Novel pharmaeutical interventions in experimental atherosclerosis and myocardial infarction

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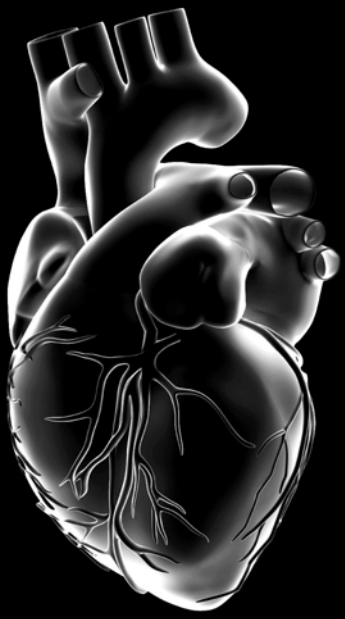

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Olmesartan and Pravastatin Additively Reduce Development of Atherosclerosis in APOE*3Leiden transgenic mice

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Abstract

Objective This study was designed to investigate the effect of the angiotensin II receptor blocker olmesartan alone, or in combination with standard treatment with a statin, pravastatin, on atherosclerosis development in APOE*3Leiden transgenic mice.

Methods and Results Four groups of 15 mice received an atherogenic diet alone (plasma cholesterol 17.4 ±2.7 mM) or supplemented with either 0.008% (w/w) olmesartan (9.3 mg/kg/d) (plasma cholesterol 16.4 ±3.9 mM), 0.03% (w/w) pravastatin (35 mg/kg/d) (plasma cholesterol 14.6 ±2.6 mM), or the combination of both (plasma cholesterol 14.5 ±2.9 mM) for six months. Treatment with olmesartan or pravastatin reduced the development of atherosclerosis as compared to the control group (-46% and -39%, respectively). Pravastatin also reduced the severity of the lesions. As compared to control the combination of both treatments almost fully prevented atherosclerosis (-91%, $p<0.001$) and strongly reduced lesion number (-69%), lesion severity (-79%), number of macrophages (-89%) and T lymphocytes (-86%) per cross-section. Treatment with olmesartan alone and in combination with pravastatin inhibited the adhesion of monocytes to the vessel wall (-22%; $p<0.05$ and -25%; $p<0.01$, respectively), and reduced the relative quantity of macrophages in the lesions (-38%; $p<0.05$ and -26%; N.S., respectively) as compared to control.

Conclusion Olmesartan reduced atherosclerosis development mainly by decreasing monocyte adhesion and the relative amount of macrophages, whereas pravastatin inhibited the progression of atherosclerosis to more advanced lesions, reflecting different anti-atherosclerotic modes of action of the two drugs. Combination therapy with olmesartan and pravastatin additively reduced atherosclerosis development, resulting in less and less severe lesions.

Introduction

Atherosclerosis is a complex disease in which foam cell formation and vascular remodeling, next to oxidation and inflammation, play an important role[1]. Since atherosclerosis is considered to be a multifactorial disease, there is broad consensus that medical treatment should have different approaches. Cholesterol accumulation in macrophages, which leads to foam cell formation, is a crucial stage in the development of atherosclerotic lesions. Therefore, reduction of high plasma cholesterol appears to be the first choice approach for medical treatment in preventing atherosclerosis development. Reduction of plasma cholesterol levels by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, is a widely used therapy in primary and secondary prevention of cardiovascular disease[2]. Angiographic clinical trials, like REGRESS[3] and MAAS[4], clearly demonstrate that statins significantly reduce progression of coronary atherosclerosis and decrease the occurrence of new cardiovascular events in patients with coronary artery disease. Large clinical trials like CARE[5], WOSCOPS[6], LIPID[7] have shown significant benefit from pravastatin in both primary and secondary prevention of coronary events. There is growing evidence that statins, independent of their cholesterol lowering capacities, have anti-inflammatory activity as well[8-10].

Angiotensin II, the major effector molecule in the Renin-Angiotensin-System (RAS), is known to play a pivotal role in the regulation of blood pressure and electrolyte homeostasis. Besides its vasoconstrictive effect by binding to the angiotensin II type I receptors (AT1) on vascular smooth muscle cells (VSMCs), angiotensin II has proinflammatory actions by stimulating the production of cytokines and of reactive oxygen species. These can activate nuclear factor-κB (NF-κB) resulting in its translocation into the nucleus where it regulates the transcription of genes encoding for cytokines, chemokines and adhesion molecules, which are all involved in the recruitment of monocytes/macrophages and leukocytes to sites of inflammation in the vascular wall[11,12]. It is known that angiotensin II plays an important role in the development of atherosclerosis[13,14]. Strong links between hypercholesterolemia and the production and expression of angiotensin II and AT1 have been described[15,16]. Clinical intervention studies like SAVE[17] and SOLVD[18] with angiotensin-converting enzyme (ACE) inhibitors and ELITE[19] with an angiotensin II receptor blocker (ARB), showed a reduction in myocardial infarction and sudden cardiac death. In clinical studies it was observed that olmesartan is a very potent anti-hypertensive drug with minimal adverse effects[20]. Olmesartan also significantly reduced vascular microinflammation in patients with essential hypertension[21].

The purpose of this study was to investigate whether the angiotensin II receptor blocker (ARB) olmesartan has additional or synergistic anti-atherosclerotic effects, when it is used together with the HMG-CoA reductase inhibitor pravastatin in APOE*3Leiden transgenic mice. APOE*3Leiden mice are a well-established model for

hyperlipidemia and atherosclerosis[9,22,23]. The mice have a human-like lipoprotein profile in which, upon feeding a cholesterol-containing diet, elevated plasma cholesterol and triglyceride levels are mainly confined to the VLDL/LDL-sized lipoprotein fraction. In contrast to other mouse models for hyperlipidemia, i.e. LDL receptor deficient[24] and ApoE deficient mice[25], APOE*3Leiden mice have relatively mildly increased plasma cholesterol levels, and respond well to statin treatment by reduction of both the apoB-containing lipoproteins and atherosclerosis. In this mouse model the human atherosclerotic situation can be mimicked both with regard to the development of atherosclerosis as well as to the response on therapy[9,22,26-28].

Methods

Mice

Female heterozygous APOE*3Leiden transgenic mice (16 to 18 weeks of age), characterized by ELISA for human apoE[23], were used. Animal experiments were approved by the Institutional Animal Care and Use Committee of The Netherlands Organization for Applied Scientific Research (TNO). Animals were bred by TNO.

Diets

During a 3 week run-in period, all animals received a semi-synthetic high fat cholesterol diet (HFC) containing 40.5% sucrose, 15% cacao butter and 0.5% (w/w) cholesterol. After randomization into 4 groups on the basis of age, body weight, plasma cholesterol and triglyceride levels, the mice received HFC diet alone (control group) or supplemented with either 0.008% (w/w) olmesartan (9.3 mg/kg/d), with 0.03% (w/w) pravastatin (35 mg/kg/d), or 0.008% (w/w) olmesartan plus 0.03% (w/w) pravastatin. The mice receiving olmesartan in the diet showed a small reduction in food intake, probably causing the slight decrease in plasma cholesterol levels. After 18 weeks of treatment, both diets containing olmesartan were adjusted to 0.005% (w/w) (5.8 mg/kg/d) to reduce the hypotensive effect and increase the food intake. The pravastatin concentration was raised to 0.04% (w/w) (47 mg/kg/d) to obtain equal cholesterol exposures (i.e. plasma cholesterol levels x total time in weeks) between the control and olmesartan groups and between the pravastatin and combination groups. Since the olmesartan group had a significant lower total cholesterol exposure after 24 weeks of treatment, this group was sacrificed 2 weeks later. Olmesartan and pravastatin were provided by Sankyo Company, Ltd. The animals received food and water *ad libitum*. Body weight and food intake were monitored during the study.

Lipid and lipoprotein analysis and plasma SAA

After a 4-hour fasting period from 9 a.m. to 1 p.m., EDTA plasma was collected (Sarstedt,

Nümbrecht, Germany). Total plasma cholesterol (Roche Diagnostics, No-1489437) and triglyceride (Roche Diagnostics, No-1488872) levels were measured. Lipoprotein profiles were obtained by FPLC[23]. Serum amyloid A was determined by ELISA (Biosource International, Nivelles, Belgium)[9,28].

Systolic blood pressure

To evaluate the effect of olmesartan, the systolic blood pressure was measured in all groups after 4, 13 and 20 weeks of treatment using the Blood Pressure System for Rats and Mice (RTBP1001, Harvard Apparatus, Holliston, MA, USA). The mice were trained every day, seven days before measurement. For each mouse the blood pressure was measured three times during one session[27].

Histological assessment of atherosclerosis

After the six-months treatment period, the mice were sacrificed after anesthetizing and blood collection[23]. Formalin fixed and paraffin embedded sections of the entire aortic root area were haematoxylin-phloxine-saffron stained for atherosclerosis measurement[29]. For determination of severity of atherosclerosis, the lesions were classified into 5 categories[9,27,28]: I) early fatty streak, II) regular fatty streak, III) mild plaque, IV) moderate plaque, V) severe plaque. Per mouse the percentages of all lesions found in the respective categories were calculated. The total lesion area was calculated per cross-section.

In each segment used for lesion qualification, the number of monocytes adhering to the endothelium was counted. Mouse monocytes were immunostained with AIA31240 (1:3000, Accurate Chemical and Scientific, New York, USA). Macrophage area was measured after immunostaining with anti mouse CD68 (1:100, Serotec Ltd, UK). The number of T lymphocytes was counted after immunostaining with mouse anti human CD3 (Serotec Ltd, UK), cross reacting with mouse CD3, a marker for all T cell subtypes. Collagen content of the plaque was quantified morphmetrically after Sirius Red staining. Mouse smooth muscle cells were immunostained with mouse anti-human alpha actin (1:800, DAKO, Denmark), which cross reacts with mouse alpha actin. smooth muscle cells were counted in the superficial part of the lesions (e.g. the cap) in the type III, IV and V lesions. Proliferating smooth muscle cells in the cap were immunostained with anti PCNA (1:180, Calbiochem, Merck, Germany). All analyses were performed by the same operator, who was blinded for experimental group allocation.

Statistical analysis

Significance of differences was calculated by using the non-parametric Mann-Whitney U test. Each group was compared to control and the combination group was additionally compared to the pravastatin group. Differences in lesion area were corrected for differences in blood pressure, using analysis of variance and analysis of covariance. To

ensure normality, lesion area was transformed using a square-root transformation, which was used as dependent variable. The treatment group was used as the independent variable and difference in blood pressure was the covariate. P<0.05 was considered significant. All data are presented as mean ± SD.

Results

Plasma lipids and blood pressure

As presented in **table 1** the control group had a cholesterol exposure of 420 ± 34 mM*weeks, which was equal to the olmesartan group. Cholesterol exposure was decreased by 18% (p<0.001) in the pravastatin group and by 17% (p<0.001) in the combination group, as compared to the control group. Average plasma cholesterol levels were 17.5 ± 2.7 mmol/L for the control group. Although at any time point the olmesartan group did not differ from the control group, the average overall cholesterol level was slightly decreased (p<0.05). In the pravastatin and the combination group a 17% (p<0.001) reduction of the plasma cholesterol level was observed. The average plasma triglyceride level for the control group was 1.47 ± 0.47 mmol/L. Olmesartan lowered plasma triglyceride levels by 13% (p=0.001) and pravastatin by 37% (p<0.001). In the combination group triglyceride levels were decreased by 39% (p<0.001). Average systolic blood pressure, measured after 4 and 13 weeks of treatment, the blood pressures were 101 ± 6 mmHg for the control group, 83 ± 6 mmHg for olmesartan group (-18%, p<0.001), 104 ± 7 mmHg for the pravastatin group and 89 ± 4 mmHg for the combination group (-11%, p<0.001). After diet adjustment (at t=18 weeks), the blood pressures were measured again after 20 weeks of treatment (shown in **table 1**). Olmesartan alone or in combination with pravastatin decreased systolic blood pressure by 14% as compared to control and pravastatin treatment.

	Total cholesterol exposure (mM*weeks)	**Average plasma cholesterol** (mmol/L)	**Average plasma triglyceride** (mmol/L)	**Systolic blood pressure** (mmHg)
Control	420 ± 34	17.5 ±2.7	1.47 ±0.47	104 ± 4
Olmesartan	415 ± 74	16.4 ± 3.9*	1.27 ± 0.48*	90 ± 2*#
Pravastatin	346 ± 48*†	14.6 ± 2.6*	0.93 ± 0.42*	105 ± 4
Olmesartan + Pravastatin	348 ± 49*†	14.5 ± 2.9*	0.89 ± 0.45*	89 ± 3*#

Table 1 The effect of olmesartan, pravastatin and the combination of both on plasma lipids and systolic blood pressure after twenty weeks of treatment. (*p<0.05 compared to control; †p<0.05 compared to olmesartan; #p<0.05 compared to pravastatin)

Atherosclerosis evaluation: Lesion number, lesion area and lesion severity

Representative photomicrographs of atherosclerotic lesions found in the different groups are shown in **figure 1**. The number of lesions per cross-section in the control group was 5.9 ± 1.1 (**figure 2A**). A significant decrease of 31% was found in the olmesartan group (P<0.005) and of 34% in the pravastatin group (P<0.001). Combination treatment reduced lesion number by 69% (P<0.001), which was also significantly different from the olmesartan group (-56%; P=0.001) and pravastatin group (-54%; P<0.001). The total lesion area per section for the individual groups is shown in **figure 2B**. For the control group the total lesion area was 164.4 ± 68.8 μm^2*1000. Olmesartan significantly reduced lesion area by 46% (P<0.05) and

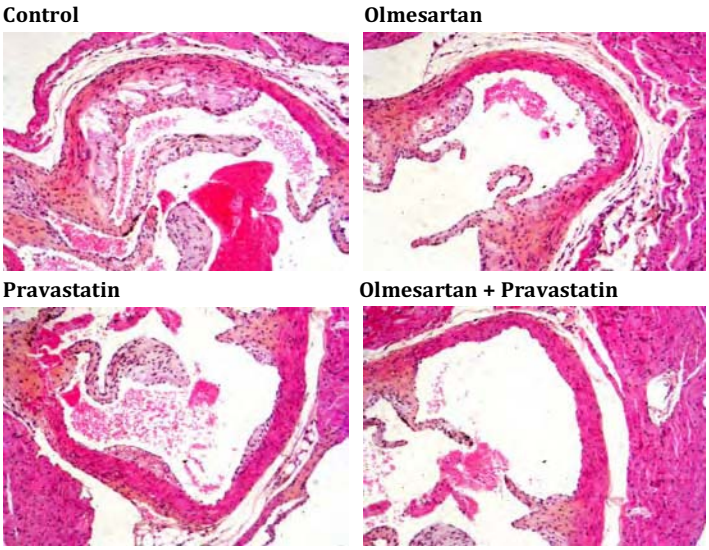

Figure 1 Representative photomicrographs of atherosclerotic lesions found in the different groups (haematoxylin-phloxine-saffron staining). The control example shows a severe lesion (type V). For both the olmesartan and pravastatin group mild and moderate lesions (type II, III and IV) are presented. The example for the combination group shows a small fatty streak (type I).

pravastatin by 39% (P<0.05). The combination therapy further inhibited the development of atherosclerosis by 91% which was highly significant compared to the control (P<0.001), the olmesartan (-83%; P=0.001) and pravastatin (-85%; P<0.001) group. For each animal the lesion severity was analyzed and the percentages of lesions belonging to the respective lesion categories were calculated. **Figure 2C** shows the percentages of type 0-III lesions (no lesions, fatty streaks and mild plaques) and type IV-V lesions (moderate and severe plaques). About 70% of lesions in the control group were type IV or type V lesions, which was 47% (N.S.) in the olmesartan group, 38% (P<0.01) in the pravastatin, and only 15% (P<0.001) in the combination group. This finding indicates that treatment with olmesartan, alone or in combination with pravastatin, interferes with the progression of lesion development, resulting in less advanced lesions.

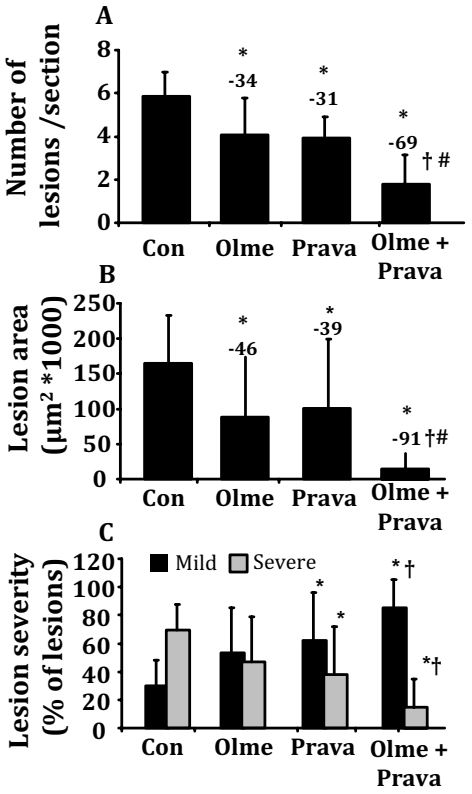

Figure 2 Effect of olmesartan, pravastatin and the combination of both on atherosclerosis. The number of lesions (A) and lesion area per cross-section (B). Severity of the atherosclerotic lesions (C) as determined by the percentages of lesions classified as mild (absence of lesions + type I-III lesions) and severe (type IV-V lesions). P<0.05 compared to control; †P<0.05 compared to olmesartan; #P<0.05 compared to pravastatin.

We also analyzed whether olmesartan had anti-atherosclerotic properties beyond its blood pressure lowering qualities. We calculated, using a univariate analysis of variance (with blood pressure as covariate), that the differences in lesion area remained significant after statistical correction for differences in blood pressure (P<0.01). This shows that olmesartan had an additional beneficial effect independent of its blood pressure lowering effect. The nature of these effects was explored in more detail below.

Systemic inflammation: plasma serum amyloid A

The liver-derived plasma inflammation marker serum amyloid A (SAA), which reflects the overall systemic inflammatory state, was measured at the beginning of the study and at sacrifice (**figure 3**). Levels at sacrifice were 32.2 ± 19.2 µg/mL in the control group, which was significantly higher (P=0.01) when compared to the levels at the start of the treatment (11.6 ± 3.5 µg/mL). As compared to control, SAA was reduced by 68% (p<0.001) in the olmesartan group, by 72% (p<0.001) in the pravastatin group, and by 64% (P<0.001) in the combination group. The pravastatin group showed a significant 22% reduction (p<0.05) in SAA levels as compared to the levels at the beginning of the study. These data emphasize the anti-inflammatory properties of both drugs.

Inflammatory cells: Monocyte adhesion and macrophage and T lymphocyte abundance

As inflammation is an important process in atherogenesis, the presence of pro-inflammatory cells was measured. In the same four sections of the aortic root used for measurement of lesion number, size and severity, the monocytes adhering to the activated endothelium were counted. In the control group on average 18.1 ± 3.2 adhering monocytes per cross-section were present (**figure 4A**). A significant reduction

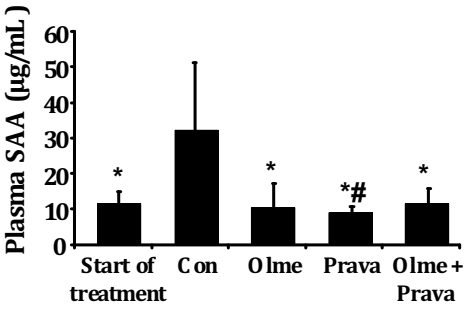

Figure 3 Effect of olmesartan, pravastatin and the combination of both on plasma levels of serum amyloid A (SAA). *P<0.05 compared to control; #P<0.05 compared to start of treatment.

of 22% (p<0.05) was observed in the olmesartan group, whereas the pravastatin group had an equal number of adhering monocytes as compared to the control group. The combination group showed a similar reduction as the olmesartan group, which was significantly different from the control group (-25%; P<0.01) and the pravastatin group (-27%; P<0.01). A resembling pattern was seen for the relative amount of macrophages in the total lesion area (**figure 4B**), in which a 38% (P<0.05) decrease was observed in the olmesartan group. No reduction was observed in the pravastatin group and there was a 26% (N.S.) decrease in the combination group. The total number of macrophages in the lesions showed a pattern comparable to the lesion area (compare **figure 4C** with **figure 2B**). In the control group on average 209 ± 67 macrophages were present per cross-section. Significant decreases of 46% (P<0.05) and of 51% (102 ± 113, P<0.01) were found in the olmesartan group and pravastatin group, respectively. An 89% (P<0.001) reduction was observed in the combination group, which was significantly lower than the olmesartan (P<0.01) and pravastatin group (p<0.001).

In the control group on average 2.1 ± 0.4 T-lymphocytes were present per cross-section (**figure 4D**). This number was lowered by 62% (P<0.05) in the olmesartan group, by 76% (P<0.01) in the pravastatin group, and by 86% (P<0.001) in the combination group. Taken figures 4A to 4C together, our data indicate that treatment with olmesartan decreases activation of the endothelium and results in less foam cell rich plaques as compared with pravastatin treatment.

Lesion composition: Collagen and smooth muscle cell content

To obtain an indication of plaque stability, the collagen content of the lesions and smooth muscle cell area in the cap of the lesions were measured. The average collagen content was 30.8 ± 10.9% in the control group. A 30% increase (39.8 ± 14.0%; N.S.) was seen in the olmesartan group, 40% (43.3 ± 10.7%; P<0.01) in the pravastatin group and 48% (45.6 ± 22.2%; N.S.) in the combination group. Smooth muscle cell area in the cap was measured and expressed as percentage of the lesion area, in those lesions that contained fibrous caps (type III, IV en V) (**figure 4E**). It was found that 1.7 ± 1.2% of the lesion area was smooth muscle cell area in the control group. A 2.6-fold (P<0.01) increase was measured in the olmesartan group. Pravastatin treatment resulted in a 3.1-fold increase (P<0.001) and a 5-fold (P<0.01) increase was observed in the combination

group. To obtain an indication about the state of differentiation of these smooth muscle cells the sections were stained for alpha-SMactin, as a marker of the differentiated, contractile phenotype and for PCNA as marker of proliferation. In all groups hardly any proliferation of smooth muscle cells was found in the cap area (data not shown). This suggests that the smooth muscle cells in the cap area are differentiated contractile smooth muscle cells, which hardly proliferate. The enhanced smooth muscle cell area per lesion area suggests an increased stability of the plaques.

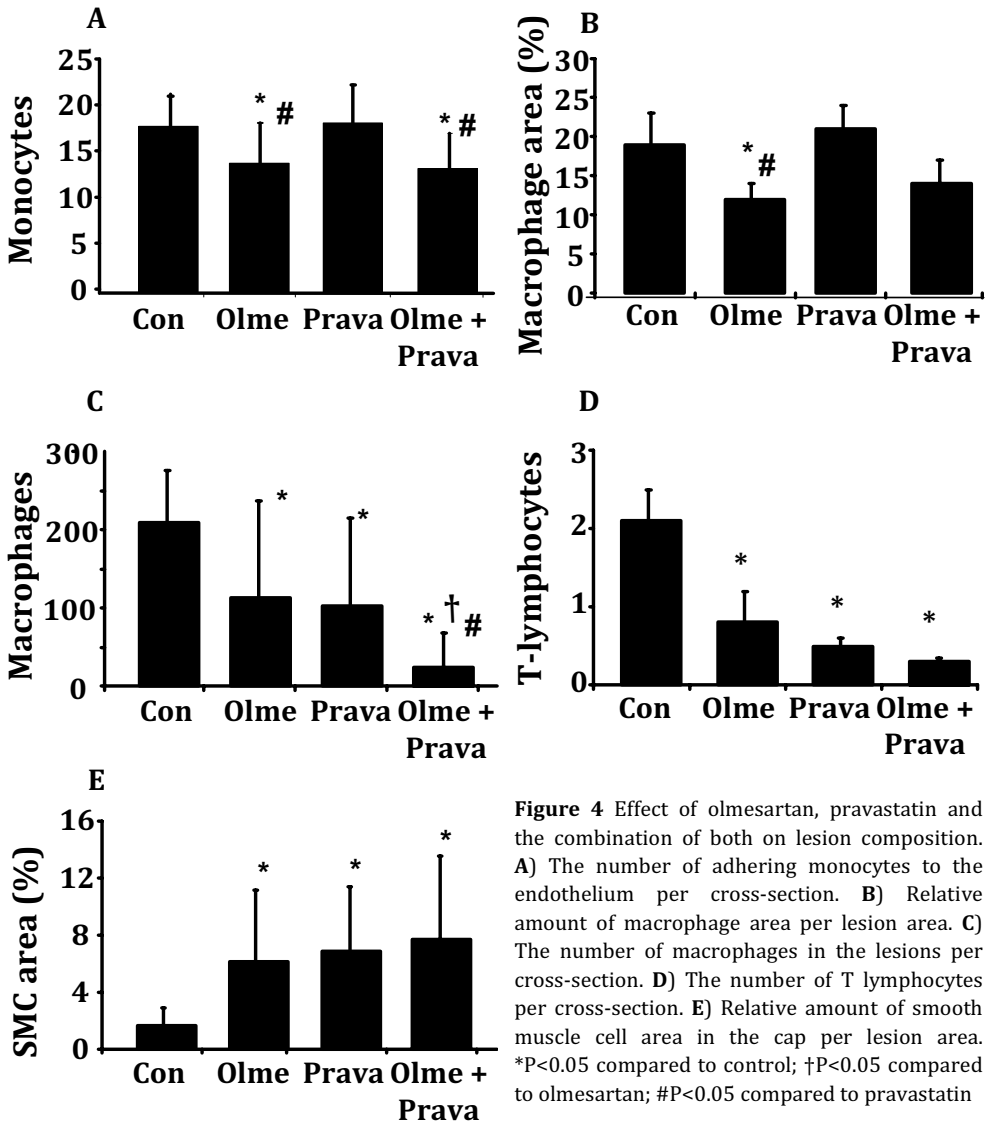

Figure 4 Effect of olmesartan, pravastatin and the combination of both on lesion composition. **A**) The number of adhering monocytes to the endothelium per cross-section. **B**) Relative amount of macrophage area per lesion area. **C**) The number of macrophages in the lesions per cross-section. **D**) The number of T lymphocytes per cross-section. **E**) Relative amount of smooth muscle cell area in the cap per lesion area. *P<0.05 compared to control; †P<0.05 compared to olmesartan; #P<0.05 compared to pravastatin

Discussion

The present study was designed to evaluate and characterize the nature of the effect of the ARB olmesartan alone or in combination with statin treatment, the latter of which can be considered as standard therapy for patients suffering from cardiovascular disease, on the development of atherosclerosis. To create a human-like condition APOE*3Leiden transgenic mice were used, since these mice respond to statins with cholesterol-lowering just as humans[9,22,26-28] and develop atherosclerotic lesions akin to their human counterparts with respect to morphological, histological and immunohistochemical characteristics[23]. In this study treatment with olmesartan resulted in an 11 to 18% reduction in blood pressure, resembling the human response (-10 to -17%) to ARB treatment[30]. Pravastatin reduced plasma cholesterol levels by 17%, which is also comparable to the about 20% decrease achieved in clinical trials[31].

Mono treatment with olmesartan inhibited atherosclerosis development, beyond and independent of the reduction achieved by its antihypertensive action alone. This finding is in line with previous studies in monkeys[32] and in apoE deficient mice[33], in which olmesartan reduced atherosclerosis but did not affect blood pressure. We investigated whether olmesartan exerts this additional anti-atherosclerotic effect via an anti-inflammatory activity by measuring plasma levels of the liver-derived inflammation marker SAA. SAA is a risk factor for cardiovascular disease, which reflects the overall inflammatory state[34]. Treatment with olmesartan reduced SAA levels to initial levels of healthy control animals (*i.e.* before the atherogenic diet was started). The decrease was observed even under conditions of increased plasma cholesterol levels, which are known to increase SAA levels[9,28,29]. In addition, histological analysis of the lesions showed anti-inflammatory features of olmesartan as characterized by reductions in the number of pro-inflammatory adhering monocytes, macrophages, and T-cells per cross-section and by a decrease in total macrophage area in the lesions. Since lesion formation starts by monocyte adherence to the activated endothelium, the above data indicate that olmesartan has an inhibiting effect on the early phase of lesion formation. The reduction in the 'soft' macrophage area, known to be prone to plaque rupture, together with the increased smooth muscle cell area of the contractile phenotype covering the lesions, suggests that olmesartan has plaque stabilizing effects.

Besides blood pressure and inflammation, elevated plasma triglyceride levels are an independent risk factor for cardiovascular disease[35]. In this study it was observed that olmesartan slightly but significantly decreased plasma triglyceride levels. A similar effect combined with an improved insulin sensitivity was observed in olmesartan-treated, fructose-fed rats[36]. Another angiotensin II receptor blocker, telmisartan, was recently shown to reduce triglyceride levels and to improve insulin sensitivity in insulin resistant rats and humans[37]. These effects were attributed to its peroxisome proliferator-activated receptor-γ (PPAR-γ) modulating abilities. PPAR-α and PPAR-γ are

expressed in the cells of the cardiovascular system and have been shown to participate in the regulation of cell growth and migration, and oxidative stress and inflammation[38]. For olmesartan no PPAR-γ activating capacity has been detected[39] until now and it needs to be investigated whether the effect on triglyceride levels and the anti-inflammatory effects of olmesartan are due to modulation of PPAR-α activity.

Mono treatment with pravastatin inhibited the progression of atherosclerosis resulting in less severe and less advanced lesions. This can not solely be attributed to the reduction in plasma lipid levels by pravastatin treatment, but also to its anti-inflammatory properties. These were exhibited in the liver as was visible by reduced plasma SAA levels to even lower concentrations than at the start of the study and histologically in the vessel wall by a reduced number of macrophages and T-cells. SAA, macrophages and T cells are considered to participate in pro-atherogenic processes of early lesion evolution and promote lesion development[40,41]. The reductions in these parameters were all comparable to the decreases achieved by olmesartan mono therapy. Pravastatin did not affect the number of adhering monocytes and the macrophage containing area in the plaques. However, it may stabilize the lesions by increasing the amount of differentiated contractile alpha-SMactin positive smooth muscle cells in the fibrotic cap like olmesartan does.

When olmesartan was combined with pravastatin the anti-atherosclerotic and anti-inflammatory activities of both drugs appeared to be additive, resulting in a significant reduction of 85% when compared to the pravastatin mono treatment. Combination treatment lowered the number of adhering monocytes and T-cells like olmesartan mono therapy, but further reduced the lesion severity and the number of macrophages. No further reduction by combination treatment was found for plasma SAA, which already was decreased by olmesartan or pravastatin alone to levels found at the start of treatment. In agreement with the present data combination treatment with candesartan/rosuvastatin[42], or valsartan/fluvastatin[43] reduced atherosclerosis to a greater extent than treatment with each drug alone in ApoE$^{-/-}$ mice. However, treatment with telmisartan plus atorvastatin did not show any additional effects on atherosclerotic progression and stability in this mouse model[44]. Our data in APOE*3Leiden transgenic mice are in line with a recent report on the effect of combination treatment in rabbits[45] and extend the latter observation of reduced atherosclerosis development by providing a more mechanistic explanations for the additive effect of both drugs.

In conclusion, the current data show that olmesartan interferes with the initiation of lesion formation, whereas pravastatin inhibits lesion progression, and both drugs have vascular and hepatic anti-inflammatory properties. The clinical study EUTOPIA[21] also points to anti-inflammatory activity of olmesartan in humans by reducing the plasma levels of C-reactive protein, interleukin-6 and tumor necrosis factor-α in patients

with essential hypertension and microinflammation. Co-treatment with pravastatin resulted in further reductions of these levels, whereas pravastatin alone did not affect these inflammatory factors. SAA levels were not measured in the latter study.

The effect of combination treatment of olmesartan with pravastatin on cardiovascular endpoints in humans has not yet been studied. However, combination therapy of atorvastatin with an ACE inhibitor was shown to be more effective in reducing cardiovascular events than statin treatment alone in a post-hoc analysis of the patients of the GREASE[46] study[47]. Similar synergistic effects of atorvastatin and anti-hypertensive treatment with the calcium channel blocker amlodipine and ACE inhibitor perindopril were recently reported in the ASCOT study[48]. The present study and the results of the mentioned clinical trails provide evidence that combination treatment of olmesartan and pravastatin may be more effective in the prevention of atherosclerosis than treatment with statins alone.

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Disclosures

None.

References

1. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-874.
2. Nixon JV. Cholesterol management and the reduction of cardiovascular risk. Prev Cardiol 2004;7:34-39.
3. Jukema JW, Bruschke AV, van Boven AJ, Reiber JH, Bal ET, Zwinderman AH, Jansen H, Boerma GJ, van Rappard FM, Lie KI, . Effects of lipid lowering by pravastatin on progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels. The Regression Growth Evaluation Statin Study (REGRESS). Circulation 1995;91:2528-2540.
4. Effect of simvastatin on coronary atheroma: the Multicentre Anti-Atheroma Study (MAAS). Lancet 1994;344:633-638.
5. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med 1996;335:1001-1009.
6. West of Scotland Coronary Prevention Study: identification of high-risk groups and comparison with other cardiovascular intervention trials. Lancet 1996;348:1339-1342.

7. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. N Engl J Med 1998;339:1349-1357.
8. Werner N, Nickenig G, Laufs U. Pleiotropic effects of HMG-CoA reductase inhibitors. Basic Res Cardiol 2002;97:105-116.
9. Kleemann R, Princen HM, Emeis JJ, Jukema JW, Fontijn RD, Horrevoets AJ, Kooistra T, Havekes LM. Rosuvastatin reduces atherosclerosis development beyond and independent of its plasma cholesterol-lowering effect in APOE*3-Leiden transgenic mice: evidence for antiinflammatory effects of rosuvastatin. Circulation 2003;108:1368-1374.
10. Liao JK, Laufs U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol 2005;45:89-118.
11. Marumo T, Schini-Kerth VB, Fisslthaler B, Busse R. Platelet-derived growth factor-stimulated superoxide anion production modulates activation of transcription factor NF-kappaB and expression of monocyte chemoattractant protein 1 in human aortic smooth muscle cells. Circulation 1997;96:2361-2367.
12. Brasier AR, Jamaluddin M, Han Y, Patterson C, Runge MS. Angiotensin II induces gene transcription through cell-type-dependent effects on the nuclear factor-kappaB (NF-kappaB) transcription factor. Mol Cell Biochem 2000;212:155-169.
13. Cheng ZJ, Vapaatalo H, Mervaala E. Angiotensin II and vascular inflammation. Med Sci Monit 2005;11:RA194-RA205.
14. Morawietz H. Beyond blood pressure: endothelial protection against hypercholesterolemia by angiotensin II type-1 receptor blockade. Hypertension 2005;45:185-186.
15. Daugherty A, Rateri DL, Lu H, Inagami T, Cassis LA. Hypercholesterolemia stimulates angiotensin peptide synthesis and contributes to atherosclerosis through the AT1A receptor. Circulation 2004;110:3849-3857.
16. Nickenig G, Wassmann S, Bohm M. Regulation of the angiotensin AT1 receptor by hypercholesterolaemia. Diabetes Obes Metab 2000;2:223-228.
17. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ, Jr., Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, . Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. N Engl J Med 1992;327:669-677.
18. Studies of left ventricular dysfunction (SOLVD)--rationale, design and methods: two trials that evaluate the effect of enalapril in patients with reduced ejection fraction. Am J Cardiol 1990;66:315-322.
19. Pitt B, Segal R, Martinez FA, Meurers G, Cowley AJ, Thomas I, Deedwania PC, Ney DE, Snavely DB, Chang PI. Randomised trial of losartan versus captopril in patients over 65 with heart failure (Evaluation of Losartan in the Elderly Study, ELITE). Lancet 1997;349:747-752.
20. Stumpe KO. Olmesartan compared with other angiotensin II receptor antagonists: head-to-head trials. Clin Ther 2004;26 Suppl A:A33-A37.
21. Fliser D, Buchholz K, Haller H. Antiinflammatory effects of angiotensin II subtype 1 receptor blockade in hypertensive patients with microinflammation. Circulation 2004;110:1103-1107.
22. Delsing DJ, Offerman EH, van Duyvenvoorde W, van der Boom H, de Wit EC, Gijbels MJ, Van der Laarse A, Jukema JW, Havekes LM, Princen HM. Acyl-CoA:cholesterol acyltransferase inhibitor avasimibe reduces atherosclerosis in addition to its cholesterol-lowering effect in ApoE*3-Leiden mice. Circulation 2001;103:1778-1786.
23. Van Vlijmen BJ, van den Maagdenberg AM, Gijbels MJ, van der Boom H, HogenEsch H, Frants RR, Hofker MH, Havekes LM. Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3-Leiden transgenic mice. J Clin Invest 1994;93:1403-1410.
24. Bisgaier CL, Essenburg AD, Auerbach BJ, Pape ME, Sekerke CS, Gee A, Wolle S, Newton RS. Attenuation of plasma low density lipoprotein cholesterol by select 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in mice devoid of low density lipoprotein receptors. J Lipid Res 1997;38:2502-2515.
25. Quarfordt SH, Oswald B, Landis B, Xu HS, Zhang SH, Maeda N. In vivo cholesterol kinetics in apolipoprotein E-deficient and control mice. J Lipid Res 1995;36:1227-1235.
26. Van Vlijmen BJ, Pearce NJ, Bergo M, Staels B, Yates JW, Gribble AD, Bond BC, Hofker MH, Havekes LM, Groot PH. Apolipoprotein E*3-Leiden transgenic mice as a test model for hypolipidaemic drugs. Arzneimittelforschung 1998;48:396-402.

27. Delsing DJ, Jukema JW, van de Wiel MA, Emeis JJ, van der Laarse A, Havekes LM, Princen HM. Differential Effects of Amlodipine and Atorvastatin Treatment and Their Combination on Atherosclerosis in ApoE*3-Leiden Transgenic Mice. J Cardiovasc Pharmacol 2003;42:63-70.
28. Verschuren L, Kleemann R, Offerman EH, Szalai AJ, Emeis SJ, Princen HM, Kooistra T. Effect of low dose atorvastatin versus diet-induced cholesterol lowering on atherosclerotic lesion progression and inflammation in apolipoprotein E*3-Leiden transgenic mice. Arterioscler Thromb Vasc Biol 2005;25:161-167.
29. Trion A, de Maat MP, Jukema JW, van der Laarse A, Maas MC, Offerman EH, Havekes LM, Szalai AJ, Princen HM, Emeis JJ. No effect of C-reactive protein on early atherosclerosis development in apolipoprotein E*3-leiden/human C-reactive protein transgenic mice. Arterioscler Thromb Vasc Biol 2005;25:1635-1640.
30. Cheung BM, Cheung GT, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large outcome trials of angiotensin receptor blockers in hypertension. J Hum Hypertens 2006;20:37-43.
31. Edwards JE, Moore RA. Statins in hypercholesterolaemia: a dose-specific meta-analysis of lipid changes in randomised, double blind trials. BMC Fam Pract 2003;4:18.
32. Koike H. New pharmacologic aspects of CS-866, the newest angiotensin II receptor antagonist. Am J Cardiol 2001;19;87:33C-36C.
33. Suzuki J, Iwai M, Li Z, Li JM, Min LJ, Ide A, Yoshii T, Oshita A, Mogi M, Horiuchi M. Effect of combination of calcium antagonist, azelnidipine, and AT1 receptor blocker, olmesartan, on atherosclerosis in apolipoprotein E-deficient mice. J Hypertens 2005;23:1383-1389.
34. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-843.
35. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;3:213-219.
36. Okada K, Hirano T, Ran J, Adachi M. Olmesartan medoxomil, an angiotensin II receptor blocker ameliorates insulin resistance and decreases triglyceride production in fructose-fed rats. Hypertens Res 2004;27:293-299.
37. Kurtz TW. Treating the metabolic syndrome: telmisartan as a peroxisome proliferator-activated receptor-gamma activator. Acta Diabetol 2005;42 Suppl 1:S9-16.
38. Schiffrin EL. Peroxisome proliferator-activated receptors and cardiovascular remodeling. Am J Physiol Heart Circ Physiol 2005;288:H1037-H1043.
39. Kurtz TW, Pravenec M. Antidiabetic mechanisms of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists: beyond the renin-angiotensin system. J Hypertens 2004;22:2253-2261.
40. O'Brien KD, McDonald TO, Kunjathoor V, Eng K, Knopp EA, Lewis K, Lopez R, Kirk EA, Chait A, Wight TN, deBeer FC, LeBoeuf RC. Serum amyloid A and lipoprotein retention in murine models of atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:785-790.
41. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685-1695.
42. Chen J, Li D, Schaefer RF, Mehta JL. Inhibitory effect of candesartan and rosuvastatin on CD40 and MMPs expression in apo-E knockout mice: novel insights into the role of RAS and dyslipidemia in atherogenesis. J Cardiovasc Pharmacol 2004;44:446-452.
43. Li Z, Iwai M, Wu L, Liu HW, Chen R, Jinno T, Suzuki J, Tsuda M, Gao XY, Okumura M, Cui TX, Horiuchi M. Fluvastatin enhances the inhibitory effects of a selective AT1 receptor blocker, valsartan, on atherosclerosis. Hypertension 2004;44:758-763.
44. Grothusen C, Bley S, Selle T, Luchtefeld M, Grote K, Tietge UJ, Drexler H, Schieffer B. Combined effects of HMG-CoA-reductase inhibition and renin-angiotensin system blockade on experimental atherosclerosis. Atherosclerosis 2005;182:57-69.
45. Kato M, Sada T, Mizuno M, Kitayama K, Inaba T, Koike H. Effect of Combined Treatment With an Angiotensin II Receptor Antagonist and an HMG-CoA Reductase Inhibitor on Atherosclerosis in Genetically Hyperlipidemic Rabbits. J Cardiovasc Pharmacol 2005;46:556-562.
46. Athyros VG, Papageorgiou AA, Mercouris BR, Athyrou VV, Symeonidis AN, Basayannis EO, Demitriadis DS, Kontopoulos AG. Treatment with atorvastatin to the National Cholesterol Educational Program goal versus 'usual' care in secondary coronary heart disease prevention. The GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study. Curr Med Res Opin 2002;18:220-228.

47. Athyros VG, Mikhailidis DP, Papageorgiou AA, Bouloukos VI, Pehlivanidis AN, Symeonidis AN, Elisaf M. Effect of statins and ACE inhibitors alone and in combination on clinical outcome in patients with coronary heart disease. J Hum Hypertens 2004;18:781-788.
48. Dahlof B, Sever PS, Poulter NR, Wedel H, Beevers DG, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes GT, Mehlsen J, Nieminen M, O'Brien E, Ostergren J. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial. Lancet 2005;366:895-906.