Apolipoprotein E Polymorphism and Plasma Cholesterol Response to Probucol

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Probucol has been shown to be an effective and well-tolerated cholesterol-lowering drug. However, response in terms of cholesterol reduction has been shown to vary significantly among individuals. The purpose of this study was to assess the role of apolipoprotein E polymorphism in determining this variation. A retrospective study of 89 hypercholesterolemic type II patients who had been treated with probucol (1 g/d) and for whom the apolipoprotein E phenotype was known was carried out. The patients were first grouped into those with heterozygous familial hypercholesterolemia (FH) and those considered to have other forms of hypercholesterolemia (non-FH). Further subclassification of the individuals in both groups as Ila or Ilb, allowed the definition of four diagnostic classes, FH Ila or Ilb and non-FH Ila or Ilb. Among these classes there was no significant heterogeneity for the relationship between response and age or sex. After correction for between-class heterogeneity in duration of probucol treatment, comparison of individuals with the apo E3/3 phenotype with those carrying the e4 allele showed significant differences in cholesterol reduction both absolute change and percent change. Further contrasts between diagnostic and apo E genotype stratifications of these data showed that the FH patients carrying the e4 allele had the greatest reduction in cholesterol level.

MATERIALS AND METHODS

Subjects

The clinical records of 131 hypercholesterolemic type II patients of our lipid clinic who had been given probucol were surveyed. Having satisfied the following criteria, 89 were entered into this study: the patients had high plasma cholesterol levels and LDL-C > 190 mg/dL, probucol (1g/d) was the only lipid-lowering drug administered and there were no cases with confounding secondary factors such as oral synthetic hormone replacement (estrogens or thyroxin), ileal bypass, or diabetes. A stable baseline period with the patients taking no lipid-lowering drugs but on a prescribed diet, appropriate to the hyperlipidemia phenotype, was chosen to include the three or four cholesterol measurements that immediately preceded the probucol period. In ten cases, however, because other therapeutic regimens had been tried in the interval, this was not available and the baseline had been obtained 2.7 ± 1.0 years previously. In five of these cases, however, one or two cholesterol values were available prior to the administration of probucol which were very similar to the baseline obtained earlier, or with cessation of probucol, cholesterol levels rose to the range of the previously obtained baseline. There was no clustering of these ten subjects in any of the diagnostic or genotype subgroups: 5 FH, 5 non-FH with almost equal distribution of Ila and Ilb classifications. Seven subjects had the E3/3 phenotype and the three apo E4/3 individuals were in the group of subjects for whom at least one cholesterol observation was available just prior to institution of probucol treatment. Further, cholesterol response to probucol was not extreme in these ten subjects; all showed moderate reductions. The baseline averaged 14 ± 6 weeks. In order to have enough determinations to assess the effect of probucol, the period of observation was selected as the first five or six cholesterol measurements after institution of the drug and this averaged 28 ± 12 weeks. Dietary therapy.

continued during the probucol period and was monitored throughout by regular consultations with a dietitian and as a routine, by body weight fluctuation.

The diagnosis of familial hypercholesterolemia (FH) was confirmed on the basis of raised cholesterol levels associated with the presence of tendon xanthomas (X), which in doubtful cases had been confirmed by radiologic examination, or a positive family history for premature coronary heart disease and tendon xanthomas in at least one first-degree relative. Although many of the type II patients had been classified as familial combined hyperlipidemia (FCH) due to the presence of multiple hyperlipidemic phenotypes within the same family, this information was not available for all patients, and therefore, this non-FH group included FCH and possibly polygenic hypercholesterolemia. Both FH and non-FH type II were subclassified as Ila or IIb on the basis of baseline plasma triglyceride:cholesterol ratios of <0.4 and >0.4, respectively. This resulted in four diagnostic classes: IlaX and IIbX for FH and Ila and IIb for non-FH. The clinical features of the patients and the study periods are summarized in Table 1.

All blood samples were obtained after 12 to 14 hours of fasting and drawn into Vacutainer tubes containing EDTA (1 mg/mL). Plasma were stored at 4°C until analysis within three to four days. As previously described, plasma and lipoprotein lipids were measured using an autoanalyser (ABA-100, Abbott Laboratories) after Lipid Research Clinic methods and apolipoprotein E phenotypes were determined after isoelectric focusing of VLDL separated and washed by ultracentrifugation.

Statistical Methods

The average levels of cholesterol during the baseline period and the period while the patient was on probucol were the primary variables considered. Multiple linear regression was used to investigate the contribution of age at the beginning of the probucol treatment, number of weeks on baseline, weeks on probucol, number of times cholesterol was measured during the baseline and number of measurements during the probucol period to interindividual variation in these primary variables. The one-way ANOVA was used to test for significant differences in the average value of a trait among diagnostic classes and apoE genotypes. Statistical tests were judged significant at the .05 level of probability.

Table 2. Average Age and Weeks on Baseline for Each of the Four Subgroups of Patients

<table>
<thead>
<tr>
<th>Hyperlipidemia</th>
<th>n (Males)</th>
<th>Apo E Phenotype (n)</th>
<th>Study Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>89 (36)</td>
<td>E4/4 (3)</td>
<td></td>
</tr>
<tr>
<td>FH Ila</td>
<td>34 (11)</td>
<td>2/3 (67)</td>
<td></td>
</tr>
<tr>
<td>FH IIb</td>
<td>16 (9)</td>
<td>2/2 (1)</td>
<td></td>
</tr>
<tr>
<td>Ila</td>
<td>12 (1)</td>
<td>3/2 (6)</td>
<td></td>
</tr>
<tr>
<td>IIb</td>
<td>27 (15)</td>
<td>4/2 (2)</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± SD.

Table 3. Regression Coefficients for the Relationship Between the Dependent Variables, Mean Baseline (B) Cholesterol, and Mean Probucol (PB) Cholesterol, and Concomitant Variation in Age, Weeks of Treatment, and Number of Observations During Each Period

<table>
<thead>
<tr>
<th>Concomitant</th>
<th>Baseline Cholesterol</th>
<th>Probucol Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.22</td>
<td>0.20</td>
</tr>
<tr>
<td>Weeks B</td>
<td>2.97*</td>
<td>2.06</td>
</tr>
<tr>
<td>Samples B (n)</td>
<td>-14.37</td>
<td>-6.25</td>
</tr>
<tr>
<td>Weeks PB</td>
<td>2.45*</td>
<td>1.01</td>
</tr>
<tr>
<td>Samples PB (n)</td>
<td>5.68</td>
<td>1.05</td>
</tr>
<tr>
<td>R²</td>
<td>0.03*</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Significantly different from zero at the .01 level of probability.

RESULTS

We began our analyses of these data by considering the distribution among the diagnostic classes and apo E genotypes of age at the beginning of the probucol treatment, weeks on baseline, number of measurements during the baseline period, weeks on probucol, and the number of measurements during the probucol period. We found no statistically significant differences among genotypes for the average of any of these concomitants. However, the average age of the patients and weeks on baseline varied significantly among the four diagnostic classes. Table 2 summarizes these comparisons.

The comparison of the average age of patients with FH (44.2 years) with that of the non-FH patients (52.1 years) explains the major fraction of the variability among diagnostic classes. The non-FH individuals were followed significantly longer (4 more weeks) during the baseline period than those with FH. This difference in length of time, however, was not associated with a significant difference in the number of measurements during the baseline.

We next turned to determining whether these concomitants were associated with individual average cholesterol levels during baseline or the probucol periods. Twenty-one percent (P < .001) of the interindividual variability in average cholesterol for the baseline period and 8% (P > .10) for the probucol period were associated with variability in these five concomitants. As summarized in Table 3, a stepwise regression established that weeks on baseline and weeks on probucol explain a major fraction of the variability in baseline cholesterol, R² = 17.6. Although the effect of these predictors on the average cholesterol level during the probucol period was not statistically significant, the longer that the patient was followed the lower the average cholesterol.
Table 4. A Description of the Cholesterol Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Range of Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw unadjusted data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>363.47</td>
<td>74.75</td>
<td>229-598</td>
</tr>
<tr>
<td>Probucol</td>
<td>313.01</td>
<td>65.81</td>
<td>195-520</td>
</tr>
<tr>
<td>Change</td>
<td>-50.46</td>
<td>41.65</td>
<td>71-144</td>
</tr>
<tr>
<td>% Change</td>
<td>-13.39</td>
<td>11.00</td>
<td>24.5-39.0</td>
</tr>
<tr>
<td>Adjusted data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>363.47</td>
<td>67.85</td>
<td>206-577</td>
</tr>
<tr>
<td>Probucol</td>
<td>313.01</td>
<td>63.38</td>
<td>185-489</td>
</tr>
<tr>
<td>Change</td>
<td>-50.46</td>
<td>39.20</td>
<td>61-138</td>
</tr>
<tr>
<td>% Change</td>
<td>-13.52</td>
<td>10.34</td>
<td>20.2-38.3</td>
</tr>
</tbody>
</table>

The change variable - the average level during the baseline - the average level during the probucol treatment period.

The average cholesterol level in both periods. There was no evidence for significant heterogeneity (at .10 level of probability) among diagnostic classes or among the apoE genotype classes for either of the regression equations given in Table 3.

We next adjusted the individual average cholesterol levels during the baseline and during the probucol periods each separately for weeks on baseline and weeks on probucol. Table 4 presents a description of the unadjusted and adjusted data that will be considered below for the analysis of the effects of diagnosis and apolipoprotein E genotype on response to probucol. After adjustment, the average values do not change but the range is narrowed and there are small reductions in the standard deviations of each of the four variables. A paired t test of the average of the difference between individual mean cholesterol levels during baseline and probucol was significant at the .001 level of probability.

All subjects did not respond to probucol with a drop in cholesterol. Although 81 patients showed an average decrease of 58 mg/dL (adjusted data, 14.6% change), the mean plasma cholesterol increased in eight. These records were surveyed in detail. All patients showed increases in cholesterol and LDL-C and the baseline had been obtained immediately before the probucol period. Seven of these resistant patients have the E3/3 genotype (four FH type IIa, one IIa non-FH and two FH IIb). The other resistant individual is E3/2 and type IIa FH.

For comparison of the adjusted data by genotype and diagnostic classification, the one E2/2 and six E3/2 individuals were not considered because of small sample sizes. We include here only the contrast of the most frequent E3/3 genotypic class (n = 57) with the pooled class of genotypes carrying the e4 allele (E4/2 + E4/3 + E4/4, n = 25). There were highly significant differences among the diagnostic classes at both baseline and during the probucol treatment. For the baseline period, patients with FH (IIaX and IIbX) had a significantly higher (P < .001) average cholesterol (388 ± 61 mg/dL) than the non-FH group (329 ± 58 mg/dL). The differences between the mean IIaX v IIbX and IIa v IIb, during the baseline period or during the probucol treatment, were not statistically significant. Despite the differences in baseline levels, the average response of the FH group (−51 ± 39 mg/dL) was not significantly different from the average response of the non-FH group (−49 ± 37 mg/dL), −12.9 and −15.2%, respectively.

In considering response by genotype, however, although the average baseline cholesterol level of the E4- genotypes was not significantly different than the average of the E3/3 genotypes, there was a highly significant difference between genotypes on probucol. This effect is reflected in a significantly larger response for the E4- (−61 ± 36 mg/dL, −18%) than the E3/3- genotypes (−46 ± 39 mg/dL, −12%). Furthermore, as shown in Fig 1, this response is dependent on the diagnostic classification. For the patients with presumably polygenic hypercholesterolemia and familial combined hypercholesterolemia, ie non-FH, the response to probucol was not significantly different between genotypes, the E3/3 and E4- groups showing similar reductions, −47 and −54 mg/dL change, −14% and −16% respectively. P > .50. In contrast, for those with FH, the drop in cholesterol was significantly greater in those subjects with the E4 allele (−70 ± 39 mg/dL, −18%) than in those with the E3/3 genotype (−45 ± 39 mg/dL, −11%), P < .03.

**DISCUSSION**

This retrospective study confirms and extends the observations of previous trials in which probucol was shown to be a moderately effective cholesterol lowering agent in diet-resistant hyperlipidemia type II with significant inter-patient variability in response. In an attempt to investigate
genetic factors which may play a role in this variability, we first subdivided the patients into two groups; those with clearly defined familial hypercholesterolemia and those with FCH or hypercholesterolemia of undefined polygenic etiology, or non-FH. The FH patients were found to be younger and were followed an average of 4 weeks less during the baseline period. Both groups were found to have significantly elevated cholesterol levels at baseline, and notwithstanding the higher baseline cholesterol levels of the FH group during baseline, both FH and non-FH groups responded to probucol with an average drop of approximately 50 mg/dL, again with significant interpatient variability and with no differences between the IIa or IIb sub classifications. For the eight patients whose cholesterol and LDL-C levels showed an average rise with probucol, five had been diagnosed as FH IIa, two as FH IIb, and one as FH non FH.

When response was considered in terms of apo E genotype, however, the genotypes with the e4 allele were found to have a significantly greater reduction in cholesterol level than the E3/3 genotype. Furthermore, when diagnostic classification was considered in addition to apo E genotype, this effect was shown to be due to the significantly greater response of the FH patients having the e4 allele; E3/3 FH, non-FH patients with the e4 allele and the non-FH E3/3 patients all responding with smaller reductions in cholesterol level.

This study was not designed to investigate changes in cholesterol associated with the individual lipoprotein classes. However, in view of the lowering effect of probucol on both LDL and HDL cholesterol,26-27 we were interested in the contribution of potential reductions in HDL-C to the observed overall changes in plasma cholesterol levels. We were able to obtain one or two lipoprotein cholesterol profiles in both the baseline and probucol periods for 30 of the 82 subjects whose cholesterol values were considered in the contrasts of genotype by diagnostic classification: in the FH group, 11/35 E3/3 and 5/11 E4/-, and in the non-FH group, 8/22 E3/3 and 6/14 E4/- individuals. The initial HDL-C levels for all subjects were low and the changes induced chemical modifications of LDL which result in an altered lipoprotein conformation is crucial in determining lipoprotein metabolism. That the non-FH individuals did not respond as well can be explained by the fact that the probucol enhances the metabolism of LDL by mechanisms independent of the native or modified-LDL receptor. Apolipoprotein E is a major determinant responsible for mediating the high affinity binding of lipoproteins to the LDL receptor as well as the hepatic apo E receptor.28 The major isoforms are known to differ in their ability to interact with these receptors; the E2 form and various E2 mutants having as little as 2% of the normal E3 binding which results in varying degrees of decreased remnant catabolism. The E4 isoform has equivalent binding to E3; the structural modification of an additional positively charged residue in E4 at site 112 having no apparent effect on binding.28 Recently, however, it has been reported that E4 VLDL is catalyzed more rapidly in E3/3 individuals and independently of the LDL receptor.29 Thus, taken with the probucol induced chemical modifications of LDL which result in an altered and enhanced LDL catabolism, it is possible that an additionally enhanced catabolism of VLDL, both processes being independent of the LDL receptor, explains the greater response of FH-e4 carrying individuals in the present study. A presumption being that in the face of defective receptors (FH), the role of apo E possibly as a determinant of lipoprotein metabolism that the non-FH individuals did not respond as well can be explained by the fact that the underlying genetic etiology of their disease involves many gene loci rather than a monogenic receptor defect. The polygenic nature of the non-FH hypercholesterolemia likely masks the apo E response that is observed in FH patients. The use of probucol as a probe provides further evidence for the role of genetic factors in determining the homeostasis that establishes individual serum cholesterol levels. Genetic factors must be considered in our efforts to understand the mechanism(s) of action of probucol and in our selection of intervention strategies to lower an individual's cholesterol level.
REFERENCES


38. Bradley WA, Gianiturco SH: Apo E is necessary and sufficient for the binding of large triglyceride-rich lipoproteins to the LDL receptor; apo B is unnecessary. J Lipid Res 27:40-48, 1986