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REVIEW

Reinventing the ACE inhibitors: some old and new implications of ACE inhibition

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Since their inception, angiotensin-converting enzyme (ACE) inhibitors have been used as first-line therapy for the treatment of cardiovascular and renal diseases. They restore the balance between the vasoconstrictive salt-retentive and hypertrophy-causing peptide angiotensin II (Ang II) and bradykinin, a vasodilatory and natriuretic peptide. As ACE is a promiscuous enzyme, ACE inhibitors alter the metabolism of a number of other vasoactive substances. ACE inhibitors decrease systemic vascular resistance without increasing heart rate and promote natriuresis. They have been proven effective in the treatment of hypertension, and reduce mortality in congestive heart failure and left ventricular dysfunction after myocardial infarction. They inhibit ischemic events and stabilize plaques. Furthermore, they delay the progression of diabetic nephropathy and neuropathy and act as antioxidants. Ongoing studies have elucidated protective roles for them in both memory-related disorders and cancer. Lastly, N- and C-domain selective ACE inhibitors have led to new uses for ACE inhibitors.
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INTRODUCTION

In recent years, stressful and fiercely competitive lifestyles and food habits have compounded the problems of hypertension. Long-standing and stressful, progressively rising hypertension can lead to many disorders, including myocardial infarction (MI), cerebrovascular events, congestive heart failure, peripheral arterial insufficiency, premature mortality[1] and renal dysfunction leading to glomerulosclerosis and kidney artery aneurysm.[2]

A number of therapies are available, but angiotensin-converting enzyme (ACE) inhibitors have been the preferred first-line therapy for hypertension, congestive heart failure, left ventricular (LV) systolic dysfunction and MI.[3,4] ACE inhibitors (ACEis) have been in use for the past two decades, and the interest in them is still growing. Recently, the discovery of domain-selective ACEis and new members of the renin–angiotensin system (RAS) (that is, angiotensin-converting enzyme 2) have again fueled the interest of researchers. Some new studies have expanded the already impressive clinical profile of ACEis. This review traces some already known and new facets of ACE inhibition and introduces new advances in the designing of a new generation of ACEis.

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

The ACE (or kininase II) is a bivalent dipeptidyl carboxyl metallopeptidase, which is a membrane enzyme in endothelial, epithelial and neuroepithelial cells and the brain; it is also present in a soluble form in blood and numerous body fluids.[5] ACE cleaves the C-terminal dipeptide from Ang I and bradykinin, thus interacting with RAS and the kallikrein–kinin system simultaneously.

It can be concluded that ACE has a pivotal role in the balance between vasodilatory and natriuretic properties of bradykinin, and vasoconstrictive and salt-retentive properties of Ang II. An increase in ACE activity disturbs this delicate balance and promotes vasoconstrictive and salt-retentive Ang II (Figure 1) and decreases vasodilatory and natriuretic bradykinin. The ACEis restore this balance by decreasing the formation of Ang II and the degradation of bradykinin (Figure 2).

A number of ACEis are currently in the market. The ACEis differ in the chemical structure of their active moieties, as well as in their potency, bioavailability, plasma half-life, route of elimination, distribution and affinity for tissue-bound ACE, and whether they are administered as prodrugs. The ACEis may be classified into three groups according to the chemical structure of their active moiety. Captopril is the prototype of the sulfhydryl-containing ACEis; others are fentiapril, pivalopril, zofenopril and alacepril. Fosinopril is the only ACE inhibitor containing a phosphinyl group as its reactive moiety. The majority of other ACEis, like lisinopril, enalapril and perindopril, contain a carboxyl moiety.[6]

CLINICAL IMPLICATIONS OF ACE INHIBITION

Essential hypertension

According to The Joint National Committee (JNC) VII, ACEis are one of the first-line drugs for hypertension. Patients with stage-I hypertension (systolic blood pressure (BP): 140–159 or diastolic BP:

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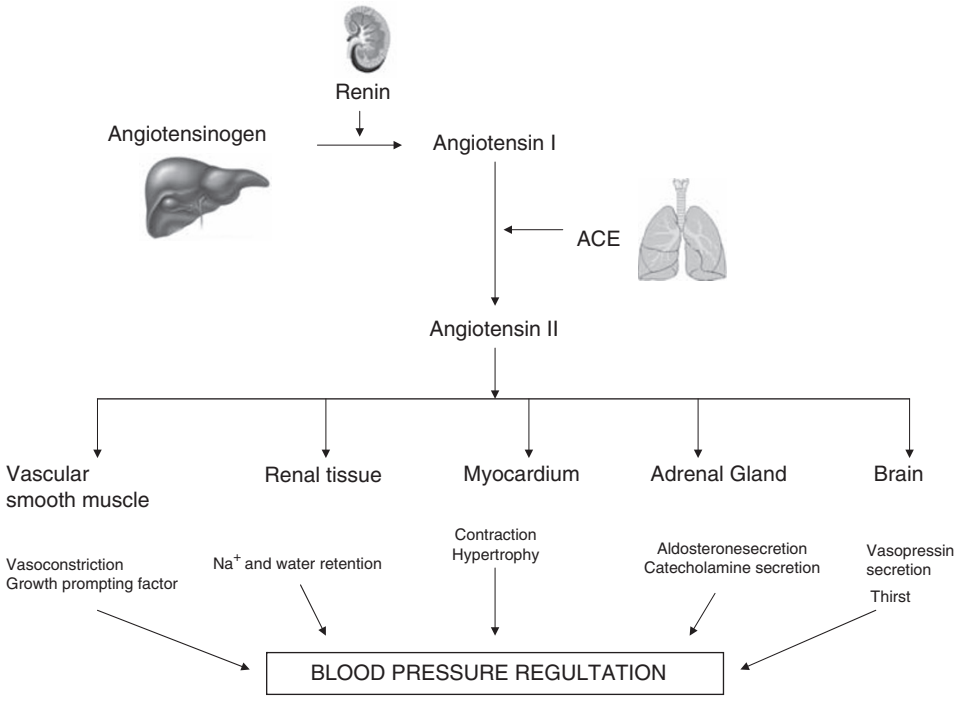

Figure 1 Classical view of the action of angiotensin-converting enzyme (ACE) on angiotensin (Ang) I and the effect of Ang II on different tissues.

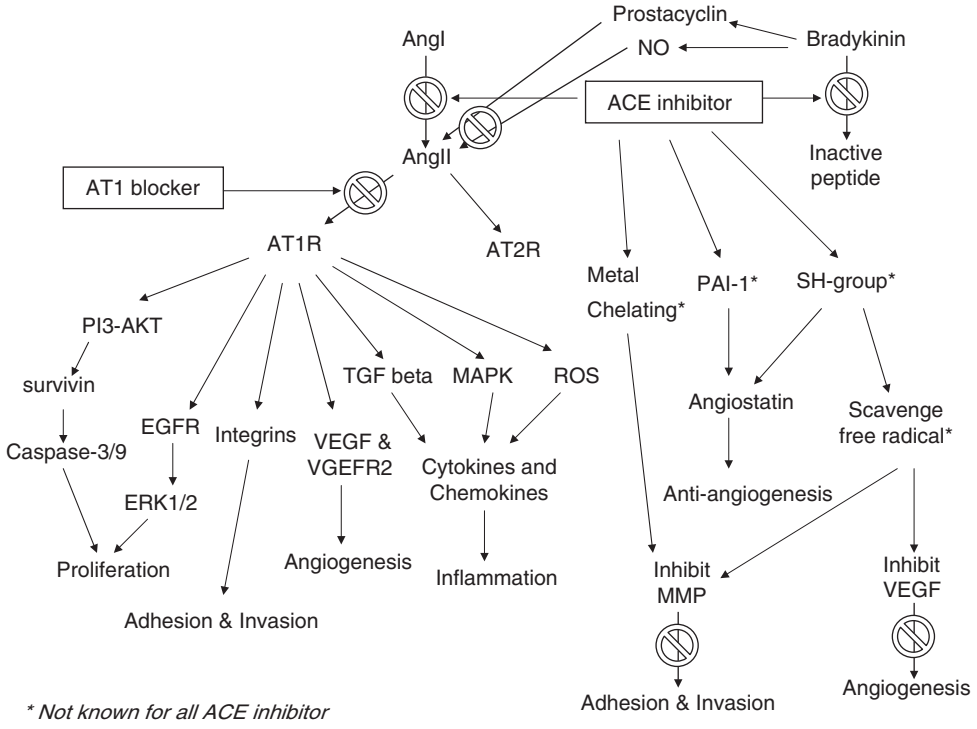

Figure 2 Effect of angiotensin-converting enzyme (ACE) inhibition on blood pressure and independent actions of angiotensin (Ang II).

90–99 mmHg) should be treated with ACEis.[7] The Antihypertensive and Lipid Lowering Therapy in Heart Attack Trial (ALLHAT) also suggests the use of ACEis as initial therapy alone or in combination with thiazide diuretics, with an overall response of 50–70% in mild to moderate disease.[8]

ACEis are effective in lowering the mean, systolic and diastolic pressures in hypertensive patients as well as in salt-depleted normotensive subjects.[9–11] The acute change in BP correlates with pretreatment plasma renin activity and angiotensin levels, such that the greatest reductions in BP are seen in patients with the highest plasma

renin activity.[12–15] In support of this, co-administration of drugs that increase plasma renin activity, such as diuretics, abolishes the racial differences in response to ACEis.[16] However, with long-term therapy, a greater percentage of patients achieve a decrease in BP, and the antihypertensive effect no longer correlates with pretreatment plasma renin activity.[13–15] The mechanism for this increased efficacy with chronic administration is not clear but may involve the kallikrein–kinin system or production of vasodilatory prostaglandins.[16]

One of the characteristics of ACEis is that they lower peripheral vascular resistance without causing a compensatory increase in heart rate[17–20] or changing baroreceptor activity.[21] They also show lack of reflex tachycardia[22] and cause inhibition of the normal tonic influence of Ang II on the sympathetic nervous system.[23] During ACE inhibition, heart rate responses to postural changes and exercise are not impaired.[24]

Atherosclerosis

In a recent study, it was observed that the antiatherosclerotic effect of quinapril is more potent than that of losartan in hypertensive patients, as quinapril reduced carotid artery intima-media thickness by a larger extent in patients with mild-to-moderate arterial hypertension than did losartan.[25]

Experimental evidence shows that ACE inhibition can retard the development of atherosclerosis. In animal models for atherosclerosis, including apolipoprotein E knockout mice,[26] Watanabe rabbits[27] and cholesterol-fed monkeys,[28] the ACEis reduce the extent of vascular lesions. Oxidatively modified low-density lipoprotein (LDL) has an important role in the development of atherosclerosis. ACE inhibition retards the development of atherosclerosis by reducing expression of LOX-1, the human endothelial receptor for oxidized LDL, in mammary artery biopsies of patients with coronary artery disease.[29] Furthermore, endothelial dysfunction in normotensive patients with coronary artery disease, hypertensive patients and patients with non-insulin-dependent diabetes mellitus is reversed by ACE inhibition.[30] The ACEis also improve endothelial function by attenuating the vasoconstrictive and superoxide radical-generating effects of Ang II while simultaneously enhancing the bradykinin-dependent induction of endothelial nitric oxide (NO) production. The ACEis promote ischemic preconditioning, probably through a bradykinin-mediated mechanism.[31] Studies *in vitro* and in humans suggest that ACEis favorably alter the fibrinolytic balance, both by decreasing Ang II, a potent stimulus for plasminogen activator inhibitor synthesis,[32,33] and by increasing bradykinin, a potent stimulus for tissue plasminogen activator secretion.[34,35] Trials like Heart Outcomes Prevention Evaluation (HOPE) suggest that ramipril, an ACE inhibitor, is beneficial in a broad range of patients with no evidence of LV systolic dysfunction or heart failure who are at high risk for cardiovascular events. Treatment with ramipril reduced the rates of death, MI, stroke, coronary revascularization, cardiac arrest and heart failure, as well as the risk of complications related to diabetes and diabetes itself.[36] In addition to decreasing Ang II and increasing bradykinin, a study demonstrated that the antiatherogenic effects of ACEis are also dependent on their active groups. Chronic treatment with sulfhydryl ACEis, like zofenopril and captopril, is more successful at reducing the susceptibility of plasma LDL to *in vitro* oxidation, formation of oxidation-specific epitopes in the arterial wall and atherogenesis in apolipoprotein E knockout mice.[37]

Congestive heart failure and LV dysfunction

Since 1987, several large, prospective, randomized, placebo-controlled trials have demonstrated that treatment with ACEis results in a reduction in overall mortality in patients with congestive heart failure due to systolic dysfunction.[38–42] The reduction in mortality has even been seen in patients with asymptomatic LV dysfunction.[43,44] This reduction in mortality results primarily from a reduction in the progression of congestive heart failure,[38,40,41] although the incidence of sudden death[40] and MI[43]may also decrease.

Although ACEis improve outcome in patients with systolic dysfunction, many patients with hypertension experience congestive heart failure due to diastolic dysfunction related to LV hypertrophy. In animal models, ACEis have been shown to reverse ventricular remodeling by blocking the trophic effects of Ang II on cardiac myocytes.[45,46] The ACEis have been shown to reverse LV hypertrophy in patients with hypertension.[19,47,48] A meta-analysis of the effects of several antihypertensive agents suggested that ACEis were the most effective agent for reducing LV hypertrophy.[49]

Myocardial infarction

Animal models and human studies demonstrated that ACEis attenuate LV remodeling after MI. Subsequently, large, randomized, controlled trials have shown that ACEis reduce cardiovascular and all-cause mortality, prevent or delay the onset of heart failure, and decrease the risk of stroke after a MI.[50] Early ACE inhibitor treatment ($\leqslant$36 h post-MI) is associated with significantly lower mortality both in the first week and in the first month after MI.[51] Long-term trials such as the Survival and Ventricular Enlargement (SAVE) trial, the Acute Infarction Ramipril Efficacy (AIRE) study and the Trandolapril Cardiac Evaluation (TRACE) study selectively enrolled high-risk patients with LV dysfunction or heart failure after MI and demonstrated a 20% reduction in mortality.[52] ACEis prevent or delay the detrimental cardiac remodeling after MI that apparently contributes to the worse prognosis after MI as shown by perindopril in a recent Perindopril and Remodeling in Elderly with Acute Myocardial Infarction (PREAMI) trial.[53] According to class I American College of Cardiology/American Heart Association guidelines,[54] The ACEis should be given to all patients with acute MI and anterior ST elevation, an ejection fraction of 40% or clinical heart failure in the absence of hypotension. Therapy should be continued for at least 6 weeks in all patients and indefinitely in patients with persistent LV dysfunction, although another study suggests a role for long-term ACE inhibition in all patients with ischemic heart disease.[55]

Diabetic nephropathy

Both RAS and increased glomerular capillary pressure have been implicated in the progression of renal dysfunction due to a number of renal diseases, including diabetic nephropathy.[56] In addition, Ang II causes mesangial cell growth and matrix production.[57,58] The ACEis reduce glomerular capillary pressure by decreasing arterial pressure and selectively dilating efferent arterioles.[59] Numerous animal studies and small clinical trials have suggested that ACEis significantly reduce the loss of kidney function in diabetic nephropathy.[60] ACEis prevent the progression of microalbuminuria to overt proteinuria.[61] A large, prospective, placebo-controlled study has shown that captopril slows the progression of nephropathy in patients with insulin-dependent diabetes mellitus, as measured by the rate of decline in creatinine clearance and the combined end points of dialysis, transplantation and death.[62] A second large-scale, prospective, double-blind study extended these observations by showing a protective effect of ACEis in patients with a variety of renal diseases, including glomerulopathies, interstitial nephritis, nephrosclerosis and diabetic nephropathy.[63] The exception was polycystic kidney disease. Importantly, the protective effect of ACE inhibition was independent of the severity of renal insufficiency.

In a recent study, it was observed that blockade of the RAS by ACEis in type-2 diabetis patients with diabetic nephropathy reduces urinary monocyte chemoattractant protein-1 levels and improves renal function. As monocyte chemoattractant protein-1 induces monocyte immigration and differentiation to macrophages, which augment extracellular matrix production and tubulointerstitial fibrosis, pharmacological reduction of Ang II may also exert its beneficial effects in diabetic nephropathy by downregulation of renal monocyte chemoattractant protein-1.[64]

Diabetic neuropathy

Angiotensin II increases oxidative stress in the kidney by stimulating NAD(P)H oxidase,[65–67] which is a primary source of reactive oxygen species in vascular tissue and a contributing factor in diabetic neuropathy.[68,69]

In addition to nephropathy and hypertension, ACEis are also effective against diabetic neuropathy. In diabetic rats, lisinopril treatment prevented nerve dysfunction.[70] In studies of human diabetes, two small clinical trials demonstrated that diabetic neuropathy was improved by treatment of patients with trandolapril or lisinopril.[71,72]

Diabetes causes impairment in vascular relaxation in response to acetylcholine and calcitonin gene-related peptide in epineurial arterioles of the sciatic nerve.[73,74] Acetylcholine-induced vascular relaxation is endothelium dependent and mediated by NO and endothelium-derived hyperpolarizing factor, and both are impaired by diabetes.[73,75,76] Moreover, impairment by diabetes of acetylcholine-mediated vascular relaxation of epineurial arterioles precedes the slowing of motor nerve conduction velocity suggesting that vascular dysfunction contributes to impaired nerve activity.[77] In a study by Coppey *et al.*,[78] it was demonstrated that the ACE inhibitor enalapril was more effective than the angiotensin 1 (AT1) receptor blocker, L-158809, at reversing diabetes-induced impairment of endoneurial blood flow and motor nerve conduction velocity. Furthermore, enalapril treatment was more capable of preventing/reversing the diabetes-induced impairment of relaxation by acetylcholine and calcitonin gene-related peptide. It was demonstrated that treatment of diabetic rats with enalapril reduced superoxide formation by 50–75% in epineurial arterioles of the sciatic nerve, and with L-158809 treatment the decrease in superoxide level in epineurial arterioles was minimal. The improvement of acetylcholine-mediated vascular relaxation may be due to increased formation of NO capable of overcoming quenching by superoxide and/or improved formation/activity of endothelium-derived hyperpolarizing factor.[79–81] In this regard, Kihara *et al.*[82] have demonstrated that treatment of diabetic rats with ACEis improves diabetic neuropathy by increasing NO synthase synthesis.

ACE inhibitors as antioxidant

In addition to reducing levels of Ang II and increasing bradykinin, ACEis have important implications for vascular oxidative stress. All major cell types of the vascular wall (endothelium, smooth muscle and fibroblasts) contain the enzyme NADPH oxidase, which is responsible for the production of superoxide anion,[83] which is activated in response to Ang II[84] and leads to both hypertension[85] and smooth-muscle cell hypertrophy.[86] Experimental models of atherosclerosis have also demonstrated activation of the local RAS and vascular NADH/NADPH oxidase activity.[87]

The ACEis represent a new antioxidant strategy that targets oxidative stress at its source. ACE inhibition, by decreasing Ang II, limits the stimulation of vascular NAD(P)H oxidase, thereby preventing the increased superoxide flux associated with activation of the RAS.[88] As superoxide reacts with NO, ACE inhibition also improves NO bioactivity, as has been observed in patients with coronary artery disease[89] and in experimental models for hypertension.[90] The ACEis limit the formation of H_2O_2, which is responsible for smooth muscle cell proliferation, and this slows the progression of carotid intimal thickening as described in the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE).[91]

The antioxidant effect of ACEis is BP independent. *In vivo*, ACE inhibition by zofenopril for 6 weeks in Watanabe heritable hyperlipidemic rabbits reduced plasmatic LDL oxidation and atherosclerosis.[92] *In vitro*, a number of ACEis (thiols and nonthiols) showed a direct inhibitory effect on LDL oxidation in the presence of copper.[93] Angiotensin II can increase 12-lipoxygenase activity in vascular smooth muscle cells[94] and macrophage lipoxygenase activity through AT1 receptor (AT1R)-mediated mechanisms.[95] Moreover, Ang II-mediated macrophage lipid peroxidation was found to involve the action of cellular NADPH oxidase as well as 15-lipoxygenase.[96] It has been suggested that lipoxygenase and NADPH oxidase mediate the oxidative modification of LDL.[97] Thus, the antioxidative effect of ACEis seems to be mediated by decreasing the expression of the lipoxygenase enzyme.

Plaque stabilization

Angiotensin II stimulates endothelin release. Endothelin is one of the most potent coronary vasoconstrictors, and its local release might, in the presence of a susceptible atherosclerotic lesion, accelerate plaque rupture.[98,99] Hypomagnesemia has also been shown to cause an increase in coronary vascular reactivity and could potentially accelerate plaque rupture.[100,101] Ang II can trigger most of the changes reported to be associated with magnesium deficiency, including induction of pro-oxidant and proinflammatory conditions.[102] Moreover, Ang II induces the synthesis of plasminogen activator inhibitor type 1 in endothelial cells. Therefore, activated RAS accelerates the formation of coronary artery plaque and thrombus associated with hypertension. This results in destabilization of arteriosclerotic plaques.[103]

The ACEis have a role in reducing the propensity for plaque rupture. A reduction in BP and pulse rate with the use of ACEis may reduce the propensity for plaque disruption by reducing circumferential stress on the fibrous caps of lipid-rich plaques.[104] Other mechanisms of preventing plaque rupture include reduced levels of neurohumoral activation and effects on protein synthesis influencing plaque composition as Ang II stimulates vascular smooth muscle growth and proliferation. The prevention of ischemic events by ACEis also involves magnesium. Barbagallo *et al.*[105] showed that magnesium influences cellular response to ischemia and that the ability of thiol compounds, such as captopril, to ameliorate ischemic injury may be, at least in part, due to their ability to increase cytosolic free magnesium levels.

Memory-enhancing effect of ACEis

In 2002, Amenta *et al.*[106] reviewed the majority of controlled clinical trials and observed that ACEi treatment (including perindopril, captopril and lisinopril) positively influenced cognitive function independently of its BP-lowering effects, and patients treated with ACEi displayed better results than those on diuretics and β-blockers. A number of animal studies on the cognitive effects of ACEis have also corroborated this observation. Treatment with 24 mg/kg/day enalapril in streptozotocin-treated rats for 14 weeks from the onset of diabetes significantly improved memory performance in the water maze

compared to untreated diabetics.[107] Long-term administration of the ACE inhibitor captopril (400 mg/l through the drinking water), either from the time of weaning or from the age of 6 months (that is, several months after hypertension was established), improved performance in working memory spatial task in both spontaneously hypertensive rats and WKY at 24 months of age.[108]

Even in clinical situations the use of ACEis has prevented cognitive decline in patients. The Perindopril Protection Against Recurrent Stroke Study (PROGRESS), a large, randomized, double-blind, placebo-controlled trial conducted among patients with prior stroke or transient ischemic attack, also showed that ACEi has positive effects on memory. The treatment group showed a risk reduction in cognitive decline of 19%, and the composite risks of dementia with recurrent stroke and of cognitive decline with recurrent stroke declined by 34 and 45%, respectively.[109] These findings indicate that an increase in cerebral perfusion may be responsible for the observed reduction in cognitive deficit after ACEi treatment. The mechanism behind the positive effect of ACEis on memory is ambigous. The ACE is a promiscuous enzyme; apart from Ang II and bradykinin, a number of other neuropeptide substrates have been reported for ACE, including substance P, neurotensin, dynorphin and enkephalin.[110] Some of these neuropeptide substrates have memory-enhancing properties; substance P, after peripheral or central application, has reinforcing and memory-facilitating effects inducing place preference[111,112] and avoidance learning;[113,114] dynorphins have an improving role in memory-impairment paradigms, such as basal forebrain lesions[115] and mecamylamine-induced[116] and galanin-induced impairment[117] of learning and memory.

ACEi treatment has been shown to increase hippocampal blood flow and improve hippocampal long-term potentiation in diabetic rats compared with untreated diabetics.[107] These results suggest that ACEi may act through the hippocampal acetylcholine system, which is essential for memory,[118,119] to exert their positive effects on cognition.

Anticancer effect of ACEis

The RAS[120] and angiotensin receptors[121] have such an important role in cancer that the Ang II–AT1R system has begun to be viewed as a therapeutic target for cancer.[122,123] Ang II has an anti-apoptotic role involving AT1R/phosphatidylinositol 3-kinase/Akt activation and the subsequent suppression of activation of caspase-9[124] and caspase-3.[125] Ang II also reduces cell adhesion and invasion through the type 1 receptor, and this effect may be due to reduced expression of integrins, in particular $\alpha 3$ and $\beta 1$.[126] Ang II acts through AT1Rs to enhance vascular endothelial growth factor expression resulting in endothelial cell proliferation, migration and angiogenesis.[127,128] Activation of AT1R is also reported to induce inflammatory cytokines and chemokines through mitogen-activated protein kinase, reactive oxygen species and nuclear factor-κB pathways[129,130] (Figure 2). All these processes mediated by AT1 are important in the induction and progression of cancer.

Several *in vivo* studies in tumor models have revealed that ACEis inhibit tumor growth[131] and angiogenesis.[132,133] The beneficial effect of ACEis in suppressing tumor growth is primarily mediated through RAS-dependent inhibition of Ang II levels and increases in bradykinin levels.[134] Another anti-cancer activity of some ACEis is probably due to their intrinsic metal-chelating properties, which are thought to be responsible for inhibition of matrix metalloproteinase.[135,136] The direct effect of some ACEis is the reduction of plasminogen activator inhibitor and another effect is due to their free sulfhydryl group, which leads to generation of angiostatin, which in turn inhibits angiogenesis.[137] The sulfhydryl group present in ACE inhibitor also serve as a free radical scavenger.[138] The reduction of reactive oxygen species prevents subsequent activation of matrix metalloproteinase and vascular endothelial growth factor, thus preventing further invasion and angiogenesis as shown in Figure 2.

However, a few reports have suggested that ACEis may actually promote tumor growth, possibly due to their immunomodulatory activity,[139] and patients treated with ACEis have been reported to have higher rates of kidney cancers.[140] However, several other clinical studies have shown that ACEis do not increase the risk of cancer.[141,142] A prospective study in Glasgow in 5027 patients has shown that ACEis may protect against cancer[143] and are effective in managing post-anthracycline cardiotoxicity in pediatric cancer survivors without increasing any risk of cancer.[144] The role of ACE in cancer is further supported by the findings that ACE polymorphism has been found to be associated with several cancers, including prostate, gastric and breast cancer.[145–147]

Thus, several studies support anti-cancer effects of ACEis through RAS-dependent and/or -independent mechanisms, and these inhibitors may help us understand interactions between components of RAS in tumorigenesis and design new anti-cancer agents.

LOCAL RAAS: TARGET ORGAN PROTECTION AND ACEIS

Renin–angiotensin–aldosterone system (RAAS) is also present in a wide variety of organs (tissue RAAS) promoting the local synthesis of Ang II. This local RAAS system has an important role in the development of hypertensive target organ damage.[148] Apart from reducing BP, ACEis also obstruct local RAAS and protect against target organ damage, reversing or preventing its development

Experimental studies in rats demonstrated that treatment of spontaneously hypertensive rats for 12 weeks with either antihypertensive or non-antihypertensive ACEis reduced interstitial fibrosis and normalized ventricular stiffness, whereas normalization of BP and regression of cardiac hypertrophy were achieved only with an antihypertensive dosage of ACEi. They also confirmed that regression of fibrosis is independent of changes in BP and cardiac hypertrophy.[149]

The presence of local RAAS also extends to kidneys. Local activation of RAAS causes glomerular injury by further raising glomerular capillary pressure through Ang II-induced vasoconstriction of the efferent arterioles.[150] RAAS may also cause proteinuria through an effect on the expression of renal nephrin, a transmembrane protein located in glomerular podocytes. ACEis decrease proteinuria and delay the progression of renal disease in patients with nondiabetic kidney disease. In the Angiotensin-Converting Enzyme Inhibition in Progressive Renal Insufficiency (AIPRI) study, benazepril caused a 53% reduction of the overall risk in terms of reducing serum creatinine and need for dialysis in nondiabetic patients with chronic kidney disease as compared with placebo. In this trial, the benefit of ACE inhibition was greatest in patients with the highest level of proteinuria.[151]

Endothelial dysfunction seems to be a marker of disease progression and of increased risk of cardiovascular morbidity and mortality.[152] In a study by Ghiadoni *et al.*,[153] treatment with nifedipine, amlodipine, atenolol, nebivolol, telmisartan and perindopril similarly reduced BP, but only perindopril increased flow-mediated endothelium-dependent dilation in conduit arteries without modifying the response to glyceryl trinitrate.

LOOKING BEYOND ACE INHIBITION

The ACE is a promiscuous enzyme; apart from Ang II and bradykinin, a number of other neuropeptide substrates have also been reported for ACE, including substance P, neurotensin, dynorphin, and

enkephalin.[110] For this reason ACEis have diverse effects independent of their BP-lowering effect (Figure 2). Indeed, different ACEis like moexiprilate, ramipirilate, captopril, enalpril and quinaprilate amplified the effects of bradykinin in vessels that lacked measurable ACE activity[154] and also enhanced the effect of an ACE-resistant B_2-kinin receptor agonist. These findings demonstrate that ACEis selectively potentiate the B_2-receptor-mediated vascular effects of bradykinin independently of their ACE-inhibiting properties, and this might be related to differences in molecular structure.[154,155] ACEis also induce cross-talk between the transmembrane protein ACE and the B_2-kinin receptor, probably by formation of a heterodimer. This protects high-affinity receptors, blocks receptor desensitization and decreases internalization, thereby potentiating BK beyond blocking its hydrolysis.[156,157] ACEis also directly activate the bradykinin B_1 receptor by acting at the zinc-binding pentameric consensus sequence HEXXH (195–199) of the B_1 receptor, a motif that is present in the active center of ACE but absent from the B_2 receptor. ACEis, when activating the B1 receptor, elevate intracellular calcium ($[Ca^{2+}]i$) and release NO from cultured cells.[158]

ACEis also induce phosphorylation of the ACE intracellular tail (Ser^{1270}) via CK2, resulting in outside-in signaling that enhances expression of ACE and cyclooxygenase-2.[159] The effect of the ACE inhibitor on cyclooxygenase-2 is due to the transcription factor activator protein-1. This results in increased release of prostacyclin and prostaglandin E_2 by endothelial cells, which is independent of local accumulation of kinins.[160]

In addition to the kallikrein–kinin system, ACE also interacts with the opioidergic system. Hypertension and reduced pain perception have been repeatedly associated in animals and in humans. A study investigating pain threshold by hot-plate test in normotensive Wistar-Kyoto rats and spontaneously hypertensive rats after ACE inhibitor treatment observed a normalization of increased hot-plate latencies, whereas pain sensitivity was unaffected by hydralazine, which is otherwise effective at reducing BP.[161] Hypertensive patients show a reduced sensitivity to pain that is independent of the method used to induce the painful stimulus.[162,163] Takai *et al.*[163] investigated the involvement of endogenous brain Ang II in nociception in mice by using ACEis and an Ang II antagonist. Significantly longer jump latencies were obtained for the groups repeatedly treated with spirapril, trandolapril and losartan, and these antinociceptive effects were reversed by naloxone.

Clinical studies also confirm an interaction between the RAS and endogenous opioids. Guasti *et al.*,[164] using dental pain perception evaluation by means of a pulpar test, showed that the pain threshold was significantly higher in hypertensive than in normotensive subjects. However, the pain threshold and tolerance in the patients decreased to levels similar to those of normotensives during treatment with enalapril. Kalra *et al.*[165] evaluated the effect of ramipril (an ACEi) and losartan (an AT1R blocker (ARB)) on pain perception in human volunteers and observed that groups receiving either ramipril or losartan showed a decline in the threshold for maximum tolerated pain, but only ramipril (and not losartan) decreased the pain perception threshold.

ADVERSE EFFECT OF ACEIS

Like all antihypertensive agents, ACEis can cause hypotension particularly in patients with elevated plasma renin activity. Therefore, lower starting doses should be used under these conditions.[166] ACEis can cause hyperkalemia because of a decrease in aldosterone,[167] especially in patients with impaired kidney function or in patients who are taking potassium supplements (including salt substitutes) or potassium-sparing diuretics.[168,169] ACEis can cause a reversible decline in renal function in the setting of decreased renal perfusion due to bilateral renal artery stenosis,[170] severe congestive heart failure[171] or volume depletion.[172]

Coughing is a frequent side effect of ACEis. The mechanism is not known but may involve increased levels of bradykinin or substance P and stimulation of vagal C fibers.[173] Thromboxane antagonism, aspirin and iron supplementation reduce coughing induced by ACEis.[166] Angioedema, a rare but potentially life-threatening side effect of ACEis, is characterized by localized swelling of the lips, tongue, mouth, throat, nose or other parts of the face. The mechanism seems to involve bradykinin or one of its metabolites.[173]

If administered in the second or third trimester of pregnancy, ACEis can cause oligohydramnios, fetal growth retardation, pulmonary hypoplasia, joint contractures, hypocalvaria, neonatal renal failure, hypotension and death. These effects result from blockade of the conversion of Ang I to Ang II in the developing fetal kidneys leading to fetal hypotension.[174] For this reason, ACEis should be stopped once a pregnancy has been confirmed.

Adverse effects that seem to be related to the presence of a sulfhydryl group are neutropenia, nephrotic syndrome and skin rash.[175] The incidence is higher in patients who have renal insufficiency or collagen vascular disease. Skin rashes occur in 1% of patients and usually consist of a pruritic maculopapular eruption; rarely, exfoliative dermatitis has been reported. The rash seems to be dose related.[6]

ACEIS *VS.* AT1R BLOCKERS (ARBS)

Accumulating data show that ARBs are on a par with ACEis in a number of clinical benefits. However, it is now clear that ARBs and ACEis have some distinct differences in their beneficial effects as evidenced by clinical studies using ACEis and ARBs.

One factor, the effect on bradykinin, is likely to favor ACE inhibition over ARB therapy. As discussed above, ACE inhibition prevents the breakdown of bradykinin, which is a vasodilatory peptide. The effect of ACE inhibition became attenuated when bradykinin receptor antagonists were used, suggesting that bradykinin has an essential role in the antihypertensive action of ACEis.[176]

The Blood Pressure Lowering Treatment Trialist's Collaboration (BPLTT) showed comparable BP-dependent reductions in risk with ACEis and ARBs, but only ACEis elicited a statistically significant BP-independent reduction in relative risk of coronary disease events of 9%, whereas no such effect was reported with ARBs.[177] Similarly, Strauss and Hall[178] reported a consistent reduction in MI and cardiovascular death with ACEis, but a number of ARB trials in high-risk patients demonstrated an almost complete lack of reduction in MI and mortality despite significant reductions in BP. Nine of 11 key clinical ARB trials have even reported an excess of MI that achieved statistical significance in two cases: VALUE (VAlsartan antihypertensive Long-term Use Evaluation) and CHARM-Alternative.

However, in the case of stroke prevention, treatment with ARBs as compared with that using ACEis provides neuroprotection from focal cerebral ischemia in animal studies.[179] In this neuroprotection by ARB, AT2 receptor has an important role. Stimulation of the AT2 receptors in neurons in the brain region adjacent to the infarct area induces vasodilatation by locally synthesized NO and prostacyclin, thus improving cerebral blood flow by collateral circulation.[180,181] Selective blockade of central AT2 receptors abolishes the neuroprotective effect of ARBs.[180]

A limitation of ACEis in preventing stroke is further exhibited in PROGRESS, which showed that the ACE inhibitor perindopril caused only a 5% reduction in stroke, compared with a 43% stroke

reduction when the diuretic, indapamid, was added to the ACE inhibitor.[109] This finding can be explained on the basis of the hypothesis of Fournier *et al.*,[182] which states that in stroke prevention, diuretics, calcium antagonists and ARBs, which increase Ang II formation, have an edge over ACEis and β-blockers, which decrease Ang II formation. However, in the HOPE study, ramipril caused a 32% reduction of strokes because it reduced cardiac complications three-fold and prevented plaque destabilization, which otherwise could have caused stroke.[182]

To settle the debate regarding ACEis *vs.* ARBs, more large-scale clinical trials are needed. In the words of J McMurray,[183] we might propose that 'because ARBs are more costly than ACEis, their primary value is as an alternative for patients who cannot tolerate ACEis because of cough.'

FUTURE OF ACEIS

Owing to the pivotal role of RAS in cardiovascular pathophysiology, there is continued interest in new compounds that can target this system. Of particular interest are the vasopeptidase inhibitors. These are dual ACE–neutral endopeptidase inhibitors, and omapatrilat is the most advanced in clinical development. Neutral endopeptidase, a zinc-metallopeptidase, degrades vasodilatory and diuretic natriuretic peptides that reduce volume loading and are therefore beneficial in both hypertension and heart failure.[184] As expected, omapatrilat has shown better results than ACEis in clinical trials, but recent larger clinical trials have revealed a problematic incidence of angioedema with omapatrilat.[185,186] Both ACE and neutral endopeptidase inhibit bradykinin degradation, and bradykinin has been implicated in the angioedema associated with ACE inhibition.[187] Therefore, dual ACE–neutral endopeptidase inhibition may result in a higher incidence of angioedema.[188]

For these reasons, single-domain inhibitors of ACE offer an attractive alternative. The N- and C-domain sites of ACE hydrolyze Ang I and BK at comparable rates *in vitro*, but *in vivo* it seems that the C-domain is primarily responsible for regulating BP.[189] Ang I is hydrolyzed predominantly by the C-domain *in vivo*,[189] whereas BK is hydrolyzed by both domains.[190] Therefore, selective inhibition of the C-domain site will allow some level of BK degradation to continue, catalyzed by the N-domain. This may prevent excessive BK accumulation during angioedema.[191] Moreover, a C-selective inhibitor will have a lower propensity for excessive B_2 receptor stimulation by BK, which is maximal when both the N- and C-domains are inhibited.[192] Therefore, a highly selective C-domain inhibitor has the potential for effective BP control with reduced vasodilator-related side effects.

In contrast to a C-selective inhibitor, an N-selective inhibitor might open up new therapeutic areas. The N-domain has a minor role in BP control *in vivo*. At least three physiologically important peptides are hydrolyzed preferentially or exclusively by the N-domain: luteinizing hormone releasing hormone, Ang 1–7 and AcSDKP (acetyl-Ser-Asp-Lys-Pro).[193–195] There is increasing evidence that ACE is the principal metabolizing enzyme for AcSDKP, a natural hemoregulatory hormone.[196] AcSDKP has antiproliferative and antifibrotic activities, and it protects hematopoietic stem cells against chemotherapy-induced injury[196] and limits cardiac fibrosis.[197]

The current-generation ACEis in clinical use are essentially mixed N- and C-domain inhibitors.[198] Captopril has been noted to be modestly N-selective depending on Cl^- concentration, whereas lisinopril and enalaprilat are more C-selective[194,199] (Figure 3). Keto-ACE, originally described in 1980, was found to exhibit a 40- to 50-fold C-selectivity.[195] One of the bradykinin potentiator peptides, BPPb, was shown to be 300-fold more C-selective,[200] and the phosphinic tetrapeptide RXPA380 is 3000-fold more C-selective.[190] However, BPP-12b is 30-fold more N-selective, and the phosphinic tetrapeptide RXP407 is 1000-fold more N-selective[201–203] (Figure 3).

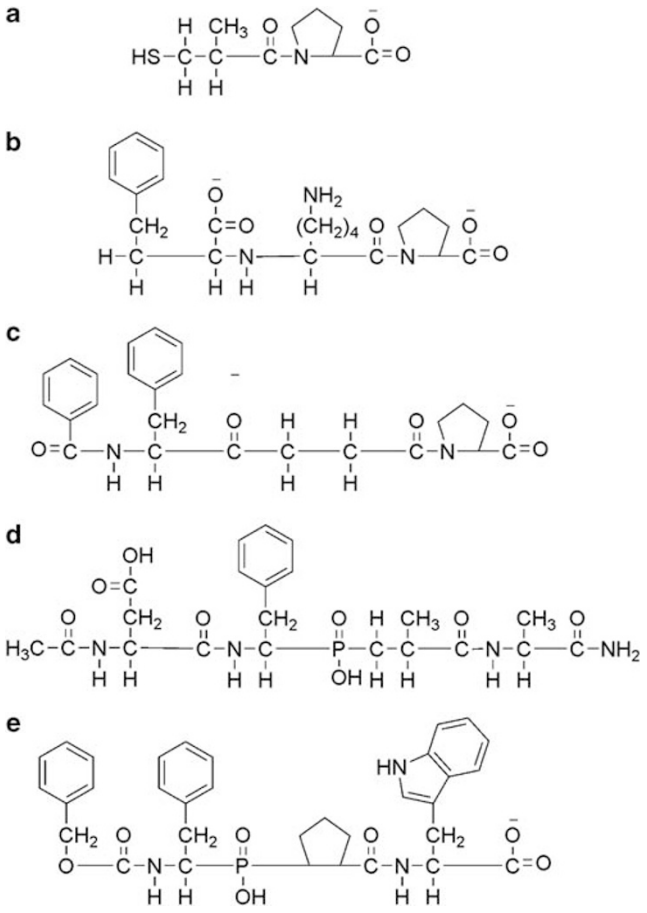

Figure 3 Structures of different angiotensin-converting enzyme (ACE) inhibitors: (**a**) captopril, (**b**) lisinopril, (**c**) keto-ACE, (**d**) RXP407 and (**e**) RXPA380.

CONCLUSION

A few years ago, it was thought that we knew all about RAS and much about its regulation. However, the clinical applications of ACEis are widening from cardiovascular to other related morbidities. Improvement of the patient's cardiovascular risk by blockade of the RAS is caused primarily by BP reduction, but additional nonhemodynamic effects contribute as well. The beneficial effects of ACEis have gone beyond ACE inhibition, and their role in noncardiac functions is also strengthening. New aspects of the RAS, such as ACE 2, continue to emerge and could become targets for new therapeutic strategies. These new facets of RAAS will continue to fuel the interest of researchers to explore this system further.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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