Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Elevated urinary angiotensinogen a marker of intrarenal renin angiotensin system in hypertensive renal transplant recipients: does it play a role in development of proteinuria in hypertensive renal transplant patients?

Aysun Aybal Kutlugun,[1] Bulent Altun,[1] Yahya Buyukasik,[2] Tuncay Aki,[3] Ercan Turkmen,[1] Mahmut Altindal,[1] Tolga Yildirim,[1] Rahmi Yilmaz[1] and Cetin Turgan[1]

1 Department of Nephrology, Hacettepe University Faculty of Medicine, Ankara, Turkey
2 Department of Hematology, Hacettepe University Faculty of Medicine, Ankara, Turkey
3 Department of Urology, Hacettepe University Faculty of Medicine, Ankara, Turkey

Keywords
angiotensinogen, hypertension, renin angiotensin system, transplantation.

Correspondence
Aysun Aybal Kutlugun, Department of Nephrology, Hacettepe University Faculty of Medicine, Sihhiye, Ankara, Turkey. Tel.: +90-0-312-3051710; fax: +90-0-312-3113958; e-mail: draysunaybal@yahoo.com

Conflict of Interest
The authors have declared no conflicts of interest.

Received: 27 May 2011
Revision requested: 19 June 2011
Accepted: 21 August 2011
Published online: 16 September 2011

doi:10.1111/j.1432-2277.2011.01338.x

Summary

The aim of this study was to evaluate the relationship of local intrarenal renin angiotensin system (RAS) with hypertension and proteinuria in renal transplant recipients. Sixty-nine nondiabetic renal transplant recipients (39 male, mean age: 36.3 ± 11.5 years) were included in this study. All patients were in stable condition with GFR greater than 30 ml/min/1.73 m^2; (MDRD). Hypertension was defined to be present if there was a recorded diagnosis of hypertension, systolic blood pressure >130 mmHg and/or diastolic blood pressure >80 mmHg according to ambulatory blood pressure monitoring. None of the hypertensive patients were receiving RAS blockers. Spot urine samples were obtained to measure urinary angiotensinogen (AGT) using human AGT-ELISA, urinary creatinine and protein levels. The demographic properties and laboratory findings were similar between hypertensive and normotensive transplant recipients. Urinary AGT–creatinine ratio (UAGT/UCre) was significantly higher in hypertensive patients compared with the normotensives (8.98 ± 6.89 μg/g vs. 5.48 ± 3.33 μg/g; $P = 0.037$). Importantly, a significantly positive correlation was found between UAGT/Ucre levels and proteinuria in hypertensive patients ($P = 0.01$, $r = 0.405$). Local intrarenal RAS probably plays an important role in the development of hypertension and proteinuria in renal transplant recipients.

Introduction

Hypertension is an important and common complication after renal transplantation. Post-transplant hypertension is an independent risk factor for cardiovascular disease that is the main cause of morbidity and mortality in renal transplant recipients [1,2]. A significant correlation was found between blood pressure and the development of chronic allograft nephropathy the leading cause of graft loss [3–5]. So patient and graft survival are adversely affected in hypertensive renal transplant recipients.

The frequency of hypertension in renal transplant patients has been reported as high as 50–90% [6]. The etiology of post-transplant hypertension is not clearly understood because of its multifactorial nature that includes pre- and post-transplant conditions. Although hypertension is a modifiable risk factor, it is poorly controlled in renal transplant patients [6].

The renin–angiotensin system is considered a hormonal system that regulates blood pressure and fluid balance. Despite this, locally produced RAS in most tissues and paracrine and autocrine functions of tissue RAS have been shown recently [7,8]. Experimental and clinical studies

based on the RAS blockage have provided evidence for the presence of blood pressure-independent renoprotective effects of the RAS blockers [9–12]. Moreover, angiotensin II (Ang II) level in renal tissue was found 1000 times greater than plasma values [13]. These findings suggest that intrarenal RAS is an independent system and regulated distinct from circulating RAS. Therefore, assessment of intrarenal RAS activation is essential to investigate the pathophysiological mechanisms of hypertension and renal diseases.

Recently, urinary AGT, measured with an ELISA, has been shown to reflect intrarenal RAS status in hypertensives and chronic kidney disease patients [14,15]. Angiotensinogen is the only known substrate for renin which is rate-limiting enzyme of RAS. Plasma AGT is synthesized by the liver, but it cannot undergo glomerular filtration because of high molecular weight [16]. Hence, it is suggested that the source of urinary AGT is locally produced AGT by proximal tubular cells [17,18]. The aim of this study was to investigate firstly the relationship between urinary AGT levels, hypertension and daily protein excretion in renal transplant recipients.

Materials and methods

Patients

Sixty-nine adult, nondiabetic renal transplant recipients (RTRs) (23 cadaveric allograft, 46 living-related allograft, 39 male, 30 female; mean age 36.3±11.5 years) that transplanted for at least 6 months (mean ± SD: 48.7 ± 57.7 months) were included in the study. Patients were selected from a total population of 273 patients treated at our center. All patients were in stable condition with GFR greater than 30 ml/min/1.73 m^2; Patients who were receiving RAS blockers, who had serum creatinine >1.5 mg/dl and had daily urinary protein excretion >1 g were excluded in this study. Using medical records, we obtained data on patient demographics (age, gender, body mass index, donor source, dialysis modality, duration of dialysis before the transplantation and time since transplantation), history of hypertension, number of antihypertensive medications, dosage of immunosuppressive drugs, serum creatinine, serum albumin, hemoglobin levels and serum lipid profiles. Except one, all patients were receiving triple immunosuppressive therapy with prednisolone (2.5–15 mg/day), calcineurin inhibitors/sirolumus, and azathioprine (1–3 mg/kg/day)/mycophenolate mofetil (30 mg/kg/day). Adjusted dose of calcineurin inhibitors/sirolumus was according to the measurement of blood levels.

Patients were divided into two groups based on the presence of hypertension. Hypertension was defined to be present if there was a recorded diagnosis of hypertension or systolic blood pressure >130 mmHg and/or diastolic blood pressure >80 mmHg according to ambulatory blood pressure monitoring.

The study protocol was approved by the Local Ethical Committees and written informed consent was obtained from each subject.

Measurements

Morning spot urine samples were collected from all patients to measure urinary AGT, urinary creatinine, albumin and protein levels. Urinary concentration of AGT was measured with human AGT-ELISA kits (Uscn Life Science Inc., Wuhan, China). Urinary creatinine level was measured with Jaffe's method. Urinary albumin and protein levels were quantified with nephelometry (Immage 800; Beckman Coulter, USA) and automated turbidimetric benzalkonium chloride method, respectively, in routine practice. Glomerular filtration rate of patients was calculated with 4-variable MDRD formula [19]. Spot urine protein–creatinine ratio and albumin–creatinine ratio was used for quantitation of daily protein and albumin excretion, respectively.

Ambulatory blood pressure monitoring

Twenty-four hour ambulatory blood pressure monitoring (ABPM) was performed using a spacelab (Redmond, WA, USA) oscillometric BP device. Blood pressure was measured every 30 min for 24 h. Measurements were considered sufficient to include if >85% of the readings were successful. Mean 24-h, day-time (06:00–22:00) and night-time (22:00–06:00) BPs were recorded in all patients.

Statistical analysis

The SPSS program version 16.0 (SPSS Inc., Chicago, IL, USA) was used for analysis. Results are presented as means ± SDs. Shapiro–Wilks test was used to evaluate normality. Hypertensive and normotensive groups were compared by Student's *t*-test for parametric variables and Mann–Whitney *U*-tests for nonparametic variables. Chi-square test was used to assess differences in categorical variables among groups. To determine relation between UAGT/Ucre and clinical parameters, Pearson correlation coefficients and Spearman correlation coefficients were used for parametric variables and nonparametric variables, respectively. A *P*-value of <0.05 was considered statistically significant.

Results

Thirty-nine (56.5%) of whole population had hypertension and 24 (61.5%) of hypertensives were receiving antihypertensive medications. The average number of

antihypertensive drugs per patient was 1.36 ± 0.64. Seven of hypertensive patients who were on antihypertensives required combination antiypertensive drugs for controlling blood pressure, and the remaining were receiving one drug. Fifteen (38.5%) of hypertensive patients had new-defined hypertension according to ABPM. The most prescribed antihypertensive medication was calcium channel blockers, followed by carvedilol, beta-blockers and alpha-blockers. Mean blood pressure levels of normotensives and hypertensives are presented in Table 1.

Table 1. Blood pressure levels (mmHg) in hypertensive and normotensive patients.

	Hypertensive group (n = 39)	Normotensive group (n = 30)	P
Office-SBP	124 ± 14	117 ± 14	0.007
Office-DBP	83 ± 9	77 ± 10	0.012
24-h SBP	130 ± 13	119 ± 9	<0.001
24-h DBP	84 ± 8	75 ± 7	<0.001
Daytime SBP	131 ± 13	120 ± 9	<0.001
Daytime DBP	85 ± 8	76 ± 7	<0.001
Night-time SBP	127 ± 15	116 ± 12	0.001
Night-time DBP	80 ± 9	72 ± 9	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure.

There were no significant differences in demographic findings and laboratory parameters between renal transplant patients with and without hypertension (Table 2).

Mean UAGT/Ucre ratio of the total patients was 7.45 ± 5.86 μg/g (1.33–27.0 μg/g). Level of UAGT/Ucre was significantly higher in hypertensive renal transplant patients compared with normotensive renal transplant patients (8.98 ± 6.89 μg/g vs. 5.48 ± 3.33 μg/g; P = 0.037) (Fig. 1). There was no significant difference in UAGT/Ucre between hypertensive renal transplant patients who were on antihypertensives and who had new-defined hypertension (8.22 ± 6.31 μg/g vs. 10.19 ± 7.81 μg/g; P = 0.45). In the evaluation of the UAGT/Ucre in hypertensive patients according to type of calcineurin inhibitor, UAGT/Ucre was lower in the tacrolimus group than cyclosporine group, but this difference did not reach statistical significance (6.95 ± 5.37 μg/g vs. 9.14 ± 6.82 μg/g; P = 0.28).

The UAGT/Ucre levels showed no significant correlation with age, gender, serum creatinine, GFR, BMI, systolic and diastolic blood pressures, dialysis modality, duration of dialysis before the transplantation and time since transplantation. A positive correlation was observed between UAGT/Ucre levels and urinary protein excretion in hypertensive patients (P = 0.01, r = 0.405), but this is not found in normotensives (P = 0.35, r = 0.176).

Table 2. Comparison of the demographic data and laboratory parameters between hypertensive patients and normotensive patients.

	Hypertensive group (n = 39)	Normotensive group (n = 30)	P
Age (years)	36 ± 11	36 ± 12	0.86
Sex (male/female)	23/16	16/14	0.82
Duration of transplantation (months)	44.0 ± 52.6	54.8 ± 64.1	0.23
Type of dialysis (HD/PD/pre-emptive) (%)	30/6/3	23/4/3	0.93
Duration of dialysis (months)	25.8 ± 28.5	42.6 ± 41.2	0.16
Donor source (living-related/cadaveric)	27/12	19/11	0.80
Body mass index (kg/m^2)	25.1 ± 4.4	24.2 ± 5.0	0.41
Dose of steroid (mg/day)	6.9 ± 2.8	6.0 ± 3.1	0.16
CNI (cyclosporine/tacrolimus)	25/12	20/9	0.93
Dose of cyclosporine (mg/kg/day)	1.97 ± 0.71	2.52 ± 1.24	0.13
Dose of tacrolimus (mg/kg/day)	0.07 ± 0.04	0.07 ± 0.01	0.66
Serum creatinine (mg/dl)	1.20 ± 0.29	1.13 ± 0.33	0.11
Glomerular filtration rate (ml/min)	65 ± 18	69 ± 18	0.28
Serum albumin (g/dl)	4.6 ± 0.3	4.6 ± 0.4	0.92
Total cholesterol (mg/dl)	195 ± 36	189 ± 31	0.72
HDL-C (mg/dl)	57 ± 14	58 ± 17	0.83
LDL-C (mg/dl)	112 ± 32	101 ± 29	0.62
Triglycerides (mg/dl)	166 ± 77	140 ± 49	0.33
Hemoglobin (g/dl)	13.7 ± 2.2	13.5 ± 2.0	0.81
Proteinuria (μg/mg)	182 ± 120	162 ± 108	0.59
Albuminuria (μg/mg)	46 ± 59	38 ± 60	0.38
Microalbuminuria (%)	43.6	33.3	0.54
Patients on AH medication (n)	24	0	<0.01

HD, hemodialysis; PD, peritoneal dialysis; CNI, calcineurin inhibitor; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein-cholesterol; AH, antihypertensive.

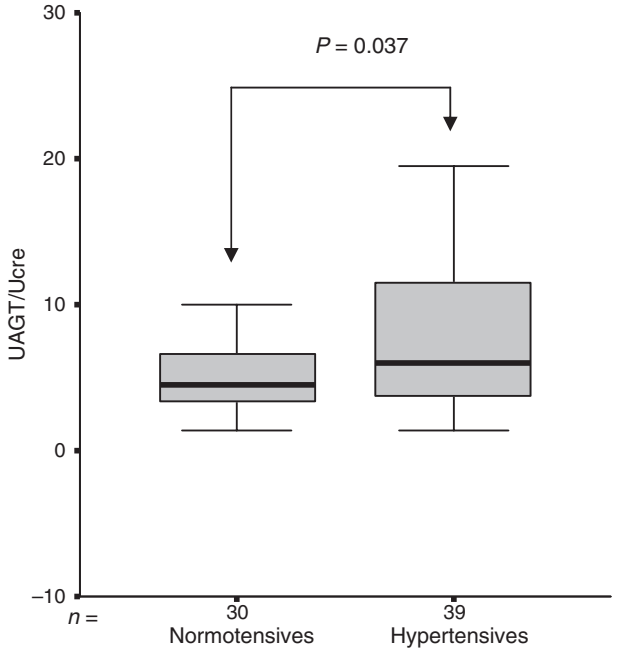

Figure 1 Mean UAGT/Ucre levels in hypertensive and normotensive renal transplant recipients.

Discussion

In this study, we demonstrated that: (i) urinary AGT levels were higher in hypertensive RTRs than in normotensive RTRs; (ii) urinary AGT levels correlated with daily protein excretion in hypertensive RTRs, but not in normotensive RTRs. To our knowledge, this is the first study that evaluated the relation between urinary AGT levels and hypertension or protein excretion in renal transplant patients. Previous studies examined the urinary AGT levels as an index of intrarenal local RAS status in essential hypertension [14] and renal diseases such as IgAN [20], diabetic nephropathy [21] and chronic glomerulonephritis [22].

Angiotensinogen is the precursor of angiotensins and the substrate for renin that is known as the rate-limiting enzyme of the RAS. The most amount of circulating AGT is produced by liver. Because of the high molecular weight of AGT, it cannot be filtered through the glomerular basement membrane [16]. Urinary AGT comes from AGT in the kidney that is locally produced and secreted into the tubular lumens directly by proximal tubular cells [18]. Further, no correlation has been found between plasma AGT and urinary AGT levels suggest that the source of urinary AGT is the locally formed AGT in the kidney [18]. Angiotensin II is the most important product of the RAS. Increased intrarenal AngII activitiy is associated with the development of hypertension and renal injury [23–25]. Positive correlation between tubulointerstitial Ang II concentrations and systolic blood pressure has been shown in salt sensitive hypertensive rats [24].

In recent years, it has been demonstrated by clinical and experimental studies that urinary AGT levels show the intrarenal Ang II activity. Kobori *et al.* [18] showed a positive correlation between urinary AGT and intrarenal Ang II levels in Ang II-dependent hypertensive rats. In addition, elevated urinary AGT was associated with a more intense immunostaining of renal AngII and type I collagen in patients with CKD [15]. Similarly, urinary AGT was significantly correlated with intrarenal angiotensinogen gene expression and AngII immunoreactivity in patients with normotensive IgAN [20]. These findings may suggest that the urinary AGT levels can be used to evaluate the intrarenal RAS status in hypertensive or renal patients.

Kobori *et al.* [14] investigated urinary AGT levels in hypertensive patients after they found that urinary AGT was a marker for intrarenal status in Ang II-dependent hypertensive rats. Elevated urinary AGT levels in 70 hypertensive patients, compared with controls, have been reported. In addition, they demonstrated that urinary AGT was significantly correlated with SBP and DBP in hypertensive patients. Despite the finding that urinary AGT levels were higher in hypertensive RTRs compared with normotensive RTRs in our study, we failed to show any correlation between urinary AGT and SBP or DBP. The difference between nontransplanted hypertensives and hypertensive RTRs is probably related to immunosuppressive treatment. Urinary AGT levels were not related with age, race, gender, BMI, eGFR and serum creatinine in both study groups including nontransplanted hypertensive patients [14] and RTRs.

One of the most important findings of the present study was the significant positive correlation between urinary AGT and daily protein excretion in hypertensive RTRs. Consistent with our findings, urinary AGT was correlated with proteinuria in nontransplanted hypertensive patients not treated with RAS blockers [14]. The well known association between the intrarenal RAS activation and proteinuria has been shown by numerous studies which have conclusively demonstrated the blood pressure-independent anti-proteinuric effects of the RAS blockers [11,12]. Locally produced Ang II induces proteinuria via TGF-B dependent glomerulosclerosis and podocyte injury [26]. In addition, the expression of other cytokines such as TNFα, VEGF that causes proteinuria in different mechanisms are also stimulated by Ang II [27,28]. In an experimental model, it was shown that incubation of glomeruli with an ARB, irbesartan, reduced the cytokines in a dose dependent manner [28]. Furthermore, local RAS activation is associated with reduced

nephrin expression which is an important mechanism underlying the development of proteinuria [29]. Despite the positive correlation between urinary AGT and proteinuria in hypertensive patients, it was suggested that increased urinary AGT was not as a nonspecific result of proteinuria [18,22].

In this study, although the difference was not statistically significant, urinary AGT levels were higher in cyclosporine treated patients compared with those receiving tacrolimus. Cyclosporine induced chronic allograft nephropathy is closely associated with local RAS activation [30]. Shang *et al.* [31] performed immunohistochemical staining for renin and Ang II on renal allograft biopsy specimens from patients with cyclosporine related chronic nephropathy. Despite the plasma renin and Ang II levels were not significantly increased, markedly positive staining for renin and Ang II of renal tissues were observed in that study, so it was suggested that intra-renal local RAS has a central role in the pathogenesis of cyclosporine induced adverse events on kidney. In addition, beneficial effects of RAS blockage in chronic cyclosporine nephrotoxity were shown in an experimental study by Yang *et al.* [30].

The most important limitation of this observational and cross-sectional study was the small sample size. Despite this limitation, a significantly elevated urinary AGT level and a positive correlation between urinary AGT and daily protein excretion were demonstrated in hypertensive RTRs. To the best of our knowledge, this is the first study reporting the relation of urinary AGT and hypertension in renal transplant patients.

In conclusion, measurement of urinary AGT levels can help to evaluate intrarenal local RAS activation that may be one possible mechanism of hypertension in RTRs. Further prospective controlled studies in larger patient groups are required to exhibit the role of local RAS activation and its diagnostic markers in hypertension in RTRs.

Authorship

AAK: performed research/study, collected data, analyzed data, wrote the paper. BA: designed research/study. YB: studied the ELISA kits. TA, RY and CT: contributed important reagents. ET, MA and TY: collected data.

Funding

None.

References

1. Howard RJ, Patton PR, Reed AI, *et al.* The changing causes of graft loss and death after kidney transplantation. *Transplantation* 2002; **73**: 1923.
2. Ojo AO, Hanson JA, Wolfe RA, *et al.* Long-term survival in renal transplant recipients with graft function. *Kidney Int* 2000; **57**: 307.
3. Mange KC, Cizman B, Joffe M, *et al.* Arterial hypertension and renal survival. *JAMA* 2000; **283**: 633.
4. Opelz G, Döhler B. Collaborative Transplant Study. Improved long-term outcomes after renal transplantation associated with blood pressure control. *Am J Transplant* 2005; **5**: 2725.
5. Opelz G, Wujciak T, Ritz E. Association of chronic kidney graft failure with recipient blood pressure. Collaborative Transplant Study. *Kidney Int* 1998; **53**: 217.
6. Kasiske BL, Anjum S, Shah R, *et al.* Hypertension after kidney transplantation. *Am J Kidney Dis* 2004; **43**: 1071.
7. Bader M, Ganten D. Update on tissue renin–angiotensin systems. *J Mol Med* 2008; **86**: 615.
8. Q Velez JC. The importance of the intrarenal renin–angiotensin system. *Nat Clin Pract* 2008; **5**: 89.
9. Lewis EJ, Hunsicker LG, Bain RP, *et al.* The effect of angiotensin-converting enzyme inhibition on diabetic nephropathy. *N Engl J Med* 1993; **329**: 1456.
10. Ruiz-Ortega M, Gonzalea S, Seron D, *et al.* ACE inhibition reduces proteinuria, glomerular lesions and extracellular matrix production in a normotensive rat model of immune complex nephritis. *Kidney Int* 1995; **48**: 1778.
11. Mifsud SA, Allen TJ, Bertram JF, *et al.* Podocyte foot process broadening in experimental diabetic nephropathy: amelioration with renin-angiotensin blockade. *Diabetologia* 2001; **44**: 878.
12. Weir MR. Effects of renin-angiotensin system inhibition end-organ protection: can we do better? *Clin Ther* 2007; **29**: 1803.
13. Nishiyama A, Seth DM, Navar LG. Renal interstitial fluid concentrations of angiotensins I and II in anesthetized rats. *Hypertension* 2002; **39**: 129.
14. Kobori H, Alper Jr AB, Shenava R, *et al.* Urinary angiotensinogen as a novel biomarker of the intrarenal renin-angiotensin system status in hypertensive patients. *Hypertension* 2009; **53**: 344.
15. Yamamoto T, Nakagawa T, Suzuki H, *et al.* Urinary angiotensinogen as a marker of intrarenal angiotensin II activity associated with deterioration of renal function in patients with chronic kidney disease. *J Am Soc Nephrol* 2007; **18**: 1558.
16. Rohrwasser A, Morgan T, Dillon HF, *et al.* Elements of a paracrine tubular reninangiotensin system along the entire nephron. *Hypertension* 1999; **34**: 1265.
17. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev* 2007; **59**: 251.
18. Kobori H, Nishiyama A, Harrison-Bernard LM, Navar LG. Urinary angiotensinogen as an indicator of intrarenal angiotensin status in hypertension. *Hypertension* 2003; **41**: 42.

19. Levey AS, Bosch JP, Lewis JB, *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in Renal Disease Study Group (PDF). *Ann Intern Med* 1999; **130**: 461.
20. Nishiyama A, Konishi Y, Ohashi N, *et al.* Urinary angiotensinogen reflects the activity of intrarenal renin-angiotensin system in patients with IgA nephropathy. *Nephrol Dial Transplant* 2011; **26**: 170.
21. Saito T, Urushihara M, Kotani Y, *et al.* Increased urinary angiotensinogen is precedent to increased urinary albumin in patients with type 1 diabetes. *Am J Med Sci* 2009; **338**: 478.
22. Kobori H, Ohashi N, Katsurada A, *et al.* Urinary angiotensinogen as a potential biomarker of severity of chronic kidney diseases. *J Am Soc Hypertens* 2008; **2**: 349.
23. Kobori H, Ozawa Y, Suzaki Y, *et al.* Enhanced intrarenal angiotensinogen contributes to early renal injury in spontaneously hypertensive rats. *J Am Soc Nephrol* 2005; **16**: 2073.
24. Franco M, *et al.* Renal angiotensin II concentration and interstitial infiltration of immune cells are correlated with blood pressure levels in saltsensitive hypertension. *Am J Physiol Regul Integr Comp Physiol* 2007; **293**: R251.
25. Mezzano S, Droguett A, Burgos ME, *et al.* Renin-angiotensin system activation and interstitial inflammation in human diabetic nephropathy. *Kidney Int Suppl* 2003; **86**: S64.
26. Li JZ, Zhou CH, Yu L, *et al.* Renal Protective Effects of Blocking the Renin-Angiotensin System. *Hypertens Res* 1999; **22**: 223.
27. Lee EY, Shim MS, Kim MJ, *et al.* Angiotensin II receptor blocker attenuates overexpression of vascular endothelial growth factor in diabetic podocytes. *Exp Mol Med* 2004; **36**: 65.
28. Vieitez P, Gómez O, Uceda ER, Vera ME, Molina-Holgado E. Systemic and local effects of angiotensin II blockade in experimental diabetic nephropathy. *J Renin Angiotensin Aldosterone Syst* 2008; **9**: 96.
29. Langham RG, Kelly DJ, Cox AJ, *et al.* Proteinuria and the expression of the podocyte slit diaphragm protein, nephrin, in diabetic nephropathy: effects of angiotensin converting enzyme inhibition. *Diabetologia* 2002; **45**: 1572.
30. Yang CW, Ahn HJ, Kim WY, *et al.* Influence of the renin-angiotensin system on epidermal growth factor expression in normal and cyclosporine-treated rat kidney. *Kidney Int* 2001; **60**: 847.
31. Shang MH, Yuan WJ, Zhang SJ, *et al.* Intrarenal activation of renin angiotensin system in the development of cyclosporine A induced chronic nephrotoxicity. *Chin Med J (Engl)* 2008; **121**: 983.