Probucol Treatment Affects the Cellular Composition but Not Anti-Oxidized Low Density Lipoprotein Immunoreactivity of Plaques From Watanabe Heritable Hyperlipidemic Rabbits

Kevin O'Brien, Yutaka Nagano, Allen Gown, Toru Kita, and Alan Chait

Use of the antioxidant probucol has been associated with a reduction in the development of atherosclerotic lesions in Watanabe heritable hyperlipidemic (WHHL) rabbits, an animal model of familial hypercholesterolemia. In this study, atheromatous lesions from control or probucol-treated WHHL rabbits were probed with monoclonal antibodies to evaluate whether use of this drug either affected the presence or distribution of epitopes recognized by an antibody against oxidized low density lipoprotein or altered the cellular makeup of lesions. Although probucol-treated animals had much less aortic atherosclerosis than did controls, equivalent immunoreactivity for the anti-oxidized LDL antibody (OXL 41.1) was demonstrated in atherosclerotic lesions of both groups of animals, consistent with a role for oxidative modification of lipoproteins in atherogenesis in this animal model. Use of smooth muscle cell-specific (HHF-35) and macrophage-specific (RAM-11) antibodies demonstrated that lesions from probucol-treated animals were significantly smaller \((p<0.01)\) and less cellular \((p<0.05)\) than were control lesions. In addition, smooth muscle cells were the predominant cell type in lesions from probucol-treated animals, whereas macrophages predominated in lesions from controls \((p<0.01)\). These findings are consistent with a reduction of monocyte/macrophage recruitment into or retention in lesions in probucol-treated animals, or with probucol-induced alterations in the production of growth factors or cytokines that might influence the cellular makeup of atherosclerotic lesions. (Arteriosclerosis and Thrombosis 1991;11:751-759)

The early cellular lesion of atherosclerosis consists of lipid-laden foam cells that have been shown to be derived primarily from macrophages. These foam cells are characteristic of both the tendinous xanthomata and the atherosclerotic plaques of patients homozygous for the familial hypercholesterolemia (FH) trait. Several years ago, Brown and Goldstein observed that FH homozygotes lack functional cell-surface receptors that recognize and internalize unmodified low density lipoprotein (LDL). This led to the discovery that macrophages possess another class of receptors, scavenger receptors, which allow them to incorporate large amounts of certain modified forms of LDL such as acetyl LDL and oxidized LDL. Several in vitro lines of evidence suggest that oxidatively modified LDL may play an important role in foam cell formation and, therefore, in the development and progression of atherosclerosis. These include the observations that oxidatively modified LDL has an increased rate of uptake by, is a chemotactrant for, and inhibits the mobility of macrophages. Minimally oxidized LDL also stimulates the production of a chemotactic factor by endothelial cells and increases monocyte binding to endothelial cells. In addition, recent in vivo studies have demonstrated the pres-
ence, in arterial lesions from Watanabe heritable hyperlipidemic (WHHL) rabbits (an animal model for FH), epitopes recognized by antibodies to an oxidatively modified form of LDL, malondialdehyde (MDA)-modified LDL.9-11

Further indirect evidence implicating a role for oxidatively modified LDL in the development of atherosclerosis comes from studies in which probucol was administered to young WHHL rabbits.12-13 In one study, short-term (6-month) probucol treatment was associated with a dramatic reduction in the rate of development of atherosclerosis, despite only a modest reduction in total cholesterol levels.12 The other study showed a similar dramatic effect of probucol therapy on the development of atherosclerotic plaques, even when the minimal hypocholesterolemic effect of probucol was controlled for by the use of lovastatin. Because the beneficial effect of probucol was shown to be independent of its hypocholesterolemic action, the dramatic decrease in atherosclerosis seen13 was attributed primarily to probucol's well-recognized antioxidant properties.14 However, probucol recently has been shown to have additional potentially antiatherogenic properties, such as the ability to decrease production of interleukin-1, a cytokine that mediates inflammation by activating thymocytes and that induces proliferation of aortic smooth muscle cells.15

We recently have characterized an antibody, OXL 41.1, which has been shown by both enzyme-linked immunosorbent assay (ELISA) and Western blot to react with oxidatively modified LDL but not to react with unmodified LDL.16 The OXL 41.1 antibody also reacts with certain other modified forms of LDL, such as acetyl LDL, MDA-conjugated LDL, and cell-modified LDL, all of which are recognized by the macrophage scavenger receptor. This antibody gave a pattern of immunoreactivity with atheromatous lesions from WHHL rabbits that was similar to that obtained with an anti-oxidized LDL antibody described elsewhere.10,11

In the present study, we used this antibody against oxidatively modified LDL (OXL 41.1), an antibody that recognizes an epitope at the LDL receptor–binding domain of unmodified LDL (MB-47), and antibodies that are specific for muscle actin isotypes (HHF-35) and rabbit macrophages (RAM-11) to stain sections of aortas from probucol-treated and control WHHL rabbits to be described in greater detail elsewhere. In view of the antioxidant properties of probucol, the following questions were addressed: 1) Can oxidatively modified LDL be detected in atherosclerotic plaques of both probucol-treated and control WHHL rabbits? 2) Is oxidatively modified LDL present in treated or control animals in areas of the aorta without atherosclerotic involvement? 3) Is there a difference in the location or amount of oxidized LDL as compared with unmodified LDL in lesions from probucol-treated versus control animals? 4) Since chronic inflammation and smooth muscle cell proliferation, both of which can be affected by interleukin-1, occur in atherosclerosis and since interleukin-1 production can be modulated by probucol,16 the question of whether the macrophage or the smooth muscle cell content of atherosclerotic lesions differed between probucol-treated and control animals was also addressed.

Methods

Animals

Homozygous WHHL rabbits were raised in Kyoto by mating heterozygous and/or homozygous female WHHL rabbits with homozygous male WHHL rabbits. At 2 months of age, 13 rabbits were divided into two groups (control and probucol treated). Control rabbits (three female and four male) were fed standard rabbit chow for 12–16 months, while probucol-treated rabbits (three female and three male) were given rabbit chow enriched with 1% (wt/wt) probucol for 15–17 months. The amount of diet for each animal was restricted to 100 g/day during the study period. Water was supplied ad libitum. At the time the animals were sacrificed (at the age of 14–19 months), blood samples and aortas were taken for analysis.

Calculation of Area With Atheromatous Plaque

Thoracic aortas were opened longitudinally, and photographs of the inner surfaces were taken. Then, the photographs were copied onto graph paper with magnification (×2), and atheromatous plaques were delineated. The numbers of small squares surrounded by the line were counted on the graph paper, and the percentages of the areas of atheromatous plaque were calculated. Each thoracic aorta was divided in two sections (aortic arch and descending portion) at the level of the first intercostal artery, and the percentage of each part involved with atherosclerosis also was calculated.

Collection of Aortic Specimens for Immunocytochemistry

Small portions of the thoracic aorta were taken from each animal at the level of the first intercostal artery. Aortic specimens to be used for immunofluorescence were placed in phosphate-buffered saline (PBS) with 25 μM butylated hydroxytoluene (BHT) and 50 μM diethylenetriaminepentaacetic acid (DTPA) to inhibit ex vivo oxidative modification, and then embedded in OCT (Miles Laboratories, Inc., Kankakee, Ill.) and snap frozen in liquid N2-cooled isopentane. These specimens were stored in the gas phase of a liquid N2 freezer until use to minimize the possibility of oxidation during processing.

Additional pieces of aorta to be used for single-label immunofluorescent studies were fixed in methanol–Carney's fixative containing 25 μM BHT and 50 μM DTPA and stored at 4°C. After transfer to 100% methanol, the specimens were embedded in paraffin.
**Immunofluorescence on Tissue Sections**

Single-label immunofluorescence was performed as follows. Six-micron-thick frozen sections were cut, air dried, and stored under N₂ at 4°C overnight. The sections were washed in PBS, followed by PBS/0.1% bovine serum albumin (BSA)/0.01% Triton X, and then again in PBS. The primary antibodies, MB-47 (an immunoglobulin G [IgG] monoclonal antibody against native LDL and a generous gift of Linda J. Curtiss) and OXL 41.1 (an IgM monoclonal antibody against oxidatively modified LDL), were diluted 1:100 in PBS/1% BSA and then incubated with adjacent tissue sections for 30 minutes at room temperature. The washing sequence was repeated, and fluorescein isothiocyanate (FITC)–conjugated anti-mouse IgG (Organon Teknika-Cappel, Durham, N.C., for anti-LDL antibody MB-47) or FITC-conjugated anti-mouse IgM (Zymed Laboratories, Inc., South San Francisco, Calif., for anti-oxidized LDL antibody OXL 41.1), diluted in PBS/1% BSA, was added for a 30-minute incubation at room temperature.

For double-label immunofluorescence, the above procedure was performed with MB-47 and rhodamine isothiocyanate (RITC)–conjugated anti-mouse IgG (Organon Teknika-Cappel), followed by OXL 41.1 and FITC-conjugated anti-mouse IgM on the same tissue section. In two animals (one probucol-treated and one control), double-label studies also were performed using FITC–anti-mouse IgG and RITC–anti-mouse IgM.

**Alkaline Phosphatase Immunocytochemistry on Paraffin-Embedded Sections**

The avidin–biotin immunooalkaline phosphatase method of Ormanns and Schaffer¹⁷ was employed, with reagent and substrate obtained from Vector Laboratories (Burlingame, Calif.).

**Analysis of Plaque Size, Cellularity, and Predominant Cell Type**

Atherosclerotic lesions from adjacent tissue sections stained with RAM-11 (anti-macrophage) and HHF-35 (anti-muscle actin) were examined to determine lesion size, cellularity, and predominant cell type. Each lesion was evaluated by two independent observers blinded to treatment group, for each of the following characteristics, with an interobserver agreement of nearly 100%.

**Lesion size.** A size score of 1 to 3 was assigned to each lesion by determining whether the length of the lesion base was less than or equal to 1 mm (score=1), greater than 1 mm to less than or equal to 2 mm (score=2), or greater than 2 mm (score=3).

**Lesion cellularity.** Lesion cellularity scores of 1 to 3 were determined by standard histological grading criteria,¹⁸ with a score of 1 corresponding to “low” cellularity and scores of 2 and 3 corresponding to “intermediate” and “high” cellularity, respectively.

**Lesion type.** Lesion type was determined by use of a semiquantitative scale of 1 to 3, corresponding to tertiles of the proportion of RAM-11 (anti-macrophage) antibody-positive cells to total cell-specific (RAM-11 or HHF-35) antibody-positive cells.¹⁶ Therefore, a score of 1=0–33% of cells RAM-11–positive (or 100–67% HHF-35–positive), 2=34–66% RAM-11–positive (or 66–34% HHF-35–positive), and 3=67–100% RAM-11–positive (or 33–0% HHF-35–positive). Lesions with a score of 1 would therefore consist predominantly of smooth muscle cells (“smooth muscle rich”), those with a score of 2 have approximately equivalent numbers of smooth muscle cells and macrophages (“mixed”), and lesions with a score of 3 have a predominance of macrophages (“macrophage rich”).

**Statistical Analysis of Lesion Characteristics**

Because lesions obtained from a single animal cannot be considered independent observations,¹⁹ a mean score for each characteristic (lesion size, cellularity, and type) was obtained for each animal by averaging the individual scores for all lesions from that animal. Student’s t test then was performed to determine the significance of differences in mean scores for lesion size, type, and cellularity between probucol-treated and control groups.

**Results**

**Plasma Lipids and Lipoproteins and Atherosclerotic Involvement of Control and Probucol-Treated Watanabe Heritable Hyperlipidemic Rabbits at End of Experiment**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n=7)</th>
<th>Probucol-treated (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>15.7±1.8</td>
<td>18.0±1.1</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3,543±783</td>
<td>2,742±225</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>372±95</td>
<td>445±79</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>302±104</td>
<td>314±106</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>13.7±3.5</td>
<td>6.3±2.2</td>
</tr>
<tr>
<td>VLDL cholesterol (mg/dl)</td>
<td>46.8±32.5</td>
<td>124±72</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>228±58</td>
<td>511±306</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.6±0.7</td>
<td>7.1±0.8</td>
</tr>
</tbody>
</table>

All values are reported as mean±SD. LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein.

There were no important differences between probucol-treated and control WHHL rabbits in baseline characteristics at the end of the study (Table 1). Despite probucol treatment, at the end of the experiment mean total serum cholesterol level in the treated group (445±79 mg/dl [mean±SD]) was similar to that of the control group (372±95 mg/dl (p=NS). Probucol-treated animals had higher triglyceride levels and lower high density lipoprotein levels than did controls; this is an expected effect of probucol treatment.¹²,¹³,²⁰ Although total cholesterol levels were not different between groups, probucol treatment was associated with a marked decrease (as compared with controls) in the amount of atheroscle-
FIGURE 1. Photomicrographs of double-label immunofluorescence of lesions from a representative control Watanabe heritable hyperlipidemic (WHHL) rabbit immunoreacted with MB-47 (upper left panel) and OXL 41.1 (upper right panel), and from a probucol-treated WHHL rabbit immunoreacted with MB-47 (lower left panel) or OXL 41.1 (lower right panel). No difference was seen in the amount of OXL 41.1 immunoreactivity in control (upper right panel) versus treated (lower right panel) animals. Lesions could be found in which OXL 41.1 stained the intima diffusely (upper right panel) or in which OXL 41.1 staining was more prominent in the luminal portion of the intima (lower right panel), but each pattern of staining could be found in lesions from both control and probucol-treated animals. Original magnification, ×100.

Distribution of Oxidized Low Density Lipoprotein and Unmodified Low Density Lipoprotein Immunoreactivity in Atherosclerotic Plaques of Probucol-Treated and Control Animals

Adjacent sections of rabbit aorta were cut from frozen sections and examined by indirect immunofluorescence using either antibody OXL 41.1 or antibody MB-47. Although some areas of atherosclerotic plaque appeared to stain with both antibodies, other areas would stain with either OXL 41.1 or MB-47, but not both. In some lesions, MB-47 staining appeared to localize toward the base of a plaque, while OXL 41.1 staining was more prevalent near the luminal surface of the plaque. However, an opposite staining pattern with MB-47 localization toward the surface of the lesion and OXL 41.1 localization toward the base also could often be found (Figure 1). The presence of a particular staining pattern was not animal specific, as both patterns often could be found in different plaques from the same animal.

Furthermore, both patterns were seen with equal frequency in the control and probucol-treated animals. Although probucol-treated animals had significantly less atherosclerosis than did controls, treatment with probucol did not appear to affect either the relative amount or distribution of oxidatively modified LDL immunoreactivity present in atherosclerotic plaques (Figure 1). Thus, epitopes immunoreactive with an antibody to oxidatively modified LDL were present in the atherosclerotic plaques of probucol-treated animals. Furthermore, neither OXL 41.1 nor MB-47 immunoreactivity could be detected in sections of aorta without atherosclerotic plaque in either probucol-treated or control animals.

Because staining of adjacent sections of tissue cannot definitively address the question of whether colocalization of epitopes recognized by the different antibodies exists, we made use of the fact that MB-47...
is a murine IgG antibody and OXL 41.1 is a murine IgM antibody to perform double-labeling studies on the same tissue section. Single frozen tissue sections therefore were incubated with both OXL 41.1 and MB-47 and then were incubated with FITC-conjugated goat anti-mouse IgM antibody to localize OXL 41.1 and RITC-conjugated anti-mouse IgG antibody to localize MB-47. These studies (Figure 1) confirmed several findings from the single-labeling experiments: 1) Although colocalization of MB-47 and OXL 41.1 staining occurred in some parts of an atherosclerotic plaque, dissociation of staining was found in other parts of the same plaque. 2) Patterns of staining in which the base of the lesion showed predominantly MB-47 staining with heavier localization of OXL 41.1 toward the lesion's luminal surface or the opposite pattern could both be found. 3) The above findings were not animal specific, age specific, or treatment group specific.

Cell-Specific Staining

Methanol–Carnoy's-fixed, deparaffinized sections were labeled with either RAM-11 (macrophage specific) or HHF-35 (muscle cell specific) antibodies. Some lesions contained predominantly macrophages and few smooth muscle cells (Figure 2, left panels), while other lesions contained predominantly smooth muscle cells and few macrophages (Figure 2, right panels). In some lesions, approximately equal proportions of macrophages and smooth muscle cells were observed.

Twenty-seven lesions from seven control WHHL rabbits and 29 lesions from six probucol-treated WHHL rabbits were evaluated, and lesion size, cellularity, and cell-type scores were assigned as described earlier. Average scores were calculated for each of the three characteristics in each animal, and the average score for each characteristic was determined for the probucol-treated and control groups (Table 2). On average, probucol-treated animals had smaller lesions than did control animals, as indicated by a lower score for average lesion size ($p<0.01$). Similarly, group cellularity scores demonstrated that lesions from probucol-treated animals were significantly less cellular ($p<0.05$) than were lesions from control animals. Finally, a highly significant association between lesion cell-type score and treatment group was observed. Probucol-treated animals had a
predominance of "smooth muscle-rich" lesions, while "macrophage-rich" lesions predominated in control animals (*p*<0.01).

The finding that probucol treatment was associated with smaller, less cellular, and more smooth muscle-rich lesions, while control animals had larger, more cellular, and more macrophage-rich lesions suggested that the characteristics of size, cellularity, and predominant cell type are interrelated. To examine the significance of relations between these characteristics, the average lesion size scores for each of the 13 animals (treated and control) were plotted against each animal's average lesion cellularity score (Figure 3, upper panel). Similar comparisons were made for cellularity score versus lesion-type score (Figure 3, middle panel) and for size score versus cell-type score (Figure 3, lower panel). Correlation coefficients and levels of significance were calculated for each of the three comparisons. Significant correlations were seen between lesion size and cellularity scores (*p*<0.05; Figure 3, upper panel) and between lesion cellularity and cell-type scores (*p*<0.01; Figure 3, middle panel). The comparison between lesion size and cell-type scores (*p*=0.10; Figure 3, lower panel) did not reach statistical significance. Thus, these comparisons support the contention that more-cellular lesions tended to be larger and more smooth muscle rich, while less-cellular lesions tended to be smaller and to have a predominance of smooth muscle cells.

**Discussion**

Probucol is effective in decreasing the extent of atherosclerosis in WHHL rabbits when therapy is started early in life. We have discovered (Y. Nagano et al, unpublished observations) that when probucol therapy is started early, its effect in ameliorating atherogenesis is sustained and is independent of an effect on serum total and LDL cholesterol levels. Furthermore, when probucol was added at a later stage, it also was associated with less atherosclerosis at the end of the experiment than was seen in control animals. Thus, probucol appears to have its major effect on the prevention of lesion initiation and progression.

It is not known which of probucol's pharmacological actions is responsible for its antiatherogenic effect. Probucol treatment has been shown to reduce LDL and high density lipoprotein cholesterol levels in humans and to have similar effects in rabbits. However, probucol is also a powerful antioxidant, and because several lines of evidence support a role for oxidatively modified LDL in the pathogenesis of atherosclerosis, it is likely that probucol exerts its antiatherogenic effect by decreasing or preventing oxidative modification of lipoproteins.

The present study used a monoclonal antibody, OXL 41.1, which recognizes oxidatively modified LDL, LDL adducted to lipid peroxidation by-products, and acetyl LDL to determine whether probucol treatment was associated with a qualitative difference in the presence or location of immunoreactivity against this antibody in atherosclerotic lesions from WHHL rabbits. The effect of probucol on the cellular composition of plaques was also determined by using two cell-specific monoclonal antibodies, RAM-11, which recognizes rabbit macrophages, and HHF-35, which, in this context, is specific for smooth muscle cells. Aortic tissue was obtained from WHHL rabbits that participated in the long-term arm of a probucol-treatment protocol. The aortic specimens examined were obtained from the same portion of aorta in each animal and were handled so as to minimize the possibility of ex vivo oxidation.

The probucol-treated animals in this study had about one third as much aortic atherosclerosis as did controls. This benefit of treatment was seen despite the fact that mean cholesterol levels for probucol-treated and control animals were essentially the same and is strong evidence that probucol's hypocholesterolemic action was not responsible for the decreased atherosclerosis seen with treatment. This is consistent with the findings of previous studies. An unexpected finding was the demonstration that an antibody, OXL 41.1, which is directed against oxidatively modified LDL, reacted equally with epitopes present in atherosclerotic plaques of both control and probucol-treated WHHL rabbits. Although every effort was made to ensure that ex vivo oxidation of the aortic specimens did not occur, it is possible that some of the LDL present in lesions was oxidized after the animals were sacrificed. Two observations support the view that OXL 41.1 was in fact recognizing oxidatively modified LDL in frozen sections. First, double-label immunofluorescence clearly showed that OXL 41.1 and MB-47 staining were confined to the intima and were not found in either nonlesioned areas or in the media. If OXL 41.1 had recognized oxidatively modified proteins other than LDL, one would not have expected to see OXL 41.1 staining confined only to the intima. Second, if ex vivo oxidation of LDL had occurred, then one would have expected to see OXL 41.1 staining only in those portions of the intima that contained native LDL at the time the animals were killed. Therefore, the finding that significant portions of the intima have OXL 41.1 staining but little MB-47 staining (Figure 1, lower panels) argues against significant ex vivo oxidative modification of LDL.

The demonstration of OXL 41.1-reactive epitopes in the lesions of the probucol-treated animals sug-
gests that although probucol may protect against lesion formation and progression by inhibiting oxidative modification of lipoproteins, protection against oxidation is not complete. Recent studies have suggested that probucol itself becomes oxidized before lipoprotein becomes oxidized, which then could be followed by the initiation and subsequent progression of atheromatous lesions. Thus, it is conceivable that oxidative modification of lipoproteins is a prerequisite for atherogenesis in this animal model. However, the presence of such proteins in plaques of probucol-treated animals raises the possibility that probucol's antioxidant properties alone do not fully account for the drug's antiatherogenic effect. In any event, the presence of such epitopes in plaques is consistent with a proposed role for oxidatively modified LDL in the pathogenesis of atherosclerosis.

The differences observed in the cellular makeup of the atherosclerotic lesions in the two groups of animals also was somewhat surprising. Although there was overlap between the control and treated animals, as a group, lesions from probucol-treated animals were significantly smaller, less cellular, and were predominantly smooth muscle rich as compared with control lesions, which were, as a group, larger, more cellular, and predominantly macrophage rich. One explanation for these findings may relate to the severity of atherosclerosis, in that larger lesions and more extensive atherosclerosis may be associated with the presence of larger numbers of macrophages.

Figure 3. Scatterplots showing comparisons of lesion size, cellularity, and cell-type scores of control (●) and probucol-treated (○) Watanabe heritable hyperlipidemic rabbits. Comparison of lesion-size and cellularity scores (upper panel) demonstrates significant correlation (p < 0.05) between increasing lesion size and increasing lesion cellularity. Similarly, comparison of lesion cellularity and cell-type scores (middle panel) shows significant (p < 0.01) relation between increases in the proportion of macrophages in lesions and lesion cellularity. Comparison of lesion size and cell-type scores (lower panel) does not reach statistical significance (p = 0.10).
An alternate possibility is that there is a difference in the pathogenesis of predominantly macrophage-rich versus predominantly smooth muscle cell-rich lesions and that probucol selectively inhibits the formation of macrophage-rich lesions. Either possibility is consistent with the finding of significant correlations between increasing size and increasing cellularity and between increasing cellularity and increasing macrophage content of lesions. These findings raise questions as to possible mechanisms that might account for differences in the cellular makeup of the plaques in these two groups of animals. It is conceivable that probucol inhibits macrophage attachment and/or chemotaxis, possibly by reducing the amount of chemotactic factors, such as oxidized LDL\textsuperscript{22} in the developing plaque. Another possibility is that probucol treatment might affect the output of growth factors or cytokines that influence either macrophage recruitment or function, or smooth muscle cell chemotaxis or proliferation. Berliner and colleagues have recently reported that minimally modified LDL, which is characterized by having a thiochromatic–acid–reactive substance content of about 5 nmol/mg cholesterol and that is internalized via the LDL receptor, can induce aortic endothelial cells and smooth muscle cells to produce a monocyte chemotactic protein,\textsuperscript{6} to promote monocyte adhesion to endothelial cells,\textsuperscript{7} and to induce the expression of monocyte colony stimulating factor\textsuperscript{23} by endothelial cells. Thus, it is possible that probucol decreases macrophage content in plaques by decreasing the amount of minimally modified LDL present in plasma or in plaques, thereby decreasing the minimally modified LDL–induced expression of monocyte-specific cytokines and adhesion proteins by endothelial cells. As demonstrated in the present study, extensively oxidized LDL epitopes are present in plaques of probucol-treated rabbits, suggesting that probucol’s antioxidant activity is overwhelmed in the sequestered microenvironment of the plaque. In the plaque, however, where probucol levels are continuously maintained, the drug may be much more effective at preventing minimal modification of LDL. Therefore, the decrease in macrophage content of plaques seen with probucol treatment is consistent with an inhibition of LDL modification in plasma. Alternatively, probucol has been reported to inhibit the production of interleukin-1\textsuperscript{1,15} which could potentially alter the distribution of cells in the lesion, and a reduction in this cytokine might be expected to lead to less smooth muscle cell proliferation and fewer smooth muscle cell–rich lesions in the treated group. Thus, further studies are needed to evaluate the mechanisms that account for our observations and whether they are specific for probucol or occur with other antioxidants.

Acknowledgments

The authors wish to thank Marilyn Skelly for technical assistance and Marianne Kolden and Kristi McIntyre for assistance with typing.

References

13. Carew TE, Schwedt DC, Steinberg D: Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: Evidence that antioxidants in vivo can selectively inhibit low density lipoprotein modification 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
18. Garcia RI, Coltrera MD, Gown AM: Analyses of proliferative grade using anti-PCNA/cyclin monoclonal antibodies in fixed,

KEY WORDS • probucol • monoclonal antibodies • Watanabe heritable hyperlipidemic rabbits
Probucol treatment affects the cellular composition but not anti-oxidized low density lipoprotein immunoreactivity of plaques from Watanabe heritable hyperlipidemic rabbits.

K O'Brien, Y Nagano, A Gown, T Kita and A Chait

Arterioscler Thromb Vasc Biol. 1991;11:751-759
doi: 10.1161/01.ATV.11.3.751

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/11/3/751

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/