Comparison of Converting Enzyme Inhibitor and Calcium Channel Blocker in Hypertensive Glomerular Injury

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The protective effect of converting enzyme inhibitors in experimental hypertensive glomerular injury is associated with decreased systemic arterial and glomerular capillary pressure. Although calcium channel blockers effectively lower systemic blood pressure, their effect on glomerular capillary pressure and on hypertensive glomerular injury is uncertain. We compared equihypotensive treatment with the calcium antagonist TA 3090 or the converting enzyme inhibitor captopril in post-salt hypertensive Dahl salt-sensitive (DS) rats for up to 5 weeks after five sixths nephrectomy. Before the nephrectomy, all rats demonstrated hypertension (mean 177 mm Hg), proteinuria (mean 175 mg/day), and mild glomerulosclerosis (mean injury score 35). Rats treated with captopril or TA 3090 demonstrated a significant and equivalent decrease in systolic blood pressure compared with untreated rats at 2, 3, and 5 weeks after five sixths nephrectomy; however, only captopril reduced proteinuria. Final proteinuria was actually increased in rats treated with TA 3090 compared with untreated rats. Glomerular injury score was significantly decreased in captopril-treated compared with untreated rats at 2 weeks (33±9 versus 117±10, p<0.05) and 5 weeks (46±9 versus 94±24, p<0.05), whereas treatment with TA 3090 delayed but did not prevent progressive glomerular injury (2-week score 35±7, p<0.05 versus untreated; 5-week score 109±19, p=NS versus untreated). Thus, in hypertensive DS rats after subtotal nephrectomy, treatment with a converting enzyme inhibitor reduced systemic blood pressure, proteinuria, and glomerulosclerosis. However, equihypotensive treatment with a calcium channel blocker did not reduce proteinuria and delayed but did not prevent glomerulosclerosis. Thus, in the rat similar reductions in systemic blood pressure with these two classes of agents have disparate effects on the progression of chronic renal failure. (Hypertension 1990;16:452-461)

Systemic hypertension complicates the course of most patients with chronic renal failure. It has been recognized for some time that uncontrolled hypertension may accelerate deterioration of renal function in these patients. On the other hand, adequate blood pressure control has clearly been shown to slow the rate of disease progression. Although the mechanisms by which systemic hypertension accelerates glomerular injury are difficult to determine in humans, a large body of experimental evidence in various animal models of hypertension now suggests that hypertensive glomerular injury results from free transmission of elevated systemic pressures to the glomerulus and that it is elevated glomerular capillary hydraulic pressure, rather than systemic hypertension itself, that induces progressive injury. Effective autoregulation of the preglomerular resistance vessels, preventing transmission of elevated systemic pressures to the glomerular capillaries, underlies the observation that glomerular injury does not invariably develop in the face of systemic hypertension.

Early workers demonstrated that after the removal of about three quarters of the renal mass in the rat, the initially normal remaining nephrons developed progressive glomerulosclerosis and the rats developed proteinuria and azotemia. Micropuncture studies in this species have revealed that the remnant nephrons undergo early functional changes consisting of increases in glomerular capillary pressures and plasma flows. In the remnant kidney model in the rat, treatment with converting...
enzyme inhibitor (CEI) results in normalization of both systemic blood pressure and glomerular capillary pressure. These hemodynamic effects are associated with decreased proteinuria and less evidence of glomerulosclerosis. Because treatment with CEI lowers not only systemic blood pressure but glomerular capillary pressure as well, it is possible that these agents will be more effective in preventing the progression of chronic renal failure than other antihypertensive agents that do not have this specific renal hemodynamic effect. Preliminary studies have indeed suggested that the CEIs may have particular advantages over other antihypertensive regimens in slowing or arresting progressive renal failure in rats and patients, perhaps because of these beneficial hemodynamic effects.

The calcium channel blockers (CCBs) are in widespread clinical use as antihypertensive agents in diverse patient groups including those with chronic renal failure. These drugs are effective antihypertensive agents in patients with renal impairment and have not been demonstrated to have adverse short-term effects on renal function. However, the effect of long-term calcium channel blockade on the course of progressive renal failure in experimental models or patients is currently unknown and is a question of potential clinical importance. Experimentally, the CCBs have been demonstrated to have unique renal hemodynamic effects. Moderate reductions in systemic blood pressure by these agents are associated with maintenance of or increases in glomerular filtration rate (GFR) due to specific vasodilatation of the preglomerular or afferent resistance vessels. Consequently, at least in the rat, some investigators have reported that glomerular capillary pressure is not reduced when the calcium antagonists are given acutely or chronically. In these reports, reduction in systemic pressure was balanced with afferent vasodilatation and glomerular capillary pressure maintained. However, preliminary reports by other investigators in other experimental models and patients have noted a decrease in glomerular capillary pressure when CCBs are given acutely or chronically. Thus, at this time, the effects of the CCBs on glomerular capillary pressure remain uncertain. A further consideration in the use of CCBs in the setting of hypertensive glomerular injury is that the normal renal autoregulatory response to changes in renal perfusion pressure, a potentially protective mechanism, is impaired by these agents. Given the above discussion, it is apparent that the hemodynamic profile of the calcium channel blockers is such that it is possible that they would not be effective in protecting against hypertensive glomerular injury.

The effects of CCBs on glomerular injury in experimental models of hypertension have been controversial. Harris and coworkers demonstrated increased metabolic stresses in remnant nephrons after subtotal renal ablation in the rat, and postulated that these stresses may be detrimental and contribute to progressive injury. In this model, decreasing hypermetabolism by treatment with a CCB appeared to protect the kidney. This same group treated rats with nonhypotensive doses of verapamil for up to 15 weeks after renal ablation and noted improved survival. Although the degree of glomerulosclerosis was diminished in rats receiving verapamil, proteinuria was not reduced and renal function, other than creatinine clearance, was not reported. Other investigators have been unable to demonstrate a long-term protective effect of CCBs against progressive glomerular injury in the remnant kidney model.

The effect of adequate, chronic antihypertensive treatment with CCBs on the course of glomerular injury in animal models has been difficult to study because of a lack of an effective means of drug delivery. Rats will not drink water to which CCBs have been added, and the addition of these agents to the diet leads to decreased food intake and inaccurate drug dosing (J.P. Tolins and L. Raij, unpublished observations). We have developed a technique for antihypertensive drug delivery in the rat that allows the administration of multiple daily doses in a carefully controlled manner. This technique, combined with the availability of very potent and long-lasting CCBs, has recently made it technically possible to adequately study the effect of long-term administration of CCBs on progressive renal injury.

After subtotal renal ablation, the Dahl salt-sensitive (DS) rat with post-salt hypertension rapidly develops progressive renal injury characterized by pronounced hypertension, heavy proteinuria, decline in glomerular filtration rate, and histological evidence of glomerulosclerosis. Using this model of accelerated hypertensive glomerular injury, we compared the effects of long-term treatment with equipotensive doses of the converting enzyme inhibitor captopril or the CCB TA 3090. The latter agent is a 1,5-benzothiazepine calcium antagonist that has increased potency and duration of hypotensive action, thus allowing sustained blood pressure control in this experimental model.

Methods

Studies were performed on male DS rats (Brookhaven National Laboratories, Upton, N.Y.). Weanling rats were allowed to equilibrate for 2 weeks on standard rat chow (Rodent Lab Chow, Purina Mills, St. Louis, Mo.) and tap water ad libitum; after which time all rats were given chow supplemented with 4% NaCl (Purina Mills) for 8 weeks. At this time, after administration of brevital anesthesia (100 mg/kg i.p.), rats underwent surgical removal of the right kidney and infarction of approximately two thirds of the left kidney by ligation of two or three branches of the left renal artery. The right kidney was processed for morphological studies as described below. A gastrostomy tube was placed at the same time for subsequent antihypertensive drug administration. A length of PE-100 polyethylene tubing with a flared end was secured in the stomach with a purse-string suture, and the exit site from the
stomach was wrapped with a small square of sterile gauze to ensure adhesion to the parietal peritoneum. The tubing was tunneled through the abdominal wall and subcutaneously to exit the skin between the scapulae. The tube was flushed with water (1 ml) after placement and after each use. Rats were allowed to recover for 3 days while consuming rat chow that contained 6% protein (ICN Nutritional Biochemical Lab., Cleveland, Ohio) ad libitum, and serum creatinine measured on distal tail vein blood samples (Creatinine Analyser 2, Beckman Instruments Inc., Fullerton, N.J.). Rats were randomly assigned into experimental groups matched for serum creatinine to ensure equivalent reduction in renal mass, placed on standard rat chow and tap water ad libitum, and housed in individual cages for the duration of the study.

**Study 1**

Three groups of rats were studied (n=8 in each group). Group 1 rats received tap water (2.5 ml) by gastric tube at 8:00 AM and 5:00 PM. Group 2 rats received captopril (generous gift of ER Squibb & Sons, Princeton, N.J.) (50 mg/kg in 1.5 ml, followed by 1.0 ml water), and group 3 rats received TA 3090 (generous gift of Marion Laboratories, Kansas City, Mo.) (100 mg/kg in 1.5 ml, followed by 1.0 ml water) at 8:00 AM and 5:00 PM. Drug therapy was begun on day 4 after renal ablation, after rats had been randomly assigned into experimental groups. Doses of TA 3090 and captopril that conferred strictly equivalent reductions in systolic blood pressure in this model, as measured in the awake rat by the tail-cuff method, were determined in pilot studies (data not shown). Doses of antihypertensive agents were then specifically chosen to ensure equivalent reduction in systemic blood pressure during the experimental periods in the two treatment groups. After 8 weeks of high salt diet (before renal ablation), and again 10 days after starting drug treatment (13 days after renal ablation), rats had awake systolic blood pressures measured by the tail-cuff method (Programmed Electro-Sphygmomanometer, Narco Bio-Systems, Inc., Houston, Tex.). All blood pressures were measured before the morning dose of antihypertensive medication or vehicle; thus, these measurements represent the nadir of drug effect and presumably the highest blood pressure of the previous 24-hour period. Therefore, the blood pressures in the various treatment groups are directly comparable. More frequent blood pressure measurements were not possible because of the stress of this procedure (warming and restraint) with demonstrated loss of several animals during our pilot studies. At these same time points, rats were placed in metabolic cages for 24-hour urine collection. Urinary total protein excretion was assayed by the Coomassie dye method (Bio-Rad Laboratories, Richmond, Calif.). After overnight fast, tail vein blood was assayed for cholesterol and triglycerides by standard laboratory techniques. Fourteen days after beginning drug treatment, renal hemodynamics were measured by clearance techniques, as described below, and the remnant kidney was weighed and processed for histological evaluation of glomerular injury.

**Study 2**

Three groups of DS rats (n=8 or 9 in each group) with post-salt hypertension underwent subtotal renal ablation and placement of a gastric tube as described above. Twice daily rats were given water (group 4), captopril (group 5), or TA 3090 (group 6) as described above, starting on day 4 after ablation. Systolic blood pressure was determined before nephrectomy and 3 and 5 weeks after nephrectomy. Urinary protein excretion rate was measured before nephrectomy and 3 weeks after nephrectomy. Rats were examined daily and, when clinical signs of impending death were noted (ruffled fur, listlessness, lack of movement, weight loss, poor food intake), rats were killed and the remnant kidney weighed and processed for histological evaluation. In our experience with this model, these clinical signs are excellent predictors of impending death. All surviving rats were similarly killed 5 weeks after renal ablation at a time when two thirds of the group 4 rats had died. In studies 1 and 2, all gastric tubes functioned well without complication for the initial 2–3 weeks. After this time, some rats required surgical revision of the gastric tube with either local (1% lidocaine) or general (Brevital) anesthesia.

**Clearance Studies**

Rats in study 1 underwent clearance studies for determination of GFR and renal plasma flow (RPF) 14 days after drug treatment was begun, as previously described. Briefly, surgical preparation was performed on a heated table, with rectal temperature maintained between 37.0 and 37.5°C. A tracheotomy was performed after administration of anesthesia (Inactin, 100 mg/kg i.p.). A double-lumen PE-50 catheter was placed in the left internal jugular vein for infusion. A PE-50 catheter was inserted into the left femoral artery for blood pressure monitoring (transducer model TNF, Gould Inc., Oxnard, Calif.) and blood sampling. The left ureter was cannulated with a PE-10 catheter and timed urine samples collected in preweighed plastic vials for gravimetric determination of urine flow rate. To determine GFR and RPF, a solution of normal saline containing [3H-methoxy]-inulin (4 μCi/ml) and para-aminohippurate (PAH) (20 mg/ml) was infused at a rate of 1.2 ml/hr after a priming dose of 0.5 ml. To maintain euvoledna, 5% bovine serum albumin in normal saline was given at 0.5 ml/hr after a priming dose of 1% of body weight. After surgical preparation, rats were allowed to equilibrate for 30 minutes before two clearance periods were begun. Two 50 μl blood samples were drawn at the midpoint of each 20-minute clearance period. Radioactivity was determined in serum and urine by liquid scintillation counting, and PAH levels in serum and urine were
TABLE 1. Physical and Biochemical Parameters of Rats in Study 1

<table>
<thead>
<tr>
<th>Group</th>
<th>3d Scr (mg/dl)</th>
<th>Body wt (g)</th>
<th>Hct (%)</th>
<th>Right kidney wt (g)</th>
<th>Remnant kidney wt/body wt (x10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>1.52±0.13</td>
<td>367±12</td>
<td>34±3</td>
<td>1.15±0.13</td>
<td>3.16±0.38</td>
</tr>
<tr>
<td>Group 2 (captopril)</td>
<td>1.49±0.13</td>
<td>418±7*</td>
<td>41±1*</td>
<td>1.34±0.10</td>
<td>3.22±0.25</td>
</tr>
<tr>
<td>Group 3 (TA 3090)</td>
<td>1.50±0.14</td>
<td>398±7*</td>
<td>40±1*</td>
<td>1.00±0.08</td>
<td>2.50±0.18*</td>
</tr>
</tbody>
</table>

3d Scr, serum creatinine 3 days after renal ablation and before initiation of antihypertensive treatment; Body wt, final body weight; Hct, hematocrit; remnant kidney wt, remnant kidney weight.

*p<0.05 versus group 1.

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determined by autoanalyzer (Technicon Corp., Tarrytown, N.Y.). GFR and RPF were calculated by standard formulas. Renal blood flow (RBF) was calculated by dividing RPF by 1-hematocrit and renal vascular resistance by dividing mean arterial pressure (MAP) by RBF.

Morphology

The renal tissue obtained for histopathology was fixed by immersion in buffered formaldehyde solution, sectioned at 3 µm and stained with the periodic acid–Schiff technique. All tissues were evaluated by a single investigator (L.R.) without knowledge of the group to which the rat belonged. Glomerulosclerosis was defined as the disappearance of cellular elements from the tuft, collapse of capillary lumens, and folding of the glomerular basement membrane with the entrapment of amorphous material. A semiquantitative scoring technique evaluating the percentage of glomeruli with sclerosis and the extent of involvement of each glomerulus was used as previously described.7 Mean glomerular volume for each sample was determined by the point counting technique of Weibel.32

Statistical Analysis

Data are presented as mean±SEM. Parameters between groups were compared by analysis of variance and subsequent Scheffe’s test, and parameters within one group at different time points were compared by analysis of variance for repeated measures (STATVIEW 512, Brainpower Inc., Calabasas, Calif.). Differences were considered significant for p<0.05.

Results

Study 1

Rats tolerated the surgical procedures well, and all gastric tubes functioned normally during the 14-day treatment period. One control rat (group 1) died at the time of the final clearance study. Two TA 3090–treated rats (group 3) died, one shortly after random assignment to the group and the other at the time of the final clearance. All rats treated with captopril (group 2) survived. Physical and biochemical parameters of rats in study 1 are shown in Table 1. There was no difference in serum creatinine, measured 3 days after renal ablation and before beginning treatment, among the three groups, implying equivalent reduction in renal mass at the start of the experimental period. Rats treated with either antihypertensive drug had a higher final body weight and hematocrit than untreated rats. Remnant kidney weight was numerically increased in captopril-treated rats compared with untreated and TA 3090–treated rats, the latter difference achieving borderline statistical significance (significant at p<0.05 by post hoc Fisher test). When factored for body weight, it can be seen that treatment with calcium channel blockade tended to reduce the renal hypertrophic response to subtotal renal ablation, whereas captopril was without effect on this parameter.

Systolic blood pressures measured immediately before subtotal renal ablation and 10 days after random assignment into experimental groups are shown in Figure 1. As can be seen, all rats had a similar degree of hypertension before renal ablation. Treatment with either captopril or TA 3090 by twice daily gastric instillation resulted in significant, sustained reduction of systolic blood pressure compared with untreated rats. Furthermore, the hypotensive effect was identical with either drug. Because these blood pressures were measured before the morning drug dose, they reflect blood pressure in the presence of trough drug levels and thus, presumably, the highest blood pressure attained in a given 24-hour period. It is clear that the experimental protocol achieved the goal of equivalent reduction of blood pressure in both treatment groups.

Urinary excretion rates for total protein before renal ablation and 10 days after random assignment

FIGURE 1. Bar graph showing awake systolic blood pressure before renal ablation and 10 days after initiation of antihypertensive treatment in rats of study 1. Rats were treated with water vehicle (Group I), captopril (Group II), or TA 3090 (Group III). p<0.05 versus water vehicle group.
into experimental groups are shown in Figure 2. After induction of post-salt hypertension, before renal ablation, the DS rat develops pronounced proteinuria, which was present to a similar degree in all three groups. Untreated rats had persistent massive proteinuria despite high grade renal ablation, implying an increase in leakage of protein per remaining nephron. As can be seen, captopril treatment (group 2) markedly decreased urinary protein excretion. Despite equivalent reduction in systemic blood pressure in rats treated with TA 3090 (group 3), proteinuria was not decreased compared with untreated rats. Thus, equihypotensive treatment with converting enzyme inhibitor or CCB had markedly different effects on proteinuria: urinary protein excretion rates were decreased only with the former agent.

Renal hemodynamic parameters, measured 14 days after random assignment into experimental groups, are shown in Table 2. MAP measured in the anesthetized rat after surgical preparation was significantly decreased in captopril-treated rats (group 2) compared with untreated rats (group 1). Rats receiving TA 3090 (group 3) had a numerically decreased MAP (19 mm Hg) under these conditions, compared with untreated rats, but this did not achieve statistical significance. As described above, systolic blood pressure in the awake, unoperated rat was significantly decreased in captopril-treated rats (group 2) and equivalently reduced in both treatment groups. Thus, in the setting of a clearance preparation, the greater reduction in MAP in the captopril-treated group most likely reflects the importance of the renin-angiotensin II system in the response to surgical trauma. Captopril-treated rats had significant preservation of GFR compared with untreated rats, whereas treatment with TA 3090 resulted in a modest but not significant increase in GFR. RPF, as estimated by the clearance of PAH, was significantly increased, to an equivalent degree, in rats receiving either captopril or TA 3090. As can be seen, treatment with either captopril or TA 3090 resulted in renal vasodilatation, as reflected in the markedly decreased RVR, compared with untreated rats. Thus, in the hypertensive DS rat with subtotal renal ablation, reduction in systemic blood pressure with captopril resulted in significant preservation of GFR and renal vasodilatation. Equivalent reduction in systemic blood pressure with TA 3090 resulted in similar directional changes in renal hemodynamics; however, the effect on GFR was proportionately not as great as the vasodilatory effect.

Nephrectomy specimens taken at the time of renal ablation showed histological changes that primarily affected the glomeruli and were characterized by mild-to-moderate increases in mesangial matrix. In addition, some glomeruli were affected by focal areas of glomerulosclerosis (mean glomerular injury score of 35±2, n=24). The tubules and interstitium appeared well preserved. Proteinaceous casts were present in occasional tubules, mostly at the corticomedullary junction. The intrarenal vessels appeared thickened. Glomerular injury scores for study 1 rats 14 days after renal ablation are shown in Figure 3A. The kidneys of untreated (group 1) rats showed diffuse histological changes that affected glomeruli, tubules, and interstitium. The glomeruli showed diffuse mesangial expansion and widespread areas of glomerulosclerosis (injury score 117±10). Tubulointerstitial infiltrates consisting of mononuclear cells were easily detectable, particularly surrounding areas of tubular atrophy. The intrarenal vessels were markedly thickened. In group 2 rats, treated with captopril, the renal structures were well preserved. Mesangial expansion appeared less prominent than in glomeruli of the companion nephrectomy specimens taken at the time of renal ablation. The capillary loops were open and dilated. Focal areas of glomerulosclerosis were observed in few glomeruli. The glomerular injury score was significantly reduced compared with untreated rats (33±9, p<0.05). The

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Bar graph showing urinary protein excretion rate in rats of study 1 before renal ablation and 10 days after initiation of antihypertensive treatment. Rats were treated with water vehicle (Group I), captopril (Group II), or TA 3090 (Group III). *p<0.05 versus water vehicle group. tp<0.05 captopril-treated versus TA 3090-treated group.

**Table 2. Renal Hemodynamic Parameters of Rats In Study 1, 14 Days After Initiation of Antihypertensive Drug Treatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>GFR (ml/min)</th>
<th>RPF (ml/min)</th>
<th>FF</th>
<th>RVR (mm Hg-min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>177±9</td>
<td>0.42±0.13</td>
<td>1.83±0.60</td>
<td>0.24±0.02</td>
<td>123±38</td>
</tr>
<tr>
<td>Group 2 (captopril)</td>
<td>136±5*</td>
<td>0.84±0.09*</td>
<td>4.21±0.42*</td>
<td>0.20±0.02</td>
<td>22±3*</td>
</tr>
<tr>
<td>Group 3 (TA 3090)</td>
<td>158±7</td>
<td>0.63±0.08</td>
<td>3.82±0.33*</td>
<td>0.17±0.02*</td>
<td>26±2*</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; RVR, renal vascular resistance.

*p<0.05 versus group 1.
FIGURE 3. Bar graphs showing renal morphological evaluation in rats of study 1: Glomerular injury score (panel A) and glomerular volume (panel B). *p<0.05 versus water vehicle (Group I). †p<0.05 captopril-treated (Group II) versus TA 3090-treated (Group III) group.

tubules were intact and there were no interstitial cellular infiltrates. Thus, captopril arrested the progression of glomerulosclerosis after renal ablation. In rats treated with TA 3090 (group 3) histological evidence of glomerular injury was absent to the same degree as in group 2 (injury score 35±7, p<0.05 versus group 1, p=NS versus group 2). Thus, at 14 days after renal ablation, calcium channel blockade provided equivalent protection to captopril, despite the lack of effect of TA 3090 on proteinuria.

Glomerular volumes for the rats in study 1, 14 days after renal ablation, are shown in Figure 3B. As can be seen, captopril had no effect on glomerular volume compared with untreated rats. On the other hand, treatment with TA 3090 (group 3) significantly decreased glomerular volume compared with both untreated (group 1) and captopril-treated (group 2) rats. Thus, the protective effect of TA 3090 on glomerular injury 14 days after renal ablation was associated with decreased renal hypertrophy and glomerular volume.

Study 2
In this study, we evaluated the effect of long-term treatment with strictly equihypotensive doses of captopril or TA 3090 on survival and glomerular injury in hypertensive DS rats after subtotal renal ablation. Physical and biochemical parameters of rats in study 2 are shown in Table 3. Body weights initially (before ablation) were similar in all three experimental groups. By 4 weeks after initiation of treatment, untreated rats (group 4) had lost a significant amount of weight. Rats receiving TA 3090 (group 6) maintained before-ablation weight, whereas in captopril-treated rats (group 5) body weight significantly increased over this period. Final remnant kidney weights, determined at the end of the study or time of death, were similar in all three treatment groups (group 4 versus group 5 versus group 6: 2.70±0.33 versus 2.87±0.24 versus 3.24±0.14 g kidney wt/g body wt×10³, respectively, p=NS). Glomerular volumes, determined on these same kidney specimens, were also similar in all three groups (group 4 versus group 5 versus group 6: 1.78±0.20 versus 1.83±0.18 versus 1.74±0.15 μm³×10³, respectively, p=NS). Thus, the reduction in kidney weights and glomerular volumes in rats treated with CCBs observed at 14 days after ablation was not sustained during long-term treatment with TA 3090.

Serum cholesterol and triglycerides were measured after overnight fast, before beginning high salt diet, before ablation, and at 4 weeks after random assignment into experimental groups. As can be seen, serum cholesterol was similar in all three groups before induction of hypertension and increased slightly, although not significantly, after 8 weeks of 4% salt diet. At 4 weeks after renal ablation, cholesterol had increased significantly compared with initial values in all groups. This increase was modest in untreated rats (group 4) and rats receiving captopril (group 5), and there was no difference between these two groups. Rats receiving TA 3090 had a dramatic increase in serum cholesterol to a level significantly higher than in groups 4 or 5. Triglycerides were not different among the three groups at any time point during the experimental protocol. Serum creatinine 3 days after renal ablation was similar in all three

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>Serum cholesterol (mg/dl)</th>
<th>3d Scr (mg/dl)</th>
<th>Final Scr (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prenephrectomy</td>
<td>Initial</td>
<td>Preablation</td>
<td>4-wk</td>
</tr>
<tr>
<td>Group 4 (control)</td>
<td>386±11</td>
<td>65±2</td>
<td>73±2</td>
<td>110±21*</td>
</tr>
<tr>
<td>Group 5 (captopril)</td>
<td>386±12</td>
<td>424±14*†</td>
<td>60±2</td>
<td>72±3</td>
</tr>
<tr>
<td>Group 6 (TA 3090)</td>
<td>393±8</td>
<td>380±26</td>
<td>59±2</td>
<td>65±2</td>
</tr>
</tbody>
</table>

* p<0.05 versus initial or preablation.
† p<0.05 versus group 4.
‡ p<0.05 versus group 5.

Body wt, body weight; 3d Scr, serum creatinine 3 days after renal ablation.
groups, reflecting equivalent reduction in functioning renal mass. At 4 weeks after random assignment into experimental groups serum creatinine in surviving rats tended to be highest in untreated rats (group 4) and lowest in rats receiving captopril (group 5), with TA 3090–treated rats (group 6) having intermediate values; however, these differences did not achieve statistical significance.

As shown in Figure 4, all three groups of rats had a similar degree of systolic hypertension before renal ablation. At 3 and 5 weeks after initiation of drug therapy, rats receiving either captopril (group 5) or TA 3090 (group 6) demonstrated significant and equivalent reductions in blood pressure compared with untreated rats. Thus, both treatment regimens achieved the goal of equivalent control of systemic hypertension when chronically administered by gastric instillation twice daily.

When the experiment was ended, 5 weeks after random assignment into experimental groups, six of nine (67%) untreated rats had died. These rats became progressively ill and were killed when they were obviously moribund by clinical observation. In rats treated with TA 3090, one rat of eight died (12.5%), and this death was within 24 hours of gastric tube revision and not associated with preoperative signs of clinical illness. In rats treated with captopril, three of eight rats died (37.5%), and again all three deaths were associated with surgical revision of the gastrosomy tube. Thus, all deaths in the rats receiving antihypertensive drug therapy were postoperative and unrelated to the natural course of this disease model. It is clear that treatment with either captopril or TA 3090 significantly improved survival to an equivalent degree when compared with untreated rats.

Urinary protein excretion rates for rats in study 2, measured before nephrectomy and 3 weeks after random assignment into experimental groups, are shown in Figure 5. As in study 1, untreated rats demonstrated pronounced glomerulosclerosis (glomerular injury score 94 ± 24). Rats receiving captopril demonstrated a significant decrease in glomerular injury score (46 ± 9, \( p < 0.05 \) versus group 4) 5 weeks after random assignment into treatment groups. However, treatment with TA 3090 for this same time period did not significantly prevent glomerulosclerosis: the glomerular injury score was similar to that seen in untreated rats level of proteinuria (180 ± 23 mg/day) despite reduction in nephron number. In rats receiving captopril (group 5), proteinuria was markedly decreased at 3 weeks (54 ± 16 mg/day, \( p < 0.05 \) versus prenephrectomy, \( p < 0.05 \) versus group 4). Group 6 rats, treated with TA 3090, markedly increased urinary protein excretion rates to levels significantly higher than both untreated and captopril-treated rats (3-week protein excretion rate, 333 ± 64 mg/day, \( p < 0.05 \) versus group 4 and group 5). Thus, reduction in systemic blood pressure by treatment with a CCB was associated with an actual increase in urinary protein excretion, whereas equivalent reduction in blood pressure with a converting enzyme inhibitor was associated with a pronounced and significant fall in proteinuria.

Results from morphological analysis of renal tissue from rats in study 2 are shown in Figure 6. As in study 1, untreated rats demonstrated pronounced glomerulosclerosis (glomerular injury score 94 ± 24). Rats receiving captopril demonstrated a significant decrease in glomerular injury score (46 ± 9, \( p < 0.05 \) versus group 4) 5 weeks after random assignment into treatment groups. However, treatment with TA 3090 for this same time period did not significantly prevent glomerulosclerosis: the glomerular injury score was similar to that seen in untreated rats.
(109±19, p<0.05 versus group 5, p=NS versus group 4). Thus, it can be seen that the protective effect of TA 3090 observed in study 1, 2 weeks after renal ablation, was not sustained during long-term follow-up despite a reduction in blood pressure equivalent to that seen in captopril-treated rats.

**Discussion**

DS rats are genetically predisposed to develop hypertension when fed a high salt diet. Micro-puncture studies in intact, hypertensive DS rats have demonstrated an elevated single nephron GFR due to an increase in glomerular capillary plasma flow and an increase in glomerular capillary hydraulic pressure. Thus, in this strain of rat, the elevated systemic blood pressure is freely transmitted to the glomerulus, and development of progressive glomerular injury due to glomerular capillary hypertension would be predicted. In the current study, systemic hypertension in the DS rat was associated with pronounced proteinuria and definite structural evidence of glomerular injury consisting of mild mesangial expansion and focal areas of glomerulosclerosis. These changes were present in the intact animals at the time of renal ablation and reflect the genetic vulnerability of this strain of rats to hypertensive glomerular injury.

The course of renal disease in the hypertensive DS rat after subtotal renal ablation has not been previously reported. We have demonstrated that, after high grade renal ablation, the untreated DS rat develops pronounced systemic hypertension, continued massive proteinuria, and by 14 days after ablation, impaired renal function with widespread areas of glomerulosclerosis (study 1). When followed for up to 5 weeks after renal ablation (study 2), untreated rats have persistent hypertension and proteinuria. These rats had a high mortality rate, with two thirds of the rats dying by 5 weeks. Although severe glomerular injury was present in the untreated rats of study 2 at the time they were killed, final serum creatinine was not markedly elevated. Therefore, it is unlikely that the untreated rats were dying of complications of renal failure and uremia. Postmortem examination was not done, other than evaluation of renal morphology; however, the high mortality rate most likely reflects the adverse systemic effects of prolonged uncontrolled hypertension, such as stroke. Thus, subtotal renal ablation in the DS rat with post-salt hypertension represents a model of accelerated hypertensive glomerular injury characterized by proteinuria, depressed renal function, glomerulosclerosis, and in the untreated rat, a high mortality rate.

We used this model to directly compare the potential renal protective effects of equihypotensive regimens of two classes of antihypertensive agents in common clinical use: CEIs and CCBs. We administered the antihypertensive agents by gastric instillation twice daily, assuring accurate drug delivery when compared with providing the drugs in food or drinking water. Blood pressure was measured in conscious rats approximately 15 hours after the evening dose and thus at a time of nadir drug effect. Doses of captopril and TA 3090 were specifically chosen, based on results from pilot studies, to achieve significant and strictly equivalent reductions in systolic blood pressure in the treatment groups compared with untreated rats. This equivalent reduction in blood pressure was maintained over the course of the experimental period as determined by measurements at 2, 3, and 5 weeks. Therefore, any differences between the two agents would be unrelated to variations in degree of blood pressure control, a confounding factor in other studies using similar agents.

In the hypertensive DS rat after renal ablation, control of systemic hypertension with captopril was associated with maintenance of general health compared with untreated rats, as demonstrated by weight gain, maintenance of hematocrit, and increased survival. Rats receiving captopril had markedly decreased proteinuria compared with untreated rats at 10 and 21 days after nephrectomy, and this was associated with preservation of GFR and renal vasodilatation, measured 14 days after ablation. Furthermore, histological evaluation of renal tissue 2 and 5 weeks after ablation revealed a significant reduction in the extent of glomerulosclerosis compared with untreated rats. Thus, captopril treatment was associated with a renal protective effect, consistent with the effect of CEIs in the remnant kidney model in other strains of rats.

In the current study, the effects of captopril were directly compared with those of the CCB TA 3090. Delivery of the CCB by gastric instillation twice daily insured accurate delivery of the drug dose and enabled us to obtain a reduction in systemic blood pressure strictly equivalent to that obtained with captopril throughout the course of the study. After 2 weeks of treatment, TA 3090–treated rats had a body weight and hematocrit similar to those of rats receiving captopril. Long-term treatment with TA 3090 was associated with improved survival but impaired weight gain. Improved survival in hypertensive DS rats during treatment with oral CCB has been reported previously. The effects of calcium channel blockade on proteinuria were markedly different from those observed with converting enzyme inhibition. At 10 days after renal ablation, rats receiving TA 3090 had a urinary total protein excretion rate equivalent to that observed in untreated rats. By 21 days after ablation, proteinuria was significantly increased compared with both untreated rats and rats receiving captopril. This disparate effect on proteinuria of CEI and calcium antagonist has been previously reported in rats and patients.

As discussed above, the renal hemodynamic profiles of these two classes of antihypertensive agents are distinctly different. In particular, although it is clear that CEIs reduce glomerular capillary pressure in addition to systemic blood pressures, equivalent reduction in systemic pressure with a CCB may not lower glomerular capillary pressure because of a concomitant reduction in preglomerular resist-
vented glomerular injury without reducing glomerular capillary pressure. Thus, treatment with a calcium antagonist delayed but did not prevent the development of severe glomerulosclerosis in this model. Interestingly, Anderson and coworkers\(^1\) found a similar pattern of early protection but ultimate lack of effectiveness in a study comparing treatment with “triple therapy” or converting enzyme inhibition on the course of progressive renal injury in diabetic rats. In this study, despite equivalent reduction in systemic blood pressure with triple therapy or converting enzyme inhibition, only the latter reduced glomerular capillary pressure.

Given the lack of effect on proteinuria, the early protective effect of TA 3090 is interesting and possibly unrelated to glomerular hemodynamic factors. The CCBs have potentially beneficial metabolic effects that may be important early in the course of development of glomerulosclerosis. Raij and Keane\(^40\) showed that, in rats, verapamil was as effective as saralasin in reducing the increased mesangial traffic of macromolecules induced by subpressor doses of angiotensin II. Recent studies have also shown that CCBs inhibit mesangial cell proliferation in response to agonists such as platelet-derived growth factor or thrombin.\(^41\) CCBs have also been demonstrated to inhibit the generation of the inflammatory mediator, platelet activating factor, by endothelial cells.\(^42\) Because increased mesangial traffic, inflammation, and cellular proliferation are potentially important processes in the development of hypertensive glomerular injury, it is possible that such metabolic actions of the CCBs on these cell types may be beneficial. Indeed, in a preliminary report, Dworkin and coworkers\(^21\) demonstrated that nifedipine prevented glomerular injury without reducing glomerular pressure in rats with deoxycorticosterone-salt-induced hypertension, presumably by a beneficial metabolic action.

Recent studies suggest that compensatory renal hypertrophy may contribute to the early protective effect of calcium channel blockade seen in the current study. Based on observations in experimental models, hyperlipidemia has been proposed to be a contributing factor to the progression of chronic renal failure.\(^43\) In the current model, serum cholesterol and triglycerides were measured during the development of hypertension and 4 weeks after renal ablation. Triglyceride levels were not different between the three experimental groups at any time during the experimental protocol. Because the degree of renal injury was dramatically different, it is apparent that triglyceride levels were not important determinants of progression of glomerular injury in this model. During the development of hypertension, cholesterol levels were not markedly elevated in any group despite the development of proteinuria and mild glomerular injury. By 4 weeks after renal ablation, cholesterol levels had increased modestly in all rats but were similar in untreated rats and those treated with captopril despite pronounced differences in degree of glomerulosclerosis between these two groups. It is thus apparent that the level of serum cholesterol is not correlated with glomerular injury in this model. The rats receiving TA 3090 had the highest cholesterol levels, most likely reflecting the high level of proteinuria seen in these rats. The current study does not support a primary role for hyperlipidemia in the progression of glomerular injury.

In summary, the hypertensive DS rat with subtotal renal ablation represents a model of accelerated glomerular injury. Treatment with CEI reduced systemic blood pressure, proteinuria, and glomerulosclerosis. However, equihypotensive treatment with a CCB had no effect on proteinuria and delayed but ultimately did not prevent glomerulosclerosis. Whether these two classes of antihypertensive drugs have disparate effects on the course of progressive renal failure in patients is an important question that warrants clinical investigation. Furthermore, given the differences in the potential mechanisms of action of these two classes of agents, it is possible that CEIs and CCBs may have a complementary and perhaps synergistic effect on the kidney.

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**References**


**Key Words** • renal hypertension • glomerular function • angiotensin converting enzyme inhibitors • glomerulosclerosis • calcium channel blockers • proteinuria
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