Effect of Amlodipine–Atorvastatin Combination on Fibrinolysis in Hypertensive Hypercholesterolemic Patients With Insulin Resistance

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Background: The aim of this study was to evaluate the effect of the amlodipine–atorvastatin combination on plasma tissue plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1) activity in hypercholesterolemic, hypertensive patients with insulin resistance.

Methods: The study population included 45 patients, aged 41 to 70 years, with mild to moderate essential hypertension (diastolic blood pressure [BP] ≥95 and ≤105 mm Hg), hypercholesterolemia (total cholesterol >200 and <350 mg/dL), and insulin resistance (HOMA index >2.5) After a 4-week wash-out period, they were randomized to amlodipine (5 mg) or atorvastatin (20 mg) or their combination at the same oral dosage for 12 weeks in three cross-over periods each separated by a 4-week placebo period (3 by 3 latin square design). At the end of the placebo wash-out and of each treatment period, office BP, total cholesterol, PAI-1, and t-PA activity were evaluated.

Results: The amlodipine–atorvastatin combination, in addition to the expected hypocholesterolemic effect, produced: 1) a greater decrease in PAI-1 activity (−10.2 U/mL, P < .01 v placebo) and an even greater increase in t-PA activity (±0.26 U/mL, P < .01 v placebo) than amlodipine (−0.5 U/mL for PAI-1, P = not significant; +0.17 U/mL for t-PA, P < .01 v placebo) and atorvastatin alone (respectively, −9.9 U/mL, P < .01 v placebo and +0.08 U/mL, P < .05 v placebo); and 2) a greater systolic BP/diastolic BP mean reduction (−22/17 mm Hg, P < .005 v placebo) than amlodipine (−18/14 mm Hg, P < .01 v placebo) and atorvastatin alone (−2.8/3.8 mm Hg, P < .05 v placebo only for diastolic BP).

Conclusions: The positive effect on fibrinolytic balance and BP control observed suggests that in hypertensive, hypercholesterolemic patients with impaired fibrinolysis, the combination of amlodipine and atorvastatin could be the treatment of choice. Am J Hypertens 2004;17:823–827 © 2004 American Journal of Hypertension, Ltd.

Key Words: Amlodipine, atorvastatin, fibrinolysis, hypertension, hypercholesterolemia.

Population-based data have indicated that the two most common cardiovascular risk factors, hypertension and hypercholesterolemia, coexist in a large proportion of patients and their combination is associated with a rate of cardiovascular complications that greatly exceeds the separate contribution of any single risk factor.1,2 In hypertension, a close relationship has also been demonstrated between disorders of lipid metabolism, insulin resistance, and impaired fibrinolysis, mainly expressed as increased plasminogen activator inhibitor type 1 (PAI-1) levels and depressed tissue plasminogen activator (t-PA) activity.3–5 Endothelial dysfunction might be the pathogenetic link between these risk factors whose clustering greatly accelerates the atherogenic process and its clinical complications.6 To achieve a reduction in both cardiovascular morbidity and mortality, current hypertension treatment guidelines stress the role of total risk factor management and state not only to lower blood pressure (BP) values but also to normalize high cholesterol and improve the global risk profile of hypertensive patients.6

The 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, commonly referred to as statins,
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are the most powerful agents available for the treatment of patients with hypercholesterolemia. Statins have demonstrated a capability to reduce the rate of cardiovascular events.\(^7\)\(^-\)\(^9\) Data from the recent Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) support the view that statins protect hypertensive patients from end-organ damage, not only through cholesterol reduction but also through other pathways.\(^10\) These include a direct modulation of the endothelial function, as well as an interaction with the fibrinolytic activity.\(^11\)\(^,\)\(^12\) In this regard, evidence from in vitro studies indicate that statins positively affect the fibrinolytic system of cultured smooth muscle cells as well as endothelial cells.\(^13\)\(^-\)\(^15\) Also some in vivo studies demonstrated that statins decreased PAI-1 plasma levels and increased t-PA activity.\(^16\)\(^,\)\(^17\) Whether statin treatment may affect fibrinolytic activity has been poorly investigated in hypercholesterolemic, hypertensive patients pharmacologically treated for both risk factors.

Given this background, the present study was undertaken to evaluate the effect of the combination of the dihydropyridine calcium antagonist amlodipine and the HMG-CoA reductase inhibitor atorvastatin on plasma PAI-1 and t-PA activity in hypercholesterolemic, hypertensive patients with insulin resistance, a condition characterized by impaired fibrinolysis.\(^18\)

Methods

The study population was selected according to the following inclusion criteria: outpatients of either sex, aged 56.3 ± 5.1 years, with mild to moderate essential hypertension (diastolic BP >90 and ≤105 mm Hg), total cholesterol (TC) >200 and <350 mg/dL, and insulin resistance, as defined by HOMeostasis Model Assessment (HOMA) Index >2.5. The HOMA Index (= glucose in millimoles per liter × insulin in microunits per milliliter/22.5) has been shown to correlate well with insulin resistance using clamp techniques.\(^19\)

Patients with a diagnosis of diabetes, liver or kidney disease, cancer, major cardiovascular complication (myocardial infarction or unstable angina within 6 months, congestive heart failure), using corticosteroids or hormone replacement therapies, and having any other diseases with a poor prognosis were excluded from the study. Secondary forms of hypertension were excluded according to standard routine clinical and laboratory examination. The study protocol was approved by the local Ethical Committee and informed consent was obtained from each participant at the time of enrollment.

After an initial 4-week wash-out period, patients were randomly assigned to receive amlodipine (5 mg) or atorvastatin (20 mg) or their combination at the same oral dosage for 12 weeks in three cross-over periods each separated by a 4-week placebo wash-out period (3 by 3 latin square). At the end of the placebo wash-out and of each treatment period, office BP, TC, HDL cholesterol, LDL cholesterol, triglycerides, plasma PAI-1, and t-PA activity were evaluated. The BP measurements were obtained from each patient in the seated position using a standard mercury sphygmomanometer (Korotkov I and V). Measurements were taken in the morning before daily drug intake (ie, 24 h after dosing) and after the subject had rested 10 min in a quiet room. Three successive BP readings were obtained at 1-min intervals and averaged. For evaluation of lipid and fibrinolytic parameters, blood was always drawn in the morning, between 8 and 9 AM, after a 15-min rest and after an overnight fast, to reduce interference by the diurnal variation of the PAI-1 and tPA.\(^20\) The TC and TG were determined by the enzymatic method of the Chemedron Company (Frankfurt, Germany). The HDL cholesterol was determined by the enzymatic method of Roschblau\(^21\) after LDL and very low-density lipoprotein (VLDL) precipitation with polyethylene glycol 6000 by the method of Viikari.\(^22\) The LDL cholesterol was calculated by the formula of Friedewald et al.\(^23\)

For fibrinolytic measurements, blood samples were collected in Biopool stabilyte tubes with citrate buffer, at pH 4.5, to ensure the stability of t-PA activity without affecting the assay of PAI-1 activity. Plasma was separated within 1 h by centrifugation for 20 min at 3000 g and stored at −70°C until assay. Plasma t-PA activity was determined with a parabolic rate assay based on fibrin stimulation of the t-PA-catalyzed conversion of Glu-plasminogen to plasmin, which subsequently cleaves the fibrinogen substrate.\(^24\) The t-PA activity was expressed in international units per milliliter by reference to the World Health Organization First International Standard for t-PA coded 86/670 from the National Institutes for Biological Standard and Control, Potters Bar, England. Plasma PAI-1 activity was determined with a two-stage, indirect enzymatic assay based on the addition of excess t-PA (40 UI) to the samples and measurement of the residual t-PA activity.\(^25\) One unit of PAI-1 activity was defined as the amount of PAI-1 that inhibits 1 UI of international t-PA standard. The reagent kits for assay of t-PA and PAI-1 activities were purchased from Biopool AB, Umea, Sweden. The coefficients of variation for repeated measures of PAI-1 activity and t-PA activity in our laboratory were 5% and 8.5%, respectively.

Data are expressed as mean ± standard deviation. The statistical analysis was conducted by using SAS version 8 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) for the cross-over design (general linear model procedure) was used to analyze the results. Statistical significance was set at \(P < .05\). To verify the basic of the crossover design,\(^26\) the possibility of a carry-over or sequence effect was also investigated using the crossover ANOVA test.

Results

Forty-five patients, 22 men and 23 women, aged 41 to 70 years, were enrolled in the study and 41 patients completed it. Four patients dropped out, one because of side effects and three for lack of cooperation.
Table 1. Effect of each treatment on blood pressure, lipids, and fibrinolytic parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Atorvastatin 20 mg</th>
<th>Amlodipine 5 mg</th>
<th>Amlodipine + Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>158.3 ± 11.1</td>
<td>155.5 ± 11.2</td>
<td>140.3 ± 9.9†</td>
<td>136.1 ± 9.9†</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>96.1 ± 4.5</td>
<td>92.3 ± 4.4*</td>
<td>81.8 ± 4.1†</td>
<td>78.4 ± 4.2†</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>264.3 ± 18.2</td>
<td>187.2 ± 14.3†</td>
<td>251.6 ± 16.9</td>
<td>180.5 ± 13.9†</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>42.9 ± 5.1</td>
<td>46.2 ± 4.3*</td>
<td>43.1 ± 4.8</td>
<td>46.8 ± 4.1*</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>193.5 ± 15.3</td>
<td>119.6 ± 11.6†</td>
<td>186.8 ± 15.2</td>
<td>111.6 ± 11.5†</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>133.9 ± 39</td>
<td>113.1 ± 33</td>
<td>134.2 ± 40</td>
<td>110.5 ± 34</td>
</tr>
<tr>
<td>PAI-1 (U/mL)</td>
<td>23.1 ± 11.3</td>
<td>13.2 ± 6.7*</td>
<td>22.6 ± 11.2</td>
<td>12.9 ± 6.8*</td>
</tr>
<tr>
<td>t-PA (U/mL)</td>
<td>0.51 ± 0.22</td>
<td>0.59 ± 0.14*</td>
<td>0.68 ± 0.20†</td>
<td>0.77 ± 0.25§</td>
</tr>
</tbody>
</table>

DBP = diastolic blood pressure; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PAI-1 = plasminogen activator inhibitor type 1; SBP = systolic blood pressure; TC = total cholesterol; TG = triglycerides; t-PA = tissue plasminogen activator.

Values expressed as mean ± standard deviation.
* P < .05 v placebo; † P < .01 v placebo; ‡ P < .005 v placebo; § P < .05 v amlodipine.

The main results of the study are reported in Table 1. As expected, amlodipine monotherapy was significantly effective in reducing both systolic BP (−18 mm Hg, −11.3%, P < .01 v placebo) and diastolic BP mean values (−14.3 mm Hg, −14.8%, P < .01 v placebo). Treatment with atorvastatin alone did not affect systolic BP values (−2.8 mm Hg, −1.7%, P = not significant), whereas it significantly reduced diastolic BP values (−3.8 mm Hg, −3.9%, P < .05 v placebo), although to a lesser extent compared with amlodipine. Interestingly, combination therapy with amlodipine plus atorvastatin produced a significantly greater reduction in both systolic BP (−22.2 mm Hg, −14%, P < .005 v placebo) and diastolic BP mean values (−17 mm Hg, −18.4%, P < .005 v placebo) than either drug alone.

Amlodipine monotherapy did not modify the lipid profile of the treated patients, whereas atorvastatin significantly reduced TC (−77.1 mg/dL, −29.1%, P < .01 v placebo) and LDL cholesterol levels (−73.9 mg/dL, −38.1%, P < .01 v placebo) and increased HDL cholesterol (+3.3 mg/dL, +7.6%, P < .05 v placebo), without affecting TG levels. Adding amlodipine did not significantly modify the lipid-lowering effect of atorvastatin.

Treatment with amlodipine alone did not affect plasma PAI-1 activity (−0.5 U/mL, −2.1%, P = not significant), whereas significantly increased t-PA activity (+0.17 U/mL, +33.3%, P < .01 v placebo). Amlodipine monotherapy significantly decreased PAI-1 activity (−9.9 U/mL, −42.8%, P < .01 v placebo) and increased t-PA activity (+0.08 U/mL, +16.6%, P < .05 v placebo). The amlodipine–atorvastatin combination produced a significantly greater reduction in PAI-1 activity (−10.2 U/mL, −44.1%, P < .01 v placebo) and an even greater increase in t-PA activity (+0.26 U/mL, +50.9%, P < .01 v placebo and P < .05 v amlodipine) than either drug alone. No relationship was found between the changes in plasma PAI-1 and t-PA activity and the hypocholesterolemic effect or the BP lowering produced by the amlodipine–atorvastatin combination.

Discussion

The results of this study showed that in hypercholesterolemic, hypertensive patients with impaired fibrinolysis, the amlodipine–atorvastatin combination, beyond the expected hypocholesterolemic effect: 1) improved the fibrinolytic balance by decreasing the PAI-1 activity and particularly by increasing t-PA activity more than the single monotherapies; and 2) decreased both systolic and diastolic BP levels more than either drug alone.

The most original findings of our study were those regarding the effects of the amlodipine–atorvastatin combination on the fibrinolytic system. In agreement with some previous observations, amlodipine monotherapy did not modify PAI-1 activity, whereas it significantly increased t-PA activity. Mechanisms for such an effect are unknown, although a direct action of amlodipine on vascular endothelium is likely to play an important role. Amlodipine has been suggested to improve endothelial function, mainly through an antioxidant action. Because both PAI-1 and t-PA are synthetized in the vascular endothelium and endothelial dysfunction induces an imbalance in fibrinolysis, improving endothelial function might reverse the fibrinolytic imbalance.

The results obtained with atorvastatin monotherapy (ie, a significant decrease in PAI-1 activity and an increase in t-PA activity) confirm the findings of some in vitro and in vivo studies. Statins have been shown to reduce PAI-1 production in cultured human endothelial and smooth muscle cells and to increase t-PA production in human smooth muscle cells. In addition, they increased fibrinolytic activity in tumor necrosis factor-α-activated human peritoneal mesothelial cells and downregulated the synthesis of PAI-1 in cultured human monocytes. Although the results of in vivo studies are more controversial, in some studies statins decreased plasma PAI-1 and increased t-PA activity. The mechanism by which statins inhibit PAI-1 and increase t-PA expression appears
to be directly associated with geranylgeranylation of some cell proteins.\textsuperscript{12,14,32,33}

Interestingly, in the present study the combination of amlodipine and atorvastatin improved the fibrinolytic balance more than the single monotherapy. In particular, a greater decrease in PAI-1 activity (−44\%) and even a greater increase in t-PA activity (+51\%) were observed. These results, which were independent from the changes in TC and BP levels induced by the amlodipine–atorvastatin combination, could be related to an additive effect of the two drugs at the endothelial level.

This study also demonstrated that the use of atorvastatin in addition to amlodipine in patients with hypertension and high cholesterol levels not only improved the lipid profile by reducing TC and LDL cholesterol and increasing HDL cholesterol levels, but also significantly improved BP control. This effect, which confirms previous observations of a positive interaction between statins and antihypertensive agents,\textsuperscript{34,35} seems to be independent from the reduction in plasma cholesterol values and suggests the possibility of a positive synergistic interaction between atorvastatin and amlodipine. The rationale for such clinical synergism could involve a direct BP-lowering effect of statins, possibly related to an improvement of endothelium-mediated vasorelaxation and to reduced arterial stiffness and vasoconstriction.\textsuperscript{15,36} Atorvastatin has been demonstrated to reduce BP in untreated hypertensive patients independently of its cholesterol-lowering effect\textsuperscript{33,37} and also in the present study, atorvastatin monotherapy produced a significant reduction in diastolic BP values. Statins also seem capable of improving the sensitivity of the vessel wall to the vasodilating effect of antihypertensive drugs. Statins have been demonstrated to improve endothelium-dependent vascular function and cause a significant vasodilation.\textsuperscript{38} This could result in a significant increase in the sensitivity of the vessel wall to the vasodilating action of amlodipine. Some retrospective analyses investigating the extent of the interaction between statins and different classes of antihypertensive drugs have shown that the effect on BP control was enhanced in patients who were given statins in combination with angiotensin-converting enzyme inhibitors and calcium channel blockers, whereas no significant interactions were observed with the use of β-blockers and diuretics.\textsuperscript{3} The enhanced interaction between statins and drugs acting mainly at the level of the vascular wall (angiotensin-converting enzyme inhibitor and calcium channel blocker) support the hypothesis that treatment with statins may enhance the capability of some classes of drugs to reduce the peripheral tone and to improve the peripheral vasodilator capacity.\textsuperscript{35}

From a clinical point of view, the additional BP reduction and the increased fibrinolytic activity observed by combining amlodipine and atorvastatin could significantly contribute to reducing the global cardiovascular risk in hypertensive, hypercholesterolemic patients and improve the overall preventive action of antihypertensive and lipid-lowering therapy. Such additional properties deserve further research.

In conclusion, the positive effect exerted by the amlodipine–atorvastatin combination on fibrinolytic balance and BP control, beyond its cholesterol-lowering effect, suggest that this combination could be the treatment of choice in hypertensive patients with hypercholesterolemia and impaired fibrinolysis.

References


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