Chronic Treatment With Long-Acting Nifedipine Reduces Vasoconstriction to Endothelin-1 in Essential Hypertension

Isabella Sudano, Agostino Virdis, Stefano Taddei, Lukas Spiekler, Roberto Corti, Georg Noll, Antonio Salvetti, Thomas F. Luscher

Abstract—Essential hypertension is associated with enhanced biological activity of endothelin-1 (ET-1) and impaired endothelium-dependent vasodilatation. Dihydropyridine calcium antagonists have antioxidant activity in vitro, and they improve endothelial function in vivo. We tested whether calcium antagonists also influence the biological activity of ET-1 in essential hypertensive (EH) patients in the presence and absence of hypercholesterolemia. In 9 healthy subjects (normotensive [NT] subjects; age: 48.3 ± 7.6 years; blood pressure: 118 ± 8.6/69 ± 5.4 mm Hg) and 21 EH subjects (age: 50.0 ± 7.8 years; blood pressure: 164.4 ± 5.4/103.8 ± 4.4 mm Hg), we studied forearm blood flow and its modification induced by intrabrachial administration of ET-1, phenylephrine, acetylcholine, and sodium nitroprusside at baseline and after 24 weeks of treatment with a nifedipine gastrointestinal therapeutic system (30 to 60 mg per day). At baseline, the first dose of ET-1 (0.5 μg/100 mL of forearm tissue per minute) caused a slight vasodilatation in NT but not in EH subjects, whereas the following higher doses caused a comparable dose-dependent vasoconstriction in EH and NT subjects. The effect of acetylcholine was significantly reduced in EH as compared with NT subjects. In contrast, sodium nitroprusside and phenylephrine had similar effects in NT and EH subjects. After chronic treatment with the nifedipine gastrointestinal therapeutic system, the vasoconstrictor effect induced by both ET-1 and phenylephrine was significantly blunted, whereas the response to acetylcholine was significantly increased and the vasodilatation to sodium nitroprusside unchanged. Hypercholesterolemic EH subjects showed a further reduced response to acetylcholine compared with normcholesterolemic EH subjects, and the nifedipine gastrointestinal therapeutic system restored the vasodilatation to acetylcholine in this subgroup. In conclusion, in EH subjects, chronic treatment with a long-acting dihydropyridine calcium antagonist not only exhibits a blood pressure–lowering effect but also reduces ET-1–induced vasoconstriction and improves endothelium-dependent vasodilatation. Those vasculoprotective effects may importantly contribute to a reduction in major clinical events seen during treatment with these compounds. (Hypertension. 2007;49:285-290.)

Key Words: endothelin-1 ■ calcium antagonists ■ free radicals ■ antioxidants ■ nitric oxide ■ hypertension ■ essential

The endothelium plays a protective role for the vascular system, because it prevents the adhesion of circulating blood cells, keeps the vasculature in a vasodilated state, and inhibits the proliferation and migration of vascular smooth muscle; NO and endothelin (ET-1) reciprocally interact to exert these effects. It has been documented that endothelial function is impaired in essential hypertensive (EH) patients.1–6 Endothelial dysfunction in EH patients seems to be characterized by a reduction of NO bioavailability, as well as by an enhanced biological activity of ET-1.7–9 These alterations could be involved in the pathogenetic mechanism leading to the development of atherosclerosis and to a rising in cardiovascular risk.10 Therefore, an important target for antihypertensive therapy should be not only blood pressure reduction but also restoration or at least improvement of endothelial function.

Dihydropyridine calcium antagonists do improve endothelial function11–13 and, in an acute study, have also been shown to reduce the vasoconstriction to exogenous ET-1.14 It is not yet known whether such an effect is mediated by blood pressure reduction alone or by some actions exerted by dihydropyridine calcium antagonists.

Therefore, the present study tests the chronic effect of dihydropyridine calcium antagonists on endothelial function and, in particular, on the vasoconstriction induced by ET-1. Moreover, we aimed to characterize the effect of that class of antihypertensive drugs in reversing endothelial function in hypertensive patients with and without hypercholesterolemia, an additional risk factor for atherosclerosis.

Methods

Patients

The study population included 9 normotensive (NT) control subjects and 21 matched EH patients. In accordance with institutional
### Experimental Model

Forearm blood flow (FBF) studies were performed at 8:00 AM after overnight fasting, with the subjects lying supine in a quiet, air-conditioned room (22°C to 24°C). A polyethylene cannula (21-gauge, Abbot) was inserted into the brachial artery under local anesthesia (2% lidocaine). The cannula was connected through stopcocks to a pressure transducer (model MS20, Electromedics) for determination of systemic mean arterial blood pressure (one-third pulse pressure plus diastolic pressure), heart rate (model VSM1, Physiocontrol), and intra-arterial infusions. FBF was measured by strain-gauge venous plethysmography (LOOSCO, GL LOOS). Circulation to the hand was occluded 1 minute before each measurement of FBF by inflating a pediatric cuff around the wrist at suprasystolic blood pressure. Earlier work had determined the sensitivity and reproducibility of the method. Forearm volume was determined by the water-displacement method, and the drug infusion rate was adjusted for each subject according to his or her forearm volume. Thus, drug infusion rates were normalized to 100 mL of forearm tissue by alteration of the drug concentration in the solvent. Drugs used were infused through 3-way stopcocks at concentrations that had no systemic effects.

### Study Design

We evaluated the effect of a dose–response curve to intra-arterial ET-1 (cumulative increase in infusion rates: 0.5, 25, and 50 μg/100 mL of forearm tissue per minute for 5 minutes each dose) and to phenylephrine (0.03, 0.1, 0.3, and 1 μg/100 mL of forearm tissue per minute for 5 minutes each dose). Endothelium-dependent forearm vasodilation was evaluated by a dose–response curve to intra-arterial acetylcholine (cumulative increase in infusion rates: 0.15, 0.45, 1.5, 4.5, and 15 μg/100 mL of forearm tissue per minute for 5 minutes each dose), whereas endothelium-independent vasodilation was assessed by sodium nitroprusside (1, 2, and 4 μg/100 mL of forearm tissue per minute for 5 minutes each dose), a direct smooth muscle cell relaxant compound.

The acetylcholine, sodium nitroprusside, and phenylephrine infusions were given in randomized sequence, and 30 minutes of recovery was allowed between each experimental intervention. Because of the long half-life, ET-1 was always tested as the last infusion.

After the baseline FBF study, patients were given 30 mg of nifedipine gastrointestinal therapeutic system (GITS) once daily for 4 weeks. After ensuring that no adverse clinical or biochemical effects had occurred, the dose was increased to 60 mg once daily for the remainder of the 20-week active treatment. Additional clinic visits were scheduled every 4 weeks for the total duration of the study. The FBF study was repeated ≥3 days (mean: 3.3±0.3) after the end of the 24-week chronic active treatment.

Blood pressure measurements were performed in our outpatient unit by a standard mercury sphygmomanometer. Blood pressure values were determined as the mean of 3 measurements made at 2-minute intervals after the patients had been seated for 10 minutes.

### Drugs

Acetylcholine HCl (Farmiga S.p.A.), ET-1 (Clinalfa AG), sodium nitroprusside (MaiDr.); and phenylephrine (Farmiga S.p.A.) were obtained from commercially available sources and diluted freshly to the desired concentration by adding normal saline. Sodium nitroprusside was dissolved in glucosate solution and protected from light by aluminum foil.

### Data Analysis

Data were analyzed in terms of changes in FBF and forearm vascular resistance (calculated as the ratio between intra-arterial mean pressure and FBF and expressed as standard units). Because arterial blood pressure did not change significantly during the FBF study, increments in FBF were taken as evidence of local vasodilation as decrements as evidence of local vasoconstriction. Differences between 2 means were compared by paired or unpaired Student’s t test, as appropriate. Responses to acetylcholine, ET-1, phenylephrine, and...
sodium nitroprusside were analyzed by ANOVA for repeated measures. Wilcoxon’s test was used to check the statistical significance of the difference between nonparametric values. Results were expressed as mean±SD. Differences were considered statistically significant at a value of P<0.05. Computations for the statistical method described were performed using the SAS System.

Results

The demographic, baseline hemodynamic, and humoral characteristics for the NT subjects and EH patients are summarized in Table 1. Age, sex, glycemia, and smoking history were similar in both study groups and within the reference range, whereas blood pressure differed (Table 1). Nine EH patients had elevated plasma cholesterol values (Table 1). Between the 2 latter subgroups, lipid profile was similar.

In EH patients, administration of nifedipine significantly decreased blood pressure values from 164.4±5.4/103.8±4.4 mm Hg to 126.9±9.5/81.2±2.4 mm Hg (P<0.001 versus baseline) after 24 weeks of treatment. However, the response to the first dose of ET-1 causes a slight vasodilatation in NT subjects but not in hypertensive patients (FBF from 2.9±1.0 to a maximum of 17.6±2.2 mL/100 mL forearm tissue per minute; Figure 2) as compared with hypertensive patients with normal cholesterol levels (FBF from 2.9±1.0 to a maximum of 17.6±2.2 mL/100 mL forearm tissue per minute; Figure 2). Contralateral FBF did not change.

TABLE 2. Clinical Characteristics of the Hypertensive Patients Included in the Study Before and After Treatment With Nifedipine GITS

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>HT (n=21) Before Treatment</th>
<th>HT (n=21) After Treatment</th>
<th>HT (n=21) After 3-Day Therapy Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>164.4±5.4</td>
<td>126.9±9.5</td>
<td>154.0±6.3</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>103.8±4.4</td>
<td>81.2±2.4</td>
<td>96.9±3.3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>65.0±6.0</td>
<td>63.5±4.6</td>
<td>69.1±7.8</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>222.2±44.1</td>
<td>217.0±41.7</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>43.3±11.6</td>
<td>53.7±16.6</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>121.9±55.9</td>
<td>124.7±68.4</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>154.5±43.2</td>
<td>138.3±37.4</td>
<td></td>
</tr>
</tbody>
</table>

BP indicates blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are presented as mean±SD.

FBF Study

Basal Vascular Responses

The response to ET-1, phenylephrine, acetylcholine, and sodium nitroprusside in EH patients and in NT subjects, in terms of FBF and forearm vascular resistance, are reported in Table 3. ET-1 caused a dose-dependent vasoconstriction, which was comparable to the one obtained in NT controls (Figure 1).

However, the response to the first dose of ET-1 causes a slight vasodilatation in NT subjects but not in hypertensive patients (FBF from 3.0±0.9 to 3.2±1.1 mL/100 mL forearm tissue per minute in NT subjects versus 3.1±0.7 to 2.5±0.9 mL/100 mL forearm tissue per minute in hypertensive subjects P=0.021; Figure 1). Vasoconstrictor response to phenylephrine resulted in similar responses in EH patients as compared with NT controls (Figure 1). The dose-dependent response to acetylcholine was significantly (P=0.02) reduced in EH patients as compared with NT subjects (Figure 2), whereas the dose-dependent vasodilatation to sodium nitroprusside was similar in both groups (data not shown).

The responses to sodium nitroprusside, phenylephrine, and ET-1 were not significantly different in normocholesterolemic and hypercholesterolemic hypertensive subgroups. However, the response to acetylcholine was significantly reduced (P=0.032) in hypertensive subjects with high cholesterol (FBF from 2.9±1.3 to a maximum of 14±0.4 mL/100 mL forearm tissue per minute; Figure 2) as compared with hypertensive patients with normal cholesterol levels (FBF from 2.9±1.0 to a maximum of 17.6±2.2 mL/100 mL forearm tissue per minute; Figure 2).

TABLE 3. Effect of 24-Week Treatment With Nifedipine GITS on FBF and Forearm Vascular Resistance in EH Patients and Baseline Data Obtained in NT Subjects

<table>
<thead>
<tr>
<th>Vascular Parameters</th>
<th>EH Patients</th>
<th>NT Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Maximal Doses</td>
</tr>
<tr>
<td>FBF, mL/100 mL forearm tissue per minute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET-1</td>
<td>3.1±0.7</td>
<td>1.4±0.5</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>3.2±0.8</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>3.0±0.8</td>
<td>13.9±5.4*</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>2.8±0.9</td>
<td>15.7±6.5</td>
</tr>
<tr>
<td>Forearm vascular resistance, SU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET-1</td>
<td>39.1±7.3</td>
<td>90.5±22.1</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>39.0±7.5</td>
<td>89.9±22.8</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>45.8±8.4</td>
<td>9.3±4.0*</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>43.9±7.3</td>
<td>8.6±3.6</td>
</tr>
</tbody>
</table>

FVR indicates forearm vascular resistance; SU standard unit.

*P<0.05 or less for hypertensive patients vs NT subjects.
†P<0.05 or less for before vs after treatment.
Effect of Chronic (24-Week) Nifedipine GITS Administration

Chronic nifedipine treatment lasted for 24 weeks and led to a significant and persistent blood pressure reduction. At the end of the treatment period, nifedipine was withdrawn for 3 days before the final study. At that time, patients again became hypertensive (Table 2).

The vasoconstrictor effect induced by both ET-1 and phenylephrine was significantly blunted ($P<0.001$) by treatment with nifedipine GITS (Figure 1). Moreover, vascular

![Figure 1: FBF responses ($\Delta\%$) to ET-1 and phenylephrine in normotensive subjects ($n=9$, $\triangle$), and hypertensive patients ($n=21$) before ($\bigcirc$) and after ($\blacksquare$) treatment with nifedipine GITS. $^*P<0.05$ or less for normotensive subjects vs hypertensive patients; $\dagger P<0.05$ or less for hypertensive patients before vs after treatment.](image1)

![Figure 2: FBF responses to acetylcholine in normotensive subjects ($n=9$, $\triangle$) and hypertensive patients with normal ($n=9$) cholesterol (left) before ($\bigcirc$) and after ($\blacksquare$) treatment with nifedipine GITS and hypertensive patients with high cholesterol ($n=12$; right), before ($\bigcirc$) and after ($\blacksquare$) treatment with nifedipine GITS. $^*P<0.05$ or less for normotensive subjects vs hypertensive patients; $\dagger P<0.05$ or less for hypertensive patients before vs after treatment.](image2)
responses to acetylcholine significantly increased ($P<0.001$) as compared with pretreatment values, whereas the vasodilating effect of sodium nitroprusside was unchanged (Figure 2). This increase in acetylcholine-mediated vasodilation was significantly more pronounced ($P=0.01$) in hypertensive patients with high cholesterol as compared with normcholesterolemic hypertensive patients (Figure 2). Contralateral FBF did not significantly change during the entire study (data not shown).

Discussion

This study showed that, in hypertensive patients, a chronic oral treatment with a long-acting dihydropiridine calcium antagonist, nifedipine GITS, at clinically used dosages, reduces the vasoconstriction induced by ET-1 and phenylephrine and improves the endothelium-dependent vasodilation. This effect was seen after the treatment had been withdrawn for 3 days in the presence of blood pressure values that were similar to those at baseline. Therefore, this suggests that this effect is unlikely because of the blood pressure reduction in agreement with previous evidence demonstrating that blood pressure normalization, per se, is not a maneuver sufficient to increase endothelium-dependent vasodilation in the forearm circulation of EH patients. Previous studies have shown that blockade of ET-1 activity with endothelin receptor blockers leads to an improvement in endothelium-dependent vasodilation in patients with hypertension, suggesting that an increased ET-1 activity may play a role in the pathophysiology of this abnormality.

Previous data in human forearm circulation showed that intra-arterial infusion but not oral administration of nifedipine can reduce ET-induced vasoconstriction in NT subjects. The present study is the first demonstrating that oral administration of nifedipine, at clinically used dosages, is effective in diminishing the vasoconstrictor effect of ET infusion in hypertensive patients.

In EH patients, ET-1 only caused dose-dependent vasoconstriction, which was found to be more pronounced, even if not significantly different as compared with NT controls. However, as shown previously, the response to the first dose of ET-1 caused a slight vasodilatation in NT subjects but not in hypertensive patients suggesting a dysfunction of ET-mediated endothelial-dependent vasodilation.

Because the mechanisms involved are known, as in certain blood vessels, such as the porcine coronary artery, ET receptors on vascular smooth muscle are linked to voltage-operated Ca$^{2+}$ channels via G proteins. This may explain why calcium antagonists reduce ET-induced vasoconstriction in these vessels and are similarly effective in the human coronary artery. In the human internal mammary artery, the contractile effects induced by ET are mediated via a cascade of activation of phospholipase C and diacylglycerol and, ultimately, formation of inositol triphosphate, which, in turn, releases Ca$^{2+}$ from the sarcoplasmic reticulum, thus increasing cytosolic Ca$^{2+}$. In those vessels, calcium antagonists do not markedly affect the response to ET.

A nifedipine effect mediated by an interaction with intracellular Ca$^{2+}$ handling is confirmed by the results obtained with phenylephrine. Infusion of the adrenergic agonist induced a dose-dependent vasoconstrictor response similar in EH patients and in NT controls. After chronic nifedipine GITS administration, the vasoconstrictor effect induced by phenylephrine was significantly blunted. This agrees with previous evidence showing that nifedipine, as well as other calcium antagonists, like diltiazem, amldopidine, verapamil, and nitrendipine, reduced the vasoconstrictor effect of phenylephrine or other $\alpha$-agonists, such as $\alpha$-methyl-nor-adrenaline.

Another possible explanation might be related to a specific effect of nifedipine on NO availability. Chronic treatment with nifedipine GITS improved the vasodilation to acetylcholine but not that to sodium nitroprusside, confirming that nifedipine improves endothelial function in hypertensive patients. Previous data have shown that, in patients with essential hypertension, an increase in acetylcholine-induced vasodilatation is related, at least in part, to an increased NO bioavailability. Moreover, we demonstrated that nifedipine increases endothelium-dependent vasodilation by restoring NO availability, an effect probably determined by its antioxidant activity, because nifedipine decreased circulating parameters of oxidative stress and prevented the effect of the antioxidant vitamin C in patients with essential hypertension.

In NT subjects, where NO production is preserved, the effect of ET-1 in maintaining vascular tone is very modest, whereas in EH patients, where basal NO production is reduced, a vasoconstrictor component of ET-1 seems to be much more evident. Thus, the possibility exists that the overall vascular effect of ET-1 on vascular tone is partially dependent on the integrity of the NO pathway. Therefore, nifedipine GITS by increasing NO bioavailability may play a role in reducing the ET-1-mediated vasoconstriction.

The effect of nifedipine on acetylcholine-induced vasodilation was more evident in hypercholesterolemic hypertensive subjects, whereas no difference was observed concerning the ET-mediated vasoconstriction between EH patients with normal or high cholesterol. These data are in line with a previous study, which showed that, in subjects without advanced atherosclerosis, hypercholesterolemia is not associated with increased endogenous ET-1 activity above that observed in the resistance vessels of healthy individuals, whereas hypertension is characterized by increased ET-1 activity.

Limitations of the Study

In this study we did not evaluate the mechanisms underlying the effect of nifedipine GITS on endothelin-induced vasoconstriction, but on the basis of previous data from our group, we may speculate that the increased NO bioavailability and an antioxidative effect may have a role in the effect of nifedipine on ET-induced vasoconstriction together with the direct effect on intracellular Ca$^{2+}$ handling. Another limitation of the study could be the fact that we selected patients in which the blood pressure response to nifedipine GITS was $>10\%$. This selection was done to be able to leave these patients with a monotherapy for the whole study. The patients excluded from the study were, therefore, patients who needed to add another drug to nifedipine GITS to normalize their blood pressure.
Perspectives
These results suggest that nifedipine and possible calcium antagonists not only lower blood pressure values but also can have beneficial effects on vascular endothelium by reducing ET-mediated vasoconstriction and increasing endothelium-dependent vasodilation, thereby offering considerable potential in the prevention and/or treatment of atherosclerosis.

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Disclosures
None.

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