Published in final edited form as:

J Investig Med. 2013 February ; 61(2): 256–264. doi:10.231/JIM.0b013e31827c28bb.

Cardinal Role of the Intrarenal Renin-Angiotensin System in the Pathogenesis of Diabetic Nephropathy

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Abstract

Diabetes mellitus is one of the most prevalent diseases and is associated with increased incidence of structural and functional derangements in the kidneys, eventually leading to end-stage renal disease in a significant fraction of afflicted individuals. The renoprotective effects of renin-angiotensin system (RAS) blockade have been established; however, the mechanistic pathways have not been fully elucidated. In this review article, the cardinal role of an activated RAS in the pathogenesis of diabetic nephropathy is discussed with a focus on 4 themes: 1) Introduction to RAS cascade, 2) Intrarenal RAS in diabetes, 3) Clinical outcomes of RAS blockade in diabetic nephropathy, and 4) Potential of urinary angiotensinogen (AGT) as an early biomarker of intrarenal RAS status in diabetic nephropathy. This review article provides a mechanistic rationale supporting the hypothesis that an activated intrarenal RAS contributes to the pathogenesis of diabetic nephropathy, and that urinary AGT levels provide an index of intrarenal RAS activity.

Keywords

diabetic nephropathy; renin-angiotensin system; angiotensinogen; kidney

Diabetes affects 220 million people worldwide, including 24 million Americans, and is the 6th leading cause of death in the US. It is associated with increased incidence of functional and structural alterations in the kidneys, eventually leading to end-stage renal failure in many patients. Diabetic nephropathy (DN) is the most common cause of end-stage renal failure in the US, accounting for 45% of patients starting dialysis ^{1, 2}. Type 2 diabetes mellitus (T2D) is the most common type of diabetes accounting for 90–95% of all diagnosed cases of diabetes and affecting 8% of the US population ^{3, 4}. Obesity has been identified as the principal risk factor associated with the rising prevalence of T2D ⁵. The epidemic proportions of obesity and diabetes justify the enormous effort to identify novel pathways and mechanisms involved in their prevention and treatment. Diabetes is a chronic and debilitating disease that is characterized by progressive albuminuria, declining glomerular

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filtration rate (GFR), functional and structural deterioration of the kidney, and increased risk of cardiovascular disease.

RENIN-ANGIOTENSIN SYSTEM (RAS) CASCADE

The importance of the RAS in the regulation of blood pressure (BP) and fluid and electrolyte homeostasis has been well recognized ^{6, 7}. As indicated in Figure 1, the balance between vasoconstrictor and vasodilator effects is determined by the actions of angiotensin II and angiotensin 1–7. The formation of angiotensin II is dependent upon the substrate availability of AGT, angiotensin I and the activities of renin, angiotensin converting enzyme (ACE), ACE2, and ACE-independent enzymatic pathways including serine proteases such as chymase. Angiotensin 1–7 can be formed directly from angiotensin II hydrolyzed by ACE2 or indirectly from angiotensin I via an intermediate step of the formation of angiotensin 1–9 hydrolyzed by ACE2 and ACE in sequence. The actions of angiotensin II are determined by signaling via angiotensin II type 1 (AT1) and type 2 (AT2) receptors ⁸ and the putative angiotensin 1–7 receptor, Mas ^{9, 10}.

INTRARENAL RAS IN DIABETES

Emerging evidence has demonstrated the importance of local RAS ¹¹ in the brain ¹², heart ¹³, adrenal glands ¹⁴, vasculature ^{15, 16}, and kidneys ^{6–8, 17}. In particular, the renal RAS is unique because all of the components necessary to generate intrarenal angiotensin II are present along the nephron in both interstitial and intratubular compartments (Figure 2) ^{7, 10}. AGT has been localized primarily at the mRNA level ¹⁸, and immunoreactive AGT ⁷ has been found in the proximal tubules. Detailed localization of the AGT in the proximal tubular segments was controversial, however, divergent localization of AGT mRNA and protein was reported recently ^{19, 20}. The proximal convoluted tubules and proximal straight tubules exhibit positive immunostaining for AGT (Figure 3). Furthermore, weak expression of AGT protein was also observed in glomeruli and vasa recta, whereas the distal tubules and collecting ducts are negative ^{21–25}. AGT mRNA is found strongly in the proximal straight tubules. Recent evidence suggests that AGT is constitutively secreted in the proximal straight tubule as in the liver ²⁶. Renin mRNA and renin-like activity have been demonstrated in cultured proximal tubular cells, and low concentrations of renin have been detected in proximal tubule fluid in rats ^{27–30}. Moreover, there is abundant expression of ACE mRNA ³¹ and protein ^{32, 33} on brush border membranes of proximal tubules of human kidney. Finally, ACE is also present in proximal and distal tubular fluid but is greater in proximal tubule fluid ³⁴. ACE2 protein is found in proximal tubule cells, glomerular podocytes ³⁵, and tunica media of renal arterioles ³⁶. In addition, experimental studies have shown that intrarenal ACE-independent, serine-protease-dependent pathways have an increased role in the conversion of angiotensin I to angiotensin II in diabetic models, thus influencing renal hemodynamics ³⁷.

Data concerning intrarenal RAS states in diabetes are inconsistent ^{38–40}. Although various studies support an association between RAS and DN, direct measurements have failed to establish that intrarenal angiotensin II is consistently elevated in diabetes ⁴⁰. However, intrarenal AGT levels are elevated in patients with DN ⁴¹. In rodent diabetic models, renin content varies and ACE expression has been shown to be increased or unchanged in glomeruli and vessels ^{39, 42}. In the T2D mouse kidney, proximal tubule ACE immunostaining is decreased, while ACE2 immunostaining is increased compared to control mice (Figure 3) ³⁷. However, AT1 receptor protein levels were significantly elevated in renal cortex from streptozotocin-induced diabetic rats compared with control rats associated with downregulation of AT2 receptors ^{39, 42}. The cortical collecting ducts of streptozotocin-induced diabetic kidneys displayed a striking increase in AT1 receptor immunostaining

intensity relative to control kidneys (Figure 3)³⁹. Moreover, it was recently shown that prorenin expression is elevated in the cortical collecting ducts of type 1 diabetic (T1D) rats⁴³. Furthermore, studies in models of T2D show increased intrarenal angiotensin II levels and AGT mRNA levels which are prevented by treatment with an angiotensin II receptor blocker (ARB)⁴⁴. Finally, increases in renal cortical AGT (Figure 3) and angiotensin II levels associated with increased reactive oxygen species (ROS) and renal injury have been observed in Zucker diabetic fatty obese rats compared to control lean rats^{46, 47}.

Recent studies have identified a major role for intrarenal ACE-independent formation of angiotensin II in T2D. Park et al.³⁷ reported that afferent arteriole vasoconstriction in control kidneys that is produced by angiotensin I was significantly attenuated by ACE inhibition, but not by serine protease inhibition. In contrast, afferent arteriole vasoconstriction produced by the intrarenal conversion of angiotensin I to angiotensin II was significantly attenuated by serine protease inhibition, but not by ACE inhibition in diabetic kidneys³⁷. Therefore, there appears to be a switch from ACE-dependent to serine protease-dependent angiotensin II formation in the T2D kidney. It has been suggested that chymase may be responsible for serine protease-dependent angiotensin II formation in the diabetic kidney⁴⁸. It is plausible that pharmacological targeting of these serine protease-dependent pathways may provide further protection from diabetic renal vascular disease.

CLINICAL OUTCOMES FOR RAS BLOCKADE IN DN

ARBs and ACE inhibitors retard the development and progression of renal dysfunction in human studies^{49–52}. Table 1 summarizes major clinical trials concerning the effects of RAS inhibition in the development and progression of renal dysfunction in both diabetic and non-diabetic renal disease. In the BENEDIC Trial⁵³, patients with diabetes with no history of microalbuminuria were randomized to trandolapril vs. placebo over median of 3.6 years follow up. Trandolapril resulted in a significant decrease in the development of microalbuminuria and limited the progression of renal dysfunction. This reduction was still significant even after adjusting for BP reduction by trandolapril. In the AASK trial⁵⁴, the reduction of microalbuminuria by ACE inhibitors was also shown in the non-diabetic renal population in which patients were randomized to ramipril vs. amlodipine. The benefit of ARB therapy in patients with DN has been studied in 2 large trials. In the RENAAL trial⁵⁵ and the IDN Trial⁵⁶, evaluating patients with T2D with nephropathy, the addition of ARB to standard therapy resulted in improvements in all causes of mortality, progression to end stage renal disease, and doubling of serum creatinine. In the first direct comparison of ARB with an ACE inhibitor, the DETAIL trial⁵⁷ evaluated patients with T2D randomized to either enalapril or telmisartan. Telmisartan was not inferior to enalapril in the primary end point of change in baseline estimated GFR. Recently, the ONTARGET Trial⁵⁸ showed that ARBs and ACE inhibitors were equally effective in improving renal outcome (dialysis, doubling of serum creatinine, and death) and the number of events for the composite outcome was similar for telmisartan and ramipril. In the dual therapy group, even though there was a significant reduction in proteinuria, there was an increase in side effects and worsening renal outcomes. The beneficial effect of ARB was also confirmed in a most recent mega-trial^{59,60}. The ROADMAP trial is a randomized, double-blind, multicenter study conducted in Europe, including 4,447 patients with diabetes and at least one additional cardiovascular risk factor, but no evidence of renal dysfunction. The participants were randomized to receive either olmesartan at 40 mg/day (N = 2,232) or placebo (N = 2,215), and all were allowed to take additional non-RAS antihypertensives to reach target BP (<130/80 mm Hg), until the predefined number of adjudicated microalbuminuria events occurred at a median follow-up of 3.2 years. The primary end point was time to onset of albuminuria. The results show there was a cumulative incidence of microalbuminuria of

8.2% with olmesartan and 9.8% with placebo; the primary end point, time to onset of microalbuminuria, was delayed by 23% with olmesartan (hazard ratio 0.77, $P = 0.01$), with the majority of this effect being BP independent. What is missing, however, is an actual marker to test for the efficacy of treatment. If indeed, the major factor initiating the DN is an inappropriate increase in intrarenal RAS, then it would seem particularly worthwhile to have a direct means of evaluating the status of the intrarenal RAS in patients and the efficacy of treatment in reducing or arresting RAS activation in the kidneys.

URINARY AGT AS A NEW BIOMARKER OF INTRARENAL RAS STATUS IN DIABETES

Clinically, microalbuminuria is the most commonly used early marker of DN⁶¹. DN is thought to be a unidirectional process from microalbuminuria to end-stage renal failure⁶². However, recent studies demonstrate that a large proportion of DN patients revert to normoalbuminuria and that one-third of them exhibit reduced renal function even in the microalbuminuria stage⁶³. It is claimed that urinary inflammatory markers are high in microalbuminuric T1D having diminished renal function, but not in microalbuminuric T1D patients with stable renal function. However, no single marker has been sufficient to represent the whole panel⁶⁴. Therefore, a more sensitive and more specific marker for activation the RAS in DN would be highly advantageous.

AGT is the only known substrate for renin, which is the rate-limiting enzyme of the RAS. Because the level of AGT is close to the Michaelis-Menten constant for renin, not only renin levels but also AGT levels can control the activity of the RAS, and upregulation of AGT levels may lead to elevated angiotensin peptide levels^{65, 66}. Recent studies on experimental animal models and transgenic mice have documented the involvement of AGT in the activation of the RAS^{67–75}. Genetic manipulations that lead to overexpression of the AGT gene have consistently been shown to cause hypertension^{76, 77}. In human genetic studies, a linkage has been established between the AGT gene and hypertension^{78–81}. Enhanced intrarenal AGT mRNA and/or protein levels have also been observed in multiple experimental models of hypertension and diabetes including angiotensin II-dependent hypertensive rats^{25, 82–86}, Dahl salt-sensitive hypertensive rats^{87, 88}, and spontaneously hypertensive rats⁸⁹ as well as in kidney diseases including DN^{44, 46, 47, 90–92}, IgA nephropathy^{93, 94}, and radiation nephropathy⁹⁵. In addition, models of T1D and T2D and patients with metabolic syndrome also exhibit increases in intrarenal AGT and urinary AGT excretion^{44–47, 96, 97}. Thus, AGT plays an important role in the development and progression of hypertension and kidney diseases and may be particularly useful as a predictor of developing kidney disease^{7, 17}.

In rodents, urinary excretion rates of AGT provide a specific index of the intrarenal RAS status and are correlated with kidney angiotensin II levels in angiotensin II-dependent hypertensive rats (Figure 2)^{25, 83–86}. Because of its potential importance, a direct quantitative method to measure urinary AGT using human AGT ELISA was recently developed⁹⁸. Using this system, urinary excretion rates of AGT have been used as an index of intrarenal RAS status in patients with chronic kidney disease^{99–102} and in patients with hypertension^{103, 104}. Recently, 2 clinical studies showed the potential of urinary AGT levels as a novel biomarker of intrarenal RAS status in diabetes mellitus^{105, 106}.

To demonstrate that the administration of an ARB interferes with the vicious cycle of high glucose-ROS-AGT-angiotensin II-AT1 receptor-ROS by suppressing ROS and inflammation, 13 hypertensive DN patients who received ARBs were recruited and evaluated before and at 16 weeks after treatment¹⁰⁵. Urinary AGT, albumin, 8-hydroxydeoxyguanosine, 8-epi-prostaglandin F2 alpha, monocyte chemoattractant protein-1

(MCP-1), interleukin-6, and interleukin-10 were assessed. ARB treatment reduced the BP and urinary levels of AGT, albumin, 8-hydroxydeoxyguanosine, 8-epi-prostaglandin F2 alpha, MCP-1, and interleukin-6; while increasing urinary interleukin-10 levels. The reduction of urinary AGT correlated with the reduction of BP and urinary levels of albumin, 8-hydroxydeoxyguanosine, 8-epi-prostaglandin F2 alpha, MCP-1, and interleukin-6 and the increased urinary interleukin-10 levels. These results suggest that the mechanisms by which ARBs exert their renoprotective effect may involve the suppression of intrarenal AGT levels in association with reduced anti-inflammatory and anti-oxidant effects in patients with T2D (Figure 4) ¹⁰⁵.

To determine if urinary AGT levels can be dissociated from urinary albumin or protein excretion rates in T1D juveniles, early phase studies were performed in control and diabetic juveniles ¹⁰⁶. Of the 55 juveniles recruited, 34 were patients with T1D and 21 were gender- and age-matched control subjects. Since the primary focus of the study was comparison between characteristics of normoalbuminuric patients with T1D and those of control subjects, 6 microalbuminuric patients with T1D (urinary albumin-creatinine ratio > 30 mg/g) were excluded. Consequently, 49 urine and plasma samples were analyzed. None of them received treatment with RAS blockade. Neither urinary albumin-creatinine ratios nor urinary protein-creatinine ratios were significantly increased in these patients with T1D compared to control subjects, suggesting that these patients were in their pre-microalbuminuric phase of DN. However, urinary AGT-creatinine ratios were significantly increased in these patients compared to control subjects ($12.1 \pm 3.2 \mu\text{g/g}$ vs. $4.2 \pm 0.7 \mu\text{g/g}$, $P = 0.0454$). Importantly, the AGT increase was not observed in plasma ($26.3 \pm 1.3 \mu\text{g/ml}$ vs. $29.5 \pm 3.3 \mu\text{g/ml}$, $P = 0.3148$) (Figure 5). These data indicate that urinary AGT levels are increased in T1D subjects and that increased urinary AGT levels precede the increased urinary albumin levels, suggesting a possibility that urinary AGT levels serve as a very sensitive early marker of intrarenal RAS activation and may be one of the earliest predictors of DN in patients with diabetes ¹⁰⁶.

CONCLUSIONS

The complicated and pleiotropic actions of an activated RAS in pathogenesis of DN continue to receive recognition from emerging and ongoing studies. Clearly, the use of ARBs and ACE inhibitors has become common practice in treating patients with diabetes. Since RAS activation plays such a central role in the development and progression of DN, there has been extensive interest in the potential hope for reduction in morbidity and mortality by using agents that block one or more steps in the RAS. Accordingly, the assessment of urinary AGT as an early biomarker of the status of the intrarenal RAS may be of substantial importance. It may be particularly helpful in serving as a means to determine efficacy of the treatment to reduce intrarenal angiotensin II levels.

Acknowledgments

Sources of Funding

The authors' laboratories are supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK072408), National Center for Research Resources (P20RR017659), National Heart, Lung, and Blood Institute (R01HL026371), and by the American Heart Association Grant-in-Aid (GRNT2250875 & GRNT3020018).

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Figure 1.

Enzymes in the RAS cascade. Serine Proteases * includes kallikrein and tonin (a kallikrein like serine protease). Serine Proteases ** includes chymase and cathepsin G (a lysosomal enzyme with properties analogous to chymase and chymotrypsin).

Figure 2.

Intrarenal RAS components and urinary AGT as a potential biomarker for intrarenal RAS status. PT: proximal tubules, DT: distal tubules, CD: collecting ducts.

Figure 3.

A and B: ACE immunohistochemical localization of renal cortex of control (A) and T2D db/db (B) mice. ACE protein is localized to the proximal tubule brush border and endothelial cells of the renal vasculature and glomerular capillaries. ACE immunostaining is reduced in the diabetic kidney.

C and D: ACE2 immunohistochemical localization of renal cortex of control (C) and T2D db/db (D) mice. ACE2 protein is localized to the proximal tubule brush border and is increased in the diabetic kidney (from Park et al. ³⁷).

E and F: Representative light photomicrographs illustrating the immunohistochemical distribution of AGT in kidneys from control (E) and T2D rats (F). Prominent proximal tubular localization of AGT is found in the rat renal cortex. Distal tubules exhibit negative staining. AGT immunoreactivity was more intense in kidneys from diabetic rats (F) compared with control rats (E) (from Miyata et al. ⁴⁷).

G and H: Representative light photomicrographs illustrating the immunohistochemical distribution of AT1 receptors in kidneys from control (G) and T1D rats (H) utilizing a monoclonal antibody. In the cortex, immunostaining for AT1 receptor protein was evident in proximal tubules and cortical collecting ducts (asterisks), with fainter staining of glomeruli (g). AT1 receptor immunoreactivity was more intense in cortical collecting ducts in kidneys from diabetic rats (G) compared with control rats (H) (from Harrison-Bernard et al. ³⁹).

Scale bar, 100 μ m

Figure 4.

Correlations of urinary AGT with the reduction rates of BP (B) and urinary levels of albumin (A), 8-hydroxy-deoxyguanosine (C), and 8-epi-prostaglandin F₂ alpha (D) by treatment with ARB in patients with T2D (from Ogawa et al. ¹⁰⁵).

Figure 5.

Urinary albumin-creatinine ratio (A) or urinary protein-creatinine ratio (B) were not increased in these patients with T1D compared to control subjects, suggesting that these patients were in their pre-microalbuminuric phase of diabetic nephropathy. However, UAGT/UCre was significantly increased in these patients compared to control subjects (C). Importantly, the AGT increase was not observed in plasma (D) (from Saito et al. ¹⁰⁶).

Table 1

Clinical trials for RAS blockade in diabetic nephropathy

Trials	Sample Number (Total Number)	Subjects	Major Outcomes
BENEDICT (Bergamo Nephrologic Diabetes Complications Trial) ⁵³	1,204 subjects	Patients with diabetes with no history of microalbuminuria were randomized to trandolapril, 2 mg/day plus verapamil, 180 mg/day, trandolapril alone, 2 mg/day, verapamil alone, 240mg/day, or placebo.	In subjects with type 2 diabetes, the use of trandolapril plus verapamil and trandolapril alone decreased the incidence of microalbuminuria.
AASK (African American Study of Kidney Disease and Hypertension) ⁵⁴	1,094 subjects	Patients with hypertensive renal disease were randomly assigned to receive amlodipine, 5 to 10 mg/day, ramipril, 2.5 to 10 mg/day, or metoprolol, 50 to 200 mg/day, with other agents.	Ramipril, compared with amlodipine, retards renal disease progression in patients with hypertensive renal disease and proteinuria.
RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan Study) ⁵⁵	1,513 subjects	Patients with type 2 diabetes were randomly assigned to receive losartan, 50 to 100 mg, or placebo.	Losartan reduced doubling of serum creatinine concentration and end-stage renal disease in patients with type 2 diabetes.
IDNT (Irbesartan Diabetic Nephropathy Trial) ⁵⁶	1,715 subjects	Hypertensive patients with nephropathy due to type 2 diabetes were assigned to receive irbesartan, 300 mg/day, amlodipine, 10 mg/day, or placebo.	Irbesartan reduced doubling of serum creatinine concentration, end-stage renal disease, mortality in patients with type 2 diabetes.
DETAIL (Diabetics Exposed to Telmisartan And Enalapril) ⁵⁷	250 subjects	Patients with type 2 diabetes and early nephropathy were assigned to receive either telmisartan, 80 mg/day, in 120 subjects) or enalapril, 20 mg/day.	Telmisartan was not inferior to enalapril in the primary end point of change in baseline estimated GFR.
ONTARGET (Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial) ⁵⁸	25,620 subjects	Patients with established atherosclerotic vascular disease or with diabetes with end-organ damage were randomly assigned to ramipril, 10 mg/day, telmisartan 80 mg/day, or to a combination of both drugs.	Both ARBs and ACE inhibitors were equally effective in improving outcome (dialysis, doubling of serum creatinine, and death) and the number of events for the composite outcome was similar for telmisartan and ramipril. In the dual therapy group, even though there was a significant reduction in proteinuria, there was an increase in side effects and worsening renal outcomes.
ROADMAP (Randomized Olmesartan and Diabetes Microalbuminuria Prevention) ⁵⁹	4,447 subjects	Patients with type 2 diabetes were randomly assigned to receive olmesartan, 40 mg/day, or placebo. Additional antihypertensive drugs were used as needed to lower blood pressure to less than 130/80 mm Hg.	Olmesartan was associated with delayed onset of microalbuminuria, even though blood-pressure control in both groups was excellent according to current standards. The higher rate of fatal cardiovascular events with olmesartan among patients with preexisting coronary heart disease is of concern.