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Renal impairment, hypertension and plasma urotensin II

Ari Mosenkis¹, Radhakrishna R. Kallem¹, Theodore M. Danoff², Nambi Aiyar², Jonathan Bazeley¹ and Raymond R. Townsend¹

¹Renal, Electrolyte and Hypertension Division, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA and ²GlaxoSmithKline, King of Prussia, PA, USA

Correspondence and offprint requests to: Radhakrishna R. Kallem; E-mail: krishna.kallem@uphs.upenn.edu

Abstract

Background. Human urotensin II (UII) is a potent mammalian vasoconstrictor thought to be produced and cleared by the kidneys. Conflicting data exist regarding the relationship between UII concentrations, kidney function and blood pressure (BP). We measured the associations between kidney function [including end-stage renal disease (ESRD)] and levels of BP with plasma concentrations of UII.

Methods. Ninety-one subjects were enrolled. Thirty-one subjects had ESRD (undergoing haemodialysis), 30 subjects had chronic kidney disease (CKD) and 30 control subjects had no kidney disease. Plasma UII concentrations were measured by radioimmunoassay.

Results. Mean plasma UII concentrations were highest in controls, lower in subjects with ESRD and lowest in subjects with non-ESRD CKD ($P < 0.0001$). UII concentrations correlated negatively with serum creatinine ($P = 0.0012$) and CKD stage, and positively with creatinine clearance ($P = 0.013$). In ESRD subjects, plasma UII ($P = 0.008$) increased after dialysis, while SBP ($P = 0.007$), DBP ($P = 0.009$), serum creatinine ($P < 0.0001$) and serum urea nitrogen ($P < 0.0001$) decreased. UII concentrations were lower in patients with a history of hypertension (HTN) ($P = 0.016$). Age, race and gender did not appear to be associated with UII concentration. However, the distribution

of African American race and male gender appear to be associated with increasing stages of chronic kidney disease.

Conclusions. These data suggest a potential vasodilatory role of UII in humans with kidney disease or hypertension. The reduction in UII levels in CKD also suggests either reduced production or greater clearance, or both, of UII.

Keywords: CKD; diabetes; dialysis; hypertension; urotensin II

Introduction

Hypertension (HTN) is highly prevalent (>80%) in patients with chronic kidney disease (CKD) and end-stage kidney disease (ESRD) [1], and responsible for substantial cardiovascular mortality [2]. Mechanisms invoked for HTN in CKD include increased plasma renin activity relative to the expanded extracellular volume [3] and/or due to the inadequate clearance of vasoactive substances [4]. It is in light of this latter mechanism that we undertook this observational study.

The urotensins are a family of vasoactive peptides first isolated from various fish species nearly 30 years ago [5] and later from frogs, rodents, pigs, primates and humans [6].

The vasomotor effects of urotensin II (UII) vary greatly, depending on the species studied, interactions with other vasoactive molecules and the vascular bed used [7]. The spontaneously hypertensive rat is sensitive to a blood pressure increase from UII [8] and generated enthusiasm for targeting UII receptors as a new antihypertensive therapy [9]. Supporting this are *in vitro* studies of UII in human vessels showing potent vasoconstriction [10] (8–110-fold more potent than endothelin-1) and synergy with angiotensin II [11]. However, also in humans, *in vivo* administration of UII has produced conflicting haemodynamic results. These range from cutaneous vasodilation [12] to potent vasoconstriction [13–16], and some studies show no haemodynamic changes [17]. But little is known of renal effects in humans. Consequently, the role of UII in human HTN remains an area of active investigation.

The administration of UII [8,18] has an anti-natriuretic effect that is independent of blood pressure, suggesting a direct tubular action and a possible role in the pathogenesis of hypertension. Elevated concentrations of UII were reported in several studies of patients with CKD and ESRD [19,20] as well as in patients with HTN [19,21–23], primarily noted by Asian investigators where the aetiology of renal failure is often IgA nephropathy, which varies from the predominantly diabetes- and HTN-associated renal failure found in the USA. Moreover, in patients with ESRD, the dialysability of UII is unknown, although UII is thought to be related inversely to cardiovascular events in haemodialysis patients [24]. A high concentration of UII and its receptors has been demonstrated in the kidneys of mice, monkeys [25], humans [26,27], and CKD models of rat studies [28]. These investigations have led to the thinking that UII is synthesized, secreted and cleared by the kidneys [29], although our previous experience in two surgically anephric patients indicates that the UII must be synthesized in non-renal tissues [30].

Therefore, we conducted the present study (i) to examine the relationship between plasma UII concentrations and degree of renal function (across normal kidney function, CKD and ESRD groups) and blood pressure (BP) in the USA, (ii) to examine the relationship of plasma concentration of UII with diabetic status and proteinuria, and (iii) to evaluate the effects of a haemodialysis session on plasma concentrations of UII in ESRD patients.

Materials and methods

In this single-centre study, we enrolled 91 subjects including 31 ESRD subjects undergoing routine haemodialysis, 30 subjects with CKD and 30 healthy control subjects with no known renal disease. The study was approved by the Institutional Review Boards of the University of Pennsylvania, and Gambro Healthcare (for the ESRD subjects). Pre-dialysis CKD subjects were recruited through the General Clinical Research Center at the University of Pennsylvania. Written informed consent was obtained from all the subjects. The data were stored in a password-protected laptop computer using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Subjects included men and women more than 18 years old. The 30 subjects with CKD were defined as having CKD using the Cockcroft–Gault equation-based criteria, resulting in serum creatinine concentrations ranging from 1.1 to 3.2 mg/dL in women and from 1.4 to 4.7 mg/dL in men [31].

All subjects underwent standard seated blood pressure measurements [32]. For the ESRD subjects, BPs were measured, and blood samples were

Fig. 1. Inverse correlation of serum creatinine and plasma UII in non-dialysis subjects ($R^2 = 0.1665$; $P = 0.001$).

obtained for urea, creatinine and UII prior to treatment initiation and within 5 min of the completion of their mid-week dialysis session. The 31 ESRD subjects were treated only with haemodialysis using hollow fibre artificial kidneys, and mix of catheter and AV grafts for access. Their weights, ultrafiltration volume and duration of dialysis session were recorded. For the non-ESRD subjects, BPs and blood samples were obtained once. In the (pre-dialysis) CKD group, 24-h urine collections were used for the measurement of daily protein excretion, corrected for body surface area/1.73 m². In the normal kidney function control group, a single voided urine sample was used for the measurement of protein and creatinine, as the ratio of urine protein/creatinine approximates daily protein excretion [33]. Demographic information and medical histories were obtained from all the subjects.

Serum blood urea nitrogen and creatinine, and urine protein and creatinine were measured by standard laboratory methods. UII concentrations were measured in unextracted plasma by radioimmunoassay using human UII-specific monoclonal antibody at GlaxoSmithKline laboratories (King of Prussia, PA, USA) [34,35]. Creatinine clearances in the control group and CKD group were calculated using the Cockcroft–Gault equation using ideal body weight.

Levels of UII in plasma were measured with a specific RIA using monoclonal antibody against human UII. Briefly, 700 μ L of buffer (50 mM phosphate buffer, pH 7.4, containing 10 μ M Na-EDTA and 0.1% BSA), 100 μ L of standard human U-II (Phoenix Pharmaceuticals) or samples, 100 μ L of diluted monoclonal human U-II antibody (1:30 000) and 100 μ L [¹²⁵I] human UII (~25 000 cpm) were incubated for 16 h at 4°C. Monoclonal antibodies against human UII were generated from immunized mouse spleen at GlaxoSmithKline [34]. Bound and free ligands were separated by addition of 0.25 mL secondary goat anti-mouse antibody (BioMagnetic Goat Antimouse IgG, Qiagen). All assays were performed in duplicate. A standard curve was constructed from which the urotensin II concentrations of unknown samples were determined.

Statistical analysis

Continuous variables are reported as mean $\pm$ SE. We compared the mean UII concentration between the three groups using an analysis of variance (ANOVA) and post-hoc testing with Tukey's honestly significant difference (HSD) procedure. Mean UII concentrations of the subjects were then compared based on the positive or negative history of HTN and diabetes, the magnitude of proteinuria (in all non-ESRD subjects), and the subject's race and gender using Student's *t*-tests. We also performed a paired Student's *t*-test to examine the changes in UII concentration, systolic blood pressure (SBP), diastolic blood pressure (DBP), serum creatinine and serum urea nitrogen after a dialysis session in the 31 ESRD subjects.

Possible correlations between the independent variable, the plasma UII concentration, and dependent variables such as systolic BP and diastolic BP both within and among three groups (control, CKD and ESRD), age, and urine protein concentration were examined using bivariate fit analyses. All the statistical tests were performed using JMP IN statistical software version 4.0 (SAS Institute, Cary, NC, USA).

Table 1. Baseline characteristics and laboratory data

	Controls (n = 30)	CKD (n = 30)	ESRD (n = 31)	P-value
Demographics				
Age (years)	55.5 ± 2.3 ^b	58.4 ± 2.3	55.5 ± 2.3	NS
Males	12 (40%)	23 (77%)	17 (55%)	0.013
African American	19 (63%)	21 (70%)	30 (97%)	0.001
History of hypertension	18 (60%)	26 (87%)	29 (94%)	0.003
History of diabetes	7 (23%)	18 (60%)	11 (35%)	0.012
UII (pg/mL)	22 495 ± 652	13 773 ± 652	16 351 ± 641	<0.0001
Blood pressure				
SBP (mmHg)	129 ± 4	133.5 ± 4	146.8 ± 4	0.006
DBP (mmHg)	76 ± 2.7	78.9 ± 2.7	84.4 ± 2.6	NS across groups 0.002 between controls/CKD and ESRD
Renal function				
Serum creatinine (mg/dL)	0.9 ± 0.3	1.9 ± 0.3	8.6 ± 0.3	<0.001
Creatinine clearance (mL/min)	73.4 ± 4.1	47.1 ± 4.1	<15	<0.0001
SUN (mg/dL)	15.4 ± 2.3	29.4 ± 2.3	41 ± 2.3	<0.001
Urine protein ^a	0.14	0.92	NA	0.01

NS, not significant; NA, not available.

^aMeasured as spot protein/creatinine in controls and as 24-h urine protein (gram/total volume adjusted for body surface area/1.73 m²) in CKD subjects. No urine was collected for 2 out of the 30 CKD subjects.

^bStandard error of the mean.

Results

Demographics

Demographic characteristics were partially balanced between the groups with no differences in age, but significant differences in gender and race (Table 1). There were significant differences between the groups for BP (except for diastolic BP between the controls and CKD subjects), creatinine, urea, and creatinine clearance (not calculated for the ESRD group). No significant relationship was observed between UII concentration and age, gender or race.

Relationship between UII and degree of renal function

Among the three groups (i.e. controls, CKD and ESRD), ANOVA indicated differences at the $P < 0.0001$ level of significance. Post-hoc testing (Tukey's HSD) showed that all group means were significantly different from one another at least at $P < 0.05$ for all comparisons between groups. The mean UII concentration in the control group was 22495 ± 652 pg/mL, compared with values of 13773 ± 652 pg/mL in the CKD and 16351 ± 641 pg/mL in the ESRD groups (Table 1). A graphic depiction of the mean UII concentrations for the three groups is shown in Figure 2.

Effect of haemodialysis on blood pressure and UII concentration in ESRD subjects

Both SBP and DBP decreased after dialysis (SBP 10.2 mmHg, $P = 0.007$, and DBP 6.5 mmHg, $P = 0.009$). UII concentrations increased significantly after dialysis, from 16352 ± 763 to 18653 ± 780 pg/mL ($P = 0.008$). Levels of serum creatinine ($P < 0.0001$) and serum urea nitrogen ($P < 0.0001$) decreased after dialysis (Table 3). The duration of dialysis and ultrafiltration volume (weight removed) were not associated with the change in UII concentrations.

Relationship between UII and blood pressure

No significant correlation was observed between UII concentration and SBP or DBP for the combined groups. UII concentrations were lower, however, in subjects with a history of HTN (16896 ± 579 vs. 20087 ± 1167 pg/mL, $P = 0.016$) or diabetes (15633 ± 812 vs. 18767 ± 657 pg/mL, $P = 0.004$) as depicted in Table 2.

Relationship between proteinuria and UII concentration

UII concentrations correlated negatively with degree of proteinuria ($P = 0.024$), and were significantly lower in patients with a greater amount of proteinuria (Table 2).

Fig. 2. Concentrations of plasma UII in control, CKD and ESRD subjects. All groups are different from each other by ANOVA ($P < 0.0001$). Shown are raw values (closed circles to the left) and mean $\pm$ SE (closed circle with error bars) to right within each subject group.

Table 2. UII concentrations (pg/mL) by degree of proteinuria as measured over 24 h and adjusted for body surface area/1.73 m² in CKD subjects, and approximated by spot protein/creatinine ratio in control subjects

	Present	Absent	P-value
Degree of proteinuria			
≥0.5 g/day	14 146 ± 1278 (n = 15)	19 581 ± 755 (n = 43)	0.0006
≥1 g/day	13 845 ± 1989 (n = 7)	18 770 ± 737 (n = 51)	0.024
Medical history			
History of HTN	16 896 ± 579 (n = 73)	20 087 ± 1167 (n = 18)	0.016
History of DM	15 633 ± 812 (n = 36)	18 767 ± 657 (n = 55)	0.004

±, standard error of the mean; HTN, hypertension; DM, diabetes mellitus.

Table 3. Plasma UII levels, blood pressures, serum creatinine, and serum urea nitrogen levels before and after dialysis in 31 ESRD subjects

	Pre-dialysis	Post-dialysis	P-value
Blood pressure			
SBP (mmHg)	146.7 ± 4.5	136.5 ± 3.6	0.007
DBP (mmHg)	84.4 ± 2.7	77.9 ± 2.5	0.009
UII levels (pg/mL)	16 352 ± 763	18 653 ± 780	0.008
Serum creatinine (mg/dL)	8.6 ± 0.4	3.2 ± 0.1	<0.0001
Serum urea nitrogen (mg/dL)	41 ± 2.5	12.8 ± 0.7	<0.0001

Discussion

In the present study, we observed a positive correlation between UII concentration and renal function. We also observed that UII concentrations were lower in subjects with proteinuria, a history of HTN or a diagnosis of diabetes. In addition, UII concentrations increased in ESRD subjects after a standard haemodialysis treatment. The increase in UII concentrations after haemodialysis in light of the significant decrease in SBP and DBP suggests that UII is not dialysable by conventional haemodialysis. Since we did not collect dialysis effluent to assay-removed UII, we can only speculate that UII levels increased post-dialysis either from haemoconcentration or some combination of modest stimulation in production with reduced clearance during dialysis. One thing our paper adds to the literature is the simultaneous study of urotensin concentrations using the same assay in a spectrum of people with markedly different degrees of kidney function.

Conflicting results have been reported by researchers measuring blood or urine values of UII in patients with kidney disease, most of whom have HTN. Totsune *et al.* reported higher plasma UII concentrations in patients with CKD and ESRD compared with control subjects, although the concentrations did not correlate with serum creatinine levels [20]. The same investigators later found no differences in plasma UII concentrations of diabetic patients with and without proteinuria or the presence of HTN [36]. Matsushita *et al.* reported higher urine UII concentrations in hypertensive subjects, although, again, the UII concentrations did not correlate with serum creatinine [19]. Totsune *et al.* also reported

higher plasma and urine concentrations of UII in subjects with diabetes [37], while Langham *et al.* reported increased expression of UII and its receptor in subjects with diabetic nephropathy [38]. Also, higher plasma concentrations of UII have been found in patients with preeclampsia and eclampsia [39]. The same authors also reported high urine UII but low plasma UII in paediatric patients with relapsing minimal change nephropathy [40]. Additionally, no correlation between plasma UII concentrations and creatinine was noted (post hoc) in studies of patients with congestive heart failure [41] or cirrhosis [42]. Cheung *et al.* demonstrated high plasma UII concentrations in 62 hypertensive subjects compared with 62 normotensive controls [21], whereas others found no differences between the plasma concentrations of UII in hypertensive versus normotensive subjects [43].

Our findings differ from those of Tostune, Matsushita, Cheung and Langham. Specifically, we found UII concentrations to be lower among subjects with CKD and ESRD. UII negatively correlated with serum creatinine (Figure 1) and serum urea nitrogen, and positively correlated with creatinine clearance in the non-ESRD subjects. Also, we found no correlation between the level of BP and concentration of UII. However, UII concentrations were found to be lower in subjects with history of HTN or diabetes. The differences in patient populations between our studies and the others cited, and the assays used may be part of the explanation for this. Levels of UII in plasma were measured with a specific RIA using monoclonal antibody against human UII. As we reported [35], the anti-hU-II mAb exhibited sub-nM affinity for hU-II in the RIA. The mAb retained significant activity for the truncated peptides hU-II [4–11] and hU-II [5–11] analogues which retain the cyclic hexapeptide sequence ('CFWKYC'). It also exhibited high affinity for the urotensin-related peptide (URP) but did not cross-react with peptides such as urotensin I, endothelin, angiotensin II, somatostatin, CGRP, etc. Totsune *et al.* used commercially available anti hU-II polyclonal antibody. Data are not available about its reactivity to various UII fragments.

Studies have shown that the monoclonal antibody used in our study recognizes multiple 'U-II-like' immunoreactive fragments in the acid-acetone-extracted human plasma. It is also reported that anti-human UII polyclonal antibodies recognize both 'mature' human UII and its pre-pro-peptide isoform. Furthermore, multiple 'U-II-like' immunoreactive fragments have been described in plasma from congestive heart failure patients. The published estimates of 'U-II-like' activity in the literature have shown up to a 5000-fold variation (2–12400 pg/mL) in plasma 'U-II-like' levels [41,44]. In our study, the assay was performed using unextracted plasma as the required plasma volume is smaller. In a typical run, an acetone/HCl-extracted sample had lower values than an unextracted sample (for example, 14.3 ± 1.6 and 3.9 ± 0.6 ng/mL for the unextracted and extracted plasma samples). This suggests that, in the unextracted sample, the antibody is recognizing UII and its related molecule.

The most plausible explanation we can propose for our findings is a reduced production and perhaps greater clearance of UII in patients associated with progressive kidney function impairment as seen in some observations such as

lower SGOT levels in azotaemia [45]. When kidney function fails to the point of needing dialysis, it is possible that non-kidney tissues participate to a greater degree in UII production (and hence greater UII levels in ESRD as compared with non-ESRD CKD group) as supported by the finding of no change in plasma UII in patients without kidneys on dialysis compared with those who still have native kidneys [30]. The statistically insignificant association between UII and SBP/DBP may be due to the antihypertensive treatment of the subjects (the specific drug usage records have not been taken into consideration for the study), which is supported by the finding of an association between the UII levels and history of hypertension irrespective of the measured blood pressure values. This would be consistent with a predominantly vasodilatory effect of UII, as was demonstrated in the studies of intravenous infusions of UII in animals, and is also consistent with lower blood pressure at the end of dialysis (when UII levels are higher), though other mechanisms such as ultrafiltration of salt and reduction in vasoconstrictors are certainly at play.

In conclusion, we observed a direct association between plasma UII concentration and degree of renal function and lower UII concentrations among subjects with proteinuria or a history of HTN or diabetes. In addition, we also demonstrated an increase in UII concentrations in ESRD subjects after a standard haemodialysis treatment. These findings combined with lower blood pressure at the end of dialysis support either a vasodilator effect or potentially compensatory down-regulation of UII production in the presence of CKD and/or HTN. Clinical testing of UII antagonists in humans will help greatly to clarify the role of UII in BP regulation in humans and whether BP effects are altered when kidney function is reduced or absent [46].

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Vitamin C supplementation in kidney failure: effect on uraemic symptoms

Richard Francis Singer

The Canberra Hospital Renal Service, Australian National University Canberra, ACT, Australia

Correspondence and offprint requests to: Richard Francis Singer; E-mail: richard.singer@act.gov.au

Abstract

Background. Vitamin C (ascorbate) deficiency and symptoms consistent with deficiency (fatigue, myalgia, dyspnoea, gingivitis, cardiovascular instability and depression) are common in patients with renal failure. This study aimed to determine if supplementation with ascorbate in patients with severe renal failure improved symptoms or cardiovascular stability, or was associated with adverse effects.

Methods. The study was a 3-month, double-blind, randomized trial of ascorbic acid 250 mg or matching placebo given thrice weekly. Subjects were clinically stable and either received conventional dialysis or had an estimated glomerular filtration rate of <20 mL/min. Symptoms were measured

using the Kidney Dialysis Quality of Life-Short Form (KDQOL-SF™) symptom subscale, and the study was 80% powered to detect a change of 10 in the KDQOL-SF™.

Results. Ninety-nine subjects were randomized, and ascorbate deficiency was present in 40% at baseline. Mean symptom scores at follow-up were similar in the two groups (P-value = 0.19). There was a trend to slightly worse nausea scores in the ascorbate group after controlling for the level of baseline nausea (P = 0.09), and there was no impact on cardiovascular stability. Compliance appeared adequate at 91%, and deficiency was corrected in most (85%) of the subjects in the active treatment group.