Effects of low-dose nifedipine on urinary protein excretion rate in patients with renal disease

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Abstract

Background. The observation that proteinuria is an important determinant of the progression of renal disease has prompted numerous studies on the effects of antihypertensive agents on protein excretion. Reports on the proteinuric effects of calcium-channel blockers are quite controversial. It has been suggested that the short-acting dihydropyridine calcium-channel blocker nifedipine increases protein excretion by interference with tubular protein reabsorption.

Methods. In a randomized controlled trial 10 patients with renal disease and proteinuria were treated with a dose of 10 mg nifedipine o.d. (slow release formulation) for 1 week. The acute effects on renal and systemic haemodynamics and on urinary albumin, IgG, and β2-microglobulin excretion were investigated during a clearance study in the supine position after the first dose. After 1 week of treatment urinary protein excretion rates were measured in 24-h urine samples collected in the ambulatory patient in consecutive fractions of 4–8 h during normal daily activities.

Results. After the first dose nifedipine lowered mean arterial blood pressure in the supine position by 7±1 mmHg (<0.01), attenuated proximal tubular sodium reabsorption (fractional excretion of sodium 3.48±0.49 vs 2.62±0.35% during control, P<0.02), but did not affect proximal tubular protein reabsorption (fractional urinary excretion of β2-microglobulin 0.97±0.30 vs 0.98±0.32% during control, NS). The decrease in blood pressure was not accompanied by decreases in urinary albumin or IgG excretion rates. The selectivity index as well as GFR, RPF, and FF did not change. Continued treatment for 1 week with nifedipine did not influence 24-h protein excretion. However, we observed a rise of proteinuria during daily activities in the first 4 h after drug intake compared to the start of the study with the patients in supine position. During control measurements there was a slight increase in proteinuria. During nifedipine the increase in proteinuria was more marked and correlated with the selectivity index.

Conclusions. (1) Nifedipine 10 mg orally did not impair tubular protein reabsorption. (2) Nifedipine had no immediate antiproteinuric effect despite the observed blood pressure reduction. (3) Nifedipine increased proteinuria in ambulatory urine collections. This latter observation might explain the seemingly different effects of dihydropyridine calcium-channel blockers as reported in previous studies.

Key words: blood pressure; nifedipine; orthostasis; proteinuria; renal disease; selectivity

Introduction

Systemic hypertension and more specifically an increased intraglomerular pressure are thought to be important factors in the progression of chronic renal failure [1]. Proteinuria may be a reflection of the increased intraglomerular pressure [2]. Moreover, recent studies have demonstrated that proteinuria is an important determinant of the progression of renal insufficiency [3]. These observations have initiated numerous studies on the effects of antihypertensive drugs on proteinuria. The literature on the antiproteinuric effects of antihypertensive drugs has recently been summarized [4,5]. The available data demonstrate that nifedipine at most causes a relatively small decrease in urinary protein excretion even when lowering blood pressure to a degree comparable to other antihypertensive drugs. In some studies even an increase of proteinuria after administration of nifedipine has been observed. The reduction of proteinuria by antihypertensive drugs is most probably caused by a decrease of the intraglomerular pressure, which is the result of a lowering of the systemic blood pressure (β blockers) and/or of a preferentially dilatation of the efferent arteriole (ACE inhibitors). It is conceivable that the intraglomerular pressure may not be lowered during antihypertensive treatment with nifedipine, since calcium-channel blockers are known to blunt afferent vasoconstriction [6]. However, proteinuria is not only dependent on intraglomerular pressure but is also determined by the size and charge selectivity of...
the glomerular basement membrane and by tubular protein reabsorption. It is well known that calcium-channel blockers such as nifedipine cause natriuresis and diuresis. We and other investigators have demonstrated that nifedipine and other dihydropyridine calcium-channel blockers predominantly attenuate proximal tubular sodium reabsorption [7], thus acting at the site of tubular protein reabsorption [8]. Therefore it is conceivable that the reported effects of nifedipine on proteinuria are due to a decrease of tubular protein reabsorption.

In this short-term study we have investigated the effects of the dihydropyridine calcium-channel blocker nifedipine on urinary protein excretion in patients with renal disease and proteinuria, in relation to changes in blood pressure, renal haemodynamics, and urinary sodium excretion. Proteinuria was assessed in the supine as well as in the ambulatory patient. Albumin and IgG excretion rates were used as markers for glomerular permeability, and excretion rates of β₂-microglobulin were used for the assessment of tubular protein reabsorption.

Subjects and methods

Patients with a proteinuria of more than 1.5 g/24 h and an endogenous creatinine clearance (C_{cr}) of more than 60 ml/min participated in the study. Their renal function had been stable during the previous 3 months. Antihypertensive drugs and drugs interfering with renal function were discontinued in all patients at study entry. In one patient who had received a kidney transplant from his sister 18 months before, and who suffered from recurrence of a membranous glomerulonephritis, the immunosuppressive treatment with prednisone 10 mg o.d. and azathioprine 175 mg o.d. was continued. After a wash-out period of 2 weeks patients were randomized in a cross-over design in two groups. Patients were treated with slow-release nifedipine (Adalat Retard) 10 mg once daily for 1 week. Measurements (see below) were done after the first dose of the drug and at the end of the 1-week period. As control, similar measurements were done without drug intake. The interval between the two periods was at least 2 weeks. A dietitian instructed the patients to adhere to a diet with a stable protein and sodium intake. For reliable determination of urinary β₂-microglobulin 6 g of sodium bicarbonate were given on the days of urine collections.

The acute effects of nifedipine were studied during renal clearance studies starting at 8 o'clock in the morning. During the experiment patients were supine except when voiding. To ensure sufficient diuresis, initially an oral water load of 7 ml/kg body-weight was given. Subsequent urinary losses were replaced orally by tap water. Thereafter, intravenous catheters were placed to enable the infusion of inulin (lactofructosan, Inutest) and para-aminohippuric acid (PAH), and the withdrawal of blood. A first dose of 10 mg nifedipine was then given orally. After an equilibration period of 60 min four urine and four mid-point serum samples were collected in one 50-min and, subsequently, three 70-min intervals. Blood and urine samples were used for the determination of haematocrit, inulin, PAH, creatinine, total protein, albumin, IgG, and β₂-microglobulin. Blood pressure (BP) and heart rate (HR) were measured at 10-min intervals with an automatic device (Dinamap, Criticon Inc., Tampa, Florida). Mean values of the measurements obtained during all urine collection periods were used for analysis.

After the acute study patients were treated with nifedipine 10 mg once daily for one week. They were instructed to take the drug at 8 o'clock in the morning. Ambulatory urine collections were done during the last 2 days of the 1-week treatment period. Because of the short duration of action of nifedipine [9], collection periods of 24 h were divided in four consecutive fractions of 4, 4, 8, and 8 h respectively. Patients were asked to start the urine collections at 8 o'clock in the morning and to record the start and stop times of each collection period. In the urine samples creatinine, urea, sodium, total protein, albumin, IgG, and β₂-microglobulin were measured. Mean values of the measurements on these 2 days were used for analysis. Patients returned to the outpatient clinic 24 h after the last drug intake. BP and HR were measured for 10 min at 1-min intervals. Mean values of the last five measurements were used for analysis. Blood was drawn for the measurements of creatinine, sodium, total protein, albumin, IgG, and β₂-microglobulin.

Glomerular filtration rate (GFR) and renal plasma flow (RPF) were determined by calculating the renal clearance of inulin and PAH as described previously [10]. Filtration fraction (FF) was calculated as GFR/RPF. Fractional excretion of a substance was expressed as a percentage of GFR. The selectivity index was defined as the clearance of IgG divided by the clearance of albumin. Albumin and IgG in urine and serum were measured by immunonephelometry using specific antibodies raised in rabbits. β₂-Microglobulin was measured by radioimmunoassay (Pharmacia, Sweden). Other laboratory parameters were measured using standard (semi)automated techniques.

After testing for period and treatment period interaction, comparisons of treatment effects were done by analysis of variance [11,12]. Since the urinary excretion of proteins is non-parametric, statistical analysis on these data was done after log-transformation. Comparisons between protein excretion rates during the first 4 h of the clearance studies and the first 4 h of the ambulatory collected urine samples were done by Wilcoxon signed rank tests. Correlations were assessed by determining the Spearman rank correlation coefficient. A P value of 0.05 was considered as the level of statistical significance. Unless otherwise indicated values are given as means ± SEM.

The study protocol was approved by the hospital ethics committee. All patients gave written informed consent.

Results

We studied 10 patients (four males), two with chronic interstitial nephritis and eight with biopsy-proven glomerulonephritis. Their mean age was 37 ± 3 years. In the whole group urinary sodium and urea excretion rates at baseline averaged 198 ± 29 and 321 ± 25 mmol/24 h respectively. These values remained stable during the study. Further clinical characteristics are given in Table 1.

Acute effects of nifedipine administration

During the renal clearance studies nifedipine lowered mean arterial pressure (MAP) by 7 ± 1 mmHg (P < 0.01, Table 2). During the second and third hour
Fractional excretion of the diuretics did not significantly influence urine flow, No significant rise in heart rate was observed. During treatment with nifedipine and during control sodium reabsorption as reflected by the increase in MAP diuretics after drug administration the largest blood pressure protein excretion rates and their percentage changes collected over 24 h. No difference in proteinuria of the 4-h period immediately after drug intake for analysis. For comparison, we used the results obtained during the first four h of the acute clearance study in supine position (Table 3). Again, it is evident that despite the increase in urine flow and urinary sodium excretion, nifedipine did not increase urinary β₂-microglobulin excretion.

With respect to the other proteins, we observed that on control days, protein excretion was slightly higher when patients were ambulatory than when supine, the difference being significant for albumin. Notably, creatinine clearance was lower in five of the patients when ambulatory. The relative change in the urinary excretion of albumin correlated with the change in creatinine clearance ($r = +0.9273$, $P < 0.01$). During nifedipine the differences between ambulatory and supine proteinuria were more marked. The increase in albuminuria was more marked than the increase in IgG excretion, resulting in a significant decrease of the selectivity index (Table 3). During nifedipine, but not during control, negative correlations were found between the baseline selectivity index and the percentage changes in urinary protein excretion of sodium.

Table 2. Acute effects of nifedipine on renal haemodynamics and urinary protein excretion

<table>
<thead>
<tr>
<th></th>
<th>Nifedipine</th>
<th>Control</th>
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<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>103 ± 4</td>
<td>** 110 ± 4</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>67.7 ± 5.3</td>
<td>69.3 ± 6.4</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td>402 ± 37</td>
<td>357 ± 33</td>
</tr>
<tr>
<td>FF</td>
<td>0.18 ± 0.02</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>7.41 ± 1.14</td>
<td>7.08 ± 0.92</td>
</tr>
<tr>
<td>FE$_{\text{Na}}$ (%)</td>
<td>3.48 ± 0.49</td>
<td>* 2.62 ± 0.35</td>
</tr>
<tr>
<td>Fractional excretion of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (%)</td>
<td>0.171 ± 0.066</td>
<td>0.180 ± 0.074</td>
</tr>
<tr>
<td>IgG (%)</td>
<td>0.048 ± 0.018</td>
<td>0.047 ± 0.019</td>
</tr>
<tr>
<td>β₂-Microglobulin (%)</td>
<td>0.973 ± 0.303</td>
<td>0.984 ± 0.319</td>
</tr>
<tr>
<td>Selectivity index</td>
<td>0.264 ± 0.019</td>
<td>0.240 ± 0.020</td>
</tr>
</tbody>
</table>

Mean (± SEM) values of the four clearance periods of the renal clearance studies; *$P < 0.02$, **$P < 0.01$. MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; FE$_{\text{Na}}$, fractional excretion of sodium.

Discussion

The effects of antihypertensive drugs on proteinuria are of possible interest in view of the reported association between proteinuria and progression of renal insufficiency [3]. In this respect, the effects of calcium-channel blockers are equivocal, and in particular the results of studies assessing the impact of nifedipine seem rather conflicting [4,5]. We have studied the effects of nifedipine on urinary excretion of different proteins. We deliberately used the low dosage of 10 mg nifedipine to avoid major decreases in blood pressure and/or GFR, which could mask the effects of nifedipine on intrarenal haemodynamics or proteinuria.

Nifedipine clearly attenuated proximal tubular sodium reabsorption as reflected by the increase in fractional sodium excretion which occurred after drug intake on both day 1 and day 7. These data confirm the well-known natriuretic effect of the dihydropyridine calcium-channel blockers [7]. However, we could not demonstrate, either acutely or after 1 week of nifedipine treatment, any effect on the urinary excretion of β₂-microglobulin, a marker of tubular protein
Table 3. Urinary protein excretion rates during the first 4 h of the clearance studies (Cl study) and during the first 4 h of the ambulatory 24-h urine collections (4-h UC)

<table>
<thead>
<tr>
<th></th>
<th>Nifedipine Cl study</th>
<th>Nifedipine 4-h UC</th>
<th>Control Cl study</th>
<th>Control 4-h UC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supine</td>
<td>Ambulatory</td>
<td>Supine</td>
<td>Ambulatory</td>
</tr>
<tr>
<td>$\beta_2$M</td>
<td>162 $\pm$ 55</td>
<td>104 $\pm$ 38</td>
<td>175 $\pm$ 74</td>
<td>125 $\pm$ 63</td>
</tr>
<tr>
<td>Total protein</td>
<td>338 $\pm$ 74</td>
<td>$^*$</td>
<td>516 $\pm$ 93</td>
<td>418 $\pm$ 90</td>
</tr>
<tr>
<td>Albumin</td>
<td>284 $\pm$ 56</td>
<td>$^*$</td>
<td>441 $\pm$ 74</td>
<td>335 $\pm$ 67</td>
</tr>
<tr>
<td>IgG</td>
<td>17.1 $\pm$ 4.2</td>
<td>21.7 $\pm$ 5.3</td>
<td>17.1 $\pm$ 5.0</td>
<td>19.7 $\pm$ 5.5</td>
</tr>
<tr>
<td>SI</td>
<td>0.26 $\pm$ 0.02</td>
<td>$^*$</td>
<td>0.23 $\pm$ 0.02</td>
<td>0.24 $\pm$ 0.03</td>
</tr>
</tbody>
</table>

Results are given as means $\pm$ SEM; $^*P<0.05$ for Cl study vs 4-h UC; $\beta_2$M, $\beta_2$-microglobulin (µg/mmol creatinine); Total protein, Albumin, and IgG excretion rates (mg/mmol creatinine); SI, selectivity index.

Fig. 1. Relationship between the selectivity index at baseline and the relative increase in urinary albumin excretion in nifedipine-treated patients.

Reabsorption [13]. Therefore, an effect of nifedipine on tubular protein reabsorption is unlikely. Our findings seemingly contrast with the results of studies by other investigators [14–16], who reported an increased excretion of urinary $\beta_2$-microglobulin after administration of nifedipine. However, in one of these studies the increase in urinary excretion of $\beta_2$-microglobulin was not significant [14], and in the study of Hartmann et al. [15] the increase was only 10%, and may have to be explained by a period effect. A definite increase of $\beta_2$-microglobulin excretion was observed by Krusell et al. [16] However, in this study the increase in the urinary excretion of $\beta_2$-microglobulin paralleled the abrupt increase in urinary flow, suggesting that the increased excretion was caused by a wash-out of urine. Overall, we feel that the arguments in favour of an effect of nifedipine on tubular protein reabsorption are weak.

Blood pressure decreased after the first dose by 7 $\pm$ 1 mmHg. GFR, RPF, and filtration fraction remained stable. Similar renal effects with higher dosages of nifedipine have been reported by others [17]. The decrease in blood pressure did not cause a decrease of urinary protein excretion. In contrast, ACE inhibitors such as captopril, acutely lower blood pressure and proteinuria [18]. These data suggest that during nifedipine treatment the decrease of systemic blood pressure in the supine position does not result in a drop of the intraglomerular pressure. These findings are in line with the observation that calcium-channel blockers preferentially cause vasodilatation of the afferent arteriole, thus facilitating a transmission of the systemic pressure to the glomerulus [6].

Because of the low, once daily, dose and the short duration of action of nifedipine [9], changes in urinary sodium and protein excretion may remain undetected if only total 24-h urine is measured. Therefore ambulatory urine collections were done in several consecutive fractions. Indeed, during the first 4 h after intake of the drug urinary sodium excretion rates and urine flow were higher, while they returned to control values during the subsequent collection periods.

Compared to the first 4 h of the clearance studies in the supine position and despite a decrease of the creatinine clearance in part of the patients, a limited increase of ambulatory urinary protein excretion rates was seen during the control period. These changes in urinary protein excretion were strongly related to changes in creatinine clearance confirming results of another study on proteinuria during orthostasis [19]. However, in this study most subjects showed a decrease in proteinuria together with a decrease in creatinine clearance. When using nifedipine, the increase of ambulatory protein excretion became more prominent. This again could not be explained by an attenuation of proximal tubular protein reabsorption as evidenced by the data on $\beta_2$-microglobulin excretion. How can we then explain this effect of nifedipine? In healthy volunteers a decrease of protein excretion in the upright position was related to creatinine clearance [19], whereas other studies carried out after prolonged standing [20] or gentle walking [21] reported an increase in proteinuria. Most probably the increased proteinuria results from angiotensin-II-mediated renal haemodynamic changes, with a decrease of GFR and RPF and a marked increase in filtration fraction. In patients with proteinuria a decrease in urinary protein excretion is seen in the upright position [22,23]. The changes in proteinuria are correlated with changes in creatinine clearance, thus demonstrating that the
The "orthostatic effect" of standing, leading to an increased filtration fraction, is prevented by an even larger drop in glomerular filtration. This suggests that in these patients standing causes profound vasoconstriction of the afferent arteriole which may result from an increased sympathetic tone. In such a case, it is understandable that nifedipine increases the orthostatic proteinuria by attenuating this afferent vasoconstriction.

Compared to the first 4 h of the clearance studies in the supine position, nifedipine-induced changes in urinary excretion of albumin, IgG and total protein during the ambulatory collection periods were inversely related to the basal selectivity index. One may argue that patients with a lower selectivity index have less damaged glomeruli with better preserved afferent arteriolar vasotonus. Subsequent reduction of afferent vasoconstriction by nifedipine may then result in a larger increase of urinary protein excretion. Possibly, a higher dose with subsequently a more profound antihypertensive effect might counteract this enhanced transmission of systemic blood pressure to the glomerular capillaries. In contrast, patients with a higher selectivity index may have more severely damaged and hyperfiltrating nephrons with vasodilated afferent arterioles. In this case, treatment with nifedipine cannot affect afferent vascular tone, and changes in intraglomerular pressure and proteinuria will depend mainly on the degree of the antihypertensive effect.

In conclusion, tubular protein reabsorption as measured by β2-microglobulin excretion, was not impaired by nifedipine treatment. The reduction of systemic blood pressure after administration of the first dose was not accompanied by a decrease in urinary protein excretion. Compared to supine rest, higher protein excretion rates were seen in ambulatory collected urine after 1 week of treatment with nifedipine, and these were inversely related to the selectivity index. We suggest that these higher protein excretion rates are a consequence of an enhanced transmission of systemic blood pressure to the glomeruli by nifedipine-induced afferent vasoconstriction. Further studies are warranted to elucidate the role of orthostasis and physical activity on proteinuria during dihydropyridine treatment.

References