Probucol promotes reverse cholesterol transport in heterozygous familial hypercholesterolemia. Effects on apolipoprotein AI-containing lipoprotein particles

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Received 5 July 1999; received in revised form 8 November 1999; accepted 9 December 1999

Abstract

In order to investigate the effect of Probucol therapy on reverse cholesterol transport, apo AI-containing lipoprotein particles were isolated and characterized, and their cholesterol effluxing capacity and LCAT activity were assayed in four familial hypercholesterolemia patients before and after 12 weeks of Probucol therapy. Four major subpopulations of apo A-containing lipoprotein particles are separated before and after drug treatment; LpAI, LpAI:AII, LpAIV, LpAI:AIV:AII. Probucol reduces both total plasma and LDL-cholesterol (−17 and −14%, respectively). Apo B decreases slightly (−7.6%). Plasma HDL-cholesterol and apo AI decrease by 36.6 and 34.7%. LpAI showed a marked decrease (−46%). Moreover, plasma LCAT and CETP activities were markedly increased under Probucol treatment. Analysis of lipoprotein particles showed that Probucol induces a decrease of protein content and an increase of cholesterol and triglycerides contents. Interestingly, Probucol induces an enhancement of LCAT activity in LpAI (4.5-fold). This drug induces a trend toward greater cholesterol efflux from cholesterol-preloaded adipose cells promoted by Lp AI and Lp AIV but not by Lp AI:AII. This study confirms the hypothesis, in addition to the lowering LDL-cholesterol levels and antioxidant effects of Probucol, that HDL reduction was not an atherogenic change in HDL system but may cause an antiatherogenic action by accelerating cholesterol transport through HDL system, promoting reverse cholesterol transport from peripheral tissues. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Probucol; Familial hypercholesterolemia; Lipoprotein particle composition; CETP activity; LCAT activity; Cholesterol efflux

1. Introduction

Many epidemiological studies have indicated that the plasma level of high-density lipoprotein (HDL) is inversely correlated with the risk for coronary artery disease [1,2]. It has been established that HDL exerts its protective effect by the ‘reverse’ transport of excess cholesterol from peripheral tissues to the liver [3,4].

Probucol, 4,4′-(isopropylidene-dithio)-bis-(2,6-di-tert-butylphenol) was introduced in the early 1970s as a cholesterol-lowering drug [5], and has been the focus of many clinical investigations because it is an antioxidant that also reduces plasma cholesterol concentration in patients with hypercholesterolemia and reduces tendon xanthoma in man [6,7]. This raises a problem in attempting to find out the effects of Probucol as an antioxidant from its effects as cholesterol-lowering agent. Probucol is a unique antiatherogenic drug, producing its effect by antioxidant action rather than hypolipidaemic effect. However, the exact mechanism of its antiatherogenic effect is unclear. This drug is known to reduce not only the total plasma cholesterol and low-density lipoprotein (LDL)-cholesterol but also HDL cholesterol [7]. The reduction of HDL cholesterol...
by Probucol is contradictory to the clinical results, which demonstrated that HDL has a protective role in coronary disease. Moreover, a close correlation between the extent of xanthoma regression and HDL reduction under Probucol treatment has been reported [8].

Protein particles isolated on the basis of apolipoprotein composition may have particular physiopathological properties [3,9,10]. HDL comprises two main subclasses: those containing, as the main protein components, apo A-I and apo A-II, designated Lp AI:AII; and those containing apo A-I but not apo A-II, designated Lp AI. It has been well established that lower HDL concentration in coronary artery disease is linked with lower Lp AI levels, while Lp AI:AII levels are unaffected [11]. This observation led to the hypothesis that Lp AI may represent the antiatherogenic lipoprotein particle. This is confirmed by ‘in vitro’ studies showing that cholesterol efflux from cells is mediated by Lp AI but not by LpAI:AII [3,12]. Apo A-IV-containing particles isolated from plasma comprise two main subpopulations: those containing, as the main protein components, apo A-IV and apo A-I, designated Lp AI:AIV:AII and those that contain apo A-IV but not apo A-I, designated Lp AIV. Both subpopulations of lipoprotein containing apo A-IV express LCAT and CETP activities and promote cholesterol efflux from adipose cells [13].

To assess the effect of Probucol drug on reverse cholesterol transport, we characterized the major subclasses of HDL lipoproteins isolated on the basis of apolipoprotein composition from plasma of patients with heterozygous familial hypercholesterolemia and analyzed their ability to promote cholesterol efflux from adipose cells, before and after 12 weeks of Probucol treatment.

2. Materials and methods

2.1. Subjects and protocol

Four patients with heterozygous familial hypercholesterolemia (FH) with mean age of 43 years (range 30–59) were selected for this study. All patients were classified as familial hypercholesterolemic on the basis of the presence of tendon xanthomas and appropriate family history, and had apo E (3/3) and apo AIV (1/1) phenotypes [14,15]. No subject took vitamin E or beta-carotene, or any drug known to affect lipid metabolism. All patients were informed of the purpose of the study, which was approved by an institutional ethics review board. The patients have been treated with Probucol (1000 mg daily) for 12 weeks. Venous blood samples were obtained after an overnight fast. Blood was promptly centrifuged at 4°C for 15 min at 3000 × g to separate cells from plasma. Samples of plasma were used for analysis of lipids, apolipoproteins and isolation of lipoprotein particles.

2.2. Lipoprotein particles isolation

HDL particles were purified from total plasma from patients before and 12 weeks after Probucol therapy, by sequential immunoaffinity chromatography using antibodies against apolipoproteins, apo B, apo E, apo AI, apo AII and apo AIV, as previously described [13,16]. This resulted in the isolation of four types of lipoprotein particles: Lp AI, Lp AI:AII, Lp AIV and Lp AI:AIV:AII. To avoid interactions with apo E and apo B/E receptors, apo B- and apo E-containing particles were removed from plasma. As shown in Fig. 1, plasma samples were applied consecutively to immunosorbents at the flow rate of 10 ml/h in Tris buffer. In each case, the immunosorbent was washed with Tris buffer containing 0.5 M NaCl at a flow rate of 60 ml/h to elute non-specifically bound particles. The retained fraction was eluted with 3 M sodium thiocyanate (NaSCN) at a flow rate of 60 ml/h. The eluate was immediately filtered through a column packed with Sephadex G 25 to remove the NaSCN from the retained fraction. This procedure minimized the inactivation of LCAT. Finally, all particles were dialyzed against Tris buffer and were filtered using a 0.22-µm Millipore filter.

Fig. 1. Flow diagram of the various stages of sequential immunoaffinity chromatography resulting in the isolation of the four types of HDL particles named according to their composition in the main apolipoproteins (Apo, apolipoprotein; Lp, lipoprotein; RF, retained fraction; NRF, non-retained fraction).
The following lipoprotein particles were finally obtained, Lp AI, Lp AI:AII, Lp AIIV and Lp AI:AIV:AII.

2.3. Lipids, apolipoproteins and lipoprotein particles analysis

Total cholesterol, triglycerides and phospholipids were determined enzymatically, using kits from Boehringer-Manheim (Germany). The HDL-cholesterol level was determined enzymatically after isolation of HDL by the phosphotungstic acid–magnesium chloride method [17]. The LDL cholesterol level was determined using kit from Biome’rieux (France).

Proteins were determined by the method of Lowry [18]. Apolipoproteins were measured by specific immunoassays, using a standard type Elisa as previously described [19]. Lp AI was quantified using a differential electroimmunoassay [20]. The quantitative determination of Lp AI:AII was performed by enzyme-linked differential antibody immunosorbent assay as described [21].

2.4. LCAT and CETP activities

The LCAT activity of samples of plasma and lipoprotein particles, purified from total plasma from patients before and 12 weeks after Probucol therapy were measured using the method of Albers et al. [22]. Proteoliposome substrate containing apo AI, lecithin and [14C] cholesterol-labeled lipoproteins complexes were incubated with 20–60 μg particle protein in a shaking water bath for 5 h at 37°C. The reaction was stopped by placing samples on ice. Lipids were extracted using CHCl3/CH3OH (2:1, v/v). Esterified [14C] cholesterol and excess labeled substrate were separated by thin-layer chromatography using petroleum benzine/ diethyl ether/acetic acid, and the radioactivity of the bonds was counted. LCAT activity was expressed as a percentage of cholesterol esterified per 100 μg of protein particle per 5 h of incubation.

Cholesterol ester transfer protein (CETP) activity of the plasma samples from patients before and 12 weeks after Probucol therapy was measured using the method of Chen and Albers [22]. Proteoliposome substrate containing apo AI, lecithin and [14C] cholesterol-labeled lipoproteins complexes were incubated with 20–60 μg particle protein in a shaking water bath for 5 h at 37°C. The reaction was stopped by placing samples on ice. Lipids were extracted using CHCl3/CH3OH (2:1, v/v). Esterified [14C] cholesterol and excess labeled substrate were separated by thin-layer chromatography using petroleum benzine/ diethyl ether/acetic acid, and the radioactivity of the bonds was counted. CETP activity was expressed as percentage of [14C] cholesterol ester-HDL3 transferred per 20 μl of plasma sample per 5 h of incubation.

2.5. Cellular cholesterol efflux studies

To promote cholesterol efflux, differentiated cells Ob 1771 [3] were first maintained for 48 h in lipoprotein-deficient bovine serum at 37°C and then exposed to [3H]-cholesterol linoleate-enriched LDL for 48 h (150 μg of cholesterol per ml) in the same medium. Subsequently, cells were washed in 0.1 M phosphate buffered saline (PBS) and maintained in serum-free medium supplemented with particles purified from total plasma from patients before and 12 weeks after Probucol therapy for various times (50 μg of protein particles/ml or only 50 μg of dimyristoyl-phosphatidyl choline (DMPC) per ml as a control). Cells were then washed with PBS at 4°C and solubilized in 0.1 N NaOH. The remaining cellular cholesterol was then determined by radioactivity counting. The radioactivity appearing in the medium was a percentage of the initial cell-associated [3H]-cholesterol.

2.6. Statistical analysis

Statistical analysis was performed using the Mann Whitney U-test to evaluate the data.

3. Results

3.1. Lipid, apolipoprotein and lipoprotein particle concentrations in plasma

The results of Table 1 indicate plasma lipid, apolipoprotein and lipoprotein particle profiles of the patients before and 12 weeks after Probucol therapy. As already reported [24–28], the actual concentration of lipids, apolipoproteins and lipoprotein particles of this group showed a decrease after 12 weeks of treatment excepted for apo AIV and apo E. Total plasma cholesterol and LDL-cholesterol levels decreased by 17 and 14%, respectively, while plasma apo B concentrations and the LDL-cholesterol/apo B ratio decreased slightly (7.6 and 5.8% respectively). Triglycerides were modestly affected (−5.5%). Probucol caused consistent reduction in the HDL-cholesterol levels (−36%). The decline in HDL-cholesterol resulted probably from reductions in apo AI (−34%) and still more in Lp AI (−45%). Lp AI:AII level decreases by 20%. Apo E levels increased by 68% while that of apo CIII decreased by 25% after Probucol treatment.

3.2. Protein, lipid and apolipoprotein compositions of isolated particles

Sequential immunoaffinity chromatography was used to isolate four subclasses from plasma according to their major apolipoprotein contents: Lp AI, Lp AI:AII,
Table 1
Plasma concentration of lipids, apolipoproteins and lipoprotein particles before and after Probucol treatment

<table>
<thead>
<tr>
<th></th>
<th>Before Probucol treatment</th>
<th>After Probucol treatment</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>300 ± 19</td>
<td>250 ± 26</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>144 ± 70</td>
<td>136 ± 73</td>
<td>NS</td>
</tr>
<tr>
<td>Phosholipids</td>
<td>275 ± 30</td>
<td>250 ± 37</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>41 ± 1.9</td>
<td>26 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>228 ± 24</td>
<td>197 ± 25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apo AI</td>
<td>92 ± 6.0</td>
<td>60 ± 5.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo AII</td>
<td>28 ± 2.0</td>
<td>27 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Apo AIV</td>
<td>8 ± 0.6</td>
<td>9 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B</td>
<td>130 ± 13</td>
<td>120 ± 24</td>
<td>NS</td>
</tr>
<tr>
<td>Apo CIII</td>
<td>3.7 ± 0.7</td>
<td>2.8 ± 0.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Apo E</td>
<td>1.6 ± 0.9</td>
<td>2.7 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp AI</td>
<td>41 ± 9.0</td>
<td>22 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp AI:AII</td>
<td>50 ± 8.0</td>
<td>40 ± 6.0</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

* Values are expressed in mg/dl and are the mean ± S.D.
* Significantly different from before Probucol treatment, P<0.05. NS, no significant.

Table 2
Protein and lipid composition (mass %) and apolipoprotein composition (mass %) of isolated lipoprotein particles

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lipids</th>
<th>Apolipoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>Lp AI</td>
<td>Before Probucol</td>
<td>72.0 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>After Probucol</td>
<td>64.1 ± 5.6</td>
</tr>
<tr>
<td>Lp AI:AII</td>
<td>Before Probucol</td>
<td>63.8 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>After Probucol</td>
<td>57.5 ± 3.1</td>
</tr>
<tr>
<td>Lp AIV</td>
<td>Before Probucol</td>
<td>90.0 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>After Probucol</td>
<td>81.2 ± 4.4</td>
</tr>
<tr>
<td>Lp AI:AIV:AII</td>
<td>Before Probucol</td>
<td>81.6 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>After Probucol</td>
<td>65.6 ± 4.6</td>
</tr>
</tbody>
</table>

* The protein and lipid composition are given as % mass. The apolipoprotein composition is given by taking as 100% the total apolipoproteins determined by immunoassays. The values are mean ± S.D. of four preparations of each lipoprotein particle.

Lp AIV and Lp AI:AIV:AII. Apo E- and Apo B-containing particles were first removed, their absence was confirmed by ELISA.

Isolated particles were analyzed for their lipid and apolipoprotein composition (Table 2). It appears that protein mass of lipoprotein particles were decreased after Probucol treatment. The repartition in percentage became 64.1, 57.5, 81.2 and 65.6% for Lp AI, Lp AI:AII, Lp AIV and Lp AI:AIV:AII, respectively. These values were approximately similar to those obtained in normolipemic subjects [13]. The percentage of total cholesterol in Lp AI, Lp AIV and Lp AI:AIV:AII was higher after Probucol treatment. The percentage of triglycerides in isolated particles showed a marked increase after Probucol treatment. Apolipoprotein compositions in Lp AI were unchanged by Probucol therapy. Lp AI contains mainly a single apolipoprotein, as in normolipemic subjects, in which apo AI represents 98% of total mass of apolipoproteins [3,13]. Lp AI:AIV:AII was enriched in apo AIV after treatment, while Lp AIV contained less of apo AII.

3.3. LCAT and CETP activities

Plasma LCAT activity was significantly (P < 0.001) increased from 0.79 ± 0.2 to 3.28 ± 0.7% of esterified cholesterol/15 μl of plasma per 30 min of incubation after Probucol treatment.
Before Probucol treatment, LCAT activity in Lp AI:AII was higher than in Lp AI. The LCAT activity in Lp AI markedly (P < 0.001) increased from 4.8 ± 2.0 to 22 ± 3.0% of esterified cholesterol per 100 μg protein per 5 h of incubation after Probucol treatment (Table 3). LCAT activity in Lp AIV was also increased after Probucol treatment but less than in Lp AI (from 1.5 ± 0.5 to 1.9 ± 0.6%). However, Probucol treatment induces no changes in LCAT activity in Lp AI:AIV:AII.

Plasma CETP activity before Probucol treatment, using [14C] cholesteryl ester-HDL3 donor and LDL acceptor induced a transfer of 7.4 ± 2.1% of [14C] cholesteryl ester:20 μl of plasma per 5 h of incubation. After Probucol treatment, activity of plasma CETP induced a transfer of 21.5 ± 3.2% of [14C] cholesteryl ester:20 μl of plasma per 5 h of incubation.

3.4. Cholesterol efflux from cholesterol preloaded Ob 1771 adipose cells

Following [3H] cholesterol preloading of adipose cells by means of [3H] cholesteryl linoleate-enriched LDL, the four types of lipoprotein particles isolated before and after Probucol treatment were assayed for their ability to promote cholesterol efflux, as a function of time, at 37°C (Table 4). Probucol therapy enhances Lp AI and Lp AIV more than Lp AI:AIV:AII to promote cholesterol efflux from adipose cells. In contrast, no efflux from was promoted by Lp AI:AII and the control DMPC liposomes after Probucol treatment. Table 4 showed a trend toward greater cholesterol efflux promoting by Lp AI and Lp AIV particles isolated after Probucol treatment.

Table 3
The LCAT activity in isolated lipoprotein particles*  

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Before Probucol treatment (mg of protein particles per 5 h)</th>
<th>After Probucol treatment (mg of protein particles per 5 h)</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp AI</td>
<td>4.8 ± 2.0</td>
<td>22 ± 3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp AI:AII</td>
<td>9.3 ± 2.1</td>
<td>6.3 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp AIV</td>
<td>1.5 ± 0.5</td>
<td>1.9 ± 0.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Lp AI:AIV:AII</td>
<td>1.1 ± 0.6</td>
<td>1.1 ± 0.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Values are in a percentage of cholesterol esterified/μg of protein particles per 5 h of incubation. Values are the mean ± S.D.
* Significantly different from before Probucol treatment, P < 0.05.

Table 4
Cholesterol efflux from [3H]-Cholesterol-preloaded Ob 1771 cells before and after 12 weeks of Probucol treatment*  

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Incubation time (min)</th>
<th>[3H]-cholesterol efflux (percent of initial cell-associated cholesterol)</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before Probucol treatment</td>
<td>After Probucol treatment</td>
</tr>
<tr>
<td>Lp AI</td>
<td>30</td>
<td>26 ± 4.2</td>
<td>35 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>30 ± 4.6</td>
<td>40 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>43 ± 5.0</td>
<td>44 ± 4.9</td>
</tr>
<tr>
<td>Lp AI:AII</td>
<td>30</td>
<td>2.0 ± 0.3</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2.0 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Lp AIV</td>
<td>30</td>
<td>28 ± 6.1</td>
<td>34 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>36 ± 7.3</td>
<td>40 ± 7.0</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>46 ± 6.8</td>
<td>46 ± 6.7</td>
</tr>
<tr>
<td>Lp AI:AIV:AII</td>
<td>30</td>
<td>25 ± 5.7</td>
<td>27 ± 6.6</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>34 ± 5.8</td>
<td>36 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>40 ± 6.0</td>
<td>41 ± 6.2</td>
</tr>
<tr>
<td>Dimyristoyl phosphatidylcholine</td>
<td>30</td>
<td>2.0 ± 0.1</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
</tbody>
</table>

* The radioactivity appearing in the medium was as a percentage of the initial cell-associated [3H]-cholesterol. Values are mean ± S.D.
* Significantly different from before Probucol treatment, P < 0.05.
4. Discussion

Probucol, the only drug shown to induce xanthoma regression in FH, is a potent antioxidant, but it also lowers HDL-cholesterol levels, causing some concern [24,25]. Probucol therapy consistently reduce total and LDL cholesterol levels [6,26–30]. The antiatherogenic mechanism of this drug relate to its antioxidant action, preventing the oxidative modification of LDL, which may play a role in the alteration of arterial wall elastic properties [31–33]. It has been reported that Probucol is located in apo B and apo A families lipoprotein particles, with a preferential association with apo B-containing lipoprotein particles [34]. Recent studies on its distribution have shown that serum concentrations of Probucol are reduced during LDL-apheresis and it is mainly due to reductions in the LDL fraction [35].

The object of this study was the evaluation of Probucol therapy effect on HDL metabolism based upon their subclasses analysis according to their apolipoprotein composition. We isolated from the plasma the major apo A-containing particles Lp AI, Lp AI:AII, Lp AIV and Lp A I:AIV:AII and analyzed their lipid and apolipoprotein contents, their LCAT and CETP activities and their abilities to promote cholesterol efflux from cholesterol-preloaded adipose Ob17 cells.

The changes in levels of plasma lipids and apolipoproteins noted in these patients following Probucol treatment were similar to those previously reported [26–30,36]. The greater decrease in both total (17%) and LDL-cholesterol (14%) with a slightly decrease in plasma triglycerides and apo B could be documented in the present study, which is in agreement with previous data [26–30]. The nature of the changes in HDL-cholesterol may result in a decrease of apo A-I and Lp AI. In addition, plasma apo E concentration was increased after Probucol treatment that is of interest according to the evidence that apo E-HDL interact with the hepatic apo E receptor [37].

Our results indicate remarkably that Probucol therapy not only reduces plasma LDL and HDL-cholesterol but also induces a marked decrease of plasma Lp AI concentration. The effect on plasma HDL apolipoprotein concentrations was primarily on apo AI (34%), consistent with the dramatic reduction of Lp AI (46%). These results were in agreement with previous reported data [28,38]. This observation is of interest considering the lipid-lowering therapeutic effect of Probucol in decreasing plasma HDL$_2$ levels [25,29,30].

Probucol stimulated CETP activity after 12 weeks treatment as previously reported [28,30,39]. Moreover, the increase of CETP activity was accompanied by a greater decrease of plasma Lp AI level that became large in size and content, and triglyceride-rich particles. It is known that CETP activity is particularly associated with the large Lp AI fraction in normolipemic subjects [40]. Thus, the effect of Probucol on CETP activity may occur through alteration of CETP catabolism, or stimulation of CETP synthesis, or with both mechanisms.

In normolipemic subjects, plasma LCAT activity was associated primarily with Lp AI (72%), particularly with the large Lp AI fraction that retained 54% of the LCAT activity [40]. Our study showed an increase of plasma LCAT activity after Probucol treatment and indicated that the low HDL-cholesterol levels associated with Probucol treatment are not a priori evidence of atherosclerosis progression.

Isolated particles before Probucol treatment showed a relatively high protein content of total weight as compared to lipoprotein particles isolated from normolipemic subjects [13]. This shift to smaller, protein-rich lipoprotein particles in our patients is in agreement with the study reported by Cheung et al. [41], in which patients suffering from cardiovascular diseases with elevated plasma cholesterol have protein-rich Lp AI and Lp AI:AII particles. Thus, after Probucol treatment protein content of lipoprotein particles reached the values of normolipemic subjects. Previous results indicate that after 18 months of Probucol treatment, the HDL protein content decreased by 56% [42]. Saku et al. [43] have reported that the small Lp AI have a higher fractional catabolic rate than the large Lp A-I. So we suggest that the decrease of the Lp AI level following Probucol treatment may be the result of the decrease of small Lp AI by an enhancement of its fractional catabolic rate and increase of large Lp AI. This change in lipoprotein particle size observed after Probucol treatment may be more active functionally in reverse cholesterol transport. Thus, we suggest that the HDL cholesterol levels following Probucol treatment may reflect increased reverse cholesterol transport.

With respect to lipid composition, our results show that lipoprotein particles have indeed more lipid content after the treatment than before. The isolated Lp AI after Probucol treatment was characterized by higher cholesteryl content than the one isolated before Probucol treatment. This result is in agreement with the results reported by Franceschini et al. [30], in which a 44% increase of cholesterol in HDL$_2$ after Probucol treatment has been found. Interestingly enough and most remarkably, Probucol treatment induces an enhancement of LCAT activity in Lp AI (4.5-fold). In normolipemic subjects LCAT activity in plasma was particularly associated with the large Lp AI fraction [40]. Before Probucol treatment, LCAT activity in Lp AI was lower than in Lp AI:AII. It was shown that in normolipemic subjects, LCAT activity in Lp AI:AII was higher than in small Lp AI [40].

After removing free cholesterol by apo AI-containing lipoprotein particles, it may be esterified by LCAT and the esterified cholesterol is rapidly exchanged by triglyc-
erides. So, isolated particles after Probucol treatment contained more triglycerides than those isolated before the treatment.

The initial step of reverse cholesterol transport consists of cholesterol transfer from the cell surface to accepting particles. The cell-derived cholesterol is rapidly transferred to small HDL particle, pre-beta-1 [44]. It has been suggested that this particle initially accepts peripheral cell free cholesterol and subsequently transports it to larger HDL that contain LCAT where it can be esterified and transferred to LDL [45].

Isolated particles of Lp AI:AIV:AII after Probucol treatment showed a slight cholesterol effluxing capacity. However, isolated particles Lp AI and Lp AIV after Probucol treatment showed a trend toward greater cholesterol efflux than isolated particles before Probucol treatment. No efflux was promoted by Lp AI:AII before and after Probucol treatment. This is of with reference to the previous studies that have demonstrated a close correlation between the extent of xanthoma regression and HDL reduction [8,46]. In addition, Goldberg and Mendez [47] observed an enhancement of the HDL-mediated cholesterol efflux from cultured human skin fibroblasts incubated with Probucol.

Based on the results of lipoprotein particle characterization, LCAT and CETP activities and cholesterol efflux from cholesterol-preloaded Ob1771 adipose cells, we confirm the hypothesis that, in addition to an antioxidant effect of Probucol, the decrease in HDL-cholesterol may not be an atherogenic change, but in contrast may reflect a favorable change for HDL metabolism. This change caused by Probucol accelerates, cholesterol transport through HDL system, promoting reverse cholesterol transport from peripheral tissues.

Probucol has been reported to regress atherosclerosis in animal models and to diminish tendinous xanthomas in man. The present findings suggest that lowering LDL-cholesterol levels, activation of reverse cholesterol transport process, and antioxidant effects of Probucol may cause an antiatherogenic action.

References


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