Renoprotective Effects of Direct Renin Inhibition in Glomerulonephritis

Kayoko Miyata, PhD, Ryousuke Satou, PhD, Daisuke Inui, MD, PhD, Akemi Katsurada, MS, Dale Seth, MS, Allison Davis, MS, Maki Urushihara, MD, PhD, Hiroyuki Kobori, MD, PhD, Kenneth D. Mitchell, PhD and L. Gabriel Navar, PhD

Abstract: The development of glomerulonephritis causes glomerular injury and renal dysfunction and is thought to increase renin release, thus activating the renin-angiotensin system (RAS). The aims of this study were to demonstrate activation of the intrarenal RAS and determine the effects of direct renin inhibition (DRI) on the progression of glomerulonephritis. Rats were treated with anti-Thy1.1 antibody with or without DRI, aliskiren (30 mg/kg/d). In the glomerulonephritic rats, protein, microalbumin excretion levels, urinary angiotensinogen excretion, glomerular expansion score and intrarenal transforming growth factor-β and plasminogen activator inhibitor-1 mRNA levels were augmented compared with control rats; however, hypertension was not observed in the glomerulonephritic rats, and aliskiren treatment did not modify their blood pressure. The increases in urinary protein (94.7 ± 13.0 mg/d) and microalbumin (7.52 ± 2.6 mg/d) excretion were reduced by aliskiren (43.6 ± 4.5 mg/d of protein and 2.57 ± 0.7 mg/d of microalbumin). Furthermore, the progression of glomerular expansion and elevation of intrarenal transforming growth factor-β and plasminogen activator inhibitor-1 levels were prevented by aliskiren. Importantly, aliskiren suppressed the augmentation of urinary angiotensinogen levels, the increased angiotensinogen expression in the kidneys and the increases in Ang II levels in renal medulla induced by the anti-Thy1.1 antibody. These results suggest that DRI with aliskiren prevents intrarenal RAS activation leading to mitigation of the development of glomerulonephritis. In addition, the renoprotective effects of DRI on glomerulonephritis occur in a blood pressure-independent manner. Accordingly, treatment with aliskiren may be an effective approach to treat glomerulonephritis and other intrarenal RAS-associated kidney diseases.

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The renin-angiotensin system (RAS) plays an important role in the development of hypertension and renal diseases.[1–8] Because components of the RAS are widely distributed in the brain,[9] heart,[10] adrenal glands,[11] vasculature and kidneys,[2,12,13] the focus of interest has shifted toward the pleiotropic roles of the local RAS activation.[3,14] The intrarenal RAS not only regulates blood pressure (BP) but also contributes to renal cell proliferation and the development of glomerulosclerosis and renal fibrosis.[15–17] Indeed, previous studies have shown that angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin (Ang) II type 1 receptor (AT1R) blockers have beneficial effects in rats and humans with various renal diseases, and these effects are often considerably stronger than their suppressive effects on BP.[18–20]

Chronic kidney diseases (CKD) result in substantial renal damage and are frequently characterized by progression to end-stage renal disease. This process typically involves glomerulosclerosis and interstitial fibrosis. The mechanisms contributing to the progression of renal damage are not fully understood; however, they may be distinct from those responsible for the initial injury.[3] Glomerular hypertension, cell hypertrophy and extracellular matrix accumulation, all appear to be involved in the progression of CKD. In addition, numerous studies have implicated Ang II in this process.[21,22] Intrarenal Ang II levels are enhanced in CKD, which can stimulate glomerular cell hypertrophy and augment extracellular matrix accumulation.[17,22,23] Blockers of AT1R or synthesis inhibitors mitigate and can even prevent the renal injury in experimental models with progressive CKD.[1,3,22,24] Studies on the efficacy of ACE inhibitors in patients with nondiabetic chronic renal failure indicate that these inhibitors have beneficial effects in preserving renal function.[25]

Various glomerulonephritis in humans including lupus nephritis, IgA nephropathy and Henoch-Schonlein purpura nephritis cause glomerular damage leading to renal dysfunction.[7] Anti-Thy1.1 glomerulonephritis is an established model of glomerulonephritis.[7,16,26] In this model, the immediate binding of the antibody to the antigen in mesangial cells activates severe immunomediated mesangiolysis. It has been demonstrated that treatment with AT1R blockers mitigates the progression of anti-Thy1.1 glomerulonephritis in rats.[7,16,27–29] Obvious mesangial cell proliferation, marked extracellular matrix accumulation, adhesion to Bowman's capsule, glomerulosclerosis and tubulointerstitial fibrosis are observed in the anti-Thy1.1 glomerulonephritis. These pathological changes were less severe in rats chronically treated with ACE inhibitors or AT1R blockers.[16,27–29] These findings indicate that activation of the intrarenal RAS plays a pivotal role in the progression of glomerulonephritis.

The development of renin inhibitors has provided an opportunity to evaluate the effects of direct renin inhibition (DRI) as another means of RAS blockade. Aliskiren is the main inhibitor currently available.[30–32] The direct administration of aliskiren to kidneys by using a collagen matrix was shown to mitigate anti-Thy1.1 antibody-induced glomerulonephritis[33]; however, the effects of aliskiren on intrarenal RAS activity

From the Department of Physiology and the Hypertension and Renal Center of Excellence, Tulane University Health Sciences Center, New Orleans, Louisiana.

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M. Urushihara is now with the Department of Pediatrics, Institute of Health Biosciences at the University of Tokushima Graduate School in Kuramoto, Tokushima, Japan. H. Kobori is now with the Department of Pharmacology at Kagawa Medical University, Kagawa, Japan. D. Inui is now with the Department of Anesthesiology, Shikoku Medical Center for Children and Adults, Kagawa, Japan.

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Correspondence: L. Gabriel Navar, PhD, Department of Physiology, SL39, Tulane University Health Sciences Center, 1430 Tulane Avenue, New Orleans, LA 70112 (E-mail: navar@tulane.edu).

and its renoprotective effects when aliskiren is systemically administrated have not been established. The central hypothesis of this study is that activation of the intrarenal RAS plays a crucial role in the development of glomerulonephritis. In accordance with the hypothesis, the following aims were targeted: (1) to demonstrate the activation of intrarenal RAS[34] using urinary angiotensinogen (uAGT) as an index of intrarenal Ang II levels, in rats with anti-Thy1.1 glomerulonephritis; and (2) to demonstrate that DRI with aliskiren suppresses the activation of the intrarenal RAS in the glomerulonephritis model and prevents the progression of renal injury, thus developing further a novel strategy for treatment of glomerulonephritis. The obtained results demonstrate that the intrarenal RAS is markedly activated during the early phases of glomerulonephritis and that chronic systemic administration of aliskiren attenuates the increase in the intrarenal RAS activity and prevents the glomerular injury in anti-Thy1.1 glomerulonephritis.

MATERIALS AND METHODS

Animals

The experiments were performed on Sprague-Dawley and Fischer male rats (230–430 g). The animal experimental protocol was approved by the Animal Care and Use Committee of Tulane University. The rats were housed in a constant temperature room with 12-hour dark and 12-hour light cycle with free access to food and water.

Anti-Thy1.1 Glomerulonephritis

A monoclonal antibody against rat anti-Thy1.1, OX-7, was purchased from Cedarlane Laboratories (Burlington, NC).

Experimental Design

Rats were divided into 3 groups; control group (n = 11), anti-Thy1.1 glomerulonephritis group (n = 19) and anti-Thy1.1 glomerulonephritis treated with aliskiren (30 mg/kg/d, n = 16). One group of rats was kept in individual metabolic cages with free access to food and tap water when urine samples were collected. Administration of aliskiren was started 4 days before the injection of anti-Thy1.1 antibody using an osmotic mini pump (Alzet Osmotic Pump; Alza, Mountain View, CA). Anti-Thy1.1 (200 μg/100 g body weight) was injected via a tail vein at day 0. Rats in the control group received the same volume of vehicle (isotonic saline). Blood and tissue samples were collected at 14 days after starting treatments by conscious decapitation.

Systolic Blood Pressure and Renal Function

Systolic arterial BP was measured by a tail-cuff system as previously described. Preliminary studies demonstrated that aliskiren treatment alone does not alter systolic blood pressure (SBP) in normal rats. In this group, after 10 days of aliskiren treatment, SBP averaged 114 ± 2 mm Hg compared with control measurement of 116 ± 4 mm Hg (n = 6). Twenty-four-hour urine samples were collected at days −4, 3, 5, 7, 10 and 14. Urinary protein was measured by the Pyrogallol red method (Wako Chemical, Osaka, Japan). The level of microalbumin was determined by the rat albumin ELISA kit (ALPCO Diagnostics, Salem, NH). Plasma creatinine levels were measured by Jaffe method.

Urinary and Plasma Angiotensinogen Measurements

Urinary and plasma concentrations of AGT were measured by using a commercially available ELISA kit (IBL America, Minneapolis, MN), as routinely used in our laboratory.[35] The results were normalized based on the 24-hour urine volumes and reported as uAGT excretion.

Plasma Renin Activity Assay

The blood samples were collected into tubes containing 5.0 mmol/L EDTA, and plasma renin activity (PRA) were assayed by using a commercially available kit (DiaSorin, Stillwater, MN). PRA was expressed as nanograms per milliliter per hour of generated Ang I.

Ang II Measurements in Plasma, Kidney Cortex and Medulla

For plasma Ang II determinations, blood (1 mL) was added to 100% methanol. The supernatant was transferred, dried and then assayed. For the kidney Ang II determinations, the right kidney was sectioned into cortex and medulla. Each section was weighed and immediately minced into ice-cold 100% methanol. The tissue was homogenized with a tissue tearor and then centrifuged as mentioned above. The soluble homogenates were transferred, vacuum dried, extracted and then assayed in the same manner as is routinely done in our laboratory.

Histological Analysis

Glomerular matrix expansion was evaluated by periodic acid-Schiff staining. Kidney tissues were fixed in 10% buffered formalin for 24 hours, embedded in paraffin and cut into 4 μm sections. Twenty glomeruli in a kidney section were randomly selected, and the periodic acid-Schiff staining positive score was analyzed by using Image Pro-plus software. Thereafter, an average of the positive score in 20 glomeruli was calculated as a glomerular matrix expansion score in each animal.

Immunohistochemistry

Formalin fixed kidney sections (4 μm) were deparaffinized with xylene and dehydrated with ethanol. The samples were heated at 100°C for 60 minutes in citrate buffer, and rat AGT was detected by using a rabbit primary antibody against rat AGT (IBL America), a Vectastain ABC kit (VECTOR laboratories, Burlingame, CA) and 3,3′-diaminobenzidine substrate kits. Samples were costained with hematoxylin before analysis. The staining was performed with an Autostainer Plus (Dako, Carpinteria, CA). Immunoreactivity was semiquantitatively evaluated in a blinded test as described in Histological Analysis section.

Western Blotting

Proteins were extracted from the renal cortex, and the protein concentrations were quantified by a bicinchoninic acid protein assays kit (Pierce, Rockford, IL). For AGT, 10 μg of total protein and, for transforming growth factor (TGF)-β1, plasminogen activator inhibitor (PAI)-1 and prorenin receptor (PRR), 60 μg of total protein were separated by using a 4% to 12% SDS-PAGE (Invitrogen Life Technologies, Grand Island, NY) and then transferred to a nitrocellulose membrane. The membranes were incubated with a rabbit polyclonal anti-AGT antibody (IBL America), anti-TGF-β1 antibody (Santa Cruz Biotechnology, Dallas, TX), anti-PAI-1 antibody (Abcam, Cambridge, MA) or anti-ATP6AP2 antibody (Sigma-Aldrich, Saint Louis, MO). A secondary fluorescent-goat anti-rabbit IgG antibody (IRDye 800 CW; Li-Cor Biosciences, Lincoln, NE) was used to detect the target proteins. GAPDH or β-actin levels were also determined by using a mouse monoclonal anti-GAPDH antibody (Santa Cruz Biotechnology) or a mouse