Intensive Combination Drug Therapy of Familial Hypercholesterolemia With Lovastatin, Probucol, and Colestipol Hydrochloride

Joseph L. Witztum, MD, Debra Simmons, MD, Daniel Steinberg, MD, PhD,
William F. Beltz, PhD, Robert Weinreb, MD, Stephen G. Young, MD,
Peggy Lester, RN, Nancy Kelly, RN, and Joseph Juliano, BA

Patients with familial hypercholesterolemia (FH) have had a life-long sustained elevation of low-density lipoprotein (LDL) cholesterol levels. Consequently, there is a need to maximally lower their elevated levels, and this usually requires lowering LDL levels more than 50%. Because no single hypolipidemic drug will consistently produce such degrees of lowering, combination drug therapy with two or even three agents is required to produce the desired degree of cholesterol lowering. A prospective trial was designed to determine if combination therapy using three hypolipidemic agents could effectively lower LDL levels in 17 severely affected FH subjects. Colestipol hydrochloride (10 g b.i.d.), probucol (500 mg b.i.d.), and lovastatin (20 or 40 mg b.i.d.) were given to each patient, in varying combinations, over a 25-month period. Lovastatin (40 mg/day) uniformly lowered LDL levels 36%. Probucol lowered LDL only 14% and in a variable manner. The combination of lovastatin and probucol lowered LDL no better than lovastatin alone. Lovastatin plus colestipol lowered LDL 52%; probucol added as a third agent produced no further lowering. Lovastatin (80 mg/day) plus colestipol lowered LDL 56%. Lovastatin increased high-density lipoprotein (HDL) cholesterol levels 6%, whereas probucol decreased HDL 29%. In all patients there was an effective lowering of LDL levels, ranging from 40% to 70%. Thus, lovastatin plus colestipol is an effective hypolipidemic regimen for producing marked decreases in LDL levels in FH subjects. The addition of probucol as a third hypolipidemic agent adds little to the therapeutic regimen as measured by lowering of LDL levels. (Circulation 1989;79:16–28)

Individuals with elevated plasma levels of low-density lipoprotein (LDL) cholesterol clearly have an increased risk for developing coronary artery disease (CAD). In particular, patients with heterozygous familial hypercholesterolemia (FH) are at increased risk for CAD, as they have had a life-long sustained elevation of LDL cholesterol. Affected male heterozygotes have approximately a 50% chance of having a major coronary event by age 50, and even affected female heterozygotes incur a substantially greater risk for CAD.1 Thus, vigorous attempts to lower their elevated plasma LDL levels are indicated. In fact, one might argue that the goal of therapy in these FH patients, particularly in the adult, should be the achievement of “ideal” LDL levels, which are far below the so-called “normal” mean for the general population. Regression studies in primates,2 as well as the results of intervention trials in humans, including the recently completed Coronary Primary Prevention Trial3 and the CLAS Trial,4 clearly indicate that the beneficial effects of hypolipidemic therapy are directly related to the extent of cholesterol lowering.

From the Departments of Medicine, Division of Endocrinology and Metabolism, and Ophthalmology (R.W.), University of California, San Diego, La Jolla, California.

Supported in part by Grant HL-14197 (SCOR) from the National Heart, Lung, and Blood Institute; by Grant PHS-RR-00827 of the General Clinical Research Center; and by grants from the Merck, Sharp & Dohme Research Laboratory and the Dow Chemical Company. S.G.Y. is supported in part by a National Institutes of Health Clinical Investigator Award; W.F.B. is supported in part by a Whitaker Foundation Grant; and J.L.W. is an Established Investigator of the American Heart Association.

Address for correspondence: Joseph L. Witztum, MD, Department of Medicine, M-013D, University of California, San Diego, La Jolla, CA 92039-0613.

Received May 6, 1988; revision accepted September 12, 1988.

*All editorial decisions for this article, including selection of reviewers and the final decision, were made by a guest editor. This procedure applies to all manuscripts with authors from the University of California San Diego or UCSD Medical Center.
Plasma LDL cholesterol levels in patients with familial hypercholesterolemia are almost always well in excess of 200 mg/dl. If one accepts the premise that the goal of therapy is to lower their LDL cholesterol to levels below 130 mg/dl, then therapy in such individuals needs to lower LDL levels by more than 50%. Although occasional patients may experience 50% drops in LDL levels in response to a single agent, it is far more common to see decreases of only 25–35%. Thus, in response to bile sequestrants, LDL levels may fall 20–35%,7–10 Nicotinic acid, in the estimated 50–60% of patients who can tolerate the required doses, may produce decreases of 20–35%.11,12 Probucol will produce decreases of 10–40% in responding patients, but many patients do not respond.13 Lovastatin, at doses of 20–40 mg b.i.d. reduces LDL levels by approximately 35–43%.14–18 Thus, for many if not most patients with FH, no single drug therapy will produce the desired 50% decrease, and combination therapy will be required.

The bile acid binding resins, such as colesevelam hydrochloride, are believed to lower plasma cholesterol levels by increasing bile acid excretion. This leads to the subsequent depletion of a critical intracellular pool of hepatic cholesterol and consequent induction of hepatic LDL-receptor activity. This increased LDL-receptor activity in turn leads to increased removal of LDL and LDL precursors from plasma.19–21 Lovastatin is a hypolipidemic agent that specifically inhibits the hepatic activity of HMG coenzyme A reductase, the rate-limiting enzyme in cholesterol biosynthesis,22 and consequently leads to decreased hepatic cholesterol biosynthesis and further stimulation of hepatic LDL-receptor activity.23,24 Probucol is a highly lipophilic agent whose hypolipidemic mechanism of action in humans is uncertain. In rabbits, it appears to increase LDL removal from plasma, in part by a non–LDL receptor–mediated pathway.25 It is of particular interest because among all known hypolipidemic agents, it has been reported to be effective in reducing plasma LDL concentrations in subjects homozygous for familial hypercholesterolemia.26,27

Because these latter subjects totally lack LDL-receptor activity, it suggests that in humans probucol can lower LDL levels independently of LDL receptor activity. In theory, colestipol, lovastatin, and probucol could act synergistically to lower LDL cholesterol levels.

We now report the results of a prospective trial to determine the efficacy of lovastatin, probucol, and colestipol used in various combinations to lower plasma LDL levels in FH patients with marked hypercholesterolemia. The majority of the patients recruited for this study had previously failed to achieve acceptable LDL cholesterol levels on various combinations of bile sequestrants, nicotinic acid, or other hypolipidemic drugs. The results show that even in FH patients with severe hypercholesterolemia, marked reductions in LDL cholesterol levels can be achieved by the use of lovastatin in combination with a bile sequestrant.

### Subjects and Methods

**Human Subjects**

Seventeen patients with severe hypercholesterolemia were recruited from the Lipid Clinic of the Division of Endocrinology and Metabolism of the University of California, San Diego. These 17 subjects were selected because of severe hypercholesterolemia.

### Table 1. Clinical Characteristics of Study Subjects and Lipoprotein Values Obtained During the Control (Placebo) Period

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age/sex</th>
<th>Xanthoma/ xanthelasmas</th>
<th>CAD/PVD</th>
<th>Baseline lipoprotein values (mg/dl)</th>
<th>Total cholesterol</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>Total triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51/M</td>
<td>+</td>
<td>+</td>
<td>496</td>
<td>438</td>
<td>42</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>60/M</td>
<td>-</td>
<td>-</td>
<td>470</td>
<td>405</td>
<td>49</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>47/M</td>
<td>+</td>
<td>-</td>
<td>371</td>
<td>244</td>
<td>48</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>64/M</td>
<td>-</td>
<td>+</td>
<td>370</td>
<td>308</td>
<td>46</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>36/M</td>
<td>-</td>
<td>-</td>
<td>318</td>
<td>258</td>
<td>48</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>24/M</td>
<td>-</td>
<td>-</td>
<td>446</td>
<td>378</td>
<td>45</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>40/M</td>
<td>+</td>
<td>+</td>
<td>447</td>
<td>344</td>
<td>34</td>
<td>345</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>56/M</td>
<td>-</td>
<td>-</td>
<td>504</td>
<td>426</td>
<td>54</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>56/M</td>
<td>+</td>
<td>+</td>
<td>481</td>
<td>406</td>
<td>32</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>36/M</td>
<td>+</td>
<td>-</td>
<td>293</td>
<td>218</td>
<td>50</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>47/M</td>
<td>-</td>
<td>+</td>
<td>333</td>
<td>254</td>
<td>40</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>53/F</td>
<td>-</td>
<td>-</td>
<td>355</td>
<td>200</td>
<td>72</td>
<td>416</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>61/F</td>
<td>+</td>
<td>-</td>
<td>542</td>
<td>460</td>
<td>42</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>65/F</td>
<td>-</td>
<td>+</td>
<td>315</td>
<td>210</td>
<td>91</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>63/F</td>
<td>+</td>
<td>-</td>
<td>588</td>
<td>504</td>
<td>60</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>63/F</td>
<td>+</td>
<td>+</td>
<td>324</td>
<td>256</td>
<td>32</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; PVD, peripheral vascular disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; M, male; F, female.
terolemia and failure to achieve adequate LDL cholesterol lowering with previous hypolipidemic therapy, which most commonly included a bile sequestrant plus large doses of nicotinic acid. The control, off-therapy, lipoprotein levels and pertinent clinical characteristics of the patients are listed in Table 1. Patients were judged to have FH if they met three of the following four criteria: 1) LDL cholesterol level more than the 99th percentile for their age and sex, 2) strong family history of hypercholesterolemia, 3) strong family history of early coronary artery disease, or 4) presence of tendon xanthomata. Fourteen of the 17 patients had an unequivocal diagnosis of familial hypercholesterolemia. One subject (subject 4) had high LDL cholesterol levels and early onset of coronary artery disease but indeterminate family history. Three subjects (subjects 2, 11, and 12) had positive family histories by no xanthomas and thus were judged to probably have FH. Secondary causes of hypercholesterolemia were excluded in all subjects by standard laboratory tests. Eight patients had existing peripheral vascular disease, coronary artery disease, or were status post–coronary artery bypass graft surgery and were taking β-blockers, diuretics, or calcium channel antagonists. In these patients, the dose of these medications was established before entrance into this study and was not changed during the course of the study. All patients gave informed consent to participate in the study, which was approved by the Human Use Study Committee of the University of California, San Diego. A preliminary report of a part of this study was previously presented at a symposium.

**Study Protocol**

Before initiation of this protocol, patients discontinued all hypolipidemic agents for 3 months. All patients were already on American Heart Association Phase II or Phase III diets, and a dietary review took place during the 3 months preceding this study, with at least one follow-up visit during each of the subsequent drug periods during the remainder of the study. The study protocol can be appreciated from Figure 1. Patients came to the clinic on 30 occasions over a 25-month period. The study was divided into seven periods. Each of the first five periods was of 3 months’ duration; a sixth period lasted 6 months; and a seventh period lasted 4 months. During the first “control” period, all patients received a double placebo, one for lovastatin and one for probucol. During the second period, designated “lovastatin,” patients received lovastatin 20 mg b.i.d. and placebo for probucol. During the third period, “probucol,” patients received a placebo for lovastatin and probucol 500 mg b.i.d. During the fourth period, “lovastatin plus probucol,” patients received active lovastatin (20 mg b.i.d.) and probucol (500 mg b.i.d.). During the initial 1st year of the study, patients were blinded as to medication as well as to their plasma cholesterol results. Beginning with the fifth period, the blind was broken and patients were now maintained on lovastatin (20 mg b.i.d.) and probucol (500 mg b.i.d.), and colestipol (10 g b.i.d.) was added. During the sixth period, probucol was discontinued, and lovastatin and colestipol continued. This period was 6 months in duration to ensure that there was no carry-over hypolipidemic effect of probucol. During a seventh period, colestipol was continued,
TABLE 2. Mean Lipid and Lipoprotein Levels During Each Period of the Drug Trial

<table>
<thead>
<tr>
<th>Period</th>
<th>n</th>
<th>Total cholesterol</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>Total triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>17</td>
<td>404±98</td>
<td>325±102</td>
<td>49±15</td>
<td>154±99</td>
</tr>
<tr>
<td>2. Lovastatin</td>
<td>17</td>
<td>286±58</td>
<td>208±61</td>
<td>53±17</td>
<td>126±74</td>
</tr>
<tr>
<td>3. Probucol</td>
<td>17</td>
<td>345±89</td>
<td>280±92</td>
<td>35±10</td>
<td>148±80</td>
</tr>
<tr>
<td>4. Lovastatin and probucol</td>
<td>17</td>
<td>268±63</td>
<td>206±61</td>
<td>39±10</td>
<td>117±70</td>
</tr>
<tr>
<td>5. Lovastatin and probucol and colestipol</td>
<td>15</td>
<td>220±59</td>
<td>154±53</td>
<td>40±10</td>
<td>131±75</td>
</tr>
<tr>
<td>6. Lovastatin and colestipol</td>
<td>16</td>
<td>234±45</td>
<td>157±42</td>
<td>49±17</td>
<td>136±83</td>
</tr>
<tr>
<td>7. Lovastatin (80 mg/day) and colestipol</td>
<td>13</td>
<td>229±54</td>
<td>149±50</td>
<td>52±16</td>
<td>134±67</td>
</tr>
<tr>
<td>8. Lovastatin (80 mg/day) only</td>
<td>5</td>
<td>259±65</td>
<td>187±70</td>
<td>52±22</td>
<td>107±50</td>
</tr>
</tbody>
</table>

For each period an average lipid level was determined for each individual from two to four values obtained at monthly intervals and a mean value (±SD) computed for the group. During periods one through four, all 17 patients adhered to the study protocol. Subsequently, five subjects discontinued colestipol for varying periods and one discontinued probucol. Statistical results for the cholesterol data are given in Figures 2–4. Plasma triglyceride levels were different from control during periods two (p<0.02), four (p<0.001), and eight (p<0.05).

LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Five subjects took only lovastatin (80 mg/day) at the end of the study (period B) for at least 3 months. The mean levels of cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides (±SD) during the control period for these five subjects were 396±89, 309±100, 48±16, and 198±136 mg/dl, respectively. These values were not different from the mean baseline values of the other 12 subjects.

and the dose of lovastatin was increased to 40 mg b.i.d. It is important to emphasize that we did not randomize the order of receiving probucol and lovastatin during periods three, four, and five because probucol is known to have a long plasma half-life and to have residual effects for several months after discontinuation. On the other hand, after discontinuation of lovastatin, plasma lipoprotein levels return to baseline within 1 month. Thus, all patients received lovastatin first, then probucol, and then the combination. During period six when probucol was discontinued, the period was extended for 6 months to minimize the carry-over effect of probucol.

During the first visit (visit 0), patients were entered into the study, and a complete history and physical examination were obtained, and lipoprotein quantification and baseline screening tests (SMA 12, T4, CBC, urinalysis, and chest radiography) were performed. Ophthalmologic examination was performed during the control period and after 1 and 2 years of therapy and included best corrected visual acuity, color vision (Ishihara color plates), slit-lamp biomicroscopy, applanation tonometry, dilated fundoscopy, and visual field testing. During the entire study, subjects were seen on a monthly basis. During the first five periods, subjects were seen for an extra visit 2 weeks after change of study medication to ensure absence of toxicity of the various drug combinations. Lipoprotein quantification and chemistry screening tests for toxicity were performed at every monthly visit. Compliance to all phases of the study protocol was excellent as most of the patients in this study had been followed for many years at our Lipid Research Clinic and were highly motivated to participate in this study. All 17 patients were followed for the entire period of study. Adherence to lovastatin and probucol was monitored by pill count and in general was more than 90%. Adherence to colestipol was monitored by self-report and the amount of resin consumed (which we supplied). Overall adherence throughout the study was excellent in 12 patients. One subject (subject 17) discontinued probucol at the end of period four because of headaches. One subject (subject 9) discontinued colestipol after period five but resumed 10 g/day during the final 2 months of period seven. Four other subjects discontinued colestipol during period seven. Thus, there were five subjects who took only lovastatin 80 mg/day for 2 or more months during the course of the study (referred to as period 8 in “Discussion”). Side effects were systematically recorded at each visit and will be reported in “Results.”

Lipoprotein Quantification

Plasma was collected from each patient after an overnight fast into tubes containing EDTA (1 mg/ml) and promptly refrigerated. Plasma was separated within several hours and used within 3 days for lipoprotein quantification. Total cholesterol and triglycerides were measured by enzymatic techniques with an ABA-200 biochromatographic analyzer (Abbott Laboratories, Irving, Texas), high performance cholesterol reagent (No 236691, Boehringer-Mannheim Diagnostics, Indianapolis, Indiana), and triglyceride agent (No 6097, Abbott Laboratories). LDL and HDL cholesterol was measured according to the standardized procedures of the Lipid Research Clinic Manual. Our laboratory is under the continuous standardization program of the Centers for Disease Control, Atlanta, Georgia.
Data Analysis

Although lipid and lipoprotein analysis were done monthly throughout the study, because of variations in time that individual drug regimens took to achieve full effect and to allow the complete washout from a previous drug effect, we used only the lipoprotein values obtained during the 2nd and 3rd month of each of the first five drug periods. During period six, lipoprotein levels were obtained monthly from the 2nd to the 6th month. A preliminary analysis indicated that for the group as a whole, the lipoprotein levels obtained during months 5 and 6 were statistically similar to those obtained during months 3 and 4, and therefore, these values were used for each individual. For period seven, the four values obtained at monthly intervals were used.

A multivariate, multiple linear regression model was developed to provide a general method by which a quantitative description of the effects of the three drugs and their combinations could be estimated and the significance of the different effects could be tested. The BMDP package of statistical programs was used for all analyses.32 A detailed description of this model is presented in the "Appendix."

Results

All 17 patients who entered the protocol completed all phases of the study. The effects of the various therapeutic regimens on mean plasma lipid and lipoprotein levels are given in Table 2 and Figure 1. The effects of the various therapeutic regimens on the individual lipoprotein classes, expressed as a percentage of the control value obtained during the placebo period, are given in Figures 2–4.
plus colestipol was nearly as effective at lowering total plasma cholesterol as was lovastatin, probucol, and colestipol. In the 13 individuals who took lovastatin (80 mg/day) plus colestipol, total cholesterol levels were only decreased 4% below levels observed on lovastatin (40 mg/day) plus colestipol. The five subjects who took only lovastatin (80 mg/day) had average decrease of total cholesterol of 31%.

**Effects on Plasma Triglyceride Levels**

Lovastatin caused a significant \((p < 0.02)\) decrease in plasma triglycerides, averaging an 18% reduction. Mean plasma triglycerides were not different on probucol alone but fell during period four when lovastatin was again added \((p < 0.01)\). The addition of colestipol resulted in a slight rise in plasma triglycerides, which partly negated the lowering effect of lovastatin. In the five subjects who took only lovastatin (80 mg/day), at the end of the study, plasma triglycerides were significantly reduced again \((p < 0.05)\).

**Effects on LDL Levels**

Lovastatin (40 mg/day) was extremely effective in lowering LDL levels, producing an average decrease of 36% in these severely hypercholesterolemic patients. All 17 patients experienced reductions in LDL that ranged from 15% to 51%. In contrast, probucol alone was not nearly as effective, producing an average decrease of only 14% \((\text{range, } +11\% \text{ to } -32\%)\). In contrast to the uniform response to lovastatin, the response to probucol was quite variable (Table 3). For example, nine subjects had LDL decreases more than 15%, three subjects had decreases of 6–15%, and five subjects had no change or even an increase in mean LDL cholesterol values. When lovastatin and probucol were combined, there was no synergistic or additive effect observed, and the lowering of LDL could be solely attributable to that produced by lovastatin alone. The addition of colestipol to lovastatin plus probucol therapy produced a substantial further decrease in LDL levels \((-23\%)\), and during this period, absolute LDL cholesterol levels were decreased an average 52% below baseline values. On this combination, all 17 patients experienced marked decreases in LDL, ranging from 40% to 70% of the average values obtained during the control period. When probucol was discontinued, the mean LDL cholesterol level remained almost as low as when on triple-drug therapy. Thirteen of the 17 patients were also given lovastatin (80 mg/day) while being maintained on colestipol. The mean LDL of these patients was 56% below their baseline value. The five individuals who took only lovastatin (80 mg/day) had LDL levels 40% below their control values.

**Effects on HDL Levels**

Lovastatin therapy alone produced a small but consistent increase in HDL cholesterol levels that averaged 6%. In striking contrast, probucol therapy alone produced a 29% decrease in HDL cholesterol levels. When lovastatin was added to the probucol therapy, during period four, this reduction in HDL was partially compensated for, but HDL levels were still nearly 20% below those of control.

### Table 3. Average LDL Cholesterol Levels Obtained During Study Periods One Through Five for Individual Subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Control (mg/dl)</th>
<th>Lovastatin (mg/dl)</th>
<th>Probucol (mg/dl)</th>
<th>Lovastatin plus probucol (mg/dl)</th>
<th>Lovastatin plus probucol plus colestipol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>438</td>
<td>297</td>
<td>322</td>
<td>261</td>
<td>182</td>
</tr>
<tr>
<td>2</td>
<td>205</td>
<td>137</td>
<td>228</td>
<td>136</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>408</td>
<td>236</td>
<td>329</td>
<td>224</td>
<td>126</td>
</tr>
<tr>
<td>4</td>
<td>244</td>
<td>171</td>
<td>167</td>
<td>143</td>
<td>124</td>
</tr>
<tr>
<td>5</td>
<td>308</td>
<td>194</td>
<td>306</td>
<td>220</td>
<td>135</td>
</tr>
<tr>
<td>6</td>
<td>258</td>
<td>154</td>
<td>188</td>
<td>163</td>
<td>136</td>
</tr>
<tr>
<td>7</td>
<td>378</td>
<td>240</td>
<td>341</td>
<td>244</td>
<td>183</td>
</tr>
<tr>
<td>8</td>
<td>344</td>
<td>218</td>
<td>249</td>
<td>175</td>
<td>117</td>
</tr>
<tr>
<td>9</td>
<td>426</td>
<td>275</td>
<td>357</td>
<td>280</td>
<td>...</td>
</tr>
<tr>
<td>10</td>
<td>406</td>
<td>198</td>
<td>300</td>
<td>203</td>
<td>173</td>
</tr>
<tr>
<td>11</td>
<td>218</td>
<td>146</td>
<td>238</td>
<td>140</td>
<td>108</td>
</tr>
<tr>
<td>12</td>
<td>254</td>
<td>215</td>
<td>242</td>
<td>198</td>
<td>161</td>
</tr>
<tr>
<td>13</td>
<td>200</td>
<td>136</td>
<td>166</td>
<td>157</td>
<td>139</td>
</tr>
<tr>
<td>14</td>
<td>460</td>
<td>312</td>
<td>500</td>
<td>332</td>
<td>288</td>
</tr>
<tr>
<td>15</td>
<td>210</td>
<td>145</td>
<td>196</td>
<td>137</td>
<td>104</td>
</tr>
<tr>
<td>16</td>
<td>504</td>
<td>303</td>
<td>418</td>
<td>299</td>
<td>238</td>
</tr>
<tr>
<td>17</td>
<td>256</td>
<td>156</td>
<td>220</td>
<td>184</td>
<td>...</td>
</tr>
</tbody>
</table>

**LDL:** low-density lipoprotein.

Shown is the average LDL cholesterol level obtained during each of the first five study periods for each individual subject. For each individual, the value shown is the average LDL cholesterol obtained after 2 and 3 months of the indicated therapy.
TABLE 4. Average Drug Effects and Drug Interactions: Results of Multiple Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lovastatin alone (40 mg/day)</td>
<td>-29</td>
<td>-36</td>
<td>+6</td>
</tr>
<tr>
<td>Probucol alone</td>
<td>-15</td>
<td>-14</td>
<td>-29</td>
</tr>
<tr>
<td>Addition of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lovastatin to probucol</td>
<td>-22</td>
<td>-26</td>
<td>+13</td>
</tr>
<tr>
<td>Probucol to lovastatin</td>
<td>-6</td>
<td>(-1)</td>
<td>-24</td>
</tr>
<tr>
<td>Colestipol to lovastatin (40 mg/day)</td>
<td>-17</td>
<td>-23</td>
<td>2</td>
</tr>
<tr>
<td>Colestipol to lovastatin (40 mg/day) plus probucol</td>
<td>-16</td>
<td>-23</td>
<td>+2</td>
</tr>
<tr>
<td>Probucol to lovastatin (40 mg/day) plus colestipol</td>
<td>-5*</td>
<td>(0)</td>
<td>-18</td>
</tr>
<tr>
<td>Lovastatin (40 mg/day) to lovastatin (40 mg/day)</td>
<td>(-3)</td>
<td>-7</td>
<td>+6</td>
</tr>
<tr>
<td>= Lovastatin (80 mg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lovastatin (40 mg/day) to lovastatin (40 mg/day) plus colestipol</td>
<td>(-3)</td>
<td>-7</td>
<td>+6</td>
</tr>
<tr>
<td>Coolestipol to lovastatin (80 mg/day)</td>
<td>-17</td>
<td>-23</td>
<td>-6</td>
</tr>
</tbody>
</table>

LDL, low-density lipoprotein; HDL, high-density lipoprotein.

This table gives the average drug effect on total, LDL, and HDL cholesterol levels for individual drugs and for addition of one drug to individuals already taking one or two agents. These results are derived from a multiple regression model using log transformed data as explained in the “Appendix.” Each value represents the percent change (positive or negative) of the lipid or lipoprotein level produced by a given drug. For example, lovastatin alone produced a 36% decrease in LDL levels in the average subject. When added to a subject already on probucol (addition of lovastatin to probucol), it produced a further 26% decrease from LDL levels obtained on probucol. It was not possible to calculate the effects of colestipol alone from this model, but previous results from our laboratory and others predict a lowering of LDL cholesterol of 20–35%.7-10

All effects are significant at p < 0.01 except (p) = NS, *p < 0.05.

obtained at baseline. The addition of colestipol resulted in no further change in HDL. However, during period six, when probucol was discontinued, HDL levels returned to control levels (4–6 months after discontinuation of the probucol). In the 13 subjects who subsequently took 80 mg/day lovastatin plus colestipol, HDL levels again increased 6% (compared with control values).

Reductions in Xanthomas

Nine of 17 patients had marked xanthomas, consisting of xanthelasma or tendon xanthoma or both. Although we did not document xanthoma size by means of xerography, in at least five individuals there was dramatic and visible evidence of regression of xanthomata. In each case, the regression was first observed by the patient during the fourth period when lovastatin and probucol were being given together. Continual regression occurred throughout the remainder of the study. For example, subject 1, who had extensive tendon xanthomata, had dramatic reduction of xanthoma on his hands. This finding was of considerable interest because no reduction had been observed previously despite years of combined colestipol and nicotinic acid therapy.

Average Drug Effects and Drug Interactions

In an attempt to quantify an average effect on lipoproteins produced by each drug as well as the interaction between the different drugs, we have analyzed the data with a linear regression model (as described in detail in the “Appendix”). This analysis allows us to estimate the average effect on each lipoprotein for each drug used individually and in combination with another drug. The results are presented in Table 4. For example, it can be seen that lovastatin alone (40 mg/day) produced an average decrease in LDL cholesterol of 36%, whereas probucol produced an average decrease of only 14%. The addition of lovastatin to individuals already on probucol is predicted to lead to a further decrease in LDL cholesterol levels of 26%; however, in the converse situation, the addition of probucol to subjects already on lovastatin (40 mg/day) would produce no further decrease in LDL levels (though total cholesterol levels would fall because of a decrease in the HDL cholesterol levels). The model suggests that for individuals taking lovastatin (40 mg/day), the addition of probucol is not likely to produce further lowering of LDL levels. However, the model predicts that the addition of colestipol to lovastatin (40 or 80 mg/day) would cause a further 23% decrease in LDL cholesterol from levels achieved on lovastatin alone. This magnitude of lowering (−23%) is similar to what literature values predict for effects of colestipol alone.7-10 In patients on a regimen of lovastatin (40 mg/day), increasing the dose of lovastatin to 80 mg/day would have the effect of lowering LDL cholesterol an additional 7%. Similarly, in subjects on lovastatin (40 mg/day) plus colestipol, increasing lovastatin to 80 mg/day also would add a further 7% lowering of LDL cholesterol.

Regarding HDL cholesterol, lovastatin alone produced an average increase in HDL cholesterol of 6%, whereas probucol alone produced a 29% decrease. The addition of lovastatin to subjects already on probucol would be predicted to partially reverse this decrease in HDL cholesterol levels.

Side Effects

As noted earlier, no medically important side effects were noted with any of the medications or
combinations that necessitated discontinuation of hypolipidemic drug therapy. In four patients, persistent mild liver function abnormalities (transaminase elevations <1.5–2 times that of normal) were noted during combined drug therapy; in all four cases, the patients were noted to consume alcohol, in moderation, on a regular basis. For example, subject 16 had consumed two to three glasses of wine a day for many years and had normal transaminase levels during periods one to three. During period four, mild abnormalities in SGPT and SGOT (values, 1.5–2 times normal) were noted. When she reduced her alcohol content to only one glass of wine a day, liver functions promptly returned to normal despite continuation of lovastatin and other study medications. Similarly, in three other individuals, similar mild elevations of liver transaminases were associated with alcohol consumption because discontinuation of alcohol led to the prompt return of liver function tests to normal despite continuation of lovastatin and other medications. One subject developed alkaline phosphatase level 1.2 times that of normal at the end of period two, which persisted throughout the remainder of the study. One other subject had random, isolated elevations of transaminases that were always less than twice that of normal. No other consistent blood chemistry abnormalities could be attributable to drug therapy in any of the other patients. Subject 17 reported the occurrence of a persistent mild headache during the periods that probucol was consumed. Within 2 days after discontinuing probucol during period six, the headache abated and was not reported again during the subsequent 6-month period of follow-up. The five individuals who discontinued colestipol did so because of gastrointestinal side effects.

Best corrected visual acuity decreased in two eyes of two individuals from 20/20 to 20/25. One eye of one individual was noted to have an increase in visual acuity from 20/50 to 20/25. No changes were noted in color vision, fundoscopy, or visual field evaluation. Six of the patients had changes noted with slit-lamp biomicroscopy and external examination. Three of these had slight progression of corneal arcus in both eyes, and one patient had a new small posterior subcapsular opacity in one eye. Two eyes of two individuals had pigment on the anterior capsule of the lens, and one patient had early diffuse cortical opacities in both eyes after two years. Initially, 11 patients were found to have corneal arcus, and two had xanthelasma. There was no regression in corneal arcus but xanthelasma disappeared in one patient.

**Response of Individual Patients**

The mean data presented above have been derived from all 17 patients. However, in treating individual patients, it is important to appreciate the range of responses that can be expected. Table 3 presents the average LDL cholesterol values obtained during the first five periods for each study subject, and Figure 5 displays the response of each individual patient to the optimal combination drug therapy,
which represents the lowest average total or LDL cholesterol level obtained during periods five, six, or seven. As can be appreciated from Figure 5, during the optimal form of therapy all 17 patients had substantial decreases in their LDL cholesterol levels, averaging 53%, with a range from 35% to 70% below baseline. Fifteen of the 17 patients experienced LDL cholesterol levels on combination therapy that were below the 95th percentile, and 11 patients achieved LDL cholesterol levels below the 50th percentile for age- and sex-matched controls. In six subjects, the lowest LDL was obtained on triple-drug therapy, whereas seven subjects had their lowest on lovastatin (80 mg/day) plus colesterol.

During the period in which the lowest total cholesterol level was achieved, a mean decrease of 44% occurred, with a range of 31% to 60%. Eleven subjects had their lowest total cholesterol level during the triple-drug therapy (reflecting the decrease in HDL levels as well), whereas only four subjects had their lowest value on lovastatin (80 mg/day) plus colesterol. In all, nine subjects achieved total cholesterol levels below 200 mg/dL.

In general, the data presented above indicated that the combination of lovastatin plus colesterol was as effective as the combination of lovastatin, colesterol, and probucol for most individuals. However, there was individual variation, and in a few patients (i.e., subjects 1, 4, and 8) probucol did appear to exert a synergistic effect with other medications in lowering LDL levels.

Discussion

Several previous reports have described successful combinations of hypolipidemic agents that yielded reductions of LDL cholesterol of 35–55%. These combinations have usually included a bile sequestrant (either colesterol or cholestyramine) together with nicotinic acid,29–31 probucol,32,33 or neomycin.38 More recently, bile sequestrants have been combined with an inhibitor of cholesterol biosynthesis, compactin, or lovastatin.39–41 There are only rare reports of successful regimens that used combinations of hypolipidemic agents that did not include a bile sequestrant.42

In the present study, we sought to test a therapeutic regimen that would allow an effective lowering of LDL levels without the necessity of using a bile sequestrant. Although these agents are highly effective in lowering LDL levels and in reducing the subsequent rate of CAD,43 their use is associated with frequent side effects and problems with long-term patient acceptance and compliance.29,43 Because lovastatin and probucol theoretically work by different mechanisms, the hypolipidemic effect of the two used together should be additive, and in a preliminary study of several FH patients, this appeared to be true.29 Therefore, we designed this long-term prospective study involving 17 FH subjects. Although a synergistic effect of lovastatin and probucol was seen in an occasional patient, for the vast majority of patients the addition of probucol added little to the therapeutic regimen, as judged by lowering of LDL levels. In contrast, the addition of colesterol to subjects on lovastatin (either 40 or 80 mg/day) led to a further 23% reduction in LDL levels. Because previous reports have suggested that, at the dose used, bile sequestrants alone lower LDL levels 20% to 25%,7–10 our data suggest that the bile sequestrants and lovastatin are working by independent, or additive, mechanisms to lower LDL levels. It is also of interest that the further addition of lovastatin (40 mg/day) to a regimen of lovastatin (40 mg/day) yielded only a further 7% lowering in LDL levels. Addition of lovastatin (40 mg/day) to someone already taking colesterol plus lovastatin (40 mg/day) increased LDL lowering only 7% further, also suggesting that bile sequestrants and lovastatin lower LDL by somewhat independent modes.

Lovastatin increased HDL levels 6%, consistent with previous reports.10,14–16 However, probucol produced a profound depression in HDL levels, averaging 29%, an effect that persisted despite the simultaneous administration of lovastatin. The effect of probucol persisted long after discontinuation of the medication, and only after discontinuation of therapy for 6 months did the HDL levels become elevated again, reflecting the lovastatin effect. Although conventional wisdom would suggest that this profound lowering of HDL cholesterol levels is detrimental, data from Japan indicate that probucol-induced regression of xanthoma size in patients with homozygous FH correlates best with reduction in HDL cholesterol levels.44 Our laboratory45,46 and others47 have suggested that oxidative modification of LDL increases its uptake in macrophages and can lead to foam cell formation. Recent observations from this laboratory indicate that probucol is a potent lipid-soluble antioxidant and that LDL isolated from patients treated with conventional doses of probucol is protected from oxidative modifications in vitro.48 Furthermore, Carew et al49 and Kita et al50 have shown that probucol inhibits atherosclerosis in hypercholesterolemic rabbits despite any substantial reduction in plasma cholesterol levels. However, only further experimentation and results of clinical trials will be able to provide guidelines as to its potential as an antiatherosclerotic agent.

Of considerable interest was that nine of 17 patients in this study had marked xanthelasmas consisting of xanthelasma or tendon xanthomas or both. Although we did not document xanthoma size in any quantitative manner, in at least five individuals there was dramatic and visible evidence of regression of xanthomata. This was particularly striking in several subjects who had been followed in this clinic for many years on combinations of a bile sequestrant and nicotinic acid and in whom no evidence for xanthoma regression had occurred. Despite the fact that at the beginning of the study eight of these subjects had clinical manifestations of
CAD or peripheral vascular disease, in no individual did worsening of disease occur over the 25 months of study, nor did evidence for CAD present in any of the other nine subjects.

No subjects experienced any serious medical side effect during this combination drug study, and in no case was any hypolipidemic medication discontinued because of any serious adverse reaction. Through periodic complete ophthalmologic examinations during the 2-year period, no vision-threatening side effects were observed. We found no change in visual acuity, color vision, fundus examination, or visual field test. Only minimal changes were detected by slit-lamp biomicroscopy and external examination. Of importance, no patient developed a new lens opacity causing loss of visual acuity. However, slit-lamp biomicroscopy grading of lens opacities is a subjective examination, and minor lens opacities may not be detected with this technique.

The goal of therapy was to develop a well-tolerated combination drug regimen for high-risk patients that would lower total cholesterol levels to values below 200 mg/dl and LDL cholesterol levels to values below 130 mg/dl. Our data indicate that lovastatin (40 mg/day) plus colestipol lowered LDL levels 52%, and lovastatin (80 mg/day) plus colestipol decreased LDL levels 56%. These values are consistent with other reports of the efficacy of lovastatin and a bile sequestrant. Although substantial reductions in cholesterol and LDL values were achieved, for many of our subjects total and LDL cholesterol values remained above the goals listed above. Malloy and colleagues recently reported that the combination of lovastatin, colestipol, and nicotinic acid resulted in a 67% decrease in LDL cholesterol levels in 21 FH subjects. In their study, a mean control LDL cholesterol level of 326 mg/dl was reduced to 112 mg/dl. However, caution should be exercised in using lovastatin and nicotinic acid together as this apparently increases the possibility of lovastatin-induced myopathy.

In a recent symposium, we summarized a number of reports in which several different combinations of two hypolipidemic drugs were used to produce LDL lowering of 40–60%. Our current report and that of Malloy and colleagues are among the first to describe the use of three agents to treat patients with FH. The use of probucol as a third agent, together with lovastatin and colestipol, appears to add little as a hypolipidemic agent for the average patient. Whether probucol's antioxidant property will eventually advocate its use as an antiatherosclerotic drug awaits further experimentation and clinical trials.

Acknowledgments

We wish to acknowledge the dedication and patience of the subjects who participated in this study. Lovastatin (and placebo) were supplied by Dr. Jonathan Tobert of Merck, Sharp and Dohme Research Labs; probucol (and placebo) were supplied by Dr. Ralph Tedeschi of Merrell Dow Pharmaceuticals; and colestipol hydrochloride was supplied by Dr. Nicholas Andreadis of the Upjohn Company.

Appendix

Data were analyzed with multivariate, multiple-linear regressions performed with the 6R program. These regressions were performed on log-transformed values of plasma cholesterol, LDL cholesterol, and HDL cholesterol, and the residuals were verified as being normally distributed. Similar analyses on untransformed data did not produce normally distributed residuals. Plasma triglycerides were not considered in this analysis as preliminary analysis indicated that only lovastatin caused significant changes in this variable, and the effect was relatively small. Fixed (constant) effects estimated from the regression represent proportional changes in plasma concentrations. Percent changes for a sum of multiple effects were calculated with the formula

\[ \% \text{ change} = 100 \times (10^8 - 1) \]

where \( S = \) sum of effects. All effects that could be estimated with the current data were included in the model. For each subject, only those periods for which multiple observations were available were analyzed.

The complete statistical model initially considered was

\[
Y_{ijk} = \gamma + \theta_i + \mu X_i + (\mu \pi \gamma) X_i X_i + (\pi \Pi \gamma) X_i + (\mu \pi \kappa) X_i X_i + \epsilon_{ijk}
\]

where \( Y_{ijk} \) is the \( k \)th replicate for subject \( i \) during period \( j \). The symbols \( \gamma, \mu, \theta_i, (\mu \pi \gamma), (\mu \pi \kappa \gamma), (\mu \pi \kappa \kappa), \) and so on represent the various effects to be estimated. \( X \) represents “independent” variables used to control which effects are included in predicting each observation, and \( \epsilon_{ijk} \) is the residual error. An \( X \) has a value of 1 if the condition indicated by its subscript is satisfied (i.e., the medication indicated was actually taken). Otherwise, it has a value of 0. For example, \( X_i \) is set to 1 for all data from periods during which the subject was taking lovastatin. Similarly, \( X_i \) is set to 1 for all data for subject 2. The nomenclature used for effects is as follows. Effect \( \gamma \) is the mean of all control (period one) values. The symbol theta \( (\theta) \) refers to subject. Effects dependent on the subject always have an \( i \) subscript and may be considered samples from a population of random effects. The symbols \( \mu, \pi, \kappa \), and \( \lambda \) refer to 40 mg/day lovastatin, 1 g/day probucol, 20 g/day colestipol, and 80 mg/day lovastatin, respectively. Because one cannot take 80 mg lovastatin without taking 40 mg lovastatin, \( X_i \) can only be 1 if \( X_i \) is
also 1. Thus, effects that include $\lambda$ must be interpreted as resulting from a second 40 mg/day dosage of the drug and were always estimated as additive to the effects of the first 40 mg/day. Effects with parentheses are interactions of the variables enclosed and are included if more than one condition is met. In practice, additional independent variables must be defined for each of the interaction terms, but these are easily calculated as products of singly subscripted variables.

Some interaction effects such as the interaction of probucol and 80 mg/day lovastatin are not included in the model because these combinations were never administered. Some of the individual terms in the above model could not be estimated with the current data and were only calculated in conjunction with other effects. For example, because colestipol was never administered in the absence of lovastatin, the effect of colestipol alone could not be estimated and was always confounded by its interaction with lovastatin. Thus, only the sums of effects $k+ (\mu k)$ and $(k\theta)_2 + (\mu k\theta)_2$, could be estimated. Similarly, the interaction of colestipol and probucol could not be separated from a possible three-way interaction among lovastatin, probucol, and colestipol. Again, only the sums $(\pi k) + (\mu \pi k)$ and $(\pi k\theta)_2 + (\mu \pi k\theta)_2$, could be estimated.

Because the 13 subjects with a period seven (80 mg/day lovastatin plus colestipol) were different individuals than the five subjects with a period eight (80 mg/day lovastatin only), comparisons of these two periods must be considered an unpaired test. This test may be interpreted as a test for an interaction between the second 40 mg/day lovastatin and colestipol. This test showed no significance, and it was concluded that the effect of adding colestipol to a lovastatin regimen does not depend on whether the subject is taking 40 or 80 mg lovastatin.

For subject 13, who had a period five but no period six, the combination of effects $(k\theta)_3 + (\mu k\theta)_3 + (\pi k\theta)_3 + (\mu \pi k\theta)_3$ had to be estimated rather than the two combinations $(k\theta)_3 + (\mu k\theta)_3$ and $(\pi k\theta)_3 + (\mu \pi k\theta)_3$, as was done for the other subjects. Subject 13 was therefore excluded during tests of significance on interactions between subject and colestipol and among subject, colestipol, and probucol.

The significance of each of the effects in the above model was tested by regressing all data with and without the effect under consideration. Results were compared with Fisher’s $F$ test and parameters removed from the model in a stepwise manner if $p > 0.05$ was obtained for all dependent variables. Effects were removed in the following order: 1) $(\lambda \theta)_2$, interaction between subject and 80 mg/day lovastatin; 2) $(\mu \theta)_2$, interaction between subject and 40 mg/day lovastatin; and 3) $(\lambda \kappa)_2$, interaction between colestipol and 80 mg/day lovastatin. All other effects were found to be significant for at least one of the dependent variables. The lack of significance of the above three effects may be interpreted to indicate that all subjects reacted similarly to the addition of 40 mg/day lovastatin when already on 40 mg lovastatin, all subjects reacted similarly to the first 40 mg/day lovastatin, and the effect of the second 40 mg/day lovastatin did not depend on whether the subject was also taking colestipol.

The effects estimated from all data using the final model were then used to estimate the effects of adding a drug to another regimen. For example, the effect of adding probucol to lovastatin was estimated as $\pi + (\mu \pi)$ while the effect of adding lovastatin to probucol as $\mu + (\mu \pi)$. The statistical significance of the addition of a drug was tested by regressing the data with the appropriate sum of effects constrained to be 0. Results were compared to the full model with Fisher’s $F$ test. These data are shown in Table 4.

For each lipoprotein variable measured, a group mean percent change from the control period was calculated for each drug period with a paired Student’s $t$ test of log-transformed data. These data are shown in Figures 2–4.

References


2. Wagner WD, St Clair RW, Clarkson TB, Connor JR: A study of atherosclerosis regression in Macaca mulatta: 3. Chemical changes in arteries from animals with atherosclerosis induced for nineteen months and regressed for forty-eight months at plasma-cholesterol concentrations of 300 or 200 mg/dl. Am J Pathol 1980;100:633–650


17. The Lovastatin Study Group II: Therapeutic response toLovastatin (mevinolin) in nonfamilial hypercholesterolemia. *JAMA* 1986;256:2829–2834


44. Henriksen T, Mahoney EM, Steinberg D: Enhanced macrophage degradation of low density lipoproteins previously incubated with cultured endothelial cells: Recognition by the receptors for acetylated low density lipoproteins. *Proc Natl Acad Sci USA* 1981;78:6495–6503


49. Carew TE, Schwenke DC, Steinberg D: Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: Evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc Natl Acad Sci USA* 1987;84:7725–7729


**KEY WORDS** • familial hypercholesterolemia • lovastatin • probucol • colestipol hydrochloride • cholesterol
Intensive combination drug therapy of familial hypercholesterolemia with lovastatin, probucol, and colestipol hydrochloride.
J L Witztum, D Simmons, D Steinberg, W F Beltz, R Weinreb, S G Young, P Lester, N Kelly and J Juliano

Circulation. 1989;79:16-28
doi: 10.1161/01.CIR.79.1.16

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/79/1/16

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/