Levels of adhesion molecules do not decrease after 3 months of statin therapy in moderate hypercholesterolaemia

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ABSTRACT

Studies in animals and humans indicate a pivotal role for adhesion molecules (AMs) in the pathogenesis of atherosclerosis. Whereas an association between hypercholesterolaemia and AM expression has been suggested, it is unclear whether lowering cholesterol decreases AM expression and release. We compared the effects of a 3-month treatment with standard doses of three different statins (atorvastatin, simvastatin and pravastatin) on plasma levels of circulating AM (cAM) in 75 hypercholesterolaemic patients in a randomized clinical trial. Plasma levels of circulating (c)E-selectin, circulating intercellular adhesion molecule-1 (cICAM-1) and circulating vascular cell adhesion molecule-1 (cVCAM-1) were measured before and after 3 months of therapy. None of the statins lowered plasma cAM levels and pooled analyses of all patients showed a 1.7% [95% confidence interval (CI), 1.4–4.9%] increase in cE-selectin, a 2.1% (95% CI, 0.2–4.4%) increase in cICAM-1, and a 2.7% (95% CI, 0.6–6.1%) increase in cVCAM-1 levels. cAM levels did not decrease, even in patients with a >50% decrease (n = 19) in low-density lipoprotein cholesterol levels. This study provides strong evidence that 3 months of therapy with three different statins does not decrease cAM levels, despite normalization of cholesterol levels, and a minor decrease in C-reactive protein levels in patients with moderate hypercholesterolaemia.

INTRODUCTION

Hypercholesterolaemia is a well-known cardiovascular risk factor. Large clinical trials with hydroxy-methyl-glutaryl CoA reductase inhibitors (statins) have shown that lowering cholesterol levels decreases the incidence of cardiovascular events and the progression of atherosclerosis [1–3]. Animal studies of hyperlipidaemia indicate a pivotal role of leucocyte–endothelial adhesion molecules (AMs)
in the propagation of atherosclerosis [4]. Lysophosphatidylcholine, a component of modified low-density lipoprotein (LDL), and native LDL, has been shown to up-regulate expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [5–7]; these AMs facilitate the diapedesis of leucocytes into the plaque.

Pathological studies have also demonstrated increased AM expression in human atherosclerotic plaques [8]; however, determining whether hypercholesterolaemia directly increases AM expression is difficult in humans, because of the inability to readily assess the levels of AM expression on vascular endothelium. As circulating (c)AMs have been used as markers of atherosclerosis [9–12], clinical trials have tried to reveal a potential association between hypercholesterolaemia and AM expression by measurement of these cAMs: elevated cAM levels have been found in hypercholesterolaemic patients in some smaller studies [13], but not in larger trials [14]. Likewise, cross-sectional studies indicate rather weak or no correlations between cAM levels and plasma cholesterol levels [15–22]. Finally, pharmacological reduction of LDL–cholesterol (LDL-C) by statins was associated with a decrease in plasma levels of E-selectin in ten patients [23], but further confusion arises from divergent results of statin therapy on cICAM-1 levels [24–26].

This study had two goals: first, to investigate whether different statins have diverse effects on cAM-levels, and secondly, to determine whether lowering of cholesterol levels by statins decreases cAM release into the circulation in a study with adequate sample size (pooled analysis).

**METHODS**

**Study design**

**Interventional trial**

This research has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The study was approved by the local Ethics Committee and all patients gave written consent. The study design was randomized and double-blind in three parallel groups, and compared the effects of a 3-month treatment with atorvastatin (10 mg; Sortis®, Parke-Davis, NY, U.S.A.), simvastatin (40 mg; Zocord®, MSD, Haarlem, The Netherlands) or pravastatin (40 mg; Selektine®, Bristol-Myers-Squibb, Munich, Germany). The medication used in this study was in the form of corn-starch-filled capsules, which were identical in appearance for the three different medications. The study medication was taken once daily after the evening meal. Patients’ compliance was verified by pill counts.

**Patients and controls**

At an initial screening visit, patients completed a detailed questionnaire about their medical and treatment histories. Blood pressure was recorded and venous fasting blood samples were drawn for total cholesterol analysis. For confirmation of elevated total cholesterol levels, despite dietary fat restrictions, patients were invited to the outpatient department 2 weeks later. Patients aged 35–75 years with fasting plasma cholesterol in the range 5.2–9.1 mmol/l (201–352 mg/dl), triacylglycerol (triglyceride) levels lower than 2.9 mmol/l (257 mg/dl), and in whom dietary-fat restriction did not result in a decrease in plasma cholesterol levels, were enrolled into the study. All patients gave a medical history and underwent a physical examination and laboratory testing. Exclusion criteria were obesity (body-mass index > 30 kg/m²), hypertension, diabetes mellitus, secondary hypercholesterolaemia, known atherosclerosis (history of intermittent claudication, angina pectoris), thyroid disease, pregnancy or lactation, malignancy or other relevant abnormalities, increased plasma levels of C-reactive protein (> 5 mg/l), abnormalities seen in the results of laboratory tests or ECG, elevated serum transaminases, impaired hepatic or renal function (microalbuminuria or creatinine > 120 μmol/l), consumption of > 40 g/day of ethanol, intake of hypolipaemic, anticoagulant or anti-inflammatory drugs. Smokers (n = 26; 20±13 cigarettes/day) and women taking oral contraceptives or undergoing hormonal replacement therapy (n = 8) were not excluded from the study. Smokers were requested not to smoke for at least 2 h before blood sampling.

**Cross-sectional analysis**

The 75 hypercholesterolaemic subjects were compared with 102 normolipidemic healthy controls [49±13 years; 39 male/63 female; mean cholesterol level, 4.6 mmol/l (range, 2.5–5.1 mmol/l)]. These controls had no relevant medical history, were free of medication and had no risk factors for atherosclerosis.

**Laboratory investigations**

Samples were taken in the morning because of potential circadian variations in cAM levels [27]. Fasting serum concentrations of all lipids were measured on a Hitachi 747 chemistry analyser using reagents from Roche (Mannheim, Germany) [19]. cAM levels were measured from frozen citrated plasma stored at −80 °C as duplicates with a single batch of enzyme immunoassays (R&D Systems, Oxfordshire, U.K.) [28] at the end of the study; intra-assay variability was < 5%. Ultrasensitive C-reactive protein (CRP) was determined nephelometrically (Dade Behring, Marburg, Germany).
Statistical calculations
An *a priori* sample size calculation was based on the day-to-day variability of cAM levels (coefficient of variation = 6–8% for all cAMs) [29]. Thus, a sample size of 25 patients in each group gave an 80% probability of detecting a decrease in cE-selectin levels as small as 8–12% in each group and 5% in the pooled analysis [30]. cE-selectin was chosen as the primary outcome variable based on the report of Hackman et al. [23], which found that statin therapy only affected cE-selectin levels, but not cVCAM-1 or cICAM-1 levels.

All results are presented as means ± S.D. (baseline data) or as 95% confidence intervals (CI). The Wilcoxon test, the Kruskal–Wallis test, the Mann–Whitney U test, and the Spearman ranks correlation were calculated with the Statistica*-software (StatSoft, Tulsa, OK, U.S.A.). A *P* value < 0.05 was considered to be statistically significant for cE-selectin (main outcome variable).

RESULTS
We studied 75 patients (27 male; 48 female), with a mean age of 54 ± 9 years, mean cholesterol level of 6.7 ± 0.7 mmol/l and a mean body-mass index of 24.4 ± 2.7 kg/m². There were no differences in baseline levels of lipids, cAM levels or demographic variables between treatment groups (results not shown). Atorvastatin, simvastatin and pravastatin decreased total cholesterol levels by 28% (CI, 24–32%), 31% (CI, 26–36%) and 22% (CI, 19–26%) respectively. LDL-C levels decreased, on average, by 32–47% in the three groups (Figure 1; *P* < 0.0001 for all groups). None of the statins lowered plasma cAM levels (Figure 1); this is underscored by pooled analyses of all 75 patients (95% CI, −2 to 6%; Figure 1). In contrast, pooled analysis indicates a small decrease in CRP levels from 0.22 ± 0.28 to 0.20 ± 0.26 mg/dl (*P* = 0.033).

Subgroup analysis
cAM levels did not decrease in the 19 patients with a > 50% decrease (CI, 55–59%) in LDL-C levels (*P* > 0.05). Similarly, those 17 patients with baseline LDL-C levels > 5 mmol/l exhibited a 43% (CI, 37–49%) reduction in LDL-C levels, but no significant reduction in cAM levels. Even after exclusion of smokers, statins did not lower cICAM-1 levels.

Correlations
In agreement with Hwang et al. [15], only cE-selectin correlated very weakly (*P* < 0.05) with pretreatment levels of high-density lipoprotein–cholesterol (*r* = −0.23) or triacylglycerol levels (*r* = 0.20), but also with LDL-C (*r* = −0.23); however, the degree of changes in lipid levels did not correlate with changes in cAM levels.

DISCUSSION
Atherosclerotic plaques are characterized by high expression of AMs. A number of publications have unequivocally shown increased cAM levels in atherosclerosis [18,31], which may even predict outcome [31]. In contrast with atherosclerosis, the effect of hypercholesterolaemia on cAM levels has been a source of controversy.
We therefore aimed to clarify whether hypercholesterolaemia directly induces AM release into the circulation.

All statins demonstrated their well-known capacity to lower LDL levels effectively, but failed to reduce cAM levels, when administered over 3 months (Figure 1). The pooled analysis of all 75 treated patients even detected a reduction in high-sensitivity CRP levels by 0.02 mg/dl. Furthermore, with 25 subjects in each group, we had an 80% power to detect reductions in cE-selectin levels as small as 8–12% in each group and 5% in the pooled analysis.

Initially, this finding seems to contradict a previous trial: Hackman et al. [23] treated ten hypercholesterolaemic patients (LDL-C, 6.8 mmol/l) with atorvastatin or simvastatin, and LDL-C levels were decreased by 51% over 42 weeks. This was accompanied by a decrease in mean cE-selectin levels from 77 ng/ml to 55 ng/ml ($P \leq 0.03$), but no changes in plasma levels of cICAM-1 or cVCAM-1. In our study, not even patients with a >50% decrease in LDL-C, nor the subgroup with LDL-C levels of >5 mmol/l exhibited a decrease in cAM levels. We therefore postulate that the difference between studies in changes observed for cE-selectin, if not due to chance, could be due to the longer duration of statin therapy in Hackman’s trial [23]. The lack of an effect on cICAM-1 levels is in good agreement with a recent trial in which 50 hypercholesterolaemic patients were treated with simvastatin (20 mg/day) or pravastatin (40 mg/day) for 3 months [25]. Simvastatin had no effect on cAM in hypercholesterolaemic postmenopausal women, but decreased interleukin-6 levels by 15% [34], which is in line with our finding of a 10% decrease in CRP levels. A 20% decrease in cICAM-1 levels was reported recently in a 3-month treatment programme, in which 26 patients with type IIa hypercholesterolaemia were treated with fluvastatin (80 mg/day) [26]. Blann et al. [24] impressively demonstrated a 24% reduction in cICAM-1 levels in hypercholesterolaemic patients with peripheral artery disease after therapy with pravastatin (40 mg/day). Thus, a likely explanation for the divergent findings could be different patient groups studied with probably varying degrees of (sub)clinical atherosclerosis. An alternative explanation could be differences in the intake of antioxidants among subjects in different studies. It is probable that cholesterol does not induce pro-inflammatory reactions when sufficient antioxidants are present in the vessel wall.

Three months of treatment with three different statins does not reduce cAM levels in moderate hypercholesterolaemia. This indicates that moderate hypercholesterolaemia is not a direct stimulus for AM expression in humans. This is also supported by our cross-sectional results (Table 1), and our findings that not even 6 months of lipid apheresis lowered cAM levels in patients with familial hypercholesterolaemia [35].

Furthermore, statins did not affect the adherence of monocytes to ICAM-1 or VCAM-1, and do not influence the expression of integrins [36]. These results imply that cholesterol may not induce endothelial activation, which indirectly contradicts the classical theory of atherosclerosis, that the initial up-regulation of VCAM-1 (and to some degree ICAM-1) expression on the surface of the endothelium by LDL-C initiates monocyte recruitment to the intima.

As cAM-levels are sensitive markers for inflammation and endothelial activation [37], our results provide strong evidence that moderate hypercholesterolaemia does not increase endothelial activation.

During the same (3 months) or even shorter time-periods, angiotensin-converting enzyme inhibitors have been found to effectively reduce cAM-leve[38]. Hence, in this study we did not intend to assess whether long-term statin therapy may lower cAM levels, because a late decrease in cAM levels is unlikely to be attributed to a decrease in cholesterol levels.

In summary, the present study provides strong evidence that 3 months of therapy with three different statins does not decrease cAM levels, despite normalization of cholesterol levels, and a minor decrease in CRP levels. Hence, moderate hypercholesterolaemia does not directly increase cAM levels, and does not induce endothelial activation.

**REFERENCES**


**Table 1** Baseline values of adhesion molecules and lipids

<table>
<thead>
<tr>
<th>Hypercholesterolaemic</th>
<th>Hypercholesterolaemic</th>
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<tbody>
<tr>
<td>non-smokers</td>
<td>smokers</td>
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<tr>
<td>cE-selectin (ng/ml)</td>
<td>40 ± 14</td>
</tr>
<tr>
<td>cICAM-1 (ng/ml)</td>
<td>233 ± 66</td>
</tr>
<tr>
<td>cVCAM-1 (ng/ml)</td>
<td>508 ± 126</td>
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<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.61 ± 0.79</td>
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<tr>
<td>LDL-C (mmol/l)</td>
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<tr>
<td>HDL-C (mmol/l)</td>
<td>1.71 ± 0.46</td>
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<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.40 ± 0.43</td>
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* $P < 0.001$ versus hypercholesterolaemic non-smokers or controls. HDL-C, high-density lipoprotein cholesterol. Results are presented as means ± 95% CI.