Inhibiting Tissue Angiotensin-Converting Enzyme
A Pound of Flesh Without the Blood?

Lawrence S. Zisman, MD

“This bond doth give thee here no jot of blood.
The words expressly are ‘a pound of flesh.’”
Portia, in Shakespeare’s The Merchant of Venice

Angiotensin-converting enzyme catalyzes the formation of angiotensin II (Ang II) from Ang I but also degrades bradykinin (BK). Ang II, acting through the AT1 receptor, is a potent vasoconstrictor, stimulates norepinephrine release from sympathetic nerve terminals in the heart, and causes hypertrophy of cardiac myocytes. BK, via activation of the BK1 receptor, stimulates the release of NO and prostaglandins and may counteract Ang II–mediated effects. ACE inhibition may exert beneficial effects both by interrupting Ang II–mediated AT1 receptor signal transduction and by augmenting BK1 receptor activation. Because of the proven survival benefit from ACE-inhibitor therapy in patients with heart failure, great attention has been given to understanding the structure and function of ACE and to the design of optimally effective ACE inhibitors. In this issue of Circulation, Hornig and colleagues1 compare the effects of 2 such ACE inhibitors, enalaprilat and quinaprilat, on the peripheral circulation in patients with heart failure. To understand the implications of their work, a discussion of the ACE molecule itself, the chemical structure of ACE inhibitors, and their interaction with ACE is required. The major issue to be examined is the proposed dichotomy between high- and low-affinity tissue ACE inhibitors.

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In somatic tissues, ACE is a glycoprotein of about 140 kDa; in testicular cells, it is synthesized from the same gene but at an alternative transcription start site that results in a protein of 100 kDa.2 The somatic form of ACE has 2 homologous domains (the amino, or N-terminal, and carboxy, or C-terminal, sites) that are both catalytically active and that both require the presence of Zn2+ for activity.3 Testicular ACE contains only the C-terminal active site, whereas an ileal molecule of the ACE may be released from the cell membrane by a carboxypeptidase that cleaves the protein between Arg-663 and Ser-664 to generate the soluble or circulating form of somatic ACE. Evidence has been presented that the release of ACE from the cell membrane is a regulated process.5 Mice genetically engineered to express only the N-terminal domain of ACE have no tissue-bound ACE and are hypertensive; this finding supports the concept that tissue-bound ACE is more important than circulating ACE in the metabolism of Ang I and BK. However, this particular model does not distinguish between the importance of the C-terminal active site and the carboxy tail that anchors the enzyme in the cell membrane.6 Both enalaprilat and quinaprilat contain a phenylpropyl moiety in place of the sulfhydryl group present in captopril. The carbonyl oxygen atom of the ketomethyl group in enalaprilat and quinaprilat coordinates with the Zn2+ ion at the active site, whereas the phenyl group in both inhibitors interacts with a hydrophobic site. The distinctive tetrahydroisoquinolone moiety of quinaprilat is predicted to increase interaction at a second hydrophobic site. This additional interaction biochemically defines the “high-affinity” ACE inhibitors.

Hornig et al1 found that the ACE inhibitor quinaprilat infused directly into the brachial artery increased flow-dependent (primarily endothelium-dependent) dilation (FDD) of the radial artery in patients with heart failure. In contrast, enalaprilat had no effect on this parameter. The component of FDD mediated by NO was doubled by quinaprilat. This finding suggests that the mechanism of action of quinaprilat was to increase the local concentration of BK and thereby stimulate the release of NO.

The authors propose that the different effects of quinaprilat compared with enalaprilat arise because quinaprilat has a high affinity to tissue ACE compared with enalaprilat. What is actually meant by this proposed dichotomy? Does it refer to tissue-bound versus circulating ACE, or is it meant to apply generally to somatic ACE whether circulating or tissue-bound? Are we being asked to take a pound of flesh and not spill any blood?

It has been reported that quinaprilat displaced 50% of 125I-labeled 351A (the tyrosyl derivative of enalaprilat) from human plasma ACE at a concentration 2 orders of magnitude lower than that of enalaprilat; however, the differences in EC50 of 351A and quinaprilat for lung and cardiac ACE appeared to be far more modest.7 The equilibrium dissociation constants (Kd) of Ro 31-8472 (a derivative of cilazaprilat), 351A, and quinaprilat for displacement of 125I-351A from lung ACE were 32±7, 15±3, and 6±1 pmol/L, respectively.8 Assuming particular assay conditions, the Kd is directly related to the EC50; therefore, these latter data do not describe

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From the Rush Heart Institute, Chicago, Ill.

Correspondence to Lawrence S. Zisman, MD, The Rush Heart Institute, Center for Pulmonary Heart Disease, Rush Presbyterian St Luke’s Medical Center, 1725 W Harrison St, Suite 020, Chicago, IL 60612.

E-mail lzisman@rus.edu

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order-of-magnitude differences. In addition, these data modeled to 1 binding site and were similar to binding curves for testicular ACE. It was recognized that the affinity of 125I-351A for the N-terminal active site, in contradistinction to enalaprilat itself, was 120-fold lower than for the C-terminal active site. Thus, competition binding studies that use 125I-351A selectively describe displacement of radiolabeled inhibitor from the C-terminal active site of ACE. When 125I-Ro 31-8472 was used as the radioligand, 2 binding sites could be modeled. The K_i for quinaprilat at the N-terminal site was 1267±629 pmol/L, versus 7±1 pmol/L for the C-terminal site. In contrast, in competition binding experiments using 125I-Ro 31-8472, enalaprilat modeled to 1 active site, indicating that the interaction of this ACE inhibitor with the 2 active sites was equivalent.9 On the basis of these data, it has been proposed that ACE inhibitors with more hydrophobic moieties have a greater affinity for the C-terminal than for the N-terminal active site of ACE.

This differential affinity may be further modulated by organ type. Bevilacqua et al9 found that the affinity of captopril for the N site in human heart left ventricle was lower than that for the N site of ACE in the lung; in contrast, the affinity of the more hydrophobic ACE inhibitor delaprilat for the C site of cardiac ACE was greater than that for lung ACE. Although the mechanism for these organ-specific effects is not known, it has been proposed that differences in the glycosylation of ACE may modulate ACE-inhibitor binding. Thus, the proposed dichotomy of high- versus low-affinity tissue ACE inhibition is an oversimplification. The 2 active sites of ACE interact differently with particular ACE inhibitors, and the specific organ in which ACE is tissue-bound may also affect inhibitor affinity for the active sites of the enzyme.

Studies of recombinant full-length ACE have shown that the apparent K_m for BK is substantially lower than for Ang I, indicating more favorable kinetics for BK than for Ang I hydrolysis. Furthermore, site-directed mutagenesis demonstrated that the K_m for BK was lower than for Ang I at both the N and C active sites. These data suggest that at physiological concentrations, BK could be preferentially hydrolyzed over Ang I at both active sites.10 However, in the case of ACE purified from rat or human tissues, it appeared that the C-terminal active site was primarily responsible for the hydrolysis of BK.11 Whether the different effects on physiology observed in this study can be attributed to differences in affinity of quinaprilat for the N versus C active site of ACE is not known.

Consistent with the variable binding affinities of ACE inhibitors in different tissues, the regulation of ACE, Ang II, and BK metabolism in the peripheral circulation may be different from that in the myocardium. In contrast to the apparently lackadaisical effects of enalaprilat in the peripheral vascular bed, intracoronary enalaprilat suppresses >80% of Ang II formation, augments the response to BK, and increases coronary blood flow.12-14 Potential explanations for this difference may be related to a higher capillary-to-myocyte ratio and higher metabolic flux in myocardium than in skeletal muscle. Differences in shear stress may alter the regulation of angiotensin and BK metabolism in these vascular beds. In the rat heart, endothelial constitutive NO synthase (ecNOS) expression is lower in capillary endothelium than in epicardial coronary artery endothelium; therefore, the relative importance of increased BK concentration compared with decreased Ang II concentration as the result of ACE inhibition may vary in different regions of the myocardial vasculature.15

In failing human heart, endothelial dysfunction and changes in cardiac myocytes are closely intertwined. ACE protein expression is increased in the failing human heart, and this increase may occur as the result of ACE gene expression in both endothelial cells and cardiac myocytes.16 Thus, the pertinent question may not be simply whether one is effectively inhibiting endothelial ACE but also whether effective inhibition of cardiac myocyte ACE has been achieved. Similarly, the inducible form of NOS (iNOS) is increased in cardiac myocytes from failing human hearts relative to ecNOS.17 Although an increase in endothelial cell production of NO appears to be desirable, it does not necessarily follow that an increase in NO production mediated by iNOS in cardiac or vascular myocytes will have a beneficial or protective effect on these cells. The important role of the endothelium in modulating cardiac myocyte structure and function was recently demonstrated by Ritchie et al,18 who found that BK prevented Ang II–induced hypertrophy of cardiac myocytes in the presence of endothelial cells; however, in the absence of endothelial cells, BK had a hypertrophic effect. Whether this differential effect of BK was related to the specific cell type in which NO production occurred or whether BK signal transduction in myocytes actually stimulated NO production was not determined. The effects of BK in this system were blocked by the BK_1 receptor–selective antagonist HOE140. Nevertheless, one is tempted to speculate that BK effects on cardiac myocytes in failing human heart could be brought about by activation of BK_1 subtype receptor expression, which, like iNOS expression, is turned on by cytokines. Signaling through the BK_1 receptor may oppose effects mediated by the BK_2 receptor. It is likely that the antihypertrophic effect of BK in the presence of endothelial cells was mediated by ecNOS. This supposition is supported by the recent report that ecNOS-deficient mice develop paradoxical hyperplasia of the carotid wall after vessel ligation.19 In the context of relatively long-term NO inhibition, ACE-inhibitor administration has been shown to prevent coronary vascular remodeling and myocardial hypertrophy.20 But, as a final counterpart, captopril may also be effective in preventing such changes without affecting NOS activity.21

The acute effects of quinaprilat found in this study predict long-term changes in vascular remodeling. From a mechanistic viewpoint, studies designed to test this prediction not only in the peripheral circulation but also in the intact failing human heart would be of interest. With regard to therapeutics, it is premature to recommend the use of quinapril over enalapril in the treatment of patients with heart failure, and as the authors observe, further studies are needed.

References


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