Probucol Decreases Apolipoprotein A-I Transport Rate and Increases High Density Lipoprotein Cholesteryl Ester Fractional Catabolic Rate in Control and Human Apolipoprotein A-I Transgenic Mice

Tony Hayek, Tova Chajek-Shaul, Annemarie Walsh, Neal Azrolan, and Jan L. Breslow

Probucol effects on lipoprotein metabolism were determined in control and human apolipoprotein A-I transgenic (HuA1Tg) mice. In control mice, probucol reduced total cholesterol from 67±2 to 25±2 mg/dl by reducing high density lipoprotein (HDL) cholesterol from 46±20 to 14±1 mg/dl and low density lipoprotein (LDL) cholesterol from 11±1 to 5±1 mg/dl. Apolipoprotein (apo) A-I levels were reduced from 122±8 to 56±5 mg/dl. In HuA1Tg mice, probucol reduced total cholesterol from 121±5 to 77±3 mg/dl by reducing HDL cholesterol from 84±4 to 56±3 mg/dl and LDL cholesterol from 19±2 to 11±2 mg/dl. Human apo A-I levels were reduced from 267±13 to 144±12 mg/dl and mouse apo A-I levels from 18±2 to 9±2 mg/dl. Control animals have primarily a monodisperse HDL with a particle diameter of 10 nm. Probucol did not appear to change the particle size distribution in the control animals. The HuA1Tg mice have a polydisperse HDL with particle diameters of 10.1 and 8.5 nm. Probucol treatment of these animals resulted in HDL with particle diameters of 9.4 and 8.5 nm, apparently reducing the size of the larger particles. In vivo turnover studies revealed that the reduction in apo A-I was primarily due to a decrease in transport rate, whereas the reduction in HDL cholesterol was primarily due to an increase in HDL cholesteryl ester fractional catabolic rate. The decrease in apo A-I transport rate was not associated with a decrease in liver apo A-I mRNA levels. Due to the low HDL cholesterol levels in probucol-treated animals, there was no probucol-induced increase in HDL cholesteryl ester total transport rate. The drug-induced decrease in LDL cholesterol levels was associated with a 50% decrease in liver cholesterol synthesis. These studies show that probucol has a multifaceted effect on the lipoprotein system. (Arteriosclerosis and Thrombosis 1991;11:1295-1302)

Probucol [bis(3,5-d-tert-butyl-4-hydroxyphenylthio)propane] was approved in 1977 for use in humans as a plasma cholesterol-lowering agent. In the early 1980s, it became apparent that probucol lowered both the "harmful" low density lipoprotein (LDL) and the "good" high density lipoprotein (HDL) cholesterol fractions.1-3 In most patients it did not improve the LDL to HDL cholesterol ratio. Concern over probucol's effect on LDL cholesterol curtailed its use. However, ongoing clinical studies have shown that probucol administration is associated with regression of cutaneous and tendinous xanthomas4 as well as coronary artery stenoses.5 In cholesterol-fed and Watanabe heritable hyperlipidemic rabbits, probucol has been shown to inhibit atherosclerotic plaque formation and stimulate plaque regression, independent of its effect on plasma cholesterol concentration.6,7 These paradoxical findings have led to intense interest in the study of probucol's mechanism of action.

The antiatherogenic activity of probucol, despite its lowering of HDL, has been explained by two mechanisms. Probucol has been shown in humans to increase cholesteryl ester transfer protein (CETP) activity in plasma.8-10 It is postulated that this would increase the transfer of HDL cholesteryl esters to apolipoprotein (apo) B-containing lipoproteins,
which are then cleared by hepatic LDL receptors. Thus, cholesteryl ester transport to the liver (so-called reverse cholesterol transport) would be enhanced and atherosclerosis diminished, even though HDL cholesterol is reduced. Second, probucol is lipophilic and a potent antioxidant.\textsuperscript{11-13} It is carried in LDL and could prevent oxidative modification of LDL in the subendothelial space, which is thought to be an early pathogenic event in the formation of foam cells.\textsuperscript{14,15} By this mechanism, probucol could inhibit atherogenesis, independent of its effect on HDL metabolism.

In the current study we have used control and human apo A-I transgenic (HuAITg) mice to examine the effect of probucol on HDL metabolism. Mice do not have CETP activity in plasma, and it was of interest to examine what effect, if any, probucol would have on HDL metabolism in this species. HuAITg mice were used because, as we have shown elsewhere, they have principally human apo A-I in their plasma and their HDL pattern is human-like, with HDL\(_2\) and HDL\(_3\) subfractions, whereas control mice have only one main HDL component.\textsuperscript{16} In theory then the HuAITg mice would be a better model to study probucol’s effects on HDL particle size distribution. In these mouse model studies, we found that probucol decreased apo A-I, HDL cholesterol, and LDL cholesterol concentrations in both control and transgenic animals. The mechanisms of apo A-I reduction appeared to be decreasing the apo A-I transport rate without decreasing hepatic apo A-I mRNA levels. The decrease in HDL cholesterol was associated with an increase in HDL cholesteryl ester fractional catabolic rate but not with enhanced total transport of HDL cholesteryl ester to the liver. Probucol treatment also caused a decrease in LDL cholesteryl levels and hepatic cholesterol synthesis. The latter may cause increased hepatic LDL receptors and may account for the decrease observed in LDL cholesterol. Thus, probucol appears to have several effects on lipoprotein metabolism, which may be unrelated.

**Methods**

**Animals**

We previously described five lines of HuAITg mice.\textsuperscript{16} The animals with the highest level of human apo A-I expression, Tg 427, were used for the current studies. In these animals human apo A-I was incorporated into HDL, and plasma HDL cholesterol was directly proportional to human apo A-I expression. Transgenic (Tg) animals were (C57BL/6J×CBA/J) F1s. These were mated with the same F1 nontransgenic mice, and Tg and non-Tg littersmates were compared in the current study. Tg animals were identified both by Southern blotting analysis of tail tip DNA to identify the human apo A-I gene and by quantitative enzyme-linked immunosorbent assay (ELISA) specific for human apo A-I.\textsuperscript{17} In the Tg animals human apo A-I was expressed only in the liver.\textsuperscript{16} In control animals the endogenous apo A-I gene was expressed equally and almost exclusively in the liver and intestine. The Tg mice underexpress mouse apo A-I, with 80–90% of apo A-I in plasma being human apo A-I. The mice were used for metabolic studies at 10–15 weeks of age (24–30 g weight). They were caged in an animal room with alternating 12-hour light (7 AM to 7 PM) and dark (7 PM to 7 AM) cycles. The mice had free access to food and water. The mice were fed a regular powdered mouse chow diet (Purina Chow) with no addition (placebo) or containing 0.2% probucol (wt/wt) for 2 weeks. Probucol levels were measured at the end of the study by high-performance liquid chromatography\textsuperscript{18} by Simon Mao at Merrell Dow Pharmaceuticals Inc. Control and HuAITg mice had levels of 3.1±0.1 and 6.2±0.2 µg/ml, respectively. Probucol is lipophilic and is transported in lipoproteins. Levels are proportional to plasma lipoprotein concentrations.

**Preparation of High Density Lipoprotein Labeled in the Apolipoprotein A-I and Cholesteryl Ester Moieties**

Human apo A-I was purified and radiolabeled with iodine-125 by the Bilheimer modification of the McFarlane method as previously described.\textsuperscript{19,20} Serum from control or HuAITg mice was incubated with tritiated cholesteryl linoleyl ether (specific activity, 46.6 Ci/mm; Amersham, Amersham, UK), intralipid, and d>1.25 g/ml rabbit plasma (a source of CETP) for 16 hours at 37°C as previously described.\textsuperscript{21} The labeled HDL fraction was isolated by sequential ultracentrifugation between d=1.063 and 1.21 g/ml, dialyzed against five changes of 110.9% NaCl and 1 mM EDTA for 20 hours, and used immediately.\textsuperscript{125}I–apo A-I was mixed with the [\textsuperscript{3}H]cholesteryl linoleyl ether–labeled HDL before injection. This procedure was associated with a greater than 90% recovery of the [\textsuperscript{3}H]cholesteryl linoleyl ether in the plasma 10 minutes after its injection.\textsuperscript{21}

**In Vivo Turnover Studies**

Mice were injected intravenously (femoral vein) with either control or HuAITg mouse HDL doubly labeled with \textsuperscript{125}I–human apo A-I and [\textsuperscript{3}H]cholesteryl linoleyl ether. The injected HDL mass was less than 5% of the mouse HDL pool. Fifty microliters of blood was taken from the retro-orbital plexus at the indicated time intervals for determination of radioactivity. The fractional catabolic rates for apo A-I and HDL cholesteryl linoleyl ether were calculated from the plasma decay curves, assuming a two-pool model by the Matthews method.\textsuperscript{22}

**In Vivo Sterol Synthesis**

One hour after intraperitoneal injection of [\textsuperscript{3}H]H\(_2\)O (Amersham), mice were exsanguinated through the heart, and incorporation of [\textsuperscript{3}H]H\(_2\)O into tissue cholesterol was determined as described by Feingold and Grunfeld.\textsuperscript{23}
TABLE 1. Effect of Probucol on Lipoprotein Profile in Control and HuAITg Mice

<table>
<thead>
<tr>
<th>Group/treatment time</th>
<th>TC (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>Mouse apo A-I (mg/dl)</th>
<th>Human apo A-I (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=16)</td>
<td>Before</td>
<td>66±2</td>
<td>47±2</td>
<td>12±1</td>
<td>7±1</td>
<td>126±11</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>63±2</td>
<td>43±2</td>
<td>13±1</td>
<td>6±1</td>
<td>128±12</td>
</tr>
<tr>
<td>Probucol (n=16)</td>
<td>Before</td>
<td>67±2</td>
<td>46±2</td>
<td>11±1</td>
<td>9±2</td>
<td>122±8</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>25±2*</td>
<td>14±1*</td>
<td>5±1†</td>
<td>7±2</td>
<td>56±5*</td>
</tr>
<tr>
<td><strong>HuAITg mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n=17)</td>
<td>Before</td>
<td>119±4</td>
<td>83±3</td>
<td>17±2</td>
<td>19±2</td>
<td>16±2</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>117±4</td>
<td>81±2</td>
<td>17±2</td>
<td>19±3</td>
<td>16±2</td>
</tr>
<tr>
<td>Probucol (n=17)</td>
<td>Before</td>
<td>121±5</td>
<td>84±4</td>
<td>19±2</td>
<td>17±3</td>
<td>18±2</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>77±3*</td>
<td>56±3*</td>
<td>11±2†</td>
<td>12±2</td>
<td>9±2†</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; apo, apolipoprotein; HuAITg, human apo A-I transgenic.

* p<0.001 compared with pretreatment; † p<0.005 compared with pretreatment.

**Apolipoprotein A-I Quantification**

Human apo A-I was quantified by a sandwich ELISA with a polyclonal goat anti-human apo A-I antibody that had less than 0.01% cross reactivity with mouse apo A-I. Mouse apo A-I was quantified by rocket electroimmunoassay with a polyclonal anti-mouse apo A-I antibody prepared in cynomolgus monkeys and generously supplied by George Melchior of The Upjohn Company, Kalamazoo, Mich. This antibody had less than 0.01% cross reactivity with human apo A-I.

**Plasma Lipid and Lipoprotein Analysis**

Total cholesterol was determined enzymatically with the use of Boehringer Mannheim reagents (Indianapolis, Ind.). Lipoprotein fractions were separated by ultracentrifugation in an airfuge, as described previously. To determine the level of HDL subfractions, VLDL plus LDL plus HDL₂ (d<1.125 g/ml) in the supernatant were separated from HDL₃ in the infranatant by spinning 50 µl plasma, whose density had been preadjusted to 1.125 g/ml with a d=1.35 g/ml KBr solution and which was overlaid with 50 µl of a d=1.125 g/ml KBr solution, for 4.5 hours. HDL₃ cholesterol was calculated by subtracting HDL₂ cholesterol from HDL cholesterol. Nondenaturing gradient gel electrophoresis was performed to determine HDL particle size by the method of Nichols with 4–30% Pharmacia polyacrylamide gels (Pharmacia, Piscataway, N.J.).

**Apolipoprotein A-I mRNA Quantification**

Liver apo A-I mRNA levels were quantified by a solution hybridization/RNase protection assay with riboprobes to detect both mouse and human apo A-I mRNA as previously described. With the animals allowed free access to food and water, an open liver biopsy to remove 100 mg tissue from an anterior lobe was performed between 8 AM and 10 AM before treatment and after 2 weeks of placebo or probucol. RNA was isolated by the method of Chomczynski and Sacchi. The human apo A-I-specific riboprobe was derived from PAI-113 and corresponded to nucleotides 390–620. The mouse apo A-I mRNA-specific riboprobe was derived from a mouse apo A-I cDNA clone isolated from a C57BL mouse line cDNA library; the isolated cDNA fragment corresponded to nucleotides 650–838 of the human apo A-I cDNA and was subcloned into a PGEM1 plasmid (Promega, Madison, Wis.). T7 RNA polymerase was used for riboprobe synthesis as previously described. The human and mouse riboprobes displayed less than 60% homology. Background radioactivity for each probe was routinely less than 0.4% of total counts by the filter counting assay.

**Results**

Total plasma and lipoprotein subfraction cholesterol concentrations and apo A-I levels were determined before and after probucol administration in control and HuAITg mice (Table 1). In control mice probucol reduced total cholesterol from 67±2 to 25±2 mg/dl. This was accomplished by reducing HDL cholesterol from 46±2 to 14±1 mg/dl and LDL cholesterol from 11±1 to 5±1 mg/dl. Mouse apo A-I was also reduced from 122±8 to 56±5 mg/dl. In the HuAITg mice probucol reduced total cholesterol from 121±5 to 77±3 mg/dl. In these animals HDL cholesterol was reduced from 84±4 to 56±3 mg/dl. HDL₃ did not change and was 16±2 and 14±2 mg/dl before and after treatment, respectively. Therefore, HDL₂ appeared to be the fraction most affected by probucol treatment, decreasing from 68±6 to 42±3 mg/dl. LDL cholesterol was reduced from 19±2 to
11±2 mg/dl. Human apo A-I was reduced from 267±13 to 144±12 mg/dl. The HuAITg mice had a greatly reduced level of mouse apo A-I in their plasma compared with that in control animals (Table 1). Probucol also reduced the mouse apo A-I levels in the plasma of the HuAITg mice from 18±2 to 9±2 mg/dl. 

Nondenaturing gradient gel electrophoresis of control and HuAITg mouse plasma before and after probucol treatment was performed (Figure 1). In the control animals pretreatment HDL was monodisperse, with a particle diameter of 10 nm. Probucol treatment did not change the HDL particle size distribution in control animals. In the HuAITg animals pretreatment HDL showed two major particle diameters of 10.1 and 8.5 nm, which correspond to human HDL₂ and HDL₃. After probucol treatment there was a marked decrease in the HDL₂ particle size to 9.4 nm, without a concomitant change in the HDL₃.

To examine the metabolic mechanism behind the probucol-induced decrease in plasma apo A-I and HDL cholesterol concentrations, we performed a turnover study. HDL labeled with ¹²⁵I-apo A-I and [³H]cholesteryl ether was injected intravenously into placebo and probucol-treated animals. The plasma radioactivity decay curves for the apo A-I label are shown in Figure 2, and the calculated apo A-I fractional catabolic rates are shown in Table 2. In control and HuAITg animals probucol increased the apo A-I fractional catabolic rate by 14% and 9%, respectively (not statistically significant). These increases in fractional catabolic rate accounted for only a small fraction of the 54% and 46% decreases in total plasma apo A-I observed in control and HuAITg animals, respectively. This indicated that the major manner by which probucol decreases plasma apo A-I concentrations is by decreasing the apo A-I transport rate.

The plasma radioactivity decay curves for the HDL cholesteryl ether label are shown in Figure 3, and the calculated HDL cholesteryl ester fractional catabolic rates are shown in Table 2. As we have previously documented, the HDL cholesteryl ester fractional catabolic rate is much greater in control animals than in HuAITg mice. We have suggested that this is due to...
TABLE 2. Effect of Probucol on Apolipoprotein A-I and High Density Lipoprotein Cholesteryl Ester Fractional Catabolic Rates in Control and HuAITg Mice

<table>
<thead>
<tr>
<th>Group/treatment</th>
<th>Control mice</th>
<th>HuAITg mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n=8)</td>
<td>Probucol (n=8)</td>
</tr>
<tr>
<td>Human apo A-I</td>
<td>273±8</td>
<td>140±14*</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse apo A-I</td>
<td>128±11</td>
<td>52±8*</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A-I FCR</td>
<td>0.11±0.01</td>
<td>0.12±0.02</td>
</tr>
<tr>
<td>(pools/hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>48±2</td>
<td>54±3*</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-CE FCR</td>
<td>0.16±0.01</td>
<td>0.15±0.01†</td>
</tr>
<tr>
<td>(pools/hr)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Apo, Apolipoprotein; FCR, fractional catabolic rate; HDL-C, high density lipoprotein cholesterol; CE, cholesteryl ester; HuAITg, human apo A-I transgenic.

*p<0.001 compared with placebo; †p<0.005 compared with placebo.

The mechanism of the reduction in apo A-I levels and transport rate was next examined by measuring apo A-I mRNA levels. In control animals placebo and probucol-treated liver apo A-I mRNA levels were 29±3 and 32±2 pg/mg RNA, respectively. In HuAITg animals placebo and probucol-treated liver human apo A-I mRNA levels were 255±26 and 250±30 pg/mg RNA, respectively. In no case did the drug significantly reduce apo A-I mRNA levels. Thus, the effect of probucol on apo A-I transport rate is not transcriptional, but at the level of mRNA translation or protein secretion.

The effect of probucol on liver and small-intestine cholesterol synthesis rates was examined next. In both control and HuAITg mice, probucol reduced liver cholesterol synthesis (Table 3) by approximately 50%, without a significant effect on intestinal cholesterol synthesis. In this experiment placebo-treated HuAITg mice had a slightly lower cholesterol synthesis in the liver and intestine than did control mice. However, in a larger series of animals this difference was not significant.

FIGURE 3. Doubly labeled high density lipoprotein (HDL) plasma radioactivity decay curves for the HDL cholesteryl ether label. Control (Cont) mice treated with placebo (○) or probucol (▲) were injected intravenously with doubly labeled control mouse HDL. HuAITg mice treated with placebo (○) or probucol (●) were injected intravenously with doubly labeled HuAITg mouse HDL. Fifty microliters of blood was taken from the retro-orbital plexus at indicated time intervals (Hours) for determination of tritiated cholesteryl linoleyl ether radioactivity. Hu AI Tg, human apolipoprotein A-I transgenic.
was on the HDL cholesteryl ester moiety. As inferred with primates plasma apo A-I and HDL cholesterol probucol did not have a different effect on mouse apo A-I mRNA abundance. In HuAITg mice we have reported that plasma apo A-I and HDL cholesterol levels are proportional to the expression of the apo A-I transgene. It was therefore surprising that the concentration differences between two species were not accompanied by an increase in the HDL cholesteryl ether fractional catabolic rate. In transgenic animals with polydisperse HDL, probucol mainly appears to decrease the size and amount of the larger HDL particles. In humans larger HDL (HDL₂) contains mainly apo A-I particles. The marked effect of probucol on apo A-I transport rate would be expected to diminish the number of apo A-I molecules available for particle formation and could affect particle size, as was observed. In human studies probucol has been shown to primarily affect the size and amount of HDL₃. In metabolic studies by several groups have shown that the drug decreases the apo A-I transport rate. In the mouse model we have shown that the decrease in apo A-I transport rate was not accompanied by a decrease in hepatic apo A-I mRNA levels. Of course, in human studies hepatic apo A-I mRNA levels could not be measured. However, in studies with primates plasma apo A-I and HDL cholesterol concentration differences between two species were correlated with differences in liver and intestinal apo A-I mRNA abundance. In HuAITg mice we have reported that plasma apo A-I and HDL cholesterol levels are proportional to the expression of the transgene. It was therefore surprising that the pronounced effect of probucol on diminishing apo A-I and HDL cholesterol levels was not accompanied by a decrease in liver mouse or human apo A-I mRNA concentrations. Our mouse studies imply that probucol decreases apo A-I mRNA translatability or apo A-I protein secretion. It is also interesting that probucol did not have a different effect on mouse compared with human apo A-I levels or metabolism.

The second effect of probucol on HDL metabolism was on the HDL cholesteryl ester moiety. As inferred by turnover studies with radiolabeled HDL cholesteryl ester, probucol appeared to increase the HDL cholesteryl ether fractional catabolic rate in both control and HuAITg mice. HDL cholesteryl ester clearance is performed principally by the liver, and evidence has been presented for three mechanisms: particle uptake, selective uptake, and CETP-mediated transfer to apo B-containing lipoproteins, which are removed from the circulation by liver LDL receptors. We have previously presented evidence that in control mice, HDL cholesteryl ester is cleared by both particulate uptake and selective uptake, whereas in HuAITg animals clearance occurs only by particulate uptake. This accounts for the marked difference in HDL cholesteryl ester fractional catabolic rate between control and HuAITg animals in the placebo-treated group in the current study. After probucol treatment in both control and HuAITg animals, the HDL cholesteryl ester fractional catabolic rate increased out of proportion to the apo A-I fractional catabolic rate. This suggests that probucol increases selective uptake even in HuAITg animals. It is also possible that probucol induces a CETP-like activity in the mouse, transferring HDL cholesteryl ester to apo B-containing particles that are then cleared by hepatic LDL receptors. In any case, the decrease in HDL cholesteryl in probucol-treated mice appears to be due to an increase in HDL cholesteryl ester fractional catabolic rate. Although LDL cholesterol levels are low to begin with in mice, probucol caused about a 50% further decrease in both control and HuAITg animals. This was associated with a 50% decrease in liver sterol synthesis, as measured by incorporation of [³H]H₂O into cholesterol. There was no drug effect on intestinal sterol synthesis. In other studies, Tawara et al. found in probucol-treated mice decreased acetate incorporation into cholesterol in liver but not intestine. Freeman et al. reported that probucol-treated mice had decreased hepatic hydroxymethylglutaryl coenzyme A reductase activity, and Balasubramaniam et al. and Li et al. have separately reported similar findings in rats. In humans, Miettinen has reported that probucol reduces whole-body cholesterol synthesis, and McNamara et al. have reported a decrease in lymphocyte cholesterol synthesis in patients on probucol treatment. The decrease in liver sterol synthesis might be accompanied by increased liver LDL receptors and increased LDL clearance. This is one possible mechanism for explaining reduced LDL cholesterol levels in the drug-treated mice. In rabbits it was previously shown that probucol altered LDL structure and causes enhanced catabolism by an LDL receptor-independent mechanism. This effect of probucol could also be operative in mice. The mechanism of LDL cholesterol lowering by probucol in humans has been explored by several groups. In vivo turnover studies have shown an increased LDL fractional catabolic rate without an effect on total turnover.
Probufol appears to have a multifaceted effect on lipoprotein levels and metabolism. The current studies do not directly address the mechanism of the drug's possible antiatherosclerotic action. The mouse, perhaps because of its low ratio of LDL to HDL cholesterol, is an atherosclerosis-resistant species, and we did not undertake to see whether the drug reduced lesions. However, aspects of the drug's effects on lipoprotein metabolism may be relevant. The decrease in LDL cholesterol and decrease in liver cholesterol synthesis certainly may be antiatherogenic. With regard to the effects on apo A-I and HDL cholesterol, it is hard to link these to an antiatherogenic mechanism. The lowering of apo A-I transport rate and plasma levels would appear to be proatherogenic in view of the data correlating apo A-I levels with reduced risk of coronary heart disease. The studies in rabbits showing the antiatherogenic effects of infusing preparations of apo A-I, whose principle component is apo A-I, the increase of HDL cholesterol ester fractional catabolic rate by probufol appears at first glance to have the potential to be antiatherogenic. However, because of the lower HDL cholesterol levels in the drug-treated animals, the total HDL cholesterol ester transport rate is not increased. Thus, reverse cholesterol transport does not appear to be accelerated by the drug. From these studies and those showing that probufol prevents LDL oxidation, we suggest that the major antiatherosclerotic effect of probufol is on LDL, not HDL. Because the drug effects on these two lipoprotein fractions are not necessarily related, perhaps analogues of probufol that do not lower apo A-I and HDL cholesterol concentrations would be even more effective antiatherosclerosis agents.

References
6. Carew TE, Schwenke DC, Steinberg D: Antiatherogenic effect of probucol unrelated to its hypcholesterolemic 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 WHHL rabbit. Proc Natl Acad Sci U S A 1987;84:7725–7729.
15. Parthasarathy S: Oxidation of low-density lipoprotein by thiol compounds leads to its recognition by the acetyl LDL receptor. Biochim Biophys Acta 1987;917:337–340.
30. Law SW, Brewer HB: Nucleotide sequence and the encoded amino acids of human apolipoprotein A-I mRNA. *Proc Natl Acad Sci USA* 1984;82:992–996


**KEY WORDS** • probucol • transgenic mice • high density lipoproteins • fractional catabolic rate • transport rate
Probucol decreases apolipoprotein A-I transport rate and increases high density lipoprotein cholesteryl ester fractional catabolic rate in control and human apolipoprotein A-I transgenic mice.

T Hayek, T Chajek-Shaul, A Walsh, N Azrolan and J L Breslow

_Arterioscler Thromb Vasc Biol_. 1991;11:1295-1302
doi: 10.1161/01.ATV.11.5.1295

_Arteriosclerosis, Thrombosis, and Vascular Biology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/11/5/1295

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Arteriosclerosis, Thrombosis, and Vascular Biology_ 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 _Arteriosclerosis, Thrombosis, and Vascular Biology_ is online at:
http://atvb.ahajournals.org//subscriptions/