Renin Inhibition: What Are the Therapeutic Opportunities?

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Blockade of the renin-angiotensin system with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers has become a crucial element in cardiovascular and renal medicine. This review evaluates the potential of renin inhibition as an adjunct to therapies that depend on renin system interruption.

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Pharmacologic Interruption of the Renin-Angiotensin System

There are a number of steps (Figure 1) at which the renin system can be interrupted (1). β-Adrenergic blocking agents reduce renin release. ACE inhibitors block the conversion of Ang I to Ang II downstream from the renin step. ARB interfere with the interaction of the hormone Ang II with a specific AT1 receptor. Renin inhibition interferes with the first and rate-limiting step in the cascade, interaction of renin with its substrate angiotensinogen (AGT). No pharmacologist would have chosen the ACE step for inhibition. As pointed out by Skeggs, the rate-limiting step, blocking the catalytic effect of renin on its substrate, would have been the logical first choice. ACE inhibition developed first as an unintended consequence of the search for an explanation for the drop in BP induced by the venom of a pit viper (6). The venom was found to contain a peptide, which acted as a “bradykinin-potentiating factor”: Pharmacologic consideration suggested that an inhibitor of bradykinin metabolism might also be an inhibitor of ACE, as both substrates are cleaved by a peptidyl-dipeptide hydrolase. That reasoning proved to be correct (6).

The renin step is a very attractive target for another reason, the remarkable specificity of renin for its substrate (1). In Figure 2, the species specificity of a renin inhibitor for human and marmoset plasma renin is contrasted with the inhibition of dog and rat renin. The marmoset is a primate. In the same figure, the inhibition of human renin is contrasted with inhibition of two other aspartic proteinases, pepsin and cathepsin-D, both of which are far less responsive to the renin inhibitor. This inherent specificity has clinical implications, as it reduces the likelihood of unwanted additional interaction and thus side effects.

A third consideration involves the state of the various elements of the renin-angiotensin cascade on blockade. Both ACE inhibitors and ARB lead to a reactive renin rise and thus to an increase in the angiotensin peptides, both Ang I and Ang II in the case of ARB and Ang I in the case of the ACE inhibitors. Only renin inhibition will render the renin-angiotensin system (RAS) quiescent, suppressing all of the Ang I–derived products. Whether that is an advantage, only time and future studies can tell.
Opportunities Related to Prorenin and the Intrarenal Renin Receptor

Multiple studies have shown that the onset of microvascular disease in the patient with diabetes, both nephropathy and retinopathy, coincides with elevated concentrations of prorenin in plasma. The onset of microvascular disease and the increase in prorenin are not necessarily associated with hypertension. Moreover, in this setting, plasma active renin concentration, the most widely used index of RAS activity, is not increased—indeed, it is suppressed. The mechanism that is responsible for the increase in plasma prorenin remains obscure. Equally mysterious is the mechanism of the possible contribution of prorenin to the pathogenesis of the microvascular injury. In the case of the eye, a fascinating series of experiments identified prorenin in the human and bovine eye. Prorenin was elevated in the vitreous humor in association with proliferative retinopathy.

The relation of prorenin to total renin is different in the patient with diabetes and in other patients in whom the renin system has been activated through the use of oral contraceptives, renovascular hypertension, or treatment with an ACE inhibitor. In the patient with diabetes, the increase in prorenin exceeds substantially the increase in prorenin in the other conditions. Although the increase in prorenin in plasma has been attributed to a range of factors, including glycation of a responsible processing enzyme or autonomic dysfunction, neither factor would account directly for the association with both retinopathy and nephropathy, and they are not directly compatible with the documented suppression of renin release in the same patients.

We are reasonably confident that these issues, as interesting as they are, have never been featured in an article dealing with renin inhibition. Why have we done so? The answer involves a fascinating, possibly relevant, and potentially very important study by Nguyen et al. This group reported the identification of a renin receptor in the human kidney. This receptor is localized primarily in the mesangium of glomeruli but also in the subendothelium of arteries of the heart and kidney. Its function is fascinating. First, the binding of renin to this receptor induced a four- to five-fold increase in the catalytic efficiency of conversion of AGT to Ang I. Second, prorenin was found to bind the renin receptor, and when prorenin was bound, it was as active as renin in inducing a biologic response. Finally, receptor occupation by renin or prorenin led to an intracellular signaling process associated with activation of mitogen-activated protein kinases, ERK-1 and ERK-2, without involvement of angiotensin generation. If these actions of prorenin contribute to pathophysiology, as seems likely, and if the renin inhibitors interrupt this sequence, as seems possible, then renin inhibitors would bring to the treatment of the patient with diabetic nephropathy or retinopathy an opportunity that far surpasses what an ACE inhibitor or an Ang II antagonist can do.

Opportunities Created by Combination Therapy

Is blockade induced by an ACE inhibitor “complete”? A related term is “break through,” in which plasma Ang II concentration begins to rise toward the pretreatment level. ACE inhibition and ARB, via the short feedback loop, lead to RAS activation. The ACE inhibitors are competitive antagonists and, thus, compete with Ang I for the receptor site on the enzyme. Do Ang I levels rise as a consequence of the renin response to blockade, rise to levels that lead to a return of Ang II formation? The answer is that they often do. Can we overcome that by increasing the ACE inhibitor dose? Of course we can, except the side effects tend to become intolerable.
For related reasons, a substantial number of reports have described the influence of a combination of an ACE inhibitor or an ARB, typically with proteinuria as the end point (4,16–21). Without exception, these studies report that the combination was more effective in reducing proteinuria than either agent alone. Such studies, to be truly informative, must deal effectively with the issue of dose. To our way of thinking, none has done so. As ARB are far better tolerated than ACE inhibitors, an optimal study would involve assessing the influence of the ARB at the top of its dose-response relationship for proteinuria reduction and then ascertaining whether adding an ACE inhibitor improves the therapeutic response.

Because to date no study has been designed to meet that demanding criterion, the interpretation of the available studies must be limited. What is clear, however, is that increasing the degree of renin-system blockade at any step improves the therapeutic response. Our next challenge is to work out how best to achieve that goal. In combination therapy, it seems intuitively obvious that one of the steps blocked should be the rate-limiting step.

Opportunities Created at the Rate-Limiting Step

Most of us carry around a notion of the rate-limiting step from the course in biochemistry that we took many years ago. Enzyme-catalyzed reactions often consist of several component steps. These different controlling steps may limit the rate of the overall reaction to different extents. Each step is characterized by a rate constant. Most of us were taught that the step with the lowest rate constant is rate limiting or the determinant of the rate of the overall reaction. However, as often pointed out (22–24), this definition can be misleading. As conditions change, the different specific activity for different enzymatic reactions that determine reaction velocity can vary. As an alternative, it has been argued that for steady state, the rate-limiting step is the “most sensitive” step, i.e., the step that, if perturbed, causes the largest change in overall velocity (23). By classical criteria, the renin-ANG interaction is the rate-limiting step for the renin cascade. Figure 1 provides the necessary information. Navar et al. (48) recorded the plasma concentration of the various elements of the cascade in the rat. AGT concentration is in the mid to high pmol range. Ang I, conversely, is in a low fmol range—more than three orders of magnitude lower. Ang II concentration is slightly lower. Thus, in the comparison of the renin and ACE steps, the renin step provides the largest step down in concentration. By the newer and more flexible definition, it is not yet clear whether the renin step is always rate-limiting—although compelling data that do so indicate have been presented (25). Despite a remarkable increase in plasma renin mass after administration of the renin inhibitor aliskiren, plasma Ang I and Ang II concentration remained suppressed. Taken in all, the available data suggest that in the plasma compartment, the renin step is always rate limiting. Whether that extends to tissue compartments remains open. There may be circumstances in which the substrate concentration is very much lower and in which the ACE step or the number of AT1 receptors becomes rate limiting.

Thus, findings raise a crucial question for combination therapy: “How important, quantitatively, is the reactive renin response to blockade with an ACE inhibitor or ARB?” The effects on proteinuria of a combination of an ACE inhibitor and an ARB suggest that the reactive response is quantitatively important. We believe that there are powerful reasons for performing an experiment in which an ACE or an ARB is combined with a renin inhibitor in the patient with proteinuria. Blockade at the most sensitive step is likely to provide the best possible therapy.

Renin Inhibitors

Renin belongs to the aspartic proteinase family, which includes pepsin, cathepsin-D, and chymosin (26). This class of enzyme is named because of a crucial feature: Each enzyme has two aspartic acid residues in their active site that are necessary for catalytic activity. Three-dimensional models of human renin, based on x-ray crystal structure of other aspartic proteinases, reveal co-planar, symmetrically arranged β-carbonyl groups of the aspartic acid residues at 38 and 226, held in an intricate web of hydrogen bonds. The active site appears as a long, deep cleft that can accommodate seven amino acid units of substrate. The enzyme also contains a mobile flap that, when closed, lies across the cleft and probably holds substrate in the active site (27). Renin has a similar bilobal structure, consistent with the hypothesis that aspartic proteinases, including renin, arose from a common ancestral gene (28). Their common catalytic sequence involves hydrolysis: Water stereospecifically attacks the carbonyl of the scissile amide bond (29).

Proof of principle that blocking the renin step would produce a biologically important response first came from an immunologic approach. Although initially antisera produced against crude renin extracts were used, these were followed by polyclonal and monoclonal antibodies against purified renin for multiple species (30). Indeed, the most potent inhibitor of human renin described so far had an IC50 of 1011 M (26).

Pepstatin, a peptide derived from microbial metabolism, acts as a competitive inhibitor of most aspartic proteinases, with an IC50 of 0.05 nmol for pepsin, for example. Unfortunately, it proved to be a weak inhibitor of renin with an IC50 of 10 nmol (31). Statin, an unusual amino acid found in pepstatin, is thought to act as a “transition state” mimic, resembling the tetrahedral conformation of the natural substrate at the scissile bond (32). This amino acid proved to be an important part of the structure: Action evolution later.

The approach that followed for the early development of renin inhibitors involved two different pathways (33), as summarized in Figure 3. One was based on the hypothesis that the prosegment of prorenin is capable of inhibiting enzyme activity by preventing access to the substrate. This pathway developed only weak inhibitors. The other approach was to synthesize peptic analogs of the N-terminal amino acid sequence of the substrate AGT. Substitution of the amino acid at the AGT scissile bond (positions 10 and 11) produced octapeptide analogs that were active competitive inhibitors. The most effective, known as “renin inhibitory peptide,” had an inhibitory potency against human renin in the micromolar range and was sufficiently active to be useful for research (33). Over time, the
peptide scissile bond was replaced by other transition state mimetics, including a reduced peptide bond or hydroxy-ethylene group (26). These synthetics produced a surfeit of potent. The result was inhibitors with potency in the nM range (Figure 3).

Having achieved sufficient potency, emphasis shifted from the development of agents that would retain potency to those that would also be bioavailable (Figure 3). The best of these agents, remikiren and enalkiren, were very potent and active on parenteral administration but showed oral bioavailability that was substantially less than 10%, more often in the 1 to 2% range (33).

Over time, progress was being made in dealing with the bioavailability problem. The last three agents shown in Figure 3, FK906, zankiren, and A-74273, all showed some improvement in oral bioavailability and efficacy in animal studies and in humans (33). Unfortunately, at this crucial stage, all of the programs were canceled for commercial reasons (27). The rapid advance of the Ang II antagonists was thought by the decision makers to make ultimate commercial success unlikely.

Figure 3. Structure and potency of renin inhibitors developed approximately in sequence. Reproduced from reference 33 with permission.

**Opportunities Created by the Development of New Agents**

Most of the early renin inhibitors involved variation on the original peptide structure. The possibility that alternative approaches to the formulation of renin inhibitors based on x-ray crystallography and reconstruction of the structure of the active site would lead to novel agents has recently found remarkable success. Four groups have developed novel agents on the basis of this principle (Figure 4). The most advanced agent, aliskiren, was developed by Speedel and Novartis. Although Figure 4 lists an activity of 0.6 nM, in fact the activity might be substantially greater. It has been tested in humans (25,35) and is entering Phase III evaluation.

The agents developed by Actelion-Merck and by Pfizer (Figure 4) are much earlier in development. The agents developed by these two groups show some similarities in structure but a different level of activity in early reports. Concurrent Pharmaceuticals has also developed renin inhibitors of two major classes with sufficient activity to be of substantial interest. No information on the bioavailability of the last three groups of agents in humans has yet been reported.

**Efficacy in BP Reduction**

A number of studies on BP in various animal models have shown either equivalence of ACE and renin inhibition or a small advantage of one or the other (26). The principle that blocking renin would reduce BP was established early in humans (34). The most instructive studies have been those in which responses to an ACE inhibitor and to a renin inhibitor were assessed in the same patients.

Jeunemaitre et al. (36) compared CJP-38560A, a renin inhibitor, with captopril, both at what were considered to be maximal doses: 0.25 mg/kg of the renin inhibitor and 50 mg of captopril. Salt intake was unrestricted, presumably fairly high in a French population. The patients had a marked fall in mean BP after captopril (15.3±1.5 mmHg) but showed only a limited (6.4±1.2 mmHg) mean BP fall after the renin inhibitor. Thus, if this renin inhibitor was actually used at the maximum dose, then captopril had an additional action unrelated to renin in these selected patients.

Neutel et al. (37) reported precisely opposite findings in a comparison of intravenous placebo enalaprilate (1.25 mg intravenously) and enalkiren (in a dose range from 0.03 to 1 mg/kg) in hypertensive patients who were hydrochlorothiazide treated to activate the renin system. The acute decrease in systolic BP of 18.5±0.4 mmHg during enalkiren was substantially greater than the 12.6±0.7 mmHg during enalaprilate (P<0.01). The fall in diastolic BP during enalkiren (11.9±0.4 mmHg) was also slightly greater than during enalaprilate (9.2±0.4 mmHg; P<0.01). In the patients in whom the plasma renin activity rose most, a similar pattern emerged, but the differences were nonsignificant in the low or normal renin group. This analysis might explain the negative findings described above by Jeunemaitre et al. (18) in individuals on a liberal salt intake.

Kiowiski et al. (38) compared another renin inhibitor, R0–425892, with captopril (50 mg). The renin inhibitor was given by
mouth in a 600-mg dose. In these normal individuals, captopril decreased diastolic BP from 60 \pm 5 to 51 \pm 7 \text{mmHg}, and the renin inhibitor did not. Salt intake was described as normal. RO-42-5892 was thought to be poorly bioavailable.

Stanton et al. (35) compared the BP response to aliskiren, dose range 37.5 to 300 mg/d, with losartan at a dose of 100 mg/d. Aliskiren induced a dose-related fall in daytime ambulatory BP averaging 11.0 mmHg for the highest dose: The daytime systolic BP fall with 100 mg of losartan was an essentially identical 10.9 mmHg.

In none of the studies was the opportunity taken to add the ACE inhibitor to the renin inhibitor or the renin inhibitor to the ACE inhibitor when a nadir in BP had been reached. Taken in all, the conclusion would be that the more renin-dependent the BP level, the larger the fall induced by renin inhibition. As there are no studies that go beyond very brief intervals of drug administration, the ultimate implications of these studies for therapeutics are limited.

### Efficacy: Renal Responses

Ang II plays a crucial role in the normal control of the renal circulation and renal function. Moreover, the remarkable efficacy of ACE inhibition in limiting renal injury in patients with diabetes and other forms of nephropathy placed a high priority on examining the influence of renin inhibitors on the renal blood supply and kidney function. When our studies in humans began, there was already substantial experience in animal models to suggest that renin inhibitors would induce a robust renal vasodilator response (39–41). The hypothesis that we were testing when we first assessed the renal hemodynamic response to a renin inhibitor was straightforward (42,45): To the extent that ACE inhibitors reduce kinin degradation and thereby induce vasodilator prostaglandin formation or activation of endothelial nitric oxide release, the renal vasodilator response to ACE inhibition should exceed the response to renin inhibition. Indeed, we thought that the difference in magnitude of the renal vascular response would provide a measure of the magnitude of the contribution of other vasodilators compared with reduced Ang II formation. To our surprise, the renal vasodilator response to the renin inhibitor enalkiren exceeded expectations from our experience with ACE inhibitors (42). In a more rigorous follow-up, a three-arm design was used to compare placebo; the same renin inhibitor, enalkiren; and captopril in random order and double blind. The placebo did nothing. Captopril and enalkiren both led to striking renal vasodilation (43). The response to enalkiren again was significantly larger than the response to captopril. The magnitude of the response to the renin inhibitor enalkiren in this study confirmed our earlier observation as did a more recent study with another renin inhibitor, zankiren, that induced a larger renal vasodilator response than could have been anticipated from the ACE inhibitor experience (44).

The renal vascular response to renin inhibition at the top of the dose-response curve in these three studies is summarized in Figure 5 in a meta-analysis format (28). All studies were performed in healthy men who were \(<40\) yr of age and who were in balance on a 10-mEq sodium intake to activate the renin system. All were studied by the same group of physicians, nurses, dietitians, and technicians on the same metabolic ward.

<table>
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<td>3</td>
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<tr>
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<td>0.4</td>
<td>PRECLINICAL</td>
<td>NA</td>
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*Figure 4. Structure, activity, and stage of development of renin inhibitors in current evaluation. All were developed on the basis of x-ray crystallography and active site modeling.*
using the same techniques for measurement of p-aminohippurate clearance as the index of renal perfusion. Also shown in Figure 5 are responses to three ACE inhibitors, each study performed at the top of the dose-response curve of renal blood flow. All were performed over the same time interval and under the same conditions. In the special case of the captopril: enalkiren comparison, the same subjects were involved. By ANOVA, there was no statistical difference identified among the three ACE inhibitors or between the two renin inhibitors, but a striking difference between the ACE and renin inhibitors was highly significant ($P < 0.001$). The renal vasodilator response to renin inhibition in healthy humans under these conditions, in the range of 140 to 150 ml/min per 1.73 m$^2$, exceeded by $>0\%$ the response to ACE inhibitors, which averaged approximately 90 to 100 ml/min per 1.73 m$^2$—when theory said it should be less.

In a subsequent study, El-Amrani et al. (46) compared systematically the renal vascular response to a renin inhibitor (R042-5892), an Ang II antagonist (losartan), and an ACE inhibitor (lisinopril) in guinea pigs. Because renin structure varies with species, renin inhibitors display species specificity: The guinea pig was selected because R042-5892, a renin inhibitor developed for primates, is also effective in the guinea pig. Doses of the agents were adjusted to induce an identical, small, but unambiguous fall in BP and were administered coded and in a random sequence. This careful, elegant study design revealed remarkable differences in the overall response to the three classes of agent. Despite an identical fall in BP, the renin inhibitor induced a substantially larger increase in renal plasma flow, GFR, diuresis, and natriuresis—as in the humans (46). The authors suggested that the potentiated response to renin inhibition could reflect greater lipophilicity and tissue penetration, leading to a local, intrarenal action at the site of Ang II production. As an alternative, the guinea pig might share with humans substantial non–ACE-dependent conversion of Ang I to Ang II, perhaps via chymase (47).

Taken in all, the data suggest strongly that in the case of the kidney, renin inhibition is far more effective than ACE inhibition in blocking Ang II formation. These observations clearly could have clinical significance.

What Is the Future for Renin Inhibition?

Although for a time it seemed that all renin inhibitor development had been closed, it is now clear that the search for an orally bioavailable renin inhibitor continues (49–51). We believe that the potential of renin inhibition in human therapy has been underestimated and indeed shows substantial promise. Should clues concerning microvascular disease at the level of the kidney and the eye and the possible influence of a renin inhibitor prove to be correct, then this pathway will have led to truly important advances in treatment. Diabetic nephropathy and diabetic retinopathy are, respectively, the leading cause of ESRD and blindness in the United States and much of the rest of the world.

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