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COMMENTARY

# Chronic blockade of the (pro)renin receptor ameliorates the kidney damage in the non-clipped kidney of Goldblatt hypertension

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Renin was discovered by Robert Tigerstedt in 1898; since then, the serial enzymes and peptides involved in the renin-angiotensin system (RAS) have been discovered and investigated in numerous studies. Accumulated evidence indicates that the RAS has a crucial role in regulating the homeostasis of circulation and in maintaining blood pressure through vasoconstriction of the vasculature and the reabsorption of salt and water in the kidneys. Humans can very easily obtain abundant salt in modern life, and as a consequence we have developed hypertension, which is associated with local activation of the RAS. As a result, the RAS in humans has been recently been shown an aspect of the 'evil' to accelerate some disease processes, such as heart disease and renal failure. In other words, this beautiful and excellent system may have become a nuisance in modern life. Therefore, many inhibitors of the RAS have been developed to treat human hypertension as well as cardiovascular and renal disease.

The first description of RAS blockade as a therapeutic approach occurred in 1957 by Skeggs *et al.*[1] Among the components of the RAS, angiotensin II (AngII), which is one of most potent vasoconstrictors in the cardiovascular system, is generated in a step-wise enzymatic cascade. To prevent the pathophysiological actions of AngII, angiotensin receptor blockers have been utilized for not only hypertension, but also diabetes nephropathy, metabolic syndrome and heart failure in the last decade.[2] The initial enzymatic action of the RAS is the conversion of angiotensinogen (ATG) to angiotensin I (AngI) by renin. The plasma concentration of AngI is only approximately twice that of AngII, even when the RAS is activated. Surprisingly, the concentration of ATG is approximately 5000-fold higher than that of AngI.[3] This phenomenon indicates that a conversion of ATG to AngI seems to be the bottleneck in the process; in other words, this reaction is the rate-limiting step of the RAS cascade. Therefore, utilizing the concept of renin inhibition is a logical approach to the management of hypertension; the first direct renin inhibitor, aliskiren, has recently been marketed as a treatment for hypertension.[3]

Although more than 100 years have passed, since the discovery of renin, prorenin had long been believed to be an inactive precursor of renin in the RAS. Recently, the (pro)renin receptor, which binds to both renin and prorenin, was identified. Prorenin binding to (pro)renin receptors results in the cleavage of ATG into AngI and triggers the activation of (pro)renin receptor-stimulated signal transduction pathways, which are independent of the production of AngII. In the last decade, it has been reported that the intracellular signaling pathway is activated by prorenin in cardiomyocytes, mesangial cells, podocytes, distal tubular cells, vascular endothelial cells and vascular smooth muscle cells, which indicates that prorenin mediates intracellular effects in various cardiovascular and kidney cells.[4] In the kidney tissue, through extracellular signal-regulated kinase (ERK)1/2 stimulation, (pro)renin receptors upregulate inflammatory mediators, such as cyclooxygenase-2, interleukin-1β and tumor necrosis factor-α.[5–7] Non-proteolytic activation of prorenin involves a conformational change that results in the unfolding of the prosegment without any cleavage. Although this reversible open conformation has only been achieved *in vitro* under low pH or cold temperature conditions, it suggested that non-proteolytic activation is possible, but not yet defined *in vivo*.[8,9]

As a 43-amino acid prosegment covers the active site cleft and protects the site of the enzymatic activity that converts ATG to AngI, prorenin is usually inactive even at high concentrations in the plasma. Under physiological conditions, the plasma prorenin/renin ratio is ~10/1 (see ref. 10). Although high prorenin levels are found in the maternal plasma during pregnancy, prorenin obviously does not cause any tissue damage.[11] Human diabetic patients also display three- to seven-fold higher prorenin/renin ratios in the blood,[12] which may be associated with the disease progression of interstitial accumulation of AngII in the kidney. Therefore, Suzuki *et al.*[9] utilized the handle region peptide (HRP) of the prorenin molecule for studies on diabetic animals.

The peptide inhibitor, HRP, was designed as a decoy for prorenin on the basis of the prorenin 'handle region' sequence described by Suzuki *et al.*,[9] which interacts with the (pro)renin receptor that was cloned by Nguyen *et al.* in 2002 (see ref. 13). In a series of seminal experiments, the beneficial effects of HRP were revealed in the kidney damage

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models of experimental diabetes. For example, long-term treatment with HRP in streptozotocin-induced diabetic rats prevented the development of proteinuria and glomerulosclerosis, despite hyperglycemia.[14] A continuous administration of HRP was also able to reverse the established diabetic nephropathy.[15] Moreover, Ichihara *et al.*[16] also revealed the efficacy of HRP in angiotensin converting enzyme (ACE) inhibitor-treated diabetic mice that were deficient in the AT1a receptor. Despite the insensitivity to AngII in these mice, they still developed glomerulosclerosis, which was consistent with observations that RAS blockers are unable to completely prevent the kidney damage associated with diabetes.[17,18] Thus, it is noteworthy that HRP could efficiently block the progression of glomerulosclerosis in the AT1a receptor knock-out mice, meaning that the (pro)renin receptor has a critical role in diabetic nephropathy.[16]

After the epoch-making reports by Ichihara *et al.*,[14] we wondered whether HRP could prevent any kind of kidney disease. Regrettably, the nephropathy that occurred acutely in the clipped kidney of Goldblatt hypertensive rats that were treated with HRP for 2 weeks did not improve.[19] Feldt *et al.*[20] also could not reduce mortality and nephrosclerosis with HRP in hypertension models, such as double-transgenic rats overexpressing human renin and ATG, whereas a direct renin inhibitor such as aliskiren may improve kidney damage. It can be argued that such hypertensive models not only display high prorenin levels, but also high plasma renin and AngII levels. As HRP blocks only prorenin and not renin binding to (pro)renin receptors, HRP apparently exerts its effect only in diseases that are associated with high prorenin and low renin levels. Susic *et al.*[21] were also able to confirm only a mild beneficial effect of HRP on cardiac hypertrophy in spontaneously hypertensive rats. Therefore, it seems unreliable that HRP function is promoted only in conditions of upregulated prorenin.

As described above, it is unfortunate that HRP did not evoke the protective effect on hypertensive nephrosclerosis in Goldblatt rats even at concentrations as high as 10 mmol/l.[19] There is much confusion concerning the mode of action of HRP, and the basis of the discrepancy between the *in vitro* and *in vivo* data is unknown. Overall, the HRP results in the animal models were disappointing, although Ryuzaki *et al.*[22] clearly showed beneficial effects of HRP in the two-kidneys, one-clip (2KIC) model of hypertensive nephrosclerosis in an issue of *Hypertension Research*. They carefully investigated the long-term effect of HRP on the slowly progressive nephropathy that occurred in the non-clipped kidney of the two-kidneys, one-clip renovascular hypertension model. Although HRP treatment did not reduce the blood pressure in Goldblatt rats, the long term administration of HRP for 12 weeks significantly ameliorated the glomerulosclerosis and tubulointerstitial damage with reduction in the AngII levels and transforming growth factor (TGF)-β mRNA levels in the non-clipped kidneys. Yet, in the clipped kidney, activated prorenin levels, AngII levels, and TGF-β mRNA levels were similar between the HRP- and scramble-peptide-treated animals. As the ischemic changes that occurred in the clipped kidneys may be mainly affected by hypoxia-inducible factors,[23] HRP may not evoke the beneficial effects on the clipped kidneys. In contrast, the long-term treatment with HRP prevents the glomerular and tubular damage that develop chronically in the non-clipped kidneys in the 2K1C models. In other words, the only difference between the previous report[20] and the study done by Ryuzaki *et al.*[22] is simply in the longer duration of administration of HRP in the Goldblatt hypertensive model, which suggests that the effect of HRP was dependent on the treatment length, but not on the dosage of HRP. In contrast to aliskiren, because HRP did not reduce the blood pressure, the effect of HRP is only expected in chronic administration and therefore was not observed in the acute phase of the Goldblatt hypertensive model.

Aliskiren, a direct oral renin inhibitor, competitively inhibited the renin activities of receptor-bound forms of both renin and prorenin *in vitro*.[24] Nussberger *et al.*[25] reported that both aliskiren and irbesartan significantly prevented atherosclerosis progression in ApoE knockout mice in the 2K1C renovascular hypertension model. In human cultured podocytes, aliskiren is the most potent inhibitor of intracellular AngII levels among RAS inhibitors and does not affect (pro)renin receptor signals, such as ERK phosphorylation.[26] Moreover, a recent clinical study indicates that an administration of aliskiren in addition to losartan reduced albuminuria and renal dysfunction in patients with type 2 diabetes, hypertension and nephropathy.[27] In clinical medicine, diabetic patients sometimes show orthostatic hypotension caused by inappropriate RAS inhibition; therefore, inhibition of prorenin would be a good approach for the treatment of diabetic nephropathy in the absence of hypertension. Based on this, HRP is still an interesting pharmacological tool for exploring the pathophysiological roles of the RAS in various diseases. Taken together, the combination therapy of RAS blockade in addition to the inhibition of prorenin action might prevent unprecedented organ damage in various types of cardiovascular and renal diseases in the near future.

Finally, the (pro)renin receptor has been recently shown to be identical to the vacuolar $H^+$-ATPase, which was formerly discovered in adrenal chromaffin cells,[28] and subsequently the (pro)renin receptor's name was changed to (P)RR/ATP6ap2.(see ref. 29) Recent data, mainly from fish and frog models, revealed that (P)RR/ATP6ap2 contributes to essential functions in cellular physiology and signaling that are independent of the RAS.[30,31] It is unknown whether an excessive inhibition of (P)RR/ATP6ap2 in combination with aliskiren, angiotensin receptor blockers and/or angiotensin-converting enzyme inhibitors might cause unknown adverse effects because the conditional (P)RR/ATP6ap2 knock-out mice could not survive more than 3 weeks, which suggests that vacuolar $H^+$-ATPase-associated protein is essential for cell survival independent of the RAS.[32] Thus, at least, pregnant women, neonates and infants should basically avoid these inhibitors. Future prospective studies and additional evidence are needed to assess whether inhibition of renin and/or prorenin are effective and safe in cardiovascular and kidney disease.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Skeggs LT, Kahn JR, Lentz K, Shumway NP. The preparation, purification, and amino acid sequence of a polypeptide renin substrate. *J Exp Med* 1957; **106**: 439–453.
2 Sofue T, Kiyomoto H. Angiotensin II receptor blocker is a renoprotective remedy for metabolic syndrome. *Hypertens Res* 2009; **32**: 735–737.
3 Hollenberg NK. Direct renin inhibition and the kidney. *Nat Rev Nephrol* 2010; **6**: 49–55.
4 Hitom H, Liu G, Nishiyama A. Role of (pro)renin receptor in cardiovascular cells from the aspect of signaling. *Front Biosci* 2010; **2**: 1246–1249.
5 Kaneshiro Y, Ichihara A, Takemitsu T, Sakoda M, Suzuki F, Nakagawa T, Hayashi M, Inagami T. Increased expression of cyclooxygenase-2 in the renal cortex of human prorenin receptor gene-transgenic rats. *Kidney Int* 2006; **70**: 641–646.
6 Huang J, Siragy HM. Glucose promotes the production of interleukine-1beta and cyclooxygenase-2 in

mesangial cells via enhanced (Pro)renin receptor expression. *Endocrinology* 2009; **150**: 5557–5565.
7 Matavelli LC, Huang J, Siragy HM. (Pro)renin receptor contributes to diabetic nephropathy through enhancing renal inflammation. *Clin Exp Pharmacol Physiol* 2009; **37**: 277–282.
8 Pitarresi TM, Rubattu S, Heinrikson R, Sealey JE. Reversible cryoactivation of recombinant human prorenin. *J Biol Chem* 1992; **267**: 11753–11759.
9 Suzuki F, Hayakawa M, Nakagawa T, Nasir UM, Ebihara A, Iwasawa A, Ishida Y, Nakamura Y, Murakami K. Human prorenin has 'gate and handle' regions for its non-proteolytic activation. *J Biol Chem* 2003; **278**: 22217–22222.
10 Danser AH, Derkx FH, Schalekamp MA, Hense HW, Riegger GA, Schunkert H. Determinants of interindividual variation of renin and prorenin concentrations: evidence for a sexual dimorphism of (pro)renin levels in humans. *J Hypertens* 1998; **16**: 853–862.
11 Sealey JE, Wilson M, Morganti AA, Zervoudakis I, Laragh JH. Changes in active and inactive renin throughout normal pregnancy. *Clin Exp Hypertens A* 1982; **4**: 2373–2384.
12 Deinum J, Ronn B, Mathiesen E, Derkx FH, Hop WC, Schalekamp MA. Increase in serum prorenin precedes onset of microalbuminuria in patients with insulin-dependent diabetes mellitus. *Diabetologia* 1999; **42**: 1006–1010.
13 Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, Sraer J-D. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest* 2002; **109**: 1417–1427.
14 Ichihara A, Hayashi M, Kaneshiro Y, Suzuki F, Nakagawa T, Tada Y, Koura Y, Nishiyama A, Okada H, Uddin MN, Nabi AH, Ishida Y, Inagami T, Saruta T. Inhibition of diabetic nephropathy by a decoy peptide corresponding to the 'handle' region for nonproteolytic activation of prorenin. *J Clin Invest* 2004; **114**: 1128–1135.
15 Takahashi H, Ichihara A, Kaneshiro Y, Inomata K, Sakoda M, Takemitsu T, Nishiyama A, Itoh H. Regression of nephropathy developed in diabetes by (Pro)renin receptor blockade. *J Am Soc Nephrol* 2007; **18**: 2054–2061.
16 Ichihara A, Suzuki F, Nakagawa T, Kaneshiro Y, Takemitsu T, Sakoda M, Nabi AH, Nishiyama A, Sugaya T, Hayashi M, Inagami T. Prorenin receptor blockade inhibits development of glomerulosclerosis in diabetic angiotensin II type 1a receptor-deficient mice. *J Am Soc Nephrol* 2006; **17**: 1950–1961.
17 Onozato ML, Tojo A, Goto A, Fujita T, Wilcox CS. Oxidative stress and nitric oxide synthase in rat diabetic nephropathy: effects of ACEI and ARB. *Kidney Int* 2002; **61**: 186–194.
18 Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK. Aliskiren combined with losartan in type 2 diabetes and nephropathy. *N Engl J Med* 2008; **358**: 2433–2446.
19 Muller DN, Klanke B, Feldt S, Cordasic N, Hartner A, Schmieder RE, Luft FC, Hilgers KF. (Pro)renin receptor peptide inhibitor 'handle-region' peptide does not affect hypertensive nephrosclerosis in Goldblatt rats. *Hypertension* 2008; **51**: 676–681.
20 Feldt S, Maschke U, Dechend R, Luft FC, Muller DN. The putative (pro)renin receptor blocker HRP fails to prevent (pro)renin signaling. *J Am Soc Nephrol* 2008; **19**: 743–748.
21 Susic D, Zhou X, Frohlich ED, Lippton H, Knight M. Cardiovascular effects of prorenin blockade in genetically spontaneously hypertensive rats on normal and high-salt diet. *Am J Physiol Heart Circ Physiol* 2008; **295**: H1117–H1121.
22 Ryuzaki M, Ichihara A, Oshima Y, Sakoda M, Kurauchi-Mito A, Narita T, Kinouchi K, Murohashi-Bokuda K, Nishiyama A, Itoh H. Involvement of activated prorenin in the pathogenesis of slowly progressive nephropathy in the non-clipped kidney of two kidneys, one-clip hypertension. *Hypertens Res* 2011; **34**: 301–307.
23 Nangaku M, Eckardt KU. Hypoxia and the HIF system in kidney disease. *J Mol Med* 2007; **85**: 1325–1330.
24 Biswas KB, Nabi AH, Arai Y, Nakagawa T, Ebihara A, Ichihara A, Watanabe T, Inagami T, Suzuki F. Aliskiren binds to renin and prorenin bound to (pro)renin receptor *in vitro*. *Hypertens Res* 2010; **33**: 1053–1059.
25 Nussberger J, Aubert JF, Bouzourene K, Pellegrin M, Hayoz D, Mazzolai L. Renin inhibition by aliskiren prevents atherosclerosis progression: comparison with irbesartan, atenolol, and amlodipine. *Hypertension* 2008; **51**: 1306–1311.
26 Sakoda M, Ichihara A, Kurauchi-Mito A, Narita T, Kinouchi K, Murohashi-Bokuda K, Saleem MA, Nishiyama A, Suzuki F, Itoh H. Aliskiren inhibits intracellular angiotensin II levels without affecting (pro)renin receptor signals in human podocytes. *Am J Hypertens* 2010; **23**: 575–580.
27 Persson F, Lewis JB, Lewis EJ, Rossing P, Hollenberg NK, Parving HH. Impact of baseline renal function on the efficacy and safety of aliskiren added to losartan in patients withtype 2 diabetes and nephropathy. *Diabetes Care* 2010; **33**: 2304–2309.
28 Ludwig J, Kerscher S, Brandt U, Pfeiffer K, Getlawi F, Apps DK, Schägger H. Identification and characterization of a novel 9.2-kDa membrane sector-associated protein of vacuolar proton-ATPase from chromaffin granules. *J Biol Chem* 1998; **273**: 10939–10947.
29 Sihn G, Rousselle A, Vilianovitch L, Burckle C, Bader M. Physiology of the (pro)renin receptor: Wnt of change? *Kidney Int* 2010; **78**: 246–256.
30 Cruciat CM, Ohkawara B, Acebron SP, Karaulanov E, Reinhard C, Ingelfinger D, Boutros M, Niehrs C. Requirement of prorenin receptor and vacuolar H+-ATPase-mediated acidification for Wnt signaling. *Science* 2010; **327**: 459–463.
31 Amsterdam A, Nissen RM, Sun Z, Swindell EC, Farrington S, Hopkins N. Identification of 315 genes essential for early zebrafish development. *Proc Natl Acad Sci USA* 2004; **101**: 12792–12797.
32 Kinouchi K, Ichihara A, Sano M, Sun-Wada GH, Wada Y, Kurauchi-Mito A, Bokuda K, Narita T, Oshima Y, Sakoda M, Tamai Y, Sato H, Fukuda K, Itoh H. The (pro)renin receptor/ATP6AP2 is essential for vacuolar H+-ATPase assembly in murine cardiomyocytes. *Circ Res* 2010; **107**: 30–34.