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Ratio of Prorenin to Plasma Renin Acitivity as a Surrogate Marker for Local Renin Angiotensin Activity of the Kidneys in Renal Diseases

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ABSTRACT

Human plasma contains a substantial amount of prorenin, which converts to active renin to produce angiotensin II. The conversion rate varies under physiological conditions, yielding different ratios of prorenin to plasma renin activity (PRA ; prorenin/PRA ratio), whose clinical significance is unclear. The present study addresses whether the prorenin/PRA ratio predicts the activity of the local renin angiotensin system (RAS) of the kidney in various renal diseases. The subjects for the study were healthy volunteers ($n=16$) and patients with diabetic nephropathy (DN, $n=50$), chronic glomerulonephritis (GN, $n=69$), or nephrosclerosis (NS, $n=16$). We found that plasma prorenin levels in patients with DN were higher than those in patients with GN or NS or in healthy subjects. Furthermore, plasma prorenin levels and PRA were correlated in the 3 patient groups. The prorenin/PRA ratio was significantly higher in patients with DN (83.5×10^{-3}/hr), GN (67.1×10^{-3}/hr), or NS (27.8×10^{-3}/hr) than in healthy subjects (10.1×10^{-3}/hr). Treatment with telmisartan, an angiotensin receptor blocker, in 10 patients with DN produced significant reductions in the prorenin/PRA ratio which were positively correlated with reductions in daily urinary protein excretion. Our findings suggest that the prorenin/PRA is a useful clinical surrogate marker to estimate the activity of the local RAS in the kidneys of patients with primary renal diseases. Moreover, this marker might be used to evaluate RAS inhibitors for their capacity to block local RAS activity in the kidney. (Jikeikai Med J 2004 ; 51 : 105-11)

Key words : prorenin, plasma renin activity, diabetic nephropathy, glomerulonephritis, nephrosclerosis, telmisartan

INTRODUCTION

The kidneys are considered the primary source of prorenin, a biologically inactive precursor of active renin, because most prorenin granules are located in the juxtaglomerular apparatus of the kidneys. Recent studies suggest that prorenin is a clinical marker for the progression of diabetic nephropathy (DN) and diabetic vascular complications[1-5]. Moreover, in a 10-year longitudinal follow-up study of patients with diabetes, an increase in prorenin preceded the development of DN[6]. These data indicate that prorenin might be used to predict the progressive nature of DN. Normally, circulating levels of renin (equivalent to plasma renin activity : PRA) in patients with DN are normal or low, suggesting that the systemic renin angiotensin system (RAS) is suppressed. However, mounting evidence suggests that intrarenal angiotensin II or renin mRNA is increased in the diabetic kidney, contributing to intraglomerular

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hypertension and accelerating disease progression[7–9]. These data suggest an activated local RAS in DN. The discrepancy between systemic and local RAS in DN is known as paradoxical suppression[10].

Unfortunately, there has been no clinical measure for estimating the local RAS activity of the kidney. The lack of such a measure, together with the recently developed antibody-activating direct prorenin assay to more accurately measure circulating prorenin[11], motivated us to investigate whether local RAS activity of the kidney in primary renal diseases could be estimated by measuring the prorenin/PRA ratio. To prove this clinical hypothesis, we also investigated the relation between telmisartan-induced reductions in daily urinary protein excretion and changes in the prorenin/PRA ratio. Our results suggest that the prorenin/PRA might be used to estimate local RAS activity in patients with various renal diseases.

Patients and Methods

1. *Patients*

Patients in this study had DN ($n=50$; 27 men and 23 women; mean age, 60±12 years), chronic glomerulonephritis (GN; $n=69$; 33 men and 36 women, mean age, 58±11 years), or nephrosclerosis (NS; $n=16$; 8 men and 8 women; mean age, 65±9 years). These conditions were diagnosed on the basis of clinical information and laboratory tests performed at the physician's discretion. Diagnoses in patients with GN and in some patients with DN were confirmed with renal biopsy. Treatments consisted of that for patients at a predialysis stage ($n=40$), hemodialysis (HD, $n=53$), or peritoneal dialysis (PD, $n=42$). Patients at a predialysis disease stage had serum creatinine concentrations of 2 to 8 mg/dl and were treated on an outpatient basis. Because there was no difference in the levels of PRA or prorenin among the 3 groups (Table 1), all data from patients receiving each treatment were pooled as a function of each disease category.

2. *Treatment plans*

Hypertension (140/90 mmHg or higher) was diagnosed on the basis of the Joint National Committee on Hypertension/World Health Organization criteria. Patients received antihypertensive therapy at their physician's discretion. For ethical reasons, drugs that can directly affect prorenin or PRA profiles such as angiotensin-coverting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), diuretics, and β-blockers were continued. The ARB telmisartan was not included in the conventional regimens of any patients because of the following protocol, in which the effect of telmisartan on changes in urinary protein excretion were evaluated. With dietary control (salt restriction 5 to 7 g/day) and antihypertensive treatment, blood pressures in most patients had been stabilized at less than 140/90 mmHg. All patients with diabetes satisfied the World Health Organization diagnostic criteria for diabetes and were regularly treated in the outpatient clinic with dietary therapy for diabetes mellitus and with exercise therapy, with or without oral hypoglycemic agents or insulin preparations.

In 10 patients with chronic renal insufficiency (6 men and 4 women; 5 with DN, 3 with GN, and 2 with NS; mean age, 65±10 years; serum creatinine level, 1.4±0.3 mg/dl) who had never been treated with

Table 1. Prorenin and PRA levels in patients enrolled in the study

n	GN (69)	DN (50)	NS (16)	Healthy (16)
Prorenin (pg/ml)	176±138	310±308*	173±103	100±86
PRA (ng/ml/hr)	2.4±2.5	3.7±4.2	2.4±2.5	

*: $p=0.018$ by Sheffe's method vs. others.

n	Predialysis (32)	HD (59)	PD(44)
Prorenin (pg/ml)	365±325	170±158	278±267
PRA (ng/ml/hr)	4.7±3.8	2.5±3.6	2.9±3.0

either ARBs or ACE inhibitors, prorenin/PRA ratios were compared before and after treatment with 20 mg telmisartan for more than 8 weeks. The research protocol was approved by the ethics committee of the Saiseikai central hospital, and all patients gave informed consent. The purpose of this trial was to observe changes in daily urinary protein excretion in association with changes in the prorenin/PRA ratio in response to treatment with the ARB telmisartan.

3. *Measurement of prorenin and PRA*

Blood samples were obtained between 10 a.m. and 12 p.m. with the subject in the sitting position after more than 30 minutes' rest. Sera from healthy control subjects and patients with primary renal diseases were assayed for prorenin and other biochemical variables. Control subjects ($n=16$; 9 men and 6 women; mean age, 44 ± 16 years) had results that were negative or within normal limits on a routine medical checkup, including urinalysis, hematologic studies, and blood pressure measurement, and had never had any severe illnesses. Informed consent for prorenin analysis was obtained from each subject before the study.

4. *Antibody-activating direct enzyme kinetic assay of human prorenin*

Immunoreactive step of human prorenin has been described elsewhere[10]. Briefly, serum samples or various concentrations of standard recombinant human prorenin was mixed with antibodies against the peptide profragment # 2 (pf # 2, LKERGVDMARLGPEWSQPMC) and pipetted into wells of a 96-well enzyme-linked immunosorbent assay plate coated with an antibody against the peptide profragment # 1 (pf # 1: LPTDTTTFKRIFLKRC). The plate was then incubated at 40° C for at least 20 hours to activate the prorenin sandwich with anti-pf # 1 and anti-pf # 2 antibodies to the highest level. The antibody against pf # 1, of the N-terminal amino acid sequence of human renin, completely activated human prorenin, as reported previously. The peptide pf # 2, which represents amino acids 23 to 41 of the profragment (prosegment) of prorenin, was conjugated with keyhole limpet hemocyanin. The anti-pf # 2 antiserum was raised in rabbits and purified to IgG by ammonium sulfate precipitation followed by ion exchange chromatography on diethylaminoethyl cellulose.

5. *PRA measurement*

PRA was measured with radioimmunoassay. Briefly, angiotensin I was generated in plasma samples at 37°C after addition of renin substrate. The concentration of angiotensin I formed was measured with radioimmunoassay, and PRA in a sample is expressed as the angiotensin I concentration generated per 1-mL sample in 1 hour (ng/mL/hr). The lower limit of detection was 0.1 ng/mL/hr.

6. *Statistical analyses*

Student's t-test, the Chi-square test, and stepwise regression analysis were applied as necessary using the SAS statistical software program (SAS Institute, Cary, NC, USA). Data are presented as means±SD, unless otherwise indicated.

Results

Plasma concentrations of prorenin in patients with DN were significantly higher than those in patients with GN or NS or in healthy subjects (Table 1). Prorenin levels in patients with GN were similar to those in patients with NS. However, prorenin levels did not differ between the 3 treatment groups (predialysis, HD, and PD).

Plasma prorenin concentrations and PRA were strongly correlated in all disease groups (Fig. 1). The relation of prorenin to PRA can be expressed with the equation $y=ax+b$, where y is prorenin level and x is PRA; the slope, a, was 83.5 in DN, 67.1 in GN, and 27.8 in NS. In healthy subjects, the relation between prorenin and PRA was expressed as $y=10.1x+60.8$ (data not shown), in which the slope (10.1) was much small than that in patients with renal diseases.

In response to treatment with telmisartan, PRA

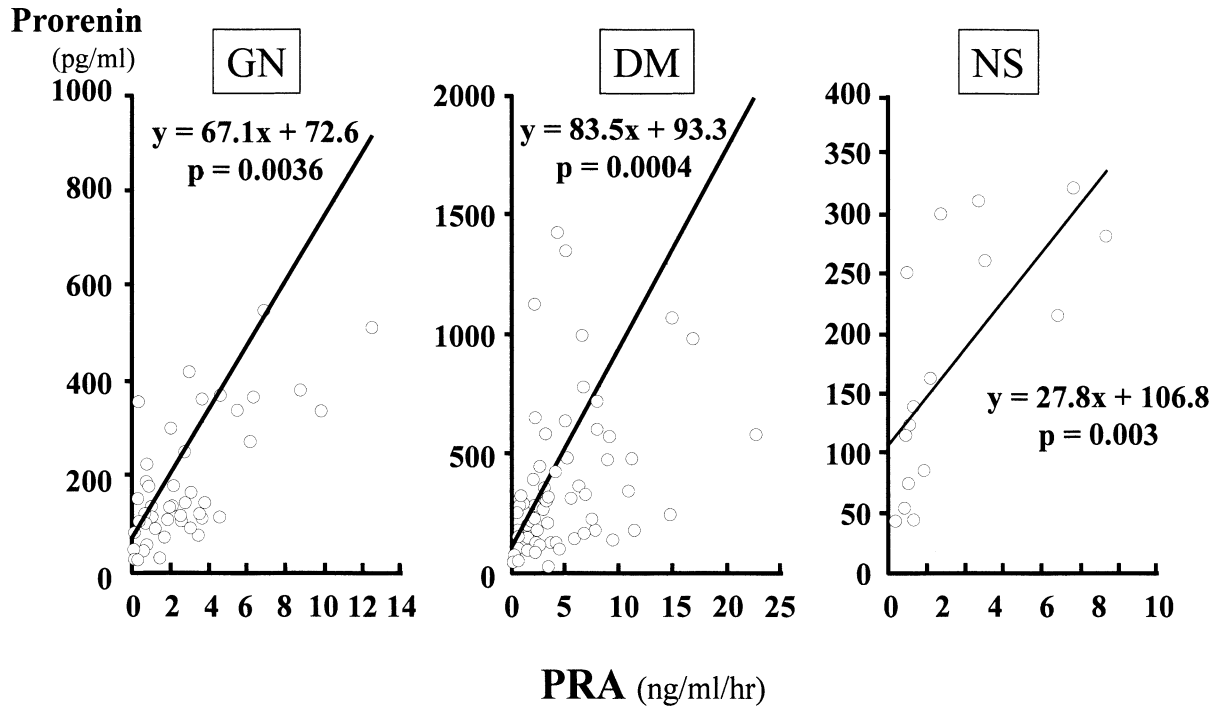

Fig. 1. Prorenin and PRA in patients with renal diseases

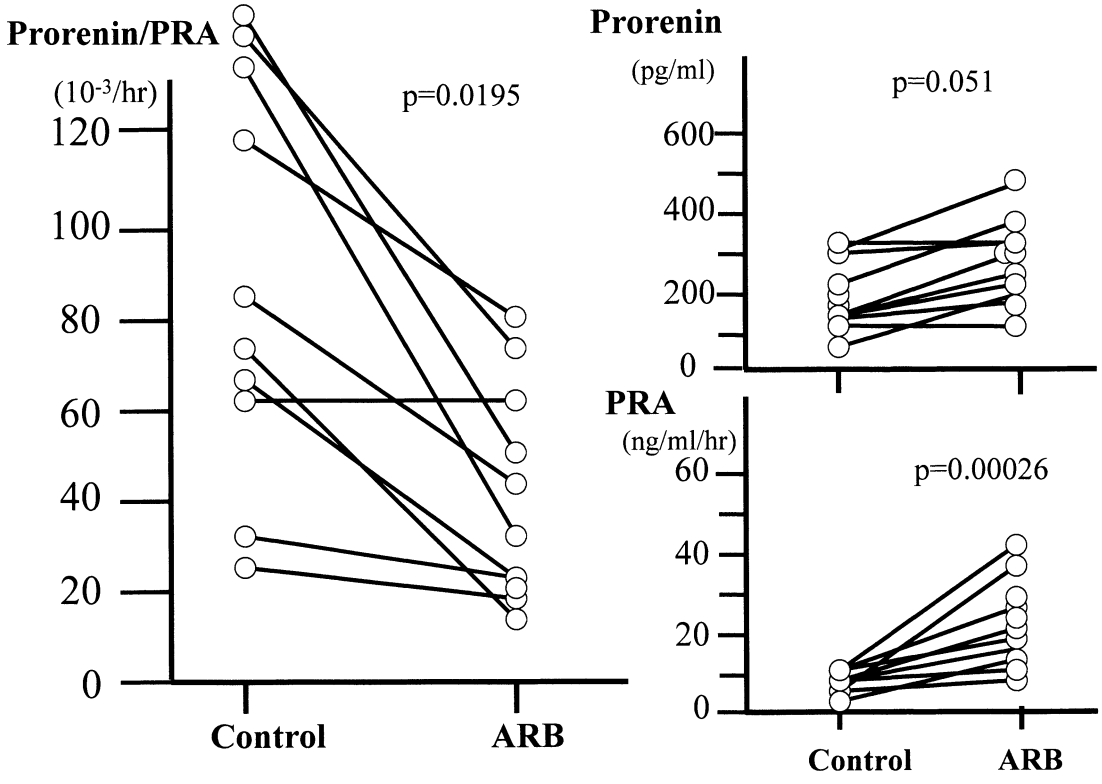

Fig. 2. Effect of telmisartan on the prorenin/PRA ratio and levels of prorenin and PRA.

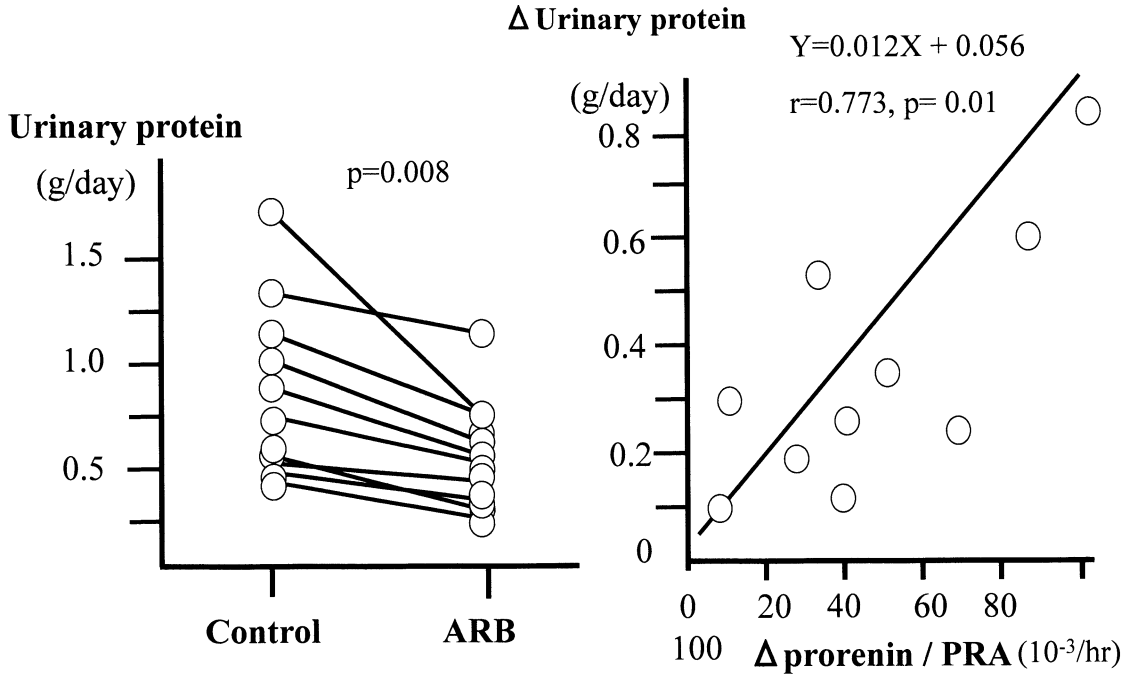

Fig. 3. Telmisartan-induced reduction in urinary protein and the relationship between Δprorenin/PRA ratio and Δurinary protein excretion

increased significantly (Fig. 2 right bottom, from 5.4±4.0 to 21.0±10.3 ng/ml/hr, $n=10$, $p=0.00026$), whereas prorenin levels remained unchanged (Fig. 2 right top, from 235±66 to 281±71 pg/ml, $n=10$, $p=0.051$). As a result of these changes, the prorenin/PRA ratio was significantly decreased (Fig. 2 left, from 92±47 to 50±22 pg/ml, $n=10$, $p=0.019$).

Daily urinary protein excretion decreased in the 10 patients treated with telmisartan (from 0.95±0.49 to 0.58±0.30 g/day, $n=10$, $p=0.008$; Fig. 3 left). The magnitude of the telmisartan-induced decrease in prorenin/PRA was strongly and positively correlated with the reduction in daily urinary protein excretion ($r=0.773$, $n=10$, $p=0.01$; Fig. 3 right).

Discussion

Measurements of PRA can be used to assess the activity of systemic RAS, but there is no surrogate marker to estimate the activity of local RAS. Although other sources prorenin have not been ruled out, the kidneys are considered the principal source of prorenin in the systemic circulation as well as the principal source of PRA[12,13]. The clinical significance of the prorenin/PRA ratio is unclear, as prorenin is biologically inactive and, thus, the ratio does not appear to have a physiological role. However, the conversion of prorenin to active renin reduces the prorenin/PRA ratio. We assume that if the local RAS were activated, the conversion from prorenin to renin (PRA) in the systemic RAS would be suppressed through compensatory mechanisms, resulting in a greater increase in the prorenin/PRA ratio. On the basis of this hypothesis, we started to view prorenin/PRA as a possible marker for evaluating local RAS.

The present study had several significant findings. First, we found that the prorenin/PRA ratio was higher in patients with major renal diseases, DN, GN, and NS, than in healthy subjects. The prorenin/PRA ratio was highest in patients with DN (83.5), moderately high in patients with GN (67.1), and slightly high in patients with NS (27.8) compared with that in healthy subjects (10.1). Second, administration of the ARB telmisartan reduced both the prorenin/PRA ratio and urinary protein excretion, with which these two variables were positively correlated. These findings lead us to speculate that: 1) the conversion from prorenin to PRA varies with the cause of primary

renal disease, and 2) there is a link between the prorenin/PRA ratio and the amount of daily urinary protein excretion.

To our knowledge, the use of the prorenin/PRA ratio as a marker for local RAS activity has not been previously proposed. We believe that the prorenin/PRA ratio is useful for evaluating local RAS activity in the kidney because a link between the reduction in the prorenin/PRA ratio and the reduction in urinary protein excretion supports, at least in part, the notion that prorenin/PRA influences urinary protein excretion through the intraglomerular pressure of the kidney. Therefore, the prorenin/PRA ratio might reflect the local RAS activity of the kidneys independent of prorenin conversion or prorenin processing. A previous study of the PRA/prorenin ratio, the reciprocal of the prorenin/PRA ratio, has shown that changes in PRA/prorenin might result from changes in the proportion of prorenin converted to renin[14]. In addition, the increased prorenin levels found in DN have been attributed to the reduced capacity of the prorenin-to-renin conversion factor[15]. Inhibition of prorenin processing also enhances plasma prorenin levels in an animal model of diabetes[16]. Therefore, the increased prorenin/PRA ratio in patients with DN, GN, or NS might be due to changes leading to either decreased conversion or reduced processing of prorenin. If the prorenin/PRA ratio is confirmed to be a surrogate marker for local RAS activity, this relation might then suggest that activation of the local RAS in the kidney is greatest in patients with DN, followed by that in patients with GN and NS. This possibility is consistent with the clinical evidence that hyperfiltration is also greatest in DN, followed by that in GN and NS, since renal hyperfiltration is associated with intraglomerular hypertension and proteinuria. In fact, the degree of protein excretion from the kidney is extremely high in DN, moderately high in GN, and high in NS[17]. However, whether changes in the prorenin/PRA ratio are a primary cause or a secondary response in renal diseases remains unclear. Further in vivo or in vitro study is needed to confirm whether the prorenin/PRA ratio is a surrogate marker for the local RAS activity of the kidney.

No information is available about the effect of ARBs on prorenin. Previously, ACE inhibitors have been shown to increase prorenin levels[18,19], a change that which might be related to increased PRA in the systemic circulation. The present study showing that telmisartan increases prorenin levels (Fig. 2; the increase is nearly statistically significant: $p=0.051$) is consistent with previous studies of ACE inhibitors. This response probably involves inhibition by telmisartan of the negative feedback mechanism for regulating prorenin, as is the case with ACE inhibitors.

In conclusion, we propose that the prorenin/PRA ratio can be used, at least in part, as a functional surrogate marker for predicting the degree of activation in hyperfiltratable kidneys. This marker might be used to assess the ability of ARBs and ACE inhibitors to inhibit the local RAS of the kidney.

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