Hypertension frequently coexists with other cardiovascular risk factors, such as hypercholesterolemia, and their combination is associated with a greater rate of cardiovascular events. Recent clinical data support that treatment of hypertensive patients with a combination of antihypertensive and lipid-lowering therapies leads to a higher reduction in the incidence of cardiovascular events. In the Anglo-Scandinavian Cardiac Outcomes Trial–Blood Pressure Lowering Arm (ASCOT-BPLA), an optimal prevention of cardiovascular events was reached in patients who were randomly assigned to atorvastatin and the amlodipine treatment. However, the potential underlying mechanisms of these vascular protective effects are not fully elucidated. Because experimental studies have shown that statins and calcium channel blockers have antitherosclerotic effects, the effect of atorvastatin alone or in combination with amlodipine was analyzed in the protein secretion profile of atherosclerotic plaques that were cultured ex vivo. In this respect, the addition of atorvastatin and amlodipine to atherosclerotic plaques normalized the levels of the different released proteins to that obtained from healthy arteries. This review highlights recent clinical and experimental studies that support that a combined treatment of hypertensive patients with both statins and calcium channel blockers could promote a higher reduction in their global cardiovascular risk profile and associated mortality. As an example, the application of a proteomic approach to assess the modulation by atorvastatin alone or in combination with amlodipine on the proteins that are released by atherosclerotic plaques has allowed the identification of novel therapeutic targets by which these drugs could promote their additive/synergic effects.

Nephrologists traditionally have been involved in the care of patients with hypertension, because this disorder is both cause and consequence of kidney disease. However, hypertension frequently coexists with other cardiovascular risk factors, such as hypercholesterolemia, and their combination is associated with a greater rate of cardiovascular events (1). Clinical and experimental studies that were performed in the past few years suggested that when treating hypertensive patients, all of these factors should be considered to achieve a higher reduction in cardiovascular morbidity and mortality on these patients.

Combined Antihypertensive and Lipid-Lowering Therapies in the Clinical Practice

A large number of clinical trials have demonstrated that treatment of either hypercholesterolemia or hypertension leads to a reduction in the incidence of cardiovascular events. Moreover, subgroup analyses that were performed in the Heart Outcomes Prevention Evaluation (HOPE) study showed the benefits of angiotensin-converting enzyme inhibitors in patients who received concomitant statin therapy (2). Similarly, in the Heart Protection Study (HPS), simvastatin diminished cardiovascular events in patients who used angiotensin-converting enzyme inhibitors (3).

The Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) studies have shed new light on this matter. The ASCOT Blood Pressure Lowering Arm (ASCOT-BPLA) analyzed 19,257 patients who had hypertension and at least three other cardiovascular risk factors and were randomly assigned to therapy with the calcium channel blocker amlodipine, adding perindopril when necessary, or to a β blocker, atenolol, using bendrofluamide as a second drug (4). By the end of the trial, 78% of patients were taking the second antihypertensive drug, and the amlodipine/perindopril regimen was superior to atenolol/atenolol/bendroflumethiazide as a second drug (4). The end of the trial, 78% of patients were taking the second antihypertensive drug, and the amlodipine/perindopril regimen was superior to atenolol/atenolol/bendroflumethiazide in reducing all-cause mortality, stroke, total cardiovascular events, and new-onset diabetes. When several variables of the ASCOT-BPLA were analyzed, BP reduction was not the only contributor to the amlodipine/perindopril reduction in cardiovascular events (5). It is interesting that although BP was the most important variable associated with the incidence of stroke, differences in HDL cholesterol were more important for coronary events. Furthermore, full adjustment for these variables as well as for body weight, glucose, triglycerides, creatinine, and potassium serum levels explained only 50 and 40% of the differences in coronary and stroke events, respectively, leaving the remaining percent-
ages to be explained potentially by other variables that were not considered in this analysis. These data suggest that BP reduction is not the only mediator in the beneficial effect of antihypertensive drugs. Accordingly, several vasculoprotective mechanisms have been demonstrated for antihypertensive drugs (6).

The ASCOT Lipid Lowering Arm (ASCOT-LLA), published in 2003, showed data of further interest (7). A total of 10,305 patients who were from the ASCOT-BPLA study and had total cholesterol concentrations of ≤6.5 mmol/L were randomly assigned to 10 mg/d atorvastatin or placebo. Treatment with the statin reduced the incidence of nonfatal myocardial infarction or fatal coronary heart disease and that of stroke, cardiovascular, and coronary events. The results of the ASCOT-LLA trial proved to be independent of the degree of BP reduction associated with lipid lowering, as both the atorvastatin and the placebo group showed a similarly good BP control. The emerging new concept that the ASCOT-LLA study addresses is that the concomitant use of statins improved the clinical evolution of hypertensive patients with moderately increased or even normal total cholesterol levels but at risk for cardiovascular events because of other accompanying risk factors. In this respect, statins have been claimed to exert a part of their beneficial actions by cholesterol-independent mechanisms (8). Remarkably, in the ASCOT-BPLA, an optimal prevention of cardiovascular events was reached in patients who were randomly assigned to atorvastatin and the amlodipine/perindopril treatment, with a reduction of 48% in the risk for fatal myocardial infarction and nonfatal coronary heart disease and 44% in the incidence of stroke. However, the underlying mechanisms of these beneficial effects have not been elucidated.

**Protective Role of Dual Therapy (Atorvastatin-Amlodipine) in Atherosclerosis**

Calcium channel blockers (CCB) have been used for decades to treat hypertension, but it also has been suggested that they interfere with the progression of atherosclerotic disease (9–11). Although the initial results for dihydropyridines in this area were weak, the appearance of amlodipine changed this view (11). In the Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT), this drug reduced carotid intima-media thickness progression and the incidence of unstable angina and revascularization in patients with coronary artery disease (12). These effects could be due to the special profile of amlodipine, which includes antioxidant, antiproliferative, and anti-inflammatory properties, among others (13–15) (Figure 1).

Figure 1. Known antiatherosclerotic mechanisms of action of statins and calcium channel blockers (CCB). The scheme represents the described similar mechanisms that potentially are involved in the vascular protection that is afforded by statins and CCB. SMC, smooth muscle cells; NO, nitric oxide; MMP, matrix metalloproteinases.

Statins, in addition to lowering lipid levels, decrease the incidence of vascular thrombotic events (2,16). Specifically, atorvastatin has demonstrated a powerful effect on clinical events and even stopped or reversed the progression of atherosclerosis (17–19). Given that statins and CCB have different mechanisms of action, it is conceivable that they may have an additive or synergic effect, not only on new plaque formation but also on inhibiting the progression of established lesions (20). Indeed, this synergic effect of lipid-lowering therapy and CCB on human coronary atherosclerosis was reported in the Regression Growth Evaluation Statin Study (REGRESS) (21). Moreover, various studies in hypertensive hyperlipidemic patients have shown that the combination of atorvastatin and amlodipine has additive effects in the improvement of arterial compliance and in the fibrinolytic balance, early markers of
vascular damage and atherosclerosis (22,23). Collectively, these studies support the clinical antiatherosclerotic advantages of the combination of both CCB and statins and, in particular, of atorvastatin with amlodipine beyond their established antihyperlipidemic and antihypertensive modes of action.

**Effect of Dual Therapy on Protein Profile of Atherosclerotic Plaques**

The beneficial effect of combining CCB with statins has been replicated in transgenic atherosclerotic mice, in which treatment with amlodipine and atorvastatin produced an additional reduction of atherosclerosis compared with that observed with either amlodipine or atorvastatin alone (24). Given that previous papers have shown that statins and amlodipine have antiatherosclerotic effects (6,8,14,24–26), it is conceivable that the synergistic effect of both drugs may be extended to these actions previously known (Figure 1). Considering this, we decided to analyze the effect of atorvastatin alone or in combination with amlodipine in the protein secretion profile of atherosclerotic plaques by following the same proteomic ap-

**Figure 2.** Novel potential therapeutic targets for atorvastatin or dual therapy in atherosclerosis. The hierarchical cluster reflects the modulation of the protein secretion profile detected by proteomic analysis for complicated atherosclerotic plaques (atheroma) that were cultured ex vivo in the presence of atorvastatin alone or in combination with amlodipine (DUAL). Following the color scale, the lower secretion levels of proteins are in the green scale, and the ones with higher secretion are red.
Atherosclerotic plaques were incubated in the presence of amlodipine (1 μmol/L). For that purpose, atherosclerotic plaques were incubated in the presence of amlodipine and the one from healthy arterial segments were compared by using two-dimensional electrophoresis and mass spectrometry. These tools have become popular in protein research because they allow the separation, detection, and identification of thousands of proteins in a single experiment and promote the characterization of new, unforeseen proteins.

In this work, significant improvements, mainly related to sample preparation protocols, that have allowed us to identify a higher number of proteins that are differentially released by atherosclerotic plaques in culture in comparison with control arterial segments have been made. In this respect, from an average of 620 spots per gel detected, we focused on the analysis of 260 proteins. According to the analysis that was done using the PD-Quest Software (BIO-RAD Laboratories, Veenendaal, The Netherlands), 217 protein spots were present at higher levels in the conditioned media of atherosclerotic plaques than in those of the control region, whereas 43 protein spots were decreased or remained unchanged. From a total of 83 proteins that were identified by mass spectrometry, 34 showed higher levels in atheroma plaque secretome, whereas another 31 proteins presented lower levels compared with the control-conditioned media. Examples of these proteins include leucine-rich α-2-glycoprotein, transferrin, apolipoprotein A-I, fibrinogen, α-1-antitrypsin, complex-forming glycoprotein HC, serum amyloid P component, heat-shock protein 27, and elastase 1. Some of these identified proteins were detected in more than one spot on the gels, suggesting the presence of posttranslational modifications and affecting to their isoelectric point and/or molecular weight. Experiments to understand the potential pathophysiologic role of these posttranslational modifications are in progress.

We hypothesized that the addition of atorvastatin alone or in combination with amlodipine to complicated atherosclerotic plaques could modulate some of the novel identified proteins that were released by the plaques ex vivo. For that purpose, atherosclerotic plaques were incubated in the presence/absence of atorvastatin (10 μmol/L) alone or in combination with amlodipine (1 μmol/L, dual), and the released proteins were analyzed and compared by two-dimensional electrophoresis. The modulation by atorvastatin or dual treatment of the identified proteins is represented in a hierarchical cluster, where differences among all experimental conditions can be observed (Figure 2). The addition of drugs to the complicated atherosclerotic segments promoted different effects in protein levels of tissue-conditioned media, but in the majority of the cases, treatment with atorvastatin alone or in combination with amlodipine normalized the levels of the different released proteins. Furthermore, in some cases, dual treatment was superior to that observed with atorvastatin alone. In this respect, treatment with the combination of atorvastatin and amlodipine but not with atorvastatin alone was able to upregulate the decreased levels that were observed for the protein β-galactoside soluble lectin in the conditioned media of atherosclerotic plaques in culture, reaching control values. It is interesting that the member of the family of β-galactoside soluble lectins galec-toxin-I was shown to inhibit leukocyte rolling and extravasation in experimental inflammation (28), a main mechanism involved in atherogenesis. Other examples are retinol-binding protein, protein disulfide isomerase, and the antioxidant protein thioredoxin peroxidase B2, probably related to the antioxidant properties that are associated to amlodipine treatment.

**Conclusion**

Clinical and experimental studies support that a combined treatment of hypertensive patients with both atorvastatin and amlodipine could promote a higher reduction in their global cardiovascular risk profile and associated mortality. In this respect, the application of a proteomic approach to assess the modulation by atorvastatin alone or in combination with amlodipine of the proteins that are released by complicated atherosclerotic plaques has allowed us to identify novel therapeutic targets by which these drugs could promote their additive/synergic effects. However, the underlying mechanisms of these beneficial effects deserve further research.

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**References**


