DRUG DISPOSITION

Clin. Pharmacokinet. 29 (1): 6-14, 1995
0312-5963/95/0007-0006/$04.50/0



# Clinical Pharmacokinetics and Efficacy of Renin Inhibitors

*Gerard A. Rongen, Jacques W.M. Lenders, Paul Smits* and *Theo Thien*

University Hospital Nijmegen, Department of Medicine, Division of General Internal Medicine, Nijmegen, The Netherlands

## Contents



## Summary

The successful introduction of angiotensin converting enzyme (ACE) inhibitors in the treatment of patients with essential hypertension or heart failure has increased interest in the (patho)physiological role of the renin-angiotensin system (RAS). ACE is not only involved in the formation of angiotensin II from angiotensin I, but also inactivates vasoactive substances such as bradykinin and substance P. Accumulation of these substances during treatment with ACE inhibitors may contribute to both their therapeutic action and certain adverse effects associated with their use, such as cough and angioneurotic oedema.

Renin inhibitors offer an alternative approach to inhibit the RAS. The major advantage of these, still experimental, drugs is their high specificity for the RAS since angiotensinogen is the only known substrate of renin. The currently available renin inhibitors are pseudopeptides that are rapidly taken up by the liver and excreted in the bile. Consequently, these drugs are subjected to a considerable first pass effect which limits their oral bioavailability. Additionally, plasma elimination half-life times are short and the duration of action is limited. Despite these shortcomings, single oral or intravenous administration results in a 80 to 90% inhibition of plasma renin activity and a slight reduction in blood pressure in patients with hypertension. The extent of blood pressure reduction is dependent on the patient's salt balance.

After 1 week of oral treatment with the renin inhibitor remikiren, the antihypertensive effect was reduced in salt-repleted hypertensive patients. Subsequent intravenous administration of the drug did not further affect blood pressure, indicating that it was not the first pass effect that was limiting the efficacy of remikiren. The reactive increase in immunoreactive renin, alternative routes of

angiotensin II formation and counteracting blood pressure regulating mechanisms may also be involved in the limited haemodynamic response to renin inhibitors.

## 1. The Renin-Angiotensin System

The renin-angiotensin system (RAS) plays an important role in cardiovascular function and regulation of water and salt excretion. Angiotensin II is the effector molecule of the RAS system. Angiotensin II is a potent vasoconstrictor, mediates renal sodium reabsorption, has growth stimulating properties, and stimulates aldosterone and vasopressin release from the adrenal cortex and hypophysis, respectively. Angiotensin II is formed by enzymatic breakdown of angiotensinogen. Several enzymes are involved in this conversion of which renin and the angiotensin converting enzyme (ACE) are probably the most important (fig. 1).[1] Renin catalyses the formation of angiotensin I from angiotensinogen. Indeed, at present angiotensinogen is the only known substrate of renin. ACE catalyses the formation of angiotensin II from angiotensin I. The functional significance of the other enzymes that are able to catalyse the formation of angiotensin II is not exactly known.

The renin-mediated conversion of angiotensinogen to angiotensin I is the rate-limiting step of the RAS and the plasma level of renin is increased in conditions of an activated sympathetic nervous system, hypovolaemia, salt depletion and reduced intrarenal blood pressure. Circulating renin primarily originates from the kidney, where it is released from the juxtaglomerular cells.[2-4] Circulating renin is primarily eliminated by the liver and to a lesser extent by the kidney.[1,5] Circulating angiotensinogen originates from the liver.[6] ACE is primarily formed in the pulmonary vascular bed, but it is also formed in other vascular beds. The essential components of the RAS (i.e. angiotensinogen, renin and ACE) are not only present in blood but also in the vascular wall and in several organs, including the heart and the kidneys.

Components of the tissue RAS may originate from the circulation. This is probably the case for renin since it has rapid access to the extravascular space where it can be trapped by renin-binding proteins.[7,8] Apart from extraction from the circulation, local production of renin in the heart, kidneys and the vascular wall has been demonstrated by molecular techniques. Also for angiotensinogen and ACE, local production has been observed in the important effector organs of the RAS.[9] At present, the primary site of angiotensin I formation is thought to be in the extravascular compartment.[10] The relevance of this phenomenon is that measurement of circulating renin or angiotensin II does not necessarily reflect the amount of angiotensin II at the receptor level. Indeed, differences between plasma and cardiac angiotensin II formation have been observed in animal models of congestive heart failure.[11]

ACE is not only involved in the formation of angiotensin II, but also metabolises substances like

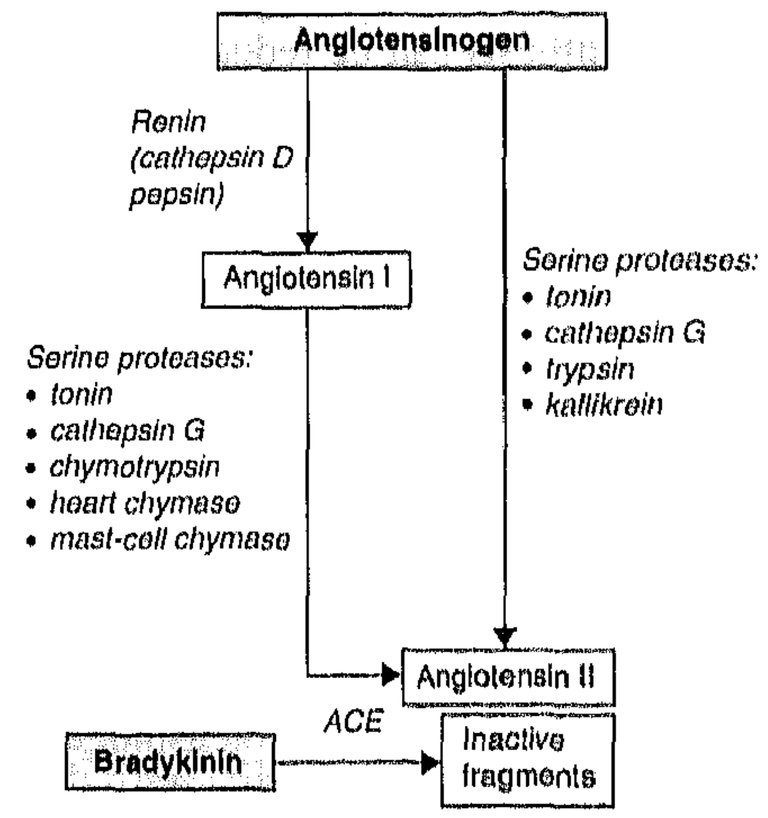



Fig. 1. Schematic representation of enzymatic degradation of angiotensinogen to angiotensin II. Angiotensin II is formed from angiotensinogen by 2 distinct metabolic routes: a renin- and angiotensin-converting enzyme (ACE)-dependent route; and a direct renin- and ACE-independent route.

bradykinin and substance P.[12] Thus, ACE inhibition not only inhibits the formation of angiotensin II, but also results in accumulation of these endogenous compounds.[12-15] Both bradykinin and substance P are well known stimulators of the release of endothelium-derived relaxing factors like nitric oxide and prostaglandins.[16,17] Apart from their effect on vascular tone, these endothelium-derived factors inhibit smooth muscle cell proliferation and inhibit thrombocyte aggregation.[18,19] Therefore, the RAS-independent effects of ACE inhibition may provide a therapeutic advantage in patients with cardiovascular disease.

Pharmacological inhibition of the RAS can be achieved at the level of renin, ACE or angiotensin II receptors. ACE inhibitors have been proven to be potent therapeutic agents in cardiovascular diseases such as hypertension and congestive heart failure.[20-22] Inhibition of the RAS with renin inhibitors or angiotensin II receptor antagonists have the potential advantage of selectivity. This review will focus on renin inhibitors and, in particular, on those that have been used in human research.

## 2. Methods to Reduce Renin Activity

In theory, there are several ways to inhibit renin activity:

- renin biosynthesis can be reduced at the DNA level or by inhibition of renin formation from prorenin
- renin release can be attenuated
- the activity of released renin can be inhibited.

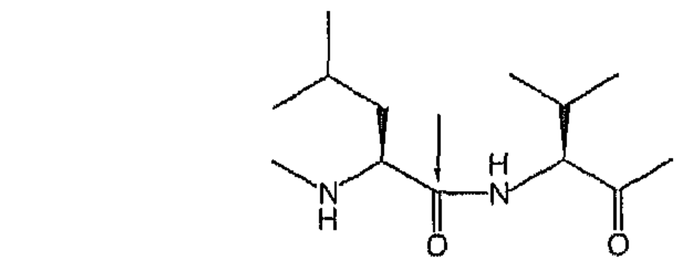
Renin release can be inhibited by β-adrenoceptor antagonists, but this is not a specific effect of these drugs.[23] Some recently developed renin inhibitors are able to attenuate the expression of the kidney renin gene and to inhibit renin release (see section 4).[24]

In animals, 2 main approaches have been adopted for inhibition of the renin-angiotensinogen reaction.[25] The first is an immunological approach by passive immunisation with anti-angiotensinogen or anti-renin antibodies or by active immunisation against renin. With this approach, a blood pressure drop could be observed in rats. In humans, this method has never been used because of the potential induction of antigen-antibody precipitation with deleterious consequences.

The second approach started with the minimal substrate of renin. By a small alteration in this octapeptide at the cleavage site (the so-called 'scissile bond'), a substrate analogue was obtained with renin-inhibiting properties.[26] Statine is thought to play an important role in the inhibitory mechanism of pepstatin, a general inhibitor of aspertyl proteases and a weak inhibitor of renin.[27] Substitution of the unusual amino acid statine or its variants at the scissile-bond, resulted in more potent and selective renin inhibitors. Hydrolysis by renin was prevented by scissile-bond modifications with nonhydrolysable pseudopeptides. The inhibitory potency was further increased by modifications at the carboxyl and amino termini of the substrate-analogue structure.[28] This approach resulted in several small pseudopeptides (fig. 2) that are able to inhibit renin specifically at low concentrations [concentration inhibiting 50% of activity ($IC_{50}$): 0.7 to 14 nmol/L].

## 3. Pharmacokinetics of Renin Inhibitors

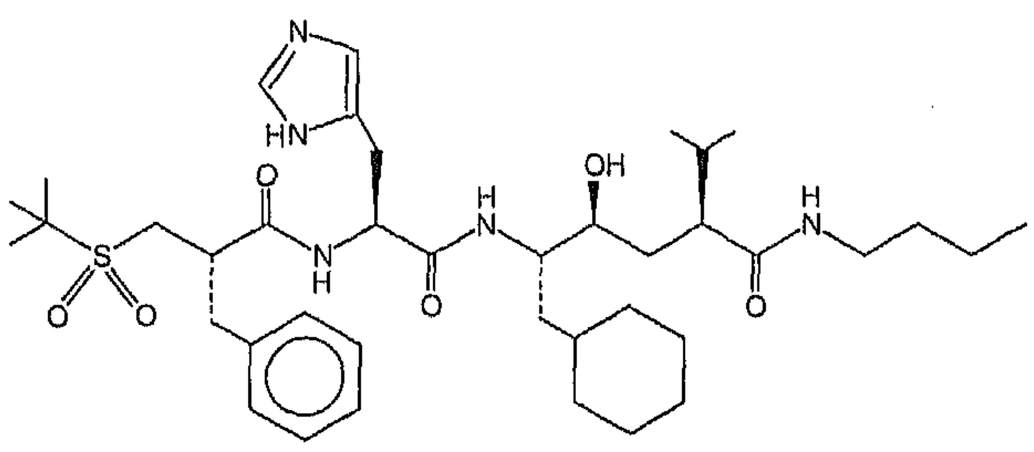
The Renin Inhibitory Peptide (RIP) was the first renin inhibitor administered intravenously to normotensive human volunteers. It reduced blood pressure. Upright tilting resulted in exaggerated hypotension and pronounced bradycardia, suggesting an aspecific action of this particular substance.[29] At present, at least 7 specific renin inhibitors have been used in human research.[30-37] For 4 of these agents, human pharmacokinetic data are available (table I).[33,38-45] All 4 peptidomimetic compounds are transition-state analogues of the cleavage site of human angiotensinogen[46] (fig. 2). Apart from enalkiren, the drugs have comparable *in vitro* potencies to inhibit renin activity. The $IC_{50}$ values in table I were obtained from purified renin. In plasma, $IC_{50}$ values are increased because of considerable protein binding resulting in a reduced free drug concentration. For example, protein binding has been reported to be 94% in the case of enalkiren.[39,40] When the potency is measured in hu-



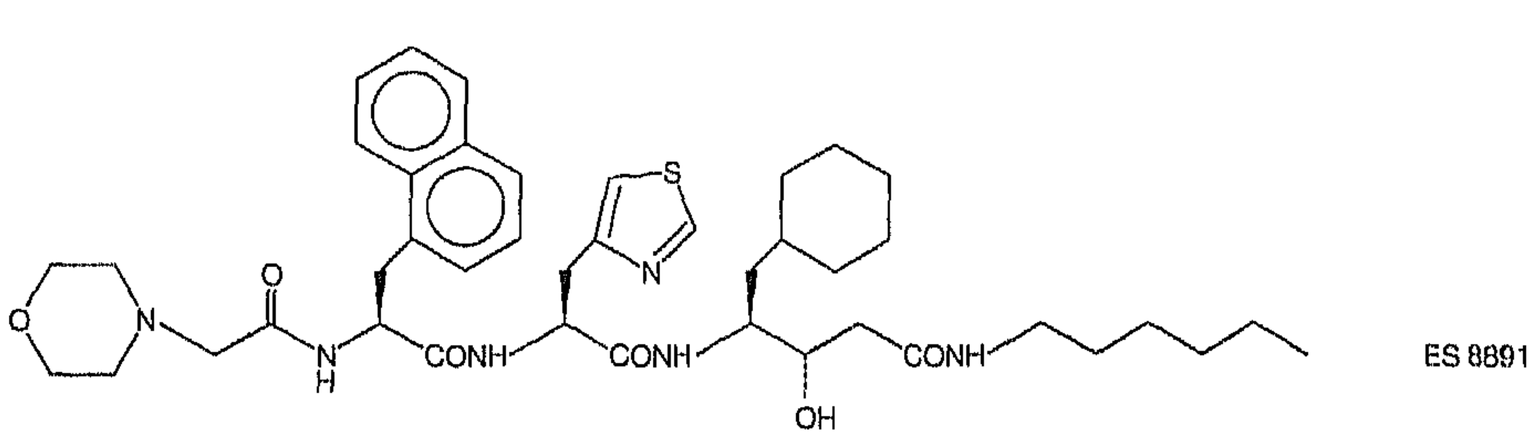
Cleavage site of angiotensinogen



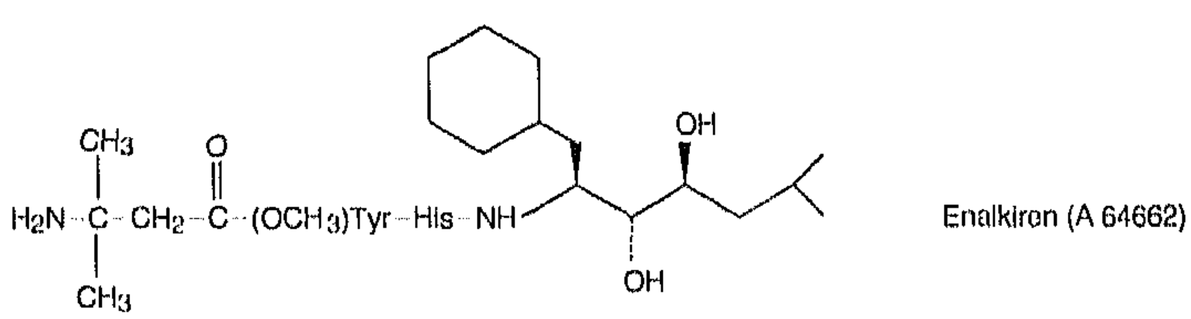
Remikiren (Ro 425892)



CGP 38560



ES 8891



Enalkiren (A 64662)

**Fig. 2.** Structural formulae of the 4 renin inhibitors that have been most extensively studied in humans, and the site of cleavage of angiotensinogen.

man plasma under almost physiological conditions, the potency of remikiren, enalkiren and CGP 38560 was 7-, 20-, and 35-fold lower, respectively, than the inhibitory potency against purified renin.[47] These compounds do not inhibit other aspertyl proteases like pepsin and cathepsin D or other proteases like ACE, indicating the high specificity of these drugs for renin.[33,48,49]

### 3.1 Oral Bioavailability

In general, the oral bioavailability of all 4 compounds is poor and highly variable. The main rea-

Table I. Pharmacokinetic data for renin inhibitors in healthy volunteers

| Renin inhibitor | $IC_{50}$a (nmol/L) | Oral bioavailability (%) | Plasma half-life (h) | | Vd (L/kg) |
|---|---|---|---|---|---|
| | | | first phase | second phase | |
| Remikiren (Ro 425892) | 0.8 | 1-2 | 0.1 | 9.4 ± 4.1 | 0.9 |
| CGP 38560 | 0.7 | <1 | 0.13 | 1.05 | In marmosets the Vd is similar to plasma volume |
| ES 8891 | 1.1 | NA[b] | ? | 0.95 ± 0.31 | |
| Enalkiren (A 64662) | 14 | undetectable | 0.25 ± 0.1 | 1.60 ± 0.43 | 0.2 |

a For human renin.

b Oral bioavailability cannot be assessed since this drug cannot be administered intravenously. Absorption indexes suggest a 10 times higher oral absorption as compared with CGP 38560.

*Abbreviations and symbol:* $IC_{50}$ = concentration required to produce 50% inhibition; NA = not available; Vd = volume of distribution; ? = unknown.

sons for this poor oral bioavailability are a low degree of intestinal absorption and a considerable first pass effect.[41,50,51] For remikiren, the high hepatic first-pass has been assumed to be the main reason for the low oral bioavailability in humans.[38] Several methods are currently under investigation to solve this major problem hindering the clinical application of renin inhibitors. Several derivatives of enalkiren have been developed that might have a better bioavailability than enalkiren itself. However, oral bioavailability of these analogues still remains below 10% in monkeys.[41,52] The use of emulsion formulations increased oral bioavailability in rats from 0.3 to 5.1% in case of a lipid soluble renin inhibitor.[53]

The intrapulmonary route of administration may be a promising alternative to oral delivery as shown in rats.[54] For the renin inhibitor U77436, 52% of the intratracheal injected drug was absorbed compared with 6 to 16% after oral administration in rats. Pulmonary absorption appeared to be slower than its intestinal counterpart in these rats resulting in a sustained release of the drug delivered by the intrapulmonary route. In this context, an intravenous study with remikiren in humans is of interest, suggesting that a sustained release of this renin inhibitor results in a more effective inhibition of the RAS than is achieved by rapid release of the same amount of drug.[55]

### 3.2 Volume of Distribution

Volumes of distribution differ considerably between the varying renin inhibitors. For remikiren, the volume of distribution is considerably higher than the plasma volume. This implies that remikiren diffuses into the extravascular compartment where it may affect the tissue RAS. This is further supported by the observation that the remikiren-induced blood pressure reduction is temporarily dissociated from the inhibition of circulating RAS.[56] Differences in tissue penetration and subsequent inhibition of tissue RAS may account for differences in efficacy between the varying renin inhibitors to reduce blood pressure, as has recently been observed in primates.[47]

### 3.3 Elimination

The liver plays an important role in the rapid elimination of renin inhibitors. A carrier mediated ATP-dependent transport system is involved in the hepatocellular uptake of hydrophilic renin-inhibiting peptides.[50,51] Subsequently, the renin inhibitor and its metabolites are excreted in the bile.[45] For remikiren, there is no indication that active metabolites are formed.[38] The longer elimination half-life of remikiren compared with the other renin inhibitors is probably due to its large volume of distribution since high systemic plasma clearance, which approaches liver plasma flow, was found.[38] During the elimination phase, a shift of remikiren from the extravascular compartment probably results in the prolonged elimination half-life in plasma. As a result of the short elimination half-lives of the currently available renin inhibitors, one single oral dosage per day does not result in drug accumulation nor is it enough to inhibit

plasma renin activity continuously as has been demonstrated in recent clinical trials with remikiren.[57,58]

## 4. Biochemical and Haemodynamic Effects

To date, no adverse effects have been observed with renin inhibitors. The efficacy of renin inhibitors can be expressed in terms of biochemical and haemodynamic effects. Although reduction in mortality and morbidity in patients with cardiovascular disease is the ultimate goal of treatment with renin inhibitors, the currently available clinical data do not allow any conclusions to be made with regard to these effect parameters. Therefore, we will confine ourselves to the biochemical and haemodynamic effects of renin inhibitors.

Several biochemical parameters are available to assess RAS inhibition of which plasma renin activity (PRA), plasma angiotensin I and II levels and plasma immunoreactive renin level are the most important. With regard to plasma renin activity, analysis is difficult when a routine assay in non-acidified plasma is used in the presence of phenylmethylsulphonyl fluoride and 8-hydroxyquiroline sulphate. These agents can cause *ex vivo* displacement of a protein-bound renin inhibitor, thereby increasing its free concentration and resulting in an overestimation of drug-induced inhibition of PRA. This problem has been solved by using angiotensin I antiserum as a trapping reagent.[59,60] Using this modified PRA assay, intravenous administration of CGP 38560, remikiren or enalkiren results in 80 to 90% inhibition of PRA. Parallel to the inhibition of PRA, the plasma angiotensin II level is reduced by 90% and amount of immunoreactive renin is increased by 200 to 400%.[31,34,35,57,60]

The increase in immunoreactive renin levels shows an interruption of the negative feedback regulation of renin release, caused by a reduction in angiotensin II levels.[61,62] After oral administration of remikiren, similar effects on PRA were observed as compared with intravenous administration, but immunoreactive renin concentrations increased only by 70 to 90%.[57,58] Plasma remikiren concentrations in these experiments were 20 times higher after intravenous administration than those achieved after oral administration. Nevertheless, comparable reductions in PRA were observed, indicating that it is not the low bioavailability but rather the associated increase in immunoreactive renin levels that is the limiting factor inhibiting plasma renin activity.[57] In this context, the property of the renin inhibitor ES 8891 to inhibit renal renin synthesis and release in marmosets is of interest.[33,63] ES 8891 has also been administered to healthy volunteers.[64] Its administration has resulted in a significant reduction in PRA and plasma levels of angiotensin I. Simultaneously measured immunoreactive renin was not affected, suggesting that this compound inhibits renal renin release in humans.[64]

With respect to the haemodynamic effects of renin inhibitors, blood pressure and heart rate are the most important parameters that have been studied at present. In healthy salt-repleted volunteers, renin inhibition does not result in a significant reduction in blood pressure, resembling the effect of ACE inhibitors.[34,38,44,65] In patients with essential hypertension, the blood pressure response to renin appears to be related to sodium intake. During salt depletion, as induced by diuretic treatment or reduced sodium intake, blood pressure homoeostasis is more dependent on the RAS. Therefore, the acute inhibition of renin results in a profound reduction in blood pressure without a concomitant increase in heart rate.[60,66] This effect on blood pressure has been observed both after intravenous and oral administration of remikiren.[67] The reduction in blood pressure is not diminished after one week of intravenous treatment.[68] In patients with heart failure, a condition that is also associated with an activated RAS, single intravenous administration of enalkiren appeared to reduce intra-arterial blood pressure.[69]

In salt-repleted patients with essential hypertension, the situation is more complex. A single oral or intravenous dose of remikiren reduced blood pressure, but to a lesser extent than the same dose

administered to salt-depleted patients. After one week of oral treatment with remikiren, the reduction in blood pressure was diminished and subsequent intravenous administration of the renin inhibitor did not result in a further reduction in blood pressure. This suggests that the mode of action, and not the low bioavailability, was the limiting factor for the reduced efficacy of the drug after prolonged treatment.[57,58]

## 5. Conclusions and Future Perspectives

The theoretical advantage of renin inhibitors in comparison with ACE inhibitors is the selectivity of the former drugs to inhibit the RAS. This is relevant in pathophysiological studies where renin inhibitors can be used as a tool to elucidate the precise role of the RAS in cardiovascular disease. In a clinical setting, renin inhibitors could replace the successful ACE inhibitors, the use of which is restricted because of adverse reactions such as cough or angioneurotic oedema that result from accumulation of substrates of ACE other than angiotensin I.[70]

The currently developed peptidomimetic renin inhibitors have a high *in vitro* and *in vivo* potency and specificity to inhibit circulating renin. The high volume of distribution of remikiren indicates significant tissue penetration. The temporal dissociation between the inhibition of circulating renin activity or angiotensin II levels and blood pressure reduction further suggests that the tissue RAS is also inhibited by this compound.

From a pharmacokinetic point of view, currently available renin inhibitors are far from ideal antihypertensive drugs because of their low oral bioavailability and rapid elimination. However, the low bioavailability cannot totally account for the disappointing effect of repeated oral administration of renin inhibitors on blood pressure in salt-repleted hypertensive patients.

From a pharmacodynamic point of view, inhibition of renin results in an increased release of active renin. The increase in plasma immunoreactive renin concentrations limits the maximal reduction in PRA that can be achieved by inhibition of renin. A direct renin-independent formation of angiotensin II from angiotensinogen has been suggested (see also fig. 1),[3] which might further decrease the efficacy of renin inhibitors to reduce the concentration of angiotensin II. Finally, blood pressure regulating mechanisms other than the RAS may contribute to the maintenance of elevated blood pressures during renin inhibition in patients with essential hypertension.

Further research is needed to develop renin inhibitors with a reproducible and high bioavailability, resulting in sustained effective plasma drug concentrations. High tissue penetration is necessary for an optimal therapeutic effect. The problem of renin release in response to renin inhibition can be solved by using compounds that also inhibit renin synthesis and release. The efficacy of renin inhibition in terms of blood pressure reduction can be augmented by reductions in salt intake or concomitant treatment with diuretics. The combination of ACE- and renin inhibitors may be of potential benefit.[71] Whether these improvements result in renin inhibitors that can compete with ACE inhibitors in the treatment of patients with hypertension or heart failure is a question that remains to be answered.

## References


1. Campbell DJ. Chapter 23: metabolism of prorenin, renin, angiotenisogen, and angiotensins by tissues. In: Robertson JIS, Nicholls MG, editors. The renin-angiotenins system. London: Gower Medical Publishing, 1993; 1-23
2. Opsahl JA, Smith KL, Murray RD, et al. Renin and renin inhibition in anephric man. Clin Exp Hypertens 1993; 15: 289-306
3. Campbell DJ, Kladis A, Duncan AM. Nephrectomy, converting enzyme inhibition, and angiotensin peptides. Hypertension 1993; 22: 513-22
4. Kurtz A, Scholz H, Della Bruna R. Molecular mechanisms of renin release. J Cardiovasc Pharmacol 1990; 16 Suppl. 4: S1-S7
5. Stella A, Zanchetti A. Chapter 22: elimination of renin, prorenin, and angiotensins by the kidney: renin in urine. In: Robertson JIS, Nicholls MG, editors. The renin-angiotensin system. London: Gower Medical Publishing, 1993; 1-8
6. Page IH, McSwain B, Knapp GM, et al. The origin of renin activator. Am J Physiol 1941; 135: 214-22
7. Schneider EG, Rostorfer HH, Nash FD. Distribution volume and metabolic clearance rate of renin in anesthetized nephrectomized dogs. Am J Physiol 1968; 215: 1115-22
8. Campbell DJ, Kladis A, Skinner SL, et al. Characterization of angiotensin peptides in plasma of anephric man. J Hypertens 1991; 9: 265-74

9. Dzau V, Pratt RE. Cardiac, vascular, and intrarenal renin-angiotensin systems in normal physiology and disease. In: Robertson JIS, Nicholls MG, editors. The renin-angiotensin system. London: Gower Medical, 1993; 42: 1-11
10. Campbell DJ. The site of angiotensin production. J Hypertens 1990; 3: 199-207
11. Hirsch AT, Talsness CE, Schunkert H, et al. Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. Circ Res 1991; 69: 475-82
12. Carretero OA, Scicli AG. Kinins paracrine hormone. Kidney International 1988; 34 Suppl. 26: S52-S59
13. Lindsey CJ, Bendhack LM, Paiva ACM. Effects of teprotide, captopril and enalaprilat on arterial wall kininase and angiotensin converting enzyme activity. J Hypertens 1987; 5 Suppl. 2: S71-S76
14. Marks ES, Bing RF, Thurston H, et al. Vasodepressor property of the converting enzyme inhibitor captopril (SQ 14 225): the role of factors other than renin-angiotensin blockade in the rat. Clin Sci 1980; 58: 1-6
15. Quilley J, Duchin KL, Hudes EM, et al. The antihypertensive effect of captopril in essential hypertension: relationship to prostaglandins and the kallikrein-kinin system. J Hypertens 1987; 5: 121-8
16. Vallance P, Collier J, Moncada S. Nitric oxide synthesised from L-arginine mediates endothelium dependent dilatation in human veins in vivo. Cardiovasc Res 1989; 23: 1053-7
17. Mitchell JA, Denucci G, Warner TD, et al. Different patterns of release of endothelium-derived relaxing factor and prostacyclin. Br J Pharmacol 1992; 105: 485-9
18. Radomski MW, Palmer RM, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. Br J Pharmacol 1987; 92: 639-46
19. Cooke JP, Rossitch Jr E, Andon NA, et al. Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. J Clin Invest 1991; 88: 1663-71
20. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med 1991; 325: 293-302
21. The SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. N Engl J Med 1992; 327: 685-91
22. Zanchetti A. The clinical role of angiotensin converting enzyme inhibitors in antihypertensive therapy in the 1990s. J Hypertens 1989; 7 Suppl. 5: S37-S40
23. Hajjali AF, Wong PC. Beta-adrenoceptor blockade in rabbit inhibits the renin-releasing effect of AT(1)-receptor antagonist losartan. J Pharmacol Exp Ther 1993; 267: 1423-7
24. Kitami Y, Hiwada K, Murakami E, et al. The effect of the renin inhibitor ES-1005 on the expression of the kidney renin gene in sodium-depleted marmosets. J Hypertens 1990; 8: 1143-6
25. Corvol P, Menard J. Renin inhibition: immunological procedures and renin inhibitor peptides. Kidney Int 1988; 34 Suppl. 26: S73-S79
26. Burton J, Cody Jr RJ, Herd JA, et al. Specific inhibition of renin by an angiotensin analog: studies in sodium depletion and renin-dependent hypertension. Proc Natl Acad Sci USA 1980; 77: 5476-9
27. Gross F, Lazar J, Orth H. Inhibition of the renin-angiotensin reaction by pepstatin. Science 1972; 175: 656
28. Hui KY, Haber E. Chapter 85: renin inhibitors. In: Robertson JIS, Nicholls MG, editors. The renin-angiotensin system. London: Gower Medical, 1993; 1-14
29. Zusman RM, Burton J, Christensen D, et al. Hemodynamic effects of a competitive renin inhibitory peptide in humans: evidence for multiple mechanisms of action. Trans Assoc Am Physicians 1983; 96: 365-74
30. Webb DJ, Manhem PJ, Ball SG, et al. A study of the renin inhibitor H142 in man. J Hypertens 1985; 3: 653-8
31. van den Meiracker AH, Admiraal PJJ, Man in 't Veld AJ, et al. Prolonged blood pressure reduction by orally active renin inhibitor Ro 42-5892 in essential hypertension. BMJ 1990; 301: 205-10
32. Glassman HN, Kleinert HD, Boger RS, et al. Clinical pharmacology of enalkiren, a novel dipeptide renin inhibitor. J Cardiovasc Pharmacol 1990; 16 Suppl. 4: S76-S81
33. Kokubu T, Hiwada K, Murakami E, et al. ES-8891, an orally active inhibitor of human renin. Hypertension 1990; 15: 909-13
34. Nussberger J, Delabays A, de Gasparo M, et al. Hemodynamic and biochemical consequences of renin inhibition by infusion of CGP 38560A in normal volunteers. Hypertension 1989; 13: 948-53
35. Denolle T, Luo P, Guyene TT, et al. Acute effects of a pseudotetrapeptide as renin inhibitor on blood pressure and renin-angiotensin system of sodium-repleted and sodium-depleted hypertensive patients. Arzneimittelforschung 1993; 43: 255-9
36. Zusman RM, Hui KY, Nussberger J, et al. R-PEP-27, a potent renin inhibitor, decreases plasma angiotensin II and blood pressure in normal volunteers. Am J Hypertens 1994; 7: 295-301
37. Ogihara T, Nagano M, Higaki J, et al. Antihypertensive efficacy of FK906, a novel human renin inhibitor. Clin Ther 1993; 15: 539-48
38. Kleinbloessem CH, Weber C, Fahrner E, et al. Hemodynamics, biochemical effects, and pharmacokinetics of the renin inhibitor remikiren in healthy human subjects. Clin Pharmacol Ther 1993; 53: 585-92
39. Gupta SK, Granneman GR, Boger RS, et al. Simultaneous modeling of the pharmacokinetic and pharmacodynamic properties of enalkiren (Abbott-64662, a new renin inhibitor), I: Single dose study. Drug Metab Dispos Biol Fate Chem 1992; 20: 821-5
40. Gupta SK, Granneman GR, Packer M, et al. Simultaneous modeling of the pharmacokinetic and pharmacodynamic properties of enalkiren (Abbott-64662, a renin inhibitor), II: a dose-ranging study in patients with congestive heart failure. J Cardiovasc Pharmacol 1993; 21: 834-40
41. Kleinert HD, Rosenberg SH, Baker WR, et al. Discovery of a peptide-based renin inhibitor with oral bioavailability and efficacy. Science 1992; 257: 1940-3
42. Weber C, Birnbock H, Leube J, et al. Multiple dose pharmacokinetics and concentration effect relationship of the orally active renin inhibitor remikiren (Ro 42-5892) in hypertensive patients. Br J Clin Pharmacol 1993; 36: 547-54
43. Weber C, Roos B, Birnboeck H, et al. Effect of food on the oral bioavailability of the renin inhibitor remikiren (Ro 42-5892) [abstract]. Br J Clin Pharmacol 1993; 36: 177P-8P
44. de Gasparo M, Cumin F, Nussberger J, et al. Pharmacological investigations of a new renin inhibitor in normal sodium-unrestricted volunteers. Br J Clin Pharmacol 1989; 27: 587-96
45. Cumin F, Schnell C, Richert P, et al. Pharmacokinetics and tissue distribution of the renin inhibitor N-(2-(R)-benzyl-3-tert-butyl-sulfonyl-propionyl)-His-ChaeVal-n-butylamine in marmosets. Drug Metab Dispos Biol Fate Chem 1990; 18: 831-5
46. Corvol P, Chauveau D, Jeunemaitre X, et al. Human renin inhibitor peptides. Hypertension 1990; 16: 1-11

47. Clozel JP, Fischli W. Comparative effects of three different potent renin inhibitors in primates. Hypertension 1993; 22: 9-17
48. Wood JM, Criscione L, de Gasparo M, et al. CGP 38 560: orally active, low molecular weight renin inhibitor with high potency and specificity. J Cardiovasc Pharmacol 1989; 14: 221-6
49. Clozel JP, Fischli W. Discovery of remikiren as the first orally active renin inhibitor. Arzneimittelforschung 1993; 43: 260-2
50. Ziegler K, Seeberger A. Hepatocellular uptake of peptides, I: Carrier-mediated uptake of hydrophilic linear peptides with renin inhibitory activity into isolated rat liver cells. Biochem Pharmacol 1993; 45: 909-16
51. Seeberger A, Ziegler K. Hepatocellular uptake of peptides, II: Interactions between hydrophilic linear renin-inhibiting peptides and transport systems for endogenous substrates in liver cells. Biochem Pharmacol 1993; 45: 917-25
52. Kleinert HD, Stein HH, Boyd S, et al. Discovery of a well-absorbed, efficacious renin inhibitor, A-74273. Hypertension 1992; 20: 768-75
53. Kararli TT, Needham TE, Griffin M, et al. Oral delivery of a renin inhibitor compound using emulsion formulations. Pharm Res 1992; 9: 888-93
54. Sheehy AM, Hoover JL, Rush BD, et al. Intrapulmonary delivery of renin inhibitory peptides results in sustained release because of saturable transport. Pharm Res 1993; 10: 1548-51
55. Doig JK, MacFadyen RJ, Meredith PA, et al. Neurohormonal and blood pressure responses to low-dose infusion of an orally active renin inhibitor, Ro 42-5892, in salt-replete men. J Cardiovasc Pharmacol 1992; 20: 875-80
56. van den Meiracker AH, Admiraal PJJ, Derkx FHM, et al. Comparison of blood pressure and angiotensin responses to the renin inhibitor Ro 42-5892 and the angiotensin converting enzyme inhibitor enalapril in essential hypertension. J Hypertens 1993; 11: 831-8
57. Rongen GA, Lenders JWM, Kleinbloesem CH, et al. Efficacy and tolerability of the renin inhibitor Ro 42-5892 in patients with hypertension. Clin Pharmacol Ther 1993; 54: 567-77
58. Kobrin I, Viskoper RJ, Laszt A, et al. Effects of an orally active renin inhibitor, Ro 42-5892, in patients with essential hypertension. Am J Hypertens 1993; 6: 349-56
59. Schalekamp MA, Derkx FH, van den Meiracker AH. Renin inhibitors, angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists: relationships between blood pressure responses and effects on the renin-angiotensin system. J Hypertens 1992; 10 Suppl.: S157-S164
60. Jeunemaitre X, Menard J, Nussberger J, et al. Plasma angiotensins, renin, and blood pressure during acute renin inhibition by CGP 38 560A in hypertensive patients. Am J Hypertens 1989; 2: 819-27
61. Menard J, Guyene T, Chatellier G, et al. Renin release regulation during acute renin inhibition in normal volunteers. Hypertension 1991; 18: 257-65
62. Camenzind E, Nussberger J, Juilerat L, et al. Effect of renin response during renin inhibition: oral Ro 42-5892 in normal humans. J Cardiovasc Pharmacol 1991; 18: 299-307
63. Ii Y, Murakami E, Hiwada K. Effect of renin inhibitor, ES-8891, on renal renin secretion and storage in the marmoset: comparison with captopril. J Hypertens 1991; 9: 1119-25
64. Murakami E, Kokubu T, Ii Y, et al. Effects of repeated oral administration of renin inhibitor ES-8891 on the renin-angiotensin system in human subjects. Hypertens Res 1992; 15: 41-4
65. Delabays A, Nussberger J, Porchet M, et al. Hemodynamic and humoral effects of the new renin inhibitor enalkiren in normal humans. Hypertension 1989; 13: 941-7
66. Weber MA, Neutel JM, Essinger I, et al. Assessment of renin dependency of hypertension with a dipeptide renin inhibitor. Circulation 1990; 81: 1768-74
67. van den Meiracker AH, Admiraal PJ, Man in 't Veld AJ, et al. Prolonged blood pressure reduction by orally active renin inhibitor RO 42-5892 in essential hypertension. BMJ 1990; 301: 205-10
68. Boger RS, Glassman HN, Cavanaugh JH, et al. Prolonged duration of blood pressure response to enalkiren, the novel dipeptide renin inhibitor, in essential hypertension. Hypertension 1990; 15: 835-40
69. Neuberg GW, Kukin ML, Penn J, et al. Hemodynamic effects of renin inhibition by enalkiren in chronic congestive heart failure. Am J Cardiol 1991; 67: 63-6
70. Morice AH, Lowry R, Brwon MJ, et al. ACE and the cough reflex. Lancet 1987; ii: 1116-8
71. Mento PF, Holt WF, Murphy WR, et al. Combined renin and converting enzyme inhibition in rats. Hypertension 1989; 13: 741-8

Correspondence and reprints: Dr *Jacques W.M. Lenders*, University Hospital Nijmegen, Department of Medicine, Division of General Internal Medicine, PO Box 9101, 6500 HB Nijmegen, The Netherlands.