Renoprotective Role of Nifedipine During Gentamicin Therapy: Randomized Controlled Trial

Jadranka Vlašić-Matas, Zvonko Rumboldt, Deni Karelovich

Divisions of Nephrology and Pharmacology at Department of Internal Medicine, and Department of Obstetrics and Gynecology, Split University Hospital and School of Medicine, Split, Croatia

Aim. To investigate the protective effect of nifedipine, a dihydropyridine calcium-channel blocker, on renal function (glomerular and tubular) in patients treated with gentamicin, an aminoglycoside antibiotic.

Methods. Thirty-two patients with gentamicin-sensitive upper urinary tract infection have been screened and randomized to two groups. The placebo group was given gentamicin and placebo, and the intervention group gentamicin and nifedipine. Gentamicin was given in slow intravenous injection every 12 hours for 10 days, and nifedipine 10 mg orally, 3 times a day.

Results. Nifedipine administration during gentamicin therapy promoted primarily the glomerular filtration. In 62% of the patients treated with nifedipine, creatinine clearance increased significantly by the end of the study. In the placebo group, 69% of the patients had a creatinine clearance significantly below the baseline at the end of the study. The decrease in creatinine clearance by more than 50% from the initial values was found in 2 patients (1 in each group). There was a significant increase in γGT/creatinine clearance ratio in both groups at the end of therapy, indicating that nifedipine did not prevent the brush-border membrane enzyme release caused by gentamicin.

Conclusion. Nifedipine has positive effects on renal hemodynamics in patients treated with gentamicin. Most likely, the mechanism of action is an increase in glomerular filtration caused by preglomerular vasodilation.

Key words: gentamicin; glomerular filtration; nifedipine; randomized controlled trials; tubular toxicity

The role of calcium-channel blockers as protective and therapeutic agents has been examined in a number of studies. It is well known that calcium antagonists cause renal vasodilation, which is greatly dependent on the actual vascular tone (1). Recent studies indicate that calcium antagonists selectively vasodilate preglomerular arterioles, leading to an increase in renal blood flow, glomerular filtration rate, and glomerular pressure (2). In contrast to ACE inhibitors and other vasodilator agents, calcium-channel blockers primarily attenuate the component of renal vascular resistance responsible for autoregulation (2). Calcium antagonists also block the afferent arteriolar vasconstrictor elicited by angiotensin II, and do not influence the efferent arteriolar vasoconstriction evoked by this peptide. These data support the premise that there is a fundamental difference between the intracellular calcium activation mechanisms in afferent and efferent arterioles, with voltage-gated channel being of predominant importance in afferent arterioles (2-5).

Vasoconstrictor responses mediated by tubuloglomerular feedback are abolished by calcium antagonists, indicating that the tubuloglomerular feedback effector mechanism may require transmembrane calcium influx into the smooth muscle cells of the afferent arterioles (2-5). However, unlike other vasodilators, calcium antagonists also prevent sodium retention and facilitate renal vasodilation (6). The cytoprotective effect of calcium-channel blockers is another feature of potential clinical relevance (7). There are two major mechanisms by which these agents may act protectively. Firstly, they increase blood flow by counteracting vasoconstriction, and thus the oxygen supply to the injured tissue. This is the protective action against cyclosporin-induced vasoconstriction in transplant recipients, but may also apply to renal-artery stenosis and contrast-mediated nephrotoxicity. Secondly, calcium antagonists may protect against cellular calcium "overload". This is relevant to settings such as acute renal failure, tubular necrosis, and other forms of renal drug toxicity (7,8).
The role of calcium-channel blockers in reducing aminoglycoside-induced acute renal failure is unclear, because of contradictory results from experimental studies. The dihydropyridine derivative nifedipine was shown to have protective effect in a rat model of gentamicin-induced nephrotoxicity (9).

The present study aimed to evaluate the effects of a dihydropyridine calcium-channel blocker, nifedipine on functional renal values (glomerular and tubular) in patients exposed to gentamicin.

**Patients and Methods**

The study was performed at the Department of Nephrology, Split University Hospital, from April 1994, until June 1995. It was a randomized, prospective, double-blind study, which took ten days per patient. The Hospital Ethics Committee approved the study protocol.

**Study Protocol**

Hospitalized patients of both sexes, aged 18-80, with gentamicin-sensitive infection in upper urinary tract were included in the study. All patients had flank pain and/or costovertebral angle tenderness; temperature higher than 38.0°C, and pyuria.

The patients with a creatinine clearance under 30 ml/min.; patients on ACE inhibitor therapy, and rheumatic and other drugs that could detent renal function were not included in the study. Furthermore, individuals with active heart failure, ischemic heart disease, and gout as well as patients with symptomatic angina pectoris were not included in the study.

All 34 patients who met the entry criteria and provided informed consent were included in the study.

**Assignment**

For randomization, we used sealed envelopes, which contained either placebo or nifedipine. The envelopes were numbered from 1 to 34 and each contained 30 tablets. Half of the envelopes contained placebo, and the other half nifedipine. Before the beginning of the therapy, the samples for bacterial analysis were taken and standard laboratory tests (urea, creatinine, and electrolytes) were performed. The urine samples for functional renal tests (creatinine clearance, osmolality, total proteins, β2-microglobulin, γ-GT, Na, K in 24 h urine) were collected within 24 hours from the onset of the therapy.

The gentamicin serum level was measured on day 3 and day 8 of the therapy, whereas the biochemical and urinary tests were repeated at the end of the study (Fig. 1).

**Clinical Parameters**

Clinical parameters, including arterial blood pressure and diuresis, were monitored daily.

**Analytical Methods**

Blood samples for the determination of gentamicin level were taken before the morning dose (concentration at the end of the aminoglycosic interval). Analysis was done by HPLC (flame photometric detection) on the Abbott TDx apparatus. Gentamicin was administered by slow intravenous injection (over 3 min.), every 12 hours for 10 days. In patients over 60 years of age, and those with impaired renal function (serum creatinine level greater than or equal to 120 μmol/l; or creatinine clearance less than or equal to 60 ml/min) the daily dose was 3 mg/kg, whereas in patients with normal renal function it was 4 mg/kg. Dosing was later modified according to creatinine clearance (standard nomograms) and the serum drug concentration (10).

All patients were on a free, salt-unrestricted diet. Prior to the initiation of therapy the samples for bacterial analysis were taken and standard laboratory tests (urea, creatinine, and electrolytes) were performed. The urine samples for functional renal tests (creatinine clearance, osmolality, total proteins, β2-microglobulin, γ-GT, Na, K in 24 h urine) were collected within 24 hours from the onset of the therapy.

The gentamicin serum level was measured on day 3 and day 8 of the therapy, whereas the biochemical and urinary tests were repeated at the end of the study (Fig. 1).

**Statistical Analysis**

Results were analyzed by use of Statistica for Windows, release 5.0 (StatSoft, Inc, Tulsa, OK, USA). For statistic analysis we used Chi-square test, Mann-Whitney's test, and Wilcoxon's test, depending on the nature of the data (12). p-values lower than 0.05 were considered significant.
Results

Thirty-two patients were included in the study, 16 treated with gentamicin and placebo (placebo group), and 16 with gentamicin and nifedipine (intervention group). There were 9 women and 7 men in the placebo group, and 5 women and 11 men in the intervention group, acceptably homogeneous according to sex (P²=1.14; p=0.28). Other clinical data of the patients are presented in Table 1.

On day 10, urea decreased in both groups. This reduction was statistically significant in the intervention group (p=0.014), but not in the placebo group (p=0.064). Similarly, the creatinine levels in both groups decreased, but this drop was again statistically significant in patients from the intervention group (p=0.039) (Fig. 2).

A slightly increased creatinine level at the end of the therapy was found in 5 patients in the placebo group, and in 4 patients in the intervention group. The highest creatinine increase in one patient in the placebo group and in the intervention group was 35 µmol/L and 31 µmol/L, respectively, neither of which fulfilled the criteria for a nephrotoxic reaction (13).

Intravenous creatinine clearance values at the onset of the study were similar in both groups (Table 1). In the placebo group, the creatinine clearance was lower (p=0.027) at the end of therapy, whereas in the group receiving gentamicin and nifedipine (Fig. 3) there was an increase (p=0.035). The decrease in the creatinine clearance was found in 11/16 of the patients in the placebo group. A deterioration of more than 50% (48 mL/min decrease) was found in a single patient. The intervention group showed a decrease in creatinine clearance in 6/16 of the patients. A patient in this group had a deterioration of more than 50% (54 mL/min decrease) as well. Urinary β₂-microglobulin and 24 h proteinuria were similar in both groups at the end of the therapy (Table 2). Urine osmolality showed no significant difference between the two groups. Natriuria in the placebo group decreased (p=0.540), whereas it sig-

### Table 1. Baseline age (years), blood pressure (BP, mmHg) and body weight (kg) of the study patients (mean±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (n=16)</th>
<th>Intervention (n=16)</th>
<th>Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.5±10.5</td>
<td>57.5±10.7</td>
<td>1.870</td>
<td>0.071</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>138.2±21.9</td>
<td>149.1±26.5</td>
<td>1.170</td>
<td>0.240</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>85.9±13.3</td>
<td>90.9±11.1</td>
<td>0.660</td>
<td>0.510</td>
</tr>
<tr>
<td>Body weight</td>
<td>75.1±11.5</td>
<td>78.6±16.4</td>
<td>0.396</td>
<td>0.692</td>
</tr>
</tbody>
</table>

*Mann-Whitney's test.*

### Table 2. Urinary tests in the placebo group (patients treated with gentamicin and placebo) and intervention group (patients treated with gentamicin and nifedipine) before and after therapy (mean±SD)

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Parameters in 24h urine</th>
<th>before</th>
<th>after</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo group</td>
<td>Creatinine clearance (mL/min)</td>
<td>67.2±24.7</td>
<td>57.2±23.7</td>
<td>2.21</td>
<td>0.027*</td>
</tr>
<tr>
<td></td>
<td>Osmolarity (mOsm/kg)</td>
<td>421.9±121.5</td>
<td>474.8±158.5</td>
<td>0.84</td>
<td>0.400</td>
</tr>
<tr>
<td></td>
<td>β₂-microglobulin (mg/day)</td>
<td>13.1±19.7</td>
<td>10.3±16.4</td>
<td>0.28</td>
<td>0.776</td>
</tr>
<tr>
<td></td>
<td>Proteinuria (mg/day)</td>
<td>813.1±660.0</td>
<td>562.0±730.0</td>
<td>1.66</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td>Na⁺ (mmol/day)</td>
<td>138.1±78.5</td>
<td>119.6±48.0</td>
<td>0.62</td>
<td>0.540</td>
</tr>
<tr>
<td></td>
<td>K⁺ (mmol/day)</td>
<td>37.6±16.6</td>
<td>43.4±21.0</td>
<td>1.36</td>
<td>0.170</td>
</tr>
<tr>
<td></td>
<td>γGT/creatinine clearance</td>
<td>2.0±2.1</td>
<td>2.5±1.2</td>
<td>2.6</td>
<td>0.010*</td>
</tr>
<tr>
<td></td>
<td>Diuresis (mL/day)</td>
<td>1,350.0±699.7</td>
<td>1,503.1±569.3</td>
<td>1.55</td>
<td>0.121</td>
</tr>
<tr>
<td>Intervention group</td>
<td>Creatinine clearance (mL/min)</td>
<td>67.8±25.2</td>
<td>73.1±32.0</td>
<td>2.20</td>
<td>0.035*</td>
</tr>
<tr>
<td></td>
<td>Osmolarity (mOsm/kg)</td>
<td>450.3±166.7</td>
<td>416.9±151.2</td>
<td>1.38</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>β₂-microglobulin (mg/day)</td>
<td>8.0±12.0</td>
<td>5.2±6.6</td>
<td>0.78</td>
<td>0.433</td>
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<tr>
<td></td>
<td>Proteinuria (mg/day)</td>
<td>626.0±250.0</td>
<td>510.0±300.0</td>
<td>0.77</td>
<td>0.440</td>
</tr>
<tr>
<td></td>
<td>Na⁺ (mmol/day)</td>
<td>128.6±65.7</td>
<td>158.3±16.6</td>
<td>2.02</td>
<td>0.044*</td>
</tr>
<tr>
<td></td>
<td>K⁺ (mmol/day)</td>
<td>36.7±13.6</td>
<td>46.1±14.9</td>
<td>2.02</td>
<td>0.044*</td>
</tr>
<tr>
<td></td>
<td>γGT/creatinine clearance</td>
<td>1.0±1.1</td>
<td>1.5±0.7</td>
<td>2.06</td>
<td>0.039*</td>
</tr>
<tr>
<td></td>
<td>Diuresis (mL/day)</td>
<td>1,368.8±736.6</td>
<td>1,578.1±498.3</td>
<td>2.44</td>
<td>0.015*</td>
</tr>
</tbody>
</table>

*Wilcoxon’s test.  
*Asterix denotes statistically significant difference.*
creatinine (shown in only 30% of patients). This can be
associated with the rise in serum creatinine clearance in the
placebo group. On day 10, there was a significant increase in
creatinine clearance ratio in placebo (p=0.010) and intervention
(group=p=0.039) (Table 2).

Discussion
Due to their nephrotoxicity, aminoglycosides have been widely studied for many years. Most studies have reported 10-25% incidence of aminoglycoside-related nephrotoxicity, even when drug levels were maintained within the accepted therapeutic range.

In the study published by Smith et al., (13) nephrotoxicity, defined as an increase in serum creatinine above 44.2 µmol/L or as a decrease in creatinine clearance of at least 50% from the baseline was similar in both patient groups in our study. The increase in the serum creatinine was slight, never exceeding 35 µmol/L above the baseline value, whereas the decrease in the creatinine clearance above 50% of the baseline value was found in only 2 patients, one in each group.

Probably because we studied patients with acute upper urinary tract infection, the mean values of urea and creatinine at the start of therapy were higher than at the end of therapy. However, such a decrease was significantly higher in the group of patients who received nifedipine than in the placebo group.

We have also observed a significantly higher creatinine clearance in the intervention group, which can be explained by improved glomerular filtration due to nifedipine. On the other hand, the placebo group showed a significant decrease in creatinine clearance. This significant decrease in creatinine clearance in the placebo group was not associated with the rise in the serum creatinine (shown in only 30% of patients). This can be explained by the renal function reserve, which prevents the plasma creatinine increase until the glomerular filtration rate falls below 30 ml/min.

Our results are comparable to those obtained by Lee et al. (9,14), who demonstrated that nifedipine protects from the gentamicin nephrotoxicity. Administration of nitrendipine to gentamicin-treated animals preserved effective glomerular filtration, tubular function, and morphology (9,14) in those animals.

Aminoglycosides cause cellular injury by interfering with the cellular and subcellular plasma membrane transport. As aminoglycosides are taken up into the proximal renal tubule cells, they cause mitochondrial calcium overload with consequent mitochondrial damage. Decreased mitochondrial cell respiration is one of the key steps toward the loss of cellular homeostasis and eventual cell death.

Primary decline in the glomerular filtration rate due to decreased renal blood flow and/or reduced glomerular capillary ultrafiltration coefficient (Kf) is thought to be the principal mechanism for gentamicin-mediated acute renal failure. Tubular obstruction and back-leakage of the filtrate occurs when tubular necrosis is already developed, usually due to medicament overdose (16,17).

Mediators of decreased renal blood flow in acute renal failure remain controversial, but suggested factors are increased adrenergic activity, altered vascular reactivity, renin-angiotensin stimulation, tubuloglomerular feedback, elevated plasma levels of vasopressin, enhanced synthesis of thromboxane, and many others.

To explain the initiation of acute renal failure, the tubuloglomerular feedback hypothesis has been proposed by Thrail’s group (18). Ischemic and toxic cell injury affects transport activity at proximal tubules, thereby increasing the diuresis and urine output, thereby increasing the diuretic stimulus to the renal tubules, thereby decreasing the diuretic stimulus to the renal tubules, thereby decreasing the diuretic stimulus to the renal tubules.

Because angiotensin II is known to reduce Kf, there is a possibility that the renin-angiotensin system might...
be important in the pathogenesis of gentamicin-induced acute renal failure. Support for renin involvement stems from observations that renal renin activity is increased in experimental models of aminoglycoside-induced acute renal failure. Plasma renin activity inversely correlates with glomerular filtration after gentamicin administration and the plasma renin returns to normal values after recovery from acute renal failure (16).

Attempts by Luft and co-authors (16) to block the renin-angiotensin system by captopril failed to ameliorate either the tubular or glomerular morphological alterations associated with gentamicin administration. Contrary to this, Schor and al (19) reported earlier that captopril ameliorated both the decreasing glomerular filtration rate and the decrease in K1 induced by gentamicin in experimental conditions. However, a further study by Morin and al (20) documented that concurrent treatment with an ACE inhibitor (perindopril) and gentamicin produced more pronounced renal impairment than the administration of gentamicin alone.

Recent experimental studies of aminoglycoside-induced nephrotoxicity have focused on the role of calcium (which has been shown to have both beneficial and detrimental effects). Humes and al (21) have demonstrated that a diet extremely rich in calcium protects against gentamicin nephrotoxicity, as it was measured by both functional and biochemical indices of renal cell injury.

Since calcium is an effective competitive inhibitor of aminoglycoside binding to biological membranes, oral calcium supplementation was used to increase the delivery of aminoglycoside to the target site of these agents without affecting renal function (21).

According to some authors, even calcium antagonists provided cellular protection through the competition for common transport systems on the brush border membrane, thus decreasing binding and internalization of gentamicin into proximal tubule cells (14, 22).

Mild toxic injury, usually not apparent at the light microscopic level, may cause early ultrastructural transformation of the brush border and release of peripheral membrane surface components into urine (21). In our study, γGT membrane enzymuria was chosen as an index of early tubular toxicity. To allow for alterations in GFR, the urinary concentration of γGT was expressed as a ratio of the creatinine clearance, because the amount of γGT present in normal urine is proportional to the mass of the functioning nephrons (11). During our study, a statistically significant increase in this ratio was observed in both groups. Thus nifedipine did not prevent membranous enmeshed shedding caused by gentamicin.

A vast number of studies reported on the diuretic and natriuretic effect of nifedipine (6, 23, 24). Different classes of calcium channel blockers do not seem to share similar properties in their capacity to increase diuresis or natriuresis, even within the dihydropyridine group (23). Chellingsworth and Kendall (24) compared the diuretic and natriuretic effects of verapamil, diltiazem, nifedipine, and placebo in normal subjects. All four drugs exerted a natriuretic effect, but nifedipine produced significantly greater natriuresis than placebo and verapamil. This natriuretic/diuretic effect of nifedipine was confirmed in our study. Our intervention group (gentamicin-nifedipine) showed significantly higher natriuresis and diuresis on day 10 when compared with initial values. The renal mechanism by which this natriuresis is produced has not been completely understood yet. Several studies have provided evidence that dihydropyridine calcium channel blockers directly affect tubular transport. The exact site of action of these agents is not entirely clear (23).

In conclusion, the results of our study confirm a protective effect of nifedipine on renal hemodynamics manifested through an increase in glomerular filtration and most likely due to vaso dilatation of preglomerular vessels. Glomerular filtration remained preserved and even improved in 62% of cases on nifedipine therapy. Nifedipine acted primarily on the afferent arteriole and cancelled vasoconstrictive signals caused by (gentamicin-induced) tubuloglomerular feedback. We have failed to form a beneficial "cytoprotective" effect of nifedipine in toxic injuries of the kidneys.

References


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Correspondence to:
Jadranka Vlašiæ-Matas
Drage Ivaniæevića 36
21000 Split, Croatia
jadranka.vlasic-matas@st.tel.hr