulation of macrophages containing the NADPH dependent enzyme.

Studies of Other Oxidase Systems: Role of NADH/NADPH-Dependent Oxidases

Oxidases are present in virtually all mammalian cells and are often named based on their substrate preference, as with xanthine oxidase. One of the best-characterized oxidase systems is neutrophil oxidase,[10, 11] which is composed of at least five components: two cytosolic components (p47phox and p67phox), a small-molecular-weight G protein similar to rac-2, and two membrane-bound components. The last two, p22phox and gp91phox, comprise the cytochrome b558. When activated, the cytosolic components translocate to the membrane components and, upon assembly, create an active oxidase. Neutrophil oxidase is activated by phorbol esters, f-met-leu-phe, and opsonized zymozan. When activated in neutrophils, the enzyme transfers electrons from NADPH to flavins, subsequently to a heme group, and eventually to molecular oxygen. The neutrophil enzyme prefers NADPH over NADH in a ratio of 3:1. Mutations occurring in the various protein subunits of the neutrophil oxidases are associated with chronic granulomatous disease.

What does the neutrophil oxidase have to do with vascular biology? During the past 2 to 3 years, it has become apparent that both the endothelium and vascular smooth muscle contain membrane-bound oxidases that utilize NADH and NADPH as substrates for electron transfer to molecular oxygen.[12–15] These oxidases have similarities to neutrophil NADPH oxidase in that they possess flavin-binding and heme-binding regions, which are likely important in the transfer of electrons. A common component of neutrophil oxidase and vascular smooth muscle oxidase is p22phox. Dr. Kathy Griendling has recently cloned the p22phox of vascular smooth muscle and has shown that it exhibits a high homology to the neutrophil analog.[16] Inhibition of p22phox expression in vascular smooth muscle using antisense techniques results in a loss of NADH oxidase activity.

Despite these similarities, there are important differences between the vascular and neutrophil oxidases. First, the output of vascular oxidase is much lower than that of neutrophil oxidase (nmol vs. µmol/min/mg protein). Second, vascular oxidase does not exhibit "bursts" of activity as does neutrophil oxidase.[17, 18] This property of low output does not lessen the importance of the vascular oxidase system. The neutrophil oxidase system serves a bactericidal role, while vascular oxidase may have other functions, such as modulation of NO activity. Third, unlike neutrophil oxidase, smooth muscle oxidase uses NADH for electron transfer in preference to NADPH.

Regulation of Oxidase Activity by Angiotensin II: Studies in Cultured Cells and Intact Animals

A particularly important aspect of the vascular NADH/NADPH oxidase systems is that their activity is regulated by angiotensin II and certain cytokines. Studies by Dr. Griendling[12] have shown that a 4-h treatment of cultured vascular smooth muscle cells with nanomolar levels of angiotensin II

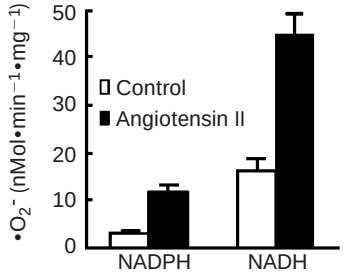

FIG. 1 Production of $\bullet O_2^-$ measured by lucigenin chemiluminescence from homogenates of control and angiotensin II-treated vascular smooth muscle cells exposed to NADH or NADPH. Exposure to angiotensin II for 4 h markedly increased $\bullet O_2^-$ production in response to both NADH and NADPH. NADH = reduced nicotinamide-adenine dinucleotide; NADPH = reduced nicotinamide-adenine dinucleotide phosphate; $\bullet O_2^-$ = superoxide anion. Adapted from Ref. No. 12 with permission.

markedly increases NADH and NADPH oxidase activity (Fig. 1).[12]

Recently, we extended these findings to an in vivo model of angiotensin II-induced hypertension.[19] We used osmotic minipumps to infuse angiotensin II subcutaneously (0.6 mg/kg/day) in Sprague-Dawley rats. To study a model of hypertension associated with low levels of angiotensin II, we also treated rats with a subcutaneous infusion of norepinephrine for a similar period of time. After 5 days the animals were sacrificed and their aortas removed for studies (by lucigenin chemiluminescence) of $\bullet O_2^-$ production. Systolic blood pressure and $\bullet O_2^-$ production are shown in Figure 2. The difference illustrated in $\bullet O_2^-$ production between angiotensin II-treated and sham-operated animals persisted in experiments in which we intentionally removed the endothelium, suggesting the vascular smooth muscle as a likely source for the increase in $\bullet O_2^-$.

Characterization of the Source of $\bullet O_2^-$ in Angiotensin II-Treated Rats

In additional experiments, we sought to characterize the enzyme systems involved in this increase in $\bullet O_2^-$ production. Diphenylene iodonium, an inhibitor of flavin-containing enzymes, normalized $\bullet O_2^-$ production in vessels removed from angiotensin II-infused animals. In contrast, $\bullet O_2^-$ production was not altered by rotenone, oxypurinol, indomethacin, or L-nitroarginine, suggesting that the source of the $\bullet O_2^-$ was not mitochondrial electron transport, xanthine oxidase, cyclooxygenase, or NO synthase. We made homogenates of tissue and examined the ability of various agents to serve as substrates for $\bullet O_2^-$ production. Arachidonic acid, xanthine, and succinate (in the presence of antimycin) had only a minimal effect on $\bullet O_2^-$ production. In contrast, both NADH and, to a lesser extent, NADPH served as substrates for $\bullet O_2^-$ production. In both cases, more than 98% of the activity occurred in the membrane and was greater in the vessels from angiotensin II-treated animals (Fig. 3).[19]

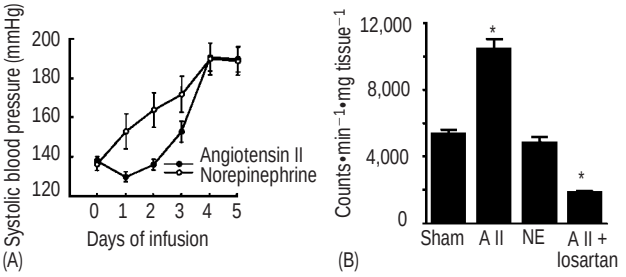

FIG. 2 (A) Effect of either angiotensin II (A II) or norepinephrine (NE) infusion on systolic blood pressures in rats (measured by tail cuff sphygmomanometry). Although blood pressure rose more rapidly in norepinephrine-treated animals, pressures after 5 days in both groups had plateaued at similar values. (B) Lucigenin counts, reflecting $\bullet O_2^-$ production from intact aortic segments from norepinephrine-infused and angiotensin II-treated animals. Sham-operated animals served as controls. Also shown is the effect of concomitant treatment with the angiotensin$_1$-receptor antagonist, losartan. Losartan treatment completely prevented (and, in fact, reduced below normal levels) the effect of angiotensin II on $\bullet O_2^-$ production. *$p < 0.01$ versus sham. Reproduced from Ref. No. 19 by copyright permission of the American Society for Clinical Investigation.

Role of Increased $\bullet O_2^-$ Production in Angiotensin II-Induced Hypertension and Changes in Vascular Reactivity

The increase in vascular smooth muscle production of $\bullet O_2^-$ caused by angiotensin II treatment was associated with a marked impairment in endothelium-dependent vascular relaxation. This impairment was not observed in vessels from rats treated with norepinephrine (Fig. 4).[19]

Because loss of NO can contribute to hypertension, we reasoned that an increase in $\bullet O^{2-}$ in the resistance circulation might contribute to the hypertension caused by infusion of angiotensin II. In a recent study,[20] we lowered endogenous steady-state levels of vascular $\bullet O^{2-}$ by treating rats with daily injections of pH-sensitive, liposome-entrapped SOD. This

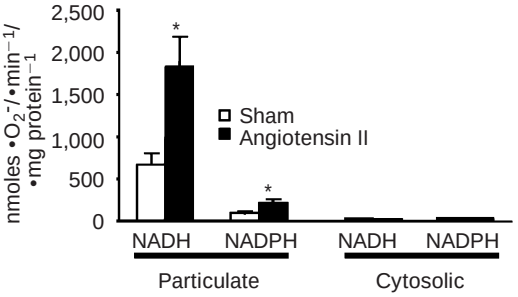

FIG. 3 Superoxide production in response to either NADH or NADPH in particulate (A) and cytosolic (B) fractions of aortic homogenates from sham-operated and angiotensin II-treated rats. The data indicate an activation of a membrane-bound NADH-dependent oxidase in aortic tissue from the angiotensin II-treated rats. *$p < 0.01$ versus sham. Reproduced from Ref. No. 19 by copyright permission of The American Society for Clinical Investigation.

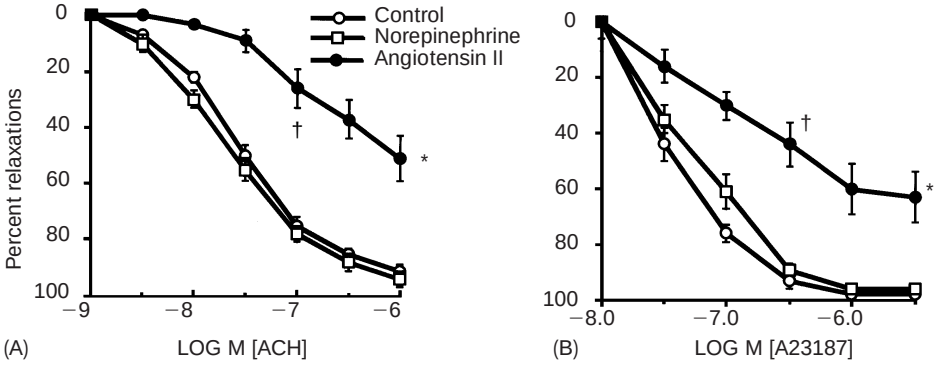

FIG. 4 Endothelium-dependent vascular relaxations to acetylcholine (ACH) and the calcium ionophore A23187 in vessels from control rats and rats infused with norepinephrine or angiotensin II. While angiotensin II and norepinephrine produced equal degrees of hypertension, only angiotensin II was associated with an impairment in endothelium-dependent vascular relaxation. * = $p < 0.05$ versus sham for % relaxation, † = $p < 0.05$ versus sham for ED_{50}. Reproduced from Ref. No. 19 by copyright permission of The American Society for Clinical Investigation.

treatment had no effect on blood pressure in either control or norepinephrine-infused rats, but lowered blood pressure by 60 mmHg in rats with angiotensin II-induced hypertension (Fig. 5). These data suggest that a portion of the hypertension in conditions of elevated angiotensin II is associated with an increase in vascular superoxide production.

These findings may provide some insight into why forms of hypertension associated with elevated plasma renin activity (and presumably elevated effects of angiotensin II) are associated with increased rates of cardiovascular events.[21] It is of interest that hypertension induced by norepinephrine infusion was not associated with an increase in vascular $\bullet O_2^-$ production and did not alter endothelial regulation of vasomotion. We also found that infusion of lower doses of angiotensin II, which had minimal effects on blood pressure, also increased NADH oxidase activity by about twofold. This result suggested that hypertension per se is not a stimulus for increased $\bullet O_2^-$ production, but that conditions in which circulating or local levels of angiotensin II are increased may have unique effects on the vessel wall independent of elevating blood pressure. Further, hypertension not associated with increases in angiotensin II and activation of vascular oxidases may be less prone to produce vascular disease.

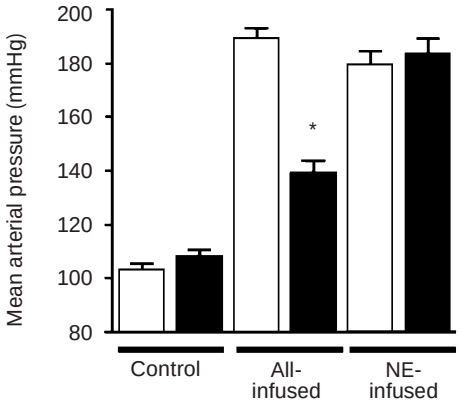

FIG. 5 Effect of liposome-entrapped superoxide dismutase (SOD) on blood pressure in control, angiotensin II-treated, and norepinephrine-treated rats. Angiotensin II (0.7 $mg \bullet kg^{-1} \bullet d^{-1}$) and norepinephrine (2.8 $mg \bullet kg^{-1} \bullet d^{-1}$) were administered subcutaneously for 5 days using osmotic minipumps, and liposome-entrapped SOD was administered daily by intravenous bolus. Blood pressure was measured by means of an indwelling arterial catheter while the rats were awake. □ = Empty liposomes, ■ = liposome-SOD. * = $p < 0.05$ versus empty liposomes.Reprinted from Ref. No. 20 with permission .

The effect of hypertension on endothelium-dependent vascular relaxation is somewhat controversial (for a review, see Ref. No. 22). Furthermore, the cause of altered endothelial regulation of vasomotion may vary in different forms of hypertension. Based on our current findings, we may speculate that animal models or human subjects with hypertension associated with elevated levels of angiotensin II might exhibit greater alterations of endothelium-dependent vascular relaxation than do hypertensive conditions associated with low-renin, low-angiotensin II states. Future studies of endothelium-dependent vascular relaxation in humans should take into account the renin-angiotensin II profiles of the subjects enrolled. Such studies may provide insight into why treatment with angiotensin-converting enzyme inhibitors or angiotensin II-receptor antagonists may have beneficial effects not seen with other drugs.[23–26]

Role of NADH/NADPH Oxidase in Nitrate Tolerance

Angiotensin II-induced hypertension is not the only situation in which vascular oxidases are activated. In other studies, we found that nitrate tolerance is in part due to an increase in vascular superoxide production.[27] Rabbits treated for 3 days with nitroglycerin demonstrated decreased vascular relaxations to nitroglycerin and cross-tolerance to NO endogenously released by acetylcholine. Tolerance to nitroglycerin was greatest when the endothelium was present. In these studies, we found that superoxide production by aortic segments from nitrate-tolerant animals was increased twofold. It is of interest

that, as with tolerance, this increase in superoxide production was greatest in vessels with intact endothelium, suggesting that a major source of the superoxide was the endothelium itself. Subsequent studies of homogenates of these vessels showed an increase in NADH-dependent oxidase activity, identical to that previously observed in angiotensin II-treated rats.[17] Because nitrate therapy is associated with an increase in plasma renin activity, we hypothesized that the increase in oxidase activity might be secondary to angiotensin II. In preliminary studies, we found that treatment with the angiotensin$_1$-receptor antagonist, losartan, completely normalized vasodilation to nitroglycerin and reduced vascular superoxide production to normal.

A related finding in these studies was that treatment with hydralazine markedly inhibited superoxide production and reduced vascular NADH oxidase activity tremendously.[17] Since hydralazine prevents nitrate tolerance, its inhibition of NADH oxidase may play a role in this phenomenon.[28]

These various studies have provided some insight into how vascular production of superoxide can play a role in regulating the bioactivity of NO, produced either endogenously or administered exogenously. Given that the levels of production of both NO and superoxide are subject to modest degrees of control, the interplay between the two may be quite dynamic. A final manner in which the levels of superoxide and NO may be modulated relates to scavenging of superoxide in the vessel wall.

Regulation of Vascular Antioxidant Defense Mechanisms

While a substantial amount has been learned about control of vascular NO and superoxide levels, less is known about what may control endogenous antioxidant defense mechanisms. The most important of these mechanisms in terms of superoxide are the SODs. There are three types of SOD—mitochondrial manganese-containing SOD, cytosolic copper-zinc SOD, and an extracellular SOD—and their regulation varies substantially. Copper-zinc SOD plays an important role in modulating the release of bioactive NO. Pharmacologic inhibition of copper-zinc SOD results in release of NO from the endothelium in an oxidatively inactive form, likely as nitrite and nitrate.[29] Thus, copper-zinc SOD plays an important role in protecting NO in the endothelium.

Based on this observation, we recently hypothesized that shear stress might modulate the expression of copper-zinc SOD.[30] This hypothesis was based on our earlier finding that endothelial NO synthase is regulated in response to shear stress. We reasoned that an increase in copper-zinc SOD might assure that any additional NO made by vessels previously exposed to high shear might be released more efficiently. We exposed human aortic endothelial cells to various levels of shear and examined the expression of copper-zinc SOD at the mRNA, protein, and activity level. The results are shown in Figures 6 and 7.[30]

It has been observed that vessels exposed to elevated shear stresses exhibit enhanced endothelium-dependent vascular relaxations.[31] Shear stress also increases the expression of endothelial cell NO synthase mRNA and protein (both approximately threefold for shears of 15 dynes/cm^2 compared with static conditions),[32] and increases the capacity of endothelial cells to release NO (approximately twofold at 15 dynes/cm^2 for 24 h compared with static conditions).[33] These findings of shear regulation of copper-zinc SOD expression suggest that augmented endothelium-dependent relaxations in vessels exposed to high shear stress may be mediated by increases in the expression of endothelial cell NO synthase and that increased expression of copper-zinc SOD might synergistically potentiate the vasorelaxant capacity of endothelium-derived NO. Although the measured increase in SOD activity was modest in these studies, even a small increase in SOD activity will markedly decrease the half-life of $\bullet O_2^-$.[34]

The distribution of hemodynamic forces is thought to influence the development of atherosclerosis substantially. In humans, regions of low shear stress are more prone to develop atherosclerosis than regions exposed to high shear stress. In experimental animals, plaque formation is greater in regions with low shear stresses, while elevated shear stresses tend to protect against plaque formation and intimal thickening.[35, 36] Differences in expression of copper-zinc SOD may in part ex-

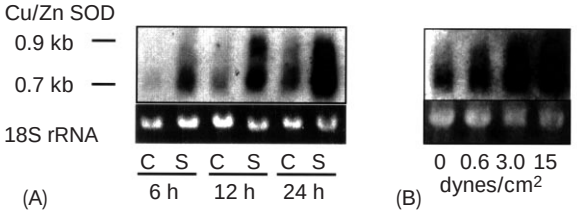

FIG. 6 Messenger RNA levels of copper-zinc SOD (Cu/Zn SOD) in human aortic endothelial cells exposed to either control conditions (C) or shear stress (S) at various times (A, 15 dynes/cm^2) or various levels (B). Reprinted from Ref. No. 30 with permission.

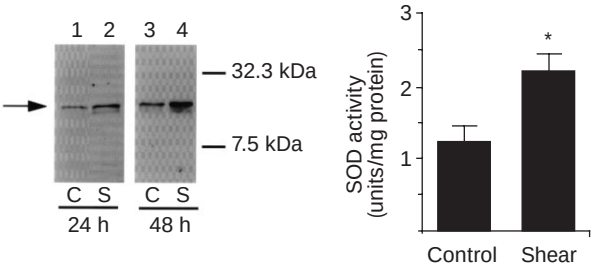

FIG. 7 Effect of shear stress on expression of copper-zinc superoxide dismutase (SOD) protein and enzyme activity (units/mg protein). Following exposure of human aortic endothelial cells to shear stress for 24 and 48 h, protein levels were examined by Western analysis. Enzyme activity was determined by the ability of the homogenates of these cells to inhibit cytochrome c reduction by superoxide generated by xanthine and xanthine oxidase. *p = 0.02. Reprinted from Ref. No. 30 with permission.

plain these observations. It is evident now that the reaction of NO and superoxide leads to the formation of peroxynitrite anion, which is protonated to form peroxynitrous acid.[37, 38] The latter can yield the hydroxyl radical and nitrogen dioxide. Peroxynitrite has been shown to produce endothelial cell injury and to oxidize sulfhydryl groups.[37] Both superoxide and the hydroxyl radical may contribute to oxidation of low-density lipoproteins.[39, 40] Recently, it has become evident that reactive oxygen species contribute to cell activation and intracellular signal transduction via redox-sensitive genes, such as vascular cell adhesion molecule-1, tissue factor, monocyte chemotactic protein-1, and others.[41–44] Preservation of the half-life of NO may also have other antiatherogenic properties, such as inhibition of platelet[45] and neutrophil[46] adhesion and inhibition of vascular smooth muscle growth.[47] These lines of evidence suggest that induction of copper-zinc SOD by shear stress might have antiatherogenic properties by reducing superoxide levels and subsequent formation of peroxynitrite.

Conclusion

The data presented in this review suggest levels of regulation not only of NO but also of superoxide and SOD in cells in the vessel wall. While none of these is dramatically altered, the effect of concomitant regulation of all three may greatly affect several aspects of vascular biology, including vasomotor tone, redox state, predisposition for disease, and gene regulation. Clearly this work is in its infancy, and a great deal more remains to be learned regarding these interactions. It is likely that specific interventions in these processes may lead to therapeutic advances in the future.

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Clin. Cardiol. Vol. 20 (Suppl. II), II-18–II-25 (1997)

Vasculoprotective and Cardioprotective Mechanisms of Angiotensin-Converting Enzyme Inhibition: The Homeostatic Balance Between Angiotensin II and Nitric Oxide

GARY H. GIBBONS, M.D.

Molecular and Cellular Vascular Biology Research Laboratory, Brigham and Women's Hospital, Boston, Massachusetts, USA

Summary: The ability of the vasculature to modify its geometry in accordance with conditions of its microenvironment—the process of vascular remodeling—is an important pathobiologic process common to vascular disorders such as atherosclerosis, restenosis after angioplasty, and hypertension. Vascular remodeling characterizes the natural history of atherosclerosis, contributes to increased vascular resistance, and may contribute to the clinical complications of hypertension. A growing body of evidence indicates that locally generated vasoactive substances such as angiotensin II and nitric oxide are important determinants of the natural history of vascular disease. In particular, angiotensin II may promote vascular lesion formation by increasing vascular cell population via increased cell growth and decreased programmed cell death, and it may also alter extracellular matrix composition. Thus, angiotensin II is a pleiotropic local mediator capable of modulating cell growth, programmed cell death, migration of vascular smooth muscle cells, and extracellular matrix modulation, all of which are biologic mechanisms of vascular remodeling and intimal formation. This is proposed to occur via a local tissue angiotensin system. Angiotensin II may also promote chronic hypertension by modulating the vascular redox state and promoting the catabolism of the endothelium–derived nitric oxide, an endogenous inhibitory vasodilator. Because angiotensin–converting enzyme (ACE) is strategically positioned to influence the activity of at least three local vasoactive systems—angiotensin II, nitric oxide, and bradykinin—blocking ACE with ACE inhibition may have profound effects on ventricular and vascular structure and function, and have particular efficacy in preventing the morbidity and mortality of vascular diseases such as hypertension and atherosclerosis.

Key words: angiotensin II, angiotensin-converting enzyme, atherosclerosis, hypertension, nitric oxide, vascular remodeling

Introduction

Advances in cardiovascular medicine have improved our capacity to prolong the lives of patients who have suffered myocardial infarctions or congestive heart failure. However, the current challenge is to develop pharmacotherapies that move beyond the treatment of symptoms toward an agenda in which interventions prevent the development of end-stage coronary heart disease. To alter the natural history of cardiovascular disease, it is important to understand the fundamental pathobiologic mechanisms that promote the morbidity and mortality associated with these disorders. This review focuses on the emerging evidence indicating that locally generated vasoactive substances such as angiotensin II (ang II) and nitric oxide (NO) are important determinants of the natural history of vascular disease. Insights into these pathobiologic processes suggest that therapeutic interventions that alter the expression of these vasoactive mediators [such as angiotensin-converting enzyme (ACE) inhibitors] may have particular efficacy in preventing the morbidity and mortality of diseases such as hypertension and atherosclerosis.

Vascular Remodeling: Clinical Implications

The vasculature is a complex, integrated organ capable of modulating its tone and structure in accordance with the conditions of its microenvironment. This ability of the vasculature to modify its geometry—the process of vascular remodeling—is now recognized as an important pathobiologic process common to various vascular disorders such as atherosclerosis, restenosis after angioplasty, and hypertension.[1] Typically, vascular remodeling involves changes in the relative dimensions

Address for reprints:

Dr. Gary H. Gibbons
Molecular and Cellular Vascular Biology Research Laboratory
Thorn–12
Brigham and Women's Hospital
75 Francis Street
Boston, MA 02115, USA

of vessel components such as the outer circumference, the lumen, the wall thickness:lumen ratio, or the intima:media ratio. These alterations in vessel structure are now considered important determinants of vascular resistance and the pathogenesis of hypertension.[1–4] Morphometric studies of hypertensive vessels in animal models and humans have documented several forms of vascular remodeling, including: (1) medial layer hypertrophy, (2) decreased vessel and/or lumen diameter, (3) expansion and/or alteration of the extracellular matrix, and (4) vessel rarefaction (microvessel occlusion). Vascular remodeling is postulated to be a critical, self–perpetuating mechanism that promotes the chronic maintenance of the hypertensive state in the setting of normal levels of vasoconstrictors and vasodilators.

In addition to contributing to increased vascular resistance, the process of vascular remodeling may also participate in the vicious cycle of events that promotes the clinical complications of hypertension. For example, changes in the vasculature of hypertensive patients observed during routine fundoscopic examination (e.g., arteriovenous nicking due to arteriolar hypertrophy) are clinical manifestations of vascular remodeling, and an association has been shown between these structural changes and the natural history of progressive hypertension.[5, 6] In addition, rarefaction in skeletal muscle beds may promote the phenomenon of insulin resistance in hypertension by compromising the delivery of insulin and glucose to skeletal muscle. Likewise, the association among lacunar infarction, subcortical white matter disease, and hypertension may relate to vascular remodeling in the cerebral microvasculature.[7] A similar process appears to occur in the coronary microcirculation and may provide a mechanism for the increased cardiac mortality noted in hypertensive patients without severe epicardial atherosclerotic disease.[8, 9] Furthermore, structural changes in the renal microcirculation may predispose to the development of nephrosclerosis in hypertension and eventual renal failure.[10, 11] Finally, vascular hypertrophy and fibrosis within the structures of conduit vessels such as the aorta may contribute to reduced vascular compliance and predispose to systolic hypertension and left ventricular hypertrophy.[12] Thus, many of the clinical sequelae of hypertension (i.e., myocardial infarction, heart failure, stroke, and renal failure) may result from vascular remodeling within the microcirculation and conduit vessels.

The natural history of atherosclerosis is also characterized by a process of vascular remodeling. The development of clinically significant vessel stenosis depends on changes in overall vessel dimensions as well as expansion of intimal lesions. Studies using intravascular ultrasound have documented the significance of vascular remodeling in the clinical progression of restenosis after angioplasty and in transplant coronary disease and atherosclerosis.[13–16] These recent studies confirm the classic morphometric studies by Glagov and others, demonstrating that the vasculature undergoes a process of compensatory enlargement to mitigate the effect of plaque expansion on lumen dimensions.[15] The locally generated factors that determine whether a vessel undergoes vascular hypertrophy, shrinkage remodeling, or enlargement remodeling are poorly characterized but may have important clinical implications in the treatment of patients with hypertension and atherosclerosis.

Vascular Homeostatic Balance

Although the histopathology of hypertensive vessels is distinct from atherosclerotic lesions, it is intriguing that the pathogenesis of vascular diseases such as hypertension and atherosclerosis share many pathobiologic mechanisms. Epidemiologic studies have established that hypertension is a potent risk factor for the development of coronary heart disease, and it is well known that the superimposition of hypertension potentiates the process of atherosclerotic lesion formation in animal models.[17] However, the mechanisms by which hypertension-promoting factors contribute to atherosclerotic vascular disease are not well defined. Both forms of vascular disease involve alterations in the regulation of vascular cell growth, programmed cell death, migration, and matrix modification. The locally generated, autocrine-paracrine mediators that regulate these processes within vessels during the pathogenesis of vascular disease remain to be further defined.

The homeostatic regulatory mechanisms governing vascular tone involve a "yin-yang" balance in which the interplay between vasoconstrictors and vasodilators modulates vessel resistance. During the pathogenesis of hypertension, this homeostatic balance becomes perturbed, so that the influence of vasoconstrictors such as ang II predominates over the influence of vasodilators such as NO. Moreover, many vasoactive substances that were originally defined as regulators of vessel tone are now recognized as pleiotropic factors that can modulate the critical cellular processes involved in vascular remodeling and lesion formation, that is, vascular cell growth, migration, and changes in extracellular matrix composition.

Mechanisms of Vascular Remodeling and Intimal Lesion Formation: Role of Angiotensin II

Much as the homeostatic regulation of vascular tone is governed by a balance between vasoconstrictors and vasodilators, the balance of growth-stimulatory and growth-inhibitory factors appears to regulate vascular remodeling and intimal lesion formation. A growing body of evidence indicates that vasoconstrictor substances (e.g., ang II) promote increased growth of vascular smooth muscle cells, whereas vasodilators (e.g., endothelium-derived NO) inhibit the growth of vascular smooth muscle cells.[1, 2]

Ang II serves as a useful archetype of a vasoactive substance that can modulate the cellular processes of vascular remodeling. It is now well documented that, in addition to the circulating renin-angiotensin system, the vessel wall expresses a paracrine vascular angiotensin system that may generate ang II locally within the vasculature.[18] Experimental studies have shown that blockade of ang II effectively reverses the changes in vascular structure associated with hypertension.[19, 20] In accordance with these in vivo studies, in vitro studies suggest that ang II can induce either hypertrophy or

hyperplasia of cultured vascular smooth muscle cells, an effect that is potentiated by mechanical forces imposed by hemodynamic stimuli.[21, 22] The angiotensin type I receptor is coupled to cellular signaling pathways such as tyrosine kinases (src- and mitogen-activated protein kinase) that mediate the induction of cell growth.[23] Ang II is a bifunctional growth factor that induces increased expression of proliferative autocrine factors such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), and of antiproliferative autocrine factors such as transforming growth factor-β1 (TGFβ1) in cultured vascular smooth muscle cells.[21] Thus, the growth response of vascular smooth muscle cells to ang II–hypertrophy versus hyperplasia—depends on the relative balance of proliferative (PDGF, bFGF) versus antiproliferative (TGFβ1) autocrine growth factors. In addition to these well-defined mediators, recent reports suggest that the induction of endothelin-1, insulin-like growth factor I, and heparin-binding epidermal growth factor may also contribute to the growth-stimulatory effects of ang II.[2] These in vitro models have provided important mechanistic insights and suggest that the net growth response to ang II is dependent on the balance of mediators within the cellular milieu.

Our knowledge of the mediators involved in vascular remodeling has been based primarily on the indirect evidence provided by pharmacologic studies that are confounded by changes in systemic hemodynamics or on in vitro studies that are limited by the artificial nature of the cell culture system. In order to define the role of these mediators in vivo, we have employed a novel experimental approach that utilizes the technology of in vivo genetic engineering to modify the expression of autocrine-paracrine factors within the vessel wall in the intact animal. We have demonstrated that transfecting the ACE gene into an intact rat carotid artery effectively increased the local expression of ACE within the vessel and thereby simulated the increase in local ACE activity observed in hypertensive vessels.[24] This increase in vascular ACE activity stimulated an increase in DNA synthesis that could be inhibited by an angiotensin type I receptor antagonist. Furthermore, the growth response stimulated by local generation of ang II induced the characteristic medial layer hypertrophy and increase in wall:lumen ratio observed in hypertensive vessels. It is noteworthy that the vascular remodeling response induced by a local increase in ACE expression occurred without effects on systemic hemodynamics or influences on the circulating renin-angiotensin system. Thus, this novel experimental approach provides the first direct evidence that the paracrine vascular angiotensin system has the capacity to induce the vascular remodeling characteristic of hypertensive vessels in vivo independent of an influence on systemic hemodynamics.

The current paradigm of the pathogenesis of vascular disease has often focused on the regulation of cell growth and matrix modifications as the critical pathobiologic processes involved in determining vessel structure. Although these processes are important, an exciting new area of research indicates that the paradigm of vascular remodeling and lesion formation must include the process of programmed cell death, or apoptosis. Apoptosis is a form of "cell suicide" in which a carefully regulated genetic program is activated that deletes a cell from a tissue without inducing an inflammatory response; it is therefore quite distinct from necrotic cell death. This powerful biologic process appears to play a crucial role in mediating changes in tissue architecture that occur during ontogeny as well as pathobiologic processes such as glomerulonephritis, acquired immunodeficiency syndrome (AIDS), and cancer. Indeed, recent studies of human vascular lesions have documented apoptosis in human atherosclerotic plaques and restenotic lesions after angioplasty.[25, 26]

Although the precise role of apoptosis as a determinant of vascular structure remains to be further defined, evidence indicates that cell-growth mediators such as PDGF are also important modulators of vascular cell programmed cell death.[27] Indeed, recent in vitro studies in our laboratory have shown that ang II is an effective inhibitor of vascular smooth muscle cell programmed cell death.[28] These in vitro observations have been confirmed by in vivo studies that have documented that the capacity of ACE inhibitors to induce the regression of vascular lesions is associated with increased apoptosis of vascular cells as well as the inhibition of cell growth.[29] Overall, these data suggest that ang II may promote vascular lesion formation by increasing the vascular cell population through two mechanisms: increased cell growth and decreased programmed cell death. One may speculate that the targeted induction of apoptosis may represent an exciting new therapeutic strategy for modifying cardiovascular tissue function and structure.

In addition to its effects on vascular cellularity, ang II may also mediate remodeling and lesion formation by altering extracellular matrix composition via its effect on thrombospondin, fibronectin, tenascin, glycosaminoglycans expression, and plasminogen activator activity.[30–32] Moreover, the migration of vascular smooth muscle cells and endothelial cells during structural modifications can be modulated by ang II.[33] Thus, ang II is a pleiotropic local mediator capable of modulating cell growth, apoptosis, migration, and matrix modulation—all the biologic mechanisms of vascular remodeling and intima formation. Similar pleiotropic effects on vascular smooth muscle cell behavior have been described for other vasoconstrictors, including norepinephrine, endothelin-1,and thromboxane.[34–36] Hence, vasoconstrictors may play an important role in determining vascular structure by influencing the various biologic mechanisms of vascular remodeling.

Mechanisms of Vascular Remodeling and Intimal Lesion Formation: Role of Nitric Oxide

Endogenous vasodilators such as NO and natriuretic peptides appear to have a countervailing influence to ang II as determinants of vascular architecture. Vasodilators generally inhibit vascular smooth muscle cell growth in in vitro models.[37,38] Recent studies suggest that vasodilators may also promote a decrease in vascular smooth muscle cellularity by inducing apoptosis.[29] Similarly, experiments performed with

intact animals have documented that the local generation of NO inhibits vascular lesion formation after vessel injury.[39] Moreover, under certain circumstances NO may alter matrix composition by modulating the activity of the metalloproteinases that degrade matrix proteins.[40] Thus, NO appears to inhibit increases in vascular smooth muscle cellularity and expansion of the extracellular matrix associated with hypertensive vascular remodeling and atherosclerotic lesion formation.

The process of vascular remodeling is particularly important as a determinant of lumen size. One of the best examples of the plasticity of the vasculature is evident from the flow-stimulated remodeling response induced by an arteriovenous shunt. The factors that induce the enlargement of lumen dimensions under these circumstances have not been characterized. However, recent experimental studies have shown that if the well-described flow-stimulated increase in NO generation[41] is prevented by pharmacologic inhibitors, the vessel chronically exposed to increased flow fails to undergo appropriate enlargement remodeling.[42] It has also been observed that chronic pharmacologic blockade of NO generation results in a hypertensive state characterized by fibrosis and shrinkage remodeling within the coronary microvasculature.[8] Taken together, these observations suggest that decreased NO generation is associated with shrinkage remodeling, whereas increased NO generation is associated with enlargement remodeling. Thus, several lines of evidence indicate that NO is an endogenous inhibitory factor that attenuates the process of occlusive vascular lesion formation characteristic of hypertensive and atherosclerotic disease.[43]

Endothelial Dysfunction: An Imbalance in Reactive Nitrogen and Oxygen Species

The endothelium is a multifactorial determinant of tissue function via its regulation of vessel tone, thrombosis, inflammation, and structure. The normal endothelium appears to have an intrinsic capacity to prevent vascular disease. However, an impairment of endothelial function manifested as abnormal endothelium-dependent vasorelaxation has been documented in a variety of vascular diseases, including hypertension and atherosclerosis in both animal models and humans.[44–46] In fact, this perturbation has been described in normotensive subjects who merely have a positive family history of risk factors such as hypertension.[47] Thus, in many cases the onset of endothelial dysfunction may precede the development of clinically evident vascular disease. Unfortunately, the molecular basis of endothelial dysfunction in vascular disease remains to be further defined.

Although there are several potential etiologies of decreased NO bioactivity, several lines of evidence suggest that increased catabolism of NO may be a principal factor in promoting endothelial dysfunction. It is important to emphasize that NO is itself a free radical—a highly reactive nitrogen species. Consequently, the biologic function of this vasoactive factor is determined in large part by the redox state of the tissue.[48] An increase in oxidative stress will mitigate the vasodilatory bioactivity of NO. A potential role for the redox state as a determinant of vascular homeostasis is demonstrated by animal model studies in which administration of antioxidants such as superoxide dismutase induced a lowering of blood pressure.[49] This antihypertensive effect is mediated in part by enhancing the bioactivity of NO. In support of this hypothesis, an increase in superoxide anion generation has been documented in the vasculature of genetically hypertensive animals compared with normotensive controls.[50] Moreover, recent in vivo studies have shown that the hypertensive state induced by infusion of ang II is due in part to increased generation of the free radical superoxide anion.[51] This ang II-stimulated increase in oxidative stress potentiates the direct vasoconstrictor effects of ang II by promoting increased catabolism of NO and endothelial dysfunction. Thus, in addition to its direct vasoconstrictive effects, ang II appears to promote chronic hypertension by modulating the vascular redox state and promoting the catabolism of the vasodilator NO.

The balance between NO and reactive oxygen species may also be an important role determinant of vessel structure. In vitro studies have documented that reactive oxygen species may function as signaling molecules that regulate vascular cell growth and programmed cell death.[52, 53] In fact, the growth-stimulatory effects of ang II on vascular smooth muscle cells appear to be mediated in part by the induction of reactive oxygen species that function as signaling molecules.[52] Similarly, the generation of reactive oxygen species may promote atherosclerosis by several mechanisms, including oxidation of low-density lipoprotein cholesterol and upregulation of leukocyte adhesion molecules and chemokines.[54] Thus, the development of endothelial dysfunction characterized by an imbalance between NO and reactive oxygen species may be an important pathogenic event in hypertension that determines the level of the blood pressure, promotes alterations in vessel structure, and contributes to clinical complications such as coronary artery disease.

One may speculate that the endothelium may be a new target for therapeutic interventions that will alter the course of vascular disease. Indeed, one of the salutary effects of antihypertensive treatment is the reversal of endothelial dysfunction.[45, 55] Future studies will further clarify the role of endothelial dysfunction in the natural history of hypertensive vascular disease and the clinical implications of reversing this abnormality.

Altering the Path of Vascular Disease: Potential Role of Angiotensin-Converting Enzyme Inhibition

As noted above, the generation of ang II is governed by both a circulating renin-angiotensin system and a tissue angiotensin system.[18] The tissue angiotensin system appears to be upregulated in the context of cardiovascular disease. Animal and human studies have documented increased expression of tissue ACE in the heart in the context of ventricular remodeling and heart failure postmyocardial infarction,[56–58] and expression of tissue ACE is increased in the vasculature in

the context of hypertension in various models.[2, 59] Moreover, we have recently documented that atherosclerotic human coronary vessels express high levels of ACE immunoreactivity and ang II within the plaque,[60] most prominently in the monocyte-macrophages that are major constituents of the plaque cellular population. Studies of human peripheral monocytes have also documented high levels of ACE expression and ang II within inflammatory cells.[61] Thus, the changes in ventricular and vascular structure observed under pathologic conditions are characterized by increased activity of a tissue angiotensin system. Given the capacity of ang II to modulate cell growth as well as programmed cell death, migration, and matrix modification, the blockade of ang II generation may have profound effects on ventricular and vascular structure and function.

Angiotensin-converting enzyme also functions as a kininase responsible for the degradation of bradykinin. Some of the most compelling evidence of the physiologic role of the kallikrein-kinin system in cardiovascular homeostasis has been provided by results in genetically engineered animal models. In these models, augmentation of kallikrein-bradykinin activity is associated with significant decreases in blood pressure.[62] Conversely, animals that lack the bradykinin type 2 receptor exhibit a hypertensive phenotype.[63] Thus, bradykinin appears to be an important modulator of vascular tone. This effect may be due in part to the fact that bradykinin is a potent inducer of NO generation. In vitro and in vivo studies have shown blocking bradykinin degradation by inhibiting ACE is an effective means of augmenting endothelial generation of NO.[64, 65]

Angiotensin-converting enzyme is strategically positioned to influence the activity of at least three local vasoactive systems—ang II, bradykinin, and NO. Accordingly, the various effects of blocking ACE on cardiovascular function and structure may be mediated in part by each or many of these factors. To the degree that vascular disease is characterized by an imbalance between a relative increase in ang II generation and a relative deficit of NO bioactivity, it is postulated that ACE inhibition may effectively restore the appropriate homeostatic balance between these vasoactive systems. This hypothesis, generated on the basis of animal model studies, has recently been tested in clinical trials. Compelling evidence indicates that long-term administration of ACE inhibitors reverses endothelial dysfunction in patients with either hypertension or atherosclerotic vascular disease.[44, 45 ,65] Thus, the beneficial effects of ACE inhibition may relate in part to changes in endothelial function that involve coordinate changes in the relative balance between ang II, bradykinin, and NO.

Angiotensin–converting enzyme inhibitors appear to have particular efficacy in reversing vascular remodeling and preventing the eventual development of hypertension in genetically predisposed animals[19, 66] and in clinical studies of patients with essential hypertension.[20, 67–69] This efficacy exists even compared with other antihypertensive agents. Such observations support the hypothesis that antihypertensive agents that reduce blood pressure and reverse the remodeling process may change the natural history of the disease.

Experimental studies suggest that alterations in microvascular structure within the kidney are important in the development of renal dysfunction and eventual organ failure in hypertensive patients, and ang II may have an important pathogenic role in the progression of this form of renovascular disease. Clinical studies have confirmed that ACE inhibitors have particular efficacy in modifying the natural history of renovascular diseases such as insulin-dependent diabetes,[70] noninsulin-dependent diabetes,[71] and various etiologies of glomerular damage.[72]

Angiotensin-converting enzyme inhibitors are the vasodilators of choice in altering the natural history of congestive heart failure due to their influence on ventricular remodeling.[73] In several clinical trials in patients with left ventricular dysfunction, ACE inhibition reduced the incidence of recurrent myocardial infarctions, indicating that ACE inhibitors may alter the natural history of coronary artery disease,[74] possibly via direct effects on coronary vascular function and structure. While the mechanisms are not well understood, animal model studies indicate that ACE inhibition within the heart enhances NO generation from coronary microvessels, an effect that is mediated via the accumulation of bradykinin.[64, 65] The clinical significance of this observation has been substantiated by the recent observation that ACE inhibition reverses endothelial dysfunction in patients with coronary atherosclerosis.[44] These findings suggest that ACE inhibition has a salutary effect on coronary blood flow and reactivity in patients susceptible to myocardial ischemia.

Other experimental studies have recently documented that ACE inhibition reduces myocardial oxygen consumption in association with an increase in NO generation.[64] Such findings are consistent with previous studies demonstrating that NO has a direct effect on muscle oxidative metabolism. These observations raise the possibility that ACE inhibition may prevent myocardial ischemia by optimizing the balance between myocardial oxygen supply and demand. Two mechanisms may be involved: enhancing blood flow and reducing myocardial oxygen demands. This response may be useful in reducing the sequelae of chronic ischemic heart disease.

Finally, the ultimate goal in altering the path of coronary heart disease is to prevent acute ischemic syndromes such as unstable angina and myocardial infarction. Pathologic studies indicate that these episodes are related to two phenomena—plaque rupture and plaque erosion.[75, 76] Plaque rupture is the most prevalent etiology of acute coronary thrombosis, accounting for 60% of cases in an autopsy series. Plaque rupture involves an inflammatory process in which leukocytes infiltrating the plaque promote increased expression and activity of metalloproteinases which may weaken the integrity of the thin fibrous cap and predispose it to rupture.[77] There are several reasons why ang II may contribute to this pathogenic process. Ang II stimulates the redox-sensitive, proinflammatory transcription factor NFkB, which, in turn, induces the coordinate up-regulation of cytokines, chemoattractants, and leukocyte adhesion molecules that promote the local inflammatory response within the vascular lesion.[78–80] Furthermore, the oxidative stress induced by factors such as ang II may pos-

sibly activate the metalloproteinases expressed within the plaque and potentially induce plaque rupture.[40, 51, 52] This proinflammatory effect of angiotensin that may predispose to plaque rupture can be counteracted by the actions of NO, which inhibits transcription factor NFkB and downregulates the expression of inflammatory cytokines, chemoattractants, and leukocyte adhesion molecules.[81, 82] Thus, the ang II-NO balance may be critical to modulating the propensity of lesions to rupture and cause acute ischemic syndromes.

In contrast to plaque rupture, the pathogenesis of plaque erosion is characterized by endothelial cell loss, exposure of the procoagulant subendothelial space, and in situ thrombosis. The cause of endothelial cell loss is unknown, but it is intriguing to speculate that this denudation of the endothelium may result from endothelial cell death by apoptosis. In this regard it is noteworthy that ang II reduces the capacity of the endothelium to regenerate by inhibiting cell replication via the angiotensin type 2 receptor.[83] Moreover, the angiotensin type 2 receptor can mediate cell loss by inducing apoptosis.[84] Conversely, recent studies indicate that NO preserves the integrity of the endothelium by enhancing regeneration[85] and preventing endothelial cell apoptosis in response to cytotoxic cytokines.[86] These findings support the concept that the maintenance of the appropriate ang II-NO balance may play an important role in vascular homeostasis and the prevention of acute ischemic events.

Clinical studies are under way that will directly test the hypothesis that chronic administration of ACE inhibitors in normotensive subjects with coronary disease will prevent ischemic events. It is hoped that these studies will move us closer to developing pharmacotherapies that modify the molecular events that eventually cause end-stage heart disease

Conclusion

The current challenge facing clinicians is to develop pharmacotherapies that move beyond the treatment of symptoms toward an agenda in cardiovascular therapeutics in which interventions actually prevent the development of end-stage coronary heart disease. The development of new strategies to alter the natural history of cardiovascular disease will be fostered by insights into the fundamental pathobiologic mechanisms that promote the morbidity and mortality of these disorders. An emerging body of evidence indicates that locally generated vasoactive substances such as ang II and NO are important determinants of the natural history of vascular disease. It is anticipated that ongoing clinical trials will extend the concept that modulating the activity of vasoactive substances generated by the endothelium has important implications for altering the course of coronary heart disease.

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Clin. Cardiol. Vol. 20 (Suppl. II), II-26–II-33 (1997)

Endothelial Dysfunction in Essential Hypertension

JULIO A. PANZA, M.D.

Cardiology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA

Summary: In the last decade, significant advances have occurred in our understanding of the presence and nature of endothelial dysfunction in a number of cardiovascular conditions, including hypertension. Endothelium-derived nitric oxide (NO) is recognized as an important mediator of endothelium-dependent vascular relaxation, and a defect in the endothelium-derived NO system—possibly decreased synthesis and/or release of NO by endothelial cells—is now known to cause the abnormal response to acetylcholine in hypertensive vessels and to account at least in part for the increased vascular resistance observed in hypertension. Extensive research by our laboratory and others to determine the nature of the defect in the NO system has found that the defect is not related to decreased availability of L-arginine, the NO precursor, or to a defect at the muscarinic receptor level or a specific G protein-dependent intracellular signal-transduction pathway; nor is it related to extracellular inactivation of NO by superoxide anion. These findings have contributed to our understanding of endothelial dysfunction in essential hypertension and have pointed out distinctions between the mechanisms leading to this vascular abnormality in hypertensive and hypercholesterolemic patients. While the exact nature of the NO system defect in hypertension is still to be clarified, the vasoconstrictive and proatherogenic effects of endothelial dysfunction probably contribute to the cardiovascular complications associated with elevated blood pressure. Continued research targeted at the identification of the precise mechanism(s) responsible for endothelial dysfunction in hypertension may lead to the development of novel therapeutic strategies to reduce the vascular complications associated with the hypertensive process.

Address for reprints:

Dr. Julio A. Panza
Cardiology Branch
National Institutes of Health
Building 10, Room 7B15
10 Center Drive, MSC-1650
Bethesda, MD 20892, USA

Key words: acetylcholine, angiotensin-converting enzyme inhibitor, endothelial function, hypertension, nitric oxide

Introduction

Endothelial dysfunction contributes to the underlying disease process of a number of conditions, including essential hypertension, hypercholesterolemia, atherosclerosis, diabetes mellitus, congestive heart failure, and pulmonary hypertension. Over the last decade, extensive research has focused on determining not only the presence but also the nature of endothelial dysfunction in patients with conditions associated with premature development of atherosclerosis. It is now known that certain aspects of the endothelial dysfunction of patients with essential hypertension differ from those of patients with other risk factors. Continuing research is attempting to determine the precise mechanism of endothelial dysfunction in various cardiovascular conditions.

Studies of endothelial dysfunction generally evaluate the vascular responses to endothelium-dependent (agents that need the presence and integrity of the endothelium to exert their relaxing effect on smooth muscle) and endothelium-independent (agents that bypass the endothelium to act directly on the smooth muscle) vasodilators such as acetylcholine and nitroglycerin or sodium nitroprusside, respectively. Because acetylcholine has been the most widely used endothelium-dependent vasodilator, it is important to recognize that its effect differs depending on the vascular bed: in epicardial coronary arteries, acetylcholine elicits a vasodilator response when the endothelium is intact; however, this is transformed into paradoxical vasoconstriction in patients with atherosclerotic coronary artery disease (CAD).[1] In resistance vessels, however, the response is vasodilation, even in conditions associated with endothelial dysfunction. Because blood pressure is largely regulated by resistance vessels (microcirculation), an appropriate assessment of endothelial dysfunction in hypertension involves measuring changes in blood flow and vascular resistance, while changes in the diameter of coronary and other conductance arteries reflect endothelial dysfunction related to atherosclerosis.[1, 2]

Thus, meaningful assessment of endothelial regulation of vascular tone in hypertensive patients requires measuring the

effects of endothelium-dependent and endothelium-independent vasodilators on the systemic microcirculation. The forearm perfusion technique provides an excellent model for this purpose because it permits the study of the human microcirculation in vivo without the confounding effects related to activation of systemic counterregulatory mechanisms of vascular homeostasis. This is achieved by measuring the forearm blood flow response (which is dependent on changes in the caliber of small resistance vessels) to pharmacologic agents directly infused into the forearm circulation via a catheter placed in the brachial artery. Blood flow is measured noninvasively by means of strain gauge plethysmography before and during the infusion of incremental doses of the agonists.

Endothelial Dysfunction and Hypertension

Over the last decade, several studies conducted at the National Institutes of Health have assessed endothelium-dependent vascular relaxation in patients with essential hypertension, defined as chronically elevated blood pressure (> 145/95 mmHg) without any apparent underlying cause in patients who had been treated with antihypertensive medication(s) for several years.[3, 4]

In our initial study, we showed that although basal forearm blood flow was not different between patients with essential hypertension and normotensive control subjects, the response to acetylcholine, an endothelium-dependent vasodilator, was significantly blunted in hypertensive patients (Fig. 1).[4] However, the response to sodium nitroprusside, an endothelium-independent vasodilator acting directly on smooth muscle cells, was preserved in patients with hypertension. The abnormal response to acetylcholine was not due to presynaptic inhibition of norepinephrine release by adrenergic nerve terminals, since significantly blunted responses to acetylcholine were observed in patients with hypertension following blockade of alpha-adrenergic receptors,[3] indicating that the abnormal response is independent of sympathetic activity.

Similar abnormal responses to acetylcholine or methacholine with preserved responses to sodium nitroprusside have been shown by many other investigators using different vascular models.[5–9]

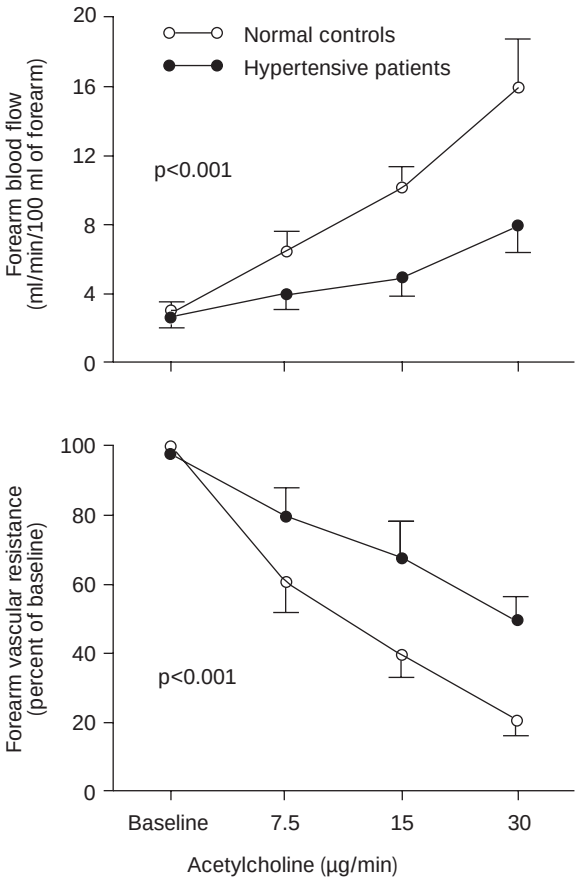

FIG. 1 Vascular responses to administration of acetylcholine in 10 normal controls and in 11 patients with hypertension. The response to acetylcholine was significantly reduced in patients with essential hypertension compared with controls. Adapted from Ref. No. 4 with permission.

Nature of Endothelial Dysfunction in Hypertension

Maintenance of vascular tone and blood flow by the endothelium is complex, involving many substances and an interplay among numerous cellular mechanisms.[10] Thus, while an impaired vasodilator response to acetylcholine indicates the presence of endothelial dysfunction, it does not identify the nature of the dysfunction. As noted above, the abnormal response to acetylcholine in patients with hypertension does not result from a different presynaptic inhibition of norepinephrine release. The role of the endothelium-derived relaxing factor nitric oxide (NO), which is synthesized from L-arginine,[11] has received extensive study. In particular, it has been hypothesized that defects in the synthesis and/or release of NO may play an important role in the endothelial dysfunction of hypertensive vessels.

Basal release of NO has been shown to be critical for the maintenance of vascular tone. Thus, when the synthesis of NO in vivo is blocked by inhibitors of NO synthase, significant vasoconstriction ensues.[12] Because NO has a very short half-life, these findings are consistent with continuous basal release of NO as an important part of the physiology of the vascular system. This role of NO in vascular homeostasis has also been demonstrated in both the resistance and conductance arteries of the coronary vascular tree.[13, 14] It is important to realize that, in addition to regulation of vascular tone, NO has other important antiatherogenic actions (including inhibition of platelet aggregation, monocyte migration, and lipid oxidation). Therefore, it is not surprising that a decreased bioavailability of NO has been shown in the coronary arteries of patients with atherosclerosis or its risk factors.[14, 15] Furthermore, that hypercholesterolemia and other risk factors impair

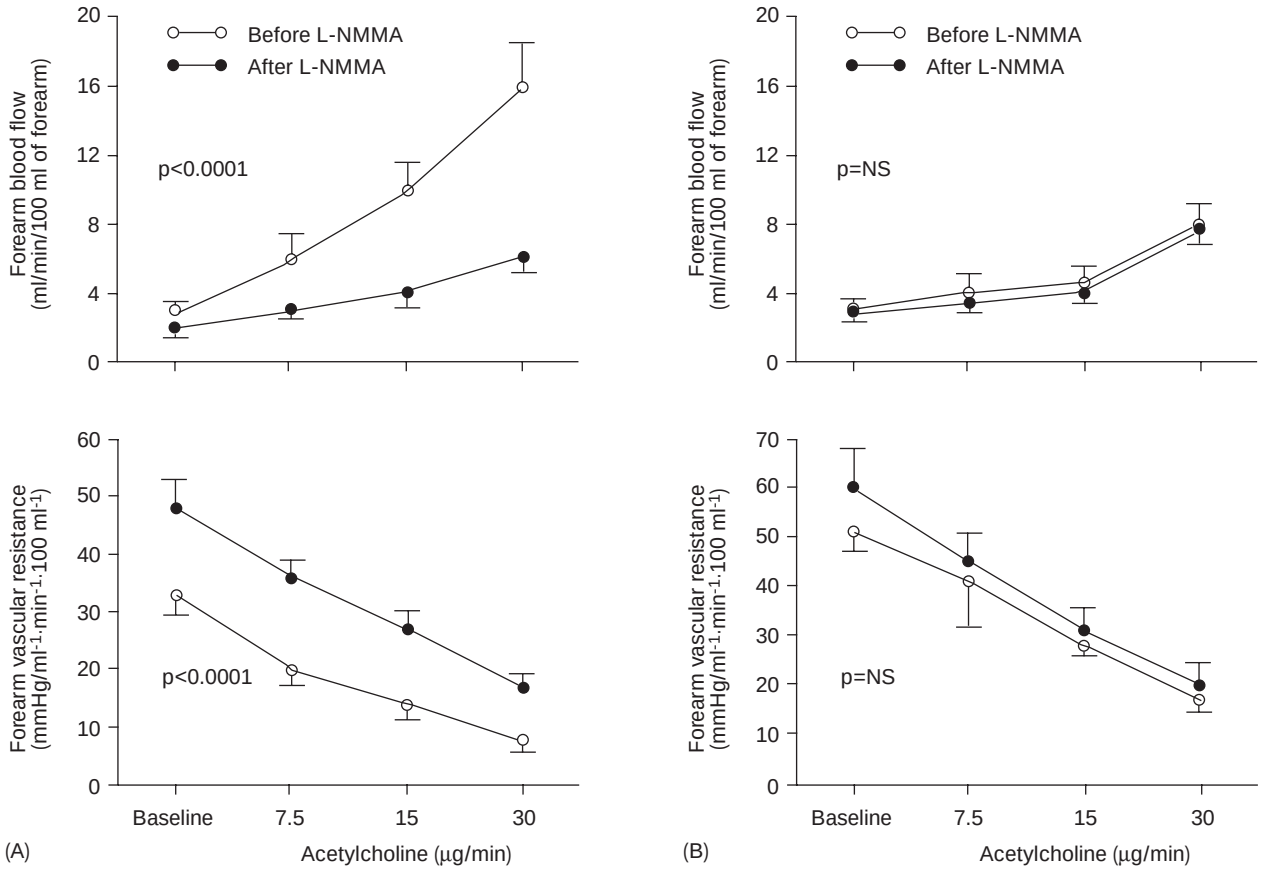

FIG. 2 Effects of L-NMMA on vascular responses to acetylcholine in 10 normal controls and 11 patients with hypertension. Infusion of L-NMMA effectively blunted the vasodilator response to acetylcholine in normotensive controls (A), but no significant change occurred in the already blunted response to L-NMMA in hypertensive patients (B). L-NMMA = N^G-monomethyl-L-arginine. Adapted from Ref. No. 4 with permission.

endothelial function even before the development of atherosclerosis suggests that NO dysfunction is at the very core of the pathogenesis of the atherosclerotic process.

Abnormal Nitric Oxide Activity in Essential Hypertension

The arginine analogue N^G-monomethyl-L-arginine (L-NMMA), which inhibits endothelial synthesis of NO, has been used to investigate the role of endothelium-derived NO in the abnormal endothelium-dependent vasodilation observed in patients with hypertension.[4, 16] In normotensive controls, basal release of NO has been indicated by a reduction in blood flow and an increase in vascular resistance during infusion of L-NMMA into the brachial artery.[4, 12, 16] Patients with hypertension also showed a vasoconstrictive response to infusion of L-NMMA, but the effect was significantly blunted, indicating that much less NO is produced/released by hypertensive vessels in the basal state.[4, 16]

The infusion of L-NMMA into the brachial artery effectively blunted the vasodilator response to acetylcholine in normotensive controls (Fig. 2A), whereas in patients with hypertension no significant change occurred during infusion of L-NMMA in the already blunted response to acetylcholine (Fig. 2B).[4] This finding suggests that NO contributes little to the vasodilator effect of acetylcholine in hypertensive vessels.

Reduced basal and stimulated NO bioactivity has also been shown in patients with hypercholesterolemia, atherosclerosis, or risk factors for atherosclerosis.[14, 15, 17] In hypercholesterolemic patients, the vascular responses to acetylcholine are blunted, and there is no significant change in this response during infusion of L-NMMA.[17] The reduction in endothelium-dependent vasodilation in these patients thus appears to be related to an attenuation in stimulated NO bioactivity.

Potential Defects in the Nitric Oxide System Contributing to Its Reduced Activity

The impaired NO activity demonstrated in patients with essential hypertension could be related to one or more abnormalities along the NO system. As mentioned previously, NO is formed using the amino acid L-arginine as a substrate in response to a variety of physiologic and pharmacologic stimuli, and is broken down primarily by superoxide anions originating both intracellularly and extracellularly.[18] Several mechanisms that may potentially account for decreased vascular activity of NO have been investigated in patients with essential

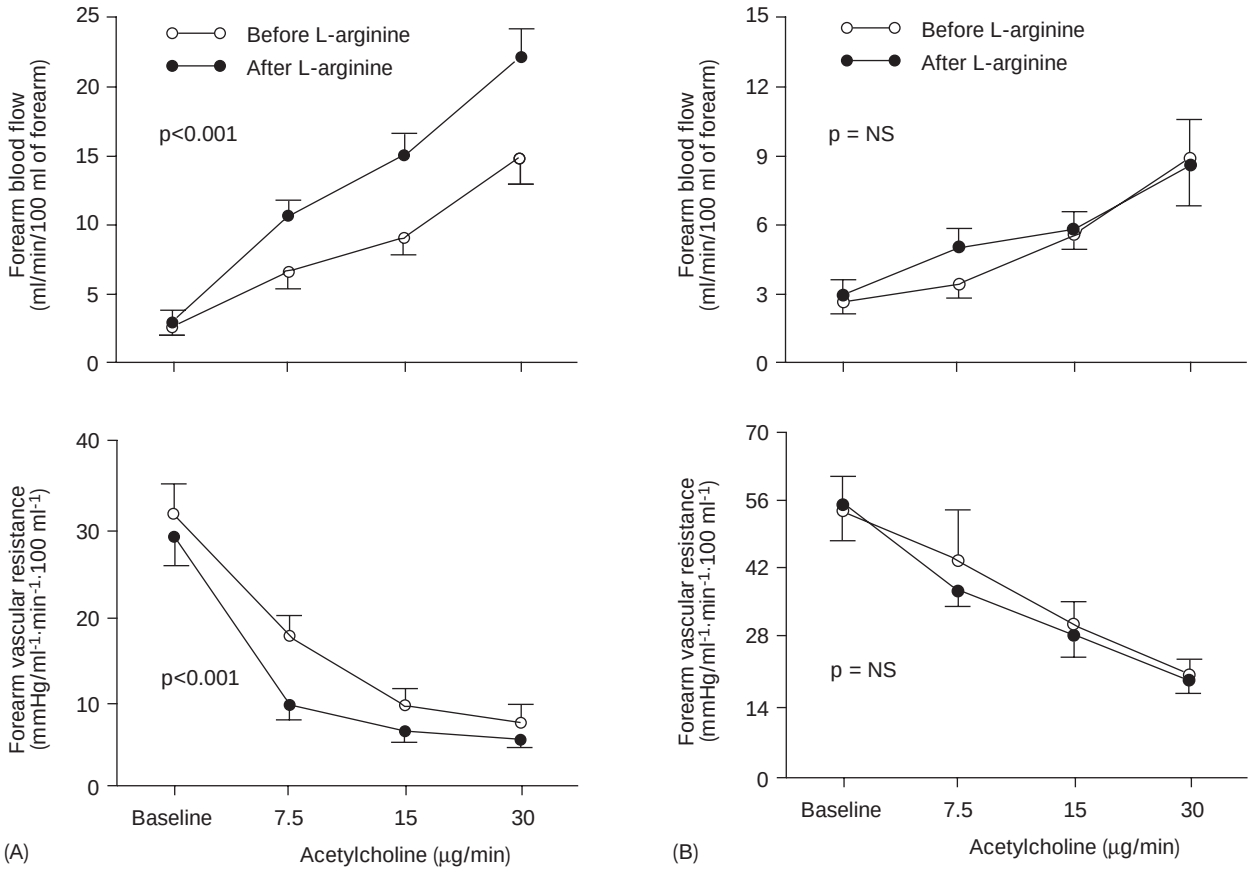

FIG. 3 Effects of L-arginine on vascular responses to acetylcholine in 12 normal controls and 14 patients with hypertension. The response to acetylcholine following L-arginine infusion in normotensive controls was augmented (A); in contrast, little change in response to acetylcholine was observed (B) following L-arginine administration to hypertensive patients. Adapted from Ref. No. 19 with permission.

hypertension in an effort to identify more precisely the nature of endothelial dysfunction that characterizes hypertensive vessels.

Substrate availability: One potential defect in the NO system that could contribute to the decreased bioactivity of NO is a decrease in the availability of its precursor, L-arginine. Studies of the vasodilator response to acetylcholine in normotensive controls showed that infusion of L-arginine into the brachial artery augmented endothelium-dependent vascular relaxation (Fig. 3A).[19] This effect apparently was specific for acetylcholine and L-arginine, as there was no difference in the response to sodium nitroprusside before and after administration of L-arginine and no change in the response to acetylcholine following administration of D-arginine, the isomer of L-arginine. In contrast to the hypothesized improvement in the endothelium-dependent vasodilator response to acetylcholine, little change was observed following L-arginine administration to patients with hypertension (Fig. 3B). These findings indicate that the defect in the endothelium-derived NO system in hypertensive vessels is likely not due to decreased availability of its precursor, L-arginine.[19]

Muscarinic receptor defect: Another postulated defect in the endothelium-derived NO system that might contribute to decreased NO activity involves an abnormality of the muscarinic receptor, which is stimulated by acetylcholine and methacholine. Some evidence has suggested that atherosclerotic coronary arteries with abnormal responses to acetylcholine may demonstrate normal vasodilation in response to substance P, a nonmuscarinic, endothelium-dependent vasodilator [20, 21] acting on a different endothelial cell receptor, a tachykinin receptor.[22, 23]

It was therefore hypothesized that, if the defect in NO activity of the hypertensive vasculature were located at the level of the muscarinic receptor, the response to substance P would be similar between patients with hypertension and normotensive controls. However, forearm blood flow studies showed a significant reduction in blood flow and vascular resistance responses to substance P in patients with hypertension compared with normotensive controls (Fig. 4). In fact, a correlation was observed between the responses to substance P and acetylcholine,[24] even though the two agonists elicit endothelial responses via different receptors. Moreover, similar responses to substance P during infusion of L-NMMA between normotensive controls and patients with hypertension indicated a reduced NO contribution to substance P-induced vasodilation in patients with hypertension. These findings

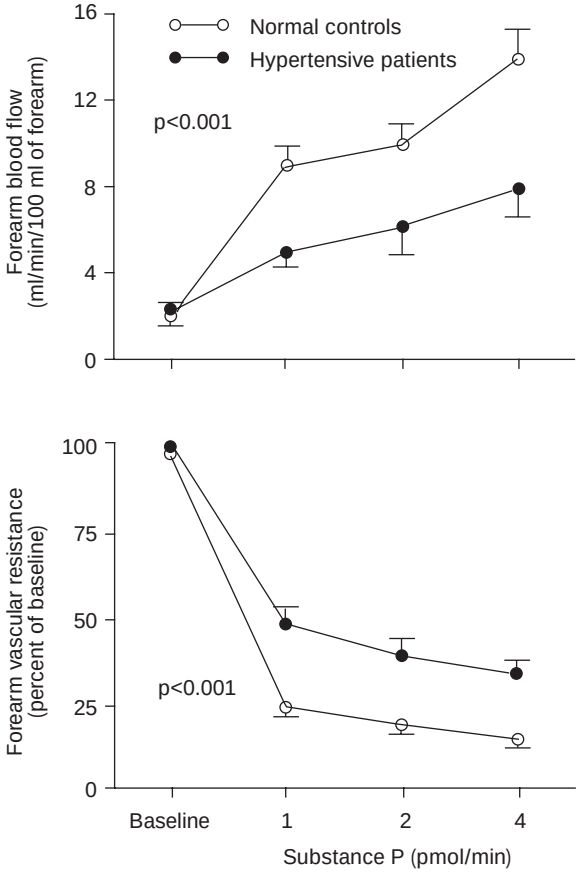

FIG. 4 Vascular responses to substance P in eight normal controls and eight hypertensive patients. A significant reduction in forearm blood flow and vascular resistance response was observed in hypertensive patients compared with normotensive controls. Reprinted from Ref. No. 24 with permission.

suggest that the cause of endothelial dysfunction in patients with hypertension is not limited to a defect at the muscarinic receptor level, but is related to a broader abnormality of endothelial cells.

Signal-transduction pathway defect: Previous studies in animal models of hypercholesterolemia have shown that, early in the disease process, only some endothelium-mediated responses are blunted. As the atherosclerotic process advances, a more generalized defect in endothelium-dependent responses is observed.[25, 26] More specifically, investigations using pertussis toxin (a selective inhibitor of certain G proteins) have demonstrated that initially only endothelium-mediated responses that require the activation of pertussis toxin-sensitive G proteins are abnormal, while those utilizing pertussis toxin-insensitive pathways are preserved. Later in the course of disease, responses mediated by pertussis toxin-insensitive G proteins also become affected, although receptor-independent endothelial responses are still intact. Eventually, as the vascular disease progresses, all endothelial responses, even those not mediated by stimulation of cell surface receptors and subsequent activation of G proteins, are abnormal. These observations suggest that the endothelial dysfunction in dyslipidemia may progress through different stages from a relatively selective defect in specific intracellular signal-transduction pathways to a more generalized abnormality of the endothelial cell.[27] These findings have been recently confirmed in patients with hypercholesterolemia without clinical evidence of atherosclerosis.[28]

Based on these observations, it was hypothesized that a similar selective defect in signal transduction was responsible for the impaired endothelial vasodilator function of patients with hypertension. Therefore, in a group of hypertensive patients, the responses to acetylcholine and bradykinin were compared with those obtained in a group of normotensive controls. In contrast to the findings in hypercholesterolemic patients, a significant reduction in forearm blood flow and responses to both acetylcholine and bradykinin was observed in patients with hypertension compared with normotensive controls (Fig. 5).[29] Thus, patients with hypertension had blunted responses to bradykinin similar to their responses to acetylcholine and substance P, indicating that endothelial dysfunction in hypertension is likely due to a more generalized

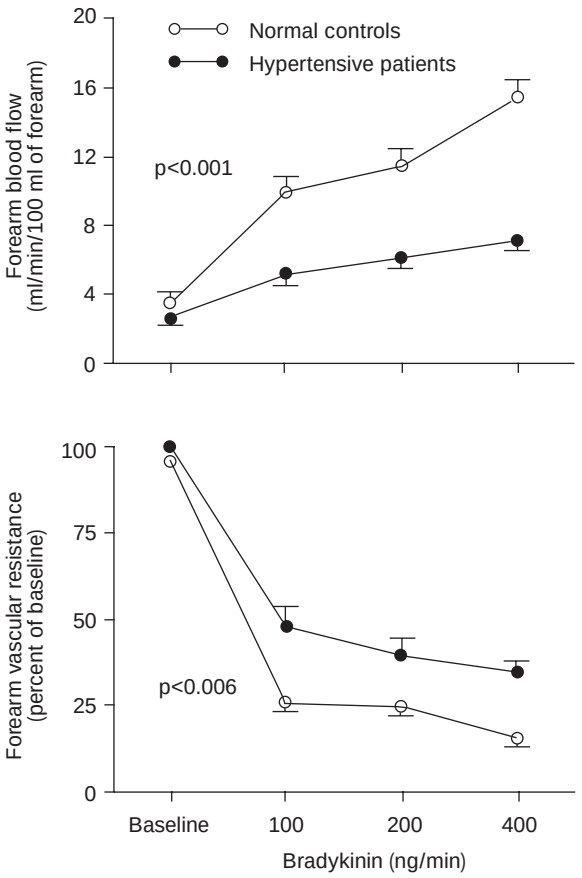

FIG. 5 Vascular responses to bradykinin in 12 normal controls and 10 hypertensive patients. As with substance P, a significant reduction in forearm blood flow and a significant increase in vascular resistance was observed in hypertensive patients compared with normotensive controls. Reprinted from Ref. No. 29 with permission.

abnormality within the endothelial cell rather than a defect in a single G protein-dependent intracellular signal-transduction pathway.

Destruction of nitric oxide by superoxide anion: A principal mechanism of NO inactivation is by superoxide anions produced by various radical-generating systems.[18, 30] Superoxide dismutase is a superoxide anion scavenger that may thus block the inactivation of NO.[10, 30] Observations from animal models of hypercholesterolemia suggest that excess generation of superoxide anion may be responsible for increased inactivation of NO resulting in impaired endothelium-dependent vascular relaxation in atherosclerotic vessels.[31–33] Similar observations have been reported in different models of hypertension, suggesting that a similar mechanism may be operative in this condition.[34, 35] If this were the case in hypertensive patients, then administration of superoxide dismutase would be expected to improve their impaired response to acetylcholine. However, vascular responses to acetylcholine were similar before and after administration of copper/zinc superoxide dismutase in both hypertensive patients and normotensive controls.[36] It must be noted that this form of the enzyme (the only one available for intravascular infusion in humans) protects only against extracellular degradation of NO due to its poor intracellular penetration. Therefore, although these results provided evidence that the defect in the NO system is not due to extracellular inactivation of NO, one cannot rule out the possibility that enhanced production of oxygen-free radical species formed within the intracellular space may contribute to a decreased bioavailability of NO.

An important intracellular source of superoxide radical is the xanthine oxidase system, which can be blocked by administration of oxypurinol. In an animal model, administration of oxypurinol normalized production of superoxide anion and improved acetylcholine-induced relaxation in hypercholesterolemic but not in normal vessels.[31] These findings suggest that endothelial cell production of superoxide anion may inactivate endothelium-derived NO, leading to endothelial dysfunction.

We recently conducted an investigation in which acetylcholine-induced vascular relaxation in hypercholesterolemic patients was improved following oxypurinol administration,[37] an observation in agreement with the results in hypercholesterolemic animal models. However, in patients with hypertension, there was no difference in the response to acetylcholine before or after administration of oxypurinol.[37] These observations suggest that the xanthine oxidase system does not significantly contribute to the endothelial dysfunction of patients with hypertension.

Effect of Antihypertensive Treatment on Endothelial Dysfunction

The observation that induction of hypertension in animal models resulted in impaired endothelium-dependent vasodilation[38] led to the hypothesis that effective antihypertensive therapy may normalize or at least improve endothelial vasodilator function. Indeed, in spontaneously hypertensive rats, treatment with an angiotensin-converting enzyme (ACE) inhibitor or a calcium-channel blocker reduced blood pressure and improved endothelial dysfunction in resistance vessels.[39] Long-term, but not short-term, treatment with an ACE inhibitor or a calcium-channel blocker improved endothelial dysfunction in a rat model of NO-deficient hypertension.[40] These studies further suggested a beneficial effect on endothelial function with antihypertensive treatment.

Studies in humans of the effects of antihypertensive treatment, including studies specifically related to the use of ACE inhibitors, have yielded negative results.[41–43] One study, however, did demonstrate an acute improvement in endothelium-dependent forearm vasodilation with ACE inhibitor treatment,[44] although it must be pointed out that in this study vasodilator responses to acetylcholine and sodium nitroprusside were measured only 1 h after oral administration of captopril, which may explain the discrepancy with the aforementioned studies using longer-term ACE inhibitor therapy.

The role of the renin-angiotensin system in controlling blood pressure and vascular reactivity has long been known. The more recent finding of a local renin-angiotensin pathway in many tissues, including blood vessels,[45, 46] suggested that treatment with an ACE inhibitor might improve endothelial function. The recently published Trial on Reversing Endothelial Dysfunction (TREND) study assessed responses to endothelium-dependent and -independent vasodilators in the coronary circulation of patients with CAD.[47] To remove confounding factors, only normotensive (or controlled hypertensive) patients without evidence of severe dyslipidemia or heart failure were enrolled. In large coronary arteries following 6 months of quinapril treatment, the vascular response to acetylcholine significantly improved compared with placebo. It must be emphasized that history of hypertension was not a predictor of improved endothelial function with quinapril, suggesting that the observed vascular effect was independent of the antihypertensive action of the ACE inhibitor. Because atherosclerosis is an important vascular complication of essential hypertension, one might speculate that chronic antihypertensive therapy with ACE inhibition may result in reduction of atherosclerotic disease in hypertensive patients by virtue of its beneficial effect on endothelial dysfunction of the macrovasculature (e.g., epicardial coronary arteries) despite the previously discussed negative effect on endothelial function of the microvasculature (e.g., forearm resistance vessels). This possibility is purely speculative at the present time and deserves further investigation in properly designed trials.

Conclusion

Over the last several years, significant progress has been made in our understanding of endothelial dysfunction in patients with hypertension. Not only has impaired endothelium-dependent vasodilation been demonstrated in patients with hypertension, but we now know that it is related largely to an abnormality in the endothelium-derived NO system.

Research has eliminated several potential defects in the NO system, including decreased availability of the NO precursor L-arginine, a defect at the muscarinic receptor level or in a single G protein-dependent intracellular signal-transduction pathway, and certain forms of inactivation of NO by superoxide anions as being responsible for impaired endothelium-dependent vasodilation in hypertensive patients. Important differences have been encountered in the responses to certain pharmacologic agents between hypertensive and hypercholesterolemic patients, suggesting that the mechanism(s) leading to the syndrome of endothelial dysfunction may be specific for each condition.

Irrespective of the specific defect in the NO pathway, the proatherogenic and vasoconstrictor effects of endothelial dysfunction probably contribute to the cardiovascular complications associated with elevated blood pressure. Treatment of the underlying pathophysiologic process of endothelial function has the potential for improving clinical outcome in patients with hypertension. In this context, continued research to identify the mechanisms responsible for endothelial dysfunction in hypertension is warranted for the eventual design of more rationalistic therapies.

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Clin. Cardiol. Vol. 20 (Suppl. II), II-34–II-37 (1997)

Endothelial Function, Fibrinolysis, and Angiotensin-Converting Enzyme Inhibition

DOUGLAS E. VAUGHAN, M.D.

Departments of Medicine and Pharmacology, Vanderbilt University and Veterans Affairs Medical Centers, Nashville, Tennessee, USA

Summary: Experimental and clinical studies with angiotensin-converting enzyme (ACE) inhibitors have suggested that these agents may reduce the risk of atherothrombotic events. Recent studies have identified the role of angiotensin II and ACE in the regulation of fibrinolysis. There is now substantial evidence that the renin-angiotensin system (RAS) plays an important role in the regulation of vascular fibrinolytic balance. This recently recognized relationship may contribute to the vasculoprotective effects of ACE inhibitors.

Key words: angiotensin-converting enzyme, fibrinolysis, plasminogen activator inhibitor type 1, tissue-type plasminogen activator

The Fibrinolytic System

The plasminogen activator, or fibrinolytic system, constitutes one of the primary endogenous mechanisms for preventing intravascular thrombosis, which is implicated importantly in the pathogenesis of myocardial infarction (MI) and other acute coronary syndromes. Fibrinolysis depends on a balance between plasminogen activators [urokinase and tissue-type plasminogen activator (TPA)] and plasminogen activator inhibitor type 1 (PAI-1), the major physiologic inhibitor of urokinase and TPA in plasma. This balance is maintained through processes that appear to be mediated largely by the endothelium. Plasminogen activators convert plasminogen to the active enzyme, plasmin, which is a protease that lyses fibrin clots. One important mechanism for regulating plasmin generation involves the formation of complexes between PAI-1 and the plasminogen activators, which prevents the conversion of plasminogen to plasmin.[1, 2] Because both TPA and PAI-1 are synthesized primarily by endothelial cells (and smooth muscle cells), the endothelium is thought to play a prominent role in maintaining vascular fibrinolytic balance.

Modest excesses or deficiencies in the fibrinolytic proteins can be associated with clinical consequences. Increased levels of PAI-1 have been associated with an increased risk of thrombosis in animal and clinical studies.[3–6] In one clinical study, low TPA activity and higher PAI-1 levels were observed in survivors of MI compared with healthy age-matched controls.[4] In another study, low TPA activity and increased PAI-1 concentrations were the only hemostatic variables associated with recurrent MI in a group of men with early coronary heart disease.[5] Imbalance of the fibrinolytic proteins can also have pathogenic consequences within the vascular wall. In vascular tissue, plasmin activates matrix metalloproteinases, which are crucial in remodeling following vascular injury through degradation of collagen and other glycoproteins that accumulate in plaques.[7] Several groups have reported increased deposition of PAI-1 in and around atherosclerotic plaques, which in turn reduces vascular plasmin activation and, subsequently, metalloproteinase activity. This reduction in plasmin activation is also associated with reduced activation of transforming growth factor-beta, which is important in suppressing the proliferation and migration of smooth muscle cells that contribute to atherosclerotic lesions.[8]

Regulatory Role of Endothelium in Fibrinolytic Balance and Role of the Renin-Angiotensin System

The role of the renin-angiotensin system (RAS) in regulating fibrinolysis was suggested by findings in two major clinical studies of angiotensin-converting enzyme (ACE) inhibitor therapy: the Survival and Ventricular Enlargement (SAVE) trial[9] and the Studies of Left Ventricular Dysfunction (SOLVD).[10] In both these studies, ACE inhibition significant-

Address for reprints:

Dr. Douglas E. Vaughan
Vanderbilt University School of Medicine
Division of Cardiology, Room 315, MRB II
2220 Pierce Ave.
Nashville, TN 37232-6300, USA

ly reduced the risk of recurrent MI and other ischemic events in patients with left ventricular dysfunction. In the SAVE trial, captopril treatment was associated with a 25% reduction in risk for recurrent MI and a 24% reduction in risk of death from cardiovascular events, severe heart failure, or MI. In SOLVD, which included more than 6,000 patients with asymptomatic left ventricular dysfunction or early congestive heart failure, enalapril was associated with an 18% reduction in risk of death from cardiovascular events and a 28% reduction in risk of death from MI.

The speculation that the endothelium serves as a link between the fibrinolytic system and the RAS has been supported by a number of experimental and clinical findings. Angiotensin II has been shown to bind to endothelial cells[11] and to stimulate dose-dependent production of PAI-1 in cultured rat vascular smooth muscle cells,[12] cultured bovine aortic cells,[13] and human endothelial cells,[13] thus demonstrating a potential link between the RAS and thrombosis. In other studies, ACE inhibition increased plasminogen activator activity in cultured bovine aortic endothelial cells[14] and decreased vascular PAI-1 expression in normal and balloon-injured rat aorta.[15] In human subjects, infusion of physiologic concentrations of angiotensin II resulted in rapid, dose-dependent, significant increases in PAI-1 levels (Fig. 1).[16] No significant changes in TPA levels were observed, indicating a selective effect of angiotensin II on PAI-1 release.

A recently identified angiotensin binding site, the angiotensin IV receptor (AT_4), appears to be the receptor on endothelial cells that mediates PAI-1 expression in response to angiotensin,[17] accounting for the observation that inhibitors of angiotensin receptors type 1 and type 2 fail to prevent endothelial production of PAI-1.[13, 17] The increase in PAI-1 expression in cultured cells is dependent on conversion of the octapeptide angiotensin to the hexapeptide angiotensin IV, which is accomplished via the effects of specific aminopeptides that are localized to the vascular surface.[17] Thus, responses to angiotensin may be mediated by an endothelial receptor specific for angiotensin IV, that is, the AT_4 receptor.

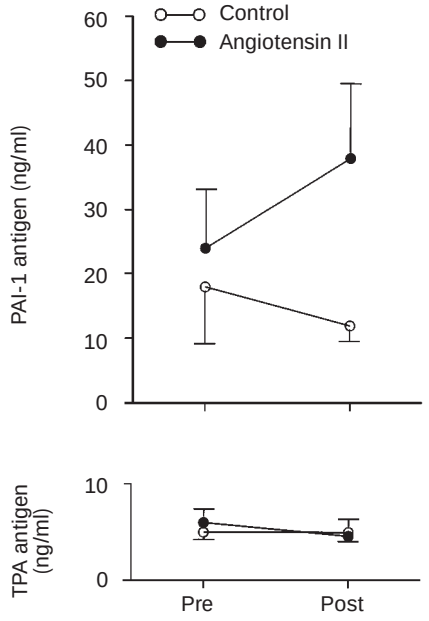

FIG. 1 Mean plasminogen activator inhibitor-1 (PAI-1) antigen levels and tissue-type plasminogen activator (TPA) antigen levels in hypertensive patients before and after infusion of angiotensin II (3 ng/kg/min for 45 min). Reprinted from Ref. No. 16 with permission.

Several clinical investigations, including recent studies designed to assess the effect of ACE inhibition on fibrinolytic factors, have provided additional evidence for the link between the RAS and the fibrinolytic system; these are reviewed below.

Angiotensin-Converting Enzyme and Fibrinolytic Balance

As suggested by the findings regarding ACE inhibition in cultured cells and in clinical studies, ACE occupies an important position in regulating the balance of fibrinolytic elements. It converts angiotensin I to angiotensin II, which is associated with stimulation of PAI-1 production. Through an independent and parallel pathway, ACE is also important in downregulating TPA production via degradation of bradykinin, a highly potent stimulator of TPA production in endothelial cells. In rats, intra-arterial administration of bradykinin results in a dose-dependent increase in plasma TPA levels.[18] In human subjects with hypertension, graded doses of bradykinin were associated with dose-dependent increases in plasma TPA levels during concomitant ACE inhibitor administration, but had no effect on TPA in the absence of ACE inhibitor administration (Fig. 2).[19] This finding confirms earlier reports that bradykinin is an extremely potent stimulus for the release of TPA in vivo. It also highlights the importance of the RAS in regulating vascular fibrinolytic balance.

Angiotensin-Converting Enzyme Inhibition and Fibrinolytic Balance

In addition to the experimental studies mentioned, clinical investigations have shown that ACE inhibition is associated with alterations in fibrinolysis. In the first study to demonstrate an effect of ACE inhibition on endogenous fibrinolysis, Wright *et al.*[20] administered captopril 75 mg/day or placebo to 15 patients beginning 8 weeks after uncomplicated MI and compared effects on fibrinolytic variables in these patients and 12 matched control subjects. The fibrinolytic variables assessed were PAI-1 antigen levels, TPA antigen levels, and PAI-1 activity. After the placebo treatment period, patients post MI had significantly higher TPA antigen and PAI-1 antigen levels and significantly greater PAI-1 activity than did controls. However, 4 weeks of ACE inhibition resulted in significant reductions in TPA antigen levels and PAI-1 activity in the 15 patients and a nonsignificant reduction in levels of PAI-1 antigen (Fig. 3).

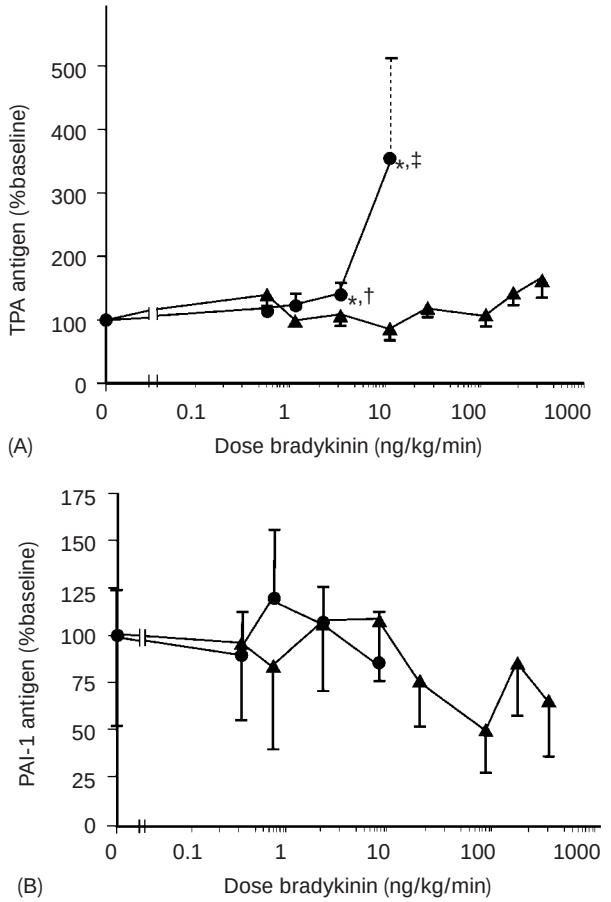

FIG. 2 Effect of bradykinin on (A) plasma TPA antigen levels and (B) levels of PAI-1 antigen in patients treated with either angiotensin-converting enzyme inhibitor (ACEI) or placebo. *$p<0.05$ versus baseline; †$p<0.05$ versus placebo; ‡$p<0.01$ versus placebo. ● = ACEI, ▲ = placebo. Reprinted from Ref. No. 19 with permission.

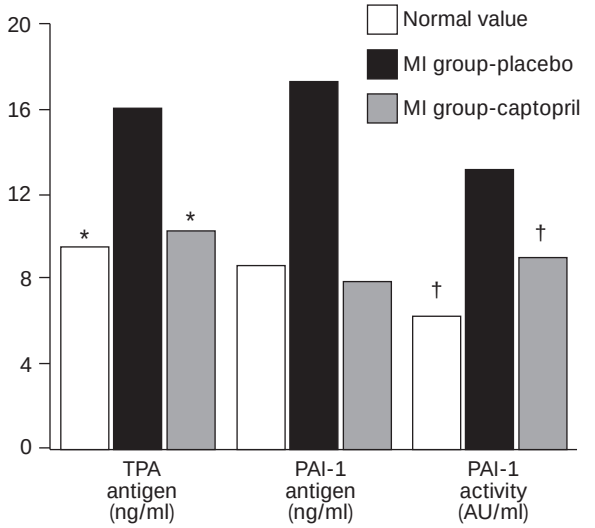

FIG. 3 Fibrinolytic variables in normal men and in patients with recent myocardial infarction (MI) after 4 weeks of placebo and 4 weeks of captopril. Adapted from data in Ref. No. 20 with permission.

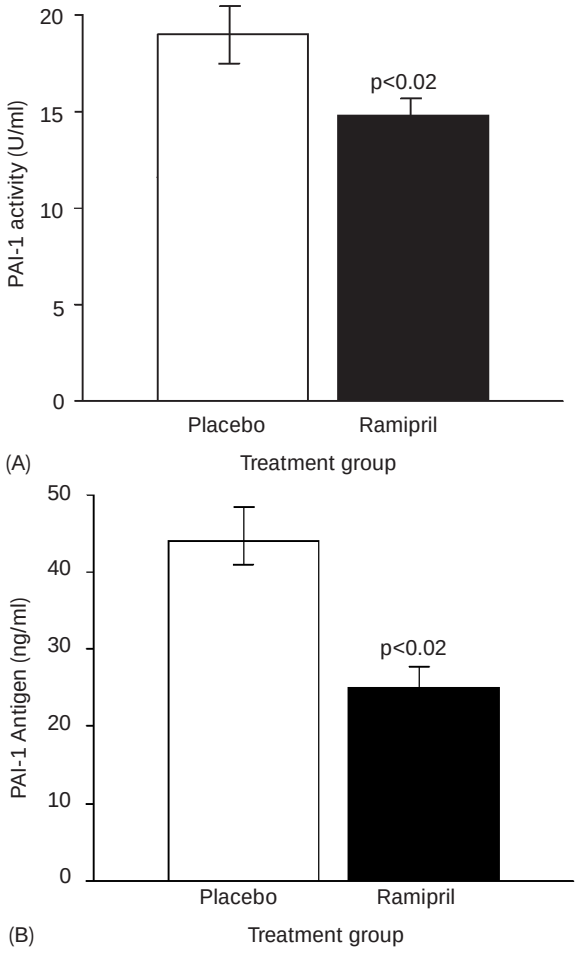

FIG. 4 Effects of ramipril and placebo on mean PAI-1 activity (A) and mean PAI-1 antigen levels (B) after 14 days of treatment. Reprinted from Ref. No. 21 with permission.

More recently, we[21] have assessed the effect of short-term ACE inhibition on fibrinolytic variables in a subset of 120 patients from the Healing and Early Afterload Reducing Therapy (HEART) study of patients with acute anterior MI and systolic blood pressure >100 mmHg. In this double-blind, placebo-controlled trial, patients were randomized to ramipril 0.625 or 1.25 mg/day titrated to 10 mg/day or placebo for 14 days. Subsequently, subjects in the placebo-treatment arm were crossed over into the high-dose ramipril arm of the study. Baseline PAI-1 activity and PAI-1 antigen and TPA antigen levels were comparable in the three groups; the ratio of PAI-1 to TPA, a measure intended as an index of fibrinolytic balance, was normal in each of the treatment groups as well. After 14 days, PAI-1 antigen levels were approximately 44% lower, and PAI-1 activity levels an average of 22% lower, in the patients treated with the ACE inhibitor (combined groups) than in placebo-treated patients (Fig. 4). In contrast, plasma TPA levels were not significantly different between the ACE inhibitor-treated patients and placebo-treated patients. Given

the significant reduction in PAI-1 activity and antigen levels, it is safe to say that ACE inhibition preserved normal fibrinolytic balance in these patients post MI. No other drugs besides ACE inhibitors have been shown to have such an impact on the plasma fibrinolytic balance during the recovery phase of acute MI. These results may help explain the beneficial effects of ACE inhibition on rates of MI and ischemic events in previous randomized trials.

Conclusion

There is accumulating evidence that the RAS interacts with the fibrinolytic system at the level of the endothelium. In fibrinolysis, both angiotensin II and ACE may be considered prothrombotic: angiotensin II because it induces PAI-1 expression, and ACE because it mediates the formation of angiotensin II and the degradation of bradykinin. Increased PAI-1 levels are associated with an increased risk of thrombotic events in humans. In experimental models, ACE inhibition is associated with reductions in PAI-1 expression in both cultured cells and tissue. These beneficial changes in fibrinolytic variables may be attributed to ACE inhibition's dual effects of inhibiting angiotensin II formation (and thus limiting the production of PAI-1) and blocking bradykinin degradation (and thereby enhancing the production of TPA by bradykinin). These mechanisms may contribute to the vasculoprotective effects of ACE inhibitors.

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Clin. Cardiol. Vol. 20 (Suppl. II), II-38–II-44 (1997)

Plaque Disruption and Coronary Thrombosis: New Insight into Pathogenesis and Prevention

PREDIMAN K. SHAH, M.D.

Division of Cardiology and the Atherosclerosis Research Center, Cedars-Sinai Medical Center, Los Angeles, California, USA

Summary: Clinical and pathologic studies have confirmed that disruption or superficial erosion of atherosclerotic plaque is the major cause of coronary thrombosis, which is the primary mechanism responsible for acute coronary syndromes of unstable angina, acute myocardial infarction, and sudden cardiac death. Serial angiographic studies have shown that nearly 60–70% of acute coronary syndromes evolve from mildly to moderately obstructive atherosclerotic plaques. The risk of plaque disruption appears to be a function of both plaque vulnerability (intrinsic factors) and extrinsic triggers, and is determined largely by the size of the lipid-rich atheromatous core, the thickness of the fibrous cap covering the core, and the presence of ongoing inflammation within and underneath the cap. Hemodynamic or mechanical stresses may precipitate plaque disruption, particularly in places where the fibrous cap is weakest, such as the shoulders. The degree of thrombosis following plaque disruption depends on the thrombogenicity of the disrupted plaque, the disturbed local rheology, and the systemic thrombotic-thrombolytic milieu. Surges in sympathetic activity (such as those provoked by sudden vigorous exercise, emotional stress, or cold weather) may also trigger plaque disruption. These observations have led to the concept of plaque stabilization as a new strategy for the prevention of acute coronary syndromes. Plaque stabilization can be achieved through pharmacologic and lifestyle-modifying interventions that alter plaque composition and/or inflammatory activity within the plaque and thus reduce its vulnerability to disruption.

Key words: atherosclerotic plaque, macrophages, metalloproteinase, plaque disruption, plaque stabilization, thrombosis

Address for reprints:

Dr. Prediman K. Shah
Division of Cardiology and the Atherosclerosis Research Center
Cedars-Sinai Medical Center
8700 Beverly Blvd., Room 5347
Los Angeles, CA 90048, USA

Introduction

Atherosclerotic vascular disease, the underlying pathology for ischemic heart disease and stroke, is the leading cause of death and disability in much of the Western world. Recent epidemiologic data indicate that ischemic heart disease is also becoming a major public health problem in developing countries such as India.[1] Coronary atherosclerosis results in a spectrum of clinical disorders, ranging from asymptomatic atherosclerosis and stable angina to acute coronary syndromes such as unstable angina, acute myocardial infarction (MI), and sudden cardiac death. It is estimated that 30 to 40% of acute coronary events occur without prior warning in persons who are unaware that they have ischemic heart disease. Therefore, prevention of acute coronary syndromes is a major focus in cardiovascular medicine. An improved understanding of the pathophysiologic basis for acute coronary syndromes can lead to more effective preventive strategies. One of these is the concept of plaque stabilization—decreasing the vulnerability of the atherosclerotic plaque to rupture or fissure.

Until recently, it was thought that the major pathophysiologic mechanism by which atherosclerosis contributes to various coronary syndromes was a slowly progressive luminal obstruction by atherosclerotic plaque that eventually resulted in decreased coronary blood flow reserve and subsequent myocardial ischemia. However, it is now generally accepted that coronary atherosclerosis progresses in a nonlinear, often abrupt fashion and that rapid progression of coronary lesions, including the sudden development of total or near-total occlusion, is due largely to thrombosis that complicates atherosclerosis.[2–13] Several autopsy, angiographic, and angioscopic studies have demonstrated that the most lethal manifestations of atherosclerosis-induced acute coronary syndromes result from coronary thrombosis that occurs either at sites of plaque disruption (60–80% of the time) or over areas of superficial endothelial erosions (20–40% of the time). Even though severely stenotic lesions are more likely, over time, to progress to total occlusion, a curious angiographic-clinical paradox is now evident: 60 to 70% of acute coronary syndromes evolve from coronary lesions that were deemed to have only mild to moderate stenosis (non-flow-limiting).[2–13] This paradox may be explained by the fact that mildly stenotic lesions outnumber severely stenotic lesions by a factor of 5 to 10.[9] It is also conceivable that the rapid transition of a mildly stenot-

ic lesion to total occlusion is more likely to result in a clinical event because the protective collaterals that normally develop over time with a severely stenotic lesion will not have had a chance to develop.[9]

Determinants of Plaque Disruption

Pathoanatomic studies of intact and disrupted plaques and in vitro studies of isolated aortic fibrous caps have demonstrated several characteristics that are more prevalent in disrupted plaques than in intact plaques. It has therefore been postulated that these characteristics can be used to identify plaques that are prone to disruption. This is the concept of plaque vulnerability or instability.

Plaque Composition

The histomorphometric characteristics associated with plaque disruption include the following: (a) a large, soft lipid core; (b) a thinned-out fibrous cap; (c) active infiltration by inflammatory cells into the plaque and fibrous cap; and (d) increased neovascularity in the plaque.

Mature atherosclerotic plaques are comprised of two main components: a lipid-rich core and an extracellular matrix consisting of collagen and other matrix proteins. Some plaque matrix is often organized as a lipid-poor, collagen-rich protective fibrous cap that separates the core from the lumen. The greater the amount of extracellular matrix, the more "stable" the plaque. More than 70% of a typical stenotic coronary plaque consists of extracellular matrix synthesized by vascular smooth muscle cells and containing primarily collagen, elastin, proteoglycans, and glycosaminoglycans.[9, 13] In contrast, the lipid core consists of soft, usually hypocellular and avascular, atheromatous "gruel," which is composed primarily of extracellular lipids (e.g., cholesterol and its esters).[14, 15]

The atheromatous lipid-rich core is thought to be derived from the necrosis or apoptosis of lipid-rich macrophages (foam cells) and possibly also from blood-borne lipoproteins trapped within the subendothelial extracellular space.[14-17] The lipid composition of the atheromatous core determines its consistency.[9] A core made up primarily of cholesterol esters is soft, whereas a core containing crystalline cholesterol is hard.[14, 15] Disrupted plaques tend to have a large, often eccentrically located, soft lipid-rich core that constitutes more than 40% of the plaque volume.[13] In vitro computer models have shown that the presence within a plaque of a large, eccentric soft lipid pool redistributes circumferential stress to the "shoulders" of the plaque, which is where 50 to 60% of plaque disruptions occur.[18] These observations suggest that a large, soft lipid core confers a mechanical disadvantage to the plaque and increases its vulnerability to disruption.

Fibrous Cap Thickness and Composition

Although fibrous caps vary widely in thickness, they are often thinnest at their shoulder regions where disruption most often occurs.[18] Disrupted fibrous caps tend to have fewer matrix-synthesizing smooth muscle cells, a lower collagen and glycosaminoglycans content, and a greater degree of infiltration by inflammatory cells (macrophages, T-lymphocytes, and mast cells) than do intact plaques.[19] Furthermore, fibrous caps infiltrated by macrophages have a lower mechanical threshold for rupture when tested in vitro.[20]

Inflammatory Response in the Plaque

Several histomorphometric studies have shown that disrupted plaques contain an active inflammatory infiltrate, commonly found in the fibrous cap and around the lipid core, with a preferential concentration at the plaque shoulders or underneath the thinned-out or disrupted fibrous cap.[21] Monocyte-derived macrophages, often bearing markers of activation, are the most abundant component of the inflammatory response, but activated T-lymphocytes and activated degranulating mast cells are also found in larger numbers in disrupted plaques.[18, 21–24]

Evidence of Matrix Dysregulation in Plaque Disruption: Role of Inflammation, Metalloproteinases, and Apoptosis

Plaque disruption results from thinning, weakening, and eventual rupture or fissure of the fibrous cap. Since the tensile strength of the fibrous cap is determined by its matrix components, specifically by the collagen content, loss of collagen matrix represents a critical step toward plaque disruption. Loss of collagen matrix could result from excessive matrix degradation or reduced matrix synthesis (i.e., matrix dysregulation).[25] Several recent studies have demonstrated that macrophages and, to a lesser extent, smooth muscle cell-derived foam cells in atherosclerotic plaques produce a family of matrix-degrading metalloproteinases (MMPs; see Fig. 1) that are capable of degrading virtually all components of the extracellular matrix.[26] Factors involved in stimulating the production or activation of MMPs in atherosclerotic plaques are not fully understood but may include interaction of macrophages with substrates, lipid ingestion by macrophages, oxidatively modified lipoproteins, oxidant stress, mechanical stress, activated T-lymphocytes, cytokines, infectious agents such as *Chlamydia*, and others.[25] In vitro experiments and in situ zymography studies have shown a net increase in matrix-degrading activity in rupture-prone regions of the atherosclerotic plaque.[27]

Data also show reduced collagen and glycosaminoglycans content and a fewer smooth muscle cells in the disrupted fibrous cap.[28] The precise mechanisms contributing to loss of smooth muscle cells remain unclear but may include inhibition of smooth muscle cell replication or increased smooth muscle cell death due to apoptosis or necrosis.[9] In vitro studies from our laboratory have shown that macrophages in cell culture as well as in the atherosclerotic plaque produce tenascin-C, a novel counteradhesive matrix protein that can induce apoptosis in vascular smooth muscle cells in vitro (unpublished data). These observations raise the tantalizing

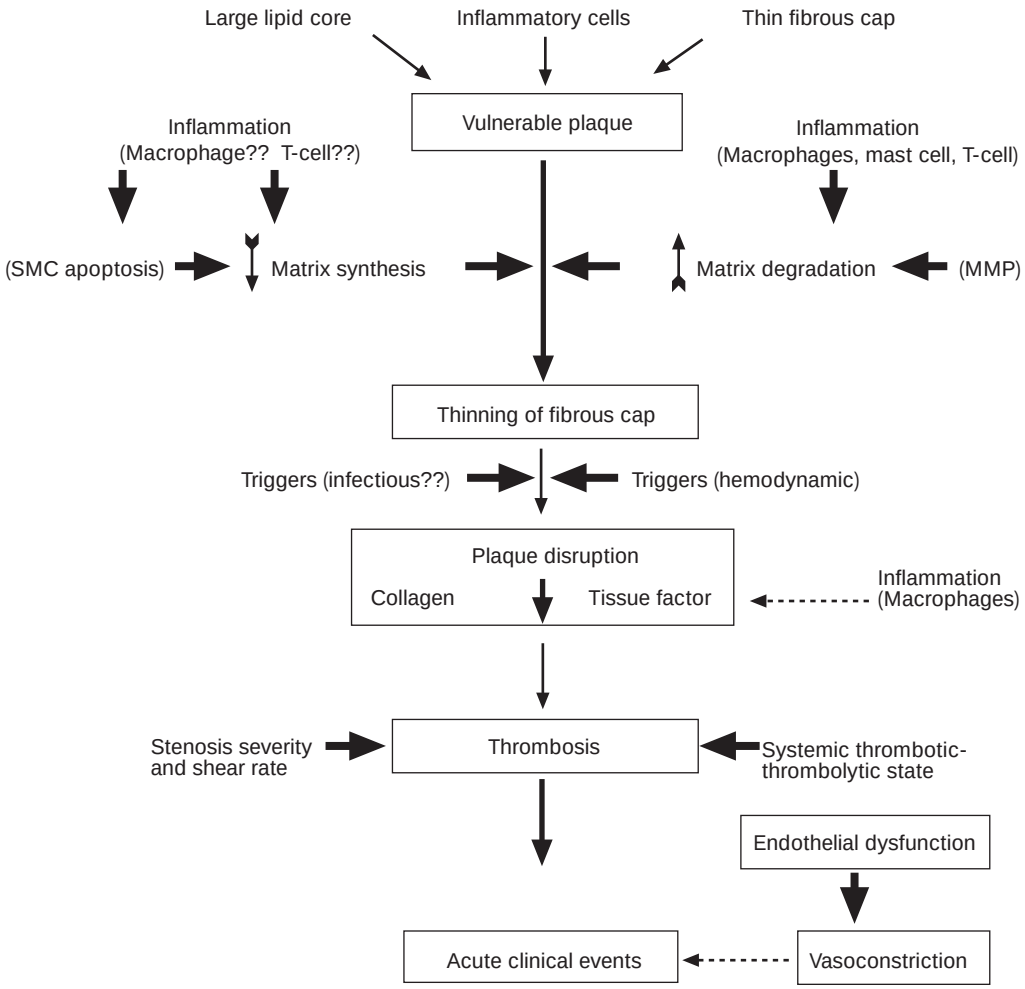

FIG. 1 Progression of a vulnerable plaque (characterized by a large lipid core, a thin fibrous cap, and the presence of inflammatory cells) to the occurrence of an acute clinical event. Inflammatory cells, such as macrophages, mast cells, and T-lymphocytes, cause decreased synthesis and/or increased degradation of matrix. The result is further thinning of the fibrous cap, which is more vulnerable to disruption caused by hemodynamic (and possibly infectious) triggers. After a plaque ruptures, the degree of thrombosis depends on the severity of stenosis, shear rate, and the systemic thrombotic-thrombolytic state. Endothelial dysfunction that causes vasoconstriction also contributes to the occurrence of acute clinical coronary events.

possibility that inflammatory cells (i.e., macrophages) may contribute not only to plaque disruption (by MMP-mediated matrix degradation) but also to smooth muscle cell death and, hence, reduced matrix synthesis.

Mechanical and Hemodynamic Triggers of Plaque Disruption

A variety of local mechanical and hemodynamic forces subject coronary plaques to constant stresses that may "trigger" disruption of unstable or vulnerable plaques, particularly at the point of their greatest weakness—the shoulder region of the fibrous cap. For example, plaques undergoing repetitive bending, compression, stretching, shear, or fluctuating pressure may develop weakness and eventually spontaneous disruption due to "cap fatigue," a phenomenon analogous to metal fatigue.[9, 29] According to Laplace's law, tensile stress correlates positively with both blood pressure and luminal diameter.[30] Consequently, plaques that are only mildly or moderately stenotic and have a larger residual lumen may be subjected to a greater circumferential stress, making them potentially more vulnerable to disruption than severely stenotic plaques.[9, 25] Maximal stress usually develops at the point where the fibrous cap is thinnest, but may also occur at other sites that have been weakened by the focal infiltration of macrophages.[18, 31, 32]

Plaques also may be sheared apart as a result of the mechanical stress that occurs when tissues with disparate properties slide against each other, causing a tear in the plaque known as "shear failure."[9, 25, 33] The ensuing pulse wave may produce cyclic changes in the size and shape of the lumen, resulting in

bending and deformation of plaques, particularly those with large atheromatous cores.[34] Cyclic bending is most likely to occur at the junction between stiff, eccentric plaques and the less diseased luminal wall (i.e., the "edge" of the plaque), ultimately causing weakening at this site that leads to spontaneous rupture.[9, 25] However, sudden and more intense bending may also trigger the rupture of a weakened cap.[9] Vasospasm, passive collapse of compliant stenoses, or bleeding from the vasa vasorum may compress the plaque by causing an increase in intraplaque pressure.[25]

There is little evidence that vasospasm alone precipitates plaque rupture or luminal thrombosis,[35, 36] or that the bleeding of small capillaries caused by rupture of the vasa vasorum would increase intraplaque pressure enough to induce plaque rupture.[25]

Plaque Disruption and Thrombosis

Plaque disruption may lead to coronary thrombosis; whether this occurs depends on the thrombogenicity of the exposed plaque components, the severity of local stenosis, and the systemic thrombotic-thrombolytic equilibrium. The main thrombogenic components of the plaque are collagen and the lipid core, although the lipid core may be more thrombogenic than the collagen matrix.[37] The greater thrombogenicity of the lipid core may be due to its high content of catalytically active tissue factor, a procoagulant transmembrane glycoprotein produced mostly by macrophages in the atherosclerotic plaque.[38] When exposed to circulating blood, tissue factor interacts with factor VIIa and forms a complex that activates factor X. Activated factor Xa initiates the thrombogenic cascade by cleaving prothrombin to thrombin, which, in turn, triggers the coagulation and platelet activation that results in thrombus formation.

Several *extrinsic* factors affect the thrombotic response to plaque disruption. These include (a) local flow disturbances related to severity of local stenosis and altered local geometry, and (b) the systemic thrombotic-thrombolytic milieu (e.g., hypercoagulable states, increased platelet aggregability, depressed endogenous fibrinolytic activity).[11, 25] Elevated fibrinogen levels, increased factor VII-mediated procoagulant activity, enhanced platelet aggregability, and depressed endogenous fibrinolysis are all associated with an increased risk of atherothrombotic vascular events.

Endothelial dysfunction may also contribute to the thrombotic consequences of plaque rupture. Normal endothelium is critical to the regulation of many vascular functions, including regulating vascular tone by releasing vasodilators (e.g., nitric oxide) and vasoconstrictors (e.g., endothelin) and maintaining a balance between thrombosis and thrombolysis by releasing antithrombotic (e.g., nitric oxide, protein C, ecto-ADPase) and prothrombotic (e.g., tissue factor, endothelin) substances as well as profibrinolytic (e.g., tissue plasminogen activator) and antifibrinolytic (e.g., plasminogen activator inhibitor-1) agents.[25] Endothelial dysfunction associated with atherosclerosis and the presence of risk factors for atherosclerosis increases the potential for excessive and paradoxical vasoconstriction, expression of adhesion molecules that recruit monocytes and other inflammatory cells into the arterial wall, and promotion of a prothrombotic and antifibrinolytic state.[39–45] Therefore, abnormalities in endothelial function may also play a role in thrombosis following plaque disruption.[11, 12]

Clinical Manifestations of Plaque Disruption

Plaque disruption does not always result in thrombosis and a clinical coronary event. In fact, plaque disruption is frequently asymptomatic, and the associated rapid plaque growth is often clinically silent. Autopsy studies have shown that 9% of ostensibly healthy persons and up to 22% of diabetic and hypertensive patients have asymptomatic disrupted plaques in their coronary arteries.[46] However, disrupted coronary plaques with and without superimposed thrombi are common in patients who die of ischemic heart disease.[22, 46–49] Of particular note is that patients who died as a result of acute coronary disease had an average of more than two disrupted plaques each; fewer than half of these were associated with luminal thrombosis sufficient to cause critical flow obstruction.[22, 47]

The clinical manifestations of plaque disruption and thrombosis vary according to the degree, location, and duration of myocardial ischemia.[9, 25] For example, a relatively stable, occlusive thrombus is likely to cause an acute Q-wave MI, whereas a nonocclusive (or transiently occlusive) thrombus is more likely to cause unstable angina or a non-Q-wave MI.[11, 25] Total or subtotal coronary occlusion may be associated with sudden cardiac death. However, coronary occlusion does not necessarily progress to MI if there is adequate collateral circulation at the time of occlusion.[2, 7] Nonetheless, plaque disruption, followed by variable degrees of hemorrhage into the plaque and luminal thrombosis, may accelerate further plaque growth and the progression of stenosis, thus accounting for the sudden, nonlinear, and unpredictable progression of coronary atherosclerosis to acute coronary syndromes.[50]

Onset of Acute Coronary Syndromes

The pathophysiologic mechanisms that underlie the onset of acute coronary events have not been definitively elucidated. However, the onset of acute coronary syndromes, particularly MI, does not occur randomly. At least half of all acute MIs are associated with "trigger" activities or conditions, which are referred to as acute risk factors.[50] These risk factors include vigorous exercise (especially in deconditioned individuals), emotional stress, earthquake, cold weather, time of day (i.e., early morning), and day of the week (i.e., Mondays).[51–56] Several pathophysiologic mechanisms may be involved in the triggering of nonrandom acute coronary events. One such mechanism is plaque disruption, most likely caused by surges of sympathetic activity that result in a sudden increase in blood pressure, pulse rate, cardiac contractility, and coronary blood flow.[56] The fact that beta blockers have demonstrated a significant beneficial effect in preventing re-

infarction provides compelling evidence that the mechanical and/or hemodynamic forces that trigger plaque disruption play a pivotal role in the sudden onset of coronary events.[9, 57, 58] Another mechanism that contributes to the onset of acute coronary syndromes is the rapid formation of thrombi at the site of previously or newly disrupted plaques; such sudden thrombus growth may be caused by changes in systemic thrombogenicity (e.g., hypercoagulability, platelet hyperaggregability, or impaired endothelial function).[9, 11, 55] A third possible mechanism is generalized or local vasoconstriction occurring around a coronary plaque.[59]

Patients with coronary heart disease have been shown to have a relatively high serologic incidence of recent *Chlamydia pneumoniae* infection.[60] Coupled with the finding of *Chlamydia* antigen in atherosclerotic plaques, this observation suggests the intriguing hypothesis that an active inflammatory or immune response, triggered by infection, may help initiate atherogenesis and possibly even plaque disruption and thrombosis.[60]

Plaque Stabilization and Prevention of Acute Coronary Syndromes

In the past several years, a number of serial angiographic trials evaluating the efficacy of lipid lowering or lifestyle modifications have demonstrated a disproportionately greater reduction in the incidence of atherothrombotic clinical events (i.e., acute coronary syndromes and strokes) than would be expected based on the relatively minor changes observed in the severity of coronary stenosis.[61–63] This clinical-angiographic paradox has led to the concept that risk factor modification, especially lipid lowering, may reduce clinical events by decreasing the incidence of plaque disruption and thrombosis through changes in plaque biology rather than by effects on the overall size or volume of the plaque or severity of stenosis.[61] This so-called "plaque stabilization" may be achieved by decreasing the lipid content of the plaque, changes in the magnitude of inflammatory cell content and activity within the plaque, improved endothelial function, or changes in circulating thrombotic-thrombolytic equilibrium.[25] Thus, plaque stabilization may reduce the vulnerability of plaques to disruption or the thrombotic response following disruption. This new therapeutic paradigm opens up a novel approach to reducing the adverse consequences of atherosclerosis by influencing plaque biology rather than plaque size or severity of stenosis.[25, 61, 64, 65]

Nonlipid-lowering interventions that may promote plaque stabilization (including improved endothelial function and reduced prothrombotic state) include angiotensin-converting enzyme inhibitors,[66–68] beta blockers, estrogens, antioxidants,[69] and cessation of cigarette smoking.

Conclusion

Considerable data from in vitro and in vivo studies of vascular biology, together with indirect evidence from clinical trials of lipid-lowering and lifestyle/risk factor-modifying interventions, provide strong support for the concept that disruption of atherosclerotic plaque and subsequent thrombosis is a key precipitating factor in potentially lethal acute coronary syndromes. Certain characteristics of plaques, including the size and composition of the lipid core, the structure and composition of the fibrous cap, and the presence of a local inflammatory process, predispose the plaque to disruption. Stresses resulting from biomechanical and hemodynamic forces acting on plaques may then trigger disruption, releasing the thrombogenic contents of the lipid core. Alterations in endothelial function may also contribute to the vulnerability of plaque to rupture and thrombosis.

Interventions aimed at decreasing plaque vulnerability to disruption—all based on the concept of plaque stabilization—may reduce the risk of acute coronary syndromes. Although not yet rigorously validated in humans, plaque stabilization may prove to be an important clinical strategy for preventing the often fatal consequences of coronary atherosclerosis.

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Clin. Cardiol. Vol. 20 (Suppl. II), II-45–II-51 (1997)

Therapeutic Interventions in Endothelial Dysfunction: Endothelium as a Target Organ

JOHN P. COOKE, M.D., PH.D.

The Section of Vascular Medicine, Falk Cardiovascular Research Center, Stanford University School of Medicine, Stanford, California, USA

Summary: Endothelial dysfunction is recognized as the initial step in the atherosclerotic process. To date, most interventions attempting to improve endothelial dysfunction have targeted one or more of the numerous risk factors that can cause endothelial damage: hypertension (angiotensin-converting enzyme inhibitors and calcium antagonists), hypercholesterolemia (lipid-lowering agents), cigarette smoking (cessation), sedentary lifestyle (increased physical activity), menopause (estrogen replacement therapy), and diabetes mellitus (control of associated metabolic abnormalities). Interventions targeted specifically to the endothelium remain speculative, as the precise mechanisms of endothelial dysfunction are still being elucidated. Several pharmacologic agents have been suggested to achieve vascular protection through mechanisms that go beyond their primary therapeutic (e.g., hypotensive or hypocholesterolemic) actions; examples of these are angiotensin-converting enzyme inhibitors or HMG-CoA reductase inhibitors. Beneficial changes to the endothelium might result from promotion of vasorelaxation, inhibition of vasoconstriction, reduction in the production of free radicals, or other mechanisms that protect the endothelium from injury.

Key words: atherosclerosis, endothelium, hypercholesterolemia, hypertension, nitric oxide

Address for reprints:

Dr. John P. Cooke
Division of Cardiovascular Medicine
Falk Cardiovascular Research Center
Stanford University School of Medicine
300 Pasteur Drive
Stanford, CA 94305–5406, USA

Introduction

That a paper can address itself to interventions targeted to the endothelium tells how far we have come since 1980, when Furchgott and Zawadzki reported that acetylcholine-induced vasodilation occurs only in the presence of an intact endothelium.[1] We now recognize that the endothelium-mediated vasodilation observed by Furchgott and Zawadzki is largely due to endothelium-derived nitric oxide (NO), a single molecule with profound effects on cardiovascular physiology. Impairment of endothelial vasodilator function is now established as a major contributor to cardiovascular disease, and accumulating evidence indicates that strategies for restoring endothelial function can have important therapeutic effects.

Risk Factors for Endothelial Dysfunction

Occupying an anatomic position that is both strategic and vulnerable, the endothelium is a target organ for the damaging effects of hypertension, diabetes, and hyperlipidemia, as well as for vascular injuries and mechanical stresses.[2]

Hypercholesterolemia and Atherosclerosis

The possible links between hypercholesterolemia, atherosclerosis, and vascular reactivity began to be examined in the 1980s. Hypercholesterolemia was recognized as a determinant in the pathogenesis of atherosclerosis, and endothelium-mediated relaxation was observed to be impaired in hypercholesterolemic vessels.[3,4]

Hypercholesterolemia enhances the response to vasoconstrictor agonists and attenuates endothelium-dependent relaxation in isolated vessels and in vivo.[5] Reduced activity of endothelium-derived NO in hypercholesterolemic vessels may be an initiating factor in atherogenesis. Endothelium-derived NO is now recognized to inhibit several pathologic processes that are critical to the development of atherosclerosis. These include monocyte adherence and chemotaxis, platelet adherence and aggregation, and vascular smooth muscle proliferation.[6]

Zeiher *et al.*[7] described a progression of endothelial dysfunction in coronary arteries that begins with hypercholesterolemia (Table I). They demonstrated a hierarchy of impairment, with progressive endothelium-mediated alterations in coronary vasomotor tone paralleling the development of early atherosclerosis, culminating in complete loss of endothelium-mediated vasodilation in atherosclerotic coronary arteries. The vasodilatory response to increased blood flow was the last function to be lost, not occurring until myointimal thickening of the arterial wall could be seen on angiography.[7] At least early in the process, endothelial dysfunction is reversible by administration of the NO precursor, L-arginine, to hypercholesterolemic individuals.[6]

Hypertension

Hypertension alters endothelial morphology and function. Platelets and monocytes interact with endothelial cells to a greater degree than in normotensive control vessels,[2] and endothelium-dependent vascular relaxation is reduced.[8]

In a number of earlier studies, antihypertensive therapy was unable to restore normal endothelium-dependent vascular relaxation in resistance vessels in patients with essential hypertension when blood pressure was normalized. The vasodilator response to acetylcholine was blunted even in patients who had received appropriate medical therapy.[9] The endothelial vasodilator dysfunction observed in subjects with essential hypertension appears to be due to a defect in the NO synthase pathway that is not reversible by administration of the NO precursor, L-arginine.[6]

Aging

The effect of aging on endothelium-dependent vasodilation of resistance coronary arteries in humans is characterized by significantly decreased coronary blood flow response to acetylcholine. In contrast, increasing age alters the response to papaverine, a direct smooth muscle dilator, only modestly.[10] Age-related decreases in the production or responsiveness of NO, increases in the production or responsiveness to vasoconstricting factors, or increased degradation of NO in the blood vessel wall may contribute to this effect.[11]

One study[12] of healthy men and women without vascular risk factors indicated that patterns of age-related vascular injury differ according to gender. Loss of flow-mediated dilation correlated with age in both men and women. The decline began in men toward the end of the fourth decade, whereas in women, flow-mediated dilation did not begin to decline until after the early fifties. By the age of 65 years, endothelial dysfunction was apparent in almost all subjects.[12]

Cigarette Smoking

Vasoconstriction,[13] platelet aggregation,[14] and increased monocyte adhesion[15] are but a few of the effects of cigarette smoking that lead to increased risk of atherosclerosis and other cardiovascular diseases. After subjects have smoked cigarettes, there is a doubling in the number of circulating endothelial cells in peripheral blood vessels (presumably reflecting increased turnover and desquamation of the endothelium).[16]

Even young, healthy, light smokers are vulnerable to endothelial damage. Endothelial dysfunction has been reported in the systemic arteries of light smokers beginning with adolescence, and physiologic abnormalities increased with increasing amount and duration of smoking. The threshold for smoking dose and endothelial dysfunction appeared to be ≥20 pack-years.[17] The endothelial vasodilator dysfunction observed in smokers is partially reversible by administration of L-arginine.[6]

Menopause

The Nurses' Health Study cohort[18] provided valuable data on some of the issues involving menopause and cardiovascular risk. Women found at highest risk of coronary heart disease were those who had undergone bilateral oophorectomy without receiving estrogen replacement therapy; those given estrogen replacement after oophorectomy demonstrated no

TABLE I Progression of endothelial dysfunction

Findings on angiography; Stage of atherosclerosis	Hierarchy of impairment
Normal coronary arteries; no risk factors for CAD (controls)	Increased epicardial artery luminal area in response to ACh, sympathetic stimulation, increased coronary flow
Normal coronary arteries; hypercholesterolemia; elevated LDL cholesterol	Selective endothelial dysfunction: vasoconstriction in response to ACh; preserved vasodilation in response to sympathetic stimulation and increased coronary flow
Angiographically normal segment of coronary artery; but disease elsewhere in coronary system	Lost ability to dilate in response to ACh and sympathetic stimulation; flow-dependent dilation intact
Diseased segment of coronary artery	Loss of endothelium-mediated vasoactive functions; vasoconstriction to sympathetic stimulation

Abbreviations: ACh = acetylcholine, CAD = coronary artery disease, LDL = low-density lipoprotein.
Adapted from data in Ref. No. 7.

excess risk, nor did women who had undergone natural menopause.

Menopause, whether natural or surgically induced, was strongly associated with an increased risk of atherosclerosis—that is, detection of calcium deposits in the abdominal aorta—in a study comprising more than 600 women.[19] The risk of atherosclerosis showed an increased trend with the number of postmenopausal years.

Diabetes Mellitus

Vascular disorders are highly prevalent in persons with diabetes and may take several forms: accelerated atherosclerosis, occurring earlier in diabetic patients than in their healthy counterparts and tending to be more severe and more diffuse;[20] thrombosis; hypertension; and hyperlipidemia.[21] The common cellular denominator in this varied pathology may be endothelial cell dysfunction.

Exposure to elevated levels of glucose may contribute to the aberrations of endothelium seen in persons with diabetes.[21] When exposed to increased concentrations of glucose in vitro, rings of isolated normal rabbit aorta are unable to relax normally in response to acetylcholine.[22] Reduced production of NO does not appear to be the cause of the impaired vasorelaxation. Rather, a vasoconstrictor prostaglandin may be elaborated in response to glucose and overcomes the normal vasodilatory effect of NO released by the endothelium. Cyclooxygenase inhibitors restored impaired acetylcholine-induced relaxation in the aortae of diabetic and normal rabbits exposed to elevated glucose in vitro.[21] In humans, the administration of vitamin C improves endothelium-dependent vasodilation, presumably by virtue of its antioxidant effects.[6]

Sedentary Lifestyle

A lack of exercise generally is considered a risk factor for atherosclerosis independent of its negative effects on body weight, blood pressure, and serum lipid values.[11] Chronic immobilization or lack of adequate physical activity, whether by choice or as a result of disease, may be associated with reduced expression of NO synthase and thereby decreased synthesis of NO.[23] So important has physical activity and exercise come to be regarded in maintaining cardiovascular integrity that the American Heart Association has issued a position statement on its benefits.[24] The statement affirms that physical inactivity is a recognized risk factor for coronary artery disease and has been related to increased cardiovascular mortality.

Asymmetric Dimethylarginine

Asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor (i.e., antagonist) of NO synthase, reduces the conversion of L-arginine to NO and citrulline. It normally circulates in plasma in humans and is usually excreted unchanged in urine. Elevated levels of ADMA inhibit endothelium-dependent vasodilation, an effect that has been reversed by administration of exogenous L-arginine.[25] Elevated circulating levels of ADMA have been observed in hypercholesterolemic rabbits,[26] in young hypercholesterolemic humans,[27] and in patients with chronic renal failure.[25]

Homocysteine

Elevated levels of homocysteine are associated with premature atherosclerosis. Indeed, nearly one third of persons with premature coronary, carotid, or peripheral arterial disease have elevated plasma levels of homocysteine.[28] Homocysteine may accelerate atherosclerosis by inducing endothelial dysfunction. Infusions of homocysteine have been shown to induce endothelial denudation. In children with homocystinuria (who are at risk for premature atherosclerosis), a dysfunction in endothelial vasodilation can be observed prior to the onset of symptoms of atherosclerosis.[29] Administration of folate (in doses > 800 µcg) is known to reduce homocysteine levels; whether this improves endothelial vasodilator function is under study.

Potential Interventions in Endothelial Dysfunction

With knowledge of endothelial mechanisms and diagnostic methods still evolving, interventions are governed by the manifestations of endothelial dysfunction rather than by the dysfunction per se. Although interventions targeted exclusively at the endothelial monolayer may be developed in the future, some currently available measures have shown promise in improving endothelial dysfunction.

Nonpharmacologic Interventions

Low-cholesterol diet: Cynomolgus monkeys fed a high-fat diet develop hypercholesterolemia and, over time, atherosclerotic lesions similar to those in humans. When placed back on a normal chow diet for several months, vascular lesions regress, with marked reduction in the amount of lipid-laden macrophages in the lesion. Moreover, dietary treatment restored impaired endothelium-dependent vascular relaxation. The mechanism by which endothelium-dependent vascular relaxation was restored by cholesterol lowering is still undefined.[30]

Functional changes and regression of atherosclerosis may occur at different rates and to different degrees in different parts of the vascular bed. Limb blood flow during regression in atherosclerotic arteries of monkeys improved to a greater degree than did hyperresponsiveness of large arteries to serotonin.[31]

Fish oil: It became known in the 1970s that consumption of large quantities of marine fish oils appeared to result in a low incidence of coronary artery disease. Fatty acids in marine fish, particularly cold water fish, differ chemically from those of land animals and those contained in vegetable oils—a greater percentage of marine-derived fatty acids are polyunsaturated, and they are less vulnerable to oxidation. Eicosapentaenoic acid and docosahexaenoic acid in marine lipids can substitute for arachidonic acid. Like arachidonic acid, they can

be converted into an active form of prostacyclin (a vasodilator and inhibitor of platelet aggregation). Unlike arachidonic acid, they are converted into an inactive form of thromboxane (the vasoconstrictor and platelet agonist). Therefore, these omega-3 fatty acids shift the balance in the arachidonic acid cascade to the side of the vasodilator/platelet antagonist prostacyclins. Some effects attributed to marine fish oils included lowered levels of triglycerides, total cholesterol, and very-low-density lipoprotein cholesterol; reduced platelet aggregation; and prolonged bleeding time.[32] Monkeys fed an atherogenic diet with half the fat-derived calories from fish oil showed evidence of reduced superoxide anion production in coronary artery endothelium after 1 h of ischemia and 2 h of reperfusion.[33] Swine fed a high-fat diet develop an endothelial vasodilator dysfunction that is reversible by treatment with fish oil.[6]

Exercise: According to a study of patients whose physical activity was limited by congestive heart failure, flow-dependent dilation can be enhanced by physical training. After 4 weeks of hand-grip training, flow-dependent dilation was restored, most likely by increased endothelial release of NO. The effect of physical training was local, however, being limited to the trained arm, and lasted for only 6 weeks.[23]

Smoking cessation: The improvements in vascular function that follow cessation of cigarette smoking partially reverses the adverse effects of cigarette smoking on the vasculature. Endothelial dysfunction improves with smoking cessation. Flow-mediated dilation was observed to be better in male former smokers than in current smokers, albeit impaired in both groups.[17]

The lipid profile also benefits from smoking cessation: high-density lipoprotein (HDL) cholesterol and apolipoprotein A-1 increase, whereas triglycerides decrease. An increase in lipoprotein lipase correlated significantly with the increase in HDL cholesterol.[34] Moreover, the increased risk of myocardial infarction conferred by smoking decreases to the level of men who never smoked within a few years after tobacco cessation.[35]

Antioxidant supplements: Because oxidation of low-density lipoprotein (LDL) cholesterol contributes to endothelial dysfunction, investigators have reasoned that a diet rich in antioxidants may be protective.[2] Results of clinical studies have not consistently shown a benefit, however. In one trial of hypercholesterolemic patients, 1 month of treatment with relatively high doses of beta-carotene and vitamin C and E supplements delayed the onset of oxidation of LDL and decreased the maximal rate of LDL oxidation, but endothelial function was still impaired.[36] Nonvitamin antioxidants, antioxidant enzymes, or concomitant reduction in LDL levels may be required to improve endothelium-dependent vasodilation in hypercholesterolemic patients.

Other investigators reported that vitamin C reversed endothelial dysfunction in the brachial circulation of patients with coronary artery disease. In a placebo-controlled, blinded study,[37] oral administration of 2 g of ascorbic acid restored endothelium-dependent vasodilation.

L-Arginine supplementation: With the recognition of NO as the major mediator of endothelium-dependent relaxation, interest began to center on L-arginine, the precursor of NO. Investigators hypothesized that increasing the availability of L-arginine might enhance synthesis of NO and thereby promote vasodilation.[5]

The first evidence that L-arginine might have an antiatherogenic effect came from a study of hypercholesterolemic rabbits whose diet was supplemented with an average sixfold increase in daily L-arginine intake.[38] Compared with lesions of hypercholesterolemic controls, atheromatous lesions in the thoracic aortae of the L-arginine-supplemented animals had markedly decreased surface area and reduced intimal thickness. Endothelium-dependent relaxation improved, even though the supplemented diet did not affect the animals' serum cholesterol levels.[38]

The potential benefits of L-arginine following arterial injury were studied 4 weeks after the iliac arteries of normocholesterolemic rabbits were denuded by a balloon catheter.[39] Administration of L-arginine during the 4-week period reduced neointimal thickening and improved acetylcholine-induced vasorelaxation. The similarity of this model to restenosis after percutaneous transluminal coronary angioplasty marks it for particular interest.

Coronary artery dimensions and blood flow in hypercholesterolemic patients and normocholesterolemic controls were compared before and after L-arginine infusion. L -arginine augmented endothelium-dependent dilatation in the coronary microcirculation of hypercholesterolemic patients who had shown impaired endothelium-dependent dilatation. No effect was observed in the normocholesterolemic controls.[40]

Essential hypertension may be a setting in which L-arginine supplementation cannot mitigate pathologic changes. Patients with essential hypertension and diminished acetylcholine-induced vasodilation did not respond with augmented endothelium-dependent vasodilation to increased availability of NO substrate.[41]

Pharmacologic Interventions

Several categories of drug used to treat cardiovascular disease have proven to ameliorate impaired endothelial vasodilation (Table II).

Lipid-lowering agents: Cholesterol-lowering therapy has been associated with a decreased risk of ischemic coronary events even in the absence of angiographic regression of atherosclerosis. Restoring coronary endothelial function may be more important to improved clinical outcome than reducing the degree of stenosis.[42]

Reversal of coronary endothelial dysfunction in patients with symptomatic coronary atherosclerosis predates changes in vascular structure. Treatment with lovastatin does not improve coronary artery endothelial responses to acetylcholine after 12 days, but does significantly improve epicardial coronary artery responses to acetylcholine at 5 1/2 months.[42] A recent report indicates that endothelial vasodilator function is improved immediately after plasmapheresis in patients with familial hypercholesterolemia.[43]

TABLE II Current agents that can reverse endothelial dysfunction

Pharmacologic strategy	Examples
Lipid-lowering agents	HMG-CoA reductase inhibitors, cholestyramine
Inhibitors of renin-angiotensin system	ACE inhibitors, angiotensin II receptor antagonists
Calcium-channel blockers	Verapamil, nifedipine
Antioxidants	Vitamin C, vitamin E
Enhancement of NO synthase pathway	Folate, arginine, estrogen
Cytoprotective agents	Superoxide dismutase, probucol
Substitutes for protective endothelial substances	Nitrovasodilators; analogs of prostacyclin

Abbreviations: ACE = angiotensin-converting enzyme, HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A, NO = nitric oxide.
Adapted from data in Ref. No. 13.

Reduction of LDL cholesterol alone failed to reverse endothelial dysfunction in coronary arteries in another study,[44] but the impairment was significantly improved when antioxidant therapy was added to the regimen. Improvement in vasomotor response to acetylcholine was significantly greater in the combined therapy (lovastatin and probucol) group than with diet or LDL cholesterol lowering alone.

Angiotensin-converting enzyme (ACE) inhibitors: The role of the renin-angiotensin system in endothelial dysfunction relates primarily to angiotensin II as a potent endothelium-derived contracting factor. Angiotensin II, the vasoactive product of angiotensin I, is produced by the action of ACE. Although this reaction takes place primarily in the lung, a tissue ACE system has also been found in endothelial cells throughout the vasculature.[13]

The vasoconstrictive effect of tissue ACE in generating angiotensin II is normally balanced by the effects of NO and prostacyclin. When the endothelium is damaged or dysfunctional, however, the countervailing effects of these endothelial vasodilators are lessened.[45]

One of the first studies to demonstrate an improvement in endothelial dysfunction with an antihypertensive agent was the Trial on Reversing ENdothelial Dysfunction (TREND).[45] TREND was conducted in 129 normotensive (or controlled hypertensive) patients with coronary artery disease to determine whether treatment with an ACE inhibitor (quinapril 40 g daily) could improve endothelial dysfunction. Angiograms performed at baseline and at 6-month follow-up showed significant improvements in endothelial vasomotor function (assessed by response to acetylcholine) in the quinapril-treated patients.

The beneficial mechanisms of quinapril in this 6-month trial probably relate to the effects of ACE inhibition on both angiotensin II and bradykinin, which is a potent vasodilator. Angiotensin-converting enzyme inhibition of angiotensin II counters its contractile effect on smooth muscle and reduces the generation of superoxide anions. In diminishing the breakdown of bradykinin, ACE inhibition enhances the bradykinin-induced release of NO by endothelial cells. In the TREND study, quinapril improved endothelial dysfunction without altering lipids or reducing blood pressure.[45]

Calcium-channel blockers: Cholesterol-fed rabbits were given a calcium antagonist at a dose too low for an antihypertensive effect. Treated rabbits had less impairment in endothelium-dependent cholinergic relaxation than untreated but hypercholesterolemic controls. Thus, treatment with a dihydropyridine calcium-channel blocker inhibited atherogenesis to a partial degree in these animals without reducing arterial blood pressure.[3] In humans, several trials of calcium-channel blockers have been concordant in showing an effect of these drugs in inhibiting the development of new lesions; however, there is no evidence that calcium-channel blockers modify existing lesions or reduce coronary events.

Estrogen replacement: Although the benefits of estrogen replacement therapy after menopause include an improved lipid profile, multiple regression analyses have indicated that only 25 to 50% of the reduction in cardiovascular events can be attributed to lipid-lowering effects.[46] The finding that estrogen receptors are localized on endothelial and smooth muscle cells of several mammalian species has suggested that the hormone may directly influence vascular function.[47, 48] More recently, estrogen receptor expression was demonstrated in human endothelial cells, suggesting that estrogen may act directly on human vascular tissue.[49]

These findings prompted several studies. For example, a trial[50] of estrogen administration in postmenopausal women with atherosclerotic coronary arteries and mild hypercholesterolemia found that estrogen improved endothelium-dependent vasodilation without any effect on lipids. After 9 weeks of estradiol therapy (1 or 2 mg/day), flow-mediated vasodilation in the brachial artery was improved. The effects of estrogen to enhance endothelial vasodilator function may be due to an antioxidant effect, or to an estrogen-induced enhancement of NO synthase expression.

Future Therapeutic Possibilities

Strategies specifically targeted to restoration of endothelial function may be expected to reverse or reduce the progression of vascular disease and to normalize vascular reactivity. A mechanistic understanding of the pathophysiology of endothelial dysfunction is required for such specific therapies to be developed. With respect to derangements of the NO synthase pathway, a number of possible mechanisms merit exploration. Reduction in the availability of the precursor, or alterations in the enzyme NO synthase, may explain the beneficial effects on

NO elaboration of supplemental L-arginine or folate (which is a precursor of tetrahydrobiopterin, a cofactor for NO synthase). Alternatively, elevated activity of α-methylase I or reduced activity of dimethylarginine dimethylaminohydrolase may explain the increased circulating levels of ADMA observed in patients with vascular disease. Observations by our group and others indicate that ADMA is an endogenous modulator of NO synthase. Finally, the expression (as well as the activity) of NO synthase can be modulated. For example, estrogen is known to increase the transcription of NO synthase, indicating proof of concept for another therapeutic strategy worth exploring.

Conclusions

Improved endothelial function appears to be possible via a variety of currently available methods, with novel approaches still to come. It seems reasonable to expect that future therapeutic strategies and agents will be directly targeted to this monolayer of cells that regulates vascular tone and structure. Early detection of endothelial dysfunction may be a useful measure to guide therapy prior to the development of symptomatic atherosclerosis.

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Clin. Cardiol. Vol. 20 (Suppl. II), II-52–II-57 (1997)

Growth Factors as a Potential New Treatment for Ischemic Heart Disease

CHRISTOPHE BAUTERS, M.D.

Service de Cardiologie B et Hémodynamique, Hôpital Cardiologique, Université de Lille, Lille, France

Summary: Growth factors such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) exert important effects on endothelial cells in vitro and in vivo. This article reviews the effect of these two growth factors on endothelial dysfunction in various animal models of vascular disease: (1) collateral circulation supplying an ischemic territory, (2) balloon injury, and (3) diet-induced experimental atherosclerosis. Endothelial dysfunction may limit the beneficial effects of collateral vessels on tissue perfusion. Administration of VEGF or basic FGF (bFGF) augments collateral development in different models of hindlimb ischemia by enhancing neovascularity and by facilitating the recovery of endothelial function in the collateral circulation. Similarly, studies performed after balloon angioplasty have demonstrated abnormal responses of previously dilated sites to endothelium-dependent agonists. Administration of VEGF or bFGF increases endothelial regrowth and normalizes endothelium-dependent responses after experimental angioplasty. Finally, endothelium-dependent relaxation is impaired in diet-induced experimental atherosclerosis. It was recently demonstrated that hypercholesterolemic rabbits treated with bFGF had significantly better endothelium-dependent responses than those not treated with bFGF. These results show that in vivo administration of the endothelial cell growth factors VEGF and bFGF leads to significant improvement in endothelium-dependent responses and supports the concept of using these growth factors as a new therapeutic strategy for patients with vascular diseases.

Key words: animal models, atherosclerosis, basic fibroblast growth factor, collateral circulation, endothelial dysfunction, vascular endothelial growth factor

Address for reprints:

Christophe Bauters, M.D.
Service de Cardiologie B
Hôpital Cardiologique
Bvd. du Professeur J. Leclercq
59037 Lille Cedex, France

Introduction

Endothelial dysfunction has been implicated in the pathogenesis of many diseases affecting the cardiovascular system. Experimental and clinical studies have shown that endothelial dysfunction may play a key role in diverse conditions such as abnormal arterial vasomotion, thrombosis, and neointimal proliferation.[1,2] Endothelial dysfunction is a characteristic feature of atherosclerotic vessels,[3,4] arteries subjected to mechanical injury,[5,6] and collateral vessels that develop in response to severe ischemia.[7,8]

Endothelial cell growth factors such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) are important growth factors for endothelial cells in vitro. Whereas VEGF is specific for endothelial cells, FGFs also potently stimulate growth of other cell types, such as smooth muscle cells.

Recent studies have demonstrated the feasibility of using endothelial cell growth factors in vivo. Basic FGF (bFGF) and VEGF increase the development of collateral vessels in ischemic models[9–11] and enhance the extent of endothelial regrowth after arterial injury (Table I).[12,13] The marked anatomic improvement associated with administration of endothelial cell growth factors has led to speculation regarding the possible effects of these factors on endothelial dysfunction.

This article briefly addresses the effects of FGF and VEGF on endothelial dysfunction in three animal models of vascular disease: (1) the collateral circulation supplying an ischemic territory, (2) regenerated endothelium after arterial injury, and (3) the atherosclerotic rabbit model. In these experimental models, in vivo administration of these endothelium-dependent growth factors leads to significant improvement in endothelial dysfunction (Table II). The mechanisms underlying this beneficial effect remain speculative but may include growth factor-induced production of nitric oxide.

Endothelial Dysfunction in the Collateral Circulation

When a major artery becomes obstructed, blood flow to the ischemic tissue often depends on collateral vessels. When spontaneous development of collateral vessels is insufficient for allowing normal perfusion of the tissue at risk, residual ischemia occurs. A growing body of evidence indicates that ab-

TABLE I Use of endothelial cell growth factors in cardiovascular disease

1. Increase the development of collateral circulation
2. Increase reendothelialization after arterial injury

normal vascular reactivity may limit the beneficial effects of collateral vessels on tissue perfusion. Previous studies have demonstrated that this abnormal reactivity occurs, at least in part, as the consequence of dysfunctional endothelium.[7, 8, 14]

The basis for the impaired endothelial regulation of collateral vessels is not known. Studies of coronary collaterals have suggested two possible explanations.[15] The first involves the possibility that the collateral circulation fails to develop at a sufficient rate to prevent ischemic damage to endothelial cells of the recipient, downstream, reconstituted microvasculature. The second suggests that receptor-mediated production or release of endothelium-derived relaxing factor (EDRF)/nitric oxide (NO) may be regulated by perfusion pressure within the recipient vasculature; compromised perfusion pressure may further compromise abnormal endothelium-dependent flow.

Administration of VEGF or bFGF augments collateral development in various models of hindlimb or myocardial ischemia.[9–11] Because persistent impairment in endothelium-dependent relaxation would constitute an important limitation of this promising therapeutic approach, we investigated the effects of VEGF therapy on endothelium-dependent blood flow in a rabbit model of hindlimb ischemia.[16] Ischemia was induced by ligation of the external iliac artery and excision of the femoral artery in one limb of New Zealand White rabbits (Day 0). Flow velocity was measured using a Doppler guidewire at rest and following injection of serotonin and of acetylcholine. In control animals studied at Days 10 and 40, serotonin induced a decrease in hindlimb blood flow (67 ± 6% from baseline and 29 ± 2% from baseline, respectively); by contrast, in animals treated with a bolus dose of VEGF into the internal iliac artery at Day 10 and studied at Day 40, serotonin induced an increase in flow (119 ± 8% from baseline; $p < 0.05$ vs. controls). Acetylcholine induced a moderate increase in flow in control animals (152 ± 15% at Day 10, 177 ± 14% at Day 40) but a major increase in flow in animals treated with VEGF (254 ± 25%; $p < 0.05$ vs. controls) (Fig. 1).[16] These data suggest that VEGF not only augments neovascularity in this animal model but also facilitates the recovery of endothelial function of the collateral circulation.

At least two mechanisms could explain an improvement in endothelial function of the collateral-dependent limb after VEGF therapy. The first possibility relates to the characteristics of flow and perfusion pressure in arterioles distal to collaterals. The improved perfusion pressure associated with VEGF therapy may have reversed endothelial dysfunction. A second and intriguing possibility relates to a direct improvement of endothelial function by VEGF. Beyond its mitogenic effects, VEGF may also modulate qualitative aspects of endothelial cell function.[17] It is tempting to speculate that VEGF may affect the phenotype of endothelial cells in the collateral-dependent limb and thereby restore normal endothelial function. Similarly, in the case of bFGF, recent in vitro studies have demonstrated that long-term administration of this endothelial cell mitogen preserves endothelial function in the coronary microcirculation perfused via collateral vessels.[18]

Endothelial Dysfunction after Arterial Injury

Percutaneous transluminal coronary angioplasty is a technique widely used in patients with atherosclerotic coronary artery disease. Restenosis occurring within the first 6 months remains the major problem limiting the long-term efficacy of the procedure. Two important mechanisms have been implicated in the pathogenesis of restenosis: neointimal hyperplasia and vessel remodeling. Neointimal hyperplasia, which results primarily from a growth response of the smooth muscle cells, is maximal at 1 to 4 weeks after the initial injury. Neointimal formation involves different steps: activation, proliferation, and migration of smooth muscle cells, and the production of extracellular matrix.[19] Arterial remodeling (i.e., changes in vessel size) also plays a major role in restenosis. The changes in vessel size may be bidirectional: some lesions show an increase (enlargement) whereas others show a decrease (constriction) in vessel size.[20]

TABLE II Summary of the effects of endothelial cell growth factors in experimental models of vascular diseases

Model	Endothelial regrowth	Endothelial function	Neointima	References
VEGF				
Arterial injury	↑	↑	↓	Asahara *et al.* (13, 28)
Atherosclerosis		↑	↓	Asahara *et al.* (34)
Collaterals		↑		Bauters *et al.* (16)
bFGF				
Arterial injury	↑	↑	↔	Meurice *et al.* (24)
Atherosclerosis		↑	↔	Meurice *et al.* (33)
Collaterals		↑		Sellke *et al.* (18)

Abbreviations: bFGF = basic fibroblast growth factor, VEGF = vascular endothelial growth factor, ↑ = increase, ↓ = decrease, ↔ = no change.

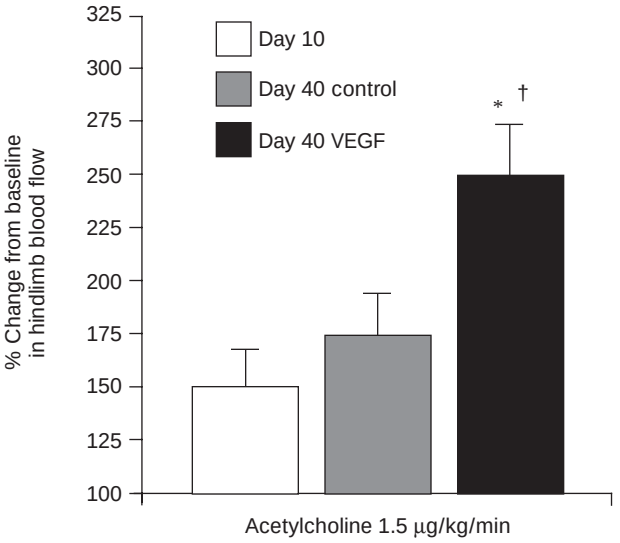

FIG. 1 Change in hindlimb blood flow in response to acetylcholine in rabbits 10 and 40 days after surgery. Note the significant difference between percent changes in hindlimb blood flow in VEGF-treated rabbits compared with control rabbits. * $p < 0.01$ versus Day 10; † $p < 0.05$ versus Day 40 control. VEGF = vascular endothelial growth factor. Reprinted from Ref. No. 16 with permission.

In the hours following experimental angioplasty, endothelial cells rapidly enter the replication cycle to restore endothelial continuity. Endothelial regeneration starts from the leading edge of the denuded area and from the ostia of collateral and/or branch arteries.[21] Even if complete reendothelialization occurs, the functional properties of the regenerated endothelium are abnormal. After vascular injury, endothelium-dependent relaxation to vasodilator agonists is depressed in arteries with regenerated endothelium while the ability of the underlying smooth muscle cells to relax or contract does not change.[5, 6] Similarly, studies performed after coronary angioplasty in men have demonstrated abnormal responses of previously dilated sites to endothelium-dependent agonists such as serotonin and acetylcholine.[22, 23]

Fibroblast growth factor and VEGF have both been used in vivo in attempts to increase endothelial regrowth after experimental arterial injury.

Fibroblast Growth Factor

Lindner *et al.*[12] first demonstrated that the in vivo administration of bFGF was associated with a significant increase in endothelial cell coverage on denuded arteries. This study provided clear evidence for the mitogenic effect of bFGF on endothelial cell replication in vivo, and further demonstrated that total endothelial cell regrowth could be achieved within 10 weeks in a rat carotid model of balloon denudation by systemic administration of bFGF. It is interesting that recombinant bFGF may achieve significant reendothelialization of denuded arteries when given at much lower doses than those used by Lindner *et al*. In a rabbit model of balloon denudation of the iliac artery, our group (Meurice *et al.*[24]) observed a significant ($p < 0.005$) increase in endothelial regrowth after administration of 2.5 µg of bFGF twice weekly for 2 weeks. Finally, the beneficial effect of FGF on endothelial regrowth is not limited to bFGF; administration of low doses of acidic FGF also promotes repair of damaged endothelium in vivo.[25]

Although these studies established the beneficial effect of FGF on endothelial cell growth in vivo, it was of critical importance to assess the function of the neoendothelium that regenerated in response to growth factor administration. As mentioned above, Meurice *et al.*[24] reported the effect of long-term (4 weeks) administration of bFGF on physiologic responses to endothelium-dependent agonists after vascular injury in a rabbit model. As stated, administration of relatively low doses of bFGF was associated with a significant increase in reendothelialization. Four weeks after denudation, endothelium-independent responses did not differ significantly between the bFGF and the control groups. In contrast, the maximal endothelium-dependent relaxation induced by acetylcholine in the bFGF-treated animals was significantly ($p < 0.05$) greater than that in the control group (Fig. 2).[24]

The mechanisms by which bFGF restored the relaxant response to acetylcholine are not completely understood, but the normalized endothelium-dependent responses observed after bFGF treatment probably do not relate solely to endothelial regrowth. Previous studies[6] that demonstrated persistent abnormal endothelium-dependent responses in rabbit iliac arteries despite complete reendothelialization suggest that bFGF, in addition to its effect on endothelial cell growth, might also modulate some qualitative aspects of endothelial cells and restore normal physiologic responses to endothelium-dependent agonists.

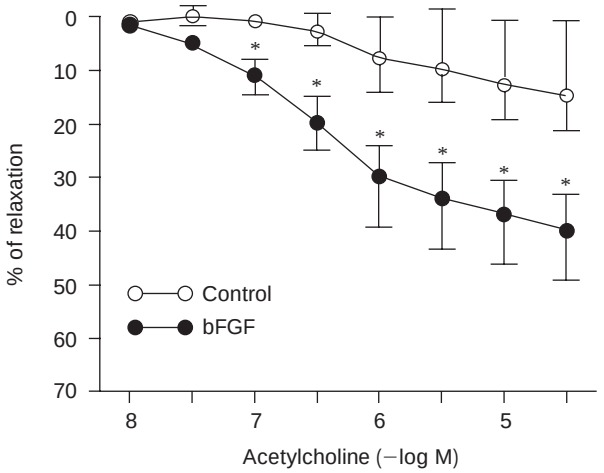

FIG. 2 Endothelium-dependent relaxation in response to acetylcholine in rabbit arteries denuded 4 weeks previously (animal model of balloon injury). The control group (top line) demonstrated almost no relaxation in response to acetylcholine, whereas the group that received bFGF showed significantly (* $p < 0.05$) increased relaxation in response to acetylcholine, indicating improvement in endothelial function. Reprinted from Ref. No. 24 with permission.

The effect of FGF administration on neointimal thickening after arterial injury is not clear. Lindner *et al.*,[26] using high doses of bFGF (12 µg/day for 2 weeks in a rat model), found an increase in neointimal thickening. By contrast, Bjornsson *et al.*,[25] using low doses of acidic FGF in the same model, observed an inhibition of neointimal thickening. Finally, in the study by Meurice *et al.*[24] discussed previously, a similar degree of neointimal thickening was observed in control and treated rabbits 4 weeks after injury. Taken together, these studies suggest that the final effect of FGF on neointimal thickening may be the consequence of a balance between stimulatory and inhibitory effects on smooth muscle cell growth. Experimental studies support the idea that certain functions of the endothelium, such as production of NO, are critical to the prevention of luminal narrowing by neointimal thickening;[27] accelerated reendothelialization may thus reduce neointimal formation. On the other hand, bFGF, as a potent growth factor for smooth muscle cells, may directly enhance neointimal formation. Variables such as the dose used, the duration of treatment, and the animal model studied may explain discrepancies among studies.

Vascular Endothelial Growth Factor

Asahara *et al.*[13] investigated the hypothesis that a single, direct application of VEGF to the intimal surface of a balloon-injured artery could accelerate reendothelialization. In this study, VEGF (100 µg) was given locally after balloon injury of the rat carotid artery. At 2 weeks and 4 weeks after injury, the extent of reendothelialization was markedly superior in the VEGF group compared with the control group (Fig. 3). It is interesting that neointimal thickening was correspondingly attenuated to a statistically significant degree ($p < 0.05$) in arteries treated with VEGF. In addition, histochemical analyses demonstrated a lower frequency of proliferating cells in the neointima of VEGF-treated animals. VEGF thus appears to be as potent as FGF in inducing endothelial regrowth; its specificity for endothelial cells may represent a potential advantage over FGF because the indirect inhibition of smooth muscle cell growth by the regenerated endothelium will not be antagonized by direct stimulation.

More recently, the effects of direct gene transfer of VEGF after angioplasty were investigated in a rabbit model.[28] In this study, New Zealand White rabbits underwent simultaneous balloon injury and gene transfer with phVEGF165, encoding the 165-amino acid isoform of VEGF. Gene expression was observed as early as 36 h post transfection and persisted through 2 weeks, before diminishing at 3 weeks. An increase in serum concentration of VEGF was observed 5 days after transfection. Planimetric analysis disclosed near-complete reendothelialization by 7 days among VEGF-transfected arteries, while the extent of reendothelialization in control arteries was < 50% complete at 7 days and remained nearly 20% incomplete at 4 weeks. A complete assessment of the consequences of reendothelialization showed that (1) treated arteries recovered near-normal endothelium-dependent responses within 1 week, whereas control arteries demonstrated persistently impaired endothelium-dependent responses at 4 weeks post injury; (2) VEGF-treated arteries had less neointimal thickening and, consequently, a greater luminal diameter on angiography than control arteries; and (3) thrombotic occlusion developed less frequently in animals transfected with phVEGF165 than in control animals.

Endothelial Dysfunction in Atherosclerotic Vessels

Diet-induced experimental atherosclerosis impairs endothelium-dependent relaxation in vitro and in vivo but has no effect on endothelium-independent relaxation.[3, 4] Several mechanisms may explain the abnormal endothelium-dependent responses in atherosclerotic vessels. Hypercholesterolemia induces extensive morphologic changes in the endothelial cell layer, including areas of deendothelialization that may reduce the total number of endothelial cells.[4] Alternatively, the thickened intima of atherosclerotic vessels could serve as a physical barrier to EDRF/NO. However, the fact that hypercholesterolemia can also impair endothelium-dependent relaxation in the absence of gross structural changes[29, 30] suggests that abnormal endothelium-dependent responses are more likely related either to reduced synthesis or enhanced destruction of EDRF/NO. This latter possibility is supported by studies showing that the EDRF/NO precursor L-arginine restores normal endothelial relaxation in hypercholesterolemic rabbits[31] and that excessive endothelial production of superoxide anion can inactivate NO in similar models.[32]

We recently tested the hypothesis that sustained administration of bFGF in hypercholesterolemic rabbits might restore

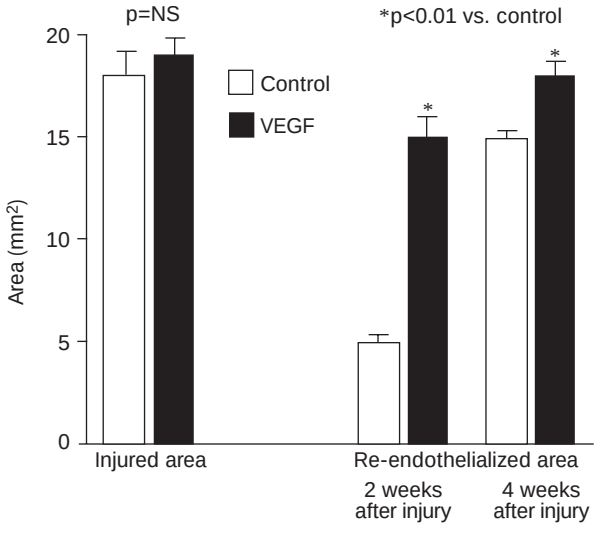

FIG. 3 Reendothelialization in rat carotid artery following balloon angioplasty. The group of rats that received VEGF showed a significantly greater degree of reendothelialization than did rats in the control group. Reprinted from Ref. No. 13 with permission.

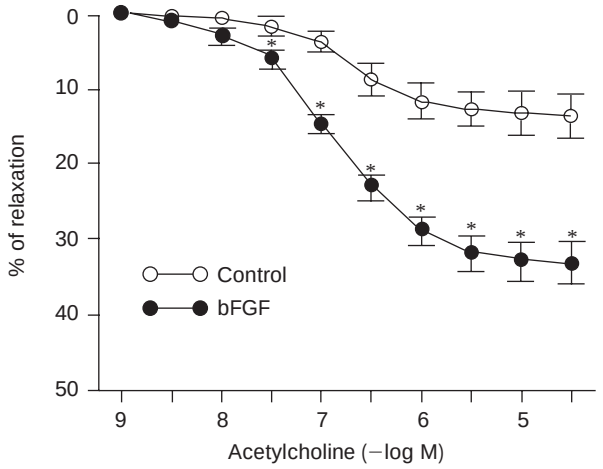

FIG. 4 Endothelium-dependent responses to acetylcholine in hypercholesterolemic rabbits treated either with placebo (control group) or twice-weekly intravenous boluses of bFGF. Hypercholesterolemic rabbits treated with bFGF had significantly (* $p < 0.05$) better endothelium-dependent relaxation than did control animals. Reprinted from Ref. No. 33 with permission.

normal physiologic responses to endothelium-dependent agonists.[33] After feeding on a 2% hypercholesterolemic diet for 6 weeks, the animals received twice-weekly intravenous boluses of either bFGF or placebo for 3 weeks and were sacrificed for assessment of in vitro vasoreactivity and for histologic analysis. Hypercholesterolemic rabbits treated with bFGF had significantly ($p < 0.05$) better endothelium-dependent responses than did untreated animals (Fig. 4). Endothelium-independent responses did not differ significantly between the two groups. A similar degree of plaque formation was observed in the two groups.

These results in the hypercholesterolemic rabbit are concordant with those of previous studies demonstrating that administration of endothelial cell growth factors may help to restore normal responses in other models of endothelial dysfunction. Although an anatomic effect on the endothelial cell cannot be excluded, our data suggest that administration of endothelial cell growth factors is also associated with functional changes at the endothelial level.

Conclusions

In vivo administration of endothelial cell growth factors leads to significant improvement in endothelium-dependent responses. This effect is observed with bFGF and VEGF in various animal models of endothelial dysfunction, such as the collateral circulation, the regenerated endothelium after arterial injury, and experimental atherosclerosis (Table II). While the precise mechanisms underlying this beneficial effect remain to be determined, the use of endothelial cell growth factors may represent a new therapeutic strategy for patients with vascular diseases.

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Clin. Cardiol. Vol. 20 (Suppl. II), II-58–II-64 (1997)

Potential Role of Angiotensin-Converting Enzyme Inhibition in Myocardial Ischemia and Current Clinical Trials

CARL J. PEPINE, M.D.

Division of Cardiovascular Medicine, University of Florida College of Medicine, Gainesville, Florida, USA

Summary: The understanding of the atherosclerotic disease process has broadened during the past few years to include the roles of the endothelium and of tissue angiotensin-converting enzyme (ACE) as regulators of a complex interaction of events that may lead to the development of atherosclerosis and, eventually, to the occurrence of clinical ischemia-related events. Several large clinical trials using ACE inhibitors have previously demonstrated a reduced risk of morbidity and mortality in patients with coronary artery disease and left ventricular dysfunction and in patients with heart failure or who had experienced an acute myocardial infarction. The effects of ACE inhibition are now being evaluated in other ongoing or recently completed trials in patients with evidence of coronary artery disease, but who have preserved left ventricular function and do not have an acute infarction. The results of these trials can be expected to enhance further our ability to intervene in the atherosclerotic process, resulting in improved outcomes in patients with coronary artery disease.

Key words: angiotensin-converting enzyme, clinical trials, endothelial dysfunction, myocardial ischemia, quinapril

Introduction

As knowledge of atherosclerotic disease has advanced during the past 15 years, research interest has increasingly focused on possible interventions to improve patient outcomes that go beyond lipid lowering. Experimental studies have greatly increased the understanding of the biologic processes that underlie the early stages of atherogenesis. The important roles played by the endothelium and by angiotensin-converting enzyme (ACE) in regulating these processes have become apparent, although the exact mechanisms are still being explored.

Address for reprints:

Dr. Carl J. Pepine
Division of Cardiovascular Medicine
University of Florida College of Medicine
1600 Archer Road
P.O. Box 100277
Gainesville, FL 32610, USA

The Atherosclerotic Disease Process

Figure 1 diagrams the hypothetical sequence of events leading to adverse outcomes in coronary artery disease (CAD). The process begins with the presence of factors such as genetic background, high levels of low-density lipoprotein (LDL) cholesterol, increased blood pressure, and environmental factors. These risk factors modify some of the normal processes in or near the endothelium (at least in part via oxidative stress), resulting in damage to the endothelium (i.e., endothelial dysfunction). This generalized endothelial dysfunction becomes localized in response to local factors, which may relate to shear turbulence, external twisting, low blood flow, and possibly other factors causing modifications in specific regions of the blood vessel. These local modifications occur in certain parts of the large and medium-sized arteries as opposed to small vessels; branch points and curves are particularly susceptible. This process ultimately contributes to the development of fatty streaks, which grow to raised intimal lesions at these locations in the vessel wall. Further growth leads to a recognized atherosclerotic plaque. The combined effects of time, the magnitude of endothelial injury, and local factors will lead eventually either to a clinical event or to clinically silent disease progression. If the duration and magnitude of various risk factors (and other factors) continue after an atherosclerotic plaque develops, the plaque may develop vulnerability to rupture or fissuring. If the fissure is small and remains localized to the superficial surface, and if local factors are favorable (e.g., little turbulence), the resultant thrombus may be quite small and eventually become incorporated into the plaque; this plaque will grow, further narrowing the blood vessel and causing silent progression or obstruction. In contrast, if the time and magnitude are greater and local factors

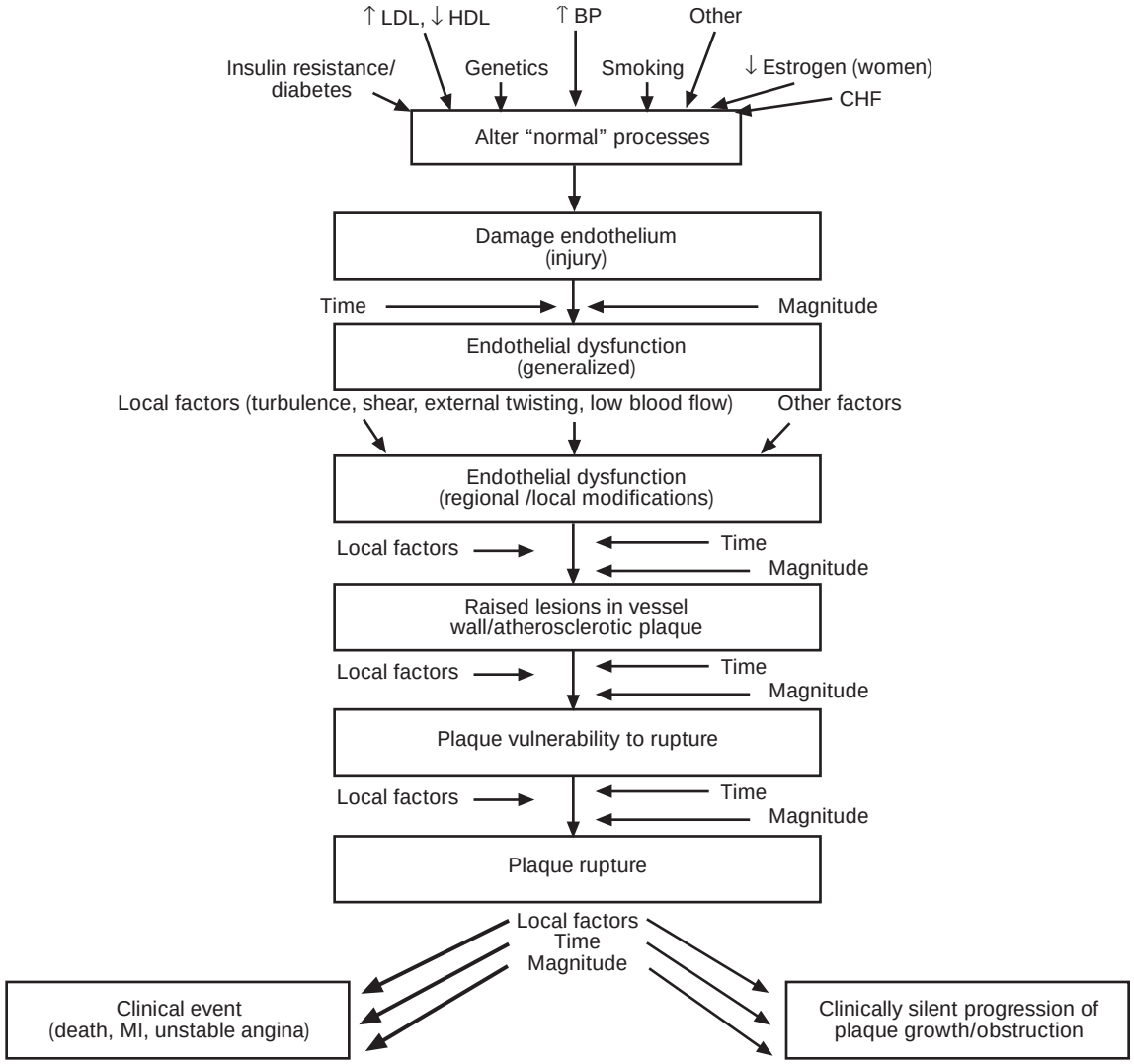

FIG. 1 Sequence of events leading to adverse outcomes in coronary artery disease. BP = blood pressure, CHF = congestive heart failure, HDL = high-density lipoprotein, LDL = low-density lipoprotein, MI = myocardial infarction.

are unfavorable (e.g., area of high turbulence, twisting of the vessel, etc.), the fissure may propagate as a dissection. The blood within the dissection will narrow the vessel, and/or the thrombus at the site of fissure/dissection may grow when local factors favor thrombosis (e.g., reduced tissue-type plasminogen activator, increased plasminogen activator inhibitor, increased platelet aggregability, etc.), causing an acute event. Clinical consequences of atherosclerosis may relate to the vulnerability of plaque to rupture, reduction of blood supply to parts of the body, or contracting myocardium. The resulting ischemic syndrome may be transient or persistent [e.g., transient ischemic attacks versus stroke, unstable angina versus acute myocardial infarction (MI)]. The clinical challenge for physicians is to try to identify patients at high risk for such events and then to modify endothelial dysfunction and factors related to this dysfunction so as to prevent the development of clinical events, such as MI, unstable angina, or death.

The Renin-Angiotensin System in Atherosclerosis

Experimental studies during the past several years have provided a wealth of data to support an important role for the renin-angiotensin system (RAS) and particularly ACE in the modulation of vasoconstrictive/relaxing factors involved in the development of atherosclerosis.[1] Figure 2 illustrates schematically the complex interactions of cellular mechanisms within the blood vessel wall that may contribute to the development of the foam cell, one of the earliest components of an atherosclerotic plaque.

The well-established antihypertensive effects of ACE inhibitors depend primarily on their ability to block the conversion of the relatively inactive peptide angiotensin I to angiotensin II, a potent vasoconstrictor. Angiotensin-converting enzyme is identical to kinase, the enzyme that degrades bradykinin, a potent stimulator of nitric oxide (NO) release.

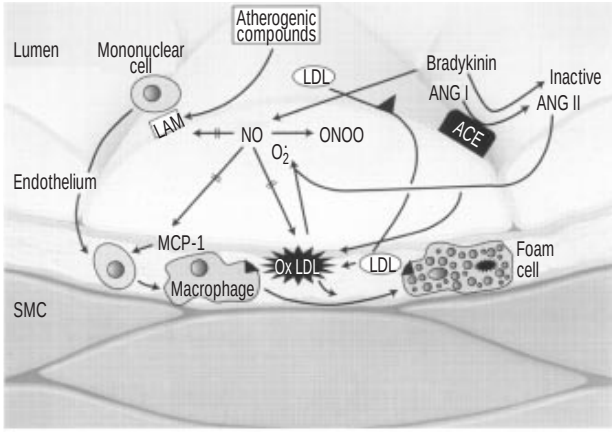

FIG. 2 Schematic illustration of the cellular processes leading to formation of a foam cell within the abluminal space. Nitric oxide (NO) apparently modulates the expression of vascular cell or leukocyte adhesion molecules (LAM) and/or chemotactic proteins such as monocyte chemotactic protein-1 (MCP-1). The vasoprotective role of NO may be inhibited by angiotensin-converting enzyme (ACE), which inactivates bradykinin. Angiotensin II (ANG II) seems to promote the oxidation of low-density lipoprotein (LDL), which is taken up by a macrophage to form the foam cell. ANG II and oxidized LDL (Ox LDL) may also act synergistically to increase the production of superoxide anion ($O^{\cdot}_2$), which inactivates NO and can directly cause contraction of smooth muscle cells (SMC). Figure courtesy of J. Turgeon, Quebec Heart Institute, 1996.

Nitric oxide (also called endothelium-derived relaxing factor) plays a crucial role in protecting the endothelium from injury. Data from experimental studies point toward many other cellular effects of angiotensin II, including stimulation of smooth muscle cell contraction, growth, proliferation, and migration; activation of monocytes and promotion of adhesion to endothelial cells; induction of endothelin formation; activation of the sympathetic nervous system; and effects acting to promote thrombosis and inhibit fibrinolysis.[2–8] Suppression of some or all of these various effects of angiotensin II may result in antiatherogenic benefits from ACE inhibitor treatment not related to blood pressure lowering. In addition, angiotensin II, by a mechanism that has not yet been fully elucidated, seems to increase the production of reactive oxygen species that either inactivate or bind NO, making it no longer available to perform its vasoprotective function (Fig. 2).[9]

A possible direct anti-ischemic effect of ACE inhibition is suggested by recent experimental studies by Zhang *et al.*[10] These investigators reported that administration of the ACE inhibitors captopril, enalaprilat, or ramiprilat to the coronary microvessels of mongrel dogs significantly increased production of nitrite (a metabolite of NO) and reduced myocardial oxygen consumption. Reductions ranged from 19% for captopril to 35% for ramiprilat. The greatest reduction in myocardial oxygen consumption occurred with ramiprilat, which had the highest affinity for tissue ACE of the three compounds studied. The authors concluded that the greatest inhibition of myocardial oxygen consumption observed with ACE inhibition was most likely secondary to stimulation of endothelial production of NO. The clinical relevance of these experimental findings in modulating the effects of cardiac ischemia may be clarified by results of the ongoing QUinapril Antiischemia and Symptoms of Angina Reduction (QUASAR) trial (see below).

Angiotensin-Converting Enzyme Inhibition in Patients with Left Ventricular Dysfunction

Beginning in the early 1990s, results of several large, controlled clinical trials were published demonstrating a benefit of ACE inhibition in patients with CAD and decreased left ventricular function or acute MI. These trials included the Studies of Left Ventricular Dysfunction (SOLVD),[11–14] the Survival and Ventricular Enlargement (SAVE) trial,[15] the Acute Infarction Ramipril Efficacy (AIRE) study,[16] the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico 3 (GISSI-3) trial,[17] the International Study of Infarct Survival 4 (ISIS-4),[18] the Survival of Myocardial Infarction Long-term Evaluation (SMILE) study,[19] the Trandolapril Cardiac Evaluation (TRACE) study,[20] and others. In SOLVD, patients (most of whom had CAD) with left ventricular ejection fractions <35%, either with overt heart failure (treatment trial) or with asymptomatic left ventricular dysfunction (prevention trial) were randomized to receive either enalapril or placebo and followed for an average of 40 months. Combined data from both study arms indicated that ACE inhibitor treatment resulted in a 23% reduction in the risk of MI and a 20% lower risk of hospitalization for unstable angina. The combined risk for any ischemia-related event (death, MI, or unstable angina) was reduced by approximately 20%. The SAVE trial enrolled patients who had experienced an MI within the previous 3 to 16 days and had an ejection fraction of <40% without overt heart failure or symptoms of myocardial ischemia. Treatment with captopril over an average follow-up of 42 months was associated with a 19% reduction in the risk of all-cause mortality, a 21% reduction in cardiac death, a 37% reduction in the development of severe heart failure, a 22% reduction in congestive heart failure requiring hospitalization, and a 25% reduction in the risk of recurrent MI. Similar trends toward reductions in ischemia-related clinical outcomes were reported in AIRE, GISSI-3, ISIS-4, SMILE, and TRACE. In general, the risk reductions reported in these studies were proportional to the duration of treatment with ACE inhibitors and to the initial risk levels of the populations enrolled.

Because the patient populations studied in these earlier trials had documented left ventricular dysfunction, it has been suggested that the improvements noted could have been due to the effects of ACE inhibition on vascular remodeling, rather than due to a direct effect on improving endothelial function.[1] The next generation of clinical trials was therefore designed to examine the question of whether the benefits of ACE inhibition could be extended to patients with CAD but with preserved or normal ventricular function. Positive results would indicate that ACE inhibition has direct vasculo-protec-

TABLE I Study designs of current clinical trials of angiotensin-converting enzyme inhibitor treatment in patients with coronary artery disease and preserved left ventricular function: Trials with clinical outcomes

Study	Design	ACE inhibitor	Follow-up (years)	Sample size	Patient age (years)	Inclusion criteria	Primary outcomes
HOPE	DB, R, PC	Ramipril (+vitamin E)	≤4	9,541	≥55	High risk for major CV event	CV disease events: MI, stroke, CV death
ALLHAT	DB, R, C	Lisinopril (± pravastatin)	6	4,230*	≥55	Hypertension, atherosclerotic CV disease, NIDDM, HDL <35 mg/dl, LVH, smoking	CV death, nonfatal MI; all-cause mortality
PEACE	DB, R, PC	Trandolapril	5	14,000	≥50	Documented CAD	CV death, MI

Acronyms for clinical trials are defined in the text. Other abbreviations: BP = blood pressure, C = comparative, CV = cardiovascular, DB = double-blind, HDL = high-density lipoprotein, LVH = left ventricular hypertrophy, NIDDM = non-insulin-dependent diabetes mellitus, PC = placebo-controlled, R = randomized. * Sample size given is for the lisinopril arm in each trial.

tive and cardioprotective effects that are not dependent on hemodynamic changes. The study designs of a number of these trials are summarized in Table I (trials with clinical outcomes) and Table II (trials using surrogate markers to measure endothelial function and/or the progression of atherosclerosis).[1, 21] Of these studies, the Trial on Reversing ENdothelial Dysfunction (TREND) is the first to be completed and published.[22]

TABLE II Study designs of current clinical trials of angiotensin-converting enzyme inhibitor treatment in patients with coronary artery disease and preserved left ventricular function: Trials with surrogate outcomes

Study	Design	ACE inhibitor	Follow-up (years)	Sample size	Patient age (years)	Inclusion criteria	Primary outcomes
TREND	DB, R, PC	Quinapril	0.5	105	18–75	PTCA within 72 h, normal BP or controlled hypertension, average cholesterol	Endothelium-dependent vasomotion assessed by Ach and QCA
QUASAR	DB, R, PC	Quinapril	0.5	400	>18	Exercise-induced and daily life ischemia	Ischemia on ambulatory ECG and exercise treadmill time
SECURE	DB, R, PC	Ramipril (+ vitamin E)	3–4	732	≥55	High risk of major CV event	B-mode ultrasound measures of carotid atherosclerosis
PART-2	DB, R, PC	Ramipril	4	600	18–75	Clinically important atherosclerotic disease <5 years	B-mode ultrasound measures of carotid atherosclerosis
SCAT	DB, R, PC	Enalapril (± simvastatin)	5	468	—	Documented CAD, total cholesterol 160–240 mg/dl	CAD progression by QCA
PROTECT	DB, R, C	Perindopril	2	800	35–65	Hypertension and ultrasonographically proven intima/media thickness ≥0.8 mm of the common carotid artery	Changes in intima/media thickness of the common carotid artery determined by ultrasound

Acronyms for clinical trials are defined in the text. Other abbreviations: Ach = acetylcholine, BP = blood pressure, C = comparative, CAD = coronary artery disease, DB = double-blind, ECG = electrocardiogram, PC = placebo-controlled, PTCA = percutaneous transluminal coronary angioplasty, QCA = quantitative coronary angiography, R = randomized.

Improved Endothelial Function in the TREND Trial

In the TREND study, which used the surrogate outcome of endothelial-dependent vasomotion in target coronary artery segments assessed by quantitative angiography following administration of acetylcholine, 129 patients with coronary atherosclerosis were randomized to receive either quinapril 40 mg or placebo once daily for 6 months after baseline assessment of documented coronary endothelial dysfunction.[22] Endothelial dysfunction was defined as no dilation (≥ 5% increase in lumen diameter) in response to acetylcholine in a target artery segment. Excluded were patients with severe dyslipidemia, uncontrolled hypertension, left ventricular dysfunction (< 40% ejection fraction), and insulin-dependent diabetes mellitus. Following the 6-month double-blind treatment phase, study drugs were discontinued for at least 72 h and coronary angiography with acetylcholine infusion was repeated using the same procedures used for the baseline measurements. The primary response variable was net change in target segment response to acetylcholine.[22]

Figure 3 compares the primary efficacy parameters in the quinapril treatment group versus the placebo group for the 105 patients who completed the study. At baseline, patients in the quinapril treatment group had a similar degree of acetylcholine-induced constriction in the target artery segments as did the placebo group. These responses had not changed after 6 months in the placebo group. However, in the quinapril group, significantly less constriction occurred (1.6 and 2.3%, respectively, for acetylcholine 10^{-6} and 10^{-4} mol/l) at 6 months compared with baseline (6.1% and 14.3%, respectively; $p < 0.014$). Expressed as net change from baseline, the quinapril group improved by 4.5 ± 3 and 12.1 ± 3% at each acetylcholine dose, whereas the responses in the placebo group did not change ($p < 0.002$).[22] Similar benefit in the response to acetylcholine was observed in the group receiving quinapril when all coronary segments (secondary response variable) were measured. The lack of a constrictor response to acetylcholine indicates that ACE inhibition with quinapril improved endothelial function. A logistic regression model was used to identify predictors of improvement in endothelial function. No clinical characteristics (e.g., age, gender, smoking history, blood pressure, serum cholesterol) were found to be associated with improvement in endothelial function; the only independent predictor of improvement was assignment to quinapril ($p = 0.022$).[22]

TREND thus represents the first randomized, double-blind, placebo-controlled clinical trial to provide a new pathophysiologic rationale for the use of ACE inhibitors in patients with CAD and without left ventricular dysfunction; that is, to attenuate endothelial dysfunction. The benefits of quinapril treatment in this study occurred in patients who showed no changes in lipid values and no reductions in blood pressure.

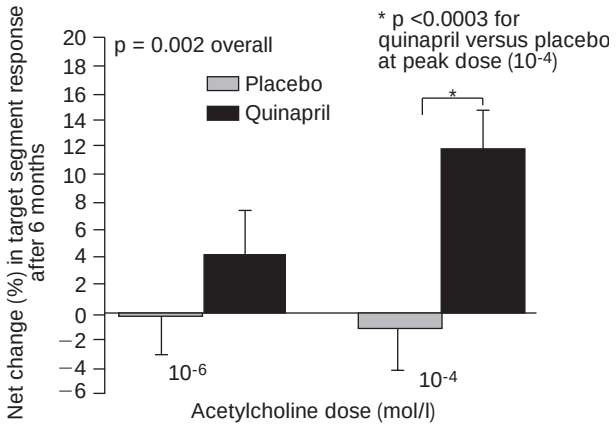

FIG. 3 Primary outcome results of the Trial on Reversing ENdothelial Dysfunction (TREND). After 6 months of treatment with quinapril, endothelial dysfunction had improved in the target segments of coronary arteries as demonstrated by vasodilator response to acetylcholine at doses of 10^{-6} and 10^{-4} mol/l. Patients who received placebo treatment continued to demonstrate a vasoconstrictive response to acetylcholine. The difference between quinapril treatment and placebo was significant ($p < 0.0003$) at the peak dose of acetylcholine (10^{-4} mol/l) and overall ($p = 0.002$). Reprinted from Ref. No. 22 with permission.

Other Trials

Trials with Clinical Outcomes

At least three large, ongoing trials are currently evaluating the effects of ACE inhibition on clinical outcomes related to CAD: death, MI, unstable angina, or the need for revascularization. Results of these trials are expected to provide important information on the potential clinical benefits of ACE inhibition on endothelial dysfunction.

Heart Outcomes Prevention Evaluation (HOPE): The HOPE trial[21,23] randomized more than 9,000 men and women to receive ramipril 10 mg/day and vitamin E 400 IU/day in a 2 × 2 factorial design. Vitamin E was chosen because of its antioxidant effects in preventing formation of oxidized LDL, a particularly atherogenic form of LDL. The primary outcomes of HOPE are listed in Table I; secondary outcomes include the number of hospitalizations for unstable angina, emergent revascularization procedures, development of congestive heart failure, cardiovascular mortality, total mortality, and nephropathy [for the subgroup of patients (36%) with diabetes]. Various substudies are planned, including one to assess left ventricular mass and function by two-dimensional quantitative echocardiography and arrhythmic activity by ambulatory electrocardiogram monitoring. Another substudy will assess risk factors for atherosclerosis using a nested case-control study design, and a third will assess renal function and microalbuminuria.

Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT): In the ALLHAT study,[24] two large, practice-based, randomized trials enrolled hypertensive patients at high risk for coronary disease. One study will compare the effects of antihypertensive therapy with one of four agents (chlorthalidone, amlodipine, doxazosin, and

lisinopril) on the combined incidence of fatal coronary heart disease and nonfatal MI. The second study randomized patients with hypertension and moderate elevations in cholesterol to receive a cholesterol-lowering diet and either a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (pravastatin) or usual care (plus one of the four antihypertensive agents) to determine whether pravastatin reduces all-cause mortality compared with usual care. The projected study population is 40,000 patients, half of whom will participate in the cholesterol-lowering trial, and enrollment will end January 30, 1998.

Prevention of Events with Angiotensin-Converting Enzyme Inhibition (PEACE): The PEACE trial,[21] begun in 1996, has the primary objective of determining whether addition of an ACE inhibitor (trandolapril, 4 mg/day) to standard therapy in patients with CAD and preserved left ventricular function will prevent cardiovascular mortality and MI. A secondary objective is to determine whether long-term ACE inhibition will reduce cardiovascular death and hospital admissions for primary cardiovascular complications such as MI, unstable angina, revascularization, cerebral vascular events, congestive heart failure, or arrhythmias.

Trials with Surrogate Outcomes

Surrogate measures of coronary or carotid atherosclerosis (e.g., endothelial dysfunction assessed by response to intracoronary acetylcholine challenge and B-mode ultrasound or progression of CAD as assessed by quantitative coronary angiography) are being evaluated in at least five ongoing clinical trials. These trials may provide further insights regarding the mechanisms by which ACE inhibitors exert their observed antiatherogenic effects.

Study to Evaluate Carotid Ultrasound Changes in Patients Treated with Ramipril and Vitamin E (SECURE): SECURE,[25] a substudy of the HOPE trial, uses the change in carotid arterial wall thickness measured by ultrasound to assess the effects of ramipril (10 and 2.5 mg/day) plus vitamin E (400 IU/day) on the rate of progression of carotid atherosclerosis in patients at high risk of cardiovascular events. Ultrasound measurements will be taken at baseline and yearly for 4 years. The primary outcome measure is the individual regression slope in the mean of maximal intimal-medial thickness measured over time, averaged from two preselected segments of the carotid artery. Secondary outcomes include measurement of the regression slope (in each patient) of the particular segment that is considered to contain the most hemodynamically significant lesion (most prone to rupture), and an evaluation of new plaque formation.

Prevention of Atherosclerotic Risk and Thrombosis (PART-2): The PART-2 trial[21] enrolled almost 500 patients with a history of coronary disease, transient cerebral ischemia, or peripheral vascular disease to receive ramipril 5 to 10 mg/day. The primary study outcome is assessment of whether ACE inhibitor treatment will slow the progression of atherosclerosis in carotid arteries and reduce left ventricular mass. B-mode ultrasound measurements of the wall thickness of the right and left common carotid arteries and M-mode ultrasound to measure left ventricular dimensions are being performed at baseline and at 2 and 4 years. Secondary study outcomes include assessment of clinical outcomes by monitoring hospital admissions, nonfatal cardiovascular and other clinical events, and all-cause mortality.

Simvastatin Coronary Atherosclerosis Trial (SCAT): Most patients with CAD have cholesterol levels either within or only slightly above normal limits. However, it is not clear whether cholesterol-lowering therapy will result in regression of atherosclerosis in these patients. The SCAT study[21] is evaluating whether dietary intervention plus enalapril with or without simvastatin will promote regression or reduce the progression of coronary atherosclerosis in patients with normal or mildly elevated cholesterol levels. Quantitative coronary angiography measurements performed at baseline and after 5 years will provide anatomic evidence of the extent of atherosclerosis. Functional measurement of disease, by mapping of body surface potential, will be performed at baseline, 2.5 years, and 5 years at one study center. Regular determinations of plasma lipid levels and clinical and laboratory evaluations are also being performed.

Perindopril Regression of Vascular Thickening European Community Trial (PROTECT): The PROTECT study[26] will compare the effects of perindopril and hydrochlorothiazide in slowing or reversing progression of increased intima/media thickness of carotid and femoral arteries in hypertensive patients. The study is being conducted at 17 clinical centers in seven European countries. A total of 800 patients with hypertension and ultrasonographically proven intima/media thickness $\geq$0.8 mm of the common carotid artery are being randomized to receive either the ACE inhibitor or the diuretic and are followed for 24 months. High-resolution duplex sonography will be used to quantify intima/media thickness at baseline and twice a year during follow-up.

Quinapril Antiischemia and Symptoms of Angina Reduction (QUASAR): The QUASAR trial is a multicenter, double-blind, placebo-controlled, parallel design study that will include approximately 450 patients with ischemic heart disease randomized to receive either quinapril or placebo. The full dosage range of quinapril—20 to 80 mg/day—will be explored. The response variables of interest include number and duration of ischemic episodes occurring during daily life determined by 48-h ambulatory electrocardiogram and the treadmill exercise time. The QUASAR trial, which has an expected completion date at the end of 1998, may provide clinical confirmation of an additional mechanism of cardioprotective benefit from quinapril (i.e., antiischemic).

Conclusion

Large clinical trials have shown that ACE inhibitors improve cardiovascular morbidity and mortality in patients with CAD and left ventricular dysfunction. Evidence from experimental studies suggests that, in addition to their antihypertensive effects, ACE inhibitors might have other an-

tiatherosclerotic and anti-ischemic effects. The TREND trial demonstrated that ACE inhibition with quinapril improved endothelial function in terms of a reduced vasoconstrictive response to acetylcholine. Other trials that are now under way can be expected to clarify further the mechanisms by which ACE inhibition might favorably affect the function and/or structure of endothelium, and QUASAR will provide data relating to possible direct anti-ischemic effects of ACE inhibition. Results of all these important trials may translate into changes in treatment patterns that could lead to improved outcomes among patients with CAD.

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