



Is the endothelium an important therapeutic target?

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The vascular endothelium modulates vascular tone and the expression of a number of vascular genes mainly by producing vasoactive autacoids such as nitric oxide (NO) and the superoxide anion (O₂⁻). Hypertension, arteriosclerosis, and ischemic heart disease are all associated with dysregulation of endothelial cell function. This is related to an imbalance in the production of endothelial NO and vascular O₂⁻, which results in the shutdown or inactivation of intrinsic antiatherogenic processes. Clinical and experimental evidence indicates that angiotensin-converting enzyme (ACE: kininase II) inhibitors are able to halt the development of endothelial dysfunction, and, in certain situations, to restore the balance in vascular autacoid production.

Keywords: endothelium; endothelial dysfunction; angiotensin II; bradykinin; nitric oxide; atherosclerosis; ischemic heart disease; hypertension; ACE inhibitor

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The vascular endothelium plays a crucial role in the modulation of vascular tone, a function that is accomplished mainly by the synthesis and release of a number of vasoactive dilating substances, including NO, prostacyclin, and the endothelium-derived hyperpolarizing factor, as well as vasoconstricting substances, such as endothelin-1, prostaglandin H₂, and O₂⁻. It can be assumed that, in the healthy vasculature, a certain level of each of these compounds contributes to the maintenance of vascular tone. In vascular disease this delicate balance is disturbed, so that a decrease in the production of a vasodilator autacoid, such as NO, or an overproduction of a vasoconstrictor substance is thought to promote resetting of vascular tone to a permanently elevated level. While this simple model contains some degree of truth, the situation is more complicated, as endothelial autacoids, especially NO and O₂⁻, modulate the expression of genes that are implicated in the atherogenic process. For example, the expression of adhesion molecules (such as E-selectin, P-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1) and the chemokine monocyte chemoattractant factor-1, which are prerequisites for monocyte infiltration into the vascular wall, is suppressed by NO. A decrease in NO bioavailability in vivo is thought to occur mainly through an increase in the vascular production of O₂⁻, which scavenges NO, although a reduction in endothelial nitric oxide

synthase (eNOS) expression and elevated circulating levels of an endogenous eNOS inhibitor have also been reported (for review see ref 1).

ACE INHIBITORS AND NO/O₂⁻

On the basis of these considerations, it is evident that the development of a therapy that halts the development of endothelial dysfunction and/or restores the balance in vascular production of NO and O₂⁻ would be of considerable interest for the treatment of cardiovascular diseases. Accumulating experimental evidence suggests that angiotensin-converting enzyme (ACE) inhibitors are able to do just that. Moreover, recent evidence suggests that they possess additional properties that may modulate at least the acute effects of this class of compounds on the cardiovascular system.

SELECTED ABBREVIATIONS AND ACRONYMS

CHO	Chinese hamster ovary
eNOS	endothelial nitric oxide synthase
Erk	extracellularly regulated kinase
HOPE	Heart Outcomes Prevention Evaluation
QUIET	QUinapril Ischemic Event Trial
TREND	Trial on Reversing ENdothelial Dysfunction

ACE INHIBITORS AND ANGIOTENSIN II

Angiotensin II is one of the most potent endogenously produced vasoconstrictors and can accentuate its effects by stimulating the production of three additional vasoconstrictors, endothelin-1, prostaglandin H_2 , and O_2^- . Regarding vascular sources of O_2^- , both the endothelium and vascular smooth muscle contain membrane-bound oxidase(s) that utilize reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) as substrates for electron transfer to molecular oxygen. These enzymes are, to a certain extent, similar to the neutrophil NADPH oxidase, with the exception that the vascular NADH/NADPH oxidase is a basally active, low-output enzyme, which does not exhibit bursts of activity following cell activation. The expression of NADH/NADPH oxidase in vascular smooth muscle cells is known to be increased within a few hours by subnanomolar concentrations of angiotensin II.² The subsequent increase in O_2^- underlies endothelial dysfunction (diminished vasodilator responsiveness to acetylcholine) in rats made hypertensive with angiotensin II, as the infusion of superoxide dismutase to inactivate O_2^- generated within the vasculature restores endothelium-dependent dilator responses.³ It is therefore tempting to suggest that ACE inhibitors could exhibit a beneficial effect by breaking this angiotensin II/ O_2^- potentiating loop. A decrease in vascular O_2^- production would, in turn, be expected to increase the half-life of endothelium-derived NO and give the appearance of restoring endothelial NO production. ACE inhibitors influence eNOS expression, and chronic ACE inhibitor therapy has been shown to enhance

eNOS expression and NO production in normotensive and spontaneously hypertensive rats. The mechanism underlying this effect is currently unknown, as little information is available regarding the effects of altered vascular O_2^- production on the expression of eNOS mRNA and protein. Interestingly, the massive increase in eNOS expression (in this case more than threefold) observed in rats treated with ramipril for 2 years was also associated with an increase in O_2^- production, an effect which may be the consequence of endothelial depletion of the essential eNOS cofactor tetrahydrobiopterin (H_4B).^{4,5} Such depletion of a cofactor may arise either as a consequence of the generation of peroxynitrite (the reaction product of NO and O_2^-), which is able to oxidize H_4B to dihydrobiopterin. Indeed, in the absence of H_4B , eNOS produces O_2^- rather than NO, and the administration of H_4B to hypercholesterolemic patients is reported to restore endothelial function.⁶ This mechanism may also be involved in the genesis of endothelial dysfunction, as H_4B alters O_2^- and NO release (and, therefore, probably ONOO⁻) in spontaneously hypertensive rats prior to the development of manifest hypertension.⁷

ACE INHIBITORS AND BRADYKININ

It is important to note that comparison of the effects of angiotensin II antagonists, renin inhibitors, and ACE inhibitors in the isolated working heart shows that only the ACE inhibitor is able to increase coronary blood flow.⁸ These and many other observations tend to suggest that it is the effect of ACE inhibition on bradykinin metabolism that underlies their beneficial effects on blood flow and NO production.⁹ Indeed, experimental evidence

has demonstrated that bradykinin and related kinins are continuously formed in the isolated heart and that ACE inhibitors elicit an approximately fivefold increase in kinin levels.¹⁰ This effect of ACE inhibitors on the heart is, as expected, dependent on kininogenase activity, and the source of this coronary bradykinin appears to be the endothelial cell layer, as its removal markedly attenuates basal and ACE inhibitor-induced kinin release.⁹ From the points mentioned above, it is clear that by inhibiting kininase II, ACE inhibitors could augment the effect of bradykinin on the endothelial cell.⁹⁻¹¹ It is, however, debatable whether enough bradykinin is available at the endothelial cell surface in the presence of a continuous flow to make this effect possible. Indeed, even if ACE inhibitors increase the local concentration of vascular bradykinin, it is unclear how this can restore endothelium-dependent vasodilatation to acetylcholine, which is routinely used in experimental and clinical assessments of endothelial function.

NOVEL ACE-INDEPENDENT EFFECTS OF ACE INHIBITORS

While generally accepted to potentiate responses to bradykinin,⁹⁻¹¹ ACE inhibitors directly elicit responses in the isolated heart, arterial segments, and cultured endothelial cells.¹² Whether the differences observed reflect the rate of endogenous bradykinin generation in the various preparations is controversial, and additional factors may determine ACE-inhibitor responsiveness. One such factor is fluid shear stress, since ACE inhibitors such as ramiprilat and moexiprilat have been reported to elicit a relaxation in superfused coronary artery rings, although neither of these inhibitors affects the tone of arterial rings



incubated under static conditions in an organ chamber.¹³ In addition, a response to ACE-inhibitor application can be detected in otherwise nonresponding preparations if these were previously acutely exposed to bradykinin.¹⁴ Whether these observations indicate that ACE inhibitors are able to liberate bradykinin stored by, or on, endothelial cells remains to be elucidated, but the efficiency of ACE inhibitors in eliciting a relaxant response does appear to be dependent on a certain basal concentration of bradykinin in the vicinity of target cells. Whether the fluid shear stress present in the superfusion systems studied is a stimulus for the production of endothelium-derived bradykinin also remains to be clarified. Shear stress-induced NO production has indeed been proposed to be mediated by endothelium-derived bradykinin, which, in an autocrine loop, stimulates the B₂ receptor, thus increasing [Ca²⁺]_i and activating eNOS.¹⁵ However, the activation of eNOS by fluid shear stress is now known to be mediated by a completely different intracellular signaling pathway than that activated in endothelial cells by bradykinin.¹⁶ Much of the experimental evidence for a role for bradykinin in the response to shear stress was interpreted under the assumption that a specific activation of the B₂ kinin receptor could be antagonized by the B₂ receptor antagonist icatibant. However, the B₂ receptor has a constitutive or basal activity, and icatibant is actually an inverse agonist at this receptor, which means that its basal activity is inhibited and the receptor is stabilized in a totally inactive form.¹⁷ Thus, the effects of icatibant on cellular responses actually reflect both the inhibition of inherent receptor activity as well as that induced by an increase in agonist concentrations. This situation has

almost certainly led to false positive results, ie, a biological or physiological role for the bradykinin has been proposed on the basis of an observed response to icatibant. Recent experimental evidence suggests that a signaling cross-talk exists between ACE and the B₂ kinin receptor, such that ACE inhibitors can elicit icatibant-sensitive responses (inositol 1,4,5-trisphosphate production, increase in [Ca²⁺]_i, activation of eNOS, and vasodilatation) by a mechanism distinct from their ability to inhibit ACE.^{9,18,19} Although pharmacological studies using various vascular preparations suggested that ACE inhibitors may exert a direct effect on the B₂ kinin receptor, this has only recently been confirmed by monitoring the sequestration of the B₂ receptor into endothelial caveolae, which are specialized microdomains of the plasma membrane.¹⁹ The ACE inhibitor ramiprilat was found to attenuate the basal flux or cycling of the kinin receptor through caveolae as well as the bradykinin-stimulated sequestration into caveolae. This latter effect was distinct from enzyme inhibition, as the synthetic ACE substrate hippuryl-L-histidyl-L-leucine, at a concentration that blocks the degradation of bradykinin by ACE, failed to affect B₂ receptor sequestration.¹⁹ In addition to this effect on the physical translocation of the receptor protein, ACE inhibitors are able to reactivate endothelial B₂ receptors that have been desensitized by exposure to high concentrations of bradykinin, resulting in an immediate secondary increase in inositol 1,4,5-trisphosphate, [Ca²⁺]_i and the activation of the extracellularly regulated kinases Erk1 and Erk2.¹⁹ A similar reactivation of B₂ signaling by enalaprilat was recently reported in response to Chinese hamster ovary (CHO) cells transfected with both ACE and the B₂

receptor.¹⁸ As the expression of ACE is reported to be essential for ACE inhibitors to reactivate B₂ kinin receptor signaling, it seems likely that some form of cross-talk exists between ACE and the B₂ receptor. One possibility is that ACE inhibitors may act in the opposite manner to icatibant and stabilize the B₂ receptor in a G-protein-coupled or basally active form. While this hypothesis is at the moment purely speculative, ACE inhibitors and peptides, such as angiotensin,¹⁻⁷ which bind to either of the two active sites on ACE, enhance bradykinin-induced responses mediated by the Gα_i subunit of heterotrimeric G-proteins. Moreover, ACE inhibitors can also directly activate Gα_i in the absence of bradykinin (author's unpublished observation). Taken together, these data provide the first clear evidence that ACE inhibitors exert effects on endothelial cells that cannot be simply attributed to the inhibition of kinase II activity and the accumulation of locally produced bradykinin.

CLINICAL EVIDENCE THAT ACE INHIBITORS RESTORE ENDOTHELIAL FUNCTION

Perhaps the most convincing evidence to date for an effect of ACE inhibitors on the endothelium is the Trial on Reversing ENdothelial Dysfunction (TREND), in which 6 months treatment with quinapril was found to improve endothelial function in subjects who had a manifest single or double coronary artery disease.²¹ As ACE inhibitors have proven to be of clinical benefit in congestive heart failure, and endothelial dysfunction is thought to be of importance in the early stages of atherosclerosis and in the pathophysiology of myocardial ischemia, a clinical trial was designed to determine whether ACE inhibitors could reduce the inci-

dence of ischemic events in patients with established coronary artery disease. The Quinapril Ischemic Event Trial (QUIET) was the first prospective, double-blind, placebo-controlled trial to investigate the long-term antiatherosclerotic effects of ACE inhibition. Unfortunately, no significant beneficial effect of quinaprilat could be detected on either ischemic incident prevention or stenosis development.²² The outcome of QUIET is in contrast to the results of a number of smaller trials in which ACE inhibition was reported to selectively improve endothelium-dependent, but not endothelium-independent, dilation and to abolish abnormal epicardial vasomotion in patients with endothelial dysfunction related to heart failure and coronary artery disease.^{23,24} The latest clinical trial, Heart Outcomes Prevention Evaluation (HOPE), with ramipril, was such a resounding success that the trial was stopped prematurely in view of the significant decrease in incidence of cardiovascular events in patients deemed to be at high risk of myocardial infarct, stroke, and complications associated with diabetes.²⁵

IS THE ENDOTHELIUM AN IMPORTANT THERAPEUTIC TARGET?

The answer is yes, as the endothelium plays a central role in vascular homeostasis, and the balance in endothelial NO/O₂⁻ production is clearly involved in the regulation of pro- and antiatherogenic vascular gene expression. Despite the disappointing results obtained to date in some of the larger clinical trials, there is a wealth of experimental evidence showing that prolonged treatment with ACE inhibitors restores endothelial function and, in some cases, reverses medial thickening and the number of monocytes detected within the subintima.²⁶

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