Genetics and therapy of familial hypercholesterolemia

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Regression of carotid and femoral intima-media thickness in familial hypercholesterolemia

Treatment with simvastatin

Submitted for publication

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Chapter 7

ABSTRACT

BACKGROUND
B-mode ultrasound imaging of arterial walls is increasingly used as a non-invasive surrogate marker of cardiovascular disease (CVD) and in particular intervention trials using this modality have shown that by reducing risk factors, progression of atherosclerosis was inhibited. The goal of our study was to investigate whether high-dose simvastatin therapy could reduce carotid and femoral artery intima-media thickness (IMT) in patients with familial hypercholesterolemia (FH) in order to prevent CVD.

METHODS AND RESULTS
After a washout period of 6 weeks, all FH patients started monotherapy with 80 mg simvastatin for the duration of two years. The primary endpoint was the change in mm of the mean combined far wall IMT of predefined carotid and femoral arterial segments at two years. A total of 153 FH patients were included and mean combined baseline IMT was 1.07 ± 0.23 mm. After treatment with 80 mg simvastatin for 2 years, this IMT decreased by -0.081 mm (95% CI; -0.109 to -0.053; p<0.0001) with its largest reduction in the femoral artery (-0.283 mm; p<0.0001). Strikingly, an actual decrease of combined IMT was seen in 69.8% of all patients.

CONCLUSIONS
High-dose simvastatin reduces arterial wall IMT in more than two thirds of the patients, with its largest effect on the femoral artery. Furthermore, FH patients treated with both statin and antihypertensive medication experienced a significantly greater benefit in terms of IMT reduction.
INTRODUCTION

Familial Hypercholesterolemia (FH) is a common autosomal dominant disorder of lipoprotein metabolism, affecting approximately 1 in 400 individuals in the Netherlands. FH patients have elevated levels of low-density lipoprotein cholesterol (LDL-C), due to mutations in the LDL-receptor gene, which consequently predispose to the development of atherosclerosis. Therefore, FH patients are at severe risk of premature cardiovascular disease (CVD). In order to modify risk in these FH patients, LDL-C levels need to be aggressively lowered by intensive lipid-lowering therapy. Methodologically, it would be preferable to investigate lipid-modifying drugs in double blind randomized placebo controlled trials. However, these studies are no longer considered ethical after the evidence obtained with lipid-lowering in non-FH patients, both with and without prior CVD history. Moreover, regression of atherosclerosis using coronary angiography has also been demonstrated to occur in FH patients treated with lipid-lowering drugs. Angiography is an invasive technique and cannot, therefore, be used in FH patients without symptoms. Fortunately, a non-invasive surrogate marker has become available in the form of B-mode ultrasound imaging of arterial walls. This technique allows for non-invasive assessment of the intima-media complex (IMT). IMT is associated with age and cardiovascular risk factors such as LDL-C, blood pressure and smoking. Moreover, intervention trials have shown that by reducing risk factors, such as LDL-C levels, progression of atherosclerosis was inhibited, or even led to regression of this process. IMT measurements are therefore now widely accepted as a standardized and validated surrogate marker for atherosclerotic vascular disease.

We were the first to demonstrate that intensive lipid lowering with 80 mg atorvastatin resulted in the actual regression of carotid IMT in the majority of FH patients, and that 40 mg of simvastatin only led to less progression of vascular wall IMT. Based on previous work in the LDL-Apheresis Atherosclerosis Regression Study (LAARS) combined with results from the Atorvastatin versus Simvastatin on Atherosclerosis Progression (ASAP) study we hypothesized that at least a 45% reduction of LDL-C would be required to reverse the atherosclerotic process in FH. We therefore also set out to evaluate the effect of simvastatin 80 mg, with its known LDL-C lowering capacity of over 45%, on the behaviour of carotid and femoral IMT over a two year intervention period. Here we present the results of our study.
METHODS

Subjects
FH patients were recruited from the Lipid Research Clinic at the University of Amsterdam. Patients were included if they met the following criteria: all patients had to have either a molecular diagnosis of FH or were diagnosed with definite FH and had to have 6 or more points, according to an algorithm (to allow standardization of the diagnosis of FH based on clinical findings, personal and familial clinical history and biochemical parameters); at least 18 years of age; and patients with a history of myocardial infarction, coronary artery bypass graft or percutaneous transluminal coronary angioplasty could be included if the physician thought it was medically allowed for the patient to have a washout period. Patients were excluded if they were pregnant or nursing women, or pre-menopausal women not using adequate contraceptives; had acute liver disease, hepatic dysfunction, or persistent elevations of serum transaminases; had hypersensitivity or intolerance to simvastatin or any of its components; had hyperlipidemia Type I, III, IV or V or homozygous FH; had a recent history of alcohol or drug abuse; had secondary hypercholesterolemia due to any cause; had inadequately controlled diabetes, unstable angina or intermediate coronary syndrome or clinically significant ventricular arrhythmia at study entry or myocardial infarction within the past 3 months; were on concurrent use of erythromycin and similar drugs affecting the cytochrome P450 enzyme or had a history of cancer.
The Institutional Review Board of the Academic Medical Center approved the protocol and written informed consent was obtained from all participants.

Study design
After a washout period of 6 weeks, patients were started on monotherapy with simvastatin 80 mg, one tablet once daily, with the intent of a study duration of 2 years. No other lipid lowering medication was allowed. Medical history, physical examination and additional risk factors for cardiovascular disease as well as laboratory analysis of lipid and lipoprotein levels and routine safety parameters were obtained in all patients. The biochemical analyses of lipid levels and safety parameters were performed at each of 8 clinic visits (at weeks: -6, 1, 6, 12, and 24, years: 1, 1½ and 2).
Biochemical analysis
Blood samples were taken in the morning after an overnight fast. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and safety parameters were routinely determined in the different laboratories and standardized by a virtual central laboratory. LDL-C was calculated using the Friedewald formula. Apolipoprotein A-I and apolipoprotein B were determined by an immunological rate-nephelometric procedure using a polyclonal goat anti-human antibody (Array protein system, Beckman Coulter, Netherlands).

Ultrasonography
All examinations were performed by the same sonographer. A Biosound Phase-2 real time scanner (Biosound Esaote, USA) equipped with a 10 MHz transducer was used. Measurements were made at baseline, after one year and two years of therapy. In both carotid arteries, three 10 mm segments were scanned: the distal portion of the common carotid artery, the carotid bifurcation and the proximal portion of the internal carotid artery. In the right common femoral artery, a 10 mm segment proximal to the branching of the deep femoral artery was scanned. The ultrasound scanning protocol was similar to that used in the ASAP study. In summary, one sonographer performed all scans. Images were stored on an optical disk and analyzed with a semi-automatic software program (Eurequa; TSA Company, Meudon, France). IMT measurements were performed on the near and far walls of the common carotid artery and the bifurcation and in the far walls of the internal carotid artery and femoral artery. Only IMT data of images of the far walls were analyzed. All ultrasound images were analyzed by one reader blinded to any patient information. Reproducibility tests were performed regularly by the sonographer and yielded a coefficient of variation of less than 5%.

Plaque was defined as an IMT ≥ 1.3 mm and/or a wall-interface displacement. When a plaque was identified in the region under investigation it was included in the measurement if there was no wall interface displacement. If the wall-interface was interrupted the plaque was separately identified by its thickness and not included in the IMT calculations.

The main outcome parameter was defined as the change in mm of the mean combined far wall IMT of predefined carotid and femoral arterial segments at two years.
Statistical analysis
Mean values of lipids and lipoproteins before and after treatment were compared using the paired sample t-test. TG levels were compared by the non-parametric Wilcoxon test, since they had a skewed distribution. The primary endpoint of the ultrasound component of the trial was defined as the change in mm of the mean combined far wall IMT of the predefined carotid and femoral arterial wall segments. Change from baseline IMT after 2 years was analyzed with covariance analysis with baseline IMT levels as covariate. The repeated IMT measurements (baseline, 1 and 2 year) were also analyzed with mixed model analysis of variance (ANOVA), in which combined far wall IMT was the dependent variable, visit fixed factor and the patients as random factor. We here report the estimated marginal means according to this mixed model. Relationships between IMT (-changes) and different parameters were described by Pearson correlation coefficients, except in the case of TG, where Spearman's rank correlation was used. Analyses were performed using the SPSS statistical package (version 10.1, Chicago; Illinois). A p-value <0.05 was considered to be statistically significant.

RESULTS

Baseline demographics
A total of 153 FH patients were recruited into the study. Of these, 14 (9.2%) dropped out of the study during the two-year treatment period: 2 died from cardiovascular events, 5 patients discontinued the study due to adverse clinical events and 7 did so for other reasons. Ages ranged from 19 to 79 years (mean age 46.2 ± 12.9 years). Slightly more males (54.9%) than females were included. The relative frequency of CVD was 24.2% with a mean age of onset of 45.5 years. The prevalence of other risk factors such as hypertension and diabetes was rather low (9.2% and 1.3%, respectively). A total of 46 patients were smokers, while 106 patients were non-smokers (of whom 51 were former smokers). Median numbers of pack-years (the number of years the patient smoked 20 cigarettes a day) were 17.0 (interquartile range: 8.0 - 24.0) in former and current smokers combined and 6.4 (interquartile range: 0.0 - 20.4) in all patients. Mean body mass index (BMI) was 25.0 ± 3.2 kg/m². Mean systolic and diastolic blood pressures were 128 ± 19 mm Hg and 79 ± 10 mmHg, respectively.
Table 1. Lipids and lipoproteins at baseline and after 2 years of therapy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (n=153)</th>
<th>End of study (n=139)</th>
<th>Change percentage</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>10.30 ± 2.03</td>
<td>6.50 ± 1.44</td>
<td>-36.2 ± 12.1 %</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>396 ± 78</td>
<td>250 ± 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>8.12 ± 2.02</td>
<td>4.45 ± 1.31</td>
<td>-44.4 ± 14.2 %</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>312 ± 78</td>
<td>171 ± 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.32 ± 0.33</td>
<td>1.40 ± 0.34</td>
<td>+7.0 ± 16.4 %</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>51 ± 13</td>
<td>54 ± 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.70 (1.10/2.40)</td>
<td>1.30 (0.90/1.70)</td>
<td>-25.0 (-45.0/-3.3)%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>150 (97/212)</td>
<td>115 (80/150)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>1.24 ± 0.22</td>
<td>1.32 ± 0.24</td>
<td>+7.6 ± 30.5 %</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>2.01 ± 0.43</td>
<td>1.24 ± 0.32</td>
<td>-37.6 ± 14.3 %</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation or as median with the interquartile range between brackets. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein; TG, triglycerides; Apo, apolipoprotein.

Lipids and lipoproteins
Mean lipid and lipoprotein levels at baseline and at two years are shown in table 1. Baseline mean TC (10.30 mmol/L or 396 mg/dl) and LDL-C (8.11 mmol/L or 312 mg/dl) levels were, as can be expected in FH patients, severely elevated. For comparison, TC and LDL-C levels in 3403 Dutch controls were 5.49 (211 mg/dl) and 3.56 mmol/L (137 mg/dl), respectively. Upon therapy with simvastatin 80 mg for the duration of two years, mean TC, LDL-C and median TG levels were significantly reduced by 36.2%, 44.4% and 25%, respectively. HDL-C levels were increased by 7.0% to a mean level of 1.40 mmol/L (54 mg/dl).

Baseline IMT measurements
Mean combined baseline IMT of the carotid and femoral far walls was 1.07 ± 0.23 mm (table 2). For comparison, a study with a similar ultrasound protocol, reported that in healthy controls without any risk factor for CVD (mean age of 45.9 years, BMI of 24.1 kg/m²) the mean combined IMT of the carotid and femoral far walls was 0.73 ± 0.20 mm, while in smoking controls without any additional risk factor for CVD (mean age of 44.3 years, BMI of 23.2 kg/m² and 23.7 pack-years) mean combined IMT of the carotid and femoral far walls was 0.86 ± 0.27 mm.
Table 2. Mean combined carotid and femoral far wall intima-media thickness (IMT) at baseline and after 2 years of therapy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (mm)</th>
<th>End of study (mm)</th>
<th>Change (mm)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean IMT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Combined far walls</td>
<td>1.07</td>
<td>0.99</td>
<td>-0.081</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(1.05 to 1.09)</td>
<td>(0.97 to 1.01)</td>
<td>(-0.053 to -0.109)</td>
<td></td>
</tr>
<tr>
<td>*Carotid far wall</td>
<td>0.92</td>
<td>0.87</td>
<td>-0.053</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.91 to 0.94)</td>
<td>(0.85 to 0.89)</td>
<td>(-0.075 to -0.031)</td>
<td></td>
</tr>
<tr>
<td>*Femoral far wall</td>
<td>1.88</td>
<td>1.60</td>
<td>-0.283</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(1.80 to 1.96)</td>
<td>(1.51 to 1.68)</td>
<td>(-0.401 to -0.166)</td>
<td></td>
</tr>
</tbody>
</table>

Estimated marginal means were reported according to the mixed-model. IMT, intima-media thickness; CI, confidence interval.

FH patients with a history of CVD compared to FH patients without CVD exhibited an increased baseline combined far wall IMT (1.19 ± 0.23 mm vs. 1.03 ± 0.22 mm, respectively; p<0.0001). The mean IMT in the femoral artery accounted for most of this difference (2.31 ± 0.81 mm vs. 1.72 ± 0.63 mm; p<0.0001) but the IMT difference for the carotid artery between patients with and without CVD was also significant (0.99 ± 0.17 mm vs. 0.90 ± 0.19 mm; p=0.01). In addition, mean baseline combined far wall IMT was correlated with increasing age (r=0.48; p<0.0001), systolic and diastolic blood pressure (r=0.33; p<0.0001 and r=0.28; p=0.001, respectively), BMI (r=0.25; p=0.002), apolipoprotein B levels (r=0.31; p<0.0001), TG levels (r=0.29; p<0.0001), TC levels (r=0.29; p<0.0001) and LDL-C levels (r=0.24; p=0.003). Mean baseline combined far wall IMT was also increased in current and former smokers compared to the patients who never smoked, although not statistically significant (1.08 and 1.04 mm, respectively; p=0.23). However, mean baseline combined far wall IMT was significantly increased in patients with ≥ 6.4 pack-years compared to those with less than 6.4 (1.11 versus 1.03 mm; p=0.03).

**The effect of simvastatin 80 mg on IMT measurements**

After 2 years of treatment with simvastatin 80 mg, mean combined far wall IMT was reduced by 0.081 mm (95% confidence interval (CI): -0.109 to -0.053 mm) to a mean of 0.99 mm (figure 1). Of all patients, 69.8% exhibited an actual decrease of IMT after two years of treatment. The extent of the IMT-change was significantly associated with baseline combined far wall IMT (r=0.53; p<0.0001) and mean change
in HDL-C at years 1 and 2 was significantly associated with change in carotid artery IMT (r= -0.24; p=0.007). In the 30 patients who were on either calcium antagonists, angiotensine-converting-enzyme (ACE) inhibitors or beta-blockers the mean combined far wall IMT was significantly more reduced compared to the patients without one of these three drugs (-0.155 mm vs. -0.069 mm; p=0.02). Lastly, change in IMT was not influenced by any other parameter, such as gender, age, previous lipid-lowering treatment, change in LDL-C or family history of premature CVD.

DISCUSSION

Baseline IMT
As in most studies, mean arterial wall IMT was severely increased in our FH patients and at least twice as thick as in controls. Moreover, mean IMT was even more increased in CVD patients compared to those without CVD. Interestingly, the largest differences between CVD and non-CVD patients were observed in the femoral artery (2.31 ± 0.81 mm vs. 1.72 ± 0.63 mm; p<0.0001), as was reported previously. These data indicate that we have recruited a large and representative cohort of FH patients with very similar baseline IMT characteristics to other study cohorts. These greatly abnormal IMT measurements must be the consequence of a very rapid progression of atherosclerotic lesions in FH patients. Wendelhag et al calculated an increased progression rate of 0.009 mm/yr in adult FH patients compared to 0.005
mm/yr in controls. In another study we have estimated a progression rate at 0.006 mm/yr in FH children versus 0.001 mm/yr in unaffected controls. The expected progression rate in the current study was 0.008 mm/yr, as calculated in a linear regression analysis relating age to mean IMT. These data combined indicate that arterial wall thickening progresses at least 2 to 3 times faster in adult FH patients than in healthy individuals.

Correlation between baseline IMT and parameters such as age, blood pressure, lipids and lipoproteins and smoking are also in line with previously reported data in FH patients.\textsuperscript{5,22,31} To the best of our knowledge, the correlation between BMI and IMT has not been observed in FH patients but only in healthy controls.\textsuperscript{31} These results support the notion that other risk factors besides LDL-C also significantly contribute to the severity of atherosclerosis in FH patients.

**The effect of simvastatin 80 mg on IMT**

Confirming our predefined hypothesis, treatment with 80 mg simvastatin not only retarded the progression rate of atherosclerosis, but even resulted in an actual reduction of the mean combined far wall IMT of the carotid and femoral arteries (-0.081 mm). Strikingly, even 1 year of treatment with simvastatin 80 mg was sufficient to detect a statistically significant decrease in mean combined far wall IMT (-0.028 mm; \(p=0.048\)). Taken together, a series of consistent data sets has now shown that the increased IMT in different groups of high risk patients is reduced by lipid lowering treatment.\textsuperscript{13,15,34} A yearly increase of carotid artery IMT of 0.03 mm is associated with an odds ratio of 3.9 for coronary events in men with coronary artery disease.\textsuperscript{35} Conversely, the reduction of approximately 0.03 mm in one year as observed in our study will likely have a significant clinical impact on the prevention of coronary artery disease.

The most striking regression was observed in the femoral artery as reported previously in two other studies.\textsuperscript{15,36} The reason for this finding could be based on the fact that baseline IMT was higher in the femoral (1.88 mm) compared to the carotid artery (0.92 mm). It may also be hypothesized that a more pronounced treatment effect in the femoral artery was observed because progression rate of IMT is higher and more sensitive to LDL-C reduction. This larger regression of femoral IMT could be more clinically relevant since in our study the correlation between IMT and CVD was better in the femoral compared to the carotid artery. Change in mean combined far wall IMT was associated with baseline combined far wall IMT, but not with change in LDL-C, in contrast to the findings in the ASAP study\textsuperscript{12} but similar to the
Carotid Atherosclerosis Italian Ultrasound Study (CAIUS). The reason for this finding could lie in the more narrow LDL-C changes between individuals in our study since monotherapy was used similar to CAIUS and in contrast to ASAP in which two different statins were used at different doses (simvastatin 40 mg vs. atorvastatin 80 mg).

Finally, in our study a statistically significant difference was observed for the effect of antihypertension treatment (calcium-antagonists, beta-blockers and ACE-inhibitors) on change in mean combined far wall IMT. Again, a larger decrease in IMT was found in the femoral artery of patients on antihypertensive treatment compared to patients without (-0.699 mm vs. -0.179 mm; p=0.006). Some other studies have also shown a treatment effect of either a calcium antagonist or an ACE-inhibitor on carotid artery IMT as summarized by Simon et al. Recently, Wiklund et al. reported an additional treatment effect of the beta-blocker metoprolol on the change in mean carotid IMT in hypercholesterolemic patients treated with statins.

These data might indicate that statin treatment and beta-blockade exert favourable synergistic effects on the arterial wall.

In conclusion, this study has proven that high dose simvastatin treatment even results in a reduction of the combined carotid and femoral far wall IMT in more than two third of the patients, with its largest effect in the femoral artery. Furthermore, FH patients treated with both statin and antihypertensive medication experienced a greater benefit in terms of IMT reduction than patients on simvastatin alone. Lastly, these effects of simvastatin on the arterial wall occurred in the light of an excellent tolerability and safety profile.

REFERENCES


