Contrasting Short-Term Effects of Nifedipine on Glomerular and Tubular Functions in Glomerulonephritic Patients

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(J. Am. Soc. Nephrol. 1994; 5:1385-1390)

ABSTRACT
The short-term effects of nifedipine on glomerular hemodynamics, sieving function, and renal tubular function were assessed in 10 patients suffering from biopsy-verified chronic glomerulonephritis. Three weeks of nifedipine treatment after 2 wk of placebo significantly reduced the blood pressure from 153 ± 6/90 to 139 ± 6/84 mm Hg (mean ± SE; P < 0.05). Renal vascular resistance was reduced from 0.54 ± 0.10 to 0.46 ± 0.08 mm Hg/mL per minute. However, GFR (44.3 ± 7 mL/min), effective RPF (265 ± 37 mL/min), and filtration fraction (0.17 ± 0.01) remained unchanged. The excretion of albumin from 1.318 ± 395 µg/min was not affected by nifedipine. The glomerular sieving estimated by use of the fractional dextran clearance technique revealed no significant change by nifedipine compared with placebo in the range of 30 to 60 Å of hydrodynamic dextran radius. Fractional proximal reabsorption (lithium clearance method) was reduced by nifedipine from 53 ± 5 to 46 ± 4% (P < 0.05). Also, the excretion of β2-microglobulin and N-acetyl-β-glucosaminidase increased from 10.98 ± 4.62 to 11.86 ± 4.74 mg/24 h (P < 0.05) and from 19.7 ± 4.2 to 25.3 ± 7.0 nmol/h per micromoles of creatinine (P = 0.05), respectively. It was concluded that nifedipine treatment acutely represses proximal tubular function but is without significant effect on glomerular sieving and albuminuria in these patients.

Key Words: Dextran clearance, glomerular barrier, sieving function, lithium, proximal

Proteinuria is an important indicator of renal disease, and heavy proteinuria is by itself a negative prognostic factor with regard to the outcome of chronic glomerulonephritis (CGN) (1,2). ACE inhibitors may reduce the proteinuria in renal disease such as diabetic nephropathy and CGN (3-5). This has been attributed to the hemodynamic effects of these drugs of reducing glomerular capillary pressure by a preferential effect on the efferent arteriole (6). However, this view may not indiscriminately hold true (7,8). We found similar effects on renal hemodynamics with the ACE inhibitors lisinopril and nifedipine in a study on patients with diabetic nephropathy, but lisinopril alone reduced the proteinuria (4). Nifedipine reduced proximal tubular reabsorption, and any possible beneficial effect of nifedipine on glomerular proteinuria could be modified by a reduction in protein reabsorption.

A beneficial effect of ACE inhibitors on glomerular size selectivity has been found in patients with immunoglobulin A nephritis, indicating an effect of these drugs on effective glomerular pore size (9). To examine whether nifedipine might have a similar beneficial effect on glomerular barrier function, we studied the effect of nifedipine treatment on neutral dextran sieving in 10 glomerulonephritic patients with overt proteinuria. The albumin excretion rate, renal hemodynamics and parameters of tubular function were also measured for evaluation of the glomerular and tubular effects of nifedipine.

PATIENTS AND STUDY PROTOCOL

Patients
Ten patients, seven men and three women, ranging from 29 to 70 yr of age (mean, 53) with a diagnosis of CGN were enrolled in the study. The duration of the renal disease averaged 11.8 yr (range, 2 to 25 yr). A renal biopsy had previously been performed in all patients; seven were classified as mesangioliproliferative glomerulonephritis (nonimmunoglobulin A), and three revealed small or uncertain changes. Inclusion criteria were proteinuria exceeding 200 µg/min and an elevated diastolic blood pressure (DBP) between 85 and 115 mm Hg. Criteria for exclusion were serum creatinine over 300 µmol/L, sequela or symptoms of cardiovascular disease, clinically important abnormal laboratory screening tests, drug abuse, pregnancy, and intolerance to calcium channel antagonists (CCA). Patients from our outpatient clinic were asked to participate in the study, they were not randomized, and all patients received a normal diet; the excretion of sodium averaged 145 (range, 37 to 242) mmol/24 h over the last 48 h preceding the acute clearance study with placebo. During nifedipine treatment, the 48-h sodium excretion preceding the acute clearance study with nifedipine remained unchanged and averaged 141 (range, 93
Contrasting Effects of Nifedipine in Glomerulonephritis

The study consisted of a 2-wk washout period. If the inclusion criteria were fulfilled at this point, patients were given placebo for 2 wk and baseline studies were performed. The dose of nifedipine tablets was 10 mg twice daily during the first week and increased to 20 mg twice daily during the next 2 wk, unless the systolic blood pressure had fallen below 120 mm Hg. The tablets used were ordinary nifedipine tablets, not the sustained-release formula. Nine of 10 patients received the higher dose. The baseline studies were repeated after 3 wk of nifedipine treatment.

Renal clearance studies were performed with the patient in a fasting state and 1 to 2 h after the morning dose of placebo or nifedipine. The patients were given an oral water load of about 20 mL/kg, and the clearance measurements were performed with a urine osmolality below 200 mosm/kg. Plasma concentrations of lithium of about 0.3 mmol/L were obtained by giving 18 mmol of lithium citrate (Lithionit®) the evening before the clearance examination. Clearance of lithium and free water were used for the estimation of proximal tubular reabsorption (see below). GFR was calculated as inulin clearance at a plasma concentration of about 200 to 300 mg/L. Inulin was measured by the resorcinol method (10). Effective RPF (ERPF) was calculated from para-aminomellipurate clearance at a plasma concentration of 20 to 40 mg/L. Para-Aminomellipurate was determined as described by Smith et al. (11). After 1 h of equilibration, three clearance periods of 30 to 45 min were obtained by spontaneous voiding. Corresponding blood samples were taken both at the start and the end of each period, with the mean of the two used for the clearance calculations.

The sieving function was estimated by clearances of neutral dextrans with different sizes. After the priming dose of inulin, a dose of 130 mg/kg of Rheomacrodex (Kabi Pharmacia, Uppsala, Sweden) was infused iv over the course of 15 min. Rheomacrodex contains various-size dextrans, 90% ranging between 10,000 and 80,000 d (molecular weight) (corresponding to a hydrodynamic or viscosity radius of 27 to 75 Å). The clearance of dextrans of different sizes was calculated after size separation by size-exclusion chromatography of serum and urine samples on a XL 16/100 column with Sephadryl S-300 HR gel packed to a bed height of 93 cm (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). The sample size applied on the column was 1 mL both for serum and undiluted urine, each separated into 20 to 30 fractions and measured in the range of 30 to 60 Å of viscosity radius (each fraction contains dextrans within a range of 2 Å radius). Dextrans were hydrolysed with sulfuric acid and measured by the anthrone method as described by Scott and Melvin (12). The column was calibrated by means of a mixture of a broad spectrum of dextrans with a well-defined composition with integral calibration for all sizes examined.

The data were calculated by a data program (Sec-soft © L. Hagel) with a simple spread sheet (Excel) as previously described (13). Sodium and potassium were measured by flame photometry; lithium was measured by flame photometry of serum and atomic absorption of urine specimens.

Standard blood tests and two consecutive 24-h urine samples for analysis of albumin, enzymes, and other proteins were also examined before and during nifedipine treatment. Urinary albumin and β2-microglobulin were determined with a radiouimmunoassay kit (Pharmacia AB, Uppsala, Sweden). N-acetyl-β-glucosaminidase (NAG) was determined by the fluorometric method of Price and Dance (14). Urinary alkaline phosphatase (ALP) was determined with a commercial kit (Boehringer, GmbH Diagnostica, Mannheim, Germany). Normal values in our laboratory are as follows: NAG, 1.5–6.4 nmol/h per micromoles of creatinine; ALP, 0.114 to 0.568 U/mmol of creatinine. Creatinine was determined with a Beckman creatinine analyzer by a rate-dependent modification of the Jaffe reaction. The osmolality of urine and plasma was measured by freezing point depression with an osmometer (Hermann Roebing, Berlin, Germany).

Calculations

Standard clearance formulas were used without Donnan correction (15). The filtration fraction is given as GFR/ERPF. Mean arterial blood pressure (MAP) was calculated as DBP + 1/3(SBP - DBP), where SBP is systolic blood pressure. Renal vascular resistance (RVR) is given as MAP/ERPF. The lithium and free water clearance method was used to calculate the proximal reabsorption of sodium as previously described by Thomsen (16) and Koomans et al. (17). The following formula was used for the calculation of fractional proximal reabsorption FPR(Li) based on the lithium clearance data.

\[
\text{FPR(Li)} = \frac{\text{AR Li}}{\text{FR Li}} = 1 - \frac{C_{Li}}{C_{\text{inulin}}} \quad \text{(Equation 1)}
\]

where ARLi is the absolute reabsorption of lithium, FRLi is filtered lithium, C_{inulin} is the inulin clearance, and C_{Li} is the lithium clearance.

Fractional reabsorption

![Graph](image)

Figure 1. Fractional proximal reabsorption during placebo and during nifedipine treatment calculated from free water clearance and the lithium clearance method. The bars represent the mean value; the vertical lines represent one SD.
Fractional proximal reabsorption calculated with free water clearance \([\text{FPR}(\text{H}_2\text{O})]\) was based on the following formula:

\[
\text{FPR}(\text{H}_2\text{O}) = 1 - \frac{(\text{C}_{\text{Na}} + \text{C}_p)}{\text{C}_{\text{main}}} \quad \text{(Equation 2)}
\]

where \(\text{C}_{\text{Na}} + \text{C}_p\) is the sum of the sodium and potassium clearances and \(\text{C}_{\text{main}}\) is the free water clearance.

**Statistics**

Wilcoxon's two-tailed test for paired observations was used to evaluate the significance of differences between the placebo and the nifedipine treatment period (18). The same method was applied to evaluate differences between the observations for each of the calculated fractional dextran clearances from 30 to 60 Å of viscosity radius. Data are presented as mean ± SD if not otherwise stated.

**RESULTS**

**Effects of Blood Pressure and Heart Rate**

Table 1 shows the effects of nifedipine on SBP, DBP, calculated MAP, and heart rate. SBP was significantly reduced by an average of 14 ± 4 mm Hg \((P < 0.01)\), DBP was reduced by an average of 6 ± 2 mm Hg \((P < 0.01)\), and calculated MAP was reduced by 9 ± 3 mm Hg \((P < 0.001)\). Heart rate increased significantly by 10 ± 9 beats/min \((P < 0.01)\).

**Effects on Renal Hemodynamics and Sodium Handling During Acute Clearance Studies**

Table 2 shows that neither GFR, ERPF, nor the calculated filtration fraction were significantly affected by nifedipine treatment. However, because of the fall in MBP, the calculated RVR was reduced by almost 15% \((P < 0.05)\).

The excretion of sodium increased significantly after nifedipine without a significant increase in the filtered sodium load. Figure 1 shows that the fractional proximal reabsorption was reduced significantly as calculated from the free water concept and the lithium clearance method. The reductions were similar with both methods, but the free water concept yielded higher values for proximal reabsorption.

**Effect on Sieving of Neutral Dextrans During Acute Clearance Studies**

The upper part of Figure 2 shows the mean clearance values of dextrans relative to that of inulin in the 30 to 60 Å range of dextran viscosity radii. Each collected fraction corresponds to a range of 2 Å. No

**TABLE 2. Renal hemodynamics and sodium handling during acute clearance studies before and during nifedipine**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERPF (mL/min)</td>
<td>265 ± 37</td>
<td>273 ± 38</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>44 ± 7</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>RVR (mm Hg/mL per min)</td>
<td>0.17 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Filtered Sodium (mmol/min)</td>
<td>6.1 ± 0.9</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>Excreted Sodium (mmol/min)</td>
<td>0.38 ± 0.09</td>
<td>0.48 ± 0.10b</td>
</tr>
</tbody>
</table>

\(^a\) Values are mean ± SE.
\(^b\) \(P < 0.05\) versus placebo.

\(^c\) \(P < 0.001\) versus placebo.

Figure 2. The upper panel displays the fractional dextran clearance, e.g., the urine-to-plasma \((U/P)\) concentration of dextran relative to the \((U/P)\) ratio of inulin as a function of dextran size, shown on the abscissa. Dextran hydrodynamic (viscosity) radii are given in Ångstroms. The curve marked with filled boxes displays the placebo period, and the curve marked with open squares represents the nifedipine period. The horizontal bars on the curve represent 1 SD. The lower panel shows the variance in fractional dextran clearance between the nifedipine and placebo treatment for each patient. Values below zero indicate an increase in dextran clearance during treatment.

**TABLE 1. Effects of nifedipine on blood pressure and heart rate**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>153 ± 6</td>
<td>139 ± 6b</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>90 ± 3</td>
<td>84 ± 4c</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>111 ± 4</td>
<td>102 ± 4c</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>83 ± 5</td>
<td>93 ± 4d</td>
</tr>
</tbody>
</table>

\(^a\) Values are mean ± SE.
\(^b\) \(P < 0.01\) versus placebo.
\(^c\) \(P < 0.001\) versus placebo.

\(^d\) \(P < 0.05\) versus placebo.
significant change was found at any point between the fractional dextran clearance curve during placebo and nifedipine. The lower curve displays the differences in fractional dextran clearance during placebo and nifedipine for each patient. Values above zero indicate a reduction in dextran clearance after nifedipine; values below zero signify an increase.

Effect on the Excretion of Albumin, Enzymes, and Other Proteins

Table 3 reveals the mean of two consecutive 24-h urine measurements during placebo and during nifedipine treatment. The excretion of the albumin, total protein, and ALP remained unchanged. By contrast, the excretion of $\beta_2$-microglobulin increased significantly by about 10% ($P < 0.05$). The absolute excretion varied substantially between the patients, ranging from about 200 to 40,000 μg/24 h, but increased in 9 of the 10 patients. The fractional excretion of $\beta_2$-microglobulin (relative to creatinine) also increased by nifedipine in 9 of the 10 patients from 4.1 to 6.7% ($P < 0.05$). The plasma concentration of $\beta_2$-microglobulin varied between 2,000 and 6,000 μg/L. Finally, the increase in NAG excretion of almost 30% also reached the level of statistical significance ($P = 0.05$).

DISCUSSION

The novel finding in this study is that nifedipine was without effect on the glomerular sieving of neutral dextrans in patients suffering from CGN. The blood pressure-lowering effect of nifedipine was moderate, but of the same magnitude as previously reported to reduce RVR with short-term CCA therapy both in diabetic nephropathy (4) and in renal transplant recipients (19). The excretion of albumin also remained unchanged, consistent with the findings of Ikeda et al. (20). They found (in contrast to captopril) no effect of nifedipine/nicardipine on albumin excretion. However, reports on the effect of CCA on proteinuria in humans have been conflicting (4,21,22). Most long-term studies with dihydropyridines favor a neutral or sometimes even a beneficial effect on proteinuria (22,23). Also, the effects of CCA therapy on GFR and RBF in humans are variable, with no effect in normotensives but of the same magnitude as previously reported to sustain ERPF and GFR after the reduction in systemic blood pressure.

It is well known that ACE inhibitors universally reduce proteinuria both in diabetic and in nondiabetic renal disease (3-5,9,24,25). This protein-saving effect has been attributed to different mechanisms, especially, a lowering of the glomerular capillary pressure. It has recently been shown that the glomerular sieving function as assessed by clearance of neutral dextrans (26-28) is improved by ACE inhibitors in humans with glomerulonephritis (9). The dextran clearance values found in our patients were similar to those found by Remuzzi et al. (9), but in contrast to their findings, we found no improvement of glomerular sieving of dextrans by nifedipine in a range of hydrodynamic (viscosity) radius from 30 to 60 Å (corresponding to a maximum of 50 Å of Stokes radius). Data from previous investigations with this method have usually been given as Stokes radius in Ångstroms. However, as discussed in a previous report, the hydrodynamic radius is a more appropriate measurement of dextran size than is the Stokes radius with regard to the size of globular proteins (13). A slight trend toward an increased leak was noted at the highest value of dextrans (50 to 60 Å). Dextrans of larger size were not examined because a previous study has shown that the method yields uncertain clearance values beyond 60 Å of viscosity radius because of the very low concentration of high-molecular-weight dextrans in urine (13). However, when the change in dextran clearance induced by nifedipine is evaluated for each patient (Figure 2, lower panel), only 6 of the 10 patients showed an increased glomerular leakage in the range of hydrodynamic radius from 50 to 60 Å. Two patients revealed small changes, and two demonstrated an improvement of sieving function. One can argue that too few patients were examined to reveal significant differences in dextran clearance induced by nifedipine. However, an improvement of glomerular sieving function by an ACE inhibitor has been demonstrated in the same number of patients (9). Any possible effect of nifedipine on glomerular leakage is therefore minor as compared with the effect of an ACE inhibitor in patients suffering from glomerulonephritis. The increase or decrease in dextran clearance was not correlated to changes in the albumin excretion rate in each patient, as demonstrated with an ACE inhibitor (9). From our data, it seems obvious that no improvement of glomerular sieving function was found with nifedipine, and consistent with this, no change in proteinuria was seen. No data on the effect of nifedipine on the dextran clearance in a control

**TABLE 3. Effects of nifedipine on 24-h excretion of albumin and other proteins**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Nifedipine</th>
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<tbody>
<tr>
<td>Albumin (μg/min)</td>
<td>1,318 ± 395</td>
<td>1,490 ± 407</td>
</tr>
<tr>
<td>Total Protein (g/24 h)</td>
<td>3.63 ± 0.94</td>
<td>3.55 ± 1.06</td>
</tr>
<tr>
<td>$\beta_2$-microglobulin (μg/24 h)</td>
<td>10.982 ± 4.620</td>
<td>11.856 ± 4.742</td>
</tr>
<tr>
<td>NAG (nmol/h per μmol of creatinine)</td>
<td>19.7 ± 4.24</td>
<td>25.3 ± 7.0^a</td>
</tr>
<tr>
<td>ALP (U/mmol of creatinine)</td>
<td>0.24 ± 0.03</td>
<td>0.28 ± 0.04</td>
</tr>
</tbody>
</table>

^a Values are mean ± SE. Normal values in our laboratory are: NAG, 1.5 to 6.4 nmol/h per micromoles of creatinine; ALP, 0.114 to 0.558 U/mmol of creatinine.

^b P < 0.05 versus placebo.

Values are mean ± SE. Normal values in our laboratory are: NAG, 1.5 to 6.4 nmol/h per micromoles of creatinine; ALP, 0.114 to 0.558 U/mmol of creatinine.

*P = 0.05 versus placebo.*
group, for example, mildly hypertensive patients, are provided. However, the patients served as their own controls. With such a study design, short-term changes in dextran excretion may be revealed (9). An increased excretion of sodium without an increase in GFR might be ascribed to a reduction in proximal tubular reabsorption. A reduction of fractional proximal sodium reabsorption derived either from the free-water clearance or the lithium clearance was found during nifedipine treatment. As previously shown, the lithium clearance method gives the best estimate of fractional proximal sodium reabsorption (15,17,29,30). Both methods indicated a reduction in proximal tubular sodium reabsorption of about 10% by nifedipine. Consistent with an effect on proximal tubular cells, nifedipine also increased the excretion of \( \beta_2 \)-microglobulin and the lysosomal enzyme NAG (31–33). The inhibitory effects of nifedipine on proximal tubular function is consistent with previous findings both in diabetic nephropathy (4) and in hypertension, probably reflecting a general effect on the drug on tubular function (34). The effect on sodium balance during the 3-wk intervention with nifedipine revealed no change in 24-h sodium excretion or body weight and is therefore probably counterbalanced over days and weeks by compensatory mechanisms not elucidated in this study.

Given the limitations as discussed above, we conclude that short-term hypotensive treatment with nifedipine is without significant effect on glomerular sieving function and albumin excretion. By contrast, nifedipine acutely reduces RVR and increases sodium excretion, probably via an effect on proximal tubular function.

ACKNOWLEDGMENTS

The financial support from Bayer Norge A/S is gratefully acknowledged. The clinical project leader Dr. Jan Abelin, Bayer Sverige AB, is especially acknowledged. We also express gratitude to Jannicke Narverud, Jean Stenström, and Liv Nilsen for laboratory assistance.

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