Interactions of Angiotensin-Converting Enzyme, Kinins and Nitric Oxide in Circulation and the Beneficial Effects of ACE Inhibitors in Cardiovascular Diseases

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Key words: angiotensin-converting enzyme inhibitors, cardiovascular disease

Abstract

Renin-angiotensin-aldosterone systems play a critical role in the development and progression of cardiovascular diseases, and inhibitors of angiotensin-converting enzyme have proven effective for the treatment of these diseases. Since angiotensin II receptor antagonists can inhibit the effects of angiotensin II via ACE-independent pathways, e.g., chymase, they were considered to be more effective than ACEIs. On the other hand, ACE inhibitors can increase bradykinin, and thus, nitric oxide, which may cause potent cardioprotection, inhibition of smooth muscle proliferation and attenuation of inflammation mechanisms. It appears that angiotensin II receptor antagonists and ACEIs may mediate cardioprotection in different ways. This is the rationale to explore the possibility of a combined administration of both drugs for the treatment of chronic heart failure and other cardiovascular pathology. In this review we try to analyze the role of ACE, kinins and chymase inhibition in the pathophysiology and treatment of cardiovascular diseases.

IMAJ 2000;2:929–934

Angiotensin-converting enzyme is a transmembrane zinc metallopeptidase that cleaves carboxy-terminal dipeptides from several peptides and is expressed in great amounts in vascular endothelial cells [1]. A soluble form of the enzyme is found in plasma, which is presumably derived from the membrane-bound form by proteolytic cleavage. ACE plays a major role in the regulation of vascular tone by converting the biological inactive decapeptide angiotensin I (ANG I) into the vasoconstrictor and proliferative octapeptide angiotensin II (ANG II). In a similar manner, ACE inactivates the vasodilatory nonapeptide bradykinin, which derives from a number of different sources [2].

Several investigators reported regulation of ACE expression/activity by nitric oxide. Chronic inhibition of endothelial NO synthase led to an up-regulation of cardiac and vascular ACE activity [3]. An inverse relationship between ACE expression/activity and the NO system was found in hypertensive rats after long-term ACE inhibition, where inhibited vascular and cardiac ACE expression and activity was associated with an up-regulated eNOS expression and increased vascular NO release [4]. Endothelium-derived or exogenously added bradykinin exerts its vasodilatory action through stimulation of endothelial B2 kinin receptors, thereby causing the synthesis and release of vasodilator substances such as endothelium-derived hyperpolarizing factor, NO and prostacyclin [5]. The effects of NO on platelets, smooth muscle cells, and cardiac myocytes are mediated by activation of soluble guanylylcyclase to synthesize cyclic guanosine monophosphate [6]. Endothelial cyclic GMP provides a negative feedback to offset further NO synthesis. Changes in the synthesis of ACE, bradykinines and NO are associated with a number of cardiovascular conditions such as hypertension, atherosclerosis or coronary heart disease, and ACE inhibitors are able to treat these diseases by accumulation of endothelium-derived kinins and the inhibition of angiotensin II [7]

Effects of ACE inhibition on NO, B_2 kinin receptors

ACE inhibition not only stimulates NO synthesis but also induces the expression of eNOS [8]. Studies with isolated human coronary arteries have provided evidence that ACE inhibitors facilitate the accumulation of locally formed bradykinin and directly affected endothelial B_2 kinin receptor signaling, resulting in an enhanced vascular response to bradykinin [9].

Angiotensin-(1-7) – a bioactive fragment of the renin-angiotensin system

Accumulating evidence suggests that Ang-(I-7) is an important component of the renin-angiotensin system. As

ACE = angiotensin-converting enzyme
ACEIs = ACE inhibitors
NO = nitric oxide
eNOS = endothelial NO synthase
Ang-(1-7) = angiotensin-(1-7)
GMP = guanosine monophosphate

the most pleiotropic metabolite of angiotensin I, it manifests actions that are most often the opposite of those described for angiotensin II. Ang-(1-7) is produced from Ang I bypassing the prerequisite formation of Ang II. The generation of Ang-(1-7) is under the control of at least three enzymes, which include neprilysin, thimet oligopeptidase, and prolyl oligopeptidase, depending on the tissue compartment. Both neprilysin and thimet oligopeptidase are also involved in the metabolism of both bradykinin and atrial natriuretic peptide. Moreover, recent studies suggest that in addition to Ang I and bradykinin, Ang-(1-7) is an endogenous substrate for angiotensin-converting enzyme. This suggests that a complex relationship exists between the enzymatic pathways forming angiotensin II and other various vasodepressor peptides from either the renin-angiotensin system or other peptide systems. The antihypertensive actions of Ang-(1-7) are mediated by an angiotensin receptor ATx, which is distinct from the pharmacologically characterized AT1 or AT2 receptor subtypes. Ang-(1-7) mediates its antihypertensive effects by stimulating synthesis and release of vasodilator prostaglandins and nitric oxide and potentiating the hypotensive effects of bradykinin [10].

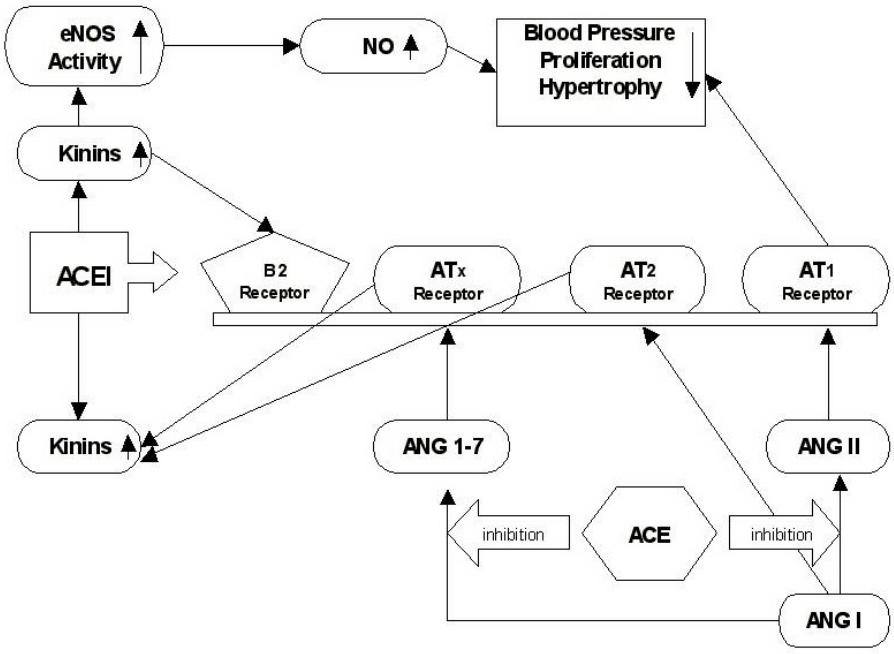

Figure 1. ACE, NO and kinins and their interaction. NEP = neutral endopeptidase.

Effects of angiotensin 1-7 on ACE and B_2 kinin receptors are now being intensively studied. The heptapeptide angiotensin 1-7 (ANG 1-7) is released from ANG I or ANG II by various post-proline cleaving endopeptidases. A marked rise in plasma level of ANG 1-7 was observed in hypertensive patients [10] following ACE blockade. Like ACE inhibitors, ANG 1-7 can potentiate the action of bradykinin on its B2 kinin receptor site by binding to the active site of ACE, independent of blocking bradykinin hydrolysis [11]. Like ANG 1-7, ANG II is able to activate the cardiovascular kinin/NO system.

All the effects of ANG II cannot be ascribed to interaction with distinct ANG II receptor subtypes. NO production induced by Ang II was prevented by subtype AT_1 as well as AT_2 ANG II receptor blockers in coronary microvessels and large coronary arteries of the dog [12]. Ang II-induced endothelial kinin/NO production favored an involvement of subtype AT_2 ANG II receptors, however this was challenged by the fact that one of five tested selective subtype AT_1 ANG II receptor blockers behaved like a subtype AT_2 ANG II receptor antagonist [13]. A possible explanation for this could be that slight conformational changes in the structure of the subtype AT_1 ANG II receptor could convert subtype AT_2 to AT_1 ANG II receptor ligands and probably vice versa [14]. Furthermore, in contrast to ANG 1-7, ANG II did not interact with ACE, since ANG II was neither a substrate nor an inhibitor of ACE. ANG 1-7 can potentiate arachidonic acid release.

Pathophysiology of ACE-kinins-NO system

In arterial hypertension

It is now demonstrated that NO plays a critical role in the maintenance of blood pressure homeostasis [11]. Shear stress as well as locally generated compounds such as bradykinin, acetylcholine, ATP and substance P were reported to be stimulators for endothelial NO production [15]. Furthermore, it was suspected from experiments on isolated blood vessels that a significant part of the blood pressure-lowering effect of ACE inhibitors *in vivo* was mediated by the accumulation of kinins.

The antihypertensive effect of ACE inhibitors could not be antagonized by icatibant in all models of experimental hypertension. The blood pressure-lowering effect of ACE inhibitors was only antagonized by icatibant in renovascular models of hypertension with increased plasma renin and Ang II activity [16]. Thus, it seemed that under these conditions endogenously increased kinins might counteract the vasoconstriction induced by high ANG II production. Moreover, it was observed in patients with renal hypertension that the blood pressure-lowering effects of an ACE inhibitor were more pronounced than in patients with primary hypertension [17].

Ang 1 = angiotensin 1

In recent clinical observations in normotensive and hypertensive subjects it was found that icatibant attenuates the blood pressure-lowering effect of an ACE inhibitor. This effect of icatibant tended to be greater in subjects with normal to high plasma renin activity than in those with low plasma renin activity. Furthermore, ACE inhibition enhanced flow-dependent endothelium-mediated dilation, 46% over baseline, in healthy normotensive humans by a bradykinin-dependent mechanism, whereas icatibant showed reduction of 33%. The activity of kinins and the bioavailability of NO play a major role in the regulation of blood pressure. The basal NO dilator mechanism appeared to be abnormal in the forearm arterial bed of untreated patients with essential hypertension. In such patients a diminished basal whole-body NO production was observed. Therefore, alterations in NO expression/activity might be related to the pathogenesis of hypertension [18].

Both mechanical strain and Ang II increases matrix gene expression and protein synthesis by human vascular smooth muscle cells. The effect of strain was attenuated by AT-1 receptor antagonism. The mechanical strain may sensitize human vascular SMC to the fibrogenic actions of Ang II, perhaps via up-regulation of the AT-1 receptor [19].

In atherosclerosis

The endothelial NO pathway is also involved in hypercholesterolemia and atherosclerosis. Chronic inhibition of eNOS impaired endothelial function and accelerated atherosclerosis in hypercholesterolemic rabbits. In patients with coronary atherosclerosis basal NO release was impaired, as suggested by a blunted response by L-NNA. Chronic administration of L-arginine improved endothelium-dependent relaxation in hypercholesterolemic rabbits, and infusion of L-arginine restored endothelial dysfunction in the coronary microcirculation and forearm resistant vessels of hypercholesterolemic patients [20].

Down-regulation of eNOS mRNA and expression in endothelial cells overlying advanced atherosclerotic lesions in human aorta was reported. In clinically manifested human atherosclerosis, carotid eNOS protein expression and NO release were markedly reduced. The reduced NO availability might also be due to an enhanced superoxide production via endothelial xanthine oxidase activation. It was found that eNOS-dependent superoxide production was enhanced in human endothelial cells incubated with native low density lipoprotein [21].

Improvement of endothelial function and morphology by ACE inhibitors was demonstrated in experimental models of hypercholesterolemia and atherosclerosis. However, the mechanism by which ACE inhibitors affect atherosclerosis is not well understood. Reduction of ANG II production may play a role. An increased ACE immunoreactivity and increased concentrations of immunoreactive ANG II were found within atherosclerotic plaques of human coronary artery segments [22].

ACE not only generates ANG II, but also inactivates kinins. Inhibition of kinin breakdown by ACE inhibitors enhanced synthesis and release of NO, which had an antiproliferative influence on vascular smooth muscle cells [23]. Vascular protection by ACE inhibition via activation of the kinin/NO pathway was supported by recent findings, which showed that the acute toxic effects of oxidized LDL were attenuated in isolated aorta from rats chronically treated with ACE inhibitor. ACE inhibition seems to reduce LDL oxidation by scavenging superoxide through enhanced NO synthesis and release. Anti-atherogenic effects of ACE inhibitors may be associated with the direct inhibition of LDL oxidation [24]

Finally, angiotensin II exerts an immunomodulatory effect on monocyte maturation, differentiation and extravasation, which may depend on the myelomonocytic phenotype. Angiotensin II may promote early atherogenesis by AT1–receptor-mediated phenotypic modification of monocytic precursors, resulting in circulation of pro-atherogenic monocytes that express activated Mac-1. Suppressing production of such pro-inflammatory monocytes, Ang II and AT 1 receptor antagonists may contribute to their clinical benefit, independent of depressor effects, and may represent a paradigm for novel, anti-inflammatory actions of these drugs [25].

In ischemic heart disease and chronic heart failure

ACE inhibitors reduce myocardial injury in conditions of acute ischemia. These effects were due not only to a decreased synthesis of ANG II, but also to the demonstrated ability of these drugs to attenuate the degradation of endogenous kinins in the heart, for which a local kallikrein-kinin system was described. That kinins play a significant role in myocardial ischemia was supported by many investigations. In the ischemic heart the enhanced generation and release of kinins seemed to have cardioprotective actions.

Drugs that blocked kinin receptors during ischemia reversed the beneficial cardioprotective effects evoked by bradykinin or ACE inhibitors. In addition, kinins seem to contribute to the immediate and delayed cardioprotective effects associated with ischemic preconditioning. The enhanced release of kinins from ischemic myocardial tissue was always correlated with an increase of NO. There is also strong evidence for a major role of endothelium-derived NO in controlling vascular smooth muscle proliferation in response to remodeling stimulus [26].

Angiotensin II is known to play a crucial role in cell migration and proliferation of vascular tissues. For example, ACE inhibitor is effective in preventing the proliferation of vascular tissue after balloon injury of vessels in rats, whose

SMC – smooth muscle cells

LDL = low density lipoprotein

vascular tissue contains only ACE as an angiotensin II forming enzyme. But a study of ACE inhibitor suppression of vascular restenosis after percutaneous transluminal coronary angioplasty in humans was negative [27]. Such species differences in the effects of ACE inhibitors on neointimal formation after injury may depend on whether or not a given species possesses angiotensin II-forming chymase in vascular tissue.

Abnormalities of vasomotor tone are characteristic of heart failure. In a rat model of chronic heart failure (normotensive rats with myocardial infarction), early and delayed ACE inhibitor treatment both increased survival and exerted similar beneficial effects on cardiac hemodynamics and remodeling [28], an effect also observed in patients [29]. In all experimental models a chronic up-regulation of vascular and cardiac eNOS expression/activity was found [30]. In L-arginine administration enhanced NO production was accompanied by an attenuation of cardiac hypertrophy in studied animals [31]. *In vitro* studies with cultured cardiomyocytes revealed that the antihypertrophic effect of bradykinin was critically dependent on endothelium-derived NO. Only when cardiomyocytes were co-cultured with endothelial cells was bradykinin able to abolish the hypertrophic effect induced by ANG II [32].

The ACE inhibitor-induced decrease of ventricular volume, myocyte size and interstitial fibrosis, as well as myocardial function, were partially blocked by icatibant [34]. Antiproliferative effects were observed under subtype AT_1 ANG II receptor blockade, probably also mediated in part by endogenous kinins via stimulation of subtype AT_2 ANG II receptors [30–33].

ACE inhibitors exert their cardioprotective actions by enhancing the oxygen supply to tissue oxygen demand during various cardiovascular stresses. These effects were blocked by icatibant and L-NNA [34]. It seems that the beneficial cardiovascular effects of ACE inhibition in addition to its hemodynamic action is related to an improvement of the cardiovascular kinin/NO pathway.

Apoptosis is emerging as a novel therapeutic approach to control intimal thickening in atherosclerosis and vascular injury. AT 1 receptor blockade may deprive smooth muscle cells of a key survival signal. Losartan-induced smooth muscle cell apoptosis is dependent on elevated plasma angiotensin II levels and activation of AT 2 receptors, which are expressed in uninjured blood vessels [35]. Thus it can be speculated that AT 1 receptor antagonists may be effective in the prevention of vascular occlusive diseases in part via the prevention of smooth muscle cell apoptosis.

Alternative angiotensin-generating pathways

Angiotensin II synthesis may occur independently of renin and ACE. The candidates that are generally assumed to replace renin and ACE are cathepsin D and chymase, respectively. Cathepsin D is a lysosomal enzyme that cleaves angiotensinogen, unlike renin, at low pH [36]. Studies in which Ang I-generating activity is quantitated as a measure for renin activity should therefore always be performed in the absence and presence of specific renin inhibitors to correct for non-renin-dependent Ang I generation. Evidence that cathepsin D is of importance *in vivo* is currently lacking. Studies in the isolated perfused heart also do not support a role for cathepsin D [37].

Chymase is a serine protease present in the interstitium, and cardiac mast cells and endothelial cells are sites of chymase biosynthesis and storage. Remarkably, chymase is the main enzyme in human heart homogenates responsible for Ang I-Il conversion. In contrast, Ang I-Il conversion in the coronary vascular bed of intact humans and pigs depends on ACE only [38]. This raises the question whether chymase is of importance *in vivo*. It was suggested that interstitial fluid contains an endogenous inhibitor of chymase, a $_1$-antitrypsin, which would normally suppress any chymase-dependent Ang I-II conversion. However, the inhibitory effect of a $_1$-antitrypsin may be limited to tissue homogenates only, since it could not be demonstrated in an intact preparation [39]. Cardiac chymase mRNA levels are unaltered in subjects with heart failure. More detailed knowledge on the *in vivo* role of chymase will be obtained once specific chymase inhibitors are available.

Conclusions

Under physiological and pathophysiological conditions, ACE, kinins and NO act independently, but activity of endothelium-derived kinins depends only on the expression and/or activity of ACE.

The components of the kallikrein-kinin-system in cardiac and vascular tissue form systemic and local kallikrein-kinin-system pathways, which involve different cell types like endothelial cells, cardiomyocytes and vascular smooth muscle cells. Kinins may contribute to the regulation of the cardiovascular system in health and disease and to the pharmacological effects of cardiovascular agents via autocrine-paracrine mechanisms. ACE inhibitors' increase of endothelial-derived kinins, and regulation of ACE is correlated with changes of the expression and activity of eNOS. There is an inverse relationship between the expression/activity of ACE and eNOS via feedback regulation.

The bioavailability of nitric oxide depends on the steady-state level of eNOS expression and functional eNOS activity. The regulation of eNOS is important in the development of hypertension, atherosclerosis and heart failure, where impaired endothelium-derived NO is observed. Both kinins and ACE inhibitors are able to up-regulate eNOS protein, enhance NO production, and reduce superoxide production generated by a dysfunctional eNOS.

ACE inhibitors can act directly on the B_2 kinin receptors; they can also cause ANG 1-7 action on subtype AT_2 ANG II receptors. These mechanisms are important in the connection between ACE, kinins and NO. The alternative angiotensin-generating pathways (cathepsin D and chymase)

do not seem to have the significant effect on tissue level *in vivo* that was previously thought. These data suggest the hypothesis that ACE inhibitors may have significant advantages over AT 1 receptor inhibitors in the treatment of different cardiovascular diseases; thus, these drugs may be used as first line agents.

The HOPE study (Heart Outcomes Prevention Evaluation) [40] shows that ACE inhibitors (such as ramipril) are effective in the prevention of major vascular events in a wide population with high cardiovascular risk, but show normal left ventricular function and high normal blood pressure. The HOPE study also suggests that Ang II may directly contribute to atherosclerosis and its clinically relevant sequelae. An ongoing study, Optimal Therapy In Myocardial Infarction with Losartan (OPTIMAAL), should provide additional information on whether Ang II mediates coronary artery disease through AT 1 receptor stimulation since it compares the effects of therapy with captopril versus losartan on mortality in patients after myocardial infarction. In the event of intolerance to ACE inhibitors, for instance cough, AT 1 inhibitors can be considered the treatment of choice. The results of the ELITE II study emphasized that AT 1 receptor antagonists can be used instead or in addition to ACE inhibitors with similar clinical results.

In the near future we might reach a clearer molecular perception of kinin actions in the cardiovascular system, as well as their role in human health and disease, which may result in an innovative treatment approach by modulation of the kallikrein-kinin-system pathways.

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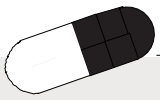
He is the summit of sex, the pinnacle of masculine, feminine and neuter. Everything that he, she and it can ever want ... This deadly, winking, sniggering, snuggling, chromium-plated, scent-impregnated, luminous, quivering, giggling, fruit-flavoured, mincing, ice-covered, heap of mother love.

William Connor Cassandra, English journalist (1909-67), on Liberace

Capsule



Kaposi sarcoma-associated herpesvirus

Kaposi sarcoma-associated herpes virus (KSHV), also known as human herpes virus 8, is a 'large' DNA virus linked to Kaposi sarcoma (KS) and disorders such as primary effusion lymphoma and multicentric Castleman disease. The incidence of KS is increased considerably in patients with AIDS. Although most KS tumor cells contain latent KSHV, only a few cells contain actively replicating, lytic KSHV. Radkov et al. report that latent nuclear antigen (LNA)-1, the main LNA of KSHV, can form a complex with the retinoblastoma susceptibility gene product (pRB), one of the cellular 'master' regulators that is often targeted by 'small' DNA tumor viruses. The authors found that LNA-1 has an oncogenic activity that may be related to its ability to bind to pRB.

The pRB protein governs aspects of cellular proliferation, differentiation, senescence and death. The anti-proliferative activity of pRB is mechanistically linked to the cell division cycle. The pRB pathway is intimately linked to the p53 tumor suppressor pathway, an essential sensor of abnormal cell function. Activation of p53 by various cellular insults can arrest the cell cycle in G1, and pRB needs to be functional for this outcome. On the other hand, pRB dysfunction can trigger an apoptotic cellular response that depends in part on p53.

Although the molecular entities that form functional interfaces with this region of pRB are unknown, they may regulate cellular senescence and/or differentiation, and their identification will be important to fully understand the tumor suppressor functions of pRB. Such studies will also shed more light on the pathogenic function of KSHV in the development of KS, a histopathologically complex and multifactorial malignancy.

Nat Med 2000;6:1091