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【 ORIGINAL ARTICLE 】

The Intrarenal Renin-angiotensin System Is Activated Immediately after Kidney Donation in Kidney Transplant Donors

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Abstract:

Objective The intrarenal renin-angiotensin system (RAS) is activated in clinical settings, such as chronic kidney disease (CKD), as well as in CKD animal models, and kidney transplant donors have a greater risk of end-stage renal disease than healthy controls. However, whether or not the intrarenal RAS is activated immediately after kidney donation in kidney transplant donors is unclear, and the mechanism underlying intrarenal RAS activation is unknown.

Methods We investigated 10 kidney transplant donors (4 men and 6 women, 58.6±9.0 years of age). Their blood pressure (BP), estimated glomerular filtration rate (eGFR), plasma angiotensinogen (AGT) and plasma angiotensin II (AngII) levels (which reflect circulating RAS activation), urinary albumin excretion, and urinary AGT excretion (which reflects intrarenal RAS activation) were evaluated before kidney donation (-1.2± 0.40 days) and after kidney donation (7.5±1.7 days).

Results The renal function after kidney donation was significantly lower than before donation. There were no significant differences in the BP during 24-h ambulatory BP monitoring, plasma AngII levels, or urinary albumin excretion after kidney donation. In contrast, the levels of plasma AGT and urinary AGT excretion were significantly increased after kidney donation. The urinary AGT excretion after kidney donation did not show a significant relationship with the systolic BP, plasma AGT, plasma AngII, or urinary albumin excretion. In addition, the percentage change in urinary AGT excretion after kidney donation was not associated with the percentage change in other clinical parameters.

Conclusion The intrarenal RAS is activated in kidney transplant donors immediately after kidney donation, independent of the systemic BP and filtration of increased plasma AGT, due to augmented inflammation.

Key words: intrarenal renin-angiotensin system, kidney transplant donor, kidney donation, renal damage, urinary angiotensinogen

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Introduction

The circulating renin-angiotensin system (RAS) plays an important role in blood pressure (BP) and sodium homeostasis (1, 2). Conversely, the activation of the intrarenal RAS plays a critical role in the pathophysiology of renal damage in some animal models as well as in patients with chronic kidney disease (CKD) or hypertension. The intrarenal RAS is independent of the circulating RAS, and urinary angiotensinogen (AGT) excretion is broadly used as a surrogate marker of intrarenal RAS activation (3-10). In addition, renal damage activates the intrarenal RAS via inflammation and oxidative stress (4, 11-14).

The remnant kidney model is characterized by progressive hypertension, proteinuria, and renal histological damage,

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such as focal segmental glomerulosclerosis (FSGS) and, ultimately, the reduction in the glomerular filtration rate (GFR), in animals subjected to major renal mass reduction. These changes are ameliorated by RAS blockers (15, 16). In this model, 5/6 nephrectomy is commonly used to induce chronic progressive renal damage. However, even unilateral nephrectomy induces the production of reactive oxygen species in the remaining kidney (17), and Muzaale et al. reported that kidney transplant donors are at a greater risk of end-stage renal disease than matched healthy non-donors in a cohort study (18). However, whether or not the intrarenal RAS is activated immediately after unilateral nephrectomy in clinical settings is unclear.

Therefore, we performed the present study to clarify whether or not urinary AGT excretion, which reflects the intrarenal RAS, increases immediately after kidney donation in kidney transplant donors who have no existing renal damage. We also investigated the factors associated with intrarenal RAS activation.

Materials and Methods

Subjects

The present study was approved by the ethics committee of Hamamatsu University School of Medicine (No. 14-049) and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients. We recruited 10 subjects who had been admitted to our hospital as kidney transplant donors and were to undergo kidney donation between July 2014 and December 2017. Subjects who had received RAS blockers were excluded, as previously described, because these drugs influence the intrarenal RAS expression (6, 9).

Study protocol

The subjects' vital signs, such as their height and body weight, were measured on pre- and post-operative days, and ambulatory BP monitoring (ABPM) was conducted at 30-min intervals for 24 hours using an automatic device (TM-2431; A and D, Tokyo, Japan). Urine samples were collected for 24 hours, and blood samples were drawn at 6:00 AM the next day, after the patients had rested in the supine position for at least 15 minutes. Thereafter, the samples were centrifuged at 3,000 rpm for 10 minutes at 4°C and stored at -80°C until analyses, as described previously (9, 19, 20). The measurements were taken 1.2±0.40 days before kidney donation and 7.5±1.7 days after kidney donation, just before discharge.

Clinical data

The subjects' serum creatinine concentrations and urinary albumin concentrations were measured in the clinical laboratory of the Hamamatsu University School of Medicine University Hospital. The estimated GFR (eGFR) was calculated based on the serum creatinine concentrations using the Japanese eGFR equation (21). The levels of plasma AGT and urinary AGT excretion were measured using an enzyme-linked immunosorbent assay (ELISA), as described previously (22). Levels of plasma angiotensin II (AngII) and serum high-sensitivity C-reactive protein (hs-CRP) were determined using a radioimmunoassay (SRL, Tokyo, Japan).

The percentage change in each parameter between pre-operation and post-operation was calculated using the following equation: (levels after nephrectomy - levels before nephrectomy)/(levels before nephrectomy)×100.

Statistical analyses

The results were expressed as the mean±standard deviation. The significance of the differences between pre- and post-operative values was determined using Student's *t*-test for paired samples. Because serum hs-CRP levels and urinary AGT and albumin excretion levels did not show a normal distribution, a logarithmic transformation was applied before Student's *t*-test was carried out. The correlations between urinary AGT excretion and clinical parameters, as well as those between the percentage change (Δ) in urinary AGT excretion and percentage change in clinical parameters, were evaluated using Pearson's product-moment correlation test. We considered all p values <0.05 to be statistically significant. Statistical analyses were performed using the IBM® SPSS® software program, version 23 (IBM, Armonk, USA).

Results

Subjects' characteristics

This study included 10 subjects (4 men and 6 women, 58.6±9.0 years of age) who underwent kidney donation. One subject had both hypertension and hyperlipidemia, and two subjects had hyperlipidemia. However, these three subjects had been well-treated by diet and exercise or by medication prior to the procedure. In addition, 1 subject had diabetes mellitus, but hemoglobin A1c was <6.5% using voglibose, an alpha glycosidase inhibitor, and the urinary albumin excretion was <30 mg/g creatinine. In addition, this subject did not suffer from diabetic retinopathy. All subjects' baseline characteristics are presented in Table 1.

Changes in clinical parameters after kidney donation

We compared the subjects' clinical parameters before and after kidney donation (Table 2). Their body weight and body mass index (BMI) were significantly reduced by the hospitalization and kidney donation. Although their BP over a 24-h period tended to be increased after kidney donation, the change was not significant. The subjects' serum creatinine levels and eGFR were dramatically increased and decreased after kidney donation, respectively, and significant differences were found between the pre- and post-operation values. Similarly, their CKD stage was increased (pre-operation: stage 2 in all 10 patients; post-operation: stage 3

Table 1. Patient Characteristics.

Age, year	58.6±9.0
Sex	Male 4
	Female 6
Past history	Appendicitis 4
	Myoma uteri 2
	Inguinal hernia 1
	Varicose vein 1
	Pregnancy induced hypertension 1
	Herpes zoster 1
Comorbidity	Diabetes mellitus 1
	Hypertension 1
	Hyperlipidemia 3
	Others 2
Use of drugs	antihyperglycemic drugs 1
	antihypertensive drugs 1
	antihyperlipidemic drugs 2

A in 6 patients and stage 3B in 4 patients). Plasma AngII levels, which reflect circulating RAS activation, did not change significantly after surgery. However, the levels of plasma AGT after kidney donation were significantly higher than those before kidney donation, as were the levels of serum hs-CRP. The urinary albumin excretion did not change significantly after kidney donation. However, the urinary AGT excretion increased significantly after kidney donation.

Relationships between urinary AGT excretion and clinical parameters after kidney donation

We investigated the correlations between urinary AGT excretion after kidney donation and post-operative clinical parameters to investigate what activates the intrarenal RAS. The urinary AGT excretion was significantly and negatively associated with the eGFR. In contrast, no significant correlations were found between the urinary AGT excretion and the BMI, BP, heart rate, plasma AGT or AngII, hs-CRP, or urinary albumin excretion (Table 3).

Relationships of percentage changes in urinary AGT excretion and clinical parameters between pre-operation and post-operation

Next, we calculated the percentage changes (Δ) between pre-operation and post-operation for the clinical parameters and investigated the relationships between the Δ of urinary AGT excretion levels and the Δ of each clinical parameter. No significant relationships were found in this regard (Table 4).

Discussion

When clinical parameters were compared between pre- and post-operation kidney transplant donors in this study, urinary AGT excretion, which is a surrogate marker of intrarenal RAS activation, was significantly increased after kidney donation. It is surprising that the intrarenal RAS was activated only 7.5±1.7 days after kidney donation. Intrarenal RAS activation induces renal damage (3-14), and this activation of the intrarenal RAS at such an early phase may explain why kidney transplant donors have a greater risk of end-stage renal disease than matched healthy non-donors.

Intrarenal RAS activation and systemic BPs are well known to be positively correlated (9). However, BPs were not elevated in the present study, despite the observed increase in intrarenal RAS activation. Two possible reasons for this discrepancy are proposed. First, although intrarenal RAS was activated in the kidney transplant donors, the urinary AGT excretion levels in the kidney transplant donors (1.65±0.69 μg/gCr in the daytime and 1.52±0.45 μg/gCr in the nighttime; data not shown) were lower than those in the CKD patients in our previous report (2.39±0.99 μg/gCr in the daytime and 2.24±1.06 μg/gCr in the nighttime) (9); this may have resulted in the intrarenal RAS activation not increasing the BP very much. Second, the significant reduction in body weight due to hospitalization and kidney donation may have limited the BP elevation despite the observed increase in intrarenal RAS activation.

Plasma AngII levels did not increase despite the increase in plasma AGT levels, and plasma renin assay (PRA) results were not significantly different after kidney donation (before kidney donation, 0.65±0.42 ng/mL/h; after kidney donation, 1.20±1.18 ng/mL/h; p=0.20; data not shown). However, the serum levels of angiotensin-converting enzyme (ACE) were significantly decreased after kidney donation (before kidney donation, 10.29±2.10 U/L; after kidney donation, 7.59±1.37 U/L; p<0.01; data not shown). This decrease in serum ACE levels may explain why the plasma AngII levels were not increased despite the elevation of plasma AGT levels.

There are several mechanisms by which the intrarenal RAS may be activated at this early phase. First, it may have been activated by the increase in urinary AGT excretion that results from an increase in the plasma AGT levels. AGT is an acute-phase protein, and its production in the liver is increased during some inflammatory conditions, such as nephrectomy (23). In the present study, the serum hs-CRP levels after kidney donation were significantly higher than those before kidney donation, so the urinary AGT levels may have been increased as a result of the increased plasma AGT, which was in turn induced by inflammation due to kidney donation. However, urinary AGT excretion was not correlated with plasma AGT or serum hs-CRP levels. In addition, Matsusaka et al. reported that AGT produced in the liver and filtered through the glomerular basement membrane is the primary source of intrarenal RAS activation (24). However, the kidney transplant donors in the present study had no glomerular damage prior to surgery, so it is unlikely that any glomerular damage subsequently occurred while plasma AGT was filtered through the glomerular basement membrane by 7.5±1.7 days after kidney donation. Therefore, the increase in urinary AGT excretion was likely not caused by the increase in plasma AGT levels. Second, the increase in urinary AGT excretion may have been

Table 2. Changes in Clinical Parameters before and after Kidney Donation.

	Before kidney donation	After kidney donation	p value
Body weight (kg)	53.8±9.1	51.7±8.9	<0.01
Body mass index (kg/m^2)	22.1±3.2	21.2±2.9	<0.01
SBP (mmHg)	122.5±16.1	124.6±14.3	0.47
DBP (mmHg)	76.0±9.7	78.1±10.8	0.28
MBP (mmHg)	91.1±11.5	93.4±11.1	0.25
Heart rate (/min)	66.6±9.8	74.5±11.2	<0.01
sCr (mg/dL)	0.71±0.11	1.09±0.17	<0.01
eGFR (mL/min/1.73 m^2)	74.4±6.4	46.8±7.2	<0.01
CKD stages			
Stage 1	0	0	
Stage 2	10	0	
Stage 3A	0	6	
Stage 3B	0	4	
Stage 4	0	0	
Stage 5	0	0	
Plasma AGT (mg/mL)	15.0±3.5	24.3±5.8	<0.01
Plasma AngII (pg/mL)	10.8±5.7	11.0±5.9	0.88
Log serum hs-CRP (ng/mL)	2.74±0.51	3.81±0.39	<0.01
Log urinary Alb/day (mg/day)	0.77±0.30	0.91±0.24	0.20
Log urinary AGT/day (mg/day)	0.86±0.41	1.77±0.42	<0.01

SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure, sCr: serum creatine, eGFR: estimated glomerular filtration rate, CKD: chronic kidney disease, AGT: angiotensinogen, AngII: angiotensin II, hs-CRP: high-sensitivity C-reactive protein, Alb: albumin

Table 3. Relationships between Urinary Angiotensinogen Excretion and Clinical Parameters after Kideny Donation.

	r	p value
Body mass index	-0.49	0.18
SBP	-0.036	0.93
DBP	-0.11	0.79
MBP	-0.11	0.77
Heart rate	0.18	0.64
eGFR	-0.70	0.036
Plasma AGT	0.49	0.18
Plasma AngII	-0.003	0.99
Log hs-CRP	0.58	0.10
Log urinary Alb/day	0.69	0.060

SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure, eGFR: estimated glomerular filtration rate, AGT: angioteninogen, AngII: angiotensin II, hs-CRP: high-sensitivity C-reactive protein, Alb: albumin

Table 4. Relationships of Percentage Changes in Urinary Angiotensinogen Excretion Levels and Clinical Parameters between Pre-operation and Post-operation.

	r	p value
ΔBody mass index	-0.41	0.31
ΔSBP	<-0.01	0.99
ΔDBP	0.30	0.52
ΔMBP	0.076	0.86
ΔHeart rate	0.54	0.16
ΔeGFR	-0.47	0.25
ΔPlasma AGT	0.31	0.45
ΔPlasma AngII	0.21	0.62
ΔLog hs-CRP	0.58	0.10
ΔLog urinary Alb/day	0.72	0.11

SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure, eGFR: estimated glomerular filtration rate, AGT: angioteninogen, AngII: angiotensin II, hs-CRP: high-sensitivity C-reactive protein, Alb: albumin

due to BP elevation. However, the subjects' systemic BP readings did not differ markedly, and the values were not correlated with the urinary AGT excretion. Therefore, it is unlikely that the increase in urinary AGT excretion was caused by BP elevation. Third, the increase in the urinary AGT excretion may have been due to hyperfiltration in the remaining kidney after kidney donation. The subjects' eGFR after kidney donation (46.8±7.2 mL/min/1.73 m^2) was on average 63%, not 50%, of that before kidney donation (74.4 ±6.4 mL/min/1.73 m^2), suggesting that glomerular hyperfiltration was induced in the remaining kidney. However, given that the subjects' urinary albumin excretion levels did not differ markedly between pre- and post-operation, and the values were not correlated with the urinary AGT excretion, it is unlikely that the increased urinary AGT excretion was caused by hyperfiltration in the remaining kidney in the present study, and it may be impossible to determine the mechanisms using kidney transplantation donors. In the near future, we will clarify, using an animal model involving the

injection of visualized AGT protein, how much AGT is filtered from the glomeruli in the remaining kidney immediately after kidney donation. Finally, the increase in AGT production in the tubule may have led to the activation of the intrarenal RAS. The proximal tubule is the main region of AGT production in the kidney, and it produces AGT aggressively under conditions of renal damage (25-27). In addition, although most AGT is produced in the liver and filtered through the glomerular basement membrane in cases of marked glomerular damage (24), slight glomerular damage, such as that occurring in benign nephrosclerosis, causes a small amount of plasma AGT to be filtered through the glomerular basement membrane (28). Therefore, AGT produced in proximal tubular cells is likely to be abundant in kidney transplant donors who are not suffering from glomerular damage. In this regard, Saito et al. reported that urinary AGT excretion precedes microalbuminuria. They suggested that the intrarenal RAS is activated in the early phase of type 1 diabetes mellitus nephropathy in a manner similar to our present results (29).

Several limitations associated with the present study warrant mention. First, the sample size was small. The sample size is a very important component of clinical studies, and it cannot be denied that the small sample size in the present study led to an incorrect conclusion. However, even with our limited sample size, we were able to demonstrate the following: There were no significant differences in the BP, plasma AngII, or urinary albumin excretion after kidney donation; the levels of urinary AGT excretion were significantly increased after kidney donation; and the levels as well as the Δ in urinary AGT excretion after kidney donation did not show a significant relationship with the levels as well or Δ in systolic BP, plasma AGT, plasma AngII, or urinary albumin excretion. Therefore, we conclude that the intrarenal RAS is activated in kidney transplant donors immediately after kidney donation, independent of the systemic BP and filtration of increased plasma AGT, due to augmented inflammation. Second, it cannot be denied that the invasiveness of the operation influenced the results in the present study. However, the degree and frequency of subjective symptoms were dramatically reduced at 7.5±1.7 days after kidney donation compared with those symptoms in the immediate post-operative period, and the kidney transplant donors had no fever at 7.5±1.7 days after kidney donation. In addition, neither the hs-CRP levels nor Δhs-CRP correlated with other clinical parameters or the Δ in other clinical parameters, except for ΔeGFR (Supplementary material 1 and 2). Furthermore, the measurements were taken just before discharge to eliminate the influence of the invasiveness of the operation, and it was impossible to extend the hospitalization in the present study. Third, although we hypothesize that the intrarenal RAS activation at such an early phase may lead to a greater risk of end-stage renal disease, the associations between the changes in urinary AGT excretion and the changes in the eGFR after donation, at 3 months, or at 1 year were not examined, as this study was performed to investigate whether or not the intrarenal RAS was activated immediately after kidney donation in kidney transplant donors. Kendi et al. showed that the urinary AGT levels for 16 kidney transplant donors were increased at 15 days, 1 month, 6 months, and 12 months, and the urinary AGT/creatinine levels at 6 months after kidney donation and the urinary protein creatinine ratio at 12 months after kidney donation showed a positive correlation. As a result, those authors concluded that increased urinary AGT levels can be the result of intrarenal RAS activation that affects the compensatory changes in the remaining kidney (30). In the future, we will perform another study to clarify the long-term effects of donation on the urinary AGT levels. Finally, whether or not the phenomena observed in this study are specific to kidney donation remains unclear. Cardiac surgery is known to increase urinary AGT excretion levels (31). However, several mechanisms, such as hemodynamic, inflammatory, metabolic, and nephrotoxic factors, are involved in increased urinary AGT excretion in cardiac surgery. Therefore, it is difficult to make a direct correlation between the urinary AGT excretion and inflammation due to cardiac surgery. We will need to investigate the urinary AGT excretion levels after surgery (e.g., laparoscopic cholecystectomy) that causes inflammation to a similar degree to kidney donation without influencing the hemodynamic or metabolic factors.

Conclusion

In summary, the intrarenal RAS is activated in kidney transplant donors immediately after kidney removal, independently of the systemic BP and filtration of increased plasma AGT, due to increased inflammation. These results may indicate a causal relationship regarding the increased risk of end-stage renal disease in kidney transplant donors.

The authors state that they have no Conflict of Interest (COI).

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