Combination Treatment of Enalapril With Nitrendipine in Rats With Renovascular Hypertension

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Abstract We have recently shown that treatment with the calcium channel blocker nitrendipine may aggravate albuminuria and glomerular injury in rats with two-kidney, one clip renovascular hypertension if arterial blood pressure is not reduced. To test whether nitrendipine also exerts its adverse renal effects when normotension is achieved, we examined the effect of combined therapy with nitrendipine and the converting enzyme inhibitor enalapril on blood pressure, albuminuria, glomerular filtration rate, and morphology of the nonclipped kidney. Rats treated with enalapril alone or in combination with the diuretic hydrochlorothiazide or rats treated with nitrendipine alone served as controls. Therapy was started 6 weeks after clipping of one renal artery. Nitrendipine alone did not reduce blood pressure but significantly increased albuminuria, diuresis, glomerular filtration rate, and glomerular volume and injury compared with untreated hypertensive controls. Increase of glomerular filtration rate, diuresis, and albuminuria was reversible after withdrawal of nitrendipine.

In rats with renovascular hypertension, we have recently shown that treatment with the calcium channel blocker (CCB) nitrendipine increases albuminuria and glomerular hypertrophy and sclerosis compared with untreated hypertensive control animals. However, in those studies nitrendipine did not normalize blood pressure. Nitrendipine alone could have accelerated the progression of glomerular injury by preglomerular dilatation, which in the absence of blood pressure reduction results in a rise of glomerular capillary pressure. The converting enzyme inhibitor (CEI) enalapril lowered blood pressure, albeit not to normal levels, and did not influence albuminuria and glomerular sclerosis.

Clinical studies suggest that the combination of CCB and CEI may be superior in decreasing proteinuria compared with the respective monotherapy. Despite the evidence for a beneficial effect of this combination on blood pressure and proteinuria in hypertensive patients, there are no data as to the effect of CCB plus CEI therapy on renal parameters in rats with experimental renal disease.

Therefore, the aim of the present study was to investigate whether the adverse renal effects of nitrendipine could be prevented by normalization of blood pressure with combined CCB and CEI therapy. In addition, we compared the effect of combined CCB and CEI therapy with a combination of CEI and the diuretic hydrochlorothiazide, which also lowered blood pressure to normotensive levels in this model. The reversibility of the adverse effects of nitrendipine was tested by withdrawal of the CCB after 6 weeks of therapy. Finally, we examined whether nitrendipine also exerts its adverse effects on renal function and structure when given to normotensive rats.

Methods

Animals and Protocol

Studies were performed in male Sprague-Dawley rats (Savo, Kisslegg, Germany) that had free access to tap water and standard rat chow (Sniff, Germany, containing 21% protein, 0.25% sodium, and 1% potassium). In rats weighing 120 to 140 g, two-kidney, one clip hypertension was induced. For this purpose, a rigid U-shaped silver clip (0.23 to 0.25 mm internal diameter) was placed around the right renal artery through a loin incision while the rat was under ketamine/xylazin anesthesia (Parke-Davis/Bayer, Berlin/Leverkusen, Germany; 100/10 mg/kg IM). The nonclipped kidney remained undisturbed. In sham-operated control rats, the same procedure was performed without application of a clip.

After 6 weeks without treatment, only hypertensive animals with systolic blood pressure (SBP) exceeding 165 mm Hg were
randomly divided into seven groups: (1) untreated hypertensive rats (HC, n=18), (2) untreated normotensive rats (NC, n=10), (3) rats treated with enalapril (100 mg/L drinking water, n=6; Merck Sharp & Dohme, Munich, Germany), (4) rats treated with nitrendipine (1 mg/g diet, n=12; Bayer, Leverkusen, Germany), (5) rats treated with enalapril and nitrendipine (ENP+NIT) (same dosage as in monotherapy, n=12), (6) rats treated with enalapril and hydrochlorothiazide (ENP+HCTZ) (enalapril same dosage as in monotherapy; hydrochlorothiazide, 50 mg/L drinking water, n=12; Sigma, Deisenhofen, Germany), and (7) rats treated with nitrendipine after 6 weeks therapy with nitrendipine (PNIT, n=9).

Normotensive control animals gained slightly more weight than hypertensive animals with or without therapy, so NC rats were food-restricted for 1 or 2 days per week.

In a pilot study we tested whether further blood pressure reduction could be achieved by the combination of nitrendipine and hydrochlorothiazide (nitrendipine, 1 mg/g food; hydrochlorothiazide, 50 mg/L drinking water, n=6). As has been shown in humans, the blood pressure-lowering effect of this combination was not significantly different from rats treated with nitrendipine alone (data not shown).

In addition, we tested whether nitrendipine exerts an effect on renal function and structure of normotensive animals as was observed in hypertensive animals. Therefore, normotensive rats without clip operation were housed for 6 weeks before being treated for 6 weeks with nitrendipine (1 mg/g food, n=6). The protocol and study design were identical to the rest of the study. Because normotensive animals treated with nitrendipine gained more body weight, an additional group of controls without food restriction (n=6) was studied for comparison with the nitrendipine-treated normotensive rats.

Before and during the treatment period, SBP was measured by tail-cuff plethysmography in awake rats. Animals were placed in individual metabolic cages (Ebeco, Castrop Rauxel, Germany), and 24-hour urine collections were made for determination of albuminuria. Albumin concentrations were measured by nephelometry (Beckman, Munich, Germany) using a rabbit anti-rat albumin antibody (Cappel Laboratories).

Renal Function
Six weeks after therapy, rats were anesthetized with thiopental (50 mg/kg IP) and placed on a heated operating table. A tracheostomy was performed, and polyethylene catheters were inserted into the right jugular vein and the ureter of the nonclipped kidney for solute infusion and sample collection. A cannula in the left carotid artery connected to a pressure transducer (Statham 23Db, Statham Laboratories, Hato Rey, Puerto Rico) allowed measurement of arterial blood pressure and collection of blood.

For replacement of fluid losses associated with anesthesia and surgery, animals received isoncotic albumin infusion with 1 mL/100 g body wt over 30 minutes. A bolus (0.2 mL/100 g body wt in 10 minutes) of a solution containing 3% inulin in 5% glucose was given, followed by an infusion rate of 0.6 mL/100 g per hour for a 60-minute control period to reach constant urine flow. Thereafter, two 30-minute clearance periods followed. Urine was collected from the left ureter in preweighed tubes, and urine volume was determined gravimetrically. Blood was collected in the middle of each clearance period for determination of inulin concentration, which was measured by anthrone reaction according to Führ et al.

Renal Morphology
At the end of the clearance periods, kidneys were removed and weighed. Kidney slices were fixed in 4% buffered formalin, embedded in paraffin, cut in 3- to 4-μm-thick sections, and stained with periodic acid–Schiff.

Histological studies were performed in a blind fashion. Glomerular injury was assessed by a semiquantitative score (grade 0 to 4) using the method of Raji and Olson as described earlier. Grade 1 represents involvement of up to 25% of the glomerulus, and grade 4 represents injury of 75% to 100% of the glomerulus. The number of glomeruli with a score of 1 was multiplied by 1, with a score of 2 by 2, 3 by 3, and 4 by 4. These numbers were summed and divided by the number of glomeruli assessed, including those with a score of zero.

Planimetric examinations of cross-sectional area were performed by means of a Zeiss drawing tube in combination with a semiautomatic interactive image-analysis system (Morphotomat 30, Zeiss, Oberkochen, Germany). Using a serpentine movement from cortex to medulla and vice versa, the outlines of 50 consecutively encountered capillary tufts were traced manually, and the mean glomerular random cross-sectional area (AG) was determined. The average glomerular tuft volume (VG) was then calculated according to Weibel as $VG = (\frac{\rho k}{\pi}) AG^2$, where $\rho = 1.38$ and $k = 1.1$ and are shape and size distribution coefficients, respectively.

In addition, the amount of tubulointerstitial damage of the clipped kidney (tubular atrophy and dilatation, interstitial fibrosis) was estimated by examining six fields (magnification x100) and semiquantitatively grading the degree of damage in each field using a scale of 0 to 4+. The mean of six determinations was calculated as an index in each tissue specimen. No tubulointerstitial abnormalities were found in normotensive controls. Therefore, statistical analysis was not performed in this group.

The guidelines of the American Physiological Society for experimental animal research were followed, and approval was obtained from the animal care committee at the University of Frankfurt.

Statistical Analysis
Data are presented as mean±SEM. Statistical analysis was performed with the Mann-Whitney-Wilcoxon test. In addition, the Kruskal-Wallis test was performed as a screening test, if appropriate. Values of $P<0.05$ were considered significant.

Results

Blood Pressure
The time course for changes in SBP before and during therapy is shown in Fig 1. At the time of randomization, SBP with renal artery stenosis was increased to 190±3 versus 114±4 mm Hg in sham-operated controls. After 6 weeks of therapy, enalapril lowered blood pressure significantly to 142±6 mm Hg, but not to normotensive control values (117±5 mm Hg). In contrast, there was no significant decrease of blood pressure after nitrendipine treatment (175±4 mm Hg) compared with HC rats (189±8 mm Hg). ENP+NIT and ENP+HCTZ decreased blood pressure to 118±3 and 115±2 mm Hg, respectively, which was not significantly different from NC rats. Seven days after withdrawal of nitrendipine, blood pressure was 170±7 mm Hg, which was not significantly different from the HC rats or nitrendipine-treated group.

Diuresis
As shown in Fig 2, urinary output throughout the course of therapy was significantly higher in nitrendipine-treated animals compared with all other groups. Although in the second and fourth weeks of therapy urinary output in rats treated with enalapril, ENP+NIT, and ENP+HCTZ was significantly increased compared with HC rats, during the sixth week, no significant difference was found. Urinary output of NC and HC rats was not significantly different. Seven days after withdrawal of nitrendipine, urinary output decreased to 17±2 mL/24 h,
Fig 1. Line graph shows time course of systolic blood pressure measured in conscious rats before and during therapy. *P<.005, **P<.001 vs hypertensive controls; +P<.0025 vs normotensive controls and nitrendipine+enalapril; P<.001 vs enalapril+hydrochlorothiazide.

which differed significantly from the nitrendipine-treated group during the sixth week of therapy (P<.001) but was not significantly different from the HC group.

Albumin Excretion

Albumin excretion is depicted in Fig 3. After 6 weeks of therapy, albuminuria in the nitrendipine-
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FIG 3. Bar graph shows albumin excretion of various groups after 6 weeks of treatment. NC indicates normotensive controls; HC, hypertensive controls; ENP, enalapril-treated group; NIT, nitrendipine-treated group; NIT+ENP, nitrendipine+enalapril-treated group; ENP+HCTZ, enalapril+hydrochlorothiazide-treated group; and PNIT, animals 7 days after withdrawal of nitrendipine. *P<.005, **P<.001 vs HC; +P<.01, ++P<.005 vs ENP.

Treated rats increased significantly (P<.005) compared with HC rats. There was a numerical decrease of albuminuria after enalapril treatment; however, this difference was not statistically significant compared with HC rats. The combination of nitrendipine and enalapril not only prevented the increase of albuminuria seen after nitrendipine alone but also decreased albuminuria significantly more than enalapril alone (P<.001 versus HC rats). A similar significant decrease of albuminuria was found with the combination therapy of ENP+HCTZ. Albuminuria of animals treated with ENP+NIT and ENP+HCTZ was not significantly different from normotensive controls but was significantly lower than enalapril alone. Seven days after withdrawal of nitrendipine, albuminuria significantly decreased compared with the nitrendipine group during the sixth week of therapy and was no longer significantly different from HC rats.

**Glomerular Filtration Rate**

As shown in Fig 4, glomerular filtration rate (GFR) factored by kidney weight was significantly higher in nitrendipine-treated rats (1.97±0.28 mL/min per gram kidney weight) than in HC rats (1.22±0.17 mL/min per gram). The combination of nitrendipine and enalapril significantly prevented this increase (1.7±0.29 mL/min per gram, not significant versus HC rats). No significant difference existed between HC rats and rats treated with enalapril (1.07±0.17 mL/min per gram) or ENP+HCTZ (1.5±0.17 mL/min per gram). After withdrawal of nitrendipine, GFR in these rats was numerically lower (0.91±0.12 mL/min per gram) than in HC rats; however, this difference was not significant. GFR of the enalapril-treated group (P<.05) and after withdrawal of nitrendipine (PNIT, P<.005) was significantly lower than in NC rats. When GFR was not factored by kidney weight, the values were for HC rats, 1.93±0.17; nitrendipine-treated rats, 3.88±0.52; ENP+NIT-treated rats, 2.89±0.35; and ENP+HCTZ-treated rats, 2.54±0.22 mL/min. The significance of the differences remained the same regardless of whether GFR data were factored by kidney weight.

**Glomerular Morphology, Nonclipped Kidney**

The effects of the different drugs on glomerular injury were similar to their effects on albuminuria. As shown in Fig 5, glomerular injury in HC rats was significantly higher compared with NC rats. Nitrendipine significantly increased glomerular lesions compared with HC rats, which persisted after withdrawal of nitrendipine. ENP+NIT not only significantly prevented the increase of glomerular injury but also decreased glomerular injury significantly compared with HC rats. Representative glomeruli with segmental injury from a nitrendipine-treated animal are

![Fig 4. Bar graph shows glomerular filtration rate factored by kidney weight of nonclipped kidney after 6 weeks of treatment. KW indicates kidney weight; NC, normotensive controls; HC, hypertensive controls; ENP, enalapril-treated group; NIT, nitrendipine-treated group; NIT+ENP, nitrendipine+enalapril-treated group; ENP+HCTZ, enalapril+hydrochlorothiazide-treated group; and PNIT, animals 7 days after withdrawal of nitrendipine. *P<.05 vs HC; #P<.005, $P<.05 vs NC.](http://hyper.ahajournals.org/)

![Fig 5. Bar graph shows glomerular injury score of nonclipped kidney in various groups. NC indicates normotensive controls; HC, hypertensive controls; ENP, enalapril-treated group; NIT, nitrendipine-treated group; NIT+ENP, nitrendipine+enalapril-treated group; ENP+HCTZ, enalapril+hydrochlorothiazide-treated group; and PNIT, animals 7 days after withdrawal of nitrendipine. *P<.05, **P<.001 vs HC; +P<.05, ++P<.01, +++P<.005 vs ENP; #P<.001 vs NIT.](http://hyper.ahajournals.org/)
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FIG 6. Top: Light micrograph of renal cortex shows two glomeruli with segmental glomerular injury from rat receiving nitrendipine. Bottom: Photomicrograph shows two glomeruli without noticeable glomerular changes from rat receiving nitrendipine and enalapril (periodic acid-Schiff stain).

showed in Fig 6, top, and glomeruli without noticeable structural changes from a rat receiving ENP+NIT in Fig 6, bottom. Enalapril did not influence glomerular changes, whereas injury was decreased significantly in the ENP+HCTZ-treated group compared with HC rats. Glomerular injury was not significantly different between ENP+NIT- and ENP+HCTZ-treated rats, but both groups had significantly less glomerular injury than enalapril alone.

Glomerular volume of HC rats (1.14±0.06 µm³×10⁶) was significantly increased compared with NC rats (0.95±0.04 µm³×10⁶) (Fig 7). Glomerular volume was significantly higher (P<.005) in the nitrendipine-treated group (1.63±0.12 µm³×10⁶) compared with HC rats. This increase persisted after withdrawal of nitrendipine (1.45±0.04 µm³×10⁶). The combination of enalapril and nitrendipine prevented the increase of glomerular volume significantly (1.16±0.07 µm³×10⁶, P<.01) compared with nitrendipine-treated rats or the PNIT group and was not significantly different from the HC group.

General Characteristics

There were no differences among the groups with regard to body weight (Table 1). Weights of the nonclipped kidneys of all treated and untreated hypertensive groups were significantly higher compared with NC rats, and no significant differences existed among the treatment groups. Weights of the clipped kidneys were significantly lower in the nitrendipine and ENP+HCTZ groups and after withdrawal of nitrendipine compared with NC rats. There was also a significant difference between the ENP+HCTZ-treated group and HC rats.

Morphology, Clipped Kidney

Table 2 shows morphological data of the clipped kidney. In the clipped kidney of HC and nitrendipine-treated rats, only few focal areas of tubular atrophy with accompanying moderate interstitial fibrosis and infiltration by mononuclear cells were found. In contrast, the clipped kidney of rats treated with enalapril, ENP+NIT, and ENP+HCTZ showed prominent and diffuse atrophy of tubules with flattened epithelium and widened or cystically dilated lumina occasionally containing cosinophilic hyaline casts. The interstitium was widened, fibrotic, and infiltrated. Periglomerular fibrosis and occasionally glomerular collapse were found. These findings are consistent with earlier reports of the effect of chronic CEI therapy on the clipped kidney in rats with renovascular hypertension.¹¹⁻¹³ No significant differences were found between HC rats and the treatment groups for glomerular volume of the clipped kidneys. The glomerular volume of the clipped kidney in the ENP+HCTZ-treated group was significantly lower than in the nitrendipine-treated group (P<.001).

Nitrendipine in Normotensive Controls

As shown in Table 3, no significant differences were found between normotensive rats treated with nitrendipine for 6 weeks and their controls for body weight, kidney weight, glomerular injury, glomerular volume, GFR, blood pressure, and albuminuria (6 weeks of therapy). However, nitrendipine significantly increased urinary output.

Discussion

This study demonstrates that the combination of nitrendipine and enalapril prevents the adverse effects of nitrendipine monotherapy on glomerular function.
TABLE 1. General Characteristics of Study Groups

<table>
<thead>
<tr>
<th>Kidney Weight, g</th>
<th>Body Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Normotensive controls</td>
<td>417±16</td>
</tr>
<tr>
<td>Hypertensive controls</td>
<td>442±16</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>400±10</td>
</tr>
<tr>
<td>Enalapril</td>
<td>440±15</td>
</tr>
<tr>
<td>Nitrendipine+enalapril</td>
<td>426±14</td>
</tr>
<tr>
<td>Enalapril+hydrochlorothiazide</td>
<td>415±11</td>
</tr>
<tr>
<td>Nitrendipine (withdrawal)</td>
<td>431±14</td>
</tr>
</tbody>
</table>

Withdrawal indicates animals 7 days after withdrawal of nitrendipine.

*P<.01 vs hypertensive controls, P<.001 vs all other groups.
†P<.05 vs normotensive controls.
‡P<.001 vs hypertensive controls.
§P<.05 vs hypertensive controls.

and structure in rats with hypertension-induced kidney disease. This combination was also superior to enalapril alone in reducing blood pressure and maintaining kidney integrity. The combination of enalapril and hydrochlorothiazide, however, was as effective with respect to blood pressure lowering, protection of kidney function, and preservation of morphology as nitrendipine and enalapril combined. Also, the increases in GFR, diuresis, and albuminuria after nitrendipine alone were reversible within a short period after withdrawal of the CCB. Finally, nitrendipine given to normotensive rats did not exert the adverse effects that were seen in the hypertensive rats.

Protective effects of CCB have been demonstrated in various animal models of renal disease. However, few studies found adverse effects of CCB on renal parameters. The effects of CCB on glomerular hemodynamics are variable, and this group of drugs may exert cytoprotective effects through prevention of calcium overloading, reduction of metabolic activity, decrease of free radical formation, and antiproliferative and antiplatelet aggregatory effects.

In addition to nonhemodynamic mechanisms, CEI may be beneficial in preventing the progression of renal disease by a hemodynamic mechanism, i.e., decrease of systemic blood pressure and lowering of glomerular capillary pressure. Because a CEI and CCB may protect renal function by different mechanisms, it might be useful to combine both drugs in the treatment of hypertension and chronic renal disease, especially in a model in which either drug alone was not successful in preventing progression of chronic renal failure.

In this study the combination of CEI and CCB was highly protective for the kidney. However, the addition of a diuretic to enalapril increased its effectiveness to a similar extent. Because triple therapy fails to normalize blood pressure in this model of renovascular hypertension, as we have shown previously, the role of blood pressure per se in the progression of renal disease in this model of hypertension is difficult to determine. It is unclear whether the protection afforded by combination therapy is achieved by blood pressure normalization or is due to the specific hemodynamic effect of enalapril of a decrease of glomerular capillary pressure. However, enalapril monotherapy failed to normalize blood pressure and also did not exert significant protective effects on the nonclipped kidney, whereas enalapril combined with a diuretic decreased both significantly; therefore, it seems that for CEI therapy, strict blood pressure control is an essential condition for inhibition of the progression of renal disease in this model of hypertension. It remains to be determined whether in other models of renal disease the theoretically attractive combination of a CEI and CCB will result in greater preservation of renal function over either agent alone or over the combination of enalapril with a diuretic, when normalization of blood pressure is also achieved by the respective monotherapies.

The beneficial effect of CEI in lowering blood pressure and preventing the progression of renal disease has been

TABLE 2. Morphology of the Clipped Kidney

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubulointerstitial Damage (Score)</th>
<th>Glomerular Volume, μm² x 10⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive controls</td>
<td>0.68±0.05</td>
<td>0.82±0.04</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>0.55±0.05</td>
<td>0.90±0.05</td>
</tr>
<tr>
<td>Enalapril</td>
<td>2.00±0.33*</td>
<td>0.73±0.06</td>
</tr>
<tr>
<td>Nitrendipine+enalapril</td>
<td>2.40±0.26†</td>
<td>0.74±0.03</td>
</tr>
<tr>
<td>Enalapril+hydrochlorothiazide</td>
<td>3.00±0.26‡</td>
<td>0.73±0.02†</td>
</tr>
</tbody>
</table>

*P<.01 vs hypertensive controls.
†P<.001 vs normotensive controls.
‡P<.01 vs nitrendipine-treated rats.
§P<.01 vs enalapril-treated rats.

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TABLE 3. Nitrendipine in Normotensive Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nitrendipine (n=6)</th>
<th>Controls (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>514±9.0</td>
<td>481±10</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>1.44±0.04</td>
<td>1.37±0.02</td>
</tr>
<tr>
<td>Glomerular injury, score</td>
<td>0.030±0.08</td>
<td>0.028±0.07</td>
</tr>
<tr>
<td>Glomerular volume, μm³ x 10⁶</td>
<td>1.34±0.11</td>
<td>1.26±0.06</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>107.5±5.0</td>
<td>118.8±3.0</td>
</tr>
<tr>
<td>Albuminuria, mg/24 h</td>
<td>0.41±0.09</td>
<td>0.32±0.12</td>
</tr>
<tr>
<td>Diuresis, mL/24 h</td>
<td>23.3±3.8*</td>
<td>13.3±0.9</td>
</tr>
<tr>
<td>Glomerular filtration rate, mL/min</td>
<td>2.33±0.27</td>
<td>2.66±0.24</td>
</tr>
</tbody>
</table>

*P<.025 vs controls.
demonstrated in many animal models (for review, see Reference 29). In most studies, CEI treatment was initiated shortly after the onset of renal disease. However, if therapy is initiated after a delay, as in the present study, CEIs are less effective in decreasing proteinuria, glomerular injury, and the progression of renal disease.30-34 Nonetheless, in two recently published studies CEI therapy failed to decrease or normalize proteinuria and glomerular damage despite the early start of therapy.35,36 Furthermore, suppression of the renin-angiotensin system by a high salt diet impairs37,38 whereas stimulation of the renin-angiotensin system by diuretics or salt restriction enhances the capacity of CEIs or angiotensin II antagonists to lower blood pressure and prevent the progression of renal disease.21,29 The angiotensin II antagonist saralasin strongly lowered blood pressure in rats with acute two-kidney, one clip hypertension, but the antihypertensive effect decreased with duration of hypertension, and saralasin was less effective in subacute and finally was ineffective in chronic renovascular hypertension, when plasma renin activity was lower than in the acute phase.40 Thus, it is not unexpected that in our study enalapril started 6 weeks after induction of renovascular hypertension lowered blood pressure only to a small extent and was without significant effect on albuminuria and glomerular injury. Addition of a diuretic enhanced the therapeutic potential of enalapril to lower blood pressure and decrease renal damage probably because of volume and/or sodium depletion with consequent stimulation of the renin-angiotensin system. A similarity can be found in the antihypertensive treatment of patients: black and elderly hypertensive patients tend to have a less-activated renin-angiotensin system and usually do not respond as well to CEI as do white or young patients with high blood pressure. However, combination with a diuretic improves the response of blood pressure to CEI in both populations.41

Besides the critical role of high blood pressure, important parameters that lead to the progression of chronic renal disease in rats are glomerular hyperfiltration and hypertrophy.14,42-44 These two factors are not invariably associated; an increase in GFR does not necessarily lead to the development of glomerulosclerosis unless there is a concomitant increase in glomerular size.43,45 Our experiments show persistence of high blood pressure, increased GFR, and increased glomerular volume in nitrendipine-treated animals. Therefore, these three variables have to be considered as possible mediators of the increased glomerular damage after nitrendipine monotherapy.

Because enalapril and nitrendipine combined lowered blood pressure and prevented the rise of GFR and glomerular volume, it is difficult to decide which factor was responsible for the increased albuminuria and glomerular injury after therapy with nitrendipine. Withdrawal of nitrendipine normalized GFR and albuminuria within days, whereas glomerular volume and blood pressure remained high, so it seems likely that the hyperfiltration per se is responsible at least in part for the increased albuminuria in nitrendipine-treated rats. In many experimental models of renal disease, glomerular sclerosis and proteinuria are associated with increased single-nephron GFR and two of its determinants, increased glomerular capillary pressure and flow, and changes in perselectivity. Attenuation of these alterations either by CEI or dietary protein restriction largely prevents proteinuria and the development of glomerular injury, indicating that hyperfiltration (and/or some hemodynamic determinant thereof) might play a role in the initiation and progression of glomerular disease.46,47

However, it should be mentioned that the role of hyperfiltration and glomerular hypertrophy in the progression of nondiabetic renal disease in humans remains to be determined.48,49 Although large randomized, controlled, and prospective clinical trials on the effect of blood pressure control on the course of renal function in patients with kidney disease are lacking, there is circumstantial evidence from epidemiologic and clinical studies that treatment of hypertension is the most important criterion for ameliorating the downhill course of renal function in patients with chronic renal failure.48,50,51

To our knowledge, this is the first experimental model of hypertension and renal disease in which an increase of glomerular volume after therapy with CCB is present. The mechanism of the increased glomerular volume in nitrendipine-treated rats remains unclear. However, some considerations apply. First, the increased glomerular volume is not a functional consequence caused by nitrendipine because it persisted after withdrawal of the drug. This is in accordance with the results of Yoshida and coworkers,43 who found no correlation between glomerular capillary pressure or single-nephron GFR and volume of individual glomeruli. Second, nitrendipine does not act as a pharmacologic growth factor for glomeruli in general, because the glomerular volume in the clipped kidney of the nitrendipine-treated rats and the glomerular size of normotensive rats were not increased after nitrendipine treatment. This is in agreement with the in vivo and in vitro data. In vivo studies found either no change52 or decrease of glomerular volume after therapy with CCB,14,43 and CCB inhibited synthesis of DNA and cell growth in mesangial cells53,54 and smooth muscle cells (for review, see Reference 55). However, one study found a weak enhancement of smooth muscle cell proliferation by nitrendipine.56

Whereas in normotensive and in the absence of afferent renal vasoconstriction CCBs have only marginal effects on renal hemodynamics, as shown in our nitrendipine-treated normotensive rats, their role in hypertensive animals and in states of angiotensin II-induced renal vascular dysfunction is different. In those pathophysiological states, CCBs are able to increase GFR by preferential preglomerular dilatation, whereas efferent arteriolar tone is preserved.57 It is therefore not surprising that nitrendipine increased GFR in the nonclipped kidney of Goldblatt rats, which have a high, probably angiotensin II-mediated, preglomerular resistance.58,59 The increase of GFR during chronic treatment in our study confirms the results of Huang,60 who found an increase of GFR in the nonclipped kidneys of anesthetized Goldblatt hypertensive rats after acute administration of nifedipine. Although an increase of GFR may be advantageous in many states of chronic renal disease, hyperfiltration could also be detrimental in the long term, especially if it is associated with an insufficient blood pressure control, as in the present study.42

Despite the increased glomerular damage, GFR in nitrendipine-treated animals was not impaired but even increased compared with all other groups. The effect of nitrendipine on the remaining intact glomeruli to hyperfiltrate probably compensated for the loss of filtering glomeruli during nitrendipine. However, after withdrawal of nitrendipine, the increased GFR was not only
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Wenzel et al. 1992;20:233-241


Acknowledgments

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References

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Because diuresis increased shortly after nitrendipine was started and decreased promptly after it was withdrawn, it seems unlikely that the increased diuresis is the consequence of structural alterations in the kidney caused by nitrendipine. Rather, it may be either a consequence of augmented filtered load secondary to increased GFR or a result of a direct effect of CCB on tubular reabsorption or both. Dihydropyridine CCBs also are known to increase urinary β2 microglobulin excretion, suggesting a decrease of tubular protein reabsorption, possibly caused by an increase in tubular flow. Such a mechanism also may contribute to the increase of albuminuria during nitrendipine in our study.

We feel that the results of this study confirm our previous hypothesis that the adverse effects of the CCB nitrendipine on glomerular function and structure are probably caused by preglomerular dilatation and insufficient blood pressure reduction, facilitating a rise of glomerular pressure. Because addition of a CEI, known for its specific glomerular pressure-lowering effects, and simultaneous blood pressure reduction to normotensive levels ameliorated the negative effects of nitrendipine. This hypothesis is further supported by the recent observation by Fenoy et al. of an increased glomerular capillary pressure after the CCB clentiazem and by the finding of an increased glomerular capillary pressure by Remuzzi and coworkers after treatment with nitrendipine in MWF/Ztm rats. Additionally, Benstein et al. recently described an increase of proteinuria and glomerular sclerosis after administration of the CCB nifedipine to rats with remnant kidneys and established injury.

In summary, the combination of nitrendipine and enalapril ameliorates the adverse renal effect of nitrendipine by lowering blood pressure to normotensive levels. Increased GFR, diuresis, and albuminuria after nitrendipine are reversible after withdrawal, whereas increased glomerular injury and glomerular volume persist. Nitrendipine does not exert its adverse effects on the kidney in normotensive animals. Addition of nitrendipine or a diuretic to enalapril increases its effectiveness in lowering blood pressure and protecting the kidney, and no significant difference exists with regard to blood pressure control and decrease of renal injury between both combination therapies. Therefore, it seems that strict blood pressure control, which is only obtained by a combination therapy including a CEI in this model of hypertension, is necessary for protection of the kidney.


Combination treatment of enalapril with nitrendipine in rats with renovascular hypertension.
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