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

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 neurohumoral 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...
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
Compared with matched Caucasians. At the cellular level, loss of NO bioavailability is attributed to excessive \( \mathbf{O}_2^+ \) and ONOO\(^-\) generation by NAD(P)H oxidase, which ultimately leads to uncoupled eNOS activity. 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. Indeed, agents that reverse eNOS uncoupling and reduce oxidative stress have been shown to lower BP while improving NO bioavailability.

**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. 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 IP3-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 AT1 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 O2·− 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 AT2 receptors reverses certain effects mediated by the AT1 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 O2·− 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 P47phox-transcriptional and -translational regulation. The increase in oxidative stress reduced eNOS activity via BH4 oxidation and Ser1177 dephosphorylation in a mineralocorticoid 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 BH4 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 athero-protective 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 AT2 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
Review of P2Y-purinoceptors

NO release through the subsequent stimulation by activating the efflux of ATP, which increases up to 4 years and pulse wave analysis in 2199 patients for measurements by radial artery applanation tonometry, aortic pressure and hemodynamic variables were measured. 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. Central aortic pressure is determined by cardiac output and peripheral vascular resistance – factors that are influenced by arterial stiffness and pressure wave reflections. 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 1-NG-monomethyl arginine (1-NMMA). Under identical conditions, other DHP- and non-DHP-type CCBs,
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 [49]. Nifedipine was also shown to stimulate NO release and scavenge $O_2^-$ in isolated cultured rabbit endothelial cells [54]. 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 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 1-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, AT1 receptor antagonists allow uninhibited stimulation of AT2 receptors, resulting in important signal transduction events and vascular benefits. AT2 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 AT2 receptor antagonist, consistent with AT2 receptor-mediated NO release [56]. In coronary arteries, activation of
AT1 receptors by a low concentration of Ang II causes vasoconstriction by increasing NAD(P)H oxidase and subsequent O2·− production. By contrast, higher concentrations of Ang II promote vasodilatation by activating AT2 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 physiologic 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 it 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 O2·− 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/p22phox 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 BH4 levels and reduced O2·− and ONOO− production [33]. As oxidation and depletion of eNOS cofactor BH4 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 AT1 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$_2^\cdot$ 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, co-administration 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 notwithstanding 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 Ser312 and Ser616 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, 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 non-genomic 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.

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### Executive summary

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<tr>
<th><strong>Differential impact of antihypertensive drugs on central versus brachial arterial blood pressure</strong></th>
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<td>Central arterial pressure may be a superior predictor of cardiovascular events as compared with conventional brachial artery recordings of hypertension.</td>
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<td>Certain antihypertensive agents have differential effects on central aortic pressure and hemodynamics despite similar changes in blood pressure over the brachial artery.</td>
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<td>The results of clinical studies showed that central aortic pressure predicted clinical outcomes for a CCB/ACE inhibitor combination but not other combination treatments.</td>
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<th><strong>Evaluation of combination therapy with RAS &amp; calcium channel blockade</strong></th>
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<td>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.</td>
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<td>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.</td>
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<th><strong>Effects of hyperglycemia, insulin resistance &amp; hypertension on endothelial function</strong></th>
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<td>A disproportionate number of people with diabetes and insulin resistance are also hypertensive, resulting in higher cardiovascular risk in this population group.</td>
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<td>These disease processes share common pathophysiologic mechanisms, including endothelial dysfunction, which lead to oxidative stress and inflammation.</td>
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<th><strong>Future perspective</strong></th>
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<td>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.</td>
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Calculated channel blockers & inhibitors of the renin–angiotensin system for treating hypertension

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 response to stimuli of vasodilation, resulting in 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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No writing assistance was utilized in the production of this manuscript.

Bibliography
Papers of special note have been highlighted as:
* of interest
** of considerable interest

** 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.


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


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


- Important review that describes the molecular basis for endothelial dysfunction.


- Initial study that reported improvement in nitric oxide release and reduction in atherogenesis with aliskiren treatment.


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


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


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


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


Calcium channel blockers & inhibitors of the renin–angiotensin system for treating hypertension


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).


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).


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.

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

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