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Original Article

Correlation among angiotensinogen level, renal function and renal injury in rats with chronic renal failure

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Abstract: Chronic renal failure is a common nephropathy. Renin-angiotensin-aldosterone system (RAS) participates in a blood pressure feedback control system. However, the relationship between RAS and renal injury in chronic renal failure remains unclear. This study analyzed the relationship between angiotensinogen (AGT) and renal function and injury. A rat chronic renal failure model was constructed. Renal pathology was observed under the microscope. Creatine (Cr), blood urea nitrogen (BUN), urine protein (UP), creatine clearance rate (Ccr), and glomerular filtration rate (GFR) levels were tested on automatic biochemistry analyzer. AGT and angiotensin II (Ang II) levels in blood and urine were examined by ELISA. Correlation was analyzed by Pearson correlation. Serum Scr, BUN, and UP levels were significantly higher, while Ccr and GFP levels were lower in the experimental group compared with controls. Their differences kept increasing following time extension ($P < 0.05$). Serum AGT and Ang II expressions were significantly lower, whereas urine AGT and Ang II levels were higher in the experimental group than that in the sham and control group, with time dependence ($P < 0.05$). Urine AGT was positively correlated with Scr, BUN, UP, Ccr, GFP, and serum and urine Ang II levels ($P < 0.05$). Renal morphology was abnormal in chronic renal failure rats with structural damage and renal dysfunction. In conclusion, abnormal AGT and Ang II levels are found in renal failure and urine AGT was positively correlated with renal function and Ang II, indicating that AGT might be a marker to evaluate renal injury in chronic renal failure.

Keywords: Chronic renal failure, angiotensinogen, renal function, renal injury

Introduction

Chronic renal failure is a clinical syndrome involving in multiple systems. It can be caused by a variety of kidney diseases, leading to persistent renal dysfunction, decline, metabolite accumulation, as well as water, electrolyte, and acid-base disturbance. Such a process is progressive and irreversible [1-3]. With the develpoment of our modern age, the incidence of chronic renal failure is also increasing which seriously affects the patient's quality of life and healthy.

The renin-angiotensin system (RAS) is involved in the whole development process of chronic renal failure. Specific RAS antagonism treatment, such as ACEI, is widely and effectively used to protect renal function and delay the process of renal insufficiency [4, 5]. As an important part of the RAS system, angiotensin (AGT) is the important and only substrate of renin. It is mainly synthetized in the liver. Therefore, circular AGT cannot be filtrated by the glomerulus but is generated by proximal renal tubular secretion, thus forming angiotensin II (Ang II) [6]. It is found that RAS can not only be produced by the endocrinium, but also derived from tissue. Organs contain multiple biological processes, including autocrine, paracrine, and intracrine functions. Thus, RAS can be found in the kidney [7]. Renin, synthetized by the special cells of afferent vessels, mainly exists in the juxtaglomerular apparatus cells in particle form. In addition to being intracellular, renin can be secreted to the circulation and from Ang II located in the renal vein, renal tissues, and juxtaglomerular apparatus [8, 9]. Nevertheless, renin can be regulated at several aspects, such as gene level, intracellular synthesis and processing, secretion, and metabolism velocity [10, 11]. Untransformed AGT can only be eliminated through the urine since it cannot be filtrated, secreted, and reabsorbed [12]. Numerous studies have focused on chronic renal failure. However, the relationship

between AGT level and renal injury caused by chronic renal failure has not been elucidated. This study aimed to investigate the relationship between AGT and renal function and injury by establishing a rat chronic renal failure model to uncover the role of AGT in renal injury in chronic renal failure.

Materials and methods

Experimental animals

A total of 90 SPF grade healthy Wistar rats aged 8-weeks and weighing 180±20 g were provided by the Laboratory Animal Center of Tianjin Medical University. There were of both males and females in each group.

Experimental drugs and instruments

AGT and Ang II ELISA kits were bought from Zhongshan Bio-tech co., Ltd.

Experimental methods

Establishment of a rat renal failure model: The rats were randomly and equally divided into three groups (30 per group, 15 male and 15 female), including an experimental group, sham group, and control. The rats in experimental group were anesthetized by 100 mg/kg pentobarbital intraperitoneal injection and positioned to expose the left kidney through the posterior peritoneum. A total of 2/3 of the renal tissue was resected and the bleeding was stopped by compression, fibrinogen, and thrombin. The right kidney was removed after one week. The rats in the sham group were treated with perirenal fat and renal fascia isolation instead of kidney resection. The rats in the normal control did not receive surgery.

Rats were used for all experiments and all procedures were approved by the Animal Ethics Committee of Tianjin Medical University.

Sample collection: The 24 h urine was collected to extract the supernatant for urine protein (UP) detection. The venous blood was extracted from the fundus venous plexus by a capillary tube. The kidney was extracted and washed with PBS at 4°C. Next, the tissue was cut into 0.5 cm × 0.5 cm for H&E staining or freezing at -80°C.

Renal morphology observation: The renal tissue was fixed in 10% formaldehyde and dehydrated. After being embedded in wax, the tissue was sliced at 3-4 μm for H&E staining. The sections were observed under the microscope.

Auto biochemical analysis: Urine and serum Scr, BUN, UP, Ccr, and GFR levels were tested on an auto biochemical analyzer.

ELISA: The venous blood was centrifuged to obtain the supernatant. The sample and diluted standard substance were added to the plate and incubated at 37°C for 30 min. After being washed five times, the plate was given 50 μl developer A and 50 μl developer B at 37°C in the dark for 15 min. Next, 50 μl stop buffer was added to the plate and measured at 450 nm.

Data analysis: The data were analyzed by using SPSS 17.0 software. The numeration data were compared by χ^2 test, while the measurement data were depicted as mean ± standard deviation and compared by ANOVA. $P < 0.05$ was considered as statistical significance.

Results

Renal morphology observation

The rats in the control group showed normal kidney and related structure without inflammatory cell infiltration. The rats in the experimental group exhibited light pink kidney tissue with volume increase, peripheral tissue adhesion, and thrombosis. Glomerulus reduction, mesangial matrix hyperplasia, sacculus adhesion, renal tubule atrophy, epithelial cell degeneration, and lumen expansion were all observed. Protein cast was found. A large amount of inflammatory cells infiltrated in the renal interstitium.

The rats in the sham group showed dark red kidney with normal glomerulus. No basement membrane thickening or sacculus adhesion was observed. Renal tubule demonstrated clear structure without atrophy. Only mild inflammatory cell infiltration was found (**Figure 1**).

Comparison of Scr, BUN, UP, Ccr, and GFR levels

To investigate the level of renal functional indicators, we tested serum Scr, BUN, UP, Ccr, and

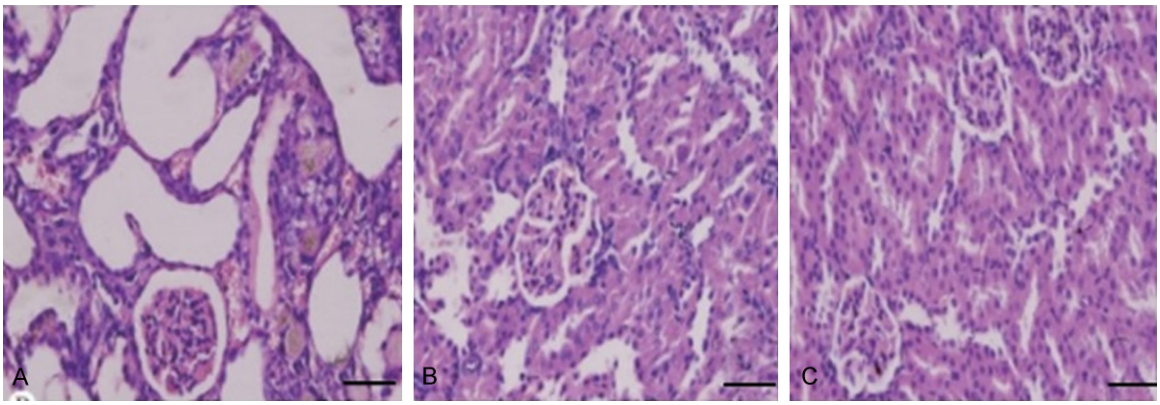

Figure 1. Renal morphology observation under the microscope. A. Experimental group; B. Sham group; C. Control. Scale bar: 100 μm.

Table 1. Serum Scr, BUN, UP, Ccr, and GFR levels comparison

Groups	Cases	Scr (×10^9/L)	BUN (g/L)	UP (mmol/L)	Ccr (μmol/L)	GFR (ml/min)
Experimental group	30					
2 weeks		51.9±5.1$^{*,\#}$	8.7±1.4$^{*,\#}$	12.3±1.1$^{*,\#}$	0.86±0.1$^{*,\#}$	18.7±0.9$^{*,\#}$
4 weeks		72.1±6.2$^{*,\#,\&}$	10.3±1.7$^{*,\#,\&}$	22.6±3.1$^{*,\#,\&}$	0.72±0.1$^{*,\#,\&}$	16.1±0.5$^{*,\#,\&}$
8 weeks		88.1±6.4$^{*,\#,\&,@}$	11.8±1.6$^{*,\#,\&,@}$	37.5±6.5$^{*,\#,\&,@}$	0.59±0.1$^{*,\#,\&,@}$	15.2±0.2$^{*,\#,\&,@}$
Sham group	30					
2 weeks		5.9±0.4	5.1±1.4	16.7±2.1	1.14±0.2	99.7±1.2
4 weeks		6.1±0.2	4.9±1.8	17.1±1.2	1.21±0.1	98.2±1.8
8 weeks		6.3±0.1	5.2±1.0	16.8±1.8	1.17±0.1	99.2±1.5
Control	30					
2 weeks		4.6±0.2	4.8±1.1	17.1±2.2	1.17±0.2	100.2±1.3
4 weeks		4.5±0.3	4.6±1.3	16.8±2.5	1.21±0.1	100.8±1.1
8 weeks		4.7±0.1	4.5±1.5	17.3±1.6	1.22±0.1	100.6±1.0

$^*P < 0.05$, compared with sham group; $^\#P < 0.05$, compared with control; $^\&P < 0.05$, compared with 2 weeks; $^@P < 0.05$, compared with 4 weeks.

GFR levels. It was found that serum Scr, BUN, and UP levels were significantly higher, while Ccr and GFR levels were significantly lower at 2 weeks after modeling compared with sham group and controls ($P < 0.05$). Their differences kept increasing following time extension ($P < 0.05$). No statistical difference was observed in serum Scr, BUN, UP, Ccr, and GFR levels between the sham group and control ($P > 0.05$) (**Table 1**).

Comparison of serum and urine AGT and Ang II

Next, we tested serum and urine AGT and Ang II levels. It was shown that serum AGT and Ang II levels were significantly lower, whereas urine AGT and Ang II levels were significantly higher in the experimental group than that in the sham and control group, with time dependence ($P < 0.05$). No statistical difference was observed in serum and urine AGT and Ang II levels between the sham group and controls ($P > 0.05$) (**Table 2**).

The relationship of serum and urine AGT with Scr, BUN, UP, Ccr, GFR, and serum and urine Ang II

To analyze the relationship of serum and urine AGT with Scr, BUN, UP, Ccr, GFR, and serum and urine Ang II, we selected the data at 8 weeks after modeling from the experimental group. It was revealed that serum AGT exhibited no correlation with serum Scr, BUN, UP, Ccr, GFR, and serum and urine Ang II ($P > 0.05$). On the contrary, urine AGT was positively correlated with Scr, BUN, UP, Ccr, GFP, and serum and urine Ang II levels ($P < 0.05$) (**Table 3**).

Table 2. Serum and urine AGT and Ang II comparison

Group	Cases	AGT		Ang II	
		Serum	Urine	Serum	Urine
Experimental group	30				
2 weeks		3.32±0.56$^{*,\#}$	16.42±11.26$^{*,\#}$	11.42±6.02$^{*,\#}$	54.39±31.67$^{*,\#}$
4 weeks		2.73±0.41$^{*,\#,\&}$	18.94±13.02$^{*,\#,\&}$	8.33±5.16$^{*,\#,\&}$	63.23±36.02$^{*,\#,\&}$
8 weeks		2.01±0.23$^{*,\#,\&,@}$	20.05±15.14$^{*,\#,\&,@}$	6.47±5.78$^{*,\#,\&,@}$	70.17±39.22$^{*,\#,\&,@}$
Sham group	30				
2 weeks		5.78±4.19	8.53±1.52	17.67±4.11	15.28±5.08
4 weeks		5.02±3.73	9.02±1.66	17.33±3.02	15.34±5.23
8 weeks		4.21±2.79	9.07±1.78	17.02±2.98	15.27±5.45
Control	30				
2 weeks		5.92±4.21	8.47±1.34	21.02±4.78	15.36±5.44
4 weeks		5.87±4.34	8.51±1.41	21.27±4.81	15.01±5.27
8 weeks		5.79±4.42	8.57±1.45	21.33±4.92	15.41±5.31

$^{*}P < 0.05$, compared with sham group; $^{\#}P < 0.05$, compared with control; $^{\&}P < 0.05$, compared with 2 weeks; $^{@}P < 0.05$, compared with 4 weeks.

Table 3. The relationship of serum and urine AGT with Scr, BUN, UP, Ccr, GFR, and serum and urine Ang II

Items	Serum AGT		Urine AGT	
	r	P	r	P
Scr	-0.105	> 0.05	0.415	< 0.05
BUN	-0.306	> 0.05	0.408	< 0.05
UP	-0.171	> 0.05	-0.667	< 0.05
Ccr	-0.078	> 0.05	0.497	< 0.05
GFR	0.039	> 0.05	0.449	< 0.05
Serum Ang II	0.044	> 0.05	0.427	< 0.05
Urine Ang II	0.042	> 0.05	0.455	< 0.05

Discussion

RAS is a blood pressure feedback control system with complex processes. As one of the important influencing factors, RAS activation participates in the occurrence and development of chronic renal failure [13]. Classic Ang II has many biological functions which are mediated by AT1 receptor, including the regulation of blood vessel contraction, the release of aldosterone, and promoting inflammation reaction, etc. The content of AT1 receptor can indirectly reflect the Ang II level [14]. Recent study suggested that RAS in the kidney does not depend on the circulating RAS regulation [15]. This study analyzed the relationship between AGT and renal function and injury by establishing a rat chronic renal failure model to detect renal function indicators, serum and urine AGT and Ang II.

In this study, the rat chronic renal failure model was treated as the experimental group to observe the morphology changes of kidney. The rats in the experimental group exhibited light pink kidney tissue with a volume increase, peripheral tissue adhesion, and thrombosis. Glomerulus reduction, mesangial matrix hyperplasia, sacculus adhesion, renal tubule atrophy, epithelial cell degeneration, and lumen expansion were observed. A large amount of inflammatory cells infiltrated in the renal interstitium. The rats in the sham group showed dark red kidney tissue with normal glomerulus. No basement membrane thickening or sacculus adhesion was observed. Renal tubule demonstrated clear structure without atrophy. Only a few inflammatory cells infiltrated. The rats in the control group showed normal kidney tissue and related structure without inflammatory cell infiltration. To investigate the level of renal functional indicators, we tested serum Scr, BUN, UP, Ccr, and GFR levels and found that serum Scr, BUN, and UP levels were significantly higher, while Ccr and GFR expressions were significantly lower at 2 weeks after modeling compared with the sham group and controls. Their differences kept increasing following time extension, revealing abnormal renal morphology and declined renal function in the chronic renal failure rats.

Next, we tested serum and urine AGT and Ang II levels. We show that serum AGT and Ang II levels were significantly lower, whereas urine AGT and Ang II levels were significantly higher in the

experimental group than that in the sham and control groups, with time dependence. This indicated that serum AGT and Ang II levels were reduced, while urine AGT and Ang II levels were increased in chronic renal failure rats. AGT is a necessary substance and part of the reaction to synthetize Ang peptide. As glycosylation globulin, AGT is mainly synthetized in the liver. It cannot pass the glomerular filtration barrier, thus urine AGT level is not related to circular AGT [16]. AGT can form Ang II under the effect of renin. However, not all the AGT in the serum and urine are involved in this process, leading to elimination of part of untransformed AGT with urine [17, 18].

Through analysis of the relationship of serum and urine AGT with Scr, BUN, UP, Ccr, GFR, and serum and urine Ang II, we found that serum AGT exhibited no correlation with serum Scr, BUN, UP, Ccr, GFR, and serum and urine Ang II. Whereas, urine AGT was positively correlated with Scr, BUN, UP, Ccr, GFP, and serum and urine Ang II levels. It has been shown that urine AGT level is a special noninvasive indicator reflecting the changes of renal function [19]. Pathology investigation revealed the positive correlation between urine AGT level and urine type IV collagen content in chronic kidney disease. It is suggested that urine type IV collagen is an important indicator to reflect the severity of renal fibrosis. Therefore, urine AGT not only reflects renal fibrosis, but is also related to the extent of chronic kidney injury [20].

Conclusion

Serum AGT and Ang II levels are significantly lower, while urine AGT and Ang II levels are significantly higher in chronic renal failure rats. Urine AGT was positively correlated with Scr, BUN, UP, Ccr, GFP, and serum and urine Ang II levels, indicating that AGT might be used a reliable marker to evaluate renal injury in chronic renal failure.

Disclosure of conflict of interest

None.

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