Determinants of Variable Response to Statin Treatment in Patients With Refractory Familial Hypercholesterolemia

Frans H. O’Neill, Dilip D. Patel, Brian L. Knight, Clare K.Y. Neuwirth, Mafalda Bourbon, Anne K. Soutar, Graham W. Taylor, Gilbert R. Thompson, Rossitza P. Naoumova

Abstract—Interindividual variability in low density lipoprotein (LDL) cholesterol (LDL-C) response during treatment with statins is well documented but poorly understood. To investigate potential metabolic and genetic determinants of statin responsiveness, 19 patients with refractory heterozygous familial hypercholesterolemia were sequentially treated with placebo, atorvastatin (10 mg/d), bile acid sequestrant, and the 2 combined, each for 4 weeks. Levels of LDL-C, mevalonic acid (MVA), 7-α-OH-4-cholesten-3-one, and leukocyte LDL receptor and hydroxymethylglutaryl coenzyme A reductase mRNA were determined after each treatment period. Atorvastatin (10 mg/d) reduced LDL-C by an overall mean of 32.5%. Above-average responders (ΔLDL-C < −39.5%) had higher basal MVA levels (34.4±6.1 μmol/L) than did below-average responders (ΔLDL-C < −23.6%, P<0.02; basal MVA 26.3±6.1 μmol/L, P<0.01). Fewer good responders compared with the poor responders had an apolipoprotein E4 allele (3 of 11 versus 6 of 8, respectively; P<0.05). There were no baseline differences between them in 7-α-OH-4-cholesten-3-one, hydroxymethylglutaryl coenzyme A reductase mRNA, or LDL receptor mRNA, but the latter increased in the good responders on combination therapy (P<0.05). Severe mutations were not more common in poor than in good responders. We conclude that poor responders to statins have a low basal rate of cholesterol synthesis that may be secondary to a genetically determined increase in cholesterol absorption, possibly mediated by apolipoprotein E4. If so, statin responsiveness could be enhanced by reducing dietary cholesterol intake or inhibiting absorption. (Arterioscler Thromb Vasc Biol. 2001;21:832-837.)

Key Words: familial hypercholesterolemia ■ statins ■ apolipoprotein E ■ cholesterol ■ hydroxymethylglutaryl coenzyme A reductase

Familial hypercholesterolemia (FH) is caused by mutations in the gene encoding the LDL receptor, resulting in either deficient gene expression or the expression of defective LDL receptors. The lack of functioning LDL receptors results in decreased catabolism of LDL and its consequent accumulation in plasma. In homozygous1 and heterozygous2 individuals, hypercholesterolemia is more severe with mutations causing null alleles than with mutations resulting in defective receptors.

Hydroxymethylglutaryl coenzyme A (HMG CoA) reductase catalyzes the rate-limiting step in cholesterol synthesis, namely, the conversion of HMG CoA to mevalonic acid (MVA), and its activity is rapidly regulated at the transcriptional, translational, and protein levels.3 HMG CoA reductase inhibitors (statins) have been very effective in treating FH, but we4 and others5,6 have reported large variations in interindividual plasma cholesterol responses to statins, irrespective of the statin and dose used. Whether the nature of the LDL receptor mutation influences the degree of cholesterol lowering achieved by statins is controversial, with evidence for5,7,8 and against6,9–12 this hypothesis. However, the extent of variability documented by Karayan et al6 in >100 patients, all with the same mutation, as well as in non-FH subjects without LDL receptor mutations suggests that other factors play a major role in determining the response to statins.

One potential determinant of statin responsiveness is the apoE genotype. Data in FH13 and non-FH14 subjects suggest that possession of an e4 allele results in a lesser reduction in LDL cholesterol (LDL-C) than is seen in those with e2 or e3 alleles, although this was not confirmed in other reports.15–17

Previously, we showed that statin responsiveness was associated with the rate of cholesterol synthesis before treatment.4 Similar findings have been reported by Miettinen et al.18 Other factors that might influence the interindividual response to statins are whether these drugs differentially affect bile acid synthesis and any variability in the rate at which they are metabolized by the cytochrome P-450 pathway.

The object of the present study was to further elucidate the mechanisms underlying the large variations observed in plasma cholesterol response when FH patients are treated with statins.
TABLE 1. Characteristics of 21 FH Heterozygotes at Recruitment

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD age, y</td>
<td>51.4±9.4</td>
</tr>
<tr>
<td>Male/female, n/n</td>
<td>15/6</td>
</tr>
<tr>
<td>White/Asian, n/n</td>
<td>19/2</td>
</tr>
<tr>
<td>Tendon xanthomas, n</td>
<td>13</td>
</tr>
<tr>
<td>CHD, n</td>
<td>13</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>6.85±1.43*</td>
</tr>
</tbody>
</table>

CHD indicates coronary heart disease.

*LDL-C at recruitment on maximal lipid-lowering therapy as described in text.

Methods

Subjects

Twenty-one subjects with refractory heterozygous FH attending the lipid clinic at Hammersmith Hospital, London, were enrolled in the present study, 19 of whom completed it. Being refractory to treatment was defined as having an LDL-C level of ≥5 mmol/L despite taking simvastatin (40 mg/d) plus at least 1 other drug, ie, cholesteramine, bezafibrate, or nicotinic acid. All patients had definite FH according to established criteria,19 including tendon xanthomas in the patient or a first-degree relative. This diagnosis was later confirmed by mutational analysis in all but 3 instances, with 1 instance proving to be a case of familial defective apol-100 (FDB).

Details of the subjects are summarized in Table 1.

The present study was approved by the Research Ethics Committee of the Hammersmith Hospitals NHS Trust, and written informed consent was obtained from all participants.

Experimental Protocol

Participants in this single-blind placebo-controlled study underwent 4 sequential treatment regimens, each of 4-week duration. All maintained their habitual diet, which was low in cholesterol and saturated fat. The treatment regimens, in chronological order, were as follows: placebo, atorvastatin (10 mg/d), bile acid sequestrant (BAS, 8 to 16 g/d cholesteramine or 10 to 20 g/d colestipol), and BAS plus atorvastatin (10 mg/d). After each treatment period, blood was collected between 9:00 and 10:00 AM after an overnight fast. Samples were assayed for levels of serum total cholesterol, HDL cholesterol, LDL-C, triglycerides, plasma MVA, plasma 7-α-hydroxylase, the rate-limiting enzyme in bile acid synthesis,23–25 7-α-OHC, mononuclear leukocyte LDL receptor mRNA, and HMG CoA reductase mRNA. Compliance with treatment was verified by tablet and sachet counts.

Analysis

Plasma MVA concentration is an indicator of the activity of HMG CoA reductase.6,20,21 MVA was extracted from fasting plasma and quantified by gas chromatography/mass spectrometry, as described previously.22 Plasma 7-α-OHC is a validated marker of the activity of cholesterol-7-α-hydroxylase, the rate-limiting enzyme in bile acid synthesis.23–25 7-α-OHC was extracted from plasma and quantified by high-performance liquid chromatography as described by Axelson et al.23

Mononuclear leukocytes were isolated from whole blood by using Ficoll-Paque density gradients adapted from Boyum.24 RNA was isolated from the samples by using an RNAgents total RNA isolation kit (Promega). Before reverse transcription, the RNA was DNase-treated (DNAAse, Promega Co). Total RNA (1.8 μg) was subsequently reverse-transcribed by using random hexamers (Roche Molecular Biochemicals). Controls without reverse transcriptase were included for all samples. The cDNA obtained was used in real-time quantitative polymerase chain reactions on the ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). The following oligonucleotide sets were used for quantifying HMG CoA reductase and LDL receptor mRNA, respectively: 5′-FAM-CCGAATCTGGTTGGGCCCAGTTG-3′ and 5′-TGGGTTGCTGACATCCTTT-3′ (primers) and 5′-FAM-

Results

After a 1-month period of treatment with atorvastatin (10 mg daily), serum LDL-C levels were reduced by an average of 32.6%, with the range varying between 46.9% and 8.9%. The mean reduction in serum LDL-C was used to arbitrarily divide the subjects into 2 groups. Eleven subjects whose LDL-C decreased by an above-average value were designated as “good responders,” whereas 8 subjects with a below-average reduction were classified as “poor responders.”

Serum lipid and lipoprotein levels at the end of each treatment regimen are shown in Table 2. Retrospective analysis showed that mean LDL-C values did not differ between the good and poor responders (9.4 and 9.8 mmol/L, respectively; P≥0.1). By definition, LDL-C levels decreased more markedly in good than in poor responders (41.9% versus 23.6%, respectively), with the difference between the 2 groups being significant (P<0.02). The sex of the individual had no significant effect on LDL-C reductions.

In contrast, there were no significant differences in LDL-C between the 2 groups when they were treated with BAS or a combination of atorvastatin and BAS. Although the difference between the groups on combination therapy was not significant, there was a trend for the good responders to show a greater reduction in LDL-C (−41.9% versus −30.9% for good versus poor responders, respectively).
TABLE 2. Serum Lipids in Good and Poor Responders Throughout the Study

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol, mmol/L</th>
<th>LDL Cholesterol, mmol/L</th>
<th>HDL Cholesterol, mmol/L</th>
<th>Triglycerides,* mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Good responders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=11)</td>
<td>11.7 ±0.3</td>
<td>9.4 ±0.3</td>
<td>1.1 ±0.1</td>
<td>2.5 ±1.2</td>
</tr>
<tr>
<td>A10</td>
<td>7.7 ±0.2</td>
<td>5.7 ±0.2</td>
<td>1.2 ±0.1</td>
<td>1.8 ±0.7</td>
</tr>
<tr>
<td>BAS</td>
<td>10.5 ±0.4</td>
<td>7.9 ±0.5</td>
<td>1.1 ±0.1</td>
<td>2.9 ±1.3</td>
</tr>
<tr>
<td>A10+BAS</td>
<td>7.8 ±0.4</td>
<td>5.5 ±0.4</td>
<td>1.2 ±0.1</td>
<td>2.2 ±0.8</td>
</tr>
<tr>
<td><strong>Poor responders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=8)</td>
<td>12.0 ±0.6</td>
<td>9.8 ±0.5</td>
<td>1.1 ±0.1</td>
<td>2.1 ±1.1</td>
</tr>
<tr>
<td>A10</td>
<td>9.5 ±0.6</td>
<td>7.5 ±0.5</td>
<td>1.1 ±0.1</td>
<td>1.7 ±0.8</td>
</tr>
<tr>
<td>BAS</td>
<td>10.9 ±0.6</td>
<td>8.6 ±0.5</td>
<td>1.1 ±0.1</td>
<td>2.4 ±1.0</td>
</tr>
<tr>
<td>A10+BAS</td>
<td>8.7 ±0.6</td>
<td>6.8 ±0.5</td>
<td>1.1 ±0.1</td>
<td>1.6 ±0.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. A10 indicates atorvastatin (10 mg/d).

*Triglycerides have been logarithmically transformed, and values given are geometric mean±approximate SD.

LDL-C in both groups with each treatment regimen are graphically represented in Figure 1.

Whereas there were no changes in HDL cholesterol, triglyceride levels were decreased by atorvastatin and increased by BAS, with the latter effect being largely eliminated when the 2 drugs were given in combination. The magnitude of these changes was similar in good and poor responders (Table 2).

As shown in Table 3, the mean MVA level in the good responders on placebo was 34.4 μmol/L, whereas in the poor responders, the mean was 26.3 μmol/L. The difference in these mean baseline MVA levels is statistically significant ($P<0.01$). On atorvastatin (10 mg/d), the mean MVA levels decreased to 22.3 μmol/L in both groups. Differences between MVA levels in the good and poor responders on BAS and the combination of BAS and atorvastatin (10 mg/d) therapy were not statistically significant.

No significant changes from baseline values were detected in 7-α-OHC levels after treatment with atorvastatin (10 mg/d) for 1 month. As expected, treatment with BAS substantially increased 7-α-OHC levels in both groups. Compared with BAS treatment alone, coadministration of atorvastatin (10 mg/d) with BAS reduced the levels of 7-α-OHC by 56% in poor responders. In comparison, the good responders showed a decrease of only 8% on combination therapy, so that both groups ended up with similar values (Table 3). Statistical analysis showed no significant difference in 7-α-OHC between the 2 groups after any of the 3 treatment regimens.

No statistically significant changes were detected in the relative LDL receptor and HMG CoA reductase mRNA levels in mononuclear leukocytes under any of the treatment regimens with the exception of LDL receptor mRNA on the statin/resin combination. Despite substantial variability, the good responders had a significant increase (44%, $P=0.05$) in their level of LDL receptor mRNA on the statin/resin combination compared with baseline values.

LDL receptor mutations were identified in all subjects, apart from 3 poor responders, 1 of whom was shown to have FDB. No mutation was detected in the other 2 subjects. As shown in Figure 2, mild, severe, and EGF domain mutations of the LDL receptor were distributed evenly among good and poor responders. A severe mutation is defined as one in which the mutant gene would produce little or no functional LDL receptor protein, whereas single amino acid substitutions in regions of the LDL receptor gene that would not be expected to have a major effect on LDL receptor function were classified as mild mutations. Mutations in the EGF precursor domain cannot be classified as either mild or severe on the basis of amino acid substitution; thus, these were assigned as a separate group.

TABLE 3. Plasma MVA and 7-α-OHC in Good and Poor Responders After Each of the 4 Treatment Regimens of the Study

<table>
<thead>
<tr>
<th></th>
<th>MVA, μmol/L</th>
<th>7-α-OHC, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Good responders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=11)</td>
<td>34.4±6.1</td>
<td>87.4±69.9</td>
</tr>
<tr>
<td>A10</td>
<td>22.3±7.4</td>
<td>72.4±39.9</td>
</tr>
<tr>
<td>BAS</td>
<td>58.1±22.9</td>
<td>217.2±259.6</td>
</tr>
<tr>
<td>A10+BAS</td>
<td>29.0±14.2</td>
<td>159.7±177.2</td>
</tr>
<tr>
<td><strong>Poor responders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=8)</td>
<td>26.3±6.1</td>
<td>87.4±52.4</td>
</tr>
<tr>
<td>A10</td>
<td>22.3±5.4</td>
<td>112.3±97.3</td>
</tr>
<tr>
<td>BAS</td>
<td>54.7±13.5</td>
<td>497.7±376.9</td>
</tr>
<tr>
<td>A10+BAS</td>
<td>25.0±8.8</td>
<td>169.7±192.2</td>
</tr>
</tbody>
</table>

Because the values were not normally distributed, the values given are the geometric mean±approximate SDs.
Possession of an e4 allele (E3/4 and E4/4) was significantly more frequent among poor responders (6 of 8) than among good responders (3 of 11, P<0.05). If the subjects are grouped according to whether they had an e4 allele or an e2 or 3 allele (E2/3, E2/4, and E3/3), the latter showed a significantly greater (P≤0.02) reduction in LDL-C after the administration of atorvastatin (Figure 3).

The ratio of urinary 6-β-hydroxycortisol to free cortisol did not differ significantly between good and poor responders at baseline (7.9±0.8 versus 8.5±1.9, respectively).

**Discussion**

These results confirm and extend our previous findings in FH patients in South Africa treated with high doses of atorvastatin, simvastatin, or pravastatin, which showed that compared with good responders, poor responders had a lower basal level and a smaller decrease of plasma MVA on statins. In the present study of British patients with refractory FH treated with low-dose atorvastatin, the spectrum of LDL receptor mutations differed from those in the South Africans, most of whom had the Afrikaner 1 or 2 mutations. However, in neither study was the extent of reduction in LDL-C on treatment attributable to the underlying LDL receptor defect, inasmuch as mild and severe mutations were evenly distributed among good and poor responders, as has been shown in several previous studies. That mild and severe mutations were evenly distributed can be deduced from the observation that baseline LDL-C levels were similar in good and poor responders. However, our group of patients was too small for any definite conclusion to be drawn about the impact of the severity of the LDL receptor mutation on therapeutic response.

We and others have justified the use of fasting plasma MVA as an index of HMG CoA reductase activity and, thus, of cholesterol synthesis. Hence, our results imply that patients in whom basal HMG CoA reductase activity is downregulated and whose rate of cholesterol synthesis is therefore low respond less well to statins than do patients with a higher rate of cholesterol synthesis. A possible explanation for these findings stems from the analysis of apoE phenotypes, which showed a significantly higher prevalence of the e4 allele among poor responders and of the e2 and e3 alleles among good responders. Subjects with an e4 allele had a significantly smaller decrease in LDL-C on atorvastatin than did those with e2 or e3 alleles. Ojala et al also observed that 6 weeks of treatment with 40 mg/d lovastatin resulted in a smaller reduction in total cholesterol in FH subjects with an E3/4 phenotype compared with those with an E3/3 phenotype (22.6% versus 27.4%, P=0.023), although this difference was no longer significant after 12 weeks of treatment.

A possible mechanism whereby apoE polymorphism might determine the response to statins comes from the work of Kesaniemi et al, who measured cholesterol absorption and synthesis in a group of middle-aged Finns. Those with an e2 allele (E2/2, E2/3, or E2/4) absorbed less and synthesized more cholesterol than did those with an e3 allele (E3/3), whereas those with an e4 allele (E3/4 or E4/4) absorbed the most and synthesized the least. In another Finnish study, compared with those with an e3 allele, subjects with an e4 allele showed a more marked increase in LDL-C after switching from a low cholesterol to a high cholesterol diet, implying greater absorption of dietary cholesterol. Because the prevalence of an e4 allele in the present study was significantly higher among the poor responders (75%) than the good responders (27%), we hypothesize that poor responders have a higher efficiency of cholesterol absorption. Higher absorption efficiency is equated with a higher uptake of chylomicron remnant cholesterol by the liver, which, in turn, will downregulate HMG CoA reductase activity because the enzyme is subject to feedback regulation by cholesterol. This is in accord with the MVA data, which indicate that the HMG CoA reductase is more downregulated at baseline in the poor responders than in the good responders. We conclude that in the poor responders, a genetically determined increase in cholesterol absorption downregulates HMG CoA reductase, as supported by the data of Miettinen et al, and renders the enzyme refractory to pharmacological inhibition. Although the lesser decrease in LDL-C of poor responders might be ascribed to poor compliance, this would not explain why their pretreatment MVA levels were significantly lower than those of good responders.

How can we reconcile the marked changes in plasma LDL and MVA on the various treatment regimens with the relative lack of change in mononuclear leukocytes? Previous studies have shown good correlation between mRNA levels of HMG

**Figure 2.** LDL-C reductions of subjects grouped according to the different mutations detected. Mild indicates mutations of the LDL receptor gene that reduce LDL receptor expression or function; severe, mutations of the LDL receptor gene that lead to nonfunctioning LDL receptors or no LDL receptors being formed; EGF-domain, mutations detected in the EGF domain; ND, no mutations detected; open triangle, good responders; and solid circle, poor responders.

**Figure 3.** Reduction in LDL-C after 1 month of atorvastatin (10 mg/d) according to apoE phenotype. Bars represent the mean reduction in each group. e4 indicates E3/4 and E4/4 phenotypes (solid circle); e2,3, E2/2, E2/3, E2/4, and E3/3 phenotypes (open circle).
CoA reductase and LDL receptor in the liver and in mononuclear leukocytes.\textsuperscript{34} Contrary to these findings, the present findings demonstrated no significant changes in mononuclear leukocyte mRNA apart from a rise in LDL receptor mRNA levels in the good responders during combined treatment with atorvastatin and BAS. An increase in LDL degradation in vitro, indicative of increased expression of LDL receptors, has previously been documented in mononuclear leukocytes obtained from FH heterozygotes treated with a BAS.\textsuperscript{35} The apparent lack of response of mononuclear leukocyte HMG CoA reductase mRNA to inhibition (statin) and stimulation (BAS) in the present study could be ascribed to various causes. One difference between the present study and that of Powell and Kroon\textsuperscript{36} is that we used FH patients, whereas they studied non-FH individuals. Alternatively, the lack of significant change in HMG CoA reductase mRNA in the mononuclear leukocytes could reflect the extent to which atorvastatin is sequestered by the liver, resulting in the absence of any inhibitory activity in plasma on the morning after the dose of the previous night, as documented for lovastatin.\textsuperscript{36} Thus, plasma MVA seems to be a better index of changes in hepatic cholesterol synthesis during lipid-lowering drug therapy than is leucocyte HMG CoA reductase mRNA, at least in FH patients.

Studies by Stein and colleagues\textsuperscript{37,38} suggested that LDL-C reductions with simvastatin in patients with raised cholesterol and triglycerides were less than those seen in patients with hypercholesterolaemia alone. However, in our FH patients, the difference between baseline triglyceride levels in the good and poor responders (2.5 versus 2.1 mmol/L) was statistically insignificant and cannot be invoked as the explanation for their variable response to statins.

Measurement of 7-\textalpha-OHC was undertaken to ascertain whether statins could induce a decrease in bile acid synthesis by limiting the availability of cholesterol, the substrate for cholesterol synthesis by therapeutic upregulation.\textsuperscript{39} The levels of 7-\textalpha-OHC, a well-authenticated marker of bile acid synthesis, did not differ significantly between the good and poor responders on atorvastatin; hence, a differential effect on bile acid synthesis cannot explain the variation in LDL response nor the differences in MVA levels. The increase in 7-\textalpha-OHC after BAS was more marked in the poor than in the good responders; this difference disappeared when they were treated with atorvastatin and BAS in combination.

Finally, we could find no difference between the 2 groups in terms of CYP3A4 activity, which suggests that the rate of metabolism of atorvastatin did not differ significantly between good and poor responders. Thus, of the various factors we investigated, only a low basal rate of cholesterol synthesis differentiated the poor from the good responders. We suggest that upregulation of cholesterol synthesis by therapeutic inhibition of cholesterol absorption might enhance their response to statins.

References


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