Regression of Carotid and Femoral Artery Intima-Media Thickness in Familial Hypercholesterolemia

Treatment With Simvastatin

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Objective: To investigate whether high-dose simvastatin therapy could reduce carotid and femoral artery intima-media thickness (IMT) in patients with familial hypercholesterolemia (FH) to prevent cardiovascular disease.

Background: Imaging of arterial walls with B-mode ultrasonography is increasingly used as a noninvasive surrogate marker of cardiovascular disease. Intervention trials using this modality have shown that by reducing risk factors, progression of atherosclerosis was inhibited.

Methods: After a washout period of 6 weeks, all patients with FH started monotherapy with simvastatin, 80 mg/d, for 2 years. The primary end point was the change (in millimeters) of the mean combined far-wall IMT of predefined carotid and femoral arterial segments at 2 years.

Results: We included a total of 153 patients with FH. Mean ± SD combined baseline IMT was 1.07 ± 0.23 mm. After treatment with simvastatin for 2 years, this IMT decreased by a mean of 0.081 mm (95% confidence interval, −0.109 to −0.053; P < .001), with its largest reduction in the femoral artery (−0.283 mm; P < .001). An actual decrease of combined IMT was seen in 69.8% of all patients.

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

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Familial hypercholesterolemia (FH) is a common autosomal dominant disorder of lipoprotein metabolism affecting approximately 1 in 400 individuals in the Netherlands. Patients with FH (hereafter referred to as FH patients) have elevated levels of low-density lipoprotein cholesterol (LDL-C), due to mutations in the LDL-receptor gene, which consequently predispose them to the development of atherosclerosis. Therefore, FH patients are at severe risk for premature cardiovascular disease (CVD). To modify risk in these FH patients, LDL-C levels need to be aggressively lowered by means of intensive therapy. Methodologically, it would be preferable to investigate drugs that modify lipid levels in double-blind randomized placebo-controlled trials. However, these studies are no longer considered ethical after the evidence obtained with the lowering of lipid levels in non-FH patients with and without a history of CVD. Moreover, regression of atherosclerosis using coronary angiography has also been demonstrated to occur in FH patients treated with drugs that lower lipid levels. Angiography is an invasive technique and cannot, therefore, be used in FH patients without symptoms. Fortunately, a noninvasive surrogate marker has become available in the form of B-mode ultrasonographic imaging of arterial walls. This technique allows for noninvasive assessment of the intima-media thickness (IMT). The IMT is associated with age and cardiovascular risk factors such as LDL-C level, blood pressure, and smoking. Moreover, intervention trials have shown that reducing risk factors such as LDL-C levels inhibited the progression of atherosclerosis or even led to regression of this process. Measurements of IMT are therefore now widely accepted as a standardized and validated surrogate marker for atherosclerotic vascular disease.
Our group\textsuperscript{12} was the first to demonstrate that intensive therapy to lower lipid levels with atorvastatin calcium, 80 mg/d, resulted in the actual regression of carotid IMT in most FH patients, and that 40 mg of simvastatin led only to less progression of vascular wall IMT.\textsuperscript{12} On the basis of previous work in the LDL-Apheresis Atherosclerosis Regression Study,\textsuperscript{16} and results from the Atorvastatin Versus Simvastatin on Atherosclerosis Progression (ASAP) study, we hypothesized that at least a 45% reduction of LDL-C levels would be required to reverse the atherosclerotic process in FH. We therefore also set out to evaluate the effect of simvastatin, 80 mg/d, with its known capacity to lower LDL-C levels by greater than 45%, on the behavior of carotid and femoral IMT during a 2-year intervention. Herein we present the results of our study.

METHODS

SUBJECTS

Familial hypercholesterolemia patients were recruited from the Lipid Research Clinic at the University of Amsterdam, Amsterdam, the Netherlands. Patients were included if they had a molecular diagnosis of FH or a diagnosis of definite FH and 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 variables),\textsuperscript{17} and were 18 years or older. We included patients with a history of myocardial infarction, coronary artery bypass graft, or percutaneous transluminal coronary angioplasty 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 premenopausal women not using adequate contraceptives; had acute liver disease, hepatic dysfunction, or persistent elevations of serum transaminase levels; 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 other drug abuse; had secondary hypercholesterolemia due to any cause; had inadequately controlled diabetes mellitus, unstable angina, intermediate coronary syndrome, clinically significant ventricular arrhythmia at study entry, or myocardial infarction within the past 3 months; were concurrently using erythromycin stearate or similar drugs affecting the cytochrome P-450 enzyme; or had a history of cancer.

The Institutional Review Board of the Academic Medical Center, University of Amsterdam, approved the protocol, and written informed consent was obtained from all participants.

STUDY DESIGN

After a washout period of 6 weeks, patients started monotherapy with simvastatin, 80 mg, 1 tablet once daily, with the intent of a study duration of 2 years. No other medication to lower lipid levels was allowed. Medical history, physical examination, additional risk factors for CVD, laboratory analysis of lipid and lipoprotein levels, and routine safety variables were obtained in all patients. The biochemical analyses of lipid levels and safety variables were performed at each of 8 clinic visits (at weeks –6, 1, 6, 12, and 24 and years 1, 1.5, and 2).

BIOCHEMICAL ANALYSIS

Blood samples were taken in the morning after an overnight fast. Levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) and safety variables were routinely determined in the different laboratories and standardized by a virtual central laboratory. Levels of LDL-C were calculated using the Friedewald formula.\textsuperscript{18} Apolipoprotein A-1 and B levels were determined by means of an immunological rate–nephelometric procedure using a polyclonal goat antihuman antibody (Array protein system; Beckman Coulter Inc, Fullerton, Calif).\textsuperscript{19}

ULTRASONOGRAPHY

All examinations were performed by the same sonographer. We used a real-time scanner (Biosound Phase-2; Biosound ESAOTE, Inc, Indianapolis, Ind) equipped with a 10-MHz transducer. Measurements were performed at baseline and after 1 and 2 years of therapy. In both carotid arteries, the following 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 ultrasonographic scanning protocol was similar to that used in the ASAP study.\textsuperscript{12,20} Briefly, the protocol included the following: One sonographer performed all scans. Images were stored on an optical disk and analyzed with a semiautomatic software program (Eurequa; TSA Company, Meudon, France).\textsuperscript{21} The 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.\textsuperscript{22} All ultrasonographic images were analyzed by 1 reader masked to any patient information. Reproducibility tests were performed regularly by the sonographer and yielded a coefficient of variation of less than 5%.\textsuperscript{23}

Plaque was defined as an IMT of at least 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 variable was defined as the change in millimeters of the mean combined far-wall IMT of predefined carotid and femoral arterial segments at 2 years.

STATISTICAL ANALYSIS

Mean values of lipid and lipoprotein levels before and after treatment were compared using the paired-sample t test. Levels of TG were compared by the nonparametric Wilcoxon test, since they had a skewed distribution. The primary end point of the ultrasonographic component of the trial was defined as the change 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 that used baseline IMT levels as the covariate. The repeated IMT measurements (at baseline and 1 and 2 years) were also analyzed with mixed-model analysis of variance, in which combined far-wall IMT was the dependent variable, visit was the fixed factor, and the patients were the random factor. We herein report the estimated marginal means according to this mixed model. Relationships between IMT (changes) and different variables were described by Pearson correlation coefficients, except in the case of TG level, for which the Spearman rank correlation was used. We performed analyses using the SPSS statistical package (version 10.0.7; SPSS Inc, Chicago, Ill). A P value of less than .05 was considered to be statistically significant. Data are expressed as mean±SD, except for data with a skewed distribution, where median with interquartile range are given.
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 2-year treatment period. Two of these died of cardiovascular events; 5 discontinued the study owing to adverse clinical events; and 7 discontinued for other reasons. Ages ranged from 19 to 79 years (mean [SD] age, 46.2 years [12.9 years]). Slightly more men (54.9%) than women were included. The relative frequency of CVD was 24.2%, with a mean age at onset of 45.5 years. The prevalence of other risk factors such as hypertension and diabetes mellitus was rather low (9.2% and 1.3%, respectively). Forty-six patients were smokers, whereas 107 patients were nonsmokers (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±SD body mass index (BMI; calculated as weight in kilograms divided by the square of height in meters) was 25.0±3.2. Mean±SD total cholesterol (5.49 mmol/L) and 137 mg/dL (3.56 mmol/L), respectively.24 After therapy with simvastatin, 80 mg/d, for the 2-year treatment period, mean total cholesterol and LDL-C values were, as can be expected in FH patients, severely elevated. For comparison, mean TC and LDL-C levels were significantly reduced by 36.2%, 44.4%, and 7.0% to a mean of 54 mg/dL (1.40 mmol/L).

LIPIDS AND LIPOPROTEINS

Mean lipid and lipoprotein levels at baseline and at 2 years are shown in Table 1. Baseline mean levels of TC (398 mg/dL [10.30 mmol/L]) and LDL-C (313 mg/dL [8.11 mmol/L]) were, as can be expected in FH patients, severely elevated. For comparison, mean TC and LDL-C levels in 3403 Dutch control subjects were 212 mg/dL (5.49 mmol/L) and 137 mg/dL (3.56 mmol/L), respectively.25 After therapy with simvastatin, 80 mg/d, for the duration of 2 years, mean TC and LDL-C and median TG levels were significantly reduced by 36.2%, 44.4%, and 25%, respectively. Levels of HDL-C were increased by 7.0% to a mean of 54 mg/dL (1.40 mmol/L).

BASELINE IMT MEASUREMENTS

Mean±SD 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 ultrasonographic protocol reported that in healthy controls without any risk factor for CVD (mean age, 45.9 years; BMI, 24.1), the mean±SD combined IMT of the carotid and femoral far walls was 0.73±0.20 mm, whereas in controls who smoked but had no additional risk factor for CVD (mean age, 44.3 years; BMI, 23.2, 23.7 pack-years), mean±SD combined IMT of the carotid and femoral far walls was 0.86±0.27 mm.25

The FH patients with a history of CVD compared with those without CVD exhibited an increased mean±SD baseline combined far-wall IMT (1.19±0.23 vs 1.03±0.22 mm; P<.001). The mean IMT in the femoral artery accounted for most of this difference (2.31±0.81 vs 1.72±0.63 mm; P<.001), but the mean IMT difference for the carotid artery between patients with and without CVD was also significant (0.99±0.17 vs 0.90±0.19 mm, respectively; P=.01). In addition, mean baseline combined far-wall IMT was correlated with increasing age (r=0.48; P<.001), systolic (r=0.33; P<.001) and diastolic (r=0.28; P=.001) blood pressure, BMI (r=0.25; P=.002), apolipoprotein B levels (r=0.31; P<.001), TG levels (r=0.29; P<.001), TC levels (r=0.29; P<.001), and LDL-C levels (r=0.24; P=.003). Mean baseline combined far-wall IMT was also increased in current and former smokers compared with the patients who never smoked, although the increase was not statistically significant (1.08 vs 1.04 mm; P=.23). However, mean baseline combined far-wall IMT was significantly increased in patients with at least 6.4 pack-years of use compared with those with less than 6.4 pack-years (1.11 vs 1.03 mm; P=.03).

EFFECT OF SIMVASTATIN ON IMT MEASUREMENTS

After 2 years of treatment with simvastatin, 80 mg/d, 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). Of all patients, 69.8% exhibited an actual decrease of IMT after 2 years of treatment. The extent of the IMT change was significantly associated with baseline combined far-wall IMT (r=0.53; P<.001), and mean change in HDL-C levels at years 1 and 2 was significantly associated with change in carotid artery IMT (r=−0.24; P=.007). In the 30 patients who were receiving calcium antagonists, angiotensin-converting enzyme inhibitors, or β-blockers, the mean combined far-wall IMT was significantly more reduced compared with the patients who were not receiving one of these 3 drugs (−0.155 vs −0.069 mm; P=.02). Change in IMT was not influenced by any other variable, such as sex, age, previous treatment for lowering of lipid levels, change in LDL-C level, or family history of premature CVD.

COMMENT

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.10,26-28 Moreover, mean IMT was even more increased in patients with CVD compared with those without CVD.
To the best of our knowledge, the correlation between BMI and IMT has not been observed in FH patients but only in healthy controls. These results support the notion that other risk factors besides LDL-C level also significantly contribute to the severity of atherosclerosis in FH patients.

**EFFECT OF SIMVASTATIN ON IMT**

Confirming our predefined hypothesis, treatment with simvastatin, 80 mg/d, retarded the progression rate of atherosclerosis and even resulted in an actual reduction of the mean combined far-wall IMT of the carotid and femoral arteries (−0.081 mm). Even 1 year of treatment with that dosage of simvastatin was sufficient to detect a statistically significant decrease in mean combined far-wall IMT (−0.028 mm; P=.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 treatment to lower lipid levels. 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. Conversely, the reduction of approximately 0.03 mm in 1 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 2 other studies.  The reason for this finding could be based on the fact that baseline IMT was higher in the femoral (1.88 mm) compared with the carotid artery (0.92 mm). It may also be hypothesized that a more pronounced treatment effect in the femoral artery was observed because the progression rate of IMT is higher and more sensitive to reduction of LDL-C levels. This larger regression of femoral IMT could be more clinically relevant, since the correlation between IMT and CVD in our study was better in the femoral compared with 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 levels. This finding is in contrast to those in the ASAP study, but similar to those of the Carotid Atherosclerosis Italian Ultrasound Study (CAIUS). The reason for this finding could lie in the narrower changes in LDL-C levels between individuals in our study, since monotherapy was used similarly to that in the CAIUS and in contrast to that in the ASAP study, in which 2 different statins were used at different dosages (simvastatin, 40 mg/d, vs atorvastatin, 80 mg/d).

Finally, in our study, a statistically significant difference was observed for the effect of antihypertension treatment (calcium antagonists, β-blockers, and angiotensin-converting enzyme inhibitors) on the change in mean combined far-wall IMT. Again, a larger decrease in IMT was found in the femoral artery of patients receiving antihypertensive treatment compared with patients receiving none (−0.699 vs −0.179 mm; P=.006).

Some other studies have also shown a treatment effect of a calcium antagonist or an angiotensin-converting enzyme inhibitor on carotid-artery IMT, as summarized by Simon et al. Recently, Wiklund et al reported an additional treatment effect of the β-blocker metoprolol suc-

### Table 2. Mean Combined Carotid and Femoral Far-Wall IMT at Baseline and After 2 Years of Therapy*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline Mean IMT (95% CI)</th>
<th>End of Study Mean IMT (95% CI)</th>
<th>Change in Mean IMT (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined far walls</td>
<td>1.07 (1.05 to 1.09)</td>
<td>0.99 (0.97 to 1.01)</td>
<td>−0.081 (−0.109 to −0.053)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Carotid artery far wall</td>
<td>0.92 (0.91 to 0.94)</td>
<td>0.87 (0.85 to 0.89)</td>
<td>−0.053 (−0.075 to −0.031)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Femoral artery far wall</td>
<td>1.88 (1.80 to 1.96)</td>
<td>1.60 (1.51 to 1.68)</td>
<td>−0.283 (−0.401 to −0.166)</td>
<td>&lt;.001</td>
</tr>
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Abbreviations: CI, confidence interval; IMT, intima-media thickness.

*Estimated marginal means were reported according to the mixed model.

Mean intima-media thickness (IMT) (with 95% confidence intervals) of the combined carotid and femoral far walls at baseline and after 1 and 2 years of treatment with simvastatin, 80 mg/d. After 1 and 2 years, mean IMT was significantly reduced (P=.048 and P<.001, respectively). The largest differences between those with and without CVD were observed in the femoral artery (mean±SD, 2.31±0.81 vs 1.72±0.63 mm; P<.001), as was reported previously. These data indicate that we have recruited a large and representative cohort of FH patients with baseline IMT characteristics that are very similar to those of 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/y in adult FH patients compared with 0.005 mm/y in controls. In another study, we have estimated a progression rate of 0.006 mm/y in children with FH vs 0.001 mm/y in unaffected controls. The expected progression rate in the current study was 0.008 mm/y in children with FH vs 0.001 mm/y in children with CHD. 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/y in adult FH patients compared with 0.005 mm/y in controls. In another study, we have estimated a progression rate of 0.006 mm/y in children with FH vs 0.001 mm/y in unaffected controls. The expected progression rate in the current study was 0.008 mm/y, 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 variables such as age, blood pressure, levels of lipids and lipoproteins, and smoking are also in line with previously reported data in FH patients. To the best of our knowledge, the correlation between BMI and IMT has not been observed in FH patients but only in healthy controls. These results support the notion that other risk factors besides LDL-C level also significantly contribute to the severity of atherosclerosis in FH patients.
Previous studies have shown that treatment to lower lipid levels can slow the progression rate of atherosclerosis. 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 thirds 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 receiving simvastatin alone. These effects of simvastatin on the arterial wall occurred in the light of an excellent tolerability and safety profile.

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