

Forum Minireview

**Drug Discovery for Overcoming Chronic Kidney Disease (CKD):
New Therapy for CKD by a (Pro)Renin-Receptor–Blocking Decoy
Peptide**Atsuhiko Ichihara^{1,*}, Mariyo Sakoda¹, Asako Kurauchi-Mito¹, Tatsuya Narita¹, Kenichiro Kinouchi¹,
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Abstract. Discovery of the (pro)renin receptor uncovered a novel function of renin/prorenin as the receptor ligands in addition to the enzyme and its precursor. The bindings of renin and prorenin to the (pro)renin receptor trigger two major pathways: the angiotensin II–dependent pathway as a result of the enzymatic activation of renin/prorenin and the angiotensin II–independent intracellular pathway involving hypertrophic, hyperplastic, and profibrotic signals. A specific blocker of the receptor was discovered through identification of the amino acid sequence of prorenin prosegment that binds to the receptor and leads to non-proteolytic conversion of prorenin to its active form. A peptide containing this sequence was found to block the binding of prorenin to its receptor. Its infusion in animal models of diabetes and low-renin hypertension significantly inhibited the development and progression of nephropathy, but (pro)renin receptor blockade had no benefit in the clipped kidney of 2K1C rats or rat models of high-renin hypertension. Since renin is still active without a (pro)renin receptor, (pro)renin-receptor blockade elicits a maximum benefit under low-renin conditions. Thus, (pro)renin-receptor blockade can be a useful therapy for chronic kidney disease with low renin levels in the plasma.

Keywords: angiotensin, diabetes, hypertension, mitogen-activated protein kinase, renin, chronic kidney disease (CKD)

Introduction

Within the kidneys, (pro)renin receptors are present not only in the mesangium, macula densa, and tubular cells (1, 2) but also in the podocytes (3). The binding of renin and prorenin to the (pro)renin receptor trigger two major pathways: the angiotensin II–dependent pathway as a result of the enzymatic activation of renin and prorenin and the angiotensin II–independent intracellular pathway via the (pro)renin receptor (1, 2, 4–6). The binding affinity of prorenin to the (pro)renin receptor is two to three times higher than that of rennin (7). The enzymatic activity (the ability to generate angiotensin I, the so-called “renin activity”) of receptor-bound renin is

four to five times higher than that of free rennin (1), but the mechanism accounting for the increased renin activity of receptor-bound renin remains undetermined.

When a specific protein binds to the “handle region” of the prosegment of prorenin, the receptor-bound prorenin gains “renin activity” without the proteolytic cleavage of the prosegment of prorenin as a result of a conformational change (8). Recombinant prorenin binds to the recombinant (pro)renin receptor in a test tube (7) and to the (pro)renin receptor expressed on the cell surfaces of COS-7 cells (9); the receptor-bound prorenin exerts “renin activity” without changing the molecular weight of prorenin. Synthetic peptides containing the amino acids sequence corresponding to the “handle” region of the prorenin prosegment (HRP) competitively inhibit prorenin binding to the receptor (7, 9). The binding of HRP to the (pro)renin receptor and its inhibition of prorenin binding to the receptor were

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clearly confirmed by a BIAcore method (10). However, since the full-length (pro)renin receptor is predominantly present in the cytoplasm around the nucleus but not on the cell surfaces of cultured cells (11), HRP failed to inhibit the intracellular prorenin's acquisition of catalytic activity by binding to the receptor in vascular smooth muscle cells (VSMCs) harvested from human-(pro)renin-receptor-transgenic rats (12) or in U937 monocytes (13). In contrast, HRP appears to inhibit the binding of prorenin to the (pro)renin receptor *in vivo*, where the full-length (pro)renin receptor is present on the cell surfaces (1, 14). This review article introduces the benefits and limitation of HRP in various conditions with chronic kidney disease.

Diabetic nephropathy

Diabetic patients have lower renin levels and 40 times higher prorenin levels than normal healthy subjects (15). Increased plasma prorenin levels in insulin-dependent diabetic patients have been reported to predict microvascular complications (16), and young diabetic patients with high plasma prorenin levels have been found to be at higher risk for retinopathy or nephropathy (17). However, the mechanism whereby the increased prorenin levels cause organ damage in patients with diabetes has remained unclear. HRP was administered to rats with streptozotocin-induced type I diabetes (18). The administration of HRP for 6 months significantly inhibited the increase in renal angiotensin II levels and the development of proteinuria and glomerulosclerosis, suggesting that the non-proteolytic activation of prorenin bound to the (pro)renin receptor plays an important role in the development of nephropathy. Although the renal mRNA expression of the (pro)renin receptor in control and diabetic rats was similar, non-proteolytically activated prorenin increased in the kidneys of the diabetic rats; this increase was inhibited by HRP. Since the mRNA expression of cathepsin B, a processing enzyme contributing to the conversion of prorenin to renin, simultaneously decreased in the kidneys of the diabetic rats, prorenin may have accumulated in their kidneys. Also, prorenin released into the circulation may have been increased in the diabetic rats. Thus, receptor-bound prorenin is elevated by the increased prorenin levels, with no change in the (pro)renin-receptor level and contributes to the development of nephropathy in diabetic animals. However, a recent study reported an increased renal expression of (pro)renin receptor in diabetic animals (19).

The administration of HRP also inhibited the development of nephropathy in angiotensin II-type 1a-receptor-deficient mice with streptozotocin-induced diabetes (20)

and led to the regression of nephropathy that had already developed in streptozotocin-induced diabetic rats (21). These results indicate that the two major (pro)renin-receptor-dependent pathways, intrarenal angiotensin II generation and renal mitogen-activated protein kinase (MAPK) activation, play important roles in the development and progression of diabetic nephropathy.

In Renin Summit 2008 Berlin (10), Prof. Wilkinson-Berka reported that HRP also has beneficial effects on diabetic retinopathy. The administration of HRP significantly inhibited the enhanced expressions of VEGFA and ICAM-1 and decreased leukostasis in the retina of diabetic animals (personal communication). Thus, (pro)renin receptor blockade can be useful therapy for diabetic microvascular complications.

Hypertensive nephrosclerosis

Elevated tissue levels of angiotensin I and II, glomerulosclerosis with proteinuria, and cardiac hypertrophy with left ventricular fibrosis have been observed in the kidneys of young hypertensive SHRsp fed a high-salt diet. Administration of HRP significantly attenuated, but did not completely suppress, these changes without affecting the development of hypertension (22, 23). These findings were recently confirmed by Susic et al. (24). The PRAM-1, identical to HRP, significantly decreased serum creatinine level, left ventricular mass, and left ventricular fibrosis and improved left ventricular function in SHR fed a high salt diet (25). Interestingly, beneficial effects of PRAM-1 were reduced in SHR fed a normal salt diet. Since an increase in dietary salt is known to decrease plasma renin levels, plasma renin may disturb the benefit of HRP. The (pro)renin receptor blockade erases the enzymatic activity of prorenin, whereas renin is still active even in the presence of a (pro)renin receptor blocker, HRP (Fig. 1A). Thus under conditions with high plasma renin levels, active renin in the plasma would contribute to the pathogenesis independently of the (pro)renin receptor. This concept was supported by a recent study that a renin inhibitor, aliskiren, but not HRP, offered benefits to double transgenic rats overexpressing the human renin and angiotensinogen genes (26). Since the plasma renin level was extremely high in this double transgenic rats, the increased angiotensin II levels caused by active renin likely contributed to the pathogenesis that occurs in this model independently of the (pro)renin receptor. In contrast, the HRP greatly benefited diabetic animals with low plasma renin levels (18, 20, 21). Thus, the efficiency of the HRP may depend on dietary salt or/and the plasma renin levels.

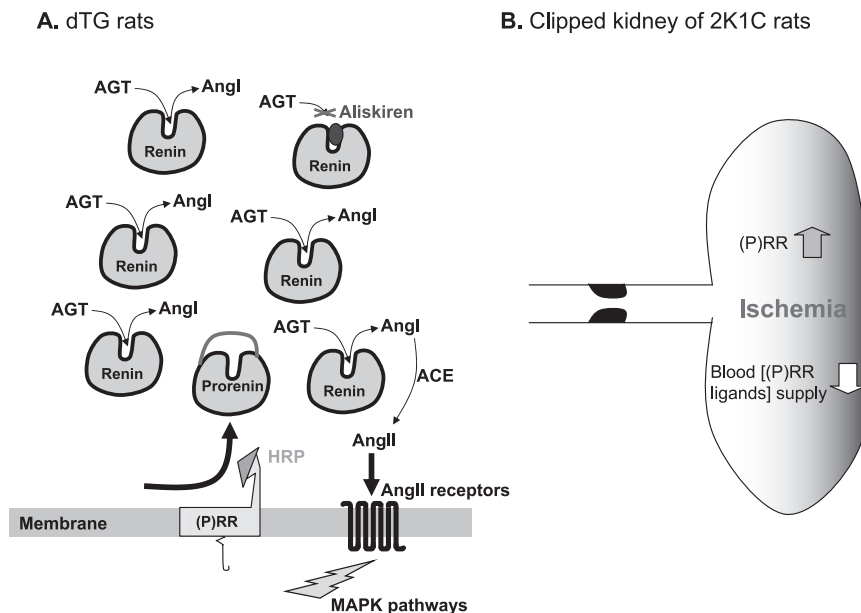


Fig. 1. Why does the handle region peptide (HRP) have no benefit in the human renin, human angiotensinogen-double transgenic (dTG) rats and clipped kidneys of two-kidneys, one-clip (2K1C) Goldblatt hypertensive rats? Although HRP inhibits the binding of renin/prorenin to the (pro)renin receptor [(P)RR], free renin unbound to (P)RR is enzymatically active enough to generate angiotensin I (AngI) from angiotensinogen (AGT). The generated AngI is converted to AngII by angiotensin-converting enzyme (ACE) and thereafter activates the mitogen-activated protein kinase (MAPK) pathways through stimulating AngII receptors. As a matter of course in the dTG rats, end-organ damage due to the increases in AngII and activated MAPK are inhibited by the renin inhibitor aliskiren, but are not influenced by HRP (26) (A). On the other hand, renal ischemia itself is a main pathogenic factor in the clipped kidneys of 2K1C rats. Even if (P)RR expression increases in the clipped kidneys (29), the supply of renin/prorenin as a (P)RR ligand is reduced by the clipping. Thus the nephropathy that occurred in the clipped ischemic kidneys appears to be independent of the (pro)renin receptor and should be treated with an intervention therapy (B).

Ischemic nephropathy

Elevated renin levels down-regulate the expression of the (pro)renin receptor via a negative feedback system (27). However, both pro-fibrotic changes in morphology and enhanced mRNA expression of the (pro)renin receptor were observed in the clipped kidneys of Goldblatt hypertensive rats with high plasma renin levels (28). This result suggests that (pro)renin-receptor-dependent mechanisms might contribute to the pathogenesis of ischemic nephropathy, although no direct evidence of such mechanisms have been obtained. A recent study showed that a short-term (14 days) administration of HRP did not improve the nephropathy that occurred acutely in the clipped kidney of Goldblatt hypertensive rats (29). Because the ischemic changes occurred in the clipped kidneys are mainly caused by hypoxia-inducible factors (30) but not by the renin-angiotensin system, any (pro)renin receptor blockers must have no beneficial effect on the clipped kidneys (Fig. 1B). In contrast, the nephropathy that develops chronically in the non-clipped kidneys of 2K1C models is dependent on the tissue renin-angiotensin system. However, the effects of HRP on the non-clipped kidneys

remain undetermined. Thus we cannot conclude that HRP is ineffective against renovascular hypertension. Further studies are needed to determine whether HRP has a benefit in the non-clipped kidneys of 2K1C models.

Future (pro)renin-receptor blockers

The HRP, which inhibits prorenin binding to the (pro)renin receptor in a test tube and to the receptor expressed on the cell surfaces of COS-7 cells but not to the intracellular receptor and degraded receptor of cultured cells, significantly inhibited the development and progression of nephropathy in animal models of diabetes and low-renin essential hypertension. However, the benefits of the handle region peptide were limited in animal models of hypertension with an extremely high plasma renin level and in ischemic kidneys. Thus, the (pro)renin receptor appears to play important roles in the pathogenesis of end-organ damage in chronic kidney disease due to diabetes and low-renin hypertension. However, since HRP is a competitive peptide inhibitor, the development of non-competitive, non-peptide (pro)renin receptor blockers that are more potent than the HRP is anticipated.

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