Effects of Treatment With Angiotensin-Converting Enzyme Inhibitor (ACEI) or Angiotensin II Receptor Antagonist (AIIRA) on Renal Function and Glomerular Injury in Subtotal Nephrectomized Rats

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The aim of this study was to determine if treatment with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor antagonists (AIIRA) might decrease urinary albumin excretion and prevent glomerular enlargement and glomerulosclerosis in subtotal (5/6) nephrectomized rats. Morphometric image analysis of glomeruli was also performed in the subtotal nephrectomized rats. The nephrectomized rats were treated with ACEI (enalapril 100 mg/l), AIIRA (L-158,809 10 mg/l) or TRX (reserpine 5 mg/l, hydralazine 80 mg/l, and hydrochlorothiazide 25 mg/l) and euthanized at 16 weeks after renal ablation. Treatments were started at 2 weeks (early treatment: Group I) or 8 weeks (later treatment: Group II) after the

ablation. ACEI and AIIRA treatments were equally and significantly effective in limiting albuminuria and progression of glomerular sclerosis. TRX was also as effective in decreasing urinary albumin excretion and preserving the renal function as ACEI or AIIRA in Group I. The improvement of albuminuria, glomerular enlargement and sclerosis after these treatments in Group II was significantly less than that in Group I. It appears that the early treatment with angiotensin converting enzyme inhibitor, angiotensin II receptor antagonist or reserpine, hydralazine and hydrochlorothiazide (TRX) may prevent glomerular injury in human patients with renal hypertension. *J. Clin. Lab. Anal.* 11:53–62. © 1997 Wiley-Liss, Inc.

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INTRODUCTION

Reduction of renal mass in the remnant kidney changes the glomerular hemodynamics in rats. Hyperfiltration and hypertrophy of the remnant nephrons might be due to increases in systemic and glomerular capillary pressure. The hemodynamic changes often lead to progressive glomerulosclerosis with persistent proteinuria in the remnant kidney (1–3). Clinically, several antihypertensive drugs have been used to prevent the progression of glomerular sclerosis in human hypertensive patients with chronic glomerulonephritis, diabetic nephropathy, or other renal diseases (3). One of the most effective drugs is angiotensin-converting enzyme inhibitors (ACEI), which vasodilate the efferent arterioles in the glomeruli (4,5). ACEI may also increase the activities of kinin and vasodilatory prostaglandins (6). These effects of ACEI may decrease the systemic and glomerular capillary pressure and preserve glomerular function. Previous studies have suggested that angiotensin II (AII) contributes to the hemodynamic changes in rats and also to the later development of glomerular injury in

the remnant kidney (7,8). In recent studies, infusion of AII in normal rats significantly increased glomerular capillary pressure, which ultimately led to progressive glomerulosclerosis (7). Lafayette et al. (9) reported that ACEI and angiotensin II receptor antagonist (AIIRA) treatments were equally effective on remnant kidney function when initiated 2 weeks after subtotal nephrectomy.

Morphometric image analysis allows quantitative evaluation of the glomerular injury by light microscopy (10) and has revealed features including expansion of the mesangium. The purpose of the present study was to determine whether ACEI or AIIRA might decrease albuminuria and prevent glomerulosclerosis based on morphometric image analysis in the subtotal nephrectomized rats.

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MATERIALS AND METHODS

Animals

Experiments were performed on adult male Sprague-Dawley (SD) rats weighing 180–220 g. All rats underwent subtotal (5/6) nephrectomy under anesthesia with sodium pentobarbital (20 mg/kg body weight, i.p.), except the sham-operated control subgroup, which received only an abdominal incision. All rats had free access to standard rat chow and tap water for 2 weeks. Systolic blood pressure (SBP) was measured by the tail cuff method (11). Rats with SBP < 160 mmHg were excluded from the study.

Experimental Groups

Early treatment group (Group I)

Subtotal nephrectomized rats were divided into three treatment subgroups: ACEI (enalapril, 100 mg/l drinking water (DW)), AIIRA (L-158,809, 10 mg/l DW), TRX (reserpine 5 mg/l DW, hydralazine 80 mg/l DW, and hydrochlorothiazide

25 mg/l DW), and tap water without drug (untreated control subgroup). ACEI and AIIRA were kindly provided by Merck Research Laboratories (NJ). In pilot studies, these doses for ACEI and AIIRA were found to maintain control of SBP equal to that of TRX treatment. Each subgroup was treated for 14 weeks. The rats were euthanized at 16 weeks after renal ablation as shown in Figure 1a. The remaining kidneys were collected and prepared for further analyses.

Later treatment group (Group II)

Rats in this group did not receive any treatment for 8 weeks after renal ablation. All these rats were divided into four subgroups and received specific treatments as described for the early treatment group (Group I) for an additional 8 weeks (Fig. 1b). At 16 weeks, these rats were euthanized and the remaining kidneys were then collected.

Clinical Parameters

In all subgroups, SBP was measured at the baseline and at monthly intervals after subtotal nephrectomy by the tail

(a) Group I (early treatment)

(b) Group II (later treatment)

Fig. 1. Experimental design.

(a) Group I (early treatment)

(b) Group II (later treatment)

* $p < 0.05$ vs untreated control subgroup**Fig. 2.** Levels of systolic blood pressure (SBP).

* $p < 0.05$ vs untreated control subgroup
 $^+ p < 0.05$ vs sham-operated control subgroup

Fig. 3. Levels of urinary albumin excretion.

TABLE 1. Creatinine Clearance at 16 Weeks After Renal Ablation

Sham-operated control subgroup	2.19 ± 0.43 (ml/min)
Untreated control subgroup	0.66 ± 0.05*
Group I	
TRX treatment	0.91 ± 0.13*
ACEI treatment	1.04 ± 0.13*
AIIRA treatment	0.87 ± 0.08*
Group II	
TRX treatment	0.72 ± 0.10*
ACEI treatment	1.04 ± 0.11*
AIIRA treatment	0.87 ± 0.09*

* $P < 0.05$ vs. sham-operated control subgroup.

cuff method in the conscious state. Urinary albumin excretion was also monitored using 24-hour urine samples collected in metabolic cages at baseline and at monthly intervals after the ablation. Albumin concentration in 24-hour urine samples was measured by the Albumin Analysis Kit (Shoei, Tokyo, Japan). Creatinine concentration in plasma and urine samples was measured by the Creatinine Analysis Kit (Shoei).

Light Microscopic Study and Glomerular Morphometric Image Analysis

Light microscopic studies were performed on the renal tissues fixed in 10% formalin and embedded in paraffin. Sections 4 μ m in thickness were stained with hematoxylin and eosin (HE) or periodic acid-Schiff (PAS). Renal specimens were analyzed by the same investigator blind to treatment.

Fifty glomeruli on a single section of the collected kidney were examined by the morphometric image analysis (10). In each glomerulus, the area of Bowman's capsules and glomerular tuft area was measured by the morphometric image analyser (IBAS image analyser, videoplan, Carl Zeiss Co., Oberkochen, Germany) connected to a Zeiss photomicroscope. Outlines of the profile as viewed on a video monitor were traced on a digitizing tablet at a magnification of $\times 1,000$. The glomerular tuft area was defined as the minimal convex polygon circumscribing all tuft components, i.e., glomerular capillary walls, mesangium, and podocytes (10). The ratio of glomerular tuft area to the area of Bowman's capsules (GT/BC) was calculated. Fifty glomeruli for each rat were also used for determining the glomerular sclerosis index (SI). SI

Fig. 4. Glomerular tuft area in the untreated control subgroup (b) was significantly larger than that in the sham-operated control subgroup (a) (PAS, $\times 400$). In Group I, glomerular sclerosis in the subgroup treated with AIIRA

(c) was significantly less than that in the untreated control subgroup (b) (PAS, $\times 400$).

Figure 4. (Continued.)

was obtained by scoring each glomerulus from 0 to 4 as described by Raiji et al. (12).

Statistical Analysis

All results were expressed as mean $\pm$ SE. Data from the groups were compared through oneway analysis of variance (ANOVA) followed by the t-test and Fisher's methods.

RESULTS

Systemic Blood Pressure (SBP)

Group I

Treatment with ACEI, AIIRA, or TRX decreased SBP to a similar extent. SBP in groups treated with ACEI, AIIRA, or TRX were significantly lower than in the untreated control subgroup from 4–16 weeks ($P < 0.05$). There were no significant differences in the SBP levels among the three treatment subgroups ($P > 0.05$) (Fig. 2a).

Group II

There were no significant differences in the SBP among ACEI, AIIRA, and TRX-treated and untreated control subgroups from 2–8 weeks, but SBP in subgroups treated with ACEI, AIIRA, or TRX at 12 and 16 weeks was significantly lower than those in the untreated control subgroup ($P < 0.05$) (Fig. 2b).

Urinary Albumin Excretion and Creatinine Clearance (CCr)

Group I

From 8 to 16 weeks after the renal ablation, a significant increase in urinary albumin excretion was observed in the untreated control subgroup as shown in Figure 3a. Urinary albumin excretion in the three treatment subgroups was significantly decreased compared with that in the untreated control subgroup at 16 weeks ($P < 0.01$). There were no significant

differences in urinary albumin excretion among such treatments and the sham-operated control subgroup throughout the experiment periods.

CCr in the untreated control subgroup was significantly lower than that in the sham-operated control subgroup (0.66 ± 0.05 vs. 2.19 ± 0.43 , $P < 0.05$). CCr in the three treatment subgroups was slightly increased compared with that in the untreated control subgroup at 16 weeks, but there was no statistical significance ($0.05 < P < 0.10$). CCr in the ACEI treatment subgroup was slightly increased compared with that in the TRX or AIIRA treatment subgroup, but there was no statistical significance ($P > 0.05$). However, CCr levels in the three treatment subgroups did not reach those in the sham-operated control subgroup (Table 1).

Group II

As shown in Figure 3b, urinary albumin excretion in all rats gradually increased until 8 weeks after ablation. However, excretion in the AIIRA or TRX treatment subgroup was markedly decreased at 12 and 16 weeks ($P < 0.05$). Urinary albumin excretion in the three treatment subgroups was significantly lower than in the untreated control subgroup at 16 weeks ($P < 0.05$).

CCr in the three treatment subgroups was slightly increased compared with that in the untreated control subgroup at 16 weeks, but there was no statistical significance ($0.05 < P < 0.10$, Table 1). CCr in the ACEI treatment subgroup was slightly increased compared with that in the TRX or AIIRA treatment subgroup, but there was no statistical significance ($P > 0.05$, Table 1).

Glomerular Tuft Area, Ratio of Glomerular Tuft Area to Area of Bowman's Capsules (GT/BC) and Glomerular Sclerosis Index (SI)

Group I

Glomerular tuft area in the untreated control subgroup was significantly larger than that in the sham-operated control

TABLE 2. Glomerular Tuft Area and Ratio of Glomerular Tuft Area to the Area of Bowman's Capsules (GT/BC)

	Glomerular tuft area (μm^2)	Ratio (GT/BC)
Sham-operated control subgroup	8729.76 ± 140.37	0.86 ± 0.05
Untreated control subgroup	$16069.92 \pm 317.50^*$	0.84 ± 0.05
Group I		
TRX treatment	$11779.36 \pm 417.14^{**}$	0.86 ± 0.05
ACEI treatment	$12311.47 \pm 519.86^{**}$	0.83 ± 0.05
AIIRA treatment	$12921.36 \pm 712.68^{**}$	0.84 ± 0.04
Group II		
TRX treatment	$15108.98 \pm 219.57^*$	0.87 ± 0.04
ACEI treatment	$13478.04 \pm 376.00^{**}$	0.86 ± 0.06
AIIRA treatment	$14162.74 \pm 354.95^{**}$	0.88 ± 0.06

* $P < 0.01$ vs. sham-operated control subgroup.

** $P < 0.05$ vs. sham-operated control subgroup.

(a) Group I (early treatment)

(b) Group II (later treatment)

* $p < 0.05$ vs untreated control subgroup

Fig. 5. Glomerular sclerosis index.

subgroup ($P < 0.05$) (Fig. 4a,b). Glomerular tuft area in the three treatment subgroups was significantly smaller than that in the untreated control subgroup ($P < 0.01$). However, there were no significant differences in GT/BC among all five subgroups (Table 2). SI in the untreated control subgroup was significantly greater than that in the sham-operated control subgroup ($P < 0.01$). SI in the three treatment subgroups was significantly lower than that in the untreated control subgroup ($P < 0.01$) (Figs. 4b,c, 5a). However, there were no significant differences in the glomerular tuft area, GT/BC or SI among the treated subgroups (Fig. 5a).

Group II

Changes of the glomerular tuft area, GT/BC and SI in all five subgroups were almost the same as in Group I (Table 2). However, decrease of the glomerular tuft area or SI in Group II was lower than that in Group I (Fig. 5).

DISCUSSION

The present study was performed to determine the potential beneficial effects of ACEI, AIIRA, or TRX in the remnant kidney model. Lafayette et al. (9) indicated that TRX treatment initiated at 2 weeks renal ablation was less effective in limiting proteinuria at 6 weeks and ineffective at 11 weeks when compared with ACEI or AIIRA treatment. However, our results are in conflict with their report. In this study, TRX treatment was as effective as ACEI or AIIRA treatment in the early treatment group in the remnant kidney (Group I). At equipotent depressor doses, TRX treatment limited the gradual increase in urinary albumin excretion and even prevented glomerulosclerosis. Therefore, it appears that in this rat model, reduction in systemic blood pressure alone was sufficient to retard urinary albumin excretion and progression of renal dysfunction when the TRX treatment is initiated in the early stages of renal injury.

Effects of angiotensin II (AII) in glomeruli are not limited to increasing vasoconstriction and glomerular capillary pressure, but extend to mesangial cell growth and proliferation, matrix production, and promotion of sodium retention (7). Baboolal et al. (13) reported that a significant increase in urinary sodium excretion was observed as the major effect of AII blockade. However, the mechanisms by which AII increases systemic and glomerular capillary pressure following renal ablation and by which the reduction of AII activity preserve the remnant glomerular structure are not well established (8). ACEI treatment has been shown to limit glomerular injury by decreasing both systemic and glomerular capillary pressure in rats with the renal ablation (14–16). Ikoma et al. (14) reported that ACEI prevented the progression only in glomeruli with early lesions in rats subjected to subtotal nephrectomy, in contrast to continued progression in glomeruli with more advanced lesions. In recent studies, AIIRA treatment, which reduces AII activity on the remnant kidney,

showed similar effects to ACEI treatment. Kakinuma et al. (15) reported that AIIRA treatment resulted in amelioration of progression of glomerulosclerosis comparable to ACEI treatment when these treatments were initiated at 8 weeks after renal ablation. In this study, ACEI and AIIRA treatments were equally and significantly effective in limiting the albuminuria and progression of glomerulosclerosis in the early treatment group (Group I). Glomerular sclerosis index (SI) was markedly decreased after treatment with ACEI or AIIRA in Group I. Morphometric image analysis showed that glomerular enlargement was also improved by ACEI or AIIRA treatment in Group I. However, the ratio of the glomerular tuft area to the area of Bowman's capsules (GT/BC) was not changed in any of the subgroups in this study. Improvement of albuminuria, glomerular enlargement, and sclerosis in the later treatment group (Group II) was significantly less than that in Group I. Creatinine clearance (CCr) tended to improve following all three treatments in Groups I and II. These findings suggest that the remnant glomerular injury following renal ablation might be caused mainly by glomerular capillary hypertension. It is postulated that early treatment with ACEI, AIIRA or TRX may prevent glomerular injury in human patients with hypertension.

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