Effect of Amlodipine Compared to Atenolol on Small Arteries of Previously Untreated Essential Hypertensive Patients

Ernesto L. Schiffrin, Qian Pu, and Jeong Bae Park

In a previous retrospective study, long-term treatment of essential hypertensive patients with a slow-release calcium channel blocker resulted in normal resistance artery structure and endothelial function, which did not occur with a β-blocker. In the present prospective study, 19 previously untreated essential hypertensive patients (aged 47 ± 2 years, 75% male) were treated for 1 year in a double-blind randomized study with the long-acting calcium channel blocker amlodipine or the β-blocker atenolol. Resistance arteries (lumen diameter, 150 to 350 μm) dissected from gluteal subcutaneous biopsies were studied on a pressurized myograph. Blood pressure (BP) control (129 ± 2/85 ± 2 mm Hg) was identical in both groups for the last 6 months of the study. After 1 year of treatment with amlodipine, the media-to-lumen ratio (M/L) of resistance arteries decreased from 7.89% ± 0.40% to 6.81% ± 0.41% (P < .05). Acetylcholine-induced endothelium-dependent relaxation tended to improve from 84.3% ± 5.5% to 90.5% ± 4.8% (P = .06), whereas sodium nitroprusside-induced relaxation was unchanged in the patients treated with amlodipine. In the β-blocker-treated group there was no significant change in M/L or acetylcholine-induced relaxation. In conclusion, treatment with the calcium channel blocker amlodipine corrected altered resistance artery structure and tended to improve endothelial function in essential hypertensive patients, whereas similar good control of BP with the β-blocker atenolol did not. Whether the vascular protective effect of amlodipine will result in improved outcomes in hypertension remains to be demonstrated. Am J Hypertens 2002;15:105–110 © 2002 American Journal of Hypertension, Ltd.

Key Words: Blood pressure, endothelium, hypertrophy, remodeling, antihypertensive therapy, calcium channel blocker, β-blocker.

Small arteries with a lumen diameter of 100 to 350 μm participate in resistance to blood flow in tissues, and consequently contribute to the elevated peripheral resistance that characterizes essential hypertension. Hence, their name resistance arteries is often used interchangeably with small arteries. The changes in structure and function that occur in these vessels not only contribute to blood pressure (BP) elevation but may also participate in some of the complications of hypertension such as some forms of stroke, myocardial ischemia, and nephroangiosclerosis. These are reasons why there has been interest in finding out whether correction of small artery structure and function may occur with antihypertensive treatment, and presumably with some agents but not others.

Studies performed during the past few years have shown that treatment of hypertensive patients with some BP-lowering drugs improves vascular structure and function, whereas other agents do not even if they lower BP effectively. Angiotensin converting enzyme (ACE) inhibitors1–5 and angiotensin receptor blockers6,7 are agents with which these potentially beneficial effects have been demonstrated. Similar studies with calcium channel blockers have been retrospective,8 where patients studied were already being treated with these agents, rather than interventional studies, with evaluation before and after treatment. Therefore, these are a weaker demonstration of effects of these agents.

To eliminate the biases introduced by the retrospective nature of the previous study of calcium channel blockers with recruitment of hypertensive patients already under treatment,8 here we tested the hypothesis that equieffective lowering of BP with the dihydropyridine calcium channel blocker amlodipine and the β-blocker atenolol for 1 year
would demonstrate that amlodipine-based therapy corrects altered resistance artery structure and endothelial function, but treatment with atenolol does not.

Methods

Patients

The protocol was approved by the Ethics Committee of the Clinical Research Institute of Montreal. Normotensive subjects and essential hypertensive patients (30 to 65 years old) provided written informed consent to participate in the study. Control subjects were sex- and age-matched subjects with systolic and diastolic BP <140 and <85 mm Hg. Twenty essential hypertensive patients were recruited. They had a past history of sitting diastolic BP >90 mm Hg on at least three occasions, and were followed in our clinic for at least 1 year with well-controlled BP. They had never received other antihypertensive agents. Diagnosis of essential hypertension had been previously established in these subjects by absence of clinical evidence of secondary hypertension, normal serum electrolytes, creatinine, urinalysis, abdominal echogram, and when indicated, renal scintiscan or computed abdominal tomography. Exclusion criteria included smoking more than five cigarettes per day, abnormal fasting blood glucose, serum creatinine concentration >150 μmol/L, and systemic diseases. It was predetermined that patients without good BP control during the last 6 months of the study would be excluded from the final analysis. Clinic sitting BP was measured after 15 min rest; diastolic BP was read as phase V of Korotkoff sounds.

Trial Design

Gluteal subcutaneous biopsies measuring 1.0 × 0.5 × 0.5 cm³ were obtained under local anesthesia (2% xylocaine) with patients on placebo and after 1 year of treatment, and once in normotensive subjects. Echocardiography was also performed before and after 1 year of treatment, and left ventricular mass was calculated using the Penn convention. Patients were randomly assigned in double blind fashion to treatment with 5 mg of amlodipine or 50 mg of atenolol. If diastolic BP was >90 mm Hg after 2 weeks, the dose of drug was doubled, and 4 weeks later, open-label hydrochlorothiazide (12.5 to 25 mg) was added if needed. After the trial was over and before the code was broken, BP control in each patient was evaluated. One patient had not achieved goal BP in the last period of the study, and this patient was excluded from the final analysis of the data.

Resistance Artery Study

Study of resistance arteries was performed by individuals unaware to which groups vessels belonged. Small arteries (lumen diameter, 150 to 350 μm) were isolated from subcutaneous tissue immediately after the biopsy, and mounted on a pressurized myograph. Vessel segments (2 mm long) were slipped onto two glass microcannulae, one of which was positioned until vessel walls were parallel, and equilibrated in physiological salt solution ([PSS]; composition, in millimoles per liter: NaCl 120, NaHCO₃ 25, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ 1.18, CaCl₂ 2.5, EDTA 0.026, and glucose 5.5) continuously bubbled with 95% air and 5% CO₂ to achieve a pH of 7.40 to 7.45 at 37°C, and pressurized to 60 mm Hg. Endothelium-dependent and -independent relaxations were assessed by measuring dilatory responses to acetylcholine (1 nmol/L to 100 μmol/L) and sodium nitroprusside ([SNP], 10 nmol/mL to 1 mmol/L) respectively, in norepinephrine ([NE], 1 μmol/L) precontracted vessels. Thereafter, vessels were deactivated with PSS + 10 mmol/L EGTA to eliminate myogenic tone before measuring structure.

Data Analysis

Results are presented as means ± SEM. Comparisons were performed by paired Student t test, one-way analysis of variance followed by Newman-Keuls test, and two-way or repeated measures analysis of variance as appropriate. P < .05 was considered statistically significant.

Results

Demographics of subjects are shown in Table 1. Only 6 of the 19 hypertensive patients had echographic left ventricular hypertrophy ([LVMI] >134 g/m² for men, 110 g/m² for women). Serum electrolytes, creatinine, lipids, and supine plasma renin activity were similar in all groups before and after treatment. Patients randomized to atenolol received an average daily dose of atenolol of 77 ± 9 mg. These hypertensive patients unexpectedly exhibited a significantly lower BP at baseline than patients randomized to amlodipine (Fig. 1 and Table 1). The statistically significant difference in BP between the subjects randomized to atenolol and those randomized to amlodipine (who received an average dose of amlodipine of 9 ± 0.7 mg daily) was tracked during the first 6 months of the study. In the final 6 months of the study BP was similar in both groups (Fig. 1). Ambulatory BP monitoring, which was also significantly higher in the amlodipine group before treatment, was similarly well controlled at the end of the year of treatment. Addition of 12.5 mg of hydrochlorothiazide in 1 atenolol-treated and 7 amlodipine-treated patients was required to achieve goal BP. Echocardiographic LVMI did not change significantly in either group of subjects.

Resistance vessels of hypertensive patients exhibited significantly greater media thickness and media-to-lumen ratio (M/L) than in normotensive subjects (Table 2). After 1 year of treatment, media thickness and M/L of resistance arteries from amlodipine-treated patients were significantly smaller than before treatment, whereas in atenolol-treated hypertensives they remained abnormal (Table 2). The 7 patients on amlodipine who required hydrochlo-
Table 1. Patient demographics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensives</th>
<th>Atenolol Before</th>
<th>Atenolol After 1 Year</th>
<th>Amlodipine Before</th>
<th>Amlodipine After 1 Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>44 ± 3</td>
<td>45 ± 2.4</td>
<td>50 ± 2.0</td>
<td>3/6</td>
<td>8/1</td>
</tr>
<tr>
<td>Hypertension history (y)</td>
<td>2.67 ± 0.5</td>
<td>3.10 ± 1.0</td>
<td>2.7 ± 1.0</td>
<td>2.4 ± 0.5</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24 ± 1.2</td>
<td>29.0 ± 1.7</td>
<td>29.5 ± 1.8</td>
<td>112 ± 4.0</td>
<td>145 ± 4.9</td>
</tr>
<tr>
<td>Clinic SBP (mm Hg)</td>
<td>112 ± 4.0</td>
<td>145 ± 4.9</td>
<td>127 ± 2.8*</td>
<td>75 ± 2.4</td>
<td>99 ± 1.7</td>
</tr>
<tr>
<td>Clinic DBP (mm Hg)</td>
<td>75 ± 2.4</td>
<td>99 ± 1.7</td>
<td>85 ± 1.1*</td>
<td>88 ± 2.8</td>
<td>114 ± 2.7</td>
</tr>
<tr>
<td>Mean BP (mm Hg)</td>
<td>88 ± 2.8</td>
<td>114 ± 2.7</td>
<td>99 ± 1.3*</td>
<td>ND</td>
<td>112 ± 2.7</td>
</tr>
<tr>
<td>ASBP (mm Hg)</td>
<td>ND</td>
<td>142 ± 3.3</td>
<td>125 ± 2.3*</td>
<td>12.3 ± 3.3</td>
<td>144 ± 3.3</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>5.6 ± 0.4</td>
<td>5.8 ± 0.4</td>
<td>5.9 ± 0.1</td>
<td>1.5 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>3.3 ± 0.4</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>92 ± 3.0</td>
<td>97 ± 4.0</td>
<td>93 ± 5.0</td>
<td>5.6 ± 0.4</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>92 ± 3.0</td>
<td>97 ± 4.0</td>
<td>93 ± 5.0</td>
<td>5.6 ± 0.4</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>15.3 ± 0.8</td>
<td>28.0 ± 0.9</td>
<td>2.1 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>LVM (%)</td>
<td>12.3 ± 18.4</td>
<td>146 ± 18.7</td>
<td>116 ± 7.8</td>
<td>4.0 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>PRA, ng Ang I mL h⁻¹</td>
<td>0.9 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

SBP = systolic blood pressure; DBP = diastolic blood pressure; BP = blood pressure; ASBP = ambulatory systolic blood pressure; ND = not done; ADBP = ambulatory diastolic blood pressure; LVMI = left ventricular mass index; PRA = plasma renin activity; Ang I = angiotensin I.

rothiazone had similar M/L to other patients in their group (8.26% ± 0.69% before, 6.75% ± 0.52% after treatment).

Endothelial function tested with acetylcholine-induced relaxation demonstrated that maximal response to acetylcholine was diminished in untreated hypertensive patients compared to normotensive subjects when hypertensives were considered as a single group (Fig. 2). Maximal acetylcholine response from untreated hypertensive patients correlated inversely with clinic systolic BP (r = −0.45, P = .03). However, the significance of the correlation was due to the amlodipine group (r = 0.66, P < .05), as there was no correlation between BP and acetylcholine responses in the atenolol group (r = 0.04), probably because the BP was lower in the latter. Maximal acetylcholine relaxation was similar in the amlodipine and atenolol groups before treatment (84.3% ± 5.6% and 86.8% ± 4.1%, respectively, P < .01 v normotensives). Although there was a trend to improvement of acetylcholine-induced relaxation in vessels from amlodipine-treated patients (to 90.5% ± 4.8%), it did not achieve significance (P = .06). However, relaxation in response to acetylcholine in the amlodipine group was no longer different from that of normotensive subjects, suggesting that some improvement of endothelial function had indeed occurred. Arteries from atenolol-treated patients did not exhibit any improvement in endothelial function (86.5% ± 2.1% acetylcholine-induced relaxation after treatment, P < .01 v normotensives). Full concentration–response curves to acetylcholine and SNP are shown in Fig. 3. Maximal SNP-induced relaxation was slightly but significantly lower in hypertensive subjects (92.7% ± 1.7% before and 89.1% ± 5.1% after treatment in the amlodipine group, and 90.8% ± 2.5% before and 88.8% ± 2.3% after treatment in the atenolol group) in comparison to normotensive individuals (98.0% ± 0.9%, P < .05 v hypertensive groups before and after treatment). Diuretic administration did not alter the results obtained with either amlodipine or atenolol (results not shown).

There was no correlation between basal lipid profile and acetylcholine-induced relaxation in either the atenolol or the amlodipine-treated groups of patients. Changes in lipid profile during the treatment period and endothelium-
dependent relaxation did not exhibit any significant correlation.

**Discussion**

We demonstrate here that patients treated with the long-acting dihydropyridine calcium channel blocker amlodipine exhibit after 1 year a significantly improved small artery structure (M/L), in contrast to the lack of effect of atenolol. This occurs in spite of better BP control in the first few months of atenolol treatment. These results extend and confirm previous results from our laboratory and others, and underline the absence of effect of atenolol on small artery structure and endothelial function in hypertensive patients.1–4,6,8 Endothelial function as measured by maximal acetylcholine-induced relaxation showed a trend toward improvement in patients treated with amlodipine, which did not achieve statistical significance. It should be noted that in contrast to previous studies from our laboratory in which BP control was identical in both arms of the study, in this one, although blindly assigned to each group, patients randomized to the amlodipine group had higher BP than those in the atenolol group, which were tracked through the first 6 months of treatment. This may explain the difference in results between the present study and our previous work.8 The period of excellent BP control achieved in the last 6 months may have sufficed to induce regression of structural remodeling of blood vessels, but may not be enough to significantly improve endothelial dysfunction.

Many agents that stimulate cell growth and apoptosis of smooth muscle cells also stimulate calcium entry into the cell and calcium release. Angiotensin II stimulates smooth muscle cell growth10 and collagen deposition11 mainly by mechanisms triggered by activation of angiotensin type 1 (AT1) receptors, which induce increases in cytosolic calcium. Some of the pathways mediating growth induced by AT1 receptors such as those mediated by Pyk2 are calcium dependent,12 and could potentially participate in the pathophysiologic phenomena that are blocked by long-acting calcium channel blockers such as amlodipine.

Although direct actions of calcium channel blockade may explain some of the effects found in the present study, other mechanisms should be considered. Angiotensin converting inhibitors, ACE inhibitors, and calcium channel blockers, as well as angiotensin receptor blockers are vasodilators, whereas atenolol may induce peripheral vasoconstriction at least of some vascular beds.13 Reduced blood flow has been shown in experimental animals to induce inward eutrophic remodeling of small arteries.14 The administration of hydrochlorothiazide to some patients could also have affected structure or function of small arteries. Mean BP tended to be higher in those patients who required hydrochlorothiazide to reach goal BP, and so was M/L of small arteries in these patients. But effects of treatment were similar to those found in the patients who did not require the diuretic. With respect to endothelium-dependent relaxation, the patients who received hydrochlorothiazide had less improvement than

![FIG. 2. Maximal response to acetylcholine-induced (upper panels) or sodium nitroprusside (SNP)-induced vasodilatation of norpinephrine constricted small arteries from atenolol and amlodipine-treated patients. *P <.05 v other groups; **P <.01 v other groups, †P <.05 v before treatment.](image-url)

| Table 2. Morphologic characteristics of resistance arteries |
|----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Parameter      | Normotensives     | Before After 1 Year | Hypertensives     | Before After 1 Year | Atenolol           | Amlodipine         |
| External diameter (μm) | 214 ± 18          | 233 ± 23          | 260 ± 17          | 221 ± 18          | 269 ± 20          | 221 ± 18          |
| Internal diameter (μm) | 191 ± 17          | 199 ± 20          | 225 ± 15          | 192 ± 16          | 237 ± 18          | 192 ± 16          |
| Media width (μm) | 11 ± 1.3†         | 16.8 ± 1.8        | 17.5 ± 1.1        | 14.7 ± 1.1        | 16.3 ± 1.2        | 14.7 ± 1.1        |
| M/L ratio (%)   | 5.9 ± 0.3†        | 8.5 ± 0.5         | 7.9 ± 0.4         | 7.9 ± 0.5         | 6.8 ± 0.4*        | 6.8 ± 0.4*        |
| MCSA (μm²)      | 8035 ± 1708       | 12,257 ± 2815     | 13,970 ± 1590     | 9963 ± 1634       | 12,933 ± 1590     | 9963 ± 1634       |

M/L = media-to-lumen ratio; MCSA = media cross-sectional area.
* P <.05 v before treatment; † P <.01 v other groups.
those who did not. Several studies have found that diuretics did not influence small artery structure\(^3,6,15\) or endothelial function\(^6\) in hypertensive patients.

Acetylcholine-induced vasodilatation of small arteries from gluteal subcutaneous tissue correlates with brachial artery flow-mediated dilatation,\(^16\) and the latter has been shown to predict coronary vasomotion.\(^17\) Although in vivo endothelial dysfunction has not been detected in the forearm of hypertensive humans in some studies,\(^18\) impairment of endothelial function is a frequent finding in hypertensive patients.\(^19,20\) Endothelial dysfunction can be demonstrated in resistance arteries from gluteal subcutaneous biopsies in at least one third of mild essential hypertensives.\(^21\) In vitro acetylcholine responses of subcutaneous small arteries from hypertensive patients were moderately impaired in response to the highest concentrations of acetylcholine, but in this particular study the difference with normotensive subjects only achieved statistical significance when all untreated hypertensive subjects were considered as one group in comparison to the normotensive individuals. Although BP was not equally well controlled in the amlodipine group and in the atenolol group, the amlodipine group exhibited a trend to improvement of acetylcholine-induced relaxation, whereas the atenolol group did not experience any change. This suggests that amlodipine treatment may have been more beneficial in relation to endothelial dysfunction than atenolol, as previously found with the slow release dihydropyridine calcium channel blocker nifedipine-GITS,\(^8\) the ACE inhibitor cilazapril,\(^1,4\) and the angiotensin receptor blocker losartan.\(^6\)

Hypercholesterolemia is associated with blunted endothelium-dependent relaxation of small subcutaneous resistance arteries, and its correction could improve endothelial dysfunction.\(^22\) In the present study there was no correlation between cholesterol and the acetylcholine-induced relaxation before treatment, or changes occurring after treatment. Thus, blood lipids probably did not play a role in the minor improvement of endothelial dysfunction in amlodipine-treated patients.

The resistance vessels studied are similar in structure to small arteries from the heart and kidney from spontaneously hypertensive rats, and respond to antihypertensive treatment in similar ways.\(^23–26\) The importance of coronary small vessel endothelial dysfunction on incidence of cardiovascular events has recently been demonstrated.\(^27\) Thus, although currently unproven, it can be expected that correction of small artery structure and function as dem-

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**FIG. 3.** Concentration–response curves of relaxation of small arteries to acetylcholine and to SNP. *P < .05 normotensive v before treatment; \(^+P < .05\) normotensive v after 1 year. Abbreviation as in Fig. 2.
onstrated in a surrogate, the glutal subcutaneous resis-
tance artery, will be associated with improved structure and function of other small vessels, and potentially larger vessels in the heart, kidney, and brain. This could contribute to improved outcomes in hypertensive patients.

In conclusion, mild to moderate essential hypertensive patients treated with amlodipine for 1 year presented improved M/L of small arteries dissected from glutal subcutaneous tissue, whereas patients treated with atenolol exhibited persistently abnormal resistance artery structure and function despite adequate BP control. Endothelial function as measured by acetylcholine-induced relaxation tended to improve with amlodipine, whereas there was no change with atenolol. Treatment with dihydropyridine calcium channel antagonists, such as amlodipine, may exert vascular protective effects, which could have beneficial consequences on outcomes in hypertension.

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References
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