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The Vascular Biology of Hypertension and Atherosclerosis and Intervention with Calcium Antagonists and Angiotensin-Converting Enzyme Inhibitors

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Summary: Recent advances in the understanding of vascular disease genesis suggest that atherosclerosis and hypertension, primary targets of therapy in the INternational VErapamil SR/trandolapril STudy (INVEST), are closely related. A unified model for the development of cardiovascular disease (CVD) is emerging from recent advances related to atherosclerosis and hypertension. The process of vascular disease appears to begin early in life, when signs of endothelial dysfunction first appear. A primary cause of CVD progression is increased oxidative stress in the endothelium caused by multiple risk factor conditions, including heredity, dyslipidemia, smoking, diabetes, and elevated systolic blood pressure (SBP >110 mmHg). The renin-angiotensin and kallikrein-kinin systems are important regulators of blood pressure and atherosclerosis. In the renin-angiotensin system, angiotensin-converting enzyme (ACE) mediates generation of angiotensin II (ang II) at local vascular sites and in the plasma and also degrades bradykinin. Information derived from INVEST will help to identify treatment strategies, such as those containing a calcium antagonist and an ACE inhibitor, that are targeted directly at the vascular disorder responsible for hypertension and atherosclerosis.

Introduction

Recent advances related to the genesis of vascular disease suggest that atherosclerosis and hypertension, primary targets of therapy in the INternational VErapamil SR/trandolapril STudy (INVEST), are closely related. These disorders share similar risk factor conditions and appear to modify vascular function and structure correspondingly. Based on these findings, a unified model for the development of cardiovascular disease (CVD) is emerging. This article will summarize the vascular biology of atherosclerosis and hypertension in the context of a unified model for CVD that is useful for the clinician. In addition, this paper will discuss therapeutic intervention with calcium antagonists and angiotensin-converting enzyme (ACE) inhibitors, focusing on the influence of intervention on vascular function and structure. Some major advances in the area of CVD will be included.

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Unifying Model for Cardiovascular Disease

During the development and progression of CVD, a number of risk factor conditions involved with both atherosclerosis and hypertension interact to increase oxidative stress in the vascular wall. These conditions include heredity, dyslipidemia, smoking, diabetes, and elevated systolic blood pressure (SBP ≥110 mmHg) (Fig. 1). The principal site of this increased oxidative stress appears to be the endothelium, the site of nitric oxide (NO) inactivation. In healthy individuals, reactive oxygen species (ROS) and their derivatives are produced and detoxified by antioxidant mechanisms. In the endothelium and other vascular cells, sources of ROS include the xanthine oxidase system, the NADH/NADPH oxidase system, and the endothelial NO synthase (eNOS) system.[1] As oxidative stress increases, CVD develops because the endogenous antioxidant defense systems are overloaded. Emerging evidence suggests that stem cells derived from bone marrow, early precursors of mononuclear cells, may be programmed to interact with ROS within the disordered endothelium. This process may result in sustained functional alterations.

The principal functional alteration is a decrease in bioavailable NO within the arterial wall, principally at the level of the endothelium. When this decrease in bioavailable NO becomes critical, normal endothelial function is altered and endothelial dysfunction results. These functional alterations may include abnormal vascular smooth muscle tone (reduced relaxation and/or increased constriction), vascular smooth muscle growth, abnormalities in blood coagulation (increased activation of platelets and coagulation), and fibrinolysis (reduced release of t-PA and uPA), as well as enhanced inflammation. These

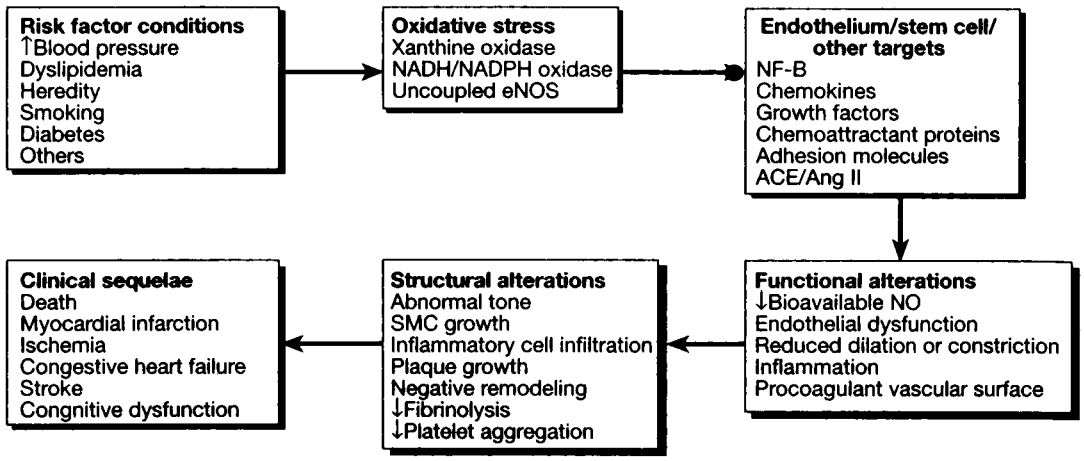

FIG. 1 The progression of cardiovascular disease. ACE = angiotensin converting enzyme, Ang II = angiotensin II, eNOS = endothelial nitric oxide synthase, SMC = smooth muscle cell, NF-B = nuclear factor Kappa B.

functional alterations lead to structural changes in the vessel (e.g., smooth muscle hypertrophy, adverse remodeling, plaque rupture, thrombus formation, and occlusion). In some cases, these functional alterations may result in clinical sequelae, such as death, myocardial infarction, stroke, ischemia, or congestive heart failure.

Renin-Angiotensin and Kallikrein-Kinin Systems

The renin-angiotensin and kallikrein-kinin systems are important regulators of blood pressure and atherosclerosis. Figure 2 shows these systems in detail. Angiotensinogen is abundant in many tissues and the circulating blood. Renin causes angiotensinogen to convert into angiotensin I (ang I) in plasma and tissue. Angiotensin-converting enzyme mediates the generation of angiotensin II (ang II) from angiotensinogen. Ang II can interact with a number of cloned AT receptors. If ang II interacts with the ang I receptor, as stated previously, vasoconstriction results. Other adverse cellular reactions include production of endothelin and superoxide, retention of sodium and water, and cell proliferation.

An angiotensin metabolite, angiotensin-(1-7), has recently been shown to exist in concentrations that are roughly equiva-

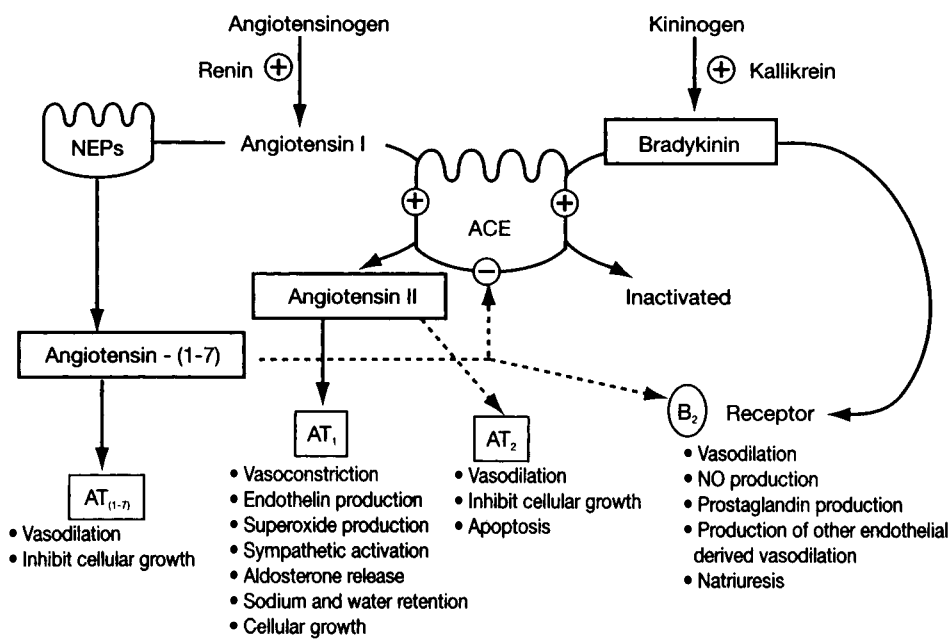

FIG. 2 The renin-angiotensin and kallikrein-kinin systems. ACE = angiotensin converting enzyme, AT_1 = angiotensin II type 1, AT(1-7) = angiotensin 1-7, AT_2 = angiotensin II type 2, NEPs = neutral endopeptidase inhibitors, NO = nitric oxide.

lent to ang II in the plasma and in tissue.[2] The antihypertensive effects of angiotensin-(1-7) suggest that it may counter regulate ang II.[3] The interaction of angiotensin-(1-7) with its receptor site results in vasodilation and growth inhibition.

Considerable evidence suggests that ACE has a greater affinity for bradykinin than for ang I.[4,5] Bradykinin interacts with its beta 2 receptor at the endothelial cell level to cause intense vasodilation and to inhibit cell proliferation. Increasing evidence suggests that bradykinin peptides that are not under the influence of ACE are available to act at the bradykinin I receptor. Therefore, these peptides also cause vasodilation and inhibit cell proliferation. Increasingly, evidence suggests that the angiotensin-renin and kallikrein-kinin systems have crucial roles in the vascular regulation of atherosclerosis and hypertension.

Angiotensin II affects blood vessel tone principally by increasing the concentration of calcium ions available for vascular smooth muscle contraction, resulting in vasoconstriction, and possibly hypertension. It also stimulates cell growth and proliferation by increasing the expression of oncogenes, which increase the number of growth factors. Angiotensin II also promotes oxidation in macrophages and endothelial cells and increases vascular permeability. Each of these reactions has a deleterious effect on blood vessels.

Local Biosynthesis of Angiotensin II

The biosynthesis of ang II from ang I occurs at local vascular sites, tissues, and in the plasma. The microcirculation present in the wall of larger blood vessels and the infiltration of mononuclear cells in the form of macrophages result in the concentration of ang II in vascular sites. Dzau recently hypothesized that ang II provides a positive feedback mechanism to allow these processes to self-perpetuate.[6] For example, increased tissue ang II results in more oxidative stress. Promotion of cytokines, adhesion molecules, smooth muscle tone, growth factors, and vascular inflammation results, causing an infiltration of granulocytes, cathepsin G, mast cells, and monocytes. Ultimately, one result is the increased tissue expression of ang II through upregulation of ACE. This mechanism may account for the perpetuation of vascular disease.

Recent data from a rat model have suggested that many, if not all, components of the renin-angiotensin system (e.g., ACE, angiotensin type 1 and type 2 [AT_1 and AT_2] receptors, and angiotensinogen) appear to be contained in the mononuclear cells and megakaryocytes of bone marrow. Renin, too, has been identified in these megakaryocytes. In addition, megakaryocytes in bone marrow stain richly for angiotensin type I receptors. These receptors activate the expression of adhesion molecules, and therefore the precursors of circulating macrophages, as well as endothelial progenitor cells, are programmed to bind with certain sites in the blood vessel wall. Several experiments suggest that such mononuclear cells in the bone marrow are programmed relative to the upregulation of ACE in the same way that endothelial cells are programmed.

This suggests that abnormal precursor cells may be present in the bone marrow compartment. These precursor cells are programmed very early in life and activated to bind with selected sites of the blood vessel wall. This mechanism, along with other mechanisms previously described, may be responsible for the promotion of vascular disease early in life.

When Does Vascular Disease Begin?

It is now clear that endothelial dysfunction is present early in life. It has been identified in children of patients who have hypertension and in children of households where there is active smoking. Traditional thinking is that foam cells and fatty streaks develop in childhood and result in intermediate type lesions. These lesions then increase in volume with the uptake of oxidized low-density lipoprotein (LDL) in the first three to four decades of a person's life. If large enough to disrupt organ blood flow, these atheroma then have the potential to rupture and produce plaque-related coronary events or cerebrovascular events; if not, they heal and become incorporated into the vessel wall as fibrous plaque.

The question regarding when this process develops recently received considerable attention based upon examination of aortae from aborted fetuses.[7] These aortae reveal considerable macrophage infiltration in the intima—early structural evidence of atherosclerosis. Special histochemistry has also shown oxidation products, revealing that these macrophages are in the earliest stages of atherosclerosis development. Evidence to date suggests that while fetal programming occurs relative to early atherosclerosis, there may also be some early regression in some sites once the child is born. The atherosclerosis process, however, redevelops at sites that may be programmed in utero. Therefore, between the ages of 3 and 10 years, atherosclerosis may be fully developed, with complicated plaque resulting between the ages of 10 and 30 years. Based upon these observations, intervention should occur during early life.

Development of Hypertension

In hypertension, current evidence suggests that endothelial dysfunction is present in early childhood. Panza *et al.* (Fig. 3A) showed that hypertensive adults had reduced blood flow augmentation in response to ascending doses of acetylcholine when compared with normotensive adults, providing evidence of endothelial dysfunction.[8] Children exhibited the same pattern in a study by Taddei *et al.* (Fig. 3B).[9] Children who did not have elevated blood pressure but had a family history of hypertension had a disordered response to acetylcholine early in life when compared with children who had no family history of hypertension. This evidence suggests that blood vessels of genetically susceptible patients may be programmed early in life to develop hypertension.

In addition, Nickenig *et al.* demonstrated the interaction within blood vessels between oxidized lipid (principally oxi-

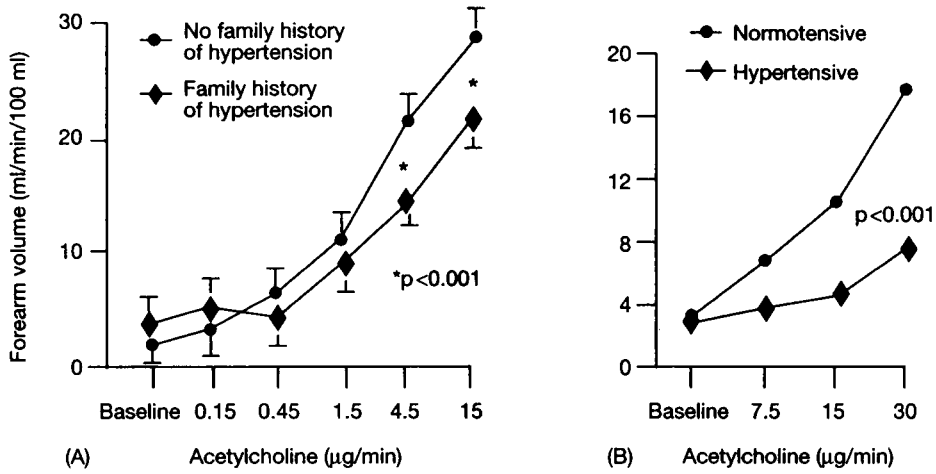

FIG. 3 The blood flow augmentation response of children with a family history of hypertension to elevated levels of acetylcholine (A) appears to mimic the response of adults with established hypertension (B). This suggests that the development of hypertension may begin at an early age. Figures reprinted from Refs. No. 8 and 9 with permission.

dized LDL) and angiotensin.[10] Patients with normal levels of cholesterol (181 ± 11 mg/dl) and patients with hypercholesterolemia (294 ± 10 mg/dl) received increasing doses of ang II, infused intravenously. Hypercholesterolemic patients had a significant increase in SBP when compared with patients with normal levels of cholesterol. When patients with hypercholesterolemia were treated with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) to reduce their cholesterol levels to normal levels and tested again, SBP responses to the same doses of ang II were normalized. The concentrations of ang II used in these studies were physiologic.

In addition, Nickenig *et al.* showed that platelet expression of the ang II type 1 (AT_1) receptor was upregulated and directly correlated to the plasma level of oxidized LDL.[10] Several studies have shown that platelet expression of the AT_1 and AT_2 receptor can be blocked with either ACE inhibitors, AT_1 receptor blockers, or statins.[11] In addition, studies have shown that platelet AT_1 receptor upregulation parallels vascular smooth muscle reactivity. Therefore, oxidized LDL appears to enhance the activity of ang II to increase blood pressure principally through enhanced vascular smooth muscle activation.

Therapeutic Strategies

The endothelium is shown in more detail in Figure 4 to show the role these processes play relative to therapeutic strategies. Regarding eNOS, multiple receptors on the surface of the endothelial cell may act to increase the availability of NO from the amino acid, L-arginine by upregulating eNOS. Nitric oxide is freely diffusible both to the abluminal surface and the luminal surface of the vessel wall. At the abluminal surface, it diffuses across the intimal space to the vascular smooth muscle cells and macrophages residing within the subintimal region. There NO interacts with G-proteins, resulting in relaxation of the vascular smooth muscle and inhibition of mononuclear cell processes.

Sufficient NO may be produced even in disease states, but it is not released in a bioactive form, largely because there is an increased generation of oxygen free radicals. This oxidative

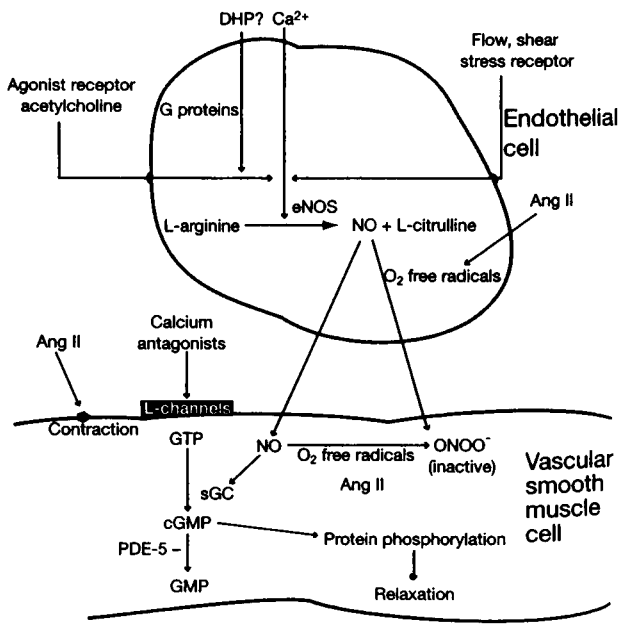

FIG. 4 Dihydropyridine induces vasorelaxation at the level of the endothelial cell via enhancement of endothelial nitric oxide release and at the level of vascular smooth muscle via inhibition of L-type calcium channels. Ang II = angiotensin II, cGMP = cyclic guanosine monophosphate, DHP = dihydropyridine, eNOS = endothelial nitric oxide synthase, GTP = guanosine triphosphate, ONOO- = peroxinitrate, PDE-5 = phosphodiesterase type 5, sGC = soluble guanylyl cyclase.

stress inactivates the NO, thus preventing it from participating in G-protein-coupled reactions. Treatment strategies that increase NO availability, either through production of more NO or reduction of ROS, are likely to have beneficial effects. One strategy is to provide more L-arginine to increase the activity of eNOS. Another is to use an antioxidant to reduce the amount of ROS. Phenolic compounds such as most calcium-channel antagonists (e.g., verapamil) are antioxidants.

Treatment strategies that influence vascular smooth muscle tone may also play a role. Calcium channels on the surface of vascular smooth muscle cells, including L-type, M-type, and others, facilitate calcium movement across the sarcolemma membrane and promote contraction. All of the calcium-channel antagonists block these calcium channels to different degrees. Verapamil, the agent studied in INVEST, has activity at both L channels and M channels. A proposed dihydropyridine calcium receptor at the endothelial cell surface may account for some of the differences reported in outcome studies using short-acting, rapid-release dihydropyridine calcium antagonists, compared with the longer-acting, slow-release, nondihydropyridine calcium antagonists, such as verapamil SR and diltiazem SR.

The angiotensin-renin system (ACE, ang II, and bradykinin) may promote both atherosclerosis and hypertension. Evidence derived from human carotid plaque and human coronary plaque removed during atherectomy has identified an abundance of ACE in plaque, particularly at the shoulders of plaque.[12] These sites may participate in plaque rupture. While ACE is present throughout the plaque and deep in the blood vessel wall, it is highly concentrated in these shoulder areas.

Immunohistochemistry has identified ACE within the macrophages of these plaques. Angiotensin-converting enzyme is responsible for the generation of ang II and the degradation of bradykinin locally. It is upregulated in atherosclerosis and hypertension. Abundant ang II is available locally at the vascular wall, while bradykinin, a potent generator of endothelial-derived vasodilators [NO, endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin (PGI-2)], is reduced. Angiotensin II is believed to be responsible for increasing oxidative stress to inactivate NO. In addition, ang II interacts with the AT_1 receptor and facilitates movement of calcium ions through channels to enhance contraction and increase inflammation. Based upon recent investigations of the involvement of ang II in vascular disease, it would seem prudent to interrupt angiotensin activity and prevent bradykinin breakdown. Therefore, an ACE inhibitor (e.g., trandolapril) would seem prudent as a therapy for atherosclerosis and hypertension. INVEST is testing this hypothesis.

Conclusion

Atherosclerosis and hypertension are the primary targets of treatment in INVEST. Recent advances suggest that these conditions commence very early in human development. New understandings of when vascular disease begins and how it progresses will lead to improved therapeutic strategies.

References

1. Hua C, Harrison DG: Endothelial dysfunction and cardiovascular disease: The role of oxidant stress. *Circ Res* 2000;87:840–844
2. Ferrario CM, Martell N, Yunis C, Flack JM, Chappell MC, Brosnihan KB, Dean RH, Fernandez A, Novikov SV, Pinillas C, Luque M: Characterization of angiotensin-(1-7) in the urine of normal and essential hypertensive subjects. *Am J Hypertens* 1998;11:137–146
3. Iyer SN, Ferrario CM, Chappell MC: Angiotensin-(1-7) contributes to the antihypertensive effects of blockade of the renin-angiotensin system. *Hypertension* 1998;31:356–361
4. Krebs LT, Hanesworth JM, Sardinia MF, Speth RC, Wright JW, Harding JW: A novel angiotensin analog with subnanomolar affinity for angiotensin-converting enzyme. *J Pharmacol Exp Ther* 2000;293:260–267
5. Meng QC, Oparil S: Purification and assay methods for angiotensin-converting enzyme. J *Chromatogr A* 1996;743:105–122
6. Dzau VJ: Theodore Cooper Lecture: Tissue angiotensin and pathobiology of vascular disease: A unifying hypothesis. *Hypertension* 2001;37:1047–1052
7. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, Palinski W: Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest* 1997;100:2680–2690
8. Panza JA, Quyyumi AA, Callahan TS, Epstein SE: Effect of antihypertensive treatment on endothelium-dependent vascular relaxation in patients with essential hypertension. *J Am Coll Cardiol* 1993;21:1145–1151
9. Taddei S, Virdis A, Mattei P, Arzilli F, Salvetti A: Endothelium-dependent forearm vasodilation is reduced in normotensive subjects with familial history of hypertension. *J Cardiovasc Pharmacol* 1992;20:S193–S195
10. Nickenig G, Baumer AT, Temur Y, Kebben D, Jockenhovel F, Bohm M: Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation* 1999;100: 2131–2134
11. Wassmann S, Laufs U, Baumer AT, Muller K, Ahlbory K, Linz W, Itter G, Rosen R, Bohm M, Nickenig G: HMG-CoA reductase inhibitors improve endothelial dysfunction in normocholesterolemic hypertension via reduced production of reactive oxygen species. *Hypertension* 2001;37:1450–1457
12. Fukuhara M, Geary RL, Diz DI, Gallagher PE, Wilson JA, Glazier SS, Dean RH, Ferrario CM: Angiotensin-converting enzyme expression in human carotid artery atherosclerosis. *Hypertension* 2000;35:353–359