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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

(aortic atherosclerosis/hypercholesterolemia/scavenger receptor/low density lipoprotein receptor deficiency)

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ABSTRACT **It has been postulated that low density lipoprotein (LDL) becomes fully atherogenic only if it first undergoes oxidative modification. The oxidatively modified form, but not native LDL, is recognized by the acetyl-LDL or "scavenger" receptor and could, therefore, be taken up rapidly by tissue macrophages to generate the fatty-streak lesion of atherosclerosis. However, there is thus far very little direct evidence for oxidative modification *in vivo*. The studies reported here take advantage of the fact that probucol is an effective antioxidant transported in lipoproteins, including LDL, and blocks the oxidative modification of LDL *in vitro*. We now show that the rate of degradation of LDL in the macrophage-rich fatty-streak lesions of the LDL receptor-deficient rabbit treated with probucol (1% by weight in the diet) is reduced to about one-half of that in the lesions of receptor-deficient rabbits not given probucol (but matched for plasma cholesterol levels). In contrast, the rates of degradation in the nonlesioned areas of the aorta were no different in probucol-treated and control animals. Most of the LDL degradation in fatty-streak lesions takes place in macrophages, whereas in nonlesioned aorta, which contains very few macrophages, the degradation is almost exclusively in endothelial cells and smooth muscle cells. Thus, the results are compatible with the postulate that the native LDL taken up and degraded by foam cells in the developing fatty-streak lesions was in part first converted to a form recognized by the scavenger receptor (by oxidative or analogous modification). Finally, and most importantly, we show that treatment with probucol significantly reduced the rate of development of fatty-streak lesions even though plasma cholesterol levels were no lower than lovastatin-treated (control) rabbits.**

Goldstein *et al.* (1) first postulated that modification of low density lipoprotein (LDL) to a form recognized by the "scavenger" or acetyl-LDL receptor may be required for lipid loading of macrophage-derived foam cells in atherosclerotic lesions. Reports from our laboratory have postulated that oxidative modification of LDL could contribute to the atherogenic process in this and in other ways (2–5). At least four mechanisms, demonstrated *in vitro*, may potentially be involved: (*i*) enhanced rates of macrophage uptake and degradation of the oxidatively modified LDL through the scavenger receptor (3); (*ii*) increased recruitment of monocytes into the intima by the chemoattractant activity of oxidatively modified LDL for circulating monocytes (4); (*iii*) retention of macrophages in the intima by inhibition of macrophage motility by oxidatively modified LDL (5); (*iv*) cellular injury caused by peroxidized lipid components of oxidatively modified LDL (6). *In vitro* studies have demonstrated that antioxidants, including α-tocopherol and butylated hydroxytoluene, can completely inhibit the oxidative modification of LDL by cultured endothelial cells, peritoneal macrophages, or smooth muscle cells (3, 7, 8). Also, our laboratory has reported that probucol, a drug that is currently in clinical use for treatment of hypercholesterolemia and that is transported in lipoproteins (9), also blocks both cell-mediated and copper ion-mediated oxidative modification of LDL (10). Most likely this effect of probucol is due to its antioxidant properties and may be quite unrelated to the mechanisms by which it lowers plasma cholesterol levels. Indeed the structure of probucol [4,4′-(isopropylidenedithio)bis(2,6-di-*t*-butylphenol)] is very similar to that of butylated hydroxytoluene (2,6-di-*t*-butyl-*p*-cresol), a widely used antioxidant.

If the "oxidative modification hypothesis" is correct, treatment with antioxidants that block modification of LDL *in vivo* might slow the progress of atherosclerosis, at least the early steps leading to the fatty-streak lesion, in which macrophage-derived foam cells contain most of the stored lipid (11). Specifically, antioxidants, by preventing the conversion of native LDL to a form recognized by the macrophage scavenger receptor, could reduce the rate of uptake and degradation of native LDL in tissue macrophages *in vivo*. To test this component of the hypothesis, we measured the uptake and degradation of LDL in receptor-deficient Watanabe heritable hyperlipidemic rabbits (WHHL rabbits) that were untreated, treated with probucol, or treated with lovastatin at doses designed to keep their cholesterol levels comparable to those in the probucol-treated group. The rate of degradation of LDL in aortic lesions was determined using the "trapped ligand" method as developed by Pittman *et al.* (12). Using this method and light microscopic autoradiography, we have shown (13) that the degradation of LDL in fatty-streak lesions of the WHHL rabbit occurs predominantly in the intima, mostly in foam cells of monocyte–macrophage origin. This result has been confirmed and extended using electron microscopic autoradiography (M. G. Rosenfeld and T.E.C., unpublished observations). Thus, degradation in fatty-streak lesions reflects primarily degradation by macrophages whereas degradation in nonlesioned

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Abbreviations: LDL, low density lipoprotein; TC, tyramine cellobiose; WHHL rabbit, Watanabe heritable hyperlipidemic rabbit.

areas represents primarily degradation by endothelial cells and smooth muscle cells. Finally, the extent of aortic lesions in all animals was evaluated by Sudan IV staining and planimetry of the aortic surface area involved in atherosclerotic lesions.

LDL degradation in lesioned areas but not in nonlesioned areas was reduced in probucol-treated rabbits, a result compatible with the hypothesis that LDL is, in part, oxidatively modified prior to uptake by macrophages in fatty-streak lesions. After about 33 weeks of treatment, the severity of aortic atherosclerosis in the probucol-treated rabbits was much less than that in untreated rabbits and, most importantly, was significantly less than that in the lovastatin-treated (control) group in which plasma cholesterol levels were the same as (or lower than) in the probucol-treated rabbits.

METHODS

Rabbits and Diets. We studied 28 WHHL rabbits from six litters over a period of 20 months. Two, 4, or 6 rabbits from each litter were assigned equally to either probucol diet or lovastatin diet (n = 11 for each diet) with the dosage of the latter drug adjusted to maintain the same cholesterol levels in the two groups. From five of the six litters studied, 1 or 2 rabbits were assigned to an additional, untreated control group. The group assignment was random except for ensuring that the sex distribution was the same in each drug-treated group.

Beginning at 6–8 weeks of age, the rabbits were fed rabbit chow supplemented with probucol (1%), chow supplemented with lovastatin (5 mg/kg of body weight), or unsupplemented chow. Pure probucol (a gift from Merrell Dow Pharmaceuticals) and lovastatin (a gift from Merck, Sharpe and Dohme) were added to the rabbit chow in diethyl ether; the diets were dried for several days before use. The daily ration was increased from 50 to 110 g as the rabbits grew. The dose of probucol was maintained at 1% in chow whereas that of lovastatin was adjusted (downward) to maintain plasma cholesterol concentrations similar to those in the probucol-fed group.

Plasma Lipids. During the interval from weaning until the start of the special diets, three baseline measurements of the plasma cholesterol concentration of each rabbit were made. Blood samples were obtained for cholesterol analysis every 2–4 weeks during the period of drug treatment until sacrifice at 9.5 months of age when the treated groups had been receiving drug for ≈33 weeks. The mean plasma cholesterol during the treatment period was calculated as the time-average of the 8–11 samples obtained over this interval except for two of the six untreated animals from which 3 samples were collected during the experimental period.

Extent of Aortic Lesions. At sacrifice the rabbits were deeply anesthetized with sodium pentobarbital (50 mg/kg). To remove trapped blood from the aorta, the systemic circulation was perfused via a large bore cannula in the apex of the left ventricle with 2 liters of isotonic phosphate-buffered saline containing 2 mM EDTA. Effluent was collected from the severed right atrium and ventricle. The entire aorta was removed and cleaned of loose adventitial tissue. The thoracic and abdominal aortas were divided 5 mm proximal to the celiac artery. The aortas were weighed, opened longitudinally, pinned flat on rubber sheets, and fixed in half-strength Karnovsky's solution for 24 hr as described (14). The aortas were stained with Sudan IV and photographed. The outlines of the arteries and the sudanophilic lesions were traced by a single experienced observer (T.E.C.) onto transparent paper from photographic prints at an enlargement of three times actual size. The tracings were made from coded photographs without knowledge of the treatment group. The areas of sudanophilic lesions and of each aortic segment were obtained by computer-assisted planimetry using a Hewlett-Packard digitizing tablet with a resolution of 25 μm. The variation in area between repeated tracings on the digitizing tablet averaged <1% for outlines of arterial segments, and for individual lesions ≥1 mm^2 in area. Such lesions contributed most of the total surface area involved. As expected, replicate determinations of areas of still smaller sudanophilic spots had somewhat greater variation. The areas of lesions within a given aortic segment were summed and the extent of lesions expressed as a percent of aortic surface area involved.

Isolation and Labeling of LDL. In a subset of 10 animals (four probucol-treated, four lovastatin-treated, and two untreated rabbits), arterial LDL degradation rates were determined at sacrifice. For this purpose, LDLs (density, 1.021–1.060 g/ml) were isolated by sequential ultracentrifugation (15) from plasma collected into EDTA after an overnight fast. LDLs were isolated from two pools of plasma from probucol-treated animals and two pools of plasma from lovastatin-treated animals. Each of the LDL preparations was doubly labeled as described (12). Briefly, LDL was directly iodinated with carrier-free Na^{131}I using Iodogen (Pierce Chemical). Subsequently, tyramine cellobiose (TC) labeled with ^{125}I was covalently linked to each LDL preparation at a ratio of 1 TC ligand per 200 kDa of LDL apoprotein (12). After dialysis against saline (0.15 M NaCl) buffered with sodium phosphate (20 mM) and containing 2 mM EDTA, 1% or less of the activity of each isotope was soluble in 10% (wt/vol) trichloroacetic acid. Radioactivity extractable into chloroform/methanol, 1:1 (vol/vol), was 10.13 ± 0.40% for ^{125}I and 1.42 ± 0.08% for ^{131}I (16). Greater than 98% of both ^{125}I and ^{131}I radioactivities was present in a single band with β mobility after electrophoresis in agarose. The labeled LDLs (250–630 cpm of ^{125}I and 140–310 cpm of ^{131}I per ng of protein) were used 2–3 days after labeling, which was 5–6 days after initial isolation.

Metabolic Studies. In the subset of 10 rabbits described above, doubly labeled LDL (6.36 ± 0.40 × 10^8 cpm of ^{125}I, 3.57 ± 0.29 × 10^8 cpm of ^{131}I) was injected intravenously 2 days before sacrifice. Drug-treated animals received homologous LDL; one control received LDL isolated from probucol-treated rabbits and the second control received LDL isolated from lovastatin-treated rabbits. Thyroid uptake of radioiodide was inhibited by injecting NaI (3 mg) at the same time as the labeled LDL. Sequential plasma samples were obtained at intervals. At sacrifice 2 days later, the systemic circulation was perfused with buffer, and the aortas were dissected, fixed as described above, stained, and photographed. The aortic arch was separated from the descending aorta 1–2 mm below the ductus scar. Sudan-positive atherosclerotic lesions and samples of macroscopically normal aorta were cut out of each aortic segment and weighed.

Chemical Analyses and Radioassay. Probucol levels in plasma were determined every 2–3 weeks by HPLC after extraction with methanol/acetone by a method supplied by Merrell Dow Pharmaceuticals. Briefly, plasma samples were extracted into methanol/acetone, 3:2 (vol/vol), with 2-pentanone bis(3,5-di-t-butyl-4-hydroxyphenyl)mercaptole as internal standard, partitioned into heptane, and analyzed by HPLC on a C_{18} reversed-phase column eluted with acetonitrile/heptane/0.1 M ammonium acetate, 92:6:2 (vol/vol). Plasma cholesterol concentrations were determined by an automated enzymatic method. The ^{125}I and ^{131}I contents of tissue and plasma samples were measured in a well-type γ scintillation counter equipped with a 3-inch crystal (Compu Gamma, LKB) with corrections for overlap of the energy spectra of the two isotopes, for background activity, and for isotopic decay.

Analysis of Whole Body and Arterial LDL Catabolism. The rate of catabolism of LDL in the whole body was assessed by calculating the fractional catabolic rate of plasma LDL from the plasma decay curve (14). Rates of catabolism of LDL by aortic tissues were determined from the accumulation of degradation products of LDL labeled with ^{125}I-labeled TC in the artery as described (14) using the arterial content of directly iodinated ^{131}I-labeled LDL as an internal standard to correct for the amount of ^{125}I-labeled TC covalently bound to intact LDL within the artery, i.e., LDL that had not yet been degraded at the time of sacrifice. In the present study, the fixation step in half-strength Karnovsky's solution served to eliminate nonprotein bound (trichloracetic acid-soluble) ^{131}I from the aortic tissue without significant loss of any intact labeled LDL or of degradation products of (^{125}I-labeled TC)-labeled LDL.

An estimate of the concentration of intact LDL in lesioned and nonlesioned aortic tissue was made by calculating the ratio of C_T^*/C_P^* where C_T^* (cpm/g) and C_P^* (cpm/ml) are the concentrations of labeled intact LDL present in the artery and plasma, respectively, at death. The ratio of C_T^*/C_P^* represents the arterial concentration of LDL expressed as a fraction of the plasma LDL concentration.

Statistical Analysis. All data are expressed as mean ± SEM. We compared data between groups by analysis of variance and, where appropriate, by independent samples *t* tests using BMDP Statistical Software (programs 2V, 3D, and 7D; see ref. 17).

RESULTS

Plasma Lipids and Lipoproteins. In contrast to our earlier results (18), the plasma cholesterol concentration in the present studies was affected very little by probucol treatment, despite the fact that plasma probucol levels (from 50 to 150 μg/ml) were in the same range as in the previous study (18). Responsiveness to probucol evidently varies among WHHL rabbits for reasons not presently understood. In any case, we had to continually decrease the level of lovastatin fed to the control group to maintain plasma cholesterol concentrations comparable to those of the probucol-treated group.

The plasma cholesterol levels of the various groups are shown in Table 1. Prior to drug treatment plasma cholesterol levels in the three groups of rabits did not differ significantly. The lovastatin-treated group had the largest change in cholesterol level relative to pretreatment values (−70 ± 25 mg/dl; $P < 0.02$) and was the only group in which the cholesterol levels during the treatment period differed significantly from pretreatment values. Untreated animals had slightly, but not significantly, higher cholesterol levels during the 33-week experimental period, while probucol-treated animals had slightly, but not significantly, lower levels during treatment. The net effect, however, was that during the 33-week drug treatment period both treated groups had significantly lower plasma cholesterol levels than did untreated controls. As intended by the design of the experiment, plasma cholesterol levels in lovastatin-treated animals and probucol-treated animals did not differ significantly, although the cholesterol levels were, if anything, slightly lower in the lovastatin group.

Table 1. Plasma cholesterol concentrations in untreated and treated WHHL rabbits

Exp. group	Cholesterol, mg/dl	
	Before treatment	During treatment
Untreated (n = 6)	736 ± 28.3*	761 ± 28.9
Lovastatin (n = 11)	688 ± 25.3	618 ± 23.2
Probucol (n = 11)	696 ± 24.5	671 ± 20.65

P values are from *t* tests of mean cholesterol concentrations during treatment. $P = 0.10$ for the probucol group vs. the lovastatin group. $P < 0.025$ for the probucol group vs. the untreated group. $P < 0.005$ for the lovastatin group vs. the untreated group.
*n = 5.

Extent of Aortic Atherosclerosis. The extent of sudanophilic lesions in the aortas of the three groups of WHHL rabbits is shown in Table 2. Probucol-treated animals had significantly less atherosclerosis than did lovastatin-treated animals. Total aortic lesion area (Table 2, column 2) was reduced by about one-half in probucol-treated animals with respect to the cholesterol-matched lovastatin-treated animals. The slowing of the atherosclerotic process was even more evident in some individual segments of the distal aorta, i.e., the descending thoracic and abdominal aortic segments. Analysis of variance of the data from all three individual segments, including the arch, also indicated significantly less extensive lesions in the probucol-treated group compared to the lovastatin-treated group. The six untreated littermates of the animals treated with lovastatin or probucol had the greatest surface area involvement, and all differences between this group and the probucol group were highly significant ($P < 0.001$). Interestingly, statistical comparison of the extent of lesions in the lovastatin-treated group with that in the untreated group indicated that the somewhat lower values in the lovastatin-treated animals bordered on being significant ($0.1 > P > 0.05$) despite only a 19% difference in the (very high) plasma cholesterol levels between the two groups (mean, 618 vs. 761 mg/dl, respectively). The latter observation emphasizes the importance of the experimental design in this study, namely, a test of the possible antioxidant antiatherosclerotic effects of probucol by comparing groups of WHHL rabbits with the same level of plasma cholesterol (i.e., probucol vs. lovastatin treatment).

Whole-Body LDL Metabolism. In a subgroup of 10 animals, studies of whole-body and arterial LDL metabolism were performed. Rabbits in each group catabolized LDL at similar fractional rates (probucol-treated rabbits, 0.019 ± 0.0002 hr^{-1}; lovastatin-treated rabbits, 0.022 ± 0.0008 hr^{-1}; untreated rabbits, 0.020 ± 0.0030 hr^{-1}). This implies that drug treatment had little if any effect on overall rates of LDL metabolism in these rabbits. This stands in contrast to the higher fractional catabolic rates in probucol-treated rabbits observed by Naruszewicz *et al.* (18), but this result is consonant with the absence of any significant effect of

Table 2. Extent of aortic lesions

Exp. group	Extent of aortic lesions, % surface area involved			
		Aortic segment		
	Total aorta	Aortic arch	Descending thoracic aorta	Abdominal aorta
Untreated (n = 6)	40.6 ± 5.1	87.5 ± 3.5	37.7 ± 8.3	16.8 ± 3.2
Lovastatin (n = 11)	27.5 ± 4.6	65.0 ± 4.9	21.6 ± 6.2	14.3 ± 3.0
Probucol (n = 11)	14.3 ± 2.1*	47.1 ± 5.3*	6.6 ± 2.0	6.4 ± 1.0

Data are expressed as mean ± SEM. The statistical analysis was performed on log-transformed data. Differences between experimental groups in extent of lesions in the total aorta were compared by *t* tests: probucol group vs. lovastatin group, $P < 0.01$; probucol group vs. untreated group, $P < 0.0005$; lovastatin group vs. untreated group, $P = 0.06$. Differences between groups in extent of lesions in the three aortic segments were compared by analysis of variance with a repeated measures design: probucol group vs. lovastatin group, $P < 0.01$; probucol group vs. untreated group, $P < 0.001$; lovastatin group vs. untreated group, $P = 0.10$.
*n = 10.

probucol on plasma cholesterol levels in the present group of rabbits.

Arterial LDL Degradation Rates. In contrast to the lack of effect of probucol treatment on overall rates of LDL metabolism, the fractional rates of LDL degradation in aortic atherosclerotic lesions were significantly and selectively decreased in rabbits treated with probucol (Table 3). Analysis of variance indicated that LDL degradation rates in lesions in all segments of aorta, treated as repeated measures, were significantly depressed in the probucol-treated animals compared to lovastatin-treated controls. (The aorta even in heavily lesioned rabbits accounts for <0.1% of total-body LDL degradation, and thus the lack of detectable effect of probucol on whole body LDL catabolism is not contradictory.) Note that the rates of LDL degradation in lesioned areas of probucol- and lovastatin-treated animals were each much greater than in adjacent nonlesioned areas. Importantly, there was no effect of probucol treatment on LDL degradation in nonlesioned areas of the aortas (Table 3). This implies that probucol affected LDL degradation very selectively in the macrophage/foam cell-rich lesions of the aorta. The calculated concentrations of intact, undegraded LDL per g(wet weight) of aortic tissue (data not shown) did not differ between the treatment groups but were greater in aortic lesions (≈8% of the plasma concentration) than in nonlesioned aorta (≈0.3% of the plasma concentration in the descending thoracic and abdominal aortas and 1% in the aortic arch).

DISCUSSION

The purpose of the present studies was to test the hypothesis that probucol, working as an antioxidant, and independently of its cholesterol-lowering effects, might slow the progress of atherosclerosis by inhibiting oxidative modification of LDL. Because probucol has been reported to lower the cholesterol level of WHHL rabbits (18), it was necessary to treat a reference group of WHHL rabbits with lovastatin at a low dose to keep the cholesterol levels of the two groups comparable. Thus, it would be possible to assess the possibility of an effect related to the antioxidant properties of probucol over and above its cholesterol-lowering effect. However, in the present study, for reasons still not understood, the cholesterol-lowering effect of probucol was less than previously observed, and the cholesterol levels in the two groups were readily matched. The effect of probucol on the extent of aortic atherosclerotic lesions was highly significant even at matched levels of plasma cholesterol (probucol group vs. lovastatin group).

Table 3. Rates of aortic degradation of LDL

Exp. group	LDL degradation, fraction of plasma LDL pool degraded × 10^5 per g of tissue per day		
	Aortic arch	Descending thoracic aorta	Abdominal aorta
	Lesions		
Untreated (n = 2)	32.2 (35.3, 29.2)	46.9 (56.6, 37.1)	19.0 (19.7, 18.4)
Lovastatin (n = 4)	30.1 ± 2.0	26.4 ± 3.0	18.1 ± 0.9
Probucol (n = 4)	18.4 ± 3.2	14.9 ± 4.5	6.49 ± 0.92
	Nonlesioned area		
Untreated (n = 2)	—	1.82 (1.52, 2.11)	1.47 (1.34, 1.59)
Lovastatin (n = 4)	2.57 ± 0.92	1.73 ± 0.15	1.74 ± 0.15
Probucol (n = 4)	2.45 ± 0.30	1.54 ± 0.08	1.71 ± 0.18

Analysis of variance with repeated measures was used to compare data between groups. For lesions, differences between groups treated with probucol vs. lovastatin were significant at $P < 0.02$. For nonlesioned area, differences between groups treated with probucol vs. lovastatin were not significant ($P = 0.76$). For probucol-treated animals, data from lesions vs. nonlesioned area were significant at $P < 0.025$. For lovastatin-treated animals, data from lesions vs. nonlesioned areas were significant at $P < 0.001$.

Although the number of animals studied with labeled LDL was small, the inhibitory effect of probucol treatment on LDL degradation in the lesions is large (≈50%) and highly significant. Indeed, the variance in observed rates of LDL degradation from animal to animal was surprisingly small. In the nonlesioned areas (containing few macrophages) the mean values were quite close to each other, providing an important internal negative control. It has been shown previously that most of the degradation of native LDL in fatty-streak lesions is attributable to foam cells (13) and that most of these foam cells are derived from the monocyte/macrophage (19). Consequently, the measurement of degradation in lesioned areas reflects primarily uptake and degradation in monocyte/macrophages. In nonlesioned areas, without significant accumulation of foam cells, the degradation is attributable to smooth muscle cells or endothelial cells. The lack of effect of probucol treatment on degradation in nonlesioned areas, then, is consistent with the hypothesis that the probucol effect is on some step or steps necessary for LDL uptake by the macrophage. This could be, as was proposed (3), an inhibition of its conversion to the oxidatively modified form recognized by the scavenger receptor. There is evidence that lipid peroxidation is an ongoing process *in vivo* (20), that LDL itself may undergo peroxidation there (21), and that peroxidized lipids are found in atherosclerotic lesions (22, 23). The plasma probucol concentrations in the present studies were similar to those measured in probucol-treated patients, whose LDL was resistant to cell-mediated and copper ion-mediated oxidation (10). In addition, preliminary results indicated that thiobarbituric acid-reactive substances in the α <1.060-g/ml lipoprotein fraction of the probucol-treated rabbits were only one-half to one-third those in untreated or lovastatin-treated rabbits.

Although the calculated rate of degradation of LDL in lesions of the probucol-treated animals was reduced whether expressed relative to surface area (data not shown) or to wet weight (Table 3), there is a caveat that should be kept in mind. If the lesions in the probucol-treated animals contained fewer macrophages per unit weight and per unit surface area, the lower values could reflect that and not a true decrease in the rate of degradation per cell. Quinn *et al.* (4, 5) have shown that oxidatively modified LDL is chemotactic for monocytes and yet inhibits the motility of resident macrophages. Preventing oxidative modification might, therefore, indeed reduce the resident arterial macrophage population. However, our autoradiographic studies using LDL labeled with ^{125}I-labeled TC (13) showed that the foam cells most active in LDL degradation were located within a few cell layers of the endothelial surface. Hence, if the lesions in the probucol-treated animals were of a certain minimal thickness and there were similar numbers of macrophages within the active inner layer, then the observed accumulation of degradation products indicates a true decrease in degradation per macrophage. In any case, the findings are consistent with observed effect of the drug in diminishing the severity of atherosclerotic lesions.

LDL degradation by macrophages could be inhibited in some other way or ways. Yamamoto *et al.* (24) have reported that probucol added in cell culture inhibits foam cell formation induced by acetyl-LDL in a subclone of U937 cells, presumably through some general effect on macrophage function. However, studies in this laboratory (S. Parthasarathy, personal communication), using resident mouse peritoneal macrophages, show no such effect except at high cytotoxic concentrations. Moreover, there was no general inhibitory effect of probucol on macrophage function *in vitro*

when the probucol was added to the macrophages in a more physiological form, i.e., in plasma from probucol-treated donors (up to 60%, vol/vol).

Thus, we suggest that the evidence presented here offers important support for the concept that probucol slows the progression of atherosclerosis by mechanisms unrelated to its cholesterol-lowering effect, perhaps by inhibiting oxidative modification of LDL to a form avidly taken up and degraded by macrophage-derived foam cells. If this interpretation is borne out, it would open up alternative avenues for preventing or slowing the development of atherosclerosis. In this context it is noteworthy that Yamamoto *et al.* (25) have observed marked regression of tendonous xanthomas during prolonged probucol treatment of patients even when the fall in their plasma cholesterol levels was quite modest. Other antioxidant compounds and compounds interfering in other ways with LDL modification should be studied for their potential antiatherosclerotic effectiveness. In principle, the combination of a cholesterol-lowering agent such as lovastatin with an inhibitor of LDL modification might be a particularly effective regimen.

Note Added in Proof. After this manuscript was submitted, Kita *et al.* (26) confirmed that LDL obtained during treatment with probucol is resistant to copper-induced oxidative modification and reported a dramatic inhibition of the progression of atherosclerosis in a limited number of probucol-treated WHHL rabbits. However, the plasma cholesterol level in their four probucol-treated rabbits was 19% lower than that in their four untreated controls, making it uncertain to what extent the antiatherogenic effect observed was due to the cholesterol-lowering effect of probucol or due to its potential antioxidant effect.

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