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No effect of atorvastatin and simvastatin on oxidative stress in patients at high risk for cardiovascular disease

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ABSTRACT

Background: Statins are thought to have anti-atherogenic effects beyond cholesterol lowering. One such mechanism may involve reduction of oxidative stress. The aim of our study was to investigate and to compare the oxidative stress lowering capacity of atorvastatin with that of simvastatin in patients at high risk for cardiovascular disease using conventional markers and sensitive markers measured by highly specific techniques such as liquid chromatography tandem mass spectrometry.

Methods: We included 30 statin-naive patients with diabetes mellitus, and/or obesity, and/or hypertension (12 male, 18 female, mean age 44.8±11.1 years), and randomised them to receive either atorvastatin 10 mg or simvastatin 40 mg daily to obtain an equimolar cholesterol reduction. Blood and urine samples were obtained at baseline and at 1, 6 and 12 weeks.

Results: Low-density lipoprotein (LDL) cholesterol and coenzyme Q10 decreased significantly in both groups. Simvastatin caused a faster initial LDL cholesterol lowering than atorvastatin (p=0.01), but the overall effect after 12 weeks of atorvastatin and simvastatin was similar. Plasma myeloperoxidase and malondialdehyde did not change during the study period in the two groups. Urinary F2-isoprostanes decreased gradually and significantly in the atorvastatin group but not in the simvastatin group, but the between-group difference was not significant. Urinary 8-hydroxy-2-deoxyguanosine did not change in the two groups.

Conclusion: This study suggested that an important role of oxidative stress lowering as possible pleiotropic effect of atorvastatin and simvastatin is questionable.

KEYWORDS

8-OHdG, cholesterol, F2-isoprostanes, oxidative stress, statins

INTRODUCTION

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are widely used for lowering plasma low-density lipoprotein (LDL) cholesterol and have a clear role in the primary prevention of cardiovascular disease (CVD) mortality and major events.[1] It was initially assumed that the reduction of cholesterol by statins was the only mechanism responsible for their beneficial effect. However, subgroup analysis of large clinical trials indicated that subjects in statin-treated arms have less cardiovascular events than subjects in placebo-controlled arms with similar serum cholesterol levels.[2] In addition, several studies have demonstrated a rapid improvement in vascular function with atorvastatin which might not solely be accounted for by the achieved initial cholesterol reduction.[3,4] Hence, it has been suggested that statins may have additional anti-atherogenic effects such as improving endothelial function, attenuating vascular

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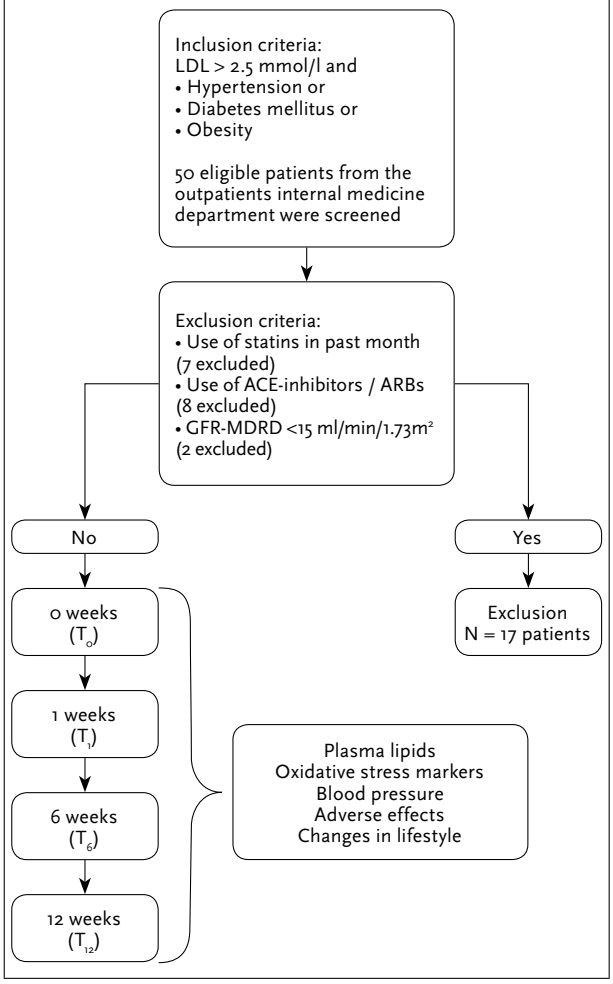
and myocardial remodelling, stabilising atherosclerotic plaques, and inhibiting vascular inflammation and oxidation.[5] One of these so-called pleiotropic effects of statins may be a reduction of oxidative stress even before the lipid-lowering effect becomes apparent and this mechanism is thought to be, at least partly, responsible for the beneficial effects which seem to occur very early in the course of the therapy.[6] Atorvastatin and simvastatin are frequently used statins and their oxidative stress lowering potential has been examined in several studies. The effects of atorvastatin versus simvastatin on oxidative stress have been examined in patients with coronary heart disease,[7] type 2 diabetes mellitus,[8] in women with polycystic ovary syndrome,[9] in patients on haemodialysis,[10] and in hyperlipidaemic subjects.[11] In these studies malondialdehyde (MDA), total peroxides, and auto-antibodies against oxidised LDL were used as markers of oxidative stress. Although MDA has been used as a marker of oxidative stress for decades, most commonly used methods to measure MDA are insufficiently sensitive and specific.[12] In addition, it is also known that lipid peroxides cannot be used as a universal marker of oxidative stress.[13] Since neither of the above-mentioned studies[7-11] examined specific oxidative stress markers, the anti-oxidative properties of atorvastatin and simvastatin remain principally unelucidated. F2-isoprostanes and 8-hydroxy-2-deoxyguanosine (8-OHdG) measured by mass spectrometry (MS) have gained recognition as better and more specific markers of oxidative lipid and DNA modifications, respectively.[14,15] Therefore, the objective of this study was to compare the oxidative stress lowering capacity of atorvastatin with that of simvastatin in a sample of patients with increased oxidative stress using sensitive markers measured by highly specific techniques on top of conventional markers.

MATERIALS AND METHODS

Subjects

In total 33 statin-naive patients were included with type 1 or 2 diabetes mellitus (according to the American Diabetes Association criteria[16]), and/or obesity (body mass index >30 kg/m^2), and/or hypertension (systolic/diastolic blood pressure >140/90 mmHg). The lowest threshold of plasma LDL cholesterol for inclusion in the study was 2.5 mmol/l. Patients who were being treated with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers were excluded because of the oxidative stress lowering properties of these agents, as were patients suffering from renal insufficiency defined as an estimated glomerular filtration rate calculated by the modification of diet in renal disease equation (GFR_{MDRD}) below 15 ml/min/1.73m^2 (*figure 1*).

Figure 1. *Flow of participants through the study.*



The study was approved by the Ethics Committee of the VU University Medical Center. Written informed consent was obtained from all participants, before randomising them into two groups. After one baseline measurement (T_0), one group received atorvastatin 10 mg once daily, the other group simvastatin 40 mg once daily. These doses were used to achieve a similar degree of LDL cholesterol lowering. The study was single blinded; the researchers were blinded while the patients and the treating physicians were not. After an overnight fast, blood and urine samples were obtained at baseline and at 1, 6 and 12 weeks (T_0, T_1, T_6 and T_{12} respectively) and side effects of the medication were registered. Urine samples were preserved at -20°C until analysis and EDTA-plasma samples were aliquoted and stored at -80°C.

Biochemical analyses

Total cholesterol, HDL cholesterol and triglycerides were measured by standard enzymatic methods (Roche, Germany). Plasma LDL cholesterol was calculated according to Friedewald's formula.[17] The total concentration of coenzyme Q10 (sum of oxidised and

reduced forms) was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described before.[18] The intra- and inter-assay coefficients of variation (CV) were 2.1% and 3.2%, respectively. A sandwich ELISA (Mercodia, Uppsala, Sweden) was used to determine myeloperoxidase (MPO) concentrations in EDTA plasma, with intra- and inter-assay CVs of 3.9% and 5.0%, respectively.[19] Total, i.e. free and protein-bound, plasma malondialdehyde (MDA) was measured by high performance liquid chromatography (HPLC) and fluorescence detection after alkaline hydrolysis and reaction with thiobarbituric acid, as previously described.[20] The intra-run and inter-run CVs of the MDA procedure were 3.5% and 8.7%, respectively. Urinary F2-isoprostanes (8-iso PGF 2α)[21] and 8-OHdG[22] concentrations were determined using LC-MS/MS. The intra- and inter-assay CVs for F2-isoprostanes were 6.8% and 8.4%, respectively, and the intra- and inter-assay CVs for 8-OHdG were 4.1% and 5.3%, respectively. To adjust for differences in analytic dilution in the urine samples, F2-isoprostanes and 8-OHdG concentrations were divided by their urine creatinine concentration. Creatinine was measured by the Jaffé reaction procedure using a commercial reagent (Roche, Germany).

Statistical analysis

In case of normally distributed variables, data are presented as mean and standard deviation (SD) or as median and interquartile range (IQR) in case of non-normally distributed variables. Differences in baseline characteristics between the two treatment groups were tested with Student's t-tests, Mann-Whitney U tests or χ^2-tests, as appropriate. Correlations between variables were expressed as Pearson's correlation coefficients. To assess the longitudinal association of treatment group with oxidative stress makers, generalised estimating equations (GEE) were used with treatment group as the independent variable, adjusted for age and sex. A p-value of less than 0.05 was considered statistically significant. All analyses were performed with SPSS version 20 (IBM SPSS Statistics, Armonk, New York).

RESULTS

After screening, we enrolled 33 subjects for this study and randomly allocated them to the atorvastatin (n=15) or simvastatin group (n=18). In the simvastatin group all data were eligible for analysis. However, in the atorvastatin group two patients were excluded because of missing urine samples and one patient never took the pills because of negative publicity about statins in the media and was therefore excluded from the study. None of the participants reported lifestyle changes such as changes in diet, smoking, medication or exercise during the study. The mean age of the remaining 30 participants was 44.8±11.1 years with 40% males. The baseline characteristics and the medications administered to the subjects are presented in *table 1*. No statistically significant differences were observed in baseline characteristics with respect to age and sex, blood pressure, and serum lipids (all p>0.05). The mean levels of LDL cholesterol, coenzyme Q10, MDA, MPO, and urinary F2-isoprostanes and 8-OHdG measured at baseline and after 1, 6, and 12 weeks are shown in *table 2* and *figure 2*, stratified by treatment allocation. No statistically significant differences were observed in baseline characteristics with respect to oxidative stress markers (all p>0.05). Simvastatin caused a faster initial LDL cholesterol lowering than atorvastatin during the first week (p=0.01). In both groups LDL cholesterol and coenzyme Q10 decreased

Table 1. *Characteristics and medications of the study population at screening and adverse side effects during the study stratified by treatment allocation*

Variable	Atorvastatin (*n*=12)	Simvastatin (*n*=18)	P-value*
Age (years)	44.0 (11.7)	45.3 (11.0)	0.75
Sex (male %)	50	30	0.46
Diabetes (yes %)	75.0†	72.2	0.67
Smoking (yes %)	16.7†	38.9	0.41
Systolic blood pressure (mmHg)	118 (14)	122 (15)	0.56
Diastolic blood pressure (mmHg)	81 (9)	79 (9)	0.52
Total cholesterol (mmol/l)	5.0 (1.0)	5.1 (1.0)	0.94
LDL cholesterol (mmol/l)	3.0 (0.8)	3.0 (0.7)	0.96
HDL cholesterol (mmol/l)	1.52 (0.49)	1.72 (0.67)	0.41
Triglycerides (mmol/l)‡	0.9 (0.8-1.3)	0.9 (0.6-1.3)	0.66
Medication			
- Insulin	8 (67%)	11 (61%)	
- Metformin	1 (8%)	3 (17%)	
- Sulphonylurea derivatives	0 (0%)	2 (11%)	
- Thiazolidinediones	0 (0%)	1 (6%)	
- Thrombocyte aggregation inhibitors	1 (8%)	2 (11%)	
- Coumarin derivatives	0 (0%)	1 (6%)	
- Dihydropirines	2 (17%)	3 (17%)	
- Thiazide diuretics	1 (8%)	2 (11%)	
Adverse effects			
- Gastrointestinal symptoms	2 (17%)	1 (6%)	
- Myalgia	3 (25%)	8 (44%)	

***P-values tested with linear or with logistic regression analyses as appropriate adjusted for age and/or sex; †n=11 (smoking status and diabetes was not recorded in one subject); ‡log-transformed prior to analysis.**

Table 2. *Changes in LDL cholesterol, coenzyme Q10 and oxidative stress markers during the study*

Variable	At baseline	At 1 week	At 6 weeks	At 12 weeks	P-value*	P-value**
LDL cholesterol, mmol/L						
- Atorvastatin group (n=12)	3.0 ± 0.8	2.4 ± 0.5	2.0 ± 0.4	2.0 ± 0.5	**<0.001**	0.15
- Simvastatin group (n=18)	2.9 ± 0.6	1.9 ± 0.5	1.6 ± 0.5	1.7 ± 0.5	**<0.001**	
Coenzyme Q10, µmol/L						
- Atorvastatin group (n=12)	0.89 ± 0.37	0.78 ± 0.26	0.74 ± 0.22	0.67 ± 0.23	**<0.001**	0.84
- Simvastatin group (n=18)	0.93 ± 0.29	0.79 ± 0.25	0.66 ± 0.20	0.62 ± 0.17	**<0.001**	
Plasma malondialdehyde, µmol/l						
- Atorvastatin group (n=12)	7.7 ± 3.3	7.1 ± 3.0	8.0 ± 3.3	8.4 ± 3.1	0.10	0.97
- Simvastatin group (n=18)	7.6 ± 2.8	7.9 ± 3.1	7.9 ± 3.1	7.1 ± 2.8	0.55	
Plasma myeloperoxidase, µg/l						
- Atorvastatin group (n=12)	56.8 ± 15.8	59.2 ± 16.9	55.4 ± 17.3	56.7 ± 14.6	0.64	0.48
- Simvastatin group (n=18)	51.9 ± 13.6	50.5 ± 13.4	48.7 ± 10.9	53.4 ± 16.0	0.32	
Urinary F2-isoprostanes, pmol/mmol creatinine						
- Atorvastatin group (n=11)	96.0 ± 39.2	87.7 ± 40.7	86.0 ± 32.6	80.0 ± 32.2	**0.023**	0.60
- Simvastatin group (n=17)	80.2 ± 42.4	80.4 ± 38.5	86.1 ± 43.6	85.5 ± 33.5	0.26	
Urinary 8-OHdG, nmol/mmol creatinine						
- Atorvastatin group (n=16)	1.34 ± 0.48	1.29 ± 0.43	1.10 ± 0.32	1.25 ± 0.41	0.16	0.08
- Simvastatin group (n=18)	1.67 ± 0.67	1.61 ± 0.65	1.61 ± 0.61	1.41 ± 0.53	0.98	

*** P-value: significance of change during study period; ** P value: significance of difference between the two treatment allocations.**

significantly during the treatment period of 12 weeks with no significant differences between the simvastatin group and the atorvastatin group (p>0.05). LDL cholesterol and coenzyme Q10 were positively and significantly associated at baseline (r=0.52, p<0.01), and at each time point during statin treatment. No significant changes in plasma MPO and MDA were observed during the treatment period or between the two treatment groups (*table 2*). Urinary F2-isoprostanes decreased gradually and significantly in the atorvastatin group but not in the simvastatin group. However, the between-group difference in F2-isoprostane reduction was not significant. There was a trend towards a greater reduction in urinary 8-OHdG in the simvastatin group versus the atorvastatin group, which was not statistically significant (p=0.08).

DISCUSSION

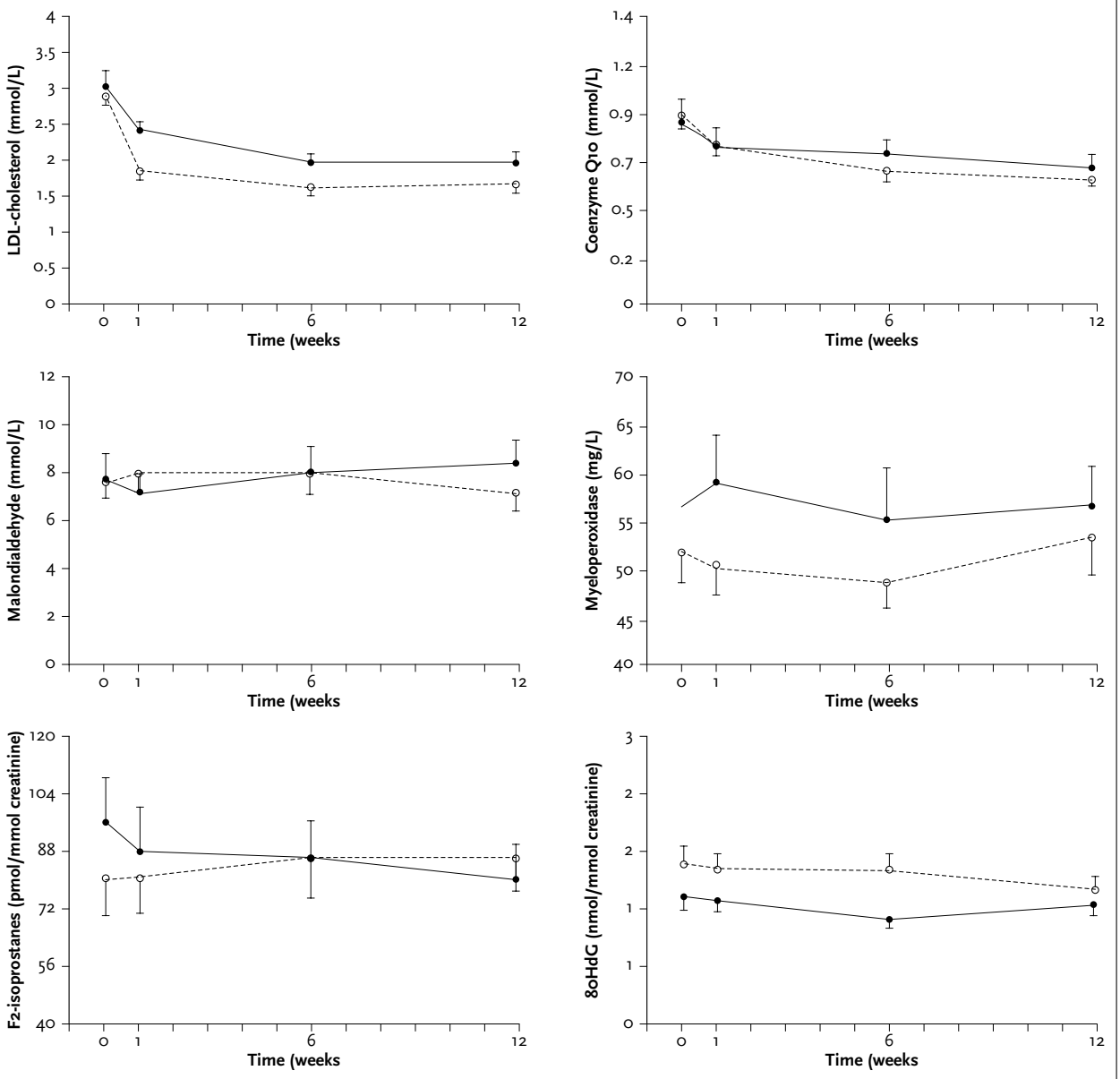
In the present study it was observed that after 12 weeks of treatment with atorvastatin and simvastatin resulted in an equimolar LDL cholesterol reduction, with simvastatin having an initial faster effect in the first week. In addition, a decrease in plasma coenzyme Q10 concentration was observed in both treatment arms. Neither atorvastatin nor simvastatin affected levels of MPO, MDA and 8-OHdG. Although F2-isoprostanes decreased significantly in the atorvastatin group, no statistical differences were observed between the two statin groups.

Effects of atorvastatin and simvastatin on coenzyme Q10 and oxidative stress markers

In addition to the cholesterol-lowering effects of statins, several studies have indicated that the beneficial effects of statins may be due to pleiotropic effects. Mevalonic acid, the product of (HMG-CoA) reductase reaction, is the precursor not only of cholesterol but also of nonsteroidal isoprenoid compounds. It is thought that many of the pleiotropic effects are mediated by inhibition of isoprenoids, such as GTP-binding proteins, which may serve as lipid attachments for intracellular signalling molecules.[23] As a result of the common biosynthetic pathway, the concentration of isoprenoid-containing plasma coenzyme Q10 may also be affected by statin treatment. Indeed, after 12 weeks of intervention, we observed a 25% and 34% reduction in plasma coenzyme Q10 levels in the atorvastatin and simvastatin group, respectively.

The principal and most studied product of polyunsaturated fatty acid peroxidation is MDA. The effect of both statins on plasma MDA concentration has been investigated in several studies. As reported by one research group, atorvastatin compared with simvastatin significantly reduced MDA levels in patients with coronary heart disease[7] as well as in patients with type 2 diabetes mellitus.[8] During statin treatment, MDA levels were also decreased in women with polycystic ovary syndrome, but no differences were observed between the atorvastatin and simvastatin group.[9] Although MDA is widely used as a proxy of oxidative damage, the validity of it has been criticised by lack of specificity and problems with post-sampling formation.[24] Furthermore, the most common MDA methods are insufficiently sensitive and are confounded by interferences.[12] To increase the specificity of the MDA assay we used HPLC with fluorescence detecting.[20] Using this technique, no significant differences in MDA concentration were seen in the two statin groups during follow-up.

Figure 2. *Changes in mean LDL cholesterol, coenzyme Q10, malondialdehyde, myeloperoxidase in plasma, and F2-isoprostanes and 8-hydroxy-2-deoxyguanosine (8-OHdG) in urine measured at baseline and after 1, 6, and 12 weeks using simvastatin (open circles) and atorvastatin (closed circles). Error bars indicate SE.*



Myeloperoxidase (MPO) is linked to oxidative stress by its role in catalysing the formation of oxidising agents.[25] Atorvastatin significantly reduced serum MPO in patients with acute coronary syndrome.[26] In contrast, Meuwese *et al.* found significantly increased MPO levels in heparin plasma after two-year treatment with atorvastatin 80 mg or simvastatin 40 mg.[27] This discrepancy can probably be explained by the type of collection tube (presence and type of anticoagulant) used to sample blood. EDTA plasma is the preferred specimen for measurement of MPO concentration as it appears unaffected by *ex vivo* release of MPO from leukocytes.[19] In the present study we did not observe differences in MPO measured in EDTA-plasma between groups or separately in either group.

The effect of statin treatment on 8-OHdG has been investigated before. In a cross-sectional evaluation of haemodialysis patients no differences in 8-OHdG levels were seen between subjects with or without statin treatment.[28] Notably, the results of this study were not stratified for the type of statin; i.e. simvastatin, atorvastatin, fluvastatin, or pravastatin. In contrast to atorvastatin, the effect of simvastatin on 8-OHdG has

not yet been studied in humans. It has been shown that atorvastatin did not have a significant effect on urinary 8-OHdG in hypercholesterolaemic patients.[29] In contrast, 8-OHdG concentrations in urine decreased significantly in patients with type 2 diabetes mellitus after one month of atorvastatin administration, but changed little at two and three months.[30] These studies all have in common that 8-OHdG was analysed by ELISA, which may not be the preferred procedure for its measurement. Commercial ELISAs seem to overestimate 8-OHdG in urine due to cross reaction of urea,[31] and therefore interpretation is adversely affected by methodological inaccuracies.[32] LC-MS/MS is reliable, sensitive and highly selective compared with other techniques and for these reasons we used this method in the present study. Using LC-MS/MS we did not observe effects on 8-OHdG urine levels upon treatment with either simvastatin or atorvastatin.

Previous studies examining the impact of statin treatment on F2-isoprostanes in urine showed inconclusive outcomes. Significantly decreased levels were found in some studies,[33-36] while other papers reported no effect on the concentration of F2-isoprostanens following statin treatment.[37-40] Although different statins were evaluated in those studies, the type of statin could not explain this disagreement. Apart from one study,[37] urinary F2-isoprostanes were determined by immuno-assays. Importantly, several studies compared immuno-assays with mass spectrometry for the measurement of F2-isoprostanes concentrations,[41-43] and without exception a poor correlation was found due to overestimating by the immuno-assay procedure. As a result, it has been concluded that an immuno-assay based method is not a valid substitute for techniques based on mass spectrometry, because both techniques are not equivalent and may not even measure the same compound. Therefore we used LC-MS/MS in the present study. After 12 weeks a slight but significant reduction was observed in the atorvastatin group, which was absent in the simvastatin group. However, the changes in F2-isoprostanes between the two treatment allocations was not significant.

Strengths and limitations of the study

There are some limitations to this study. In each group a relatively small number of subjects were examined, but clinically important effects of statins on oxidative stress would have undoubtedly been observed. In addition, the study population was rather heterogeneous, but all subjects join an increased CVD risk and benefit statin treatment. The currently most specific markers of oxidative stress, F2-isoprostanes and 8-OHdG, were measured in urine, but not in plasma. We preferred urine because both markers are less stable during handling and storage of plasma. Moreover, urine concentrations may better reflect systemic oxidative stress. Major strengths of our study were the measurement of oxidative stress markers at four different time points using sensitive and specific techniques.

In summary, simvastatin caused a faster initial LDL lowering than atorvastatin, but the overall LDL cholesterol reduction was comparable. With the exception of a slight reduction in urinary F2-isoprostanes in the atorvastatin group, no oxidative stress lowering effects of the two statins were seen during the follow-up of 12 weeks using specific markers of oxidative stress measured by selective techniques. Lowering of oxidative stress in patients at high risk for CVD may not be an important aspect in the protective pleiotropic effects of atorvastatin and simvastatin.

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DISCLOSURES

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