Combined use of calcium channel blockers and inhibitors of the renin–angiotensin system for treating hypertension

Reduced cardiovascular events and mortality have been reported for hypertensive subjects treated with dihydropyridine-type calcium channel blockers (CCBs) and inhibitors of the renin–angiotensin system (RAS) in clinical trials. Recent evidence suggests that these agents may have vascular benefits that cannot be attributed to the reduction of blood pressure alone. Dihydropyridine-type CCBs and RAS blockers have been shown to improve endothelial activity while reducing inflammation. These changes in vascular activity have been confirmed by pulse wave analyses, which show a reduced impact of pressure wave reflections on central systolic blood pressure. In this article, we examine the separate and combined effects of CCB and RAS inhibition in reducing cardiovascular risk through enhanced vascular function.

KEYWORDS: aliskiren ■ angiotensin ■ calcium channel blockers ■ endothelial dysfunction ■ hypertension ■ inflammation ■ oxidative stress ■ renin

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A number of structural and functional mechanisms have been identified in the pathogenesis of hypertensive vascular disease, including endothelial dysfunction, oxidative stress and inflammation. The vascular endothelium controls vasomotor tone through the release of signaling molecules such as nitric oxide (NO). Hypertension, together with other cardiovascular risk factors, impairs the endothelium and its responsiveness to normal stimuli. In addition, the mechanical forces inherent in hypertension activate compensatory neurohormonal mechanisms, including the renin–angiotensin system (RAS), which negatively impact the structure of the vascular wall. Antihypertensive drugs may have class-specific hemodynamic and endothelial effects that mitigate these disease processes when used separately or in specific combinations. In this article, we focus on dihydropyridine (DHP)-type calcium channel blockers (CCBs) and RAS inhibitors as a combination treatment for hypertension; however, there are other pharmacologic approaches to blood pressure (BP) reduction that are supported by outcome studies. As with any therapeutic approach, there are studies that may provide both positive and negative findings for any particular agent, depending on various design aspects such as patient characteristics and predefined end points.

Pharmacological approaches that reduce central aortic pressure and vascular resistance associated with hypertension have also been shown to reduce clinical events [1]. These agents may improve vasodilation through enhanced NO bioavailability by either increasing endogenous production through enzymatic mechanisms or by stimulating direct release by its redox congeners in a spontaneous fashion. While all antihypertensive medications lower BP, certain drug classes have pleiotropic effects that may contribute to cardiovascular risk reduction. The relative importance of BP reduction versus the mechanisms associated with vascular protection remains a subject of considerable debate [2]. The primary purpose of antihypertensive therapy is BP reduction, which continues to be the accepted basis for their clinical benefit [3]. Developing a better understanding of the mechanisms underlying hypertensive vascular disease, as well as the pleiotropic actions of antihypertensive agents, offers the potential for more targeted therapy that reduces global cardiovascular risk.

Measurements of endothelial function include changes in central circulation indices of pressure. Indeed, measures of central arterial pressure to vital organs are powerful predictors of cardiovascular events and are closely associated with vascular function [4,5]. Central aortic pressure is determined by cardiac output and peripheral vascular resistance – factors that, along with arterial stiffness, determine the timing and magnitude of pressure wave reflections. A portion of each stroke volume is delivered distally during systole, with the remainder delivered by elastic recoil of the aorta during diastole in a manner influenced by the relative elasticity of the central arteries [5]. Increases in central arterial stiffness result in the delivery of greater portions of each stroke volume during systole. The velocities of both forward and reflected waves increase, thus the reflected

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wave returns earlier to the aorta and raises central aortic pressure. Central aortic stiffness contributes directly to the generation of a wide pulse pressure with higher systolic BP (SBP) and lower diastolic BP. Antihypertensive agents produce different effects on central aortic pressure and hemodynamics despite similar effects on BP measured over the brachial artery, as evidenced by the results of recent clinical trials.

Hypertension & endothelial dysfunction

Endothelial dysfunction, a hallmark feature of hypertension, is characterized by attenuated NO bioavailability, resulting in elevated vascular resistance and reduced sensitivity to normal stimuli of vasodilation, such as shear stress and acetylcholine. This abnormality is an early event in atherogenesis and is causally related to enhanced oxidative stress. Aging, vascular injury, metabolic disorders and deficiencies in essential substrate (L-arginine) and enzyme cofactors (e.g., tetrahydrobiopterin) also contribute to reduced NO bioavailability [6,7]. In the kidney, NO induces renal arterial vasodilation, inhibits sodium reabsorption and reduces mesangial cell proliferation and extracellular matrix synthesis in response to injury [8,9]. Under disease conditions, excessive superoxide (O_2^-) generation reduces NO bioavailability in glomerular endothelial cells to form a toxic product known as peroxynitrite ($ONOO^-$) [9].

Nitric oxide is generated from the conversion of L-arginine to L-citrulline by endothelial nitric oxide synthase (eNOS), which requires calcium/calmodulin, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobiopterin (BH_4) as cofactors. Under conditions of cardiovascular risk, such as hyperlipidemia and hypertension, there is abnormal eNOS activity despite normal or even enhanced expression of the enzyme. Indeed, overexpression of eNOS in apoE knockout mice caused significantly larger atherosclerotic lesions as compared with control mice in a manner that was ameliorated with BH_4 supplementation [10]. Under conditions in which vascular tissue levels of BH_4 are deficient or lacking, eNOS becomes dysfunctional. This results in the enzymatic reduction of molecular oxygen by eNOS in a manner that is no longer coupled to L-arginine oxidation, resulting in the generation of O_2^- rather than NO [11,12]. This phenomenon is referred to as 'eNOS uncoupling'. Oxidation of BH_4 by reactive oxygen species (ROS) such as O_2^-, which is derived from NAD(P)H oxidase, also leads to eNOS uncoupling [11].

The eNOS enzyme has multiple functional regions, including a reductase domain that contains binding sites for FAD, FMN and NAD(P)H, and that is linked by a calcium/calmodulin binding site to an oxygenase domain that contains the binding sites for heme, BH_4 and L-arginine. In the presence of calcium, calmodulin and adequate L-arginine levels, electrons are transferred from NAD(P)H to the FAD and FMN redox carriers in the reductase domain to the heme moiety in the oxygenase domain, where they ultimately oxidize the guanidino group of L-arginine to liberate NO and L-citrulline. The BH_4 cofactor functions as both an allosteric and redox cofactor for eNOS. As a result, the presence of BH_4 improves the binding affinity of eNOS for L-arginine and the heme ligand. Reduced levels of BH_4 lead to eNOS uncoupling and disruption of its homodimeric configuration. In the uncoupled state, eNOS does not produce adequate levels of NO but instead generates O_2^- from molecular oxygen, which in turn reacts rapidly with available NO to form $ONOO^-$ (Figure 1). Generally, reduced NO bioavailability is the result of either reduced eNOS activity or increased eNOS uncoupling with O_2^- production and $ONOO^-$ formation.

In spontaneously hypertensive rats, for example, a pronounced loss in NO bioavailability was observed despite an increase in levels of eNOS as compared with normotensive animals [13]. This effect was even more pronounced in these animals following the induction of diabetes, as measured in both arterial and glomerular endothelial cells [9]. The basis for this paradoxical loss of NO despite increased enzyme levels is the production of O_2^- by uncoupled eNOS. A similar change in eNOS expression, as well as an increase in O_2^- and $ONOO^-$ generation, was observed in normal rats that were made hypertensive by surgical procedures such as aortic banding [14]. Glucose intolerance, as occurs in diabetes, is also believed to impair eNOS activity through enhanced oxidative stress.

Rates of hypertension are disproportionately higher in African–Americans, which leads to increased rates of cardiovascular disease as compared with other populations [15]. Under controlled experimental conditions, endothelial cells from healthy African–American donors were shown to release lower levels of NO than matched cells from Caucasians despite having higher levels of eNOS [16,17]. This is consistent with the differences observed in endothelial-dependent vasodilation in African–American subjects as

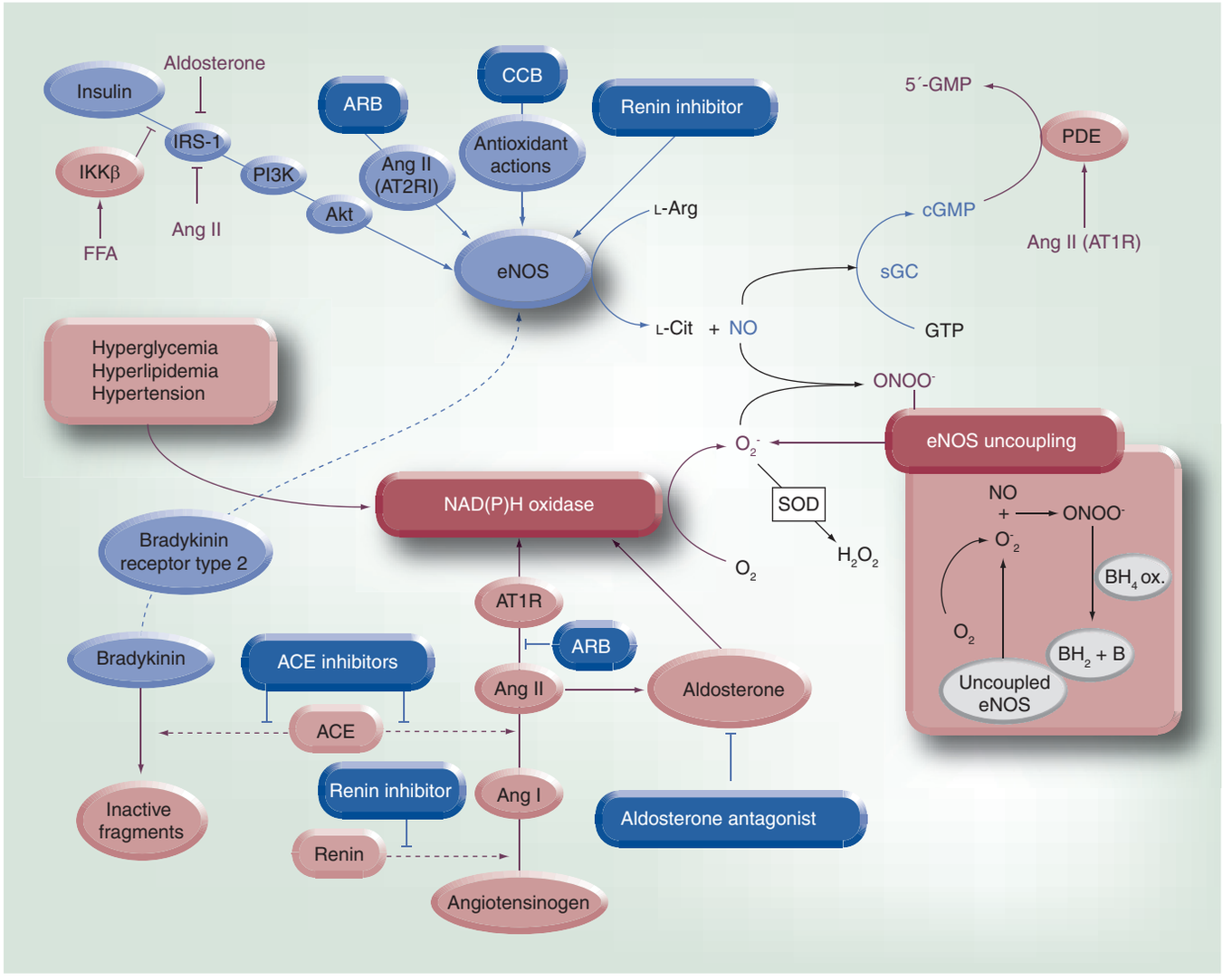

Figure 1. Conceptual rationale for the combined use of calcium channel blocker and renin–angiotensin system inhibitors based on pathophysiologic processes associated with endothelial nitric oxide synthase uncoupling, abnormal calcium influx, renin, aldosterone and angiotensin II.

ACE: Angiotensin-converting enzyme; Ang: Angiotensin; ARB: Angiotensin receptor blocker; CCB: Calcium channel blocker; eNOS: Endothelial nitric oxide synthase; FFA: Free fatty acid; ox.: Oxidation; PDE: Phosphodiesterase; SOD: Superoxide dismutase.

compared with matched Caucasians [18]. At the cellular level, loss of NO bioavailability is attributed to excessive O_2^- and $ONOO^-$ generation by NAD(P)H oxidase, which ultimately leads to uncoupled eNOS activity [16]. A hallmark feature of endothelial dysfunction in hypertension is the production of excessive ROS that react with available NO to form $ONOO^-$. By inhibiting NAD(P)H oxidase with apocynin, levels of NO bioavailability were enhanced in cells from African–Americans concomitant with reductions in $ONOO^-$ release [16]. Indeed, agents that reverse eNOS uncoupling and reduce oxidative stress have been shown to lower BP while improving NO bioavailability [9,19].

Role of the RAS in vascular disease

The RAS plays a central role in the control of vasomotor activity as well as volume and electrolyte levels. In vascular disease, activation of this complex system promotes arterial remodeling, oxidative stress and inflammation, further contributing to progressive vascular and renal dysfunction [20]. The binding of angiotensin II (Ang II) to angiotensin II type 1 (AT_1) receptors on vascular smooth muscle cells promotes vasoconstriction and leads to increased peripheral resistance. The interaction of Ang II with AT_1 receptors also facilitates sympathetic neurotransmission and stimulates aldosterone secretion from the adrenal cortex, which regulates renal function and blood volume by increasing

sodium and water reabsorption in the proximal tubules [21]. Aldosterone contributes to hypertension through enhanced restriction of renal afferent arterioles in a manner that can be blocked by enzymatic inhibitors of PKC- or IP_3-induced calcium release [22]. Thus, the RAS promotes vasoconstriction by mobilizing intracellular calcium stores, following the release of aldosterone and its interactions with G-protein-coupled receptors [22].

The interaction of Ang II with AT_1 receptors on endothelial and vascular smooth muscle cells activates signal transduction mechanisms that promote oxidative stress, inflammation, cell proliferation and fibrosis [20,23]. In particular, Ang II activates NAD(P)H oxidase in endothelial and vascular smooth muscle cells, which increases the production of O_2^- and other ROS (Figure 1) [23]. Ang II also increases the activity of the proinflammatory transcription factor, NF-κB, thereby enhancing expression of inflammatory cytokines from the vessel wall. Finally, Ang II stimulates growth factors, extracellular proteins and matrix metalloproteinases, thereby promoting proliferative and fibrotic mechanisms involved in vascular remodeling [21]. By contrast, the interaction of Ang II with AT_2 receptors reverses certain effects mediated by the AT_1 receptor by promoting normal endothelial function, NO release and anti-inflammatory activity [24].

Aldosterone also induces oxidative stress in vascular cells through NAD(P)H oxidase activation, leading to loss of NO bioavailability through its reaction with O_2^- at a rate that is several orders of magnitude faster than its removal by superoxide dismutase [25]. Spironolactone treatment was associated with an increase in endothelial-dependent forearm blood flow following stimulation with acetylcholine [26]. These data suggest an essential role for aldosterone in the RAS response to endothelial dysfunction. Moreover, spironolactone abolished an aldosterone-induced increase in the media/lumen ratio and impaired acetylcholine-induced vasorelaxation in Ang II-infused rats, which was associated with reduced vascular NAD(P)H oxidase activity and decreased plasma levels of thiobarbituric acid-reactive substances, a marker of oxidative stress [27].

In human umbilical vein endothelial cells, aldosterone increased ROS production by activating NAD(P)H oxidase, mainly via $P47^{phox}$-transcriptional and -translocational regulation. The increase in oxidative stress reduced eNOS activity via BH_4 oxidation and Ser^{1177} dephosphorylation in a mineralcorticoid receptor-dependent manner [28]. Combination therapy with eplerenone and enalapril in hyperlipidemic rabbits has also been shown to improve NO bioavailability more effectively than monotherapy [29]. This was accompanied by reduced NAD(P)H activity and increased BH_4 content as well as attenuated atherosclerotic plaque formation in the thoracic aorta [29]. In contrast to its slower genomic effects, aldosterone induces vasorelaxation through rapid, short-term effects on the endothelium. Phenylephrine-induced (α-adrenergic) vasoconstriction in rat aortic ring segments was shown to be rapidly attenuated by aldosterone through PI3K-dependent eNOS and MAPK activation [30]. These data suggest that aldosterone has beneficial effects in the vascular endothelium.

Antihypertensive agents reverse endothelial dysfunction in cardiovascular disease

A number of antihypertensive agents have been shown to improve NO bioavailability and subsequent endothelial dysfunction, including angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), direct renin inhibitors, aldosterone antagonists, certain DHP-type CCBs and vasodilating β-blockers (Figure 1) [31]. In addition to mediating normal vasomotor control, NO exerts atheroprotective effects by reducing smooth muscle cell proliferation and migration, adhesion of leukocytes to the endothelium and platelet aggregation [32]. Thus, improving NO bioavailability is an important goal in the treatment of hypertension.

Inhibitors of the RAS system enhance NO release largely by inhibiting NAD(P)H activation, an essential mechanism by which they reduce vascular resistance and hypertension. In addition to inhibiting the RAS, ACE inhibitors prevent ACE-mediated degradation of bradykinin, which is an agonist of endothelial-dependent NO release. ARBs enhance NO release by facilitating the binding of Ang II to AT_2 receptors. Direct renin inhibitors, which block the RAS at its source, have been shown to improve NO bioavailability by activating the Akt/eNOS pathway and reversing eNOS uncoupling [33]. Aldosterone antagonists also improve endothelial function by decreasing oxidative stress and increasing NO bioavailability [25]. In addition to restoring NO bioavailability, DHP-type CCBs also enhance vasodilation by increasing the antioxidant capacity and relaxation of vascular

smooth muscle cells [34,35]. Certain β-blockers promote endothelium-dependent vasodilation by activating the efflux of ATP, which increases NO release through the subsequent stimulation of P2Y-purinoceptors [36].

Differential impact of antihypertensive drugs on central versus brachial arterial BP

In light of recent clinical findings and outcome measurements, central arterial pressure may be a better predictor of cardiovascular events than conventional brachial artery recordings [4,5]. Central aortic pressure is determined by cardiac output and peripheral vascular resistance – factors that are influenced by arterial stiffness and pressure wave reflections [37]. Increased central arterial stiffness results in the delivery of greater portions of each stroke volume during systole. The velocities of both forward and reflected waves increase under these conditions while the reflected wave returns earlier to the aorta, resulting in increased central aortic pressure and left ventricular load. In accordance, central aortic stiffness contributes directly to the generation of a wide pulse pressure with higher SBP and lower diastolic BP.

Antihypertensive agents have been shown to have different effects on central aortic pressure and hemodynamics despite having similar effects on BP measured over the brachial artery. The effect of combined calcium channel and RAS blockade on central aortic pressure in hypertensive patients was tested and compared with other antihypertensive regimens in the Conduit Artery Function Evaluation (CAFE) [1], a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) [38]. In this study, patients with hypertension and at least three other cardiovascular risk factors were assigned to a CCB/ACE inhibitor regimen (amlodipine plus perindopril) or β-blocker/diuretic regimen (atenolol plus bendroflumethiazide) [38]. Central aortic pressure and hemodynamic variables were measured by radial artery applanation tonometry and pulse wave analysis in 2199 patients for up to 4 years [38]. Both antihypertensive treatment approaches produced similar reductions in brachial SBP but the CCB/ACE inhibitor regimen reduced central aortic SBP and central aortic pulse pressure to a greater extent than that achieved with the β-blocker/diuretic regimen.

In the CAFE study, differences between brachial and central aortic BP values persisted throughout the follow-up period [1]. The higher central aortic SBP and pulse pressures observed with the β-blocker/diuretic intervention were attributed to greater wave reflection (augmentation index) more than to differences in the forward wave following ejection from the left ventricle. Central aortic pulse pressure, central aortic wave augmentation and forward pressure wave height correlated with both the composite end point of cardiovascular events or procedures as well as the development of renal impairment [1]. After adjustment for age and baseline risk factors, central aortic pulse pressure remained significantly associated with the composite end point. These findings substantiated that antihypertensive agents have distinct effects on central aortic pressures and hemodynamics despite having similar effects on brachial BP. The results of CAFE showed that central aortic pulse pressure is a powerful predictor of clinical outcomes and that differences in central aortic pressures could be used to elucidate differences in clinical outcomes between the CCB/ACE inhibitor versus β-blocker/diuretic treatment approaches used in ASCOT.

In studies prior to CAFE, treatment of hypertensive subjects with either CCBs or ACE inhibitors reduced the central aortic augmentation index, consistent with a reduction in arterial stiffness and improved vessel wall elasticity [39,40]. In healthy volunteers, infusion of Ang II significantly increased the augmentation index, whereas infusion of the vasodilator nitroglycerin reduced the augmentation index [41]. In a crossover study of untreated hypertensive patients, bisoprolol produced a greater reduction in brachial SBP than other antihypertensives but increased the central aortic augmentation index [42]. In elderly patients with elevated SBP, nonvasodilating β-blockers increased the augmentation index and augmented aortic SBP, whereas CCBs, ACE inhibitors and diuretics lowered these parameters [43].

Effect of CCBs on endothelial function & cardiovascular disease

Dihydropyridine-type CCBs have favorable effects on endothelial-dependent vasodilation and NO bioavailability, independent of calcium channel modulation (Figures 1 & 2). In coronary microvessels isolated from canine cardiac tissue, amlodipine caused a dose-dependent release of nitrite, the hydration product of NO [44]. The effects of amlodipine on both nitrite release and the NO-dependent regulation of cardiac oxygen consumption were reduced with antagonists of eNOS such as L-N^G-monomethyl arginine (L-NMMA). Under identical conditions, other DHP- and non-DHP-type CCBs,

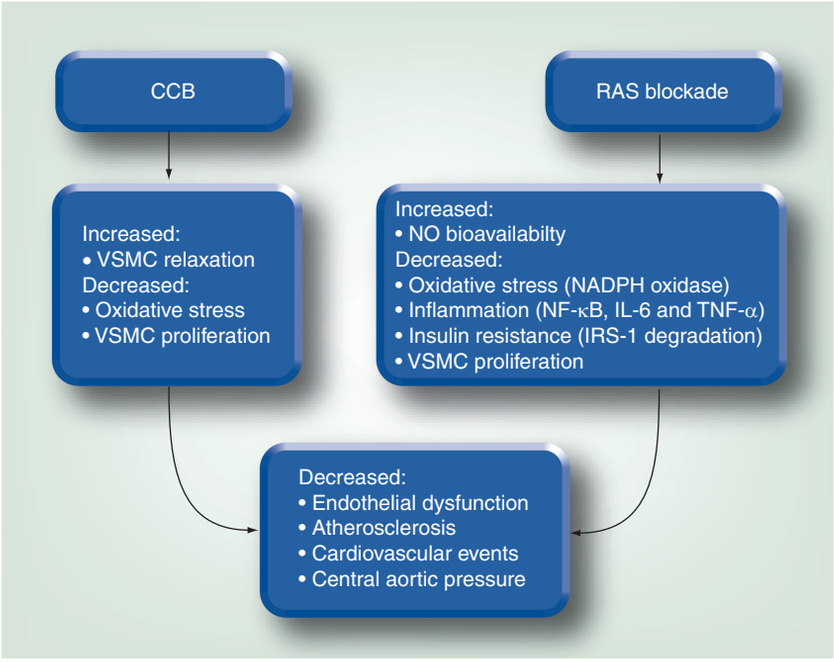

Figure 2. Contribution of pleiotropic actions for calcium channel blocker and renin–angiotensin system inhibitors to the reduction of atherosclerosis and its clinical manifestations based on clinical and experimental findings.
CCB: Calcium channel blocker; IRS-1: Insulin receptor substrate-1; NO: Nitric oxide; RAS: Renin–angiotensin system; VSMC: Vascular smooth muscle cell.

such as nifedipine and diltiazem, respectively, failed to reproduce these effects. The endothelial effects of amlodipine were similar in magnitude to those observed with ACE inhibitors [45]. Nifedipine was also shown to stimulate NO release and scavenge O_2^- in isolated cultured rabbit endothelial cells [34]. In humans, nifedipine treatment restored endothelium-dependent vasodilation and increased plasma antioxidant capacity by attenuating lipoperoxide and isoprostane levels [46]. Nifedipine significantly improved endothelium-dependent vasodilation in patients with coronary artery disease (CAD) as shown in the Elevation of Nifedipine and Cerivastatin on Recovery of Endothelial Function (ENCORE) I study [47]. The ENCORE II study showed that nifedipine, when used in combination with a statin, improved coronary endothelial function but did not slow disease progression, as defined by a reduction in plaque volume [48].

The clinical benefits of amlodipine therapy in patients with established CAD are supported by various randomized trials in patients with hypertension. Amlodipine was evaluated in 825 patients with angiographically documented CAD and controlled hypertension in the Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT) [49]. As compared with patients on placebo, amlodipine therapy (10 mg/day) significantly slowed carotid artery atherosclerosis and reduced the number of unstable angina pectoris events and coronary revascularization procedures. In the Comparison of Amlodipine versus Enalapril to Limit Occurrences of Thrombosis (CAMELOT) study, amlodipine and enalapril were examined in 1991 patients with controlled BP and angiographically documented CAD [50]. Amlodipine reduced the risk of cardiovascular events, including coronary revascularization procedures and hospitalizations for angina pectoris. In a substudy of CAMELOT, amlodipine reduced the change in atheroma volume, with significantly less progression observed in the subgroup in which SBP values were above the mean. By contrast, these same patients treated with enalapril did not experience a significant change in atheroma progression.

Effect of RAS blockade on endothelial function & atherogenesis

Agents that block the RAS, including ACE inhibitors, ARBs and direct renin inhibitors, have various atheroprotective effects on endothelial function (Figures 1 & 2). In a randomized, active-controlled trial of patients with essential hypertension, treatment with an ARB significantly improved the vasoconstrictive response to L-NMMA, indicating an increase in basal NO production, whereas diuretic treatment had no effect despite similar BP reductions [51]. RAS blockade also significantly decreased markers of monocyte and endothelial cell activation in hypertensive patients with Type 2 diabetes [52]. In a study of patients with CAD, treatment with an ACE inhibitor or ARB significantly increased flow-mediated vasodilation, primarily through increased NO bioavailability [53].

In addition to inhibitory effects on the RAS, AT_1 receptor antagonists allow uninhibited stimulation of AT_2 receptors, resulting in important signal transduction events and vascular benefits. AT_2 receptor activation reduces endothelial dysfunction by improving basal nitric oxide synthesis through eNOS activation. In hypercholesterolemic rabbits, valsartan was shown to reduce intimal thickening and endothelial dysfunction [54]. In culture studies, valsartan and losartan were found to inhibit platelet adhesion and aggregation by directly stimulating platelet NO release [55]. Other studies have shown that candesartan-induced eNOS augmentation is abolished by a specific AT_2 receptor antagonist, consistent with AT_2 receptor-mediated NO release [56]. In coronary arteries, activation of

AT_1 receptors by a low concentration of Ang II causes vasoconstriction by increasing NAD(P)H oxidase and subsequent O_2^- production. By contrast, higher concentrations of Ang II promote vasodilatation by activating AT_2 receptors through enhanced NO bioavailability [57].

RAS blockade also produces broader anti-inflammatory effects, as shown in recent model and clinical studies. In animal models of hypercholesterolemia, treatment with valsartan or benazepril was discovered to decrease the intimal lesion area, increase the lumen area and decrease macrophage proliferation [54,58]. In a recent human study, ARBs attenuated the release of proinflammatory cytokines IL-6 and TNF-α from leukocytes [59], and in a large randomized trial of patients with stage two hypertension, treatment with an ARB significantly reduced the levels of high-sensitivity C-reactive protein as compared with an ARB/diuretic combination, despite greater BP reductions with the combination [60].

Role of direct renin inhibition with aliskiren on endothelial function

Reduction of Ang II levels and renin activity with a direct renin inhibitor is also of therapeutic importance as it intervenes at an early point along the RAS pathway (FIGURE 1). Aliskiren, the first commercially available direct renin inhibitor, has been shown to have an antihypertensive potency equivalent to that of ARBs, ACE inhibitors and diuretics. Aliskiren reduces plasma renin activity in patients treated with ACE inhibitors or ARBs, allowing for a more thorough blockade of the RAS [61], and combination treatment with aliskiren and an ARB (valsartan) has been shown to significantly lower BP in humans to a greater extent than observed for each agent separately [62]. Renin and prorenin, the primary effector hormones of the RAS, exert their physiological effects by binding to the prorenin receptor [63]. These hormones directly activate intracellular signaling pathways, including MAPKs, leading to the generation of TGF-β, plasminogen activator inhibitor (PAI)-1 and heat shock protein 27 (Hsp27) [63]. The effect of aliskiren on non-Ang II-mediated activation of intracellular signaling pathways is still not fully understood, but aliskiren has been demonstrated to improve NO bioavailability and alleviate hypertension. Results obtained from current clinical trials exploring the use of aliskiren in secondary prevention (ASPIRE-HIGHER program) are expected to provide further insights into the potential pleiotropic benefits of this agent. However, it must be noted that additional clinical benefits for aliskiren remain speculative until we learn the results from these event-driven clinical trials.

The antihypertensive and renoprotective effects of aliskiren in diabetic nephropathy have been demonstrated in both human and experimental animal models, suggesting a potential benefit for aliskiren in the treatment of diabetes [64,65]. In addition, aliskiren enhances endothelial function in diabetes. In the *db*/*db* mouse model of obesity and Type 2 diabetes, aliskiren treatment for 6 weeks attenuated aortic O_2^- and restored vascular endothelial production of eNOS, thereby preserving endothelium-dependent vascular relaxation [66]. These beneficial effects were attributed to decreased activity of cardiac NAD(P)H/$p22^{phox}$ activity [66]. Moreover, aliskiren improved glucose tolerance and decreased islet fibrosis [66]. None of these effects were reproduced with hydralazine.

In hyperlipidemic rabbits, aliskiren improved NO bioavailability and attenuated atherosclerotic changes in thoracic aortae [33]. In addition, aliskiren increased vascular BH_4 levels and reduced O_2^- and $ONOO^-$ production [33]. As oxidation and depletion of eNOS cofactor BH_4 leads to eNOS uncoupling and NO breakdown, these data suggest that aliskiren prevents eNOS uncoupling. Aliskiren also upregulated Akt/eNOS phosphorylation, which is crucial to eNOS activity [33]. These findings suggest that aliskiren reversed eNOS uncoupling and thereby reduced inflammatory mediators and oxidative stress associated with plaque development in this animal model of atherogenesis.

Several lines of evidence indicate beneficial effects of aliskiren with respect to mechanisms of atherosclerosis and inflammation. In apoE knockout (*Apoe*$^{-/-}$) mice, aliskiren treatment stabilized plaque volume in a manner that could not be fully attributed to changes in BP and that was superior to other antihypertensive therapies of similar efficacy [67]. Aliskiren dramatically reduced the size of atherosclerotic lesions in fat-fed mice deficient in the LDL receptor gene (*LDLr*$^{-/-}$) [68]. Reduced levels of atherosclerosis were also observed in *LDLr*$^{-/-}$ mice repopulated with renin-deficient bone marrow; however, absence of the AT_1 receptor in the bone marrow had no effect on the size of the atherosclerotic lesion [68]. In another study, renin inhibition was shown to reduce leukocyte adhesion in mechanically injured mouse femoral arteries, consistent with an anti-inflammatory mechanism and independent of changes in BP [69].

The RAS has a strong association with obesity. For example, obesity-induced hypertension involves activation of the renin system in menopausal women [70]. In animal models, mice transfected with the human renin gene were shown to develop obesity [71] while renin-deficient mice (*Ren1c*$^{-/-}$) were lean, insulin sensitive and resistant to diet-induced obesity [72].

Evaluation of combination therapy with RAS & calcium channel blockers

Based on our understanding of their separate pharmacologic properties, certain DHP-type CCBs and RAS inhibitors have ancillary effects that enhance NO bioavailability, reduce oxidative stress and suppress inflammatory responses (Figure 2). There is growing evidence from both animal and clinical studies to support the complementary effects of these drug classes when used in combination to treat hypertension. The pleiotropic effects of both CCBs and RAS inhibitors, coupled with the efficacy of combination therapy on reducing central aortic pressure, suggest beneficial actions that underlie their ability to improve patient outcomes when used together.

Angiotensin II is a major mediator of oxidative stress, enhancing O_2^- production via NAD(P)H oxidase activation, resulting in reduced NO bioavailability. Ang II also contributes to inward calcium current modulation in the failing heart [73]. In atrial fibrillation, calcium influx via L-type voltage-sensitive calcium channels (L-VSCCs) plays a crucial role in atrial excitation-contraction coupling [74]. Ang II increases transcription of the α1C subunit of L-VSCC in atrial myocytes, while losartan and simvastatin, which inhibit NAD(P)H oxidase activity and ROS generation, attenuate L-VSCC current [75–77]. These data suggest that CCB and RAS blocker combination treatment may be more effective than monotherapy. In a cuff-induced vascular injury model, coadministration of azelnidipine and olmesartan at nonhypotensive doses significantly inhibited vascular smooth muscle cell proliferation and neointima formation in wild-type mice, while azelnidipine or olmesartan alone at these lower doses did not affect neointima formation [78].

Dahl salt-sensitive rats are known to develop hypertension, aortic hypertrophy, proteinuria and endothelial dysfunction [79]. In this animal model, amlodipine was shown to significantly reduce SBP, aortic hypertrophy and proteinuria, whereas benazepril reduced only proteinuria without lowering SBP. Treatment with either amlodipine or benazepril alone significantly improved endothelial-dependent relaxation; however, the CCB/ACE inhibitor combination was more effective than monotherapy in normalizing both SBP and proteinuria. Comparable therapeutic benefits were observed in a rat myocardial infarction model in which cardiac interstitial fluid levels of cGMP, TNF-α and NO metabolites (NOX) were measured for 5 weeks following the occlusion of the left anterior descending artery [80]. TNF-α levels increased progressively while NOX and cGMP levels were shown to decrease over the experimental time course. With amlodipine and benazepril combination therapy, TNF-α levels decreased while NOX and cGMP levels increased. By contrast, treatment with hydrochlorothiazide did not affect NO or inflammatory mediator levels. As compared with monotherapy, the benefits of the CCB/RAS blockade combination were more effective in managing cardiac ischemia.

Clinical studies show that combination treatment with amlodipine and a RAS blocker produces significantly greater reductions in BP than either agent alone [81–84]. In a randomized, crossover trial of hypertensive patients with Type 2 diabetes, amlodipine treatment significantly increased t-PA activity with no effect on plasma PAI-1 activity, while the ACE inhibitor benazepril had no effect on t-PA activity but significantly decreased PAI-1 activity; however, in combination these agents significantly increased t-PA activity and significantly decreased PAI-1 activity [85]. In hypertensive patients with at least one additional risk factor for endothelial dysfunction, the amlodipine/benazepril combination treatment versus amlodipine alone significantly increased flow-mediated vasodilation [86]. These findings suggest that CCB with ACE inhibitor combination therapy may improve endothelial function and slow the atherogenic process.

Results from the Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) study have provided strong clinical support for the hypothesis that the combination of an ACE inhibitor and a DHP-type CCB is more effective in reducing the rate of cardiovascular events than treatment involving a diuretic [87]. In this double-blind trial 11,506 patients with hypertension and at high risk for cardiovascular events were assigned to treatment with either benazepril plus amlodipine or benazepril plus hydrochlorothiazide. The primary clinical end point was the composite of death from cardiovascular causes, nonfatal

myocardial infarction, nonfatal stroke, hospitalization for angina, resuscitation after sudden cardiac arrest and coronary revascularization. After 36 months, mean BPs were similar between the two groups after dose adjustment but the number of primary outcome events in the benazepril–amlodipine group was significantly lower than in the benazepril–hydrochlorothiazide group, representing a relative risk reduction of 20%. For the secondary end point of death from cardiovascular causes, nonfatal myocardial infarction and nonfatal stroke, the benazepril–amlodipine regimen reduced relative risk by 21%. The investigators concluded that the ACE inhibitor–amlodipine combination was superior to the ACE inhibitor–diuretic combination in reducing cardiovascular events not withstanding even small differences in BP.

Effects of hyperglycemia, insulin resistance & hypertension on endothelial function

A disproportionate number of people with diabetes and insulin resistance are also hypertensive, resulting in accelerated rates of atherosclerosis and higher cardiovascular risk in this population group. These disease processes share common pathophysiologic mechanisms, including endothelial dysfunction, leading to oxidative stress and inflammation [88]. There is also a strong association between insulin resistance and hypertension in nondiabetic patients [89,90]. The insulin-signaling pathway, which includes the insulin receptor, insulin receptor substrate-1 (IRS-1), PI3K, PDK-1 and Akt, regulates glucose transporter type 4 translocation and glucose uptake in skeletal muscle and adipose tissue. The same pathway promotes vasodilation by modulating eNOS activity and NO production (FIGURE 1) [91]. Under pathologic conditions, hyperglycemia and elevated free fatty acid levels promote oxidative stress, insulin resistance and endothelial dysfunction by impairing the insulin-signaling pathway. Of note, Ser phosphorylation of IRS-1 attenuates its ability to bind and activate PI3K. As a result, mice homozygous-null for the *IRS-1* gene are not only insulin-resistant but also have a hemodynamic phenotype characterized by hypertension with impaired vasodilation [92].

Both pharmacologic and nonpharmacologic strategies have been used to restore insulin metabolism and lower BP. Interactions between the RAS and insulin signaling pathways have been demonstrated by the observation that Ang II interferes with the skeletal muscle metabolic actions of insulin/IGF, which in turn promotes vascular relaxation via increased NO release [93]. In human umbilical vein endothelial cells, Ang II increased IRS-1 phosphorylation at Ser^{312} and Ser^{616} via JNK and ERK1/2, respectively, thus impairing the vasodilatory effects of insulin mediated by the IRS-1/PI3K/Akt/eNOS pathway [94]. It has also been demonstrated in rat vascular smooth muscle cells that Ang II and aldosterone augment Src- and ROS-mediated serine phosphorylation and IRS-1 degradation while abolishing insulin-induced Akt phosphorylation [95,96]. In Ren2 transgenic rats, which harbor the mouse renin transgene and exhibit elevated tissue Ang II levels, *in vivo* insulin stimulation diminished Akt and eNOS activation in the aorta while enhancing Ang II- and NAD(P)H oxidase-derived ROS formation; these abnormalities were abrogated with ARB treatment [97].

Recently, expert committees on diabetes mellitus have advocated that primary aldosteronism be recognized as a unique form of diabetes mellitus [98]. Glucose tolerance is impaired up to 50% in patients with primary aldosteronism [99]. Increased blood glucose in primary aldosteronism patients has been attributed to not only aldosterone-induced hypokalemia and low adiponectin levels but also aldosterone-mediated impairment of glucose homeostasis and systemic insulin resistance through nongenomic actions [100]. Peroxisome proliferator activated receptor (PPAR)-γ agonists reduce Ang II-induced oxidative stress, inflammation and hypertension [101]. For example, thiazolidinediones (synthetic PPAR-γ ligands) are insulin sensitizers that increase forearm blood flow in humans [102] and reduce BP in insulin-resistant patients with hypertension [103]. Overall, RAS inhibitors that selectively activate PPAR-γ may exert beneficial effects by enhancing insulin sensitivity and reducing hypertension [104]. The combined effects of DHP-type CCBs and RAS inhibitors, including direct renin inhibitors, on vascular function and cardiovascular outcomes in patients with insulin resistance and diabetes need to be further investigated in clinical trials.

Conclusion

Hypertension is characterized by endothelial dysfunction as evidenced by impaired responsiveness to normal stimuli of vasodilation. These changes contribute to mechanical forces that activate neurohormonal processes, including the RAS, which contributes directly to atherosclerosis and its clinical manifestations.

Antihypertensive drugs have class-specific hemodynamic and physiologic effects that reverse aspects of these vascular changes, resulting in reduced cardiovascular events in randomized trials. Calcium channel and RAS blockers, including direct renin inhibitors, have pleiotropic properties that contribute additional endothelial benefits when used in combination (Figures 1 & 2). The CAFE study showed that a CCB/ACE inhibitor regimen produced greater reductions in central aortic SBP and pulse pressure than a β-blocker/diuretic regimen, despite comparable reductions in brachial SBP. The clinical benefits of a DHP-type CCB and RAS inhibitor combination, as compared with other antihypertensive regimens, were also demonstrated in the ASCOT and ACCOMPLISH trials. The basis for benefit with this combination is attributed, in part, to enhanced endothelial-dependent NO bioavailability, which slows atherothrombotic processes.

Future perspective

Additional research is needed to determine the benefits of combination antihypertensive therapy for reducing vascular injury and global cardiovascular risk, as a result of their effects on BP control as well as their pleiotropic properties. Advanced hypertensive therapy must be directed at slowing atherosclerosis, a chronic inflammatory disease

Executive summary

Role of endothelial dysfunction in hypertension

- Hypertension is characterized by reduced vasomotor control due to loss of endothelial-dependent nitric oxide (NO) release.
- Reduced NO bioavailability contributes to atherosclerosis and plaque development.
- Endothelial dysfunction is related to abnormal NO synthase activity due to reduced cofactor levels and/or increased production of reactive oxygen species.

Role of the renin–angiotensin system in hypertension

- The renin–angiotensin system (RAS) regulates vasomotor activity as well as volume and electrolyte levels.
- RAS activation promotes arterial remodeling, oxidative stress and inflammation.
- The binding of angiotensin II to angiotensin II type 1 receptors on vascular smooth muscle cells promotes vasoconstriction.
- Aldosterone release with RAS activation leads to restriction of renal afferent arterioles.

Antihypertensive agents reverse endothelial dysfunction in cardiovascular disease

- Certain antihypertensive agents have been shown to improve endothelial NO bioavailability, leading to broader vascular benefits and reduced central aortic pressure.
- Inhibitors of the RAS system enhance endothelial function by reducing NAD(P)H activation.
- Angiotensin-converting enzyme (ACE) inhibitors block ACE-mediated degradation of bradykinin, an agonist of endothelial-dependent NO release.
- Angiotensin receptor blockers enhance NO levels by facilitating the binding of angiotensin II to angiotensin II type 2 receptors.
- Direct renin inhibitors (e.g., aliskiren) enhance NO synthase activity and function.
- Aldosterone antagonists improve endothelial function by decreasing oxidative stress.
- Dihydropyridine-type calcium channel blockers (CCBs; e.g., amlodipine) enhance vasodilation through relaxation of vascular smooth muscle cells and increased NO.

Differential impact of antihypertensive drugs on central versus brachial arterial blood pressure

- Central arterial pressure may be a superior predictor of cardiovascular events as compared with conventional brachial artery recordings of hypertension.
- Certain antihypertensive agents have differential effects on central aortic pressure and hemodynamics despite similar changes in blood pressure over the brachial artery.
- The results of clinical studies showed that central aortic pulse pressure predicted clinical outcomes for a CCB/ACE inhibitor combination but not other combination treatments.

Evaluation of combination therapy with RAS & calcium channel blockade

- Based on their separate pharmacologic properties, certain dihydropyridine-type CCBs and RAS inhibitors have synergistic vascular effects that enhance NO bioavailability, reduce oxidative stress and suppress inflammatory responses.
- Results from the Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) study have validated the benefits of an ACE inhibitor/CCB combination in reducing the rate of cardiovascular events.

Effects of hyperglycemia, insulin resistance & hypertension on endothelial function

- A disproportionate number of people with diabetes and insulin resistance are also hypertensive, resulting in higher cardiovascular risk in this population group.
- These disease processes share common pathophysiologic mechanisms, including endothelial dysfunction, which lead to oxidative stress and inflammation.

Future perspective

- Future study is needed to elucidate the role of combination therapy in reducing global cardiovascular risk through reductions in central aortic pressure control and enhanced vascular activity.

evidenced by increased central aortic pressures that predict myocardial infarction, stroke and sudden death. Recent clinical trials indicate that the combination of certain DHP-type CCBs and RAS inhibitors reduce cardiovascular events in a manner that is superior to other antihypertensive regimens, as demonstrated by potent reductions in central aortic pressures despite comparable changes in brachial arterial pressure.

The 'response to injury' hypothesis proposes that atherosclerosis begins with endothelial damage that produces adaptive and innate immune responses, which propagate an arterial lesion, eventually progressing to a vulnerable plaque. Endothelial damage is evidenced by a reduced responsiveness to stimuli of vasodilation, resulting from loss of NO bioavailability. Immune cells, such as monocytes, are recruited to the injured vessel wall by adhesion molecules associated with the immune response. A number of circulating inflammatory biomarkers have been identified, including C-reactive protein, fibrinogen, cytokines and other proteins associated with the immune system. Future directions in hypertensive treatment must reduce the risk for cardiovascular events through various anti-inflammatory actions and improved endothelial function. Elucidating the basis for clinical benefit with antihypertensive combinations is an area of active research that may lead to the development of more effective approaches for the prevention and treatment of cardiovascular disease. Ultimately, these new therapeutic approaches must be tested in prospective clinical trials that lead to appropriate hypertension guidelines.

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Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Williams B, Lacy PS, Thom SM *et al.*: Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 113(9), 1213–1225 (2006).

▪▪ **Reports the results of a randomized clinical study in which measurements of central aortic pressure were demonstrated to have superior predictive value as compared to conventional brachial arterial pressure.**

2 Sever PS, Poulter NR, Elliott WJ *et al.*: Blood pressure reduction is not the only determinant of outcome. *Circulation* 113(23), 2754–2772 (2006).

3 Chobanian AV, Bakris GL, Black HR *et al.*: The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 289(19), 2560–2572 (2003).

4 O'Rourke MF, Seward JB: Central arterial pressure and arterial pressure pulse: new views entering the second century after Korotkov. *Mayo Clinic Proc.* 81(8), 1057–1068 (2006).

5 Weber T, Auer J, O'Rourke MF *et al.*: Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation* 109(2), 184–189 (2004).

6 Taddei S, Virdis A, Ghiadoni L *et al.*: Age-related reduction of NO availability and oxidative stress in humans. *Hypertension* 38(2), 274–279 (2001).

7 Funovic P, Korda M, Kubant R *et al.*: Effect of beta-blockers on endothelial function during biological aging: a nanotechnological approach. *J. Cardiovasc. Pharmacol.* 51(2), 208–215 (2008).

8 Hayakawa H, Coffee K, Raij L: Endothelial dysfunction and cardiorenal injury in experimental salt-sensitive hypertension: effects of antihypertensive therapy. *Circulation* 96(7), 2407–2413 (1997).

9 Mason RP, Kubant R, Jacob RF *et al.*: Loss of arterial and renal nitric oxide bioavailability in hypertensive rats with diabetes: effect of beta-blockers. *Am. J. Hypertens.* 22(11), 1160–1166 (2009).

▪ **Original article demonstrating the effect of multiple risk factors on endothelial dysfunction and metabolic disease.**

10 Ozaki M, Kawashima S, Yamashita T *et al.*: Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *J. Clin. Invest.* 110(3), 331–340 (2002).

11 Landmesser U, Dikalov S, Price SR *et al.*: Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J. Clin. Invest.* 111(8), 1201–1209 (2003).

12 Fukai T: Endothelial GTPCH in eNOS uncoupling and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 27(7), 1493–1495 (2007).

13 McIntyre M, Hamilton CA, Rees DD *et al.*: Sex differences in the abundance of endothelial nitric oxide in a model of genetic hypertension. *Hypertension* 30(6), 1517–1524 (1997).

14 Bouloumié A, Bauersachs J, Ling W *et al.*: Endothelial dysfunction coincides with an enhanced nitric oxide synthase expression and superoxide anion production. *Hypertension* 30(4), 934–941 (1997).

15 Burt VL, Whelton P, Roccella EJ *et al.*: Prevalence of hypertension in the US adult population: results from the Third National Health and Nutrition Examination Survey, 1988–1991. *Hypertension* 25(3), 305–313 (1995).

16 Mason RP, Kalinowski L, Jacob RF *et al.*: Nebivolol reduces nitroxidative stress and restores nitric oxide bioavailability in endothelium of black Americans. *Circulation* 112(24), 3795–3801 (2005).

17 Kalinowski L, Dobrucki IT, Malinski T: Race-specific differences in endothelial function: predisposition of African Americans to vascular diseases. *Circulation* 109(21), 2511–2517 (2004).

18 Campia U, Choucair WK, Bryant MB *et al.*: Reduced endothelium-dependent and -independent dilation of conductance arteries in African Americans. *J. Am. Coll. Cardiol.* 40(4), 754–760 (2002).

19 Mason RP, Kubant R, Jacob RF *et al.*: Effect of nebivolol on endothelial nitric oxide and peroxynitrite release in hypertensive animals: role of antioxidant activity. *J. Cardiovasc. Pharmacol.* 48(1), 862–869 (2006).

20 Duprez DA: Role of the renin-angiotensin-aldosterone system in vascular remodeling and inflammation: a clinical review. *J. Hypertens.* 24(6), 983–991 (2006).

21 Jackson EK: Renin and angiotensin. In: *Goodman & Gilman's The Pharmacological Basis of Therapeutics (11th Edition).* Brunton LL, Lazo JS, Parker KL (Eds). McGraw-Hill, NY, USA, 789–821 (2006).

22 Arima S, Kohagura K, Xu HL *et al.*: Endothelium-derived nitric oxide modulates vascular action of aldosterone in renal arteriole. *Hypertension* 43(2), 352–357 (2004).

23 Dandona P, Dhindsa S, Ghanim H, Chaudhuri A: Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. *J. Hum. Hypertens.* 21(1), 20–27 (2007).

24 Carey RM: Update on the role of the AT_2 receptor. *Curr. Opin. Nephrol. Hyperten.* 14(1), 67–71 (2005).

25 Schiffrin EL: Effects of aldosterone on the vasculature. *Hypertension* 47(3), 312–318 (2006).

26 Farquharson CA, Struthers AD: Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. *Circulation* 101(6), 594–597 (2000).

27 Virdis A, Neves MF, Amiri F *et al.*: Spironolactone improves angiotensin-induced vascular changes and oxidative stress. *Hypertension* 40(4), 504–510 (2002).

28 Nagata D, Takahashi M, Sawai K *et al.*: Molecular mechanism of the inhibitory effect of aldosterone on endothelial NO synthase activity. *Hypertension* 48(1), 165–171 (2006).

29 Imanishi T, Ikejima H, Tsujioka H *et al.*: Addition of eplerenone to an angiotensin-converting enzyme inhibitor effectively improves nitric oxide bioavailability. *Hypertension* 51(3), 734–741 (2008).

▪ **Shows an antioxidant effect associated with combined treatment with an aldosterone antagonist and angiotensin-converting enzyme (ACE) inhibitor.**

30 Liu SL, Schmuck S, Chorazcyzewski JZ *et al.*: Aldosterone regulates vascular reactivity: short-term effects mediated by phosphatidylinositol 3-kinase-dependent nitric oxide synthase activation. *Circulation* 108(19), 2400–2406 (2003).

31 Kojda G, Harrison DG: Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. *Cardiovasc. Res.* 43(3), 562–571 (1999).

32 Harrison DG: Cellular and molecular mechanisms of endothelial cell dysfunction. *J. Clin. Invest.* 100(9), 2153–2157 (1997).

▪ **Important review that describes the molecular basis for endothelial dysfunction.**

33 Imanishi T, Tsujioka H, Ikejima H *et al.*: Renin inhibitor aliskiren improves impaired nitric oxide bioavailability and protects against atherosclerotic changes. *Hypertension* 52(3), 563–572 (2008).

▪ **Initial study that reported improvement in nitric oxide release and reduction in atherogenesis with aliskiren treatement.**

34 Brovkovych VV, Kalinowski L, Muller-Peddinghaus R, Malinski T: Synergistic antihypertensive effects of nifedipine on endothelium: concurrent release of NO and scavenging of superoxide. *Hypertension* 37(1), 34–39 (2001).

35 Perez-Reyes E: Molecular physiology of low-voltage-activated T-type calcium channels. *Physiol. Rev.* 83(1), 117–161 (2003).

36 Kalinowski L, Dobrucki LW, Szczepanska-Konkel M *et al.*: Third-generation beta-blockers stimulate nitric oxide release from endothelial cells through ATP efflux: a novel mechanism for antihypertensive action. *Circulation* 107(21), 2747–2752 (2003).

▪ **Original research report, which showed that third generation β-blockers enhanced nitric oxide bioavailability through an autocrine pathway in endothelial cells, independent of β-receptor binding.**

37 Izzo JL Jr: Arterial stiffness and the systolic hypertension syndrome. *Curr. Opin. Cardiol.* 19(4), 341–352 (2004).

▪ **Valuable review of the relationship between arterial stiffness parameters and the determinants of systolic hypertension.**

38 Dahlöf B, Sever PS, Poulter NR *et al.*: Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial. *Lancet* 366(9489), 895–906 (2005).

▪▪ **Landmark clinical trial demonstrating the superior benefit of a dihydropyridine-type calcium channel blocker and ACE inhibitor combination regimen over a β-blocker and diuretic combination for the treatment of hypertensive patients with other risk factors.**

39 Chen CH, Ting CT, Lin SJ *et al.*: Different effects of fosinopril and atenolol on wave reflections in hypertensive patients. *Hypertension* 25(5), 1034–1041 (1995).

40 Hirata K, Vlachopoulos C, Adji A, O'Rourke MF: Benefits from angiotensin-converting enzyme inhibitor 'beyond blood pressure lowering': beyond blood pressure or beyond the brachial artery? *J. Hypertens.* 23(3), 551–556 (2005).

41 Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ: Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. *Hypertension* 37(6), 1429–1433 (2001).

42 Deary AJ, Schumann AL, Murfet H *et al.*: Influence of drugs and gender on the arterial pulse wave and natriuretic peptide secretion in untreated patients with essential hypertension. *Clin. Sci.* 103(5), 493–499 (2002).

43 Morgan T, Lauri J, Bertram D, Anderson A: Effect of different antihypertensive drug classes on central aortic pressure. *Am. J. Hypertens.* 17(2), 118–123 (2004).

44 Zhang X, Hintze TH: Amlodipine releases nitric oxide from canine coronary microvessels: an unexpected mechanism of action of a calcium channel-blocking agent. *Circulation* 97(6), 576–580 (1998).

▪ **Original research article describing a novel pharmacologic benefit of amlodipine on nitric oxide release from coronary microvessels, independent of calcium channel modulation.**

45 Zhang X, Xie YW, Nasjletti A *et al.*: ACE inhibitors promote nitric oxide accumulation to modulate myocardial oxygen consumption. *Circulation* 95(1), 176–182 (1997).

46 Taddei S, Virdis A, Ghiadoni L *et al.*: Restoration of nitric oxide availability after calcium antagonist treatment in essential hypertension. *Hypertension* 37(3), 943–948 (2001).

47 ENCORE Investigators: Effect of nifedipine and cerivastatin on coronary endothelial function in patients with coronary artery disease: the ENCORE I Study (Evaluation of Nifedipine and Cerivastatin On Recovery of coronary Endothelial function). *Circulation* 107(3), 422–428 (2003).

48 Luscher TF, Pieper M, Tendera M *et al.*: A randomized placebo-controlled study on the effect of nifedipine on coronary endothelial function and plaque formation in patients with coronary artery disease: the ENCORE II study. *Eur. Heart J.* 30(13), 1590–1597 (2009).

49 Pitt B, Byington RP, Furberg CD *et al.*: Effect of amlodipine on the progression of atherosclerosis and the occurrence of clinical events. *Circulation* 102(13), 1503–1510 (2000).

50 Nissen SE, Tuzcu EM, Libby P *et al.*: Effect of antihypertensive agents on cardiovascular events in patients with coronary disease and normal blood pressure: the CAMELOT study: a randomized controlled trial. *JAMA* 292(18), 2217–2225 (2004).

▪▪ **Clinical trial showing significant reductions in cardiovascular events with a dihydropyridine-type calcium channel blocker that was associated with the retardation of atheroma growth as determined by ultrasound approaches.**

51 Klingbeil AU, John S, Schneider MP *et al.*: Effect of AT_1 receptor blockade on endothelial function in essential hypertension. *Am. J. Hypertens.* 16(2), 123–128 (2003).

52 Nomura S, Shouzu A, Omoto S *et al.*: Effect of valsartan on monocyte/endothelial cell activation markers and adiponectin in hypertensive patients with Type 2 diabetes mellitus. *Thromb. Res.* 117(4), 385–392 (2006).

53 Hornig B, Landmesser U, Kohler C *et al.*: Comparative effect of ACE inhibition and angiotensin II type 1 receptor antagonism on bioavailability of nitric oxide in patients with coronary artery disease: role of superoxide dismutase. *Circulation* 103(6), 799–805 (2001).

54 de las Heras N, Aragoncillo P, Maeso R *et al.*: AT_1 receptor antagonism reduces endothelial dysfunction and intimal thickening in atherosclerotic rabbits. *Hypertension* 34(4 Pt 2), 969–975 (1999).

55 Kalinowski L, Matys T, Chabielska E *et al.*: Angiotensin II AT_1 receptor antagonists inhibit platelet adhesion and aggregation by nitric oxide release. *Hypertension* 40(4), 521–527 (2002).

56 Thai H, Wollmuth J, Goldman S, Gaballa M: Angiotensin subtype 1 receptor (AT_1) blockade improves vasorelaxation in heart failure by up-regulation of endothelial nitric-oxide synthase via activation of the AT_2 receptor. *J. Pharmacol. Exp. Ther.* 307(3), 1171–1178 (2003).

57 Zhang C, Hein TW, Wang W, Kuo L: Divergent roles of angiotensin II AT_1 and AT_2 receptors in modulating coronary microvascular function. *Circ. Res.* 92(3), 322–329 (2003).

58 Li J, Hirose N, Kawamura M, Arai Y: Antiatherogenic effect of angiotensin converting enzyme inhibitor (benazepril) and angiotensin II receptor antagonist (valsartan) in the cholesterol-fed rabbits. *Atherosclerosis* 143(2), 315–326 (1999).

59 Willemsen JM, Westerink JW, Dallinga-Thie GM *et al.*: Angiotensin II type 1 receptor blockade improves hyperglycemia-induced endothelial dysfunction and reduces proinflammatory cytokine release from leukocytes. *J. Cardiovasc. Pharmacol.* 49(1), 6–12 (2007).

60 Ridker PM, Danielson E, Rifai N, Glynn RJ: Valsartan, blood pressure reduction, and C-reactive protein: primary report of the Val-MARC trial. *Hypertension* 48(1), 73–79 (2006).

61 Campbell DJ: Interpretation of plasma renin concentration in patients receiving aliskiren therapy. *Hypertension* 51(1), 15–18 (2008).

62 Oparil S, Yarows SA, Patel S *et al.*: Efficacy and safety of combined use of aliskiren and valsartan in patients with hypertension: a randomised, double-blind trial. *Lancet* 370(9583), 221–229 (2007).

63 Nguyen G, Danser AH: The (pro)renin receptor: therapeutic consequences. *Expert Opin. Investig. Drugs* 15(10), 1131–1135 (2006).

64 Feldman DL, Jin L, Xuan H *et al.*: Effects of aliskiren on blood pressure, albuminuria, and (pro)renin receptor expression in diabetic TG(mRen-2)27 rats. *Hypertension* 52(1), 130–136 (2008).

65 Parving HH, Persson F, Lewis JB *et al.*: Aliskiren combined with losartan in Type 2 diabetes and nephropathy. *N. Engl. J. Med.* 358(23), 2433–2446 (2008).

66 Dong YF, Liu L, Kataoka K *et al.*: Aliskiren prevents cardiovascular complications and pancreatic injury in a mouse model of obesity and Type 2 diabetes. *Diabetologia* 53(1), 180–191 (2010).

67 Nussberger J, Aubert JF, Bouzourene K *et al.*: Renin inhibition by aliskiren prevents atherosclerosis progression: comparison with irbesartan, atenolol, and amlodipine. *Hypertension* 51(5), 1306–1311 (2008).

68 Lu H, Rateri DL, Feldman DL *et al.*: Renin inhibition reduces hypercholesterolemia-induced atherosclerosis in mice. *J. Clin. Invest.* 118(3), 984–993 (2008).

69 Ino J, Kojima C, Osaka M *et al.*: Dynamic observation of mechanically-injured mouse femoral artery reveals an antiinflammatory effect of renin inhibitor. *Arterioscler. Thromb. Vasc. Biol.* 29(11), 1858–1863 (2009).

70 Engeli S, Bohnke J, Gorzelniak K *et al.*: Weight loss and the renin-angiotensin-aldosterone system. *Hypertension* 45(3), 356–362 (2005).

71 Uehara S, Tsuchida M, Kanno T *et al.*: Late-onset obesity in mice transgenic for the human renin gene. *Int. J. Mol. Med.* 11(6), 723–727 (2003).

72 Takahashi N, Li F, Hua K *et al.*: Increased energy expenditure, dietary fat wasting, and resistance to diet-induced obesity in mice lacking renin. *Cell Metab.* 6(6), 506–512 (2007).

73 de Mello WC, Monterrubio J: Intracellular and extracellular angiotensin II enhance the L-type calcium current in the failing heart. *Hypertension* 44(3), 360–364 (2004).

74 van Wagoner DR, Pond AL, Lamorgese M *et al.*: Atrial L-type Ca^{2+} currents and human atrial fibrillation. *Circ. Res.* 85(5), 428–436 (1999).

75 Goette A, Staack T, Rocken C *et al.*: Increased expression of extracellular signal-regulated kinase and angiotensin-converting enzyme in human atria during atrial fibrillation. *J. Am. Coll. Cardiol.* 35(6), 1669–1677 (2000).

76 Kim YM, Guzik TJ, Zhang YH *et al.*: A myocardial Nox2 containing NAD(P)H oxidase contributes to oxidative stress in human atrial fibrillation. *Circ. Res.* 97(7), 629–636 (2005).

77 Tsai CT, Wang DL, Chen WP *et al.*: Angiotensin II increases expression of alpha1C subunit of L-type calcium channel through a reactive oxygen species and cAMP response element-binding protein-dependent pathway in HL-1 myocytes. *Circ. Res.* 100(10), 1476–1485 (2007).

78 Jinno T, Iwai M, Li Z *et al.*: Calcium channel blocker azelnidipine enhances vascular protective effects of AT_1 receptor blocker olmesartan. *Hypertension* 43(2), 263–269 (2004).

79 Zhou MS, Jaimes EA, Raij L: Benazepril combined with either amlodipine or hydrochlorothiazide is more effective than monotherapy for blood pressure control and prevention of end-organ injury in hypertensive Dahl rats. *J. Cardiovasc. Pharmacol.* 48(1), 857–861 (2006).

80 Siragy HM, Xue C, Webb RL: Beneficial effects of combined benazepril-amlodipine on cardiac nitric oxide, cGMP, and TNF-α production after cardiac ischemia. *J. Cardiovasc. Pharmacol.* 47(5), 636–642 (2006).

81 Jamerson KA, Nwose O, Jean-Louis L *et al.*: Initial angiotensin-converting enzyme inhibitor/calcium channel blocker combination therapy achieves superior blood pressure control compared with calcium channel blocker monotherapy in patients with stage 2 hypertension. *Am. J. Hypertens.* 17(6), 495–501 (2004).

82 Neutel JM, Smith DH, Weber MA *et al.*: Efficacy of combination therapy for systolic blood pressure in patients with severe systolic hypertension: the Systolic Evaluation of Lotrel Efficacy and Comparative Therapies (SELECT) study. *J. Clin. Hypertens.* 7(11), 641–646 (2005).

83 Philipp T, Smith TR, Glazer R *et al.*: Two multicenter, 8-week, randomized, double-blind, placebo-controlled, parallel-group studies evaluating the efficacy and tolerability of amlodipine and valsartan in combination and as monotherapy in adult patients with mild to moderate essential hypertension. *Clin. Ther.* 29(4), 563–580 (2007).

84 Pool J, Kaihlanen P, Lewis G *et al.*: Once-daily treatment of patients with hypertension: a placebo-controlled study of amlodipine and benazepril vs amlodipine or benazepril alone. *J. Hum. Hypertens.* 15(7), 495–498 (2001).

85 Fogari R, Preti P, Lazzari P *et al.*: Effect of benazepril amlodipine combination on fibrinolysis in hypertensive diabetic patients. *Eur. J. Clin. Pharmacol.* 59(4), 271–275 (2003).

86 Mohler ER 3rd, Herrington D, Ouyang P *et al.*: A randomized, double-blind trial comparing the effects of amlodipine besylate/benazepril HCl vs amlodipine on endothelial function and blood pressure. *J. Clin. Hypertens.* 8(10), 692–698 (2006).

87 Jamerson K, Weber MA, Bakris GL *et al.*: Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. *N. Engl. J. Med.* 359(23), 2417–2428 (2008).

▪ **Landmark clinical trial showing that an ACE inhibitor and DHP-type calcium channel blocker combination was superior to an ACE inhibitor and hydrochlorothiazide combination in reducing cardiovascular events in patients with hypertension who were at high risk for such events.**

88 Kim JA, Montagnani M, Koh KK, Quon MJ: Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 113(15), 1888–1904 (2006).

89 Ferrannini E, Natali A, Capaldo B *et al.*: Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. European Group for the Study of Insulin Resistance (EGIR). *Hypertension* 30(5), 1144–1149 (1997).

90 Saad MF, Rewers M, Selby J *et al.*: Insulin resistance and hypertension: the Insulin Resistance Atherosclerosis study. *Hypertension* 43(6), 1324–1331 (2004).

91 Zeng G, Nystrom FH, Ravichandran LV *et al.*: Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation* 101(13), 1539–1545 (2000).

92 Abe H, Yamada N, Kamata K *et al.*: Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. *J. Clin. Invest.* 101(8), 1784–1788 (1998).

93 Wei Y, Sowers JR, Nistala R *et al.*: Angiotensin II-induced NADPH oxidase activation impairs insulin signaling in skeletal muscle cells. *J. Biol. Chem.* 281(46), 35137–35146 (2006).

94 Andreozzi F, Laratta E, Sciacqua A *et al.*: Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser312 and Ser616 in human umbilical vein endothelial cells. *Circ. Res.* 94(9), 1211–1218 (2004).

95 Taniyama Y, Hitomi H, Shah A *et al.*: Mechanisms of reactive oxygen species-dependent downregulation of insulin receptor substrate-1 by angiotensin II. *Arterioscler. Thromb. Vasc. Biol.* 25(6), 1142–1147 (2005).

96 Hitomi H, Kiyomoto H, Nishiyama A *et al.*: Aldosterone suppresses insulin signaling via the downregulation of insulin receptor substrate-1 in vascular smooth muscle cells. *Hypertension* 50(4), 750–755 (2007).

97 Wei Y, Whaley-Connell AT, Chen K *et al.*: NADPH oxidase contributes to vascular inflammation, insulin resistance, and remodeling in the transgenic (mRen2) rat. *Hypertension* 50(2), 384–391 (2007).

▪ **Influential article that demonstrated the role of angiotensin II/NAD(P)H oxidase in insulin resistance and endothelial dysfunction.**

98 Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 26(Suppl. 1), S5–S20 (2003).

99 Giacchetti G, Sechi LA, Rilli S, Carey RM: The renin-angiotensin-aldosterone system, glucose metabolism and diabetes. *Trends Endocrinol. Metab.* 16(3), 120–126 (2005).

100 Lastra-Lastra G, Sowers JR, Restrepo-Erazo K *et al.*: Role of aldosterone and angiotensin II in insulin resistance: an update. *Clin. Endocrinol.* 71(1), 1–6 (2009).

▪ **Recent review that describes the role of angiotensin II and aldosterone in the pathogenesis of insulin resistance.**

101 Ryan MJ, Didion SP, Mathur S *et al.*: PPAR(gamma) agonist rosiglitazone improves vascular function and lowers blood pressure in hypertensive transgenic mice. *Hypertension* 43(3), 661–666 (2004).

102 Fujishima S, Ohya Y, Nakamura Y *et al.*: Troglitazone, an insulin sensitizer, increases forearm blood flow in humans. *Am. J. Hypertens.* 11(9), 1134–1137 (1998).

103 Nolan JJ, Ludvik B, Beerdsen P *et al.*: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N. Engl. J. Med.* 331(18), 1188–1193 (1994).

104 Benson SC, Pershadsingh HA, Ho CI *et al.*: Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 43(5), 993–1002 (2004).