Paper Title

Urinary angiotensinogen level is increased in preterm neonates

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Concise Title

Urinary angiotensinogen during renal development in neonates

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Abstract

Background

All components of the renin-angiotensin system (RAS) are abundantly synthesized in the developing kidney, suggesting that the RAS plays an important role in renal development. To examine this system in human neonates, we measured urinary angiotensinogen levels in preterm and full-term neonates, and examined the relationship between urinary angiotensinogen levels and gestational age.

Methods

Urine and plasma samples were collected from 20 preterm and 18 full-term neonates at birth. Angiotensinogen levels were measured using enzyme-linked immunosorbent assay.

Results

Plasma angiotensinogen concentrations were not increased in preterm neonates compared to that in full-term neonates (P = 0.7288). However, the urinary angiotensinogen-to-creatinine ratio was significantly higher in preterm neonates compared to that in full-term neonates (P = 0.0011). Importantly, the urinary angiotensinogen-to-creatinine ratio dropped significantly with increasing gestational age (P = 0.0010), whereas the plasma angiotensinogen concentration was not correlated with gestational age (P = 0.7814).

Conclusions

These results suggest that urinary angiotensinogen levels may indicate the involvement of intrarenal RAS activation in prenatal renal development.

Keywords

Renin-angiotensin system; neonates; renal development; gestational age; preterm

Introduction

The role of the renin-angiotensin system (RAS) in blood pressure regulation and sodium and fluid homeostasis is well recognized. The focus of interest regarding the RAS has shifted to the investigation of its role in specific tissues [1, 2]. The mechanism of the tissue RAS in the kidney is unique because the components necessary to generate Ang II are produced within the kidney [3]. Locally produced Ang II induces inflammation, cell growth mitogenesis, apoptosis, migration, and differentiation, regulates the gene expression of bioactive substances, and activates multiple intracellular signaling pathways [4].

A large number of studies have shown that the RAS plays a key role in the development of the mammalian kidney [5]. Recent studies have addressed the importance of an intact RAS cascade during postnatal kidney development using both pharmacological inhibition and genetic deletion of various RAS components. The mechanisms by which RAS supports renal development are not fully understood, but the temporal and spatial expression of different components of the system suggests a direct action on receptors expressed in the developing structures of the immature kidneys [5].

Angiotensinogen (AGT) is the only known substrate for renin that is a rate-limiting enzyme of the RAS. Recent studies showed that urinary excretion rates of AGT provided a specific index of intrarenal RAS status [6, 7]. A direct quantitative method for measuring urinary AGT using human AGT enzyme-linked immunosorbent assays (ELISA) has been developed [7]. This method demonstrated significantly increased urinary AGT levels in pediatric chronic glomerulonephritis patients who were not treated with RAS blockers compared to those in control subjects [8]. Moreover, the urinary AGT level was significantly higher in pediatric patients with type 1 diabetes in the pre-microalbuminuric phase compared to that in control subjects [9]. However, urinary AGT level in neonatal period is not yet completely documented. These data prompted us to measure urinary AGT

in neonates and investigate correlations with clinical parameters. Therefore, this study was performed to test the hypothesis that urinary AGT is a biomarker of intrarenal RAS status during renal development.

Methods

Patients and Urine Samples

The study's experimental protocol was approved by the Institutional Review Board of Tokushima University (approval number; 1425). Study participants were recruited in Tokushima University between April 1, 2012 and March 31, 2013. Neonates were excluded if they had known or suspected sepsis, severe respiratory distress syndrome, congenital heart disease, or a renal or chromosomal abnormality. Neonates expected to die within 48 hours of recruitment were also excluded. Informed consent was obtained from the parents. Demographic-perinatal characteristics including gestational age (GA), birth weight, mode of delivery, sex, and Apgar scores at 1 and 5 min were recorded for all neonates. A random spot urine and a blood samples were obtained in the few days following birth. Urine samples for analysis were obtained by collecting the urine into urine bags. The urine was stored at −20°C until biochemical analysis.

Measurements

Urinary concentrations of creatinine were measured using the Creatinine Assay Kit (BioVision, Milpitas, CA). Plasma and urinary concentrations of AGT were measured using commercially available ELISA kits (IBL, Takasaki, Gunma, Japan), as previously described [8]. The sensitivity of this assay is >0.31 ng/mL. The intra- and inter-assay coefficients of variation were 4.4% and 4.3%, respectively. The urinary level of AGT was expressed in mg/g creatinine (Cr). Plasma Cystatin C measurements were performed by ELISA

according to the manufacturer instruction (R&D, Minneapolis, MN), with intra- and inter-assay coefficients of variation <5.9%.

Statistical Analysis

Pearson correlation coefficients and Spearman correlation coefficients were used for parametric data and nonparametric data, respectively. Unpaired *t* test was performed to determine the group means. All data are presented as means ± standard error of mean (SEM). A P-value <0.05 was considered statistically significant. All computations, including data management and statistical analyses, were performed using JMP software (SAS Institute, Candler, NC) and GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Subject Profiles

The profiles of the study participants are summarized in Table 1. A total of 38 neonates, including 20 preterm and 18 full-term neonates, were recruited. The average GAs of preterm and full-term neonates were 32.0 ± 0.6 weeks and 37.7 ± 0.2 weeks, respectively. Preterm and full-term neonates weighed 1,757 ± 120 g and 2,611 ± 131 g, respectively. There were no significant differences between preterm and full-term neonates in delivery process, gender, Apgar score at 5 min, the days of sampling after birth, and plasma Cystatin C level. The average Apgar score at 1 min was significantly lower in preterm than in full-term neonates.

Urinary and Plasma AGT Levels in Preterm Neonates

The urinary AGT-to-creatinine ratio (Figure 1A) was significantly increased in preterm

neonates (3.58 ± 0.57 mg/g Cr) compared to that in full-term neonates (1.26 ± 0.26, P = 0.0011). However, an increase in plasma AGT levels was not observed (Figure 1B).

Single Regression Analysis

The urinary AGT-to-creatinine ratio (Figure 2A) was significantly inversely correlated with gestational age (r = 0.5115, P = 0.0010). However, the plasma AGT level (Figure 2B) was not correlated with gestational age (r = 0.0465, P = 0.7814).

Discussion

We compared the urinary AGT-to-creatinine ratio between preterm and full-term neonates. The urinary AGT-to-creatinine ratio was significantly higher in preterm neonates compared to that in full-term neonates. Notably, an increase in plasma AGT levels was not observed. Furthermore, the urinary AGT-to-creatinine ratio was inversely correlated with the gestational age. These data suggest that intrarenal RAS activation leads to renal development in the earlier gestation period and that increased urinary AGT levels in preterm neonates reflect this activation.

In human embryos, all components of the RAS are expressed in the kidney at as early as 5 weeks of gestation [10, 11]. Their production is precisely time-regulated, suggesting that Ang II could also exert its effects as a growth-promoting agent during kidney development [12] and levels of circulating renin and Ang II are higher during fetal life than during postnatal life [11]. During the gestation, the RAS of the fetal lamb responds to the same stimuli, such as blood volume depletion, furosemide, hypoxemia, and RAS blockade [13, 14]. In the same way, human fetuses exposed in utero to RAS blockers are severely hypotensive at birth, and sometimes develop irreversible renal lesions responsible for renal failure and anuria [15, 16]. On the other hand, inappropriate activation of the RAS during fetal life may have

deleterious consequences [17]. Thus, RAS plays an important role in kidney development.

Recently, multiple studies resulted in the accumulation of evidence indicating that urinary AGT can be a useful tool for identifying the activated intrarenal RAS in hypertension, diabetic nephropathy, and chronic kidney disease [2, 18]. We previously reported that urinary AGT levels are increased in pediatric patients with chronic glomerulonephritis and that treatment with RAS blockers suppressed urinary AGT levels [8], and increased urinary AGT levels precede increased urinary albumin levels and may be one of the earliest predictors of intrarenal RAS activation in juvenile type 1 diabetes [9]. These findings suggest that urinary AGT can be a novel biomarker of intrarenal RAS activation in pediatric patients. These data along with the present study suggest that urinary AGT levels may reflect intrarenal RAS activation associated with kidney development in neonates.

Although most circulating AGT is produced and secreted by the liver, the AGT produced in proximal tubule cells appears to be secreted directly into the tubular lumen, in addition to producing its metabolites intracellularly and secreting them into the tubular lumen [19]. Proximal tubular AGT concentrations in anesthetized rats have been reported to be in the range of 300–600 nM, which greatly exceeds free Ang I and Ang II tubular fluid concentrations [3]. Because of its molecular size (50–60 kDa), it seems unlikely that much of the plasma AGT filters across the glomerular membrane, further supporting the concept that proximal tubular cells secrete AGT directly into the tubules [20]. To determine if circulating AGT is a source of urinary AGT, the glomerular permeability of AGT was examined by multiphoton imaging [21]. The glomerular permeability of injected exogenous AGT was extremely low. By contrast, urinary excretion of endogenous AGT was significantly increased. These results indicate that the majority of urinary AGT originates from the kidney rather than from glomerular filtration [21]. Consistent with this concept, plasma AGT levels did not differ between preterm and full-term neonates despite significant

between-group differences in urinary AGT levels in the present study. Therefore, it seems highly likely that AGT in urine originates from AGT in the kidney, not from AGT in plasma. Taken together, our findings reveal physiologically significance that measuring urinary AGT in neonate is noninvasive and useful tool for the evaluation of RAS activation during kidney development independent of systemic RAS.

The relatively small sample size in this study is a potential limitation. Furthermore, the study was cross-sectional, and therefore it might be difficult to draw any causal conclusion. However, our observation demonstrates that levels of urinary AGT are increased in preterm neonates compared to that in full-term neonates, suggesting intrarenal RAS activation during kidney development. This pilot study triggered new insight into the mechanism of kidney development that requires further prospective investigation in larger multicenter studies.

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Conflict of interest

The authors declare no conflict of interests.

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Figure Legends

Figure 1.

Urinary AGT-to-creatinine ratio (Urinary AGT/Cre) and plasma AGT level in preterm and full-term neonates. The urinary AGT/Cre was significantly greater in preterm neonates compared to that in full-term neonates (A). Importantly, there was no difference in plasma AGT levels between these groups (B). Line at mean with SEM.

Figure 2.

Single regression analyses for urinary AGT-to-creatinine ratio (Urinary AGT/Cre) with gestational age (A) and plasma AGT levels with gestational age (B). The urinary AGT/Cre showed inverse correlations with gestational age, but plasma AGT levels did not. Two curves (dashed) surrounding linear regression line (solid) define the 95% confidence interval.

Figure
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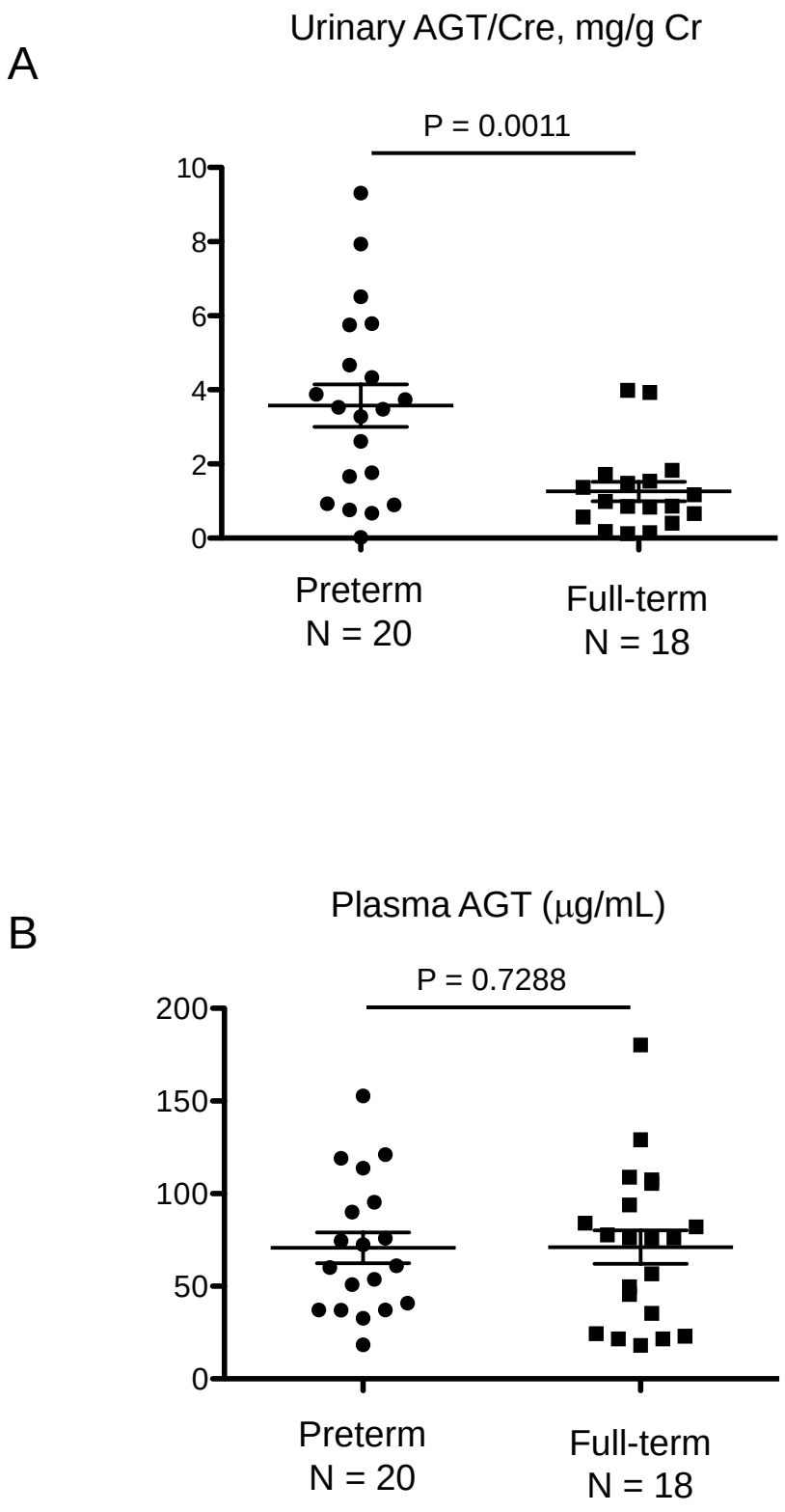
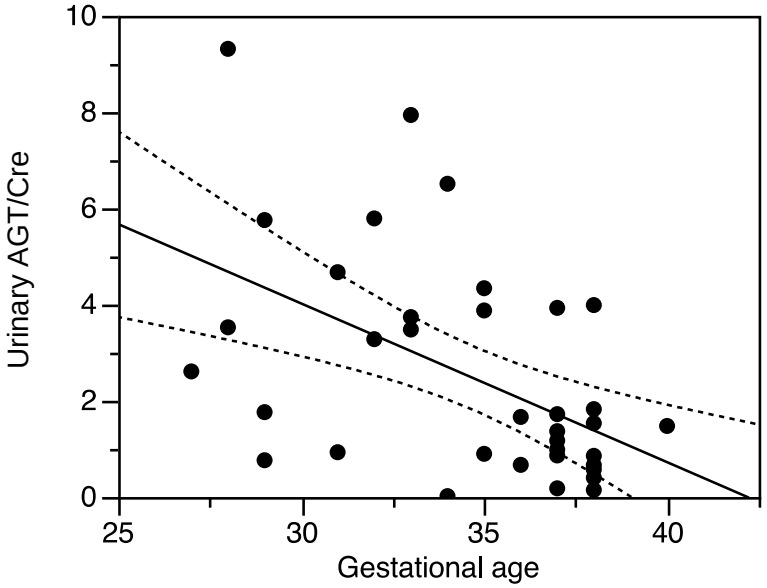


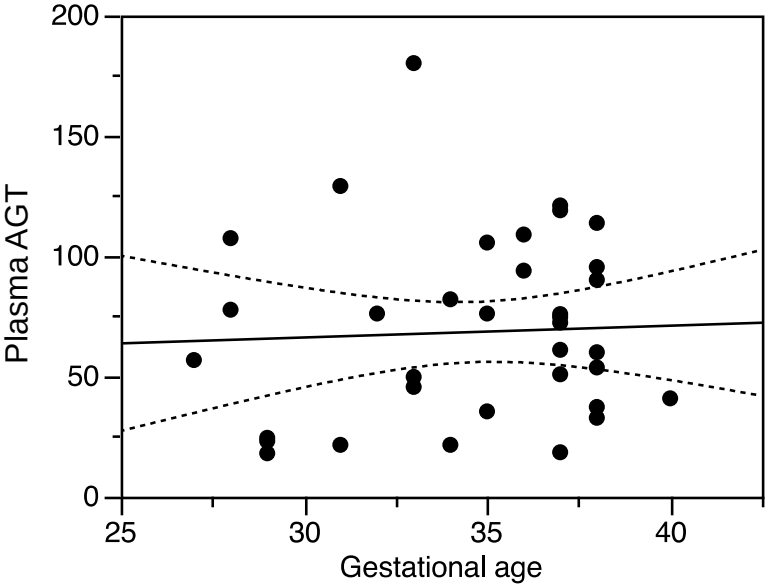
Figure 1

A



Urinary AGT/Cre = 13.91 – 0.33 Gestational age
r = 0.5115, P = 0.0010

B



Plasma AGT = 51.54 + 0.49 Gestational age
r = 0.0465, P = 0.7814

Figure 2

Table

Table 1. Subject profiles

Parameters	Preterm N = 20	Full-term N = 18	P values	$\chi 2$
Gestational age, weeks	32.0 +/- 0.6 **	37.7 +/- 0.2	< 0.01	
Birth weight, g	1,757 +/- 120 **	2,611 +/- 131	< 0.01	
Caesarean/vaginal delivery	10/10	8/10	0.73	0.12
Gender, F/M	9/11	7/11	0.70	0.15
Apgar score, 1 min	6.8 +/- 0.6 *	8.3 +/- 0.2	0.03	
Apgar score, 5 min	9.0 +/- 0.3	9.5 +/- 0.1	0.12	
Days of sampling after birth	2.5 +/- 0.3	2.0 +/- 0.3	0.25	
Plasma Cystatin C (mg/mL)	1.64 +/- 0.10	1.66 +/- 0.11	0.88	

F; Females, M; Males, *; $P < 0.05$, **; $P < 0.01$ vs. full-term.