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Abstract Intimal hyperplasia is a persistent problem after implantation of prosthetic grafts. Although the mechanisms underlying this hyperplastic response are unknown, it has been proposed that such responses may be due to chronic vascular injury similar to that of atherogenesis. Thus, the role of oxidation was explored using the potent antioxidant drug probucol. Adult New Zealand White rabbits fed a modestly (0.25%) cholesterol-enriched diet had a polytetrafluoroethylene prosthetic graft placed into the lower aorta. After the grafting procedure, a group of 11 rabbits was placed on the cholesterol-enriched diet supplemented with 1% wt/wt probucol while a control group of 10 rabbits was placed on the cholesterol-enriched diet alone. The rabbits were maintained for a further 10 weeks before histological examination of the area surrounding the graft. Although administration of probucol did not significantly alter the dimensions of lesions at the anastomotic sites, the drug promoted striking histological changes in the surrounding tissue. Both groups of rabbits had a similar intimal hyperplastic response of the aortic tissue surrounding the graft. The vascular lesions present in the

perigraft region of the control group consisted of a normal-appearing media but a thickened intima. The thickened intima contained numerous smooth muscle cells in a network of extracellular matrix. Regions in the neointima that were rich in smooth muscle cells exhibited modest staining for proliferating cell nuclear antigen. A few macrophages were present in the control group as determined by immunostaining with the monoclonal antibody RAM-11. In contrast, administration of probucol led to a marked reduction in the presence of RAM-11-staining macrophages. There was also a marked reduction in the cellularity of lesions, from 934 ± 135 nuclei/mm² in the control group versus 358 ± 32 nuclei/mm² in probucol-treated rabbits. Thus, administration of probucol did not significantly reduce the size of the intimal thickening at the relatively early interval under study, but the drug did produce cellular changes that may have beneficial effects on the maintenance of long-term patency of implanted grafts. (*Arterioscler Thromb.* 1994;14:162-167.)

Key Words • intimal thickening • prosthetic grafts • probucol • oxidation

Implantation of prosthetic grafts is now a commonly performed procedure in vascular surgery. Although this procedure is effective for the immediate restoration of arterial flow, graft failure due to an intimal hyperplastic response at the anastomotic sites commonly occurs within the first year.¹⁻³ Relatively little is known regarding the mechanisms by which this form of iatrogenic hyperplasia occurs, although it remains a major complication of several vascular interventions, including bypass surgery with vein grafts and angioplasty. Although the mechanisms of intimal hyperplasia are unknown, specific facets of the condition appear similar to those of spontaneous atherosclerosis. Therefore, there may be some commonality in the underlying cause of the two forms of the disease.

The evolution of spontaneous atherosclerotic lesions is thought to be initiated and propagated by oxidative mechanisms.⁴ Support for this concept includes the observation that oxidatively modified lipoproteins have been extracted from atherosclerotic lesions.^{5,6} In addition, immunoreac-

tivity for oxidized lipoproteins has been detected in histological sections of rabbit and human atherosclerotic lesions.⁷⁻⁹ The causal role of oxidation in the disease process is implied by the antiatherogenic efficacy of antioxidant drugs. The antiatherosclerotic properties of probucol have been observed after administration of the drug to Watanabe heritable hyperlipidemic (WHHL) rabbits soon after weaning. Under these conditions probucol reduces the extent of aortic atherosclerosis by a mechanism that is independent of its hypolipidemic effect.^{10,11} In addition, probucol administration to mature WHHL rabbits with established atherosclerotic disease results in a reduction of lesion progression.¹² A number of studies have also demonstrated that probucol reduces the extent of atherosclerosis induced by cholesterol-feeding in rabbits.¹³⁻¹⁵ This effect of probucol has also been corroborated by the similar antiatherosclerotic effects of probucol analogues¹⁶ and the antioxidants butylated hydroxytoluene¹⁷ and *N,N'*-diphenyl phenylenediamine.¹⁸

The present study was designed to determine whether probucol modulates iatrogenic intimal thickening in a manner similar to that described for spontaneous atherosclerosis. The study investigated the hyperplastic response of anastomotic aortic tissue that occurred after implantation of a prosthetic graft in rabbits fed a diet modestly enriched in cholesterol.¹⁹ Probucol did not reduce the extent of intimal thickening that developed

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10 weeks after implantation. However, probuocol markedly reduced the cell density and decreased the content of macrophages within these vascular lesions.

Methods

Animals and Graft Implantation

All animal care complied with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication No. 80-23; revised, 1985). Protocols were approved and monitored by the Washington University Animals Studies Committee.

Adult New Zealand White rabbits (2.5 to 4 kg) from a single supplier (Doe Valley Farms, Bentonville, Ark) were fed a standard laboratory chow diet enriched in 0.25% wt/wt cholesterol (Purina Mills, Inc, Richmond, Ind). All rabbits underwent an aortic interposition grafting procedure 5 weeks after initiation of the cholesterol-enriched diet.

Before the grafting procedure, the rabbits were anesthetized with a mixture of ketamine hydrochloride (40 mg/kg), acepromazine (1 mg/kg), and xylazine (20 mg/kg) administered intramuscularly. Supplemental doses of ketamine hydrochloride (40 mg/kg) were administered every 30 minutes. Penicillin (150 000 U) was administered intramuscularly before the start of surgery. With the use of aseptic techniques, the infrarenal aorta was exposed through a midline incision. Lumbar branches were ligated as required to free the distal 2 cm of aorta. Before the aorta was cross-clamped, the rabbits received 200 U/kg IV of heparin sodium. The distal aorta was then cross-clamped, and a 1-cm portion of the aorta, 1 cm proximal to the bifurcation, was removed. The distal aorta was flushed with 2.5 mL saline (0.15 mol/L) containing heparin (100 U/mL) via a 20G catheter. A 1-cm-long, 3-mm-diameter, thin-walled, polytetrafluoroethylene (PTFE) graft (WL Gore & Associates, Flagstaff, Ariz) was sewn in an end-to-end fashion into the aorta with CV-8 PTFE suture material (WL Gore & Associates) in a running fashion. Twelve stitches were placed symmetrically around the circumference of each anastomosis. Perioperative blood loss was replaced with intravenous normal saline. A circulating warm-water pad was used perioperatively to maintain body temperature.

Postoperatively the animals were randomly divided into two groups: (1) a control group received the same cholesterol-supplemented diet as preoperatively and (2) a group was fed the same cholesterol-enriched diet but supplemented with probuocol (1% wt/wt). Probuocol was administered in the diet by dissolving the probuocol in chloroform and spraying the mixture onto the chow as described.^{12,15} The chow diet fed to the control group was sprayed with chloroform without probuocol. Body weight was monitored to determine tolerance to the diets.

Plasma Lipid Characterization

Blood was drawn from a marginal ear vein for the determination of plasma lipid concentrations before each animal was started on the diet, at the time of the grafting procedure, and at 5 and 10 weeks postoperatively. Concentrations of total cholesterol, free cholesterol, phospholipids, and triglycerides in plasma were determined enzymatically with commercially available kits (Wako Pure Chemical Industries, Ltd, Richmond, Va).

Lesion Morphology

Ten weeks after graft implantation, the rabbits were anesthetized as described above and euthanized for graft removal, and the abdomen was reentered via a midline incision. The proximal abdominal aorta at the level of the diaphragm was dissected free from surrounding tissue, as were both common iliac vessels. The abdominal aorta and graft were removed en bloc to the level of the iliac vessels. Throughout this procedure

care was taken not to manipulate the graft or adjacent aorta. The aorta was gently flushed with 3 mL normal saline (0.15 mol/L) and perfusion fixed with 10 mL of 4% (wt/vol) paraformaldehyde in phosphate-buffered saline. Specimens were immersed in this fixative for a further 48 hours at 4°C. In a pilot study, this method of fixation was compared with pressure-perfusion fixing in situ, and the histological appearance of the lesions was found to be similar by light microscopy. Two grafts in the control group and one graft in the probuocol-treated group were occluded at the time of graft removal. There was no evidence of perigraft hematoma, false aneurysm, or graft infection.

After fixation the adventitia of the aorta and the granulation tissue around the graft were removed, and the aorta was sectioned longitudinally. Photographs at standardized magnification were taken of the luminal surface of each cut specimen. With a computer-assisted planimetry system (Sigma Scan; Jandel Scientific Co, San Rafael, Calif), the surface area of intimal thickening at each anastomosis was determined in a blinded fashion as described.¹⁹

For histological study one half of each specimen was embedded in paraffin, and 5- μ m-thick longitudinal sections were stained with either hematoxylin and eosin or Verhoeff's elastin stain with a van Gieson's counterstain. Sections of aorta that included the graft were examined from the iliac bifurcation to the renal artery branches. The intimal thickness in each longitudinal section was measured, in a blinded fashion, as the maximal thickness from the internal elastic lamina to the luminal surface near each proximal and distal anastomosis. Because all cuts were not exactly perpendicular to the graft wall, these intimal measurements were standardized to the thickness of the graft on each slide. As the variance in graft thickness is less than 1%, this normalization controlled for the angle of sectioning. The variance in the sectioned-graft thicknesses was similar in both treatment groups.

For characterization of specific cell types within vascular tissue, immunocytochemistry was performed with an avidin-biotin-peroxidase system (Vector Laboratories, Inc, Burlingame, Calif). A muscle-specific actin antibody (HHF-35) and an anti-rabbit macrophage antibody (RAM-11; both supplied by Dr Allen Gown, University of Washington, Seattle) were used to distinguish smooth muscle cells and macrophages within the lesions as described.^{20,21} An anti-proliferating cell nuclear antigen (PCNA; Coulter Immunology, Hialeah, Fla) was used to document the percentage of proliferating cells within the intimal hyperplastic areas. The PCNA antibody was initially tested for cross-reactivity to rabbit tissue. Comparisons were also made between frozen sections and formaldehyde-fixed sections of rabbit small intestine. Both methods of tissue fixation produced reliable and comparable immunocytochemical staining in intestinal crypts.

Cellular densities were quantified by determining the nuclear density with an image-analysis system (JAVA, Jandel Scientific Co). The entire area of intimal hyperplasia at an anastomosis on a given hematoxylin and eosin-stained longitudinal section was traced on the computer-generated digitized image. An overlay image was then created that contained only those items whose density equaled that of the hematoxylin-stained nuclei. The number of these objects, or nuclei, within the calculated area of intimal hyperplasia was then determined by the image-analysis system, thereby deriving the nuclear density within the intima on each histological section. One section from each animal in both groups was examined in this manner in a blinded fashion.

Determination of Plasma Probuocol Concentrations

Plasma probuocol concentrations were determined with a high-performance liquid chromatography method described by Satonin and Coutant.²² An internal standard of 2-pentanone-bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)-mercaptole (supplied by Merrell Dow Research Institute) was added to the plasma,

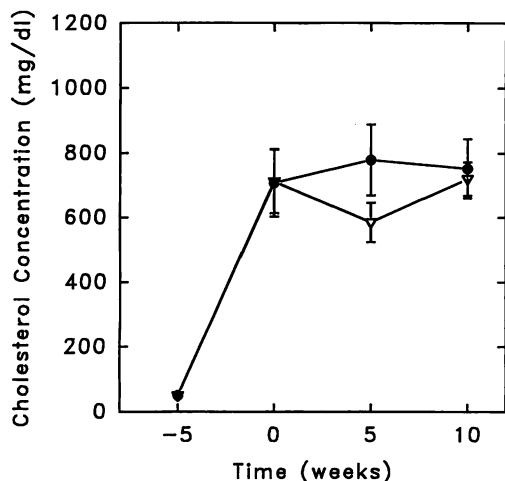


Fig 1. Line plot of plasma concentrations of total cholesterol in rabbits fed a 0.25% cholesterol-enriched diet (●) and rabbits fed the same diet that was supplemented with 1% probucol (▽) after implantation of a prosthetic graft. Both groups were fed a 0.25% cholesterol-enriched diet for 5 weeks before implantation of the prosthetic graft, which was performed at week 0. Points are the means of 10 to 11 observations, and bars are SEMs.

and the samples were extracted as described by Bligh and Dyer.²³ The extracted specimens were resuspended in methanol and injected onto a 5- μ m Hypersil ODS 250 \times 4.6-mm column with a mobile phase of acetonitrile/hexane/0.1 mol/L ammonium acetate (180:13:7, vol/vol/vol).

Statistical Analyses

The mean plasma lipid concentrations were compared with an unpaired Student's *t* test, as the data were normally distributed. Since variance ratio testing demonstrated inequality of variances, the surface areas of intimal thickening, intimal thicknesses, and cell densities were compared with the Mann-Whitney *U* test for nonparametric data. For all tests, $P > .05$ was considered statistically significant.

Results

Plasma Concentrations of Lipids and ProbucoL

All rabbits tolerated the diets and surgery well, with comparable weight gains in both groups. Body weights for the control and probucol-treated animals were 3.5 ± 0.4 kg and 3.3 ± 0.3 kg, respectively, at the start of the study and increased to 3.7 ± 0.3 kg and 3.6 ± 0.5 kg, respectively. Control rabbits had plasma total cholesterol concentrations of 708 ± 111 mg/dL (mean \pm SEM) at the time of operation, and these concentrations remained at this level throughout the rest of the study (Fig 1). ProbucoL-treated rabbits had similar plasma total cholesterol concentrations (712 ± 105 mg/dL) at the time of operation. Although the probucol-treated rabbits had slightly lower mean total cholesterol concentrations during the postoperative period (668 ± 73 mg/dL versus 745 ± 104 mg/dL in control rabbits), plasma total cholesterol concentrations were not statistically different from control ($P = .36$). Similarly, all plasma concentrations of unesterified cholesterol, phospholipids, and triglycerides were not statistically different between the two groups throughout the study.

Plasma probucol concentrations rose steadily throughout the postoperative period from an initial concentration of 26.8 ± 4.0 μ g/mL of plasma 2 weeks

Effect of ProbucoL on Intimal Lesion Dimensions at Proximal and Distal Anastomotic Sites of Aorta Adjacent to the Graft

Treatment		Area, mm ²
Area of grossly discernable lesions/site		
Proximal	Control	24.5 ± 6.0
	ProbucoL	20.5 ± 1.9
Distal	Control	15.5 ± 2.8
	ProbucoL	16.2 ± 2.3
Maximum thickness of intimal thickening/site		Thickness, mm
Proximal	Control	0.45 ± 0.06
	ProbucoL	0.37 ± 0.06
Distal	Control	0.47 ± 0.07
	ProbucoL	0.39 ± 0.05

Dimensions of the lesions were determined from the area of grossly involved intima and the maximal thickness. Data are represented as mean \pm SEM.

postoperatively to 55.2 ± 10.7 μ g/mL of plasma at the time the rabbits were euthanized.

Morphological and Histological Characteristics of Areas of Intimal Thickening

The white, glistening areas of intimal thickening were readily distinguishable from the normal aortic intima in magnified photographs of the luminal surface of each aorta, without the use of special stains. In patent grafts, the relative surface area of involvement in control rabbits was similar to that in probucol-treated rabbits (Table). Likewise, the relative area of involvement with intimal hyperplasia was not different at the proximal or distal anastomoses in either group (Table).

Histologically, the lesions in the perigraft area closely resembled the hyperplastic lesions that have been seen adjacent to prosthetic vascular grafts in humans.²⁴ The hyperplastic response began at the anastomotic site, reaching a maximal thickness 2 to 3 mm away from the anastomosis and gradually tapering to a normal-appearing, thin intima 7 to 10 mm from the graft at both anastomoses. The media appeared undisturbed, with concentric smooth muscle cells interspersed with connective tissue-containing elastic laminae. The intima was characterized by multiple layers of smooth muscle cells, as determined by immunocytochemistry with the antibody HHF-35 (Fig 2), and appeared entwined in a meshwork of connective tissue that was nearly devoid of elastin fibers on Verhoeff's-van Gieson's-stained sections as described.¹⁹ Intimal thickening in both groups was characterized by smooth muscle cell proliferation. A few cells in these regions exhibited reactivity with an anti-PCNA antibody. While the mean intimal thickness at the anastomotic site was less in probucol-treated than control rabbits, this difference did not reach statistical significance ($P = .16$, Table). However, the morphology of the lesions in the two groups was quite distinct (Fig 3). Intimal lesions in control rabbits had a markedly greater cell density than did comparable regions in probucol-treated rabbits. Intimal lesions of control rabbits had 934 ± 135 nuclei/mm² (mean \pm SEM) while the

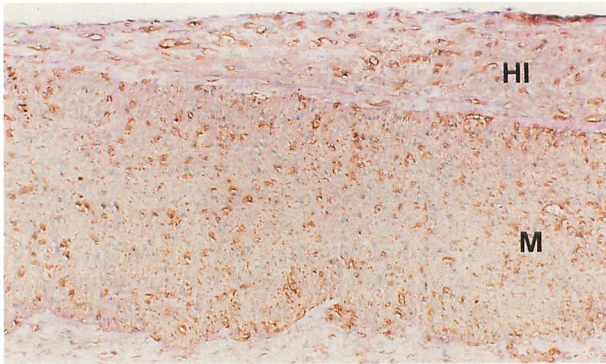


FIG 2. Photomicrograph showing an example of the smooth muscle cell content of the hyperplastic region. A section from the anastomotic region was immunostained with the monoclonal antibody HHF-35 that is specific for smooth muscle actin. Immunoreactivity was visualized by the brown peroxidase reaction product of diaminobenzidine. M indicates the medial portion of the artery; HI, the hyperplastic intima (original magnification $\times 200$).

probucol-treated rabbits had only 358 ± 32 nuclei/ mm^2 within their intimas ($P < .003$, Fig 4).

Differences in the macrophage population within the intimal hyperplastic lesions of these two groups were distinguished by immunostaining with RAM-11. Scattered macrophages were present within the intimal hyperplastic lesions of the control rabbits (Fig 5). Some

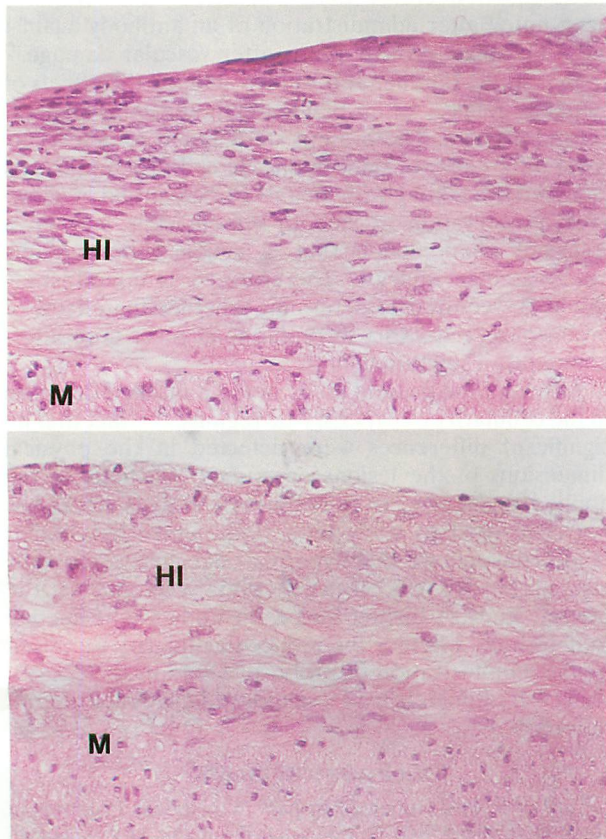


FIG 3. Photomicrographs showing examples of the histological characteristics of anastomotic regions from a rabbit fed the cholesterol-enriched diet alone (top) and a rabbit fed the cholesterol-enriched diet supplemented with 1% probucol (bottom). Sections were stained with hematoxylin and eosin. M indicates media; HI, hyperplastic intima (original magnification $\times 200$).

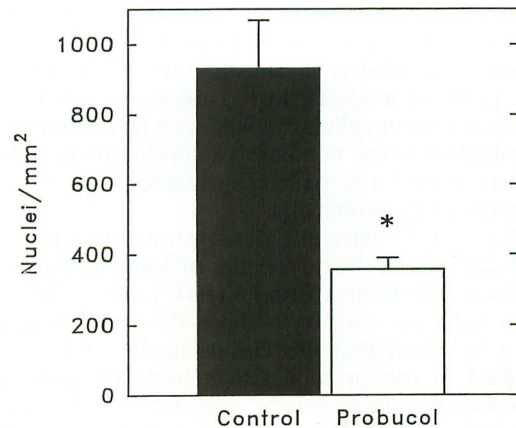


FIG 4. Bar graph of density of nuclei within randomly selected regions of hyperplastic intima of control, cholesterol-fed rabbits (■) and probucol-treated, cholesterol-fed rabbits (□). Values are the means of 10 to 11 rabbits, and bars are SEMs. *Represents a statistical difference of $P < .003$ vs the control group.

of these macrophages appeared engorged, presumably with lipids. However, multiple sections of each anastomotic area from the probucol-treated rabbits revealed few macrophages. It should be noted that changes in the populations of macrophages within the intima are based on reactivity with the monoclonal antibody RAM-11. Since the antigen that RAM-11 reacts with on macrophages has not been identified, this antibody may not be a pan-macrophage antibody. However, there are few published data to suggest that RAM-11 reacts with a selected population of macrophages.

Discussion

The present study was designed to address the question of whether probucol administration influences the hyperplastic response that is characteristic of anastomotic regions of prosthetic vascular grafts. Probucol did not reduce the dimensions of the thickened anastomotic region at the 10-week postoperative interval under study. However, probucol markedly affected the histo-

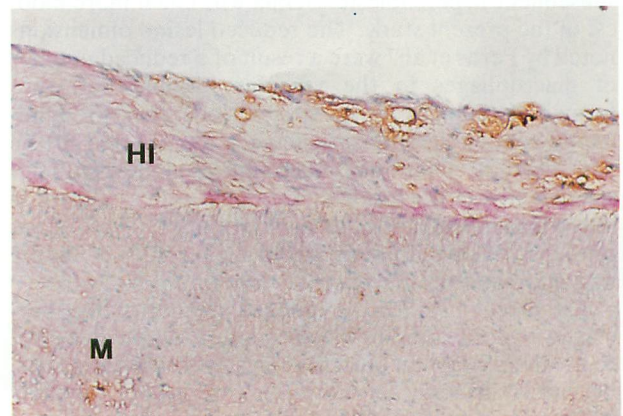


FIG 5. Photomicrograph showing example of the histological characteristics of an anastomotic region from a rabbit fed the cholesterol-enriched diet alone. Sections from anastomotic regions were immunostained with RAM-11, which reacts against rabbit macrophages. Immunoreactivity was visualized by the brown reaction product of diaminobenzidine. M indicates the medial portion of the artery; HI, the thickened intima (original magnification $\times 200$).

logical characteristics of the formed lesions. These drug-induced effects were most noticeably manifested as reduced cellularity in anastomotic regions of prosthetic grafts in modestly hypercholesterolemic rabbits. This reduction in cellularity was largely attributable to the reduction in the number of smooth muscle cells. In addition, the relative paucity of macrophages was further reduced by probucol.

O'Brien et al²⁵ have also demonstrated that probucol alters the cellular characteristics of spontaneous aortic atherosclerotic lesions from WHHL rabbits. Although their results are similar to those described herein, it should be noted that the lesions in the rabbit model described in the present article were predominantly composed of smooth muscle cells. Conversely, spontaneous lesions in WHHL rabbits are initially macrophage rich and progress through stages of increasing content of smooth muscle cells.²⁶ In addition, the altered cellularity noted in the spontaneous atherosclerotic lesions observed by O'Brien et al²⁵ was accompanied by reductions in lesion dimensions, in contrast to the results we observed for the form of iatrogenic intimal thickening that occurs at anastomotic regions of prosthetic grafts.

The effects of probucol in vascular-injury models have also been demonstrated by others. In contrast to the results of the present study, Ferns et al²⁷ have observed a reduction in neointimal thickening after balloon injury in probucol-treated, hypercholesterolemic rabbits. The vascular lesions reported by Ferns et al²⁷ were characterized by a predominance of lipid-laden macrophages. Shinomiya et al²⁸ have also observed a modest but statistically significant reduction in intimal thickening during probucol administration after balloon injury in cholesterol-fed rabbits. Discrepancies in the effects of probucol on the intimal thickening between balloon-injury studies and those in the current study may be explained by disparities in the extent of vascular injury produced by the two techniques. The study interval of 10 weeks in the present report was longer than the 5-week study of Ferns et al.²⁷ Furthermore, differences in the response to probucol may have occurred because the extent of cholesterol feeding and subsequent hypercholesterolemia was much more modest in the present study. The reduced lesion dimensions noted by Ferns et al²⁷ were a result of a reduced number of macrophages in the vascular lesions that were formed. However, in vascular lesions that predominantly contain smooth muscle cells as reported in the present study, ablation of macrophages alone would not significantly reduce lesion dimensions, as this cell type is only a minor contributor to lesion volume. Nevertheless, cytokine release from macrophages could modulate migration and proliferation of smooth muscle cells.

The mechanism for the reduced cellularity of hyperplastic regions after probucol administration does not relate to its effect on plasma cholesterol concentrations. Plasma cholesterol concentrations were approximately 700 mg/dL in both groups at the time of prosthetic graft implantation and initiation of probucol administration. There was a modest hypocholesterolemic response 5 weeks after probucol administration that was not sustained. Probucol did not produce a significant reduction in plasma cholesterol concentrations, as noted in our earlier study of cholesterol-fed rabbits.¹² This lack of a significant hypocholesterolemic response occurred de-

spite the persistence of therapeutic plasma concentrations of probucol.

A possible mechanism of probucol in promoting the changes in cellularity could be related to the inhibition of interleukin-1 production.²⁹ Ku et al²⁹ have demonstrated that the release of interleukin-1 from macrophages that is initiated by lipopolysaccharide challenge is almost totally abolished in cells that have been isolated from probucol-treated mice. Interleukin-1 is known to promote smooth muscle cell proliferation in cultures,³⁰ and this cytokine has been detected in atherosclerotic lesions.³¹ Thus, inhibition of interleukin-1 could lead to reduced numbers of smooth muscle cells in the thickened intima. Although the thickened intimal regions of probucol-treated rabbits were less cellular, there was a corresponding increase in extracellular components, so that no overall reduction in the dimensions of the lesions occurred. Since interleukin-1 induces the release of collagenases and neutral proteases,³² inhibition of the secretion of this cytokine may promote accumulation of extracellular matrix elements, as was noted for the hyperplastic regions of the probucol-treated rabbits. The presence of excessive extracellular matrix elements may restrict the migration of smooth muscle cells, a process that is considered to be a critical component of lesion formation in vascular injury that follows the initial burst of proliferative activity. Reductions in smooth muscle cell proliferation without a consequent decrease in the dimensions of lesions have been noted after administration of an antibody against basic fibroblast growth factor after vascular damage.³³ In that study, Lindner and Reidy³³ defined the effects of cytokine release after balloon-catheter injury to rat carotid arteries. The present study failed to detect any overt differences in smooth muscle cell proliferation as analyzed by staining with an anti-PCNA antibody. However, proliferation of smooth muscle cells may be a transient phenomenon that is maximal immediately after vascular injury.³³ Therefore, a temporal study would be required to determine whether the effects of probucol on cellularity were due to changes in proliferation and/or migration.

Although the present study noted a marked decrease in the cellularity of hyperplastic regions, the fact that no significant differences were detected in the physical dimensions of the lesions engenders questions of the applied significance of the observation. This model has not been used to study later time points, but similar models have shown that hyperplastic regions continually expand beyond the 10-week point explored in the present study. A further long-term study will be needed to determine whether the reduced cellularity produced by the administration of probucol noted in the present study will ultimately lead to a reduced incidence of graft failure.

Acknowledgments

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References

1. McBride W, Lange RA, Hillis LD. Restenosis after successful angioplasty: pathophysiology and prevention. *N Engl J Med.* 1988; 318:1734-1737.
2. Okadome K, Mu S, Onohara T, Fukuda A, Sungaichi K. Flow waveform analysis and long-term results of autologous saphenous vein and polytetrafluoroethylene grafts in 140 arterial reconstructions of the lower limbs. *Eur J Vasc Surg.* 1990;4:369-374.
3. Clowes AW, Gown AM, Hanson SR, Reidy MA. Mechanisms of arterial graft failure: role of cellular proliferation in early healing of PTFE prostheses. *Am J Pathol.* 1985;118:43-54.
4. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low density lipoprotein that increase its atherogenicity. *N Engl J Med.* 1989;320:915-924.
5. Daugherty A, Zweifel BS, Sobel BE, Schonfeld G. Isolation of low density lipoprotein from atherosclerotic vascular tissue of Watanabe heritable hyperlipidemic rabbits. *Arteriosclerosis.* 1988; 8:768-777.
6. Palinski W, Rosenfeld ME, Yla-Herttuala S, Gurtner GC, Socher SS, Butler SW, Parthasarathy S, Carew TE, Steinberg D, Witztum JL. Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci U S A.* 1989;86:1372-1376.
7. Haberland ME, Fong D, Cheng L. Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science.* 1988;241:215-218.
8. Boyd HC, Gown AM, Wolfbauer G, Chait A. Direct evidence for a protein recognized by a monoclonal antibody against oxidatively modified LDL in atherosclerotic lesions from a Watanabe heritable hyperlipidemic rabbit. *Am J Pathol.* 1989;135:815-825.
9. Rosenfeld ME, Palinski W, Yla-Herttuala S, Butler S, Witztum JL. Distribution of oxidation specific lipid-protein adducts and apolipoprotein-B in atherosclerotic lesions of varying severity from WHHL rabbits. *Arteriosclerosis.* 1990;10:336-349.
10. Kita T, Nagano Y, Yokode M, Ishi K, Kume N, Ooshima A, Yoshida H, Kawai C. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbits, an animal model for familial hypercholesterolemia. *Proc Natl Acad Sci U S A.* 1987;84:5928-5931.
11. Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc Natl Acad Sci U S A.* 1987;84:7725-7729.
12. Daugherty A, Zweifel BS, Schonfeld G. The effects of probucol on the progression of atherosclerosis in mature Watanabe Heritable Hyperlipidaemic rabbits. *Br J Pharmacol.* 1991;103:1013-1018.
13. Tawara K, Ishihara M, Ogawa H, Tomikawa M. Effect of probucol, pantethine and their combinations on serum lipoprotein metabolism and on the incidence of atheromatous lesions in the rabbit. *Jpn J Pharmacol.* 1986;41:211-222.
14. Kritchevsky D, Kim HK, Tepper SA. Influence of 4,4'-(isopropylidenedithio)bis(2,6-di-*t*-butylphenol) (DH-581) on experimental atherosclerosis in rabbits. *Proc Soc Exp Biol Med.* 1971;136:1216-1221.
15. Daugherty A, Zweifel BS, Schonfeld G. Probucol attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Br J Pharmacol.* 1989;98:612-618.
16. Mao SJT, Yates MT, Parker RA, Chi EM, Jackson RL. Attenuation of atherosclerosis in a modified strain of hypercholesterolemic Watanabe rabbits with use of a probucol analogue (MDL 29,311) that does not lower serum cholesterol. *Arterioscler Thromb.* 1991;11:1266-1275.
17. Bjorkhem I, Henrikssonfreyschuss A, Breuer O, Diczfalusy U, Berglund L, Henriksson P. The antioxidant butylated hydroxytoluene protects against atherosclerosis. *Arterioscler Thromb.* 1991; 11:15-22.
18. Sparrow CP, Doebber TW, Olszewski J, Wu MS, Ventre J, Stevens KA, Chao YS. Low density lipoprotein is protected from oxidation and the progression of atherosclerosis is slowed in cholesterol-fed rabbits by the antioxidant *N,N'*-diphenyl-phenylenediamine. *J Clin Invest.* 1992;89:1885-1891.
19. Baumann DS, Doblaz M, Daugherty A, Sicard G, Schonfeld G. The role of cholesterol accumulation in prosthetic vascular graft anastomotic intimal hyperplasia. *J Vasc Surg.* In press.
20. Tsukada T, Tippens D, Gordon D, Ross R, Gown A. HHF35, a muscle-actin-specific monoclonal antibody: immunocytochemical and biochemical characterization. *Am J Pathol.* 1987;126:51-60.
21. Tsukada T, Rosenfeld M, Ross R, Gown AM. Immunocytochemical analysis of cellular components in lesions of atherosclerosis in the Watanabe and fat-fed rabbit using monoclonal antibodies. *Arteriosclerosis.* 1986;6:601-613.
22. Satonin DK, Coutant JE. Comparison of gas chromatography and high performance liquid chromatography for the analysis of probucol in plasma. *J Chromatogr.* 1986;380:401-406.
23. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem.* 1959;37:911-917.
24. Eclave V, Koornick AR, Haimay M, Jacobsen JH. Intimal hyperplasia as a complication of the use of polytetrafluoroethylene graft for femoral popliteal bypass. *Surgery.* 1979;86:791-798.
25. O'Brien K, Nagano Y, Gown A, Kita T, Chait A. Probucol treatment affects the cellular composition but not anti-oxidized low density lipoprotein immunoreactivity of plaques from Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb.* 1991;11:751-759.
26. Rosenfeld ME, Tsukada T, Gown AM, Ross R. Fatty streak initiation in Watanabe heritable hyperlipidemic and comparably hypercholesterolemic fat-fed rabbits. *Arteriosclerosis.* 1987;7:9-23.
27. Ferns GAA, Forster L, Stewartlee A, Konneh M, Nouroozadeh J, Anggard EE. Probucol inhibits neointimal thickening and macrophage accumulation after balloon injury in the cholesterol-fed rabbit. *Proc Natl Acad Sci U S A.* 1992;89:11312-11316.
28. Shinomiya M, Shirai K, Saito Y, Yoshida S. Inhibition of intimal thickening of the carotid artery of rabbits and of outgrowth of explants of aorta by probucol. *Atherosclerosis.* 1992;97:143-148.
29. Ku G, Doherty NS, Wolos JA, Jackson RL. Inhibition by probucol of interleukin 1 secretion and its implication in atherosclerosis. *Am J Cardiol.* 1988;62:77B-81B.
30. Libby P, Warner SJC, Freidman GB. Interleukin-1: a mitogen for human vascular smooth muscle cells that induces the release of growth inhibitory prostanoids. *J Clin Invest.* 1988;81:487-498.
31. Ross R, Masuda J, Raines EW, Gown AM, Katsuda S, Sasahara M, Malden LT, Masuko H, Sato H. Localization of PDGF-B protein in macrophages in all phases of atherogenesis. *Science.* 1990;248:1009-1012.
32. Saklatvala J, Pilsworth LMC, Sarsfield SJ, Gavrilovic J, Heath JK. Pig catabolin is a form of interleukin-1. *Biochem J.* 1984;224: 461-466.
33. Lindner V, Reidy MA. Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. *Proc Natl Acad Sci U S A.* 1991;88:3739-3743.