Novel pharmaeutical interventions in experimental atherosclerosis and myocardial infarction

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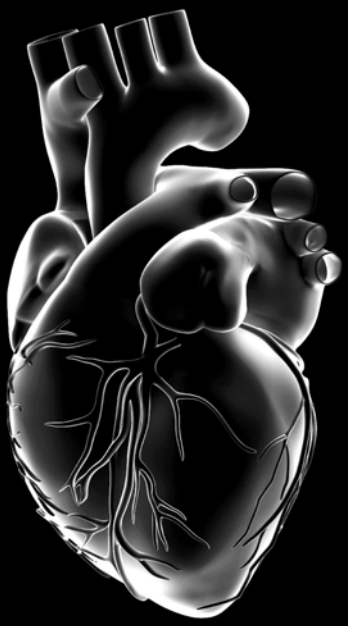

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Amlodipine and Atorvastatin in Atherosclerosis:

A review of the potential of combination therapy

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Abstract

Hypertension and hyperlipidemia are major risk factors for the development of atherosclerosis. Calcium channel blockers (CCBs) are used for decades for their established antihypertensive effects, as statins are used for a long time for their potent lipid lowering properties. Amongst others inflammation and oxidation are involved in enhanced progression of atherosclerosis and new lesion development. Therefore research has been initiated focusing on e.g. the antioxidant and anti-inflammatory properties of CCBs and statins, beyond their primary effect in order to evaluate the possible additive effects of combined treatment of CCBs with statins as anti-atherosclerotic therapy.

Clinical studies, amongst others the International Nifedipine Trial on Antiatherosclerotic Therapy (INTACT), have demonstrated that the antiatherosclerotic action of CCBs is limited to attenuation of the first stage of atherosclerogenesis (fatty streak formation or new lesion growth). The lesions that pre-existed the start of CCB therapy did not demonstrate progression or regression on angiography. However, because the mechanisms of action of lipid-lowering drugs and CCBs and their role in preventing the progression of atherosclerosis differ, it is conceivable that these two classes may have an additive or synergic effect, not only on new lesion formation but also on inhibiting the progression of established coronary atherosclerosis. Indeed, this combined effect of lipid-lowering therapy and CCBs on human coronary atherosclerosis has been reported in the Regression Growth Evaluation Statin Study (REGRESS) trial. Researchers observed a significant beneficial effect of CCBs with regard to angiographic progression and new lesion formation in patients treated with a statin, but no similar antiatherosclerotic effect in those treated with CCB alone (placebo group). This beneficial effect as a result of combining CCBs with statins has now been replicated in transgenic atherosclerotic mice, where the combination of amlodipine and atorvastatin produced an additional 60% reduction of atherosclerosis compared with that observed with the statin alone. Serum markers of atherosclerosis and vascular integrity also improved most in the combination group. Recently Mason *et al.* showed a synergistic effect of the combination of atorvastatin and amlodipine on acute NO release/endothelial function, whereas Leibovitz *et al* demonstrated that the combination of amlodipine and atorvastatin had an additive effect in improvement of arterial compliance in hypertensive hyperlipidemic patients.

Collectively, these studies support the clinical anti-atherosclerotic advantages of combination of CCBs and statins and in particular of atorvastatin with amlodipine beyond their established antihyperlipidemic and antihypertensive modes of action.

Introduction

Cardiovascular disease (CVD) is the main cause of death in the Western Society. CVD is mostly caused by atherosclerosis. Established risk factors for developing atherosclerosis are male gender, age, diabetes mellitus, genetic predisposition, high plasma lipoprotein levels, hypertension, obesity and smoking.

Hypertension is considered a 'traditional' risk factor for developing atherosclerosis, which entails a threefold risk over that of normotensive persons of the same age [1]. However, in the fast majority of cases no single reason can be found for a patient causing the hypertension. This indicates that hypertension is a very complex multifactorial disease, in which different mechanisms are involved. The last decade newer 'nontraditional' risk factors for the development of atherosclerosis are identified including inflammation and its markers, like C-reactive protein, homocysteine, oxidative stress and endothelial dysfunction, but also activation of the renin-angiotensin-aldosterone-system (RAAS)[2]. Evidence is accumulating that hypertension may be just a marker and the underlying mechanism is the risk factor for the development of atherosclerosis.

In patients with coronary atherosclerosis, disease progression is one of the main factors that determine clinical prognosis. Patients with progression of coronary atherosclerosis, do significantly worse with regard to clinical event-free survival than patients with attenuated progression[3]. Thus inhibition of the progression of atherosclerosis is almost as important as preventing atherosclerosis development. Lipid-lowering therapy has undoubtedly proven to be an effective therapeutic modality to retard the progression of coronary atherosclerosis[4]. Possible beneficial modes of action of lipid-lowering therapy include: (1) retardation of progression and induction of regression of coronary atherosclerosis[4] (2) atherosclerotic lesion-plaque stabilization[5,6] (3) restoration of endothelial dysfunction[7], (4) decreased thrombotic tendency[8], and immune system modulation [9].

Evidence indicating also that some calcium channel blockers (CCBs), which are established anti-hypertensive drugs, inhibit atherosclerosis is accumulating. Many investigations support the view that a number of key processes in atherosclerosis may be influenced by CCBs. These key processes include: (1) oxidation of circulating lipoproteins, such as LDL[10], (2) binding of monocytes to and transmigration of monocytes through the endothelial cell layer[11],(3) formation of macrophage-derived foam cells, (4) proliferation and migration of VSCMs[12], (5) binding of platelets to the endothelial cells layer and subsequent platelet aggregation[13], and (6) synthesis of matrix components, such as collagen.

In this review first the development of atherosclerosis and hypertension will be briefly described. Secondly mechanisms and effects of lipid-lowering therapy by statins and anti-hypertensive therapy by calcium channel blockers will be described. Because the mechanisms of action of lipid-lowering drugs and CCBs and their role in preventing

the progression of atherosclerosis differ, and it therefore is conceivable that these two classes may have an additive or synergic effect, it is interesting to focus on combination therapy. Most research with regard to combination therapy of CCBs and statins has been performed with amlodipine and atorvastatin and therefore the effects of the combination of these compounds on atherosclerosis will be specially highlighted.

Hypertension and atherosclerosis

In 1996 Meyer *et al.*[14] showed the effects of pressure-induced stretch and convection on low-density lipoprotein (LDL) and albumin uptake in the rabbit aortic wall. It was demonstrated that pressure-induced stretching of the arterial wall is a major determinant of arterial mass transport, and that pressure-driven convection accentuates LDL accumulation in the inner media, which may explain enhanced atherosclerosis in hypertension. Accumulation of atherogenic lipoproteins in the arterial wall is generally considered to be the first step in the development of atherosclerosis. Reactions with reactive oxygen species (ROS) [15] can oxidatively modify the lipid and apoB components of LDL trapped in the subendothelial space. Proinflammatory factors, such as oxidized LDL and ROS stimulate release of cytokines. This leads to the accumulation of mononuclear cells, migration and proliferation of SMCs and formation of fibrous tissue that eventually results in an atherosclerotic plaque.

Most, if not all, of the risk factors that are related to atherosclerosis and cardiovascular morbidity and mortality, were also found to be associated with endothelial dysfunction. Many of these risk factors, including hyperlipidemia, hypertension, diabetes and smoking are associated with overproduction of ROS or increased oxidative stress [16,17]. Measurement of endothelial (dys)function gives a proper indication about the health/condition of the endothelium. In endothelial dysfunction the homeostasis of vasoactive substances is disrupted [18]. The bioavailability of NO, which promotes vasodilation in response to hemodynamic stress, is decreased due to reduced secretion and to interaction with superoxide anion (O_2^-)[19]. The level of the vasoconstrictor factor angiotensin II, promoting the proliferation and influx of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and cytokines, and the level of the potent vasoconstrictor hormone endothelin-1 (ET-1) are increased [20]. These changes in homeostasis lead to changes in vascular structure and function [19,21]. Besides a disturbed balance of vasodilators and vasoconstrictors, endothelial dysfunction also comprises a specific state of 'endothelial activation', which is characterized by a proinflammatory, proliferative, and procoagulatory environment that favors all stages of atherogenesis[16]. As a number of these cellular and inflammatory processes are mediated by disruption of calcium homeostasis, there has been interest in the potential role of calcium channel antagonists (CCBs) as antiatherogenic agents, apart from their anti-hypertensive potential [11].

Calcium Channel Blockers

Mechanism

The mechanisms of the anti-atherosclerotic effect of CCBs, also called calcium antagonists, are not fully understood, however many pathways have been studied last decade, giving more insight in different mechanisms of CCBs. CCBs are essentially used as anti-hypertensive drugs. The primary action of calcium channel blockers is to inhibit calcium ion entry through voltage-gated transmembrane L-type channels, thus decreasing intracellular calcium concentration and inducing smooth muscle relaxation. Several important processes in atherosclerosis may be influenced by CCBs because they require calcium-dependent energy. In vitro studies have shown that CCBs can reduce lipoprotein oxidation and proliferation and migration of smooth muscle cells. However, the anti-atherosclerotic activity of CCBs probably involves many additional properties of the compounds because calcium-independent mechanisms, such as binding of monocytes to the endothelial cell layer, esterification of cholesterol in macrophages, and expression of matrix metalloproteinases in vascular endothelial cells, have also been shown to be inhibited by calcium antagonists[22]. CCB can be separated in two main groups i.e. the dihydropyridine (DHP) and the non-dihydropiridine (non-DHP) CCBs. On average these different CCBs display different anti-atherosclerotic potential, especially some DHP CCBs seem to have anti-atherosclerotic potential.

CCBs and atherosclerosis

Anti-atherosclerotic properties of CCB treatment were discovered in the 1980s. It was demonstrated that plasma membrane calcium transport in the aortic wall of rabbits with experimental atherosclerosis was increased fivefold and that CCBs were able to suppress such experimental atherosclerosis[23]. Since then CCBs have been evaluated for their anti-atherosclerotic effect in humans[24].

In Vitro

Mak *et al.*[10] demonstrated that DHP CCBs had the same protective effect against oxidative damage in bovine aortic endothelial cells like vitamin E. Because endothelial cells do not have receptors for CCBs (the L-type calcium channels are not involved in calcium influx[25]) the molecular mechanism of the antioxidant effect is less clear. Apparently, the cytoprotective effects of the DHP calcium blockers were mediated by a membrane 'chain-breaking' antiperoxidative action similar to that provided by vitamin E[10]. DHP CCB amlodipine has been shown to stimulate NO release from canine coronary microvessels in a dose dependent manner like the ACE inhibitors analaprilat and ramiprilat, whereas DHP CCB nifedipine and diltiazem did not. Amlodipine mediates NO

release and hereby may have antioxidative properties, similar to the mechanism of ACE inhibitors, by modulation of the actions or formation of kinins[26].

Oxidative-modification of LDL contributes to destructive inflammatory processes associated with atherosclerosis. It is characterized by elevations in cholesterol content and increased electro negativity, factors that contribute to aggregation and foam cell formation. Phillips and Mason[27] designed a study to test the effect of the positively charged calcium channel blocker (CCB) amlodipine on the aggregation properties of oxidized LDL lipids. Amlodipine inhibited binding of the oxidized LDL lipids in a dose-dependent fashion, in contrast to other drugs lacking a formal positive charge (including CCBs).

Amlodipine also modulates metabolism of collagens within the extracellular matrix and thus potentially has plaque stabilizing properties. It was demonstrated that CCBs, including amlodipine, specifically increased the proteolytic activity of the 72-kDa type IV collagenase and inhibited the transcription of tissue inhibitor of MMP-2[28]. Amlodipine, but not nifedipine significantly decreased interleukin-1β induced MMP-1 expression in human endothelial cells[29]. Additionally, several studies demonstrated a role for amlodipine in the remodeling of SMC membranes and in the inhibition of SMC proliferation and migration, which are hallmark features of atheroma development [11,12,29].

In vivo

Amlodipine was tested in hyperlipidemic hamsters for is antiatherogenic properties. Male Golden Syrian hamsters were subjected to a hyperlipidemic diet. At intervals ranging from 2 to 14 weeks, the animals were examined for changes in serum constituents and structural modifications of lesion-prone areas like the cardiac valves, coronary arteries and aortic arch. Amlodipine treatment of hyperlipidemic hamsters was assessed. Amlodipine exhibited an atheroprotective effect, acting as antioxidant, reducing the LDL uptake by the vessel wall and consequently, limiting the size and extent of lesion areas[30]. Recently Turgan *et al.*[31]investigated the interactions of amlodipine with major cellular antioxidants in order to elucidate the mechanisms underlying its atheroprotective effects. New Zealand white male rabbits were fed regular chow with or without 1% cholesterol and with or without amlodipine for 8 weeks. Total cholesterol, malondialdehyde and vitamin E concentrations and catalase and superoxide dismutase activities were determined in blood drawn before and after the experimental period. Aortic tissue was examined for atherosclerotic changes and aortic total cholesterol, malondialdehyde, catalase and superoxide dismutase were determined. Amlodipine seemed to exert atheroprotective effects by reducing aortic cholesterol accumulation and blood and aortic lipid peroxidation, enhancing superoxide dismutase activity both in blood and aortic tissue and suppressing the consumption of

vitamin E. On the other hand, the suppression of catalase activity in blood and the aorta interferes with the well-known antioxidant effects of amlodipine.

Atherosclerosis may result in plaque rupture resulting in a myocardial infarction. Calcium dependent factors, including cholesterol-induced changes in membrane calcium permeability and calcium deposition into lesions, may contribute to plaque formation and stability during the early and late stages of atherogenesis. Hoshida et al. evaluated the effect of amlodipine treatment on myocardial infarction size after 30-min coronary occlusion/48-h reperfusion in rabbits fed a diet with or without 1% cholesterol. Infarct size was significantly larger in cholesterol-fed rabbits (72.0 +/- 3.5%, n = 9, mean +/- S.E.M.) than in normal-fed rabbits (47.1 +/- 4.9%, n = 9, $P < 0.05$). Amlodipine treatment effectively reversed the infarct size augmentation in cholesterol-fed rabbits (46.3 +/- 6.3%, n = 9, $P < 0.05$), but did not affect infarct size in normal-fed rabbits (51.0 +/- 4.7%, n = 8). Calcium content and leukocyte accumulation were markedly elevated in the ischemic myocardium of cholesterol-fed rabbits compared with normal-fed rabbits. Amlodipine treatment effectively reversed this elevation. Acetylcholine showed a marked reduction in endothelium-dependent relaxation in the aorta of cholesterol-fed rabbits, which also was reversed by amlodipine treatment[32].

Clinical trails with CCBs, using angiography show different results. The INTACT study[33] showed that nifedipine had no effect on the progression of existing lesion but could reduce the number of new formed lesions in coronary artery diseased patients. The PREVENT study[34] demonstrated in cardiovascular diseased patients that amlodipine treatment was associated with reduction in cardiovascular morbidity, but had no effect on the angiographic progression of atherosclerosis or cardiovascular mortality. The ELSA study[35] compared the progression of atherosclerosis in hypertensive patients treated with β-blocker atenolol or DHP CCB lacidipine. Despite a smaller ambulatory blood pressure reduction, lacidipine was shown to have a greater efficacy on carotid intima-media thickness progression, which predicts cardiovascular events, and number of plaques per patient. Recently, the NICOLE study[36] evaluated the effect of nisoldipine on atherosclerosis progression in patients who had undergone coronary angioplasty. It was found that long acting nisoldipine did not retard the angiographic progression of coronary artery disease and did not affect mortality. However, it reduced the need for coronary revascularisation.

Besides the (clinical) antiatherosclerotic effects found in previous described studies Ghiadoni *et al.* [37] found reduced oxidative stress and increased plasma oxidant capacity hypertensive patients receiving amlodipine or nifedipine.

Statins

Mechanism

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase catalyses the rate-limiting step in cholesterol biosynthesis. Consequently this has been an important target for the development of cholesterol-lowering agents. Inhibition of HMG-CoA reductase leads to an upregulation of LDL-receptors in the liver mediated by the activation of Sterol Regulatory Element-Binding Proteins (SREBPs) and enhanced clearance of LDL from the circulation. HMG-CoA reductase inhibitors, or statins, were first introduced into clinical practice in the 1980s. Pravastatin and lovastatin are produced from fungal metabolites, simvastatin is semi-synthetic and fluvastatin, atorvastatin and rosuvastatin are synthetic statins[38]. Pravastatin and rosuvastatin are hydrophilic agents; other statins are highly lipophillic.

Statins and atherosclerosis

Because lipoproteins infiltrated in the vessel wall are the start of the development of atherosclerotic lesion and oxidized particles may trigger or enhance atherogenicy, statins are relevant in treatment for preventing atherosclerosis. In many clinical trails is proven that treatment with statins reduce morbidity and mortality in CVD patients. Best known, major trials in the 1990s were 4S (1994) [39], CARE (1996) [40] (1996) and LIPID (1998) [41] (1997). The 4S trial was designed to evaluate the effect of cholesterol lowering with simvastatin on mortality and morbidity in patients with coronary heart disease. 4444 Patients with angina pectoris or previous myocardial infarction and serum cholesterol 5.5-8.0 mmol/L on a lipid-lowering diet were randomised to double-blind treatment with simvastatin or placebo. Over the 5.4 years median follow-up period, simvastatin showed a reduction of major coronary events, coronary deaths and overall mortality. Other benefits of treatment included a reduction in the risk of undergoing myocardial revascularisation procedures. This study showed that long-term treatment with simvastatin is safe and improves survival in CVD patients. CARE and LIPID are both trails in which the effect of pravastatin on coronary events and death was investigated in patients with a history of myocardial infarction of unstable angina pectoris. In the CARE study 4159 patients and in the LIPID study 9014 patients participated. The mean follow-up period was 5 (CARE) and 6 (LIPID) years. In both studies was demonstrated that pravastatin reduced the fatal coronary events, myocardial infarction, stoke and overall death.

Statins and inflammation

Over the last years evidence is accumulating that not only the lipid lowering effect of the statins but also their anti-inflammatory properties may be responsible for atheroprotective effects. In 1998 Yamamoto *et al.*[42] demonstrated that fluvastatin, more

than pravastatin inhibited NAD(P)H dependent lipid peroxidation in liver microsomes. In addition fluvastatin scavenged ROS such as hydroxyl radicals and O2-. A reduction of fibrinogen and serum amyloid A was seen in ApoE*3-leiden transgenic mice treated with atorvastatin, which is a synthetic and lipophilic statin like fluvastatin [43].

Recently Hernandez-Presa *et al.*[44] investigated whether simvastatin reduced inflammation in atherosclerosis beyond its hypolipidemic effects. Rabbits with induced femoral injury and on an atherogenic diet were randomized to normolipidemic diet or to continue the atherogenic diet while receiving simvastatin or no treatment for 4 weeks. As compared with no treatment, the normolipidemic diet significantly reduced lipid levels, while simvastatin produced nonsignificant reductions. In spite of this, NF-κB binding activity in peripheral mononuclear cells was reduced in the simvastatin group as compared with no treatment and normolipidemic groups (electrophoretic mobility shift assay). NF-κB activity in the atherosclerotic lesions was also reduced by simvastatin as compared to nontreated animals, while the normolipidemic diet induced only a nonsignificant diminution (Southwestern histochemistry). Similarly, simvastatin decreased macrophage infiltration and the expression of interleukin-8 and MMP-3, while the reduction achieved by normolipidemic diet in all these parameters was again nonsignificant.

In APOE*3Leiden transgenic mice, which develop human-like atherosclerosis, Kleemann *et al*.[45] demonstrated that rosuvastatin treatment reduced atherosclerosis beyond and independent of the reduction achieved by cholesterol lowering alone. In this study mice received a high cholesterol diet with or without rosuvastatin or a normolipidemic diet. It was also shown by in situ hybridization in the aortic root, that the number of TNF-α and MCP-1 positive cells was significant reduced after rosuvastatin treatment. A same reduction was found for Serum Amyloid A and fibrinogen levels. These findings were all lipid independent, demonstrating anti-inflammatory activities of rosuvastatin.

Atorvastatin and inflammation

Several studies showed results regarding the interaction of statins and NF-κB signalling. Atorvastatin reduces activation of transcription factor NF-κB in cultured VSMCs [46] as well as in atherosclerotic lesions in the rabbit[47]. Dichtl *et al*.[48] aimed to characterize the effects of statins on the activation of transcription factors known to regulate inflammation and cell proliferation/differentiation. Simvastatin, atorvastatin, and lovastatin inhibited the binding of nuclear proteins to both the NF-κB and activator protein-1 DNA consensus oligonucleotides in human endothelial cells and VSMCs as assessed by electrophoretic mobility shift assay. Furthermore, statins inhibited DNA binding of hypoxia-inducible factor-1α.

Recently Zhao *et al.*[49] demonstrated that atorvastatin can inhibit IL-6 secretion in rabbit adipocytes, in a dose-dependent manner, possibly through upregulating

peroxisome proliferator-activated receptor (PPAR) gamma. In high cholesterol fed rabbits on two week atorvastatin treatment, IL-6 concentrations in plasma and adipocytes culture supernatant and PPARgamma mRNA expression were measured. Two weeks atorvastatin treatment resulted in significant reduction of circulating IL-6 concentrations, which was associated with IL-6 secretion in adipocytes. Meanwhile mRNA expression of PPARgamma in adipocytes was intimately related to the IL-6 secretion in adipocytes.

Thrombin, a serine protease, plays an important role in inflammation and thus the progression of atherosclerosis. Haloui *et al*. [50] demonstrated that atorvastatin could limit the pro-inflammatory response to thrombin in cultured rat aortic smooth muscle cells. The variations in expression of interleukin-6, heme oxygenase-1, p(22phox) and Mox-1 mRNAs were evaluated. Thrombin activated interleukin-6 secretion and mRNA expression in smooth muscle cells in a dose-dependent manner. The greatest effect on mRNA expression was obtained after 1 h of stimulation. Preincubation of the cells with various concentrations of atorvastatin prevented this effect. Thrombin was without effect on p(22phox) and heme oxygenase-1 mRNA expression but, after 3 hour of stimulation, induced a two-fold increase in that of Mox-1. Preincubation with atorvastatin dose-dependently down regulated this Mox-1 mRNA expression. In addition, thrombin induced NF-kappaB translocation and membrane translocation of RhoA in smooth muscle cells which were both prevented by pre-treatment of the cells by atorvastatin.

Atorvastatin in humans

Inflammation

Adhesion molecules and cytokines are involved in the pathogenesis of intimal injury in atherosclerosis. To investigate their relationship with endothelial function Nawai et al. [51] examined the effects of atorvastatin on soluble adhesion molecules, interleukin-6 (IL-6) and brachial artery endothelial-dependent flow mediated dilatation (FMD) in patients with familial (FH) and non-familial hypercholesterolemia (NFH).

A total of 74 patients (27 FH and 47 NFH) were recruited. Fasting lipid profiles, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular-cellular adhesion molecule-1 (sVCAM-1), E-selectin, IL-6 and FMD were measured at baseline, 2 weeks, 3 and 9 months post-atorvastatin treatment (FH: 80 mg/day, NFH: 10 mg/day). In both groups, compared to baseline, sICAM-1 levels were significantly reduced at 2 weeks, further reduced at 3 months and maintained at 9 months. The IL-6 levels were significantly reduced at 3 months and 9 months compared to baseline for FH and NFH. In both groups, the FMD at 2 weeks was higher than baseline, with progressive improvement up to 9 months. FMD was negatively correlated with sICAM-1 and IL-6.

Thus both low and high doses of atorvastatin lead to early progressive improvement in endothelial function in patients with primary hypercholesterolemia. sICAM-1 and IL-6 levels do reflect endothelial dysfunction in these patients.

The pleiotropic actions of statins include anti-inflammatory and antioxidant actions. To evaluate whether alternative oxidative pathways are suppressed in vivo after atorvastatin administration was examined by Shishehbor et al.[9]. Hypercholesterolemic subjects with no known coronary artery disease were evaluated at baseline and after 12 weeks of atorvastatin therapy (10 mg/d). Plasma levels of protein-bound chlorotyrosine, NO_2Tyr, dityrosine, and orthotyrosine, specific molecular fingerprints for distinct oxidative pathways up regulated in atheroma, were determined by mass spectrometry. In parallel, alterations in lipoproteins and C-reactive protein were determined. Statin therapy caused significant reductions in chlorotyrosine, NO_2Tyr, and dityrosine (30%, 25%, and 32%, respectively) that were similar in magnitude to reductions in total cholesterol and apolipoprotein B-100 (25% and 29%). Nonsignificant decreases in orthotyrosine and C-reactive protein levels were observed (9% and 11%, respectively). Statin-induced reductions in oxidation markers were independent of decreases in lipids and lipoproteins. Statins promote potent systemic antioxidant effects through suppression of distinct oxidation pathways. The major pathways inhibited include formation of myeloperoxidase-derived and nitric oxide-derived oxidants, species implicated in atherogenesis.

Plaque stability

Beside prevention and regression of atherosclerosic plaque formation, prevention of plaque rupture is another very important aspect. Coronary plaque stabilization by statin therapy in humans was investigated by Takano *et al.* [6]. They evaluated the changes in coronary plaque color and morphology by atorvastatin therapy using coronary angioscopy. Thirty-one patients with coronary artery disease were divided into either the comparison group (n = 16) or the atorvastatin group (n = 15). Before treatment and 12 months after, the color and complexity of 145 coronary plaques were determined according to angioscopic findings. The yellow score of the plaque was defined as 0 (white), 1 (light yellow), 2 (yellow), or 3 (dark yellow), and its disrupted score was defined as 0 (smooth surface) or 1 (irregular surface) and as 0 (without thrombus) or 1 (with thrombus). In each patient, the mean yellow score and mean disrupted score were calculated. Mean LDL cholesterol decreased by 45% in the atorvastatin group, whereas an increase of 9% was seen in the comparison group. The mean yellow score decreased from 2.03 to 1.13 in the atorvastatin group, whereas it increased from 1.67 to 1.99 in the comparison group. There was a good correlation between the change in the mean yellow score and the change in LDL cholesterol levels. The change in the mean yellow score and mean disrupted score differed significantly between the two groups. This is the first report clarifying detailed changes in coronary plaque by statin in humans. This study

indicated that lipid-lowering therapy changes plaque colour and morphology possibly reflecting a more stable plaque phenotype in vivo.

Revascularisation

The AVERT study[52] was designed to determine whether aggressive lipid-lowering therapy with atorvastatin is an alternative to angioplasty or other catheter-based revascularization procedures in patients with significant coronary artery disease. 341 Patients with low-density lipoprotein (LDL) cholesterol ≥ 3 mmol/L and ≥ 1 defined narrowing of a major coronary artery were randomized to atorvastatin or the indicated catheter-based revascularization and conventional care (including lipid-lowering therapy if prescribed). Ischemic events were tracked for 18 months. The primary efficacy parameter was the incidence of an ischemic event, defined as 1 of the following: cardiovascular death, cardiac arrest, nonfatal myocardial infarction, the need for coronary bypass grafting or angioplasty, cerebrovascular accident, and worsening angina verified by objective evidence requiring hospitalization (including unstable angina). Results of this study favour the use of aggressive lipid lowering over percutaneous transluminal coronary angioplasty in patients with mild to moderate coronary disease. Treatment with atorvastatin significantly reduced LDL cholesterol levels, and was associated with a 36% reduction in ischemic events and a significant delay in time to first ischemic event.

Combining Statin and Calcium Channel Blocker therapy

Advances of combination therapy

It has been demonstrated very often that statins and CCBs both have antiatherosclerotic potential via different mechanisms. Gathering their strengths might lead to additive or synergistic antiatherosclerotic effects. Important evidence for an additive effect of CCBs and statin treatment in retarding progression of coronary atherosclerosis was found in a retrospective study on data from the REGRESS-study[4]. Evaluating the effect of pravastatin on progression and regression of coronary artery disease, it was found that the combination of pravastatin and a calcium antagonist was more successful in retarding the progression of atherosclerosis than pravastatin alone. This seemed to be especially true for DHP CCBs, which tended to have stronger antiatherosclerotic properties than non-DHP CCBs [53] (**Figure 1**).

The ENCORE[54] investigators examined the effects of a statin and/or a calcium antagonist on coronary endothelial function in patients with coronary artery disease. In 343 patients the endothelial function was investigated. Thereafter, patients were randomized in a double-blind manner to a 6 month treatment with placebo, cerivastatin, nifedipine, or their combination. In the most constricted segment, nifedipine but not

cerivastatin improved endothelial dysfunction. Patients not taking ACE inhibitors showed a smaller improvement in the placebo group (6.0%), but nifedipine still had an effect (17.0%; P<0.05 versus placebo).

Analysis of all evaluable coronary segments revealed an improved endothelial function in patients receiving nifedipine and cerivastatin (P<0.05 versus placebo). Cerivastatin lowered LDL cholesterol by 35%. The ENCORE II study is designed to detect the effects of nifedipine together with a statin on coronary morphology. This may give more insights in the role of CCB combined with statin in coronary artery disease.

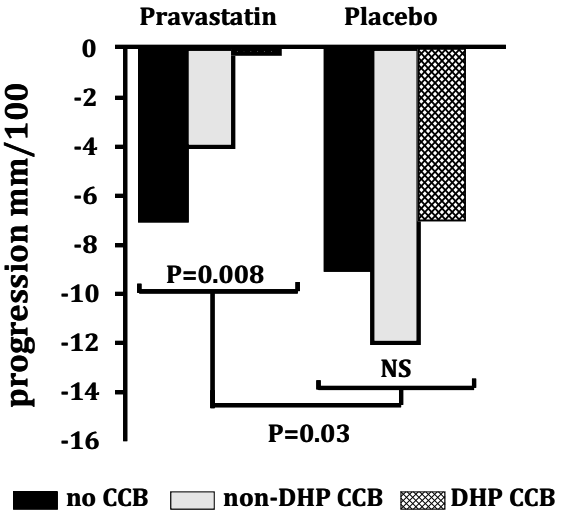

Figure 1 Angiographic progression (change of minimum obstruction diameter) in patients with and without CCB cotreatment, stratified with regard to type of CCB treatment (no CCB, non-dihydropyridine (non-DHP) CCB, and DHP CCB), in the pravastatin group and in the placebo group in the REGRESS study[4,51]. Patients in the pravastatin group had significantly less progression if cotreated with CCBs as compared with no CCB cotreatment (p=0.03), with most striking results for the DHP CCBs (hardly any progression left; p = 0.008), whereas in the placebo (no pravastatin) group no significant effect of any type of CCB treatment was observed.

Atorvastatin and amlodipine

The combination therapy of amlodipine and atorvastatin was tested in APOE*3Leiden transgenic mice, which develop human-like atherosclerosis, by Delsing *et al*[43]. 4 Groups of 15 ApoE*3Leiden mice received a high-cholesterol diet. One group received amlodipine in the diet, which had no effect on plasma cholesterol levels. Another group received atorvastatin, resulting in a decrease of plasma cholesterol by 50% by a reduction in very low density lipoprotein production. The combination group received both amlodipine and atorvastatin. After 28 weeks, atherosclerosis in the aortic root was quantified. Treatment with amlodipine alone had no significant effect on atherosclerotic lesion area, whereas atorvastatin markedly reduced atherosclerosis by 77% compared with the control group. Atorvastatin also reduced inflammation markers. The combination of amlodipine and atorvastatin tended to reduce lesion area by 61% compared with the atorvastatin-only group; this effect just did not reach statistical significance (**Figure 2**). However, after subgroup analysis, the combination therapy did reach significance in animals that respond relatively modestly to atorvastatin. Amlodipine treatment significantly reduced calcification in the lesions, whereas

atorvastatin alone had no effect. The combination of amlodipine and atorvastatin resulted in a near absence of calcium deposits in the lesions. Measurement for Serum Amyloid A resulted in significant lower levels of both in the group receiving both atorvastatin and amlodipine than in the groups receiving no drugs or only one of them. Fibrinogen levels were reduced as compared with the control group, and von Willebrand Factor, a marker for vessel wall integrity, was reduced in the combination group as compared with the group receiving amlodipine alone. Van de Poll *et al*.[55] examined the effects of amlodipine and atorvastatin on advanced atherosclerosis in APOE*3Leiden mice. They showed that the combination treatment of amlodipine and atorvastatin not only reduced lesion size compared to the control group but also reduced the area of existing lesions. The lesion areas of the mice receiving treatment were compared to the lesion area of mice which were sacrificed at the beginning of the experiment. Recently, research by Mason *et al.*[56] demonstrated that the combination of amlodipine and atorvastatin had a synergistic effect on nitric oxide release from human endothelial cells, which in vivo may lead to an improvement of endothelial function.

The ASCOT-LLA study[57] assessed benefits of cholesterol lowering in the primary prevention of coronary heart disease in hypertensive patients who are not conventionally deemed dyslipidemic. Of 19342 hypertensive patients (aged 40-79 years with at least three other cardiovascular risk factors) randomised to one of two antihypertensive regimens in the Anglo-Scandinavian Cardiac Outcomes Trial, 10305 with non-fasting total cholesterol concentrations 6.5 mmol/L or less were randomly assigned additional atorvastatin 10 mg or placebo. These patients, whom at least half were additionally treated with amlodipine, formed the lipid-lowering arm of the study. The primary endpoint was non-fatal myocardial infarction and fatal coronary heart disease. Treatment was stopped after a median follow-up of 3.3 years. A reduction of 36% (p=0.0005) of primary endpoints was seen in the atorvastatin group. This benefit emerged in the first year of follow-up. A 27% reduction of fatal and non-fatal stroke (p=0.024), 21% reduction of total cardiovascular events (p=0.0005), and a 29% reduction of total coronary events (p=0.0005) were also observed. Addition of amlodipine to the atorvastatin treatment may have accounted for these impressive positive effects.

The effects of amlodipine monotherapy and combination therapy of atorvastatin and amlodipine on arterial compliance were investigated on 21 consecutive hypertensive hyperlipidemic patients by Leibovitz *et al.*[58]. Patients were followed every month for 6 months (3 months of amlodipine therapy and 3 months of amlodipine and atorvastatin combination). During the 3 months of amlodipine monotherapy, large and small arterial compliance were improved by 26% and 38%, respectively, and the systemic vascular resistance was reduced by 10%. The addition of atorvastatin during the next 3 months improved small arterial compliance by an additional 42% and decreased the systemic vascular resistance by another 5%, but large arterial compliance

and blood pressure did not change. Thus amlodipine improved large and small arterial compliance, and the beneficial effect of atorvastatin on small arterial compliance was additive to the effect achieved by amlodipine in hypertensive hyperlipidemic patients.

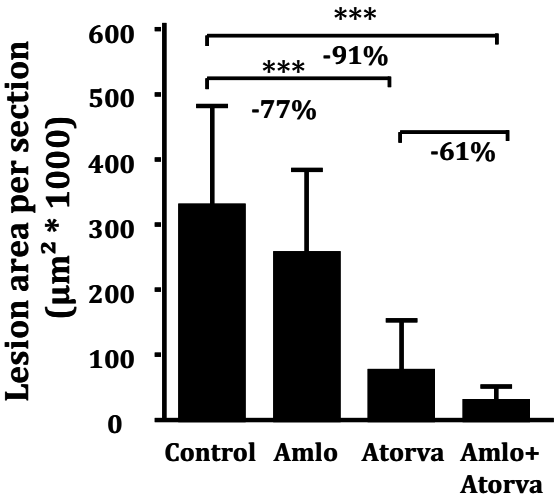

Figure 2 Lesion area per section in the aortic root of APOE*3Leiden mice after treatment with amlodipine, atorvastatin of the combination of both as described by Delsing et al. [42]. Atorvastatin reduced lesion area with 77% as compared with the control group. The combination therapy decreased the lesion area with 91% when compared with the control group and 61% (not significant) when compared to the atorvastatin monotreatment. *** P < 0.005.

Conclusion and final remarks

Previous described studies demonstrate the antiatherosclerotic properties of CCBs, especially amlodipine. Of great importance are the anti-oxidative properties[10,26] and the ability to inhibit the aggregation of oxidized LDL particles[56]. Amlodipine was also shown to stabilize plaques and to reduce the ischemic area after myocardial infarction[32]. Statins are used for years to decreased plasma cholesterol levels and thereby reduce atherosclerosis. Huge clinical trails like 4S[39], CARE[40] and LIPID[41] showed that statins reduced myocardial infarction and fatal coronary deaths. Over the last years researchers demonstrated lipid independent antiatherosclerotic properties of statins *e.g.* anti-inflammatory and antioxidative capacities[9,46-51]. It was also angiographically demonstrated that atorvastatin improved plaque stability in humans[6]. High dose treatment of atorvastatin may even be favoured above angioplasty in reducing ischemic cardiac events in patients with stable angina pectoris[52].

Because of the verifiable antiatherosclerotic properties of amlodipine and atorvastatin, it seems a logical step to combine these two types of drugs. This potent combination therapy has now been shown to result in reduced atherosclerosis, by inhibiting the progression of atherosclerosis[24,43] and even reduce lesion size[55], improvement of endothelial function[56] and arterial compliance[58]. Capacities for plaque stabilization and reduction of infarction size are suggested and should be further tested. Outcomes of the ASCOT study[59] are of great interest, since a group of approximately 2500 hypertensive patients is randomised to the combination treatment of atorvastatin

and amlodipine. The lipid lowering arm of the ASCOT study[57] has demonstrated impressive effects of atorvastatin treatment.

These results of obtained from *in vitro*, mice and clinical studies, in which the combination therapy was tested, collectively support the clinical anti-atherosclerotic advantages of combination of CCBs and statins and in particular of atorvastatin with amlodipine beyond their established antihyperlipedemic and antihypertensive modes of action.

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