Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11312–11316, December 1992
Medical Sciences

Probucol inhibits neointimal thickening and macrophage accumulation after balloon injury in the cholesterol-fed rabbit

(hypercholesterolemia/antioxidant therapy/carotid artery/deendothelialization)

GORDON A. A. FERNS, LOUISE FORSTER, ANNE STEWART-LEE, MATTHEW KONNEH, JAFFAR NOUROOZ-ZADEH, AND ERIK E. ÄNGGÅRD

The William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ, United Kingdom

Communicated by John Vane, August 31, 1992

ABSTRACT **Restenosis is a frequent long-term complication after balloon angioplasty. Although smooth muscle cells form the major constituent of the occluding lesion, macrophage-derived foam cells are usually also present in high abundance. The latter have the potential to accelerate the rate of reocclusion because they elaborate many potent cytokines and growth factors, which may act to either recruit cells into the neointima or cause neointimal cell proliferation. Macrophage-derived foam-cell formation depends upon the uptake of modified low density lipoprotein via a scavenger receptor-mediated pathway. Foam-cell formation is accompanied by the release of smooth muscle cell mitogens and chemoattractants. We have examined the effects of probucol, a lipid-soluble antioxidant, in the balloon-catheterized carotid artery of the cholesterol-fed rabbit to evaluate the importance of oxidative processes in restenosis. After 5 weeks, serum cholesterol levels were 32% lower ($P < 0.05$) in rabbits fed 1% probucol with 2% cholesterol, compared with those receiving cholesterol alone. Probucol inhibited neointimal macrophage accumulation by 68% ($P < 0.001$), reduced absolute intimal size by 51% ($P < 0.05$), and reduced the intima/media thickness ratio by 51%. These inhibitory effects were directly related to serum probucol concentrations and appeared to be unrelated to probucol's hypocholesterolemic activity. These data suggest that reactive oxygen species may be involved in the intimal response to injury and that antioxidants, such as probucol, may be therapeutically useful as inhibitors of restenosis.**

Percutaneous transluminal angioplasty is a common form of therapeutic intervention for symptomatic coronary artery disease (1). It involves the mechanical dilatation of the stenosed coronary artery by using an inflatable balloon catheter (2). Although it is a safer and less invasive procedure than coronary artery bypass surgery, percutaneous transluminal angioplasty is associated with a high long-term failure rate; 30–40% of coronary vessels reocclude within the first year of treatment (1, 3, 4). The accumulation of vascular smooth muscle cells within the intima is the major contributor to the formation of the restenotic lesion. However, intimal macrophages and macrophage-derived foam cells are also present in high abundance (5–7). In addition to causing thickening by their presence in the intima, activated macrophages express several cytokines and growth factors, such as interleukin 1, platelet-derived growth factor, insulin-like growth factor 1 (reviewed in ref. 8), and monocyte chemotactic protein 1 (9), which may further exacerbate the rate of restenosis by stimulating smooth muscle cell proliferation and the migration of medial smooth muscle cells and blood-borne monocytes into the intima. There is considerable evidence supporting the notion that macrophage-derived foam-cell formation is dependent on the oxidative modification of low density lipoprotein (LDL) and its subsequent uptake via a scavenger receptor-mediated pathway (10). Products of LDL oxidation are also reported to be chemotactic for vascular smooth muscle cells (11) and monocytes (12, 13). It is possible that agents that inhibit LDL modification will retard cholesterol uptake and thereby attenuate the processes leading to restenosis after angioplasty.

Probucol {[bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)thio]propane} is a potent lipid-soluble antioxidant (reviewed in ref. 14), which reduces atherosclerotic lesion development in Watanabe-heritable hyperlipidemic (15, 16) and cholesterol-fed rabbits (17–19). This property appears to be unrelated to its mild hypocholesterolemic effects (16), and a number of possible mechanisms for this have been proposed by Jackson *et al.* (20). We have investigated the effects of probucol therapy on neointimal lesion development in the balloon-catheterized cholesterol-fed rabbit.

MATERIALS AND METHODS

Juvenile New Zealand White rabbits (3–6 months old, weighing ≈2.7 kg) were obtained from Rosemead Rabbit (Essex, U.K.) and housed in the Biological Services Unit of St. Bartholomew's Hospital Medical College.

After 1 week on a commercial rabbit chow diet (Scientific Diet Services, Essex, U.K.), the animals were randomly allocated to one of three dietary groups: (*i*) 2% cholesterol; (*ii*) 2% cholesterol with 1% probucol in which probucol (Merrell–Dow, Cincinnati, OH) was added to the cholesterol-enriched diet by dissolving it in diethyl ether, spraying the food with probucol solution, and then air-drying the food overnight; and (*iii*) control chow, which consisted of commercial chow to which diethyl ether alone was added prior to drying. Water was allowed *ad libitum*.

One week after starting one of the above diets, animals were anesthetized with intramuscular xylazine [Rompun (Bayer, Suffolk, U.K.); 40 mg/kg of body weight] and ketamine [Vetalar (Parke–Davis); 10 mg/kg], and the left carotid bifurcation was exposed through a paramedian incision. Endothelium was removed by the passage of an inflated 2F French Fogarty Embolectomy catheter (Baxter Healthcare, Buckinghamshire, U.K.) as described (21). The validity of the procedure for endothelial cell denudation was assessed by scanning electron microscopy of several carotid arteries from animals killed immediately postoperatively (data not shown).

Samples for estimating cholesterol and probucol in serum were collected from each animal before the start of each experimental diet, peri operatively, and at the time of sacrifice. Serum was stored at −20°C prior to analysis.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein.

Four weeks after carotid deendothelialization, the animals were killed by an overdose of anesthetic and perfused via the aorta with buffered saline at a rate of 100 ml/min per kg of body weight. When the run-off from the jugular veins was clear, saline was replaced with 4% paraformaldehyde in isotonic phosphate-buffered saline (PBS) at the same flow rate for 15 min. Both carotids were dissected free and cleaned of adherent fat and fascia. The segments were rinsed in PBS and placed in 4% paraformaldehyde overnight for paraffin embedding or placed into 2.5% glutaraldehyde for subsequent electron microscopy.

Five-micrometer sections were taken for hematoxylin and eosin staining. Adjacent sections were cut for elastin staining with Verhoeff–van Geisen stain and for immunostaining with monoclonal antibodies directed against rabbit alveolar macrophages (RAM-11) and smooth muscle cell actin (HHF-35) as described by Tsukada *et al.* (22).

Serum probucol levels were measured by HPLC with a probucol analogue, 4,4′-[1-methylbutylidenebis(thio)]bis-[2,6-bis(1,1-dimethylethyl)phenol] (23), as an internal standard. Serum (50 μl) was placed into a tube with 400 μl of distilled water, 800 μl of isooctanol, and 400 μl of ethanol containing 5 μg of the probucol analogue. Tube contents were mixed and centrifuged for 5 min at 960 × *g* and 4°C, and the isooctanol layer was transferred to a glass tube, dried under nitrogen, and reconstituted in 100 μl of acetonitrile. HPLC was performed by using a Pharmacia–LKB system consisting of a Valco injector with a 10-μl sample loop, a Hypersil ODS 3 × 100 mm column (5 μm; Chromopack, Middelburg, The Netherlands), a gradient pump (model 2249), and a UV detector (model VM 2141) set at 240 nm. The solvent system used was acetonitrile/water, 94:6 (vol/vol), at a flow rate of 1 ml/min.

Serum cholesterol levels were measured by the cholesterol oxidase–peroxidase enzymatic–colorimetric method by using a cholesterol C-system kit (Boehringer Mannheim).

Intimal and medial thickness was measured with a Zeiss Axioskop microscope equipped with a ×10 Achroplan objective, a digitizing pad, and an IBM 55SX personal computer containing a VIDS V interface card and VIDS V software (Ai Cambridge, Pampisford, U.K.). Measurements were performed in duplicate on three Verhoeff-van Geisen-stained sections from the midcarotid. For each section, the intimal and medial thickness was measured at eight separate points, the intima/media ratio was calculated for each point, and the overall intima/media ratio for each section was expressed as the mean of these values.

The macrophage content of the neointima was determined by counting the proportion of RAM-11 positive cells in at least 10 high-power fields (using a ×40 Achroplan objective) of midcarotid sections taken from two separate levels. A total of more than 600 intimal cells were counted per specimen.

Tissue segments for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were postfixed in 1% osmium tetroxide as described by Ferns *et al.* (24). After dehydration in a graded ethanol series, the segments for TEM were placed in propylene oxide before embedding in Taab premix resin (Taab Laboratories Equipment, Berkshire, U.K.). Sections (1 μm) were stained with methylene blue, and areas of interest were selected for thin sectioning (800 Å) with an LKB Ultramicrotome III. The sections were placed onto copper grids and counterstained with lead citrate and uranyl acetate. They were examined with a Philips 201 TEM. Segments for SEM were critical-point-dried with CO_2 in a Bio-Rad E3000 critical point drier (V. G. Microtech, Uckfield, East Sussex, U.K.), sputter-coated with gold with an Emscope sputter coater (Emscope, Kent, U.K.), and examined with a Stereoscan 180 (Cambridge Instruments, Cambridge, U.K.) SEM at 15 kV.

Unpaired *t* and Mann–Whitney tests and linear regression analyses were performed on a Dell 316SX personal computer by using Instat software (GraphPad Software, San Diego).

RESULTS

The 2% cholesterol-enriched diet caused a significant increase in serum total cholesterol levels by 1 week, whether or not it was supplemented with probucol ($P < 0.001$) (Table 1). Serum levels of cholesterol continued to rise further over the next 4 weeks, but were lower for the animals receiving 1% probucol ($P < 0.05$) (Table 1). Each group of animals was matched for age and weight at the start of the experiment, and weight gain and final weight did not differ significantly between the groups (Table 1). Probucol levels in serum were directly related to levels of cholesterol ($r = 0.87$, $P < 0.0001$) and increased during the course of treatment (Table 1); this is in accord with the known lipophilic properties of probucol and its large volume of distribution (14).

Table 1. Mean body weights, serum cholesterol and probucol levels, and arterial-wall dimensions of rabbits undergoing carotid artery balloon angioplasty

	Dietary treatment		
	Control	2% cholesterol	2% cholesterol/ 1% probucol
Animals (*n*)	10	11	9
Mean weight (kg)			
Entry	2.84 ± 0.24	2.56 ± 0.16	2.68 ± 0.21
Sacrifice	3.35 ± 0.19	3.01 ± 0.10	3.06 ± 0.14
Mean serum cholesterol (mmol/liter)			
Entry	0.90 ± 0.13	1.58 ± 0.21	1.44 ± 0.25
1 week	1.12 ± 0.13	16.50 ± 3.18*	13.70 ± 2.63*
5 weeks	1.12 ± 0.18	39.30 ± 4.42*	26.70 ± 2.65*†
Mean serum probucol (μg/ml)			
1 week	—	—	31.2 ± 10.8
5 weeks	—	—	65.0 ± 12.7
Mean thickness 4 weeks after injury (mm)			
Intimal	0.050 ± 0.007	0.144 ± 0.029‡	0.070 ± 0.020§
Medial	0.084 ± 0.010	0.105 ± 0.009	0.099 ± 0.011
Mean intima/media ratio 4 weeks after injury	0.59 ± 0.06	1.36 ± 0.26¶	0.65 ± 0.16

Values are means ± SEM.

*, $P < 0.001$ compared to controls; †, $P < 0.05$ compared to cholesterol-fed animals with unpaired *t* tests; ‡, $P < 0.02$; ¶, $P < 0.01$ compared to controls; §, $P < 0.05$ compared to cholesterol-fed animals with Mann–Whitney tests.

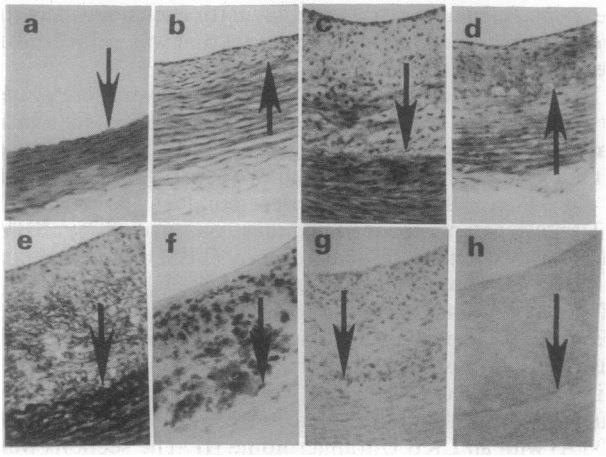

FIG. 1. (*a–d*) Hematoxylin- and eosin-stained sections of an uninjured rabbit common carotid artery (*a*) and the common carotid arteries of balloon-catheterized rabbits 4 weeks after injury from animals on a normal chow diet (*b*), a 2% cholesterol diet (*c*), and a 2% cholesterol diet supplemented with 1% probucol (*d*). (*e–h*) Balloon-injured carotid artery of an animal receiving a 2% cholesterol diet stained with Verhoeff–van Geisen elastin stain (*e*), the rabbit macrophage-specific antibody RAM-11 (*f*), or an anti-α-actin smooth muscle cell-specific antibody, HHF-35. Sections (5 μm) were deparaffinized and immunostained with RAM-11 or HHF-35 at a dilution of 1:2000 by using an ABC Vectastain kit (Vector Laboratories) and the 3,3-diaminobenzidine (Sigma) reaction product. RAM-11-positive cells are shown deep within the neointima in the animals fed a cholesterol diet. *h* is a control section in which the primary antibody was omitted during the staining procedure. Sections were counterstained with methyl green. The internal elastic lamina is marked by arrows. (×320.)

As previously demonstrated (21), balloon deendothelialization resulted in complete removal of endothelium and caused marked platelet deposition on the denuded vascular surface. Four weeks after surgery, there was considerable neointimal cell accumulation in the injured carotid arteries of all three experimental groups. At this time the neointimal layer contributed approximately 38% to the overall thickness of the arterial wall in the control animals (Fig. 1*b* and Table 1), and in this group the lesion consisted almost entirely of smooth muscle cells (HHF-35-positive cells); macrophages (RAM-11-positive cells) were only rarely observed.

Balloon injury in the cholesterol-fed animals was associated with a thicker neointima than observed in the controls (Figs. 1*c* and 2*b* and Table 1) ($P < 0.02$). In the cholesterol-fed animals, the neointima comprised more than 57% of the total vessel wall depth, and the neointima itself contained large numbers of macrophage-derived foam cells (Fig. 1 *c* and *f* and Fig. 3). In some cases macrophages were also observed below the internal elastic lamina. The mean intima/media thickness ratio in the animals receiving the 2% cholesterol supplement was increased compared with those animals receiving control chow alone ($P < 0.01$) (Table 1).

The cholesterol-fed animals that received a 1% probucol supplement had a lower macrophage content of the neointima compared with the animals receiving cholesterol alone ($P < 0.001$) (Figs. 1*d* and 2*a*). The absolute thickness of the neointima in these animals was also reduced relative to those animals receiving the cholesterol-only diet ($P < 0.05$) (Table 1). There was an inverse relationship between levels of serum probucol and intimal macrophage content (Fig. 2*c*) ($P < 0.002$, $r = -0.73$). In animals with high circulating levels of probucol, few macrophage-derived foam cells could be detected; in contrast, there was a weak positive relationship between serum cholesterol levels and intimal macrophage content (Fig. 2*d*) ($P < 0.05$, $r = 0.53$).

There were significant positive associations between absolute intimal thickness and intimal macrophage content ($P <$

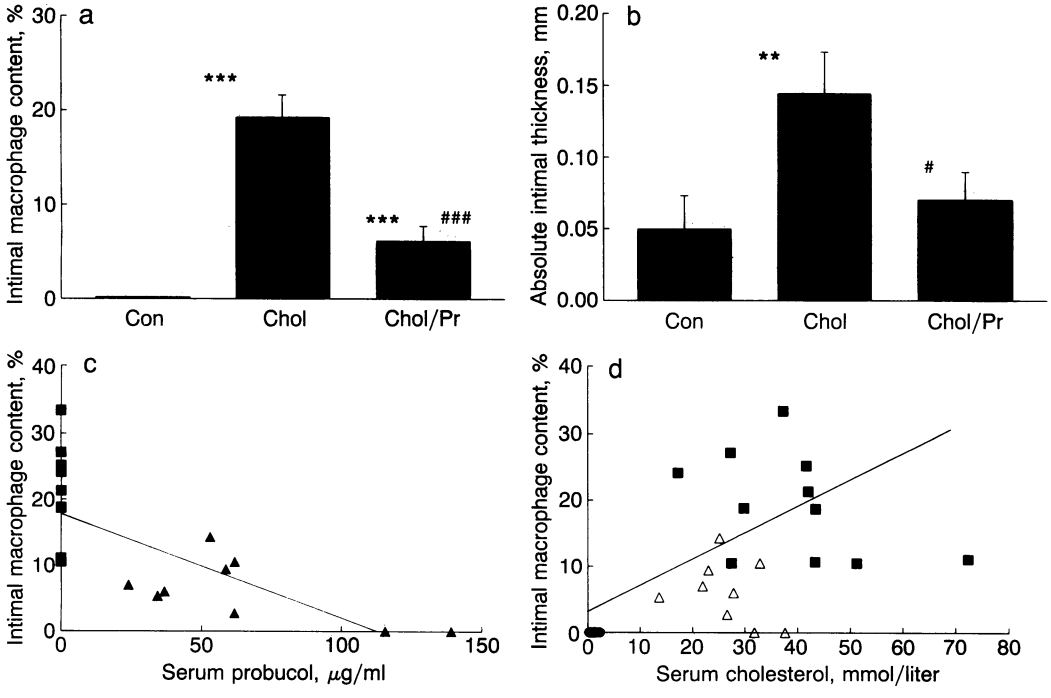

FIG. 2. Effects of probucol (Pr) on the neointimal macrophage content (*a*) and the absolute neointimal thickness (*b*) in balloon-catheterized rabbits 4 weeks after injury. ** = $P < 0.01$ and *** = $P < 0.001$, both compared to control (Con) animals. # = $P < 0.05$ and ### = $P < 0.001$, both compared to animals on 2% cholesterol (Chol). (*c*) Relationship between intimal macrophage content and serum probucol levels in 2% cholesterol-fed rabbits 4 weeks after balloon injury in animals receiving cholesterol alone (■) or cholesterol with probucol (▲). Intimal macrophage content was inversely related to serum probucol levels ($P < 0.002$, $r = -0.73$). (*d*) Relationship between intimal macrophage content and serum cholesterol levels at the time of killing in animals fed a control chow diet (●) or 2% cholesterol diets with (△) or without (■) probucol. Intimal macrophage content was directly related to serum cholesterol levels in the animals not receiving probucol ($P < 0.05$, $r = 0.53$).

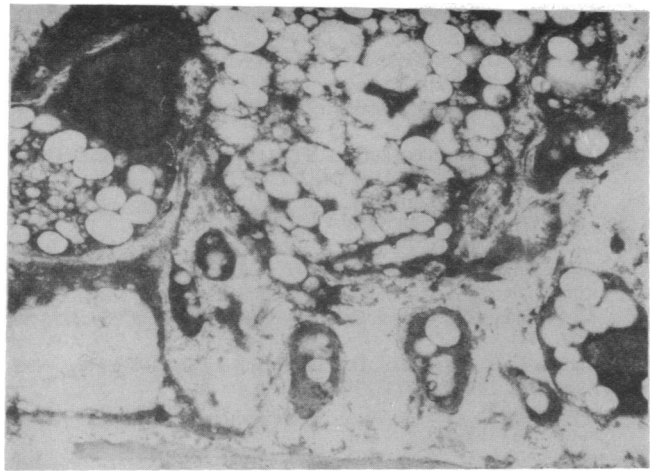

FIG. 3. A transmission electron micrograph of the intima from a balloon-catheterized rabbit fed a 2% cholesterol diet for 4 weeks after injury, showing a number of macrophage-derived foam cells with cytoplasmic lipid inclusions. (×3600.)

0.003, $r = 0.63$) (Fig. 4*a*) and between intima/media thickness ratio and intimal macrophage content ($P < 0.003$, $r = 0.64$) (data not shown) within the groups of animals fed 2% cholesterol. Probucol treatment abrogated the effects of cholesterol feeding on neointimal size (Fig. 2*b*) and there was an inverse relationship between serum probucol levels and intima/media ratio (Fig. 4*b*). Neointimal thickness did not differ significantly between the animals on cholesterol with probucol and those on a control chow diet ($P > 0.05$). The absolute size of the media did not differ significantly between the three groups.

DISCUSSION

There have been several reports of the inhibitory effects of probucol in atherogenesis (14), however this study is different in addressing the effects of this antioxidant in a model of combined mechanical and diet-induced arterial injury. Although this model differs from percutaneous transluminal angioplasty in many respects, the two procedures share a number of features in common (reviewed in ref. 25). Probucol is a potent antioxidant; its mechanism of action in preventing LDL oxidation has been attributed to its activity as a superoxide radical scavenger (26). Its efficacy as an antioxidant is directly related to its concentration in the plasma LDL fraction (27). Probucol also prevents the oxidative modification of very low density lipoprotein β (β-VLDL) (28), which is of particular relevance to the cholesterol-fed rabbit model, in which β-VLDL is the major cholesterol-rich plasma lipoprotein. Parthasarathy *et al.* (28) found that oxidation of β-VLDL facilitates its uptake and degradation by macrophages.

Steady-state levels of probucol have been achieved within approximately 14 days after the start of therapy. Using the same dietary regime that we have used (17), Daugherty *et al.* (17) found that probucol did not affect total plasma cholesterol, triglycerides, phospholipids, or β-VLDL composition. Our data indicate that probucol does have a hypocholesterolemic effect, as reported previously (reviewed in ref. 14). But this property does not appear to account for the reduction of macrophage accumulation (discussed below).

Probucol therapy reduces the proportion of intima covered by atheromatous lesions in fat-fed rabbits (17). It also inhibits lesion progression (29) and affects the cellular composition of atheromatous plaques in mature Watanabe heritable hyperlipidemic rabbits (30), though it did not promote lesion regression in these animals (31, 32). Yamomoto *et al.* (33) reported that probucol at a concentration of 103 mg/liter

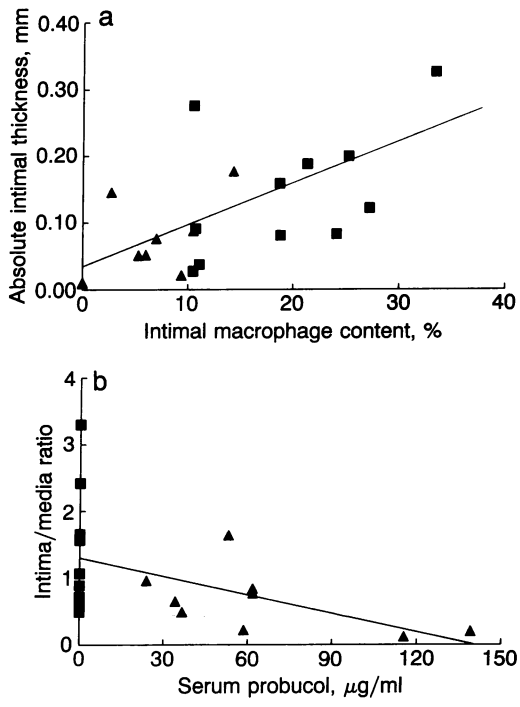

FIG. 4. (*a*) Relationship between intimal macrophage content and absolute intimal thickness in animals fed 2% cholesterol (■) or 2% cholesterol with 1% probucol (▲) ($P < 0.003$, $r = 0.64$). (*b*) Relationship between intima/media ratio and serum probucol levels in animals on a 2% cholesterol diet ($P < 0.03$, $r = -0.49$).

prevents macrophage-derived foam-cell formation *in vitro*. It is of interest that in our study, the animals with serum probucol levels in excess of this concentration had few intimal macrophages and that at lower levels the presence of macrophages was inversely related to serum probucol concentrations (Fig. 2*c*).

Gellman *et al.* (34) have shown that lowering plasma cholesterol levels with lovastatin reduces intimal hyperplasia following balloon angioplasty; they attribute this property of lovastatin to its effect on mevalonate metabolism. Our data cannot be explained by probucol's hypocholesterolemic effects alone, as those animals with the highest levels of serum probucol and fewest intimal macrophages proved to be animals with high concentrations of serum cholesterol (Fig. 2 *c* and *d*). There was a trend for intimal macrophage content to fall with increasing serum cholesterol levels within the probucol-treated group (Fig. 2*d*), though this failed to reach statistical significance ($P > 0.05$). However, when intimal macrophage content was compared for animals matched for serum cholesterol concentrations, the probucol-treated rabbits ($n = 8$, mean serum cholesterol = 28.4 ± 1.87 mmol/liter) had significantly lower values for intimal macrophage content (6.24 ± 1.81%) than animals receiving cholesterol alone ($n = 5$; mean serum cholesterol = 27.8 ± 3.88 mmol/liter, $P > 0.05$ compared with probucol-treated animals; mean intimal macrophage content = 22.8 ± 3.88%, $P < 0.01$ compared with the probucol-treated group). Hence the effects of probucol on intimal macrophage content appear to be independent of its cholesterol-lowering properties.

The mechanisms by which probucol inhibits intimal-cell accumulation and macrophage content are of particular interest. Macrophage infiltration into the neointima after balloon injury depends first on their ability to attach to the exposed subendothelium and second on their migration into the subendothelial tissues. The first property will rely in part on the activation state of the circulating monocytes and their expression of adhesion molecules. The second depends on

the expression of chemotactic agents by the cells of the damaged media and developing neointima and on the ability of monocytes to respond to these factors. It is possible that probucol inhibits the activation state of monocytes through its ability to quench reactive oxygen species. Probucol also inhibits interleukin 1 secretion from lipopolysaccharide-stimulated macrophages (35–37). Interleukin 1 is a potentially important cytokine in the balloon-injury model, as it is an indirect smooth muscle cell mitogen (38) and also induces adhesion molecule expression. Oxidized LDL itself also increases the expression of adhesion molecules on monocytic cells and stimulates their differentiation into mature macrophages (39). Probucol inhibits the oxidation of both LDL and β-VLDL (28, 40), and it may thereby inhibit these latter phenomena. Hara *et al.* (41) found that probucol enhances monocyte migration in response to f-Met-Leu-Phe and serum factors, and they propose that this may augment monocyte/macrophage egress from the vessel wall and consequently reduce monocyte accumulation. However, this is not the only possible explanation of our findings. We have recently observed that probucol inhibits monocyte adhesion to endothelial cells *in vivo* and *in vitro* (42), and this may be another means by which it inhibits monocyte recruitment both in our study and in that reported by O'Brien *et al.* (30).

Platelet-derived growth factor, a potent direct smooth muscle cell mitogen and chemoattractant, is expressed by macrophage-derived foam cells during all stages of atherogenesis (43). These cells also express monocyte chemotactic protein 1 (9), which would tend to increase the recruitment of monocytes into the tunica intima (44). Probucol slows the rate of macrophage uptake of modified LDL, and a number of possible mechanisms for this have been proposed (45).

In conclusion, probucol inhibits accumulation of macrophages in the injured carotid artery of the cholesterol-fed rabbit. This may explain in part the reduction of intimal size in animals receiving this drug. Activated macrophages/foam cells also elaborate factors that may contribute to intimal thickening including mitogens, such as platelet-derived growth factor, reactive oxygen species (46), and chemoattractants including monocyte chemotactic protein 1. Probucol may prevent macrophage activation and macrophage-derived foam cell formation, thereby suppressing monokine release. Finally, our data suggest that antioxidant therapy may be of potential benefit after percutaneous transluminal angioplasty as a means of preventing coronary artery restenosis, particularly in patients with hypercholesterolemia.

We thank Miss Kate Ong, Dr. Shurui-Li, and Dr. Jill Lewis for their expert technical assistance and Dr. Alan Gown (University of Washington, Seattle) for his gift of the mouse monoclonal antibodies. The probucol and probucol analogue were both gifts from Merrell–Dow, and we are grateful to Drs. Richard Jackson and Simon Mao for arranging this. This project was supported by a grant from Ono Pharmaceutical, Osaka, Japan. We are also grateful to the British Heart Foundation and University of London for grants to G.A.A.F.

1. Meier, B. (1991) *Annu. Rev. Med.* **42**, 47–59.
2. Gruentzig, A. (1978) *Lancet* **i**, 263.
3. Glazier, J. J., Williams, M. G., Madden, S. & Rickards, A. F. (1990) *J. R. Coll. Physicians London* **24**, 292–294.
4. Leimgruber, P. P., Roubin, G. S., Hollman, J., Cotsonis, G. A., Meier, B., Douglas, J. S., King, S. B., Jr., & Gruentzig, A. R. (1986) *Circulation* **73**, 710–717.
5. Austin, G. E., Ratliff, N. B., Hollman, J., Tabei, S. & Phillips, D. F. (1985) *J. Am. Coll. Cardiol.* **6**, 369–375.
6. Waller, B. F., Pinkerton, C. A., Orr, C. M., Slack, J. D., Van Tassel, J. W. & Peters, T. (1991) *Circulation* **83** (Suppl. 2), I28–I41.
7. Clagett, G. P., Robinowitz, M., Youkey, J. R., Fisher, D. F., Fry, R. E., Myers, S. I., Lee, E. I., Collins, G. J. & Virmani, R. (1986) *J. Vasc. Surg.* **3**, 10–23.
8. Raines, E. W. & Ross, R. (1989) in *Human Monocytes*, eds. Zembala, M. & Asherson, G. (Academic, London), pp. 247–259.
9. Yla-Herttuala, S., Lipton, B. A., Rosenfeld, M. E., Sarkioja, T., Yoshimura, T., Leonard, E. J., Witzum, J. L. & Steinberg, D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5252–5256.
10. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. & Witzum, J. L. (1989) *N. Engl. J. Med.* **320**, 915–924.
11. Autio, I., Jaakkok, O., Solakari, T. & Nikkari, T. (1990) *FEBS Lett.* **277**, 247–249.
12. Quinn, M. T., Parthasarathy, S., Fong, L. G. & Steinberg, D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2995–2998.
13. Quinn, M. T., Parthasarathy, S. & Steinberg, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2805–2809.
14. Buckley, M. M.-T., Goa, K. L., Price, A. H. & Brogden, R. N. (1989) *Drugs* **37**, 761–800.
15. Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N., Ooshima, A., Yoshida, H. & Kawai, C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5928–5931.
16. Carew, T. E., Schwenke, D. C. & Steinberg, D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7725–7729.
17. Daugherty, A., Zweifel, B. S. & Schonfeld, G. (1989) *Br. J. Pharmacol.* **98**, 612–618.
18. Kritchevsky, D., Kim, H. K. & Tepper, S. A. (1971) *Proc. Soc. Exp. Biol. Med.* **136**, 1216–1221.
19. Tawara, K., Ishihara, M., Ogawa, H. & Tomikawa, M. (1986) *Jpn. J. Pharmacol.* **41**, 211–222.
20. Jackson, R. L., Barnhart, R. L. & Mao, S. J. T. (1991) in *Hypercholesterolemia, Hypocholesterolemia, Hypertriglyceridemia*, eds. Malmendier, C. L., Alaupovic, P. & Brewer, H. B., Jr. (Plenum, New York), pp. 367–372.
21. Ferns, G. A. A., Reidy, M. A. & Ross, R. (1991) *Am. J. Pathol.* **138**, 1045–1057.
22. Tsukada, T., Rosenfeld, M. E., Ross, R. & Gown, A. M. (1986) *Arteriosclerosis* **6**, 601–613.
23. Mao, S. J. T., Yates, M. T., Rechtin, A. E., Jackson, R. L. & Van Sickle, W. A. (1991) *J. Med. Chem.* **34**, 299–302.
24. Ferns, G. A. A., Reidy, M. A. & Ross, R. (1990) *Am. J. Pathol.* **137**, 403–413.
25. Ferns, G. A. A., Stewart-Lee, A. L. & Änggård, E. E. (1992) *Atherosclerosis* **92**, 89–104.
26. Bridges, A. B., Scott, N. A. & Belch, J. J. F. (1991) *Atherosclerosis* **89**, 263–265.
27. Barnhart, R. L., Busch, S. J. & Jackson, R. L. (1989) *J. Lipid Res.* **30**, 1703–1710.
28. Parthasarathy, S., Quinn, M. T., Schwenke, D. C., Carew, T. E. & Steinberg, D. (1989) *Arteriosclerosis* **9**, 398–404.
29. Nagano, Y., Nakamura, T., Matsuzawa, Y., Cho, M., Ueda, Y. & Kita, T. (1992) *Atherosclerosis* **92**, 131–140.
30. O'Brien, K., Nagano, Y., Gown, A., Kita, T. & Chait, A. (1991) *Arterioscler. Thromb.* **11**, 751–759.
31. Daugherty, A., Zweifel, B. S. & Schonfeld, G. (1991) *Br. J. Pharmacol.* **103**, 1013–1018.
32. Brown, E. Q., Muetler, S. B., Uhlendorf, P. D., Mazar-Dootz, M. & Bocan, T. M. A. (1992) *FASEB J.* **6**, 2250 (abstr.).
33. Yamamoto, A., Takaichi, S. & Hara, H. (1986) *Atherosclerosis* **62**, 209–217.
34. Gellman, J., Ezekowitz, M. D., Sarembock, I. J., Azrin, M. A., Nochomowitz, L. E., Lerner, E. & Haudenchild, C. C. (1990) *J. Am. Coll. Cardiol.* **17**, 251–259.
35. Ku, G., Doherty, N. S., Wolos, J. A. & Jackson, R. L. (1988) *Am. J. Cardiol.* **62**, 77B–81B.
36. Ku, G., Doherty, N. S., Schmidt, L. F., Jackson, R. L. & Dinerstein, R. J. (1990) *FASEB J.* **4**, 1645–1653.
37. Akeson, A. L., Woods, C. W., Mosher, L. B., Thomas, C. E. & Jackson, R. L. (1991) *Atherosclerosis* **86**, 261–270.
38. Raines, E. W., Dower, S. K. & Ross, R. (1988) *Science* **243**, 393–396.
39. Frostegard, J., Nilsson, J., Haegerstrand, A., Hamsten, A., Wigzell, H. & Gidlund, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 904–908.
40. Parathasarathy, S., Young, S. G., Witzum, J. L., Pittman, R. C. & Steinberg, D. (1986) *J. Clin. Invest.* **77**, 641–644.
41. Hara, S., Nagano, Y., Sasada, M. & Kita, T. (1992) *Arterioscler. Thromb.* **12**, 593–600.
42. Forster, L., Ferns, G. A. A., Stewart-Lee, A., Nourooz-Zadeh, J. & Änggård, E. E. (1992) *Br. J. Pharmacol.*, in press.
43. Ross, R., Masuda, J., Raines, E. W., Gown, A. M., Katsuda, S., Sasahara, M., Malden, L. T., Masuko, H. & Stao, H. (1990) *Science* **248**, 1009–1012.
44. Cushing, S. D. & Fogelman, A. M. (1992) *Arterioscler. Thromb.* **12**, 78–82.
45. Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N., Narumiya, S. & Kawai, C. (1988) *Am. J. Cardiol.* **25**, 13B–19B.
46. Rao, G. N. & Berk, B. C. (1992) *Circ. Res.* **70**, 593–599.