AGI-1067, butanedioic acid, mono-[4-[(1-Methylthiethylidene)bis(thio)]bis-[2,6-bis(1,1-dimethylethyl)phenol]] Inhibits Compensatory Remodeling and Promotes Lumen Loss Associated with Atherosclerosis in Apolipoprotein E-Deficient Mice

Ben J. Wu,1 Nick Di Girolamo, Konstanze Beck,1 Colm G. Hanratty, Katherine Choy, Jing Y. Hou, Michael R. Ward, and Roland Stocker1

Centre for Vascular Research (B.J.W., K.B., K.C., R.S.) and Inflammatory Diseases Research Unit (N.D.G.), School of Medical Sciences, University of New South Wales, Sydney, Australia; Royal North Shore Hospital, University of Sydney, Sydney, Australia (C.G.H., M.R.W.); and Heart Research Institute, Sydney, Australia (J.Y.H.)

Received December 14, 2006; accepted February 7, 2007

ABSTRACT

Probucol [4,4′-[(1-Methylthiethylidene)bis(thio)]bis-[2,6-bis(1,1-dimethylethyl)phenol]] was withdrawn from the United States market because it failed to inhibit atherosclerosis in human femoral arteries, yet the drug was shown subsequently to inhibit atherosclerosis in human carotid arteries, and probucol monosuccinate ester is presently being tested in a phase III clinical trial as an antiatherosclerotic compound based on its antioxidant properties. Inflammatory macrophages are implicated in arterial remodeling associated with atherosclerosis, and probucol inhibits experimental atherosclerosis in part by decreasing macrophages in lesions. However, the impact of probucol on remodeling is unknown, although such knowledge could help explain why the drug’s benefit on human atherosclerosis is controversial. We therefore examined the effect of probucol on remodeling of the common carotid artery in apolipoprotein E-deficient mice. We observed that during de novo atherosclerosis, plaque growth was fully compensated by expansive remodeling, such that lumen area was unaffected. Early lesions were composed almost entirely of macrophages, and their contribution to lesion area progressively decreased thereafter. Probucol significantly decreased plaque area, expression of vascular cell adhesion molecule-1, and proliferation of intimal cells, resulting in delayed macrophage accumulation in the vessel. Probucol also decreased the production and activity of matrix metalloproteinases-2 and -9, independent of the plasmin protease system, and this was associated with an inhibition of expansive remodeling, resulting in lumen loss. These studies show that probucol attenuates compensatory remodeling associated with de novo atherosclerosis, probably via its anti-inflammatory properties. Our findings suggest that lumen volume is not a suitable surrogate to assess the antiatherosclerotic activity of probucol and related drugs.

Atherosclerosis is a disease characterized by the presence of heightened oxidative stress and inflammation (Stocker and Keaney, 2004), and there is increasing interest in novel compounds with antioxidant and anti-inflammatory activities as potential therapeutic antiatherosclerotic agents. AGI-1067 is one of these agents. It has antioxidant activities and selectively inhibits redox-sensitive endothelial and monocyte inflammatory gene expression in vitro, lowers low-density lipoprotein cholesterol, and decreases vascular cell adhesion molecule (VCAM)-1 expression in vivo and atherosclerosis in several animal models including apolipoprotein E-deficient (ApoE−/−) mice (Sundell et al., 2003; Kunsch et al., 2004). AGI-1067 also decreases restenosis after percutaneous coronary intervention in humans (Tardif et al., 2003), and the compound is presently being tested as an antiatherosclerotic drug in a phase III clinical trial. AGI-1067 is a pharmacologic derivative of the antioxidant probucol. Although introduced as a lipid-lowering drug in the
early 1970s, subsequent mechanistic studies demonstrated that, similar to AGI-1067, probucol also has anti-inflammatory activities. These include the ability to inhibit the secretion of the proinflammatory cytokine interleukin-1 by macrophages (Ku et al., 1990) and to decrease the expression of monocyte chemotactic protein 1 (Chang et al., 1995; Nakamura et al., 2002) and monocyte infiltration in vivo (Nakamura et al., 2002). Indeed, anti-inflammatory rather than antioxidant activities have been proposed recently to explain probucol’s protection against experimental atherosclerotic vascular disease (Choy et al., 2005; Wu et al., 2006). By inhibiting monocyte infiltration and macrophage foam cell accumulation in the vessel wall (Choy et al., 2005), probucol could conceivably decrease arterial matrix metalloproteinase (MMP) activity (Nakamura et al., 2002) and thus affect arterial remodeling (Galis and Khatri, 2002; Ivan et al., 2002; Kuzuya et al., 2006).

Arterial remodeling refers to a change in cross-sectional area of the blood vessel (Glagov et al., 1987) (Fig. 1) and is thought to cause the compensatory enlargement observed in human arteries with atherosclerotic disease (Pasterkamp et al., 1997). It is now also recognized that vascular remodeling can determine the lumen area of diseased blood vessels more so than atherosclerotic plaque size (Pasterkamp et al., 1997). This may help explain the controversy regarding the efficacy with which probucol inhibits atherosclerosis in humans. In particular, the PQRST study reported failure of the drug to inhibit femoral atherosclerosis in humans (Walldius et al., 1994), and for this reason, probucol was withdrawn from the United States market several years ago. However, the Fukuoka Atherosclerosis Trial study showed subsequently that probucol effectively inhibits human atherosclerosis in the carotid artery (Sawayama et al., 2002).

A potentially important difference between the two studies is the method used to assess disease progression. In the Fukuoka Atherosclerosis Trial, atherosclerosis was determined directly as the intima/media ratio (Sawayama et al., 2002), whereas in PQRST, atherosclerosis was estimated indirectly as a change in vessel lumen volume (Walldius et al., 1994). Thus, if probucol inhibited compensatory remodeling, the basis upon which the PQRST study concluded probucol to lack antiatherosclerotic activity (Walldius et al., 1994) may be uncertain.

In the present study, we therefore examined the effect of probucol on vascular remodeling during de novo atherosclerosis using the carotid artery in ApoE−/− mice. We show that probucol markedly retards the compensatory remodeling response in these arteries such that lumen loss is greater in probucol-treated than control animals. This activity of probucol seems to be due to inhibition of macrophage accumulation and the resulting decrease in MMP-2 and -9 in the affected vessel wall.

**Materials and Methods**

### ApoE−/− Mice.

As described previously (Witting et al., 2000), male C57BL/6J mice, homozygous for the disrupted apolipoprotein E gene (ApoE−/−) and originally purchased from Jackson Laboratories (Bar Harbor, ME), were used at 8 to 10 weeks of age and then fed ad libitum a high-fat diet (HFD) based on Harlan Teklad diet TD88137 (Witting et al., 2000) ± 1% (w/w) probucol (Jucker Pharma, Stockholm, Sweden) and sacrificed at 15, 30, 60, 120, 150, and 180 days. There were six to eight mice for all time points in the control and four mice for 15, 30, 60, and 150 days and 11 mice for 180 days for the probucol-treated group, and there were no probucol-treated animals for 120 days. The investigation conformed with the **Guide for the Care and Use of Laboratory Animals** published by the United States National Institutes of Health (NIH Publication 53-23, revised 1996).

### Morphometry.

Animals were perfusion fixed with 10% buffered formalin for 7 min as described previously (Witting et al., 2000), and the vessels were then immersion fixed at 4°C for a further 24 h before paraffin embedding and sectioning. To examine the atherosclerotic response in a consistent manner, carotid vessels were sectioned at the start of the bifurcation and then successively every 100 μm proximally for 500 μm. A reference site was taken at the middle of the common carotid artery, where there was no macroscopic evidence of atheroma. Sections were stained with Weigert’s hematoxylin-van Giessen, and vessel areas were measured by computed planimetry using Video-Pro 32 software (Leading Edge, Norwood, South Australia) from images obtained with a Leitz DM-IRB microscope and a Panasonic digital camera.

Lumen area (LA) and vessel area (VA) were defined as the area circumscribed by the intima-lumen boundary and the inner border of the internal elastic lamina, respectively, whereas plaque area (PA) was defined as VA – LA (Fig. 1). Measurements were carried out blinded by two assessors, and results of the two assessors were averaged. The effect of time and distance from the bifurcation on VA was examined in nondiseased segments. It was first established that there was no relationship between VA and time or site, apart from a small increase in VA at the bifurcation itself (i.e., the 0-μm site). All subsequent measurements of VA and LA at sites proximal to the

---

**Fig. 1.** Arterial remodeling and its impact on lumen area of blood vessel. A, cartoon adapted from (Ward et al., 2000) showing a nondiseased vessel in the center. If the vessel acquires atheroma (PA, black area), and there is no remodeling (arrow down), the LA (white area) decreases. If outward or positive remodeling occurs, the VA, defined as the area circumscribed by the inner border of the internal elastic lamina (i.e., the border separating gray from black areas), enlarges. If such enlargement “perfectly” compensates for the atheroma (arrow right), the lumen area remains constant. If outward remodeling is incomplete (arrow down and left), greater lumen loss results. B, histological examples of segments of nondiseased (left) and diseased vessel (right). Relative to nondiseased vessel, the vessel area (seen here as the area within the innermost black circular line) has increased but has not fully compensated for plaque area, such that lumen area is decreased.
bifurcation were normalized for VA at the midcarotid reference site (i.e., normalized VA = VA at lesion site/VA at reference site). The relationship among normalized LA, normalized PA, and normalized VA was then evaluated in animals, examining the goodness of fit of linear, biphasic, and curvilinear regression lines. This evaluation revealed that the relationship was linear. Thereafter, the mean remodeling index (ΔVA/ΔPA) for each site and time point was determined, where ΔVA = VA at lesion site – VA at reference site and ΔPA = PA at lesion site – PA at reference site. For comparisons of plaque area and remodeling index between control and probucol-treated animals, 100- and 200-μm sites were used.

**Immunohistochemistry.** Serial paraffin sections at 100 and 200 μm proximal to the bifurcation of carotid vessels (n = 3–4) were used for immunohistochemistry. Sections were deparaffinized, rehydrated, and endogenous peroxidase quenched for 15 min with 3% hydrogen peroxide. Sections were then incubated for 1 h at room temperature with the following primary antibodies: rabbit antivcym-1 mouse VCAM-1 (dilution, 1:50; Santa Cruz Biochemicals, Santa Cruz, CA), rat anti-mouse Mac-3 (macrophages; dilution, 1:200; DakoCytomation California Inc., Carpinteria, CA), rabbit anti-mouse MMP-9 (dilution, 1:50; Chemicon International, Temecula, CA), and MMP-2 (dilution 1:100, Chemicon International), and rabbit anti-mouse proliferation cell nuclear antigen (PCNA; dilution, 1:50; Santa Cruz Biochemicals) antibodies, followed by incubation with relevant secondary antibodies (dilution, 1:200) for 30 min at room temperature. Vectorstain Elite ABC reagent (Vectorstain Elite ABC Kit; 30 min; Vector Laboratories, Burlingame, CA), and 3,3′-diaminobenzidine substrate-chromogen (DakoCytomation California Inc.) were used for staining, and Harris hematoxylin was used for counterstaining. For double staining with Mac-3 and PCNA, sections were incubated with both antibodies and then successively treated with relevant secondary antibodies. Images were captured with an Olympus BX60F5 photomicroscope (Olympus, Tokyo, Japan) attached to a SPOT digital camera (Diagnostic Instruments Inc., Sterling Heights, MI). The intensity of staining with VCAM-1 was scored as follows: 1, no staining; 2, weak staining; 3, moderate staining; and 4, strong staining of the vascular cell. Plaque Mac-3- and MMP-9-positive staining areas were determined using Adobe Photoshop version 6.0 (Adobe Systems, Mountain View, CA) by tracing. To determine the total number of vascular cells, the mean numbers of nuclei present in the intima and media in three serial sections per animal (n = 3–4 for control and probucol) was determined by counting. For proliferating cells, the number of PCNA+–positive cells was determined by counting for each section and expressed as proportion of total cell numbers. For proliferating macrophages, the proportion of Mac-3+–positive cells that were also positive for PCNA was determined by manual counting. All of these analyses were carried out blinded by a single investigator.

**Biochemical Analyses of Tissue.** Carotid artery specimens were separately harvested from mice at 60 and 150 days after HFD ± 1% probucol feeding. After perfusion (Witting et al., 2000), common carotid arteries were excised between the aortic arch and the carotid bifurcation, carefully trimmed of periadventitial fat, and frozen in liquid nitrogen until use. Frozen tissues (pools from five to eight mice) were homogenized in cell lysis buffer on ice and used for zymography and plasminogen activator inhibitor (PAI-1) activity measurement. Similar carotid artery specimens (n = 4) were also harvested separately from mice at 60 days after HFD ± 1% probucol feeding and were incubated individually for 24 h at 37°C with Durbecco’s modified Eagle’s medium supplemented with 0.2% bovine serum albumin (Sigma-Aldrich, St. Louis, MO). At the end of the incubation, the conditioned media were collected and used for zymography.

**Zymography.** Equal amounts of proteins extracted from carotid arteries or present in conditioned media were loaded onto SDS-polyacrylamide gel electrophoresis gels (10% acrylamide) impregnated with gelatin (as substrate for MMP-2 and MMP-9) or casein (for plasmin) under nonreducing conditions. Gels were incubated in 2.5% Triton X-100 for 1 h after electrophoresis, then placed in MMP (50 mM Tris- HCl, pH 7.4, 10 mM CaCl2, 0.02% NaN3) or plasmin (50 mM Tris-HCl, pH 7.9, 0.02% NaN3) substrate buffer overnight at 37°C. Gels were stained with 0.1% Coomassie Blue R-250 (Sigma-Aldrich) in 40% methanol and 10% acetic acid and then destained to detect clear zones of proteolysis. Gels were digitally imaged, and bands were quantified in arbitrary units using NIH Image software.

**Tissue PAI Activity.** PAI-1 activity of the carotid arteries was determined by a two-stage, indirect enzyme assay system using polylysine-stimulated Glu-plasminogen activation (kit 101201; American Diagnostica, Greenwich, CT) according to the manufacturer’s instructions. In this assay, 1 unit of PAI-1 activity is defined as the amount of PAI-1 that inhibits 1 IU of human single chain tissue plasminogen activator.

**Results**

**Atherosclerosis in Carotid Artery of ApoE−/− Mouse Is Associated with Compensatory Outward Remodeling.** In general, atherosclerosis in carotid arteries increased with increasing duration of HFD and proximity to the bifurcation. However, there were some segments from a few mice that remained lesion free, although the number of these segments decreased with increasing duration of high-fat feeding and proximity to the bifurcation. In these carotid artery segments without measurable plaque, mean VA at sites proximal to the bifurcation was similar and independent of the distance from the bifurcation (data not shown). Similarly, there was no increase in VA with increasing duration of feeding ApoE−/− mice HFD, even if there were plaques distal to a nondiseased segment (data not shown).

In comparison with the few segments that remained lesion free, in segments with plaque, VA increased with time and proximity to carotid bifurcation (Fig. 2A). In contrast, LA remained essentially unaltered (R² = 0.002, P = 0.70, Fig. 2B), with only a small increase just at the start of the bifurcation (location 0 in Fig. 2B), whereas PA increased (Fig. 2C). These results indicate that there was significant compensatory remodeling in the prebifurcational left common carotid during atherogenesis. Indeed, at 180 days, there was a strongly positive linear relationship between VA and PA (R² = 0.70, P < 0.01, Fig. 3A). In contrast, LA remained essentially unaltered with increasing PA (R² = 0.002, P = 0.70, Fig. 3B), with the slope of the regression line and the mean remodeling index very close to 1.0. Consistent with these findings, the remodeling index remained relatively constant over time, with no significant changes observed at the sites investigated (Fig. 3C).

**Probucol Decreases Plaque Area and Compensatory Remodeling.** The size of plaques in probucol-treated mice was significantly smaller with increasing time compared with control mice (Fig. 4A). As observed in control animals, there was also a significant positive relationship between VA and PA in probucol-treated mice (R² = 0.25, P < 0.01). However, the slope of the regression line was comparatively lower (see Fig. 4, B versus A). In addition, the mean remodeling index was significantly lower than in control animals.
Fig. 2. Atherogenesis in carotid artery of ApoE−/− mice is associated with arterial remodeling. Vessel area (millimeters squared; A), lumen area (B), and plaque area (C) at reference site (R), at the bifurcation (0), and at 100, 200, 300, 400, and 500 μm proximal to the bifurcation in all carotid artery segments at 15, 30, 60, 120, and 180 days after initiation of HFD. The results shown were obtained from separate vessels of six animals for each time point, except for 180 days, where n = 8.

Fig. 3. Atherogenesis in carotid artery of ApoE−/− mice is associated with compensatory outward remodeling. Regression lines and equations for normalized PA (at lesion site/VA at reference site) versus normalized vessel area (A) or normalized lumen area in animals after 180 days of HFD feeding (B). C, remodeling index (VA at lesion site/VA at reference site/PA at lesion site/PA at reference site) in vessel segments with measurable plaque at 60, 120, and 180 days (at all time points n = 10, 19, and 36) and at the reference site (R), at the bifurcation (0), and at 100, 200, 300, 400, and 500 μm proximal to the bifurcation (n = 16, 15, 13, 8, 8, and 5, respectively).

Probucol Decreases VCAM-1 Expression, Macrophages, and MMP-2 and -9. Macrophage foam cells are a prominent component of atherosclerotic lesions in ApoE−/− mice, and these cells secrete a variety of proteases thought to promote vascular remodeling (Jormsjo et al., 2002). It has been reported that probucol reduces the aortic expression of VCAM-1 during the early stages of atherosclerosis in low-density lipoprotein receptor-deficient rabbits and that this inhibition is strongly associated with decreased intimal accumulation of macrophages (Fruebis et al., 1997). Therefore, we first determined VCAM-1 expression during the early stages of atherosclerosis (Fig. 5A) and the macrophage content in carotid arteries as atherosclerosis progressed (Fig. 5B). VCAM-1 expression increased with time, and probucol significantly inhibited this process (Fig. 5A). Macrophage content, indicated by the area staining positively for Mac-3, initially increased and then subsided 120 days post-HFD (Fig. 5, B and C). Early plaques were composed almost entirely of macrophages, whereas their contribution to plaque area progressively decreased with increasing duration of HFD (Fig. 5, B and D). Probucol significantly delayed macrophage accumulation (Fig. 5C). This was associated with a decrease in total (385 ± 29 for control and 116 ± 8.2 cells/section for probucol) and proliferating cells (109 ± 20 for control and 16 ± 2.7 cells/section for probucol). As a result, probucol decreased the proportion of intimal and medial cells positive for PCNA (Fig. 5E). Similarly, there was a decrease in the number of proliferating macrophages from 41 ± 9 cells/section in control to 6 ± 5 cells/section in probucol, as
assessed by Mac-3 staining. This resulted in a decrease in the proportion of proliferating macrophages (Fig. 5F).

Evidence suggests a key role for MMPs in vascular remodeling (Galis and Khatri, 2002). In ApoE−/− mice, arterial expansive remodeling is associated with increased macrophage-derived foam cells and MMPs (Ivan et al., 2002), and MMP-2 and MMP-9 are implied in lesion development (Kuzuya et al., 2006) and plaque rupture (Gough et al., 2006), respectively. Therefore, we next assessed the activities of MMP-2 and MMP-9 in carotid arteries of ApoE−/− mice. Expression of MMP-9 increased with time, and probucol significantly inhibited this process (Fig. 6A). Compared with MMP-9, MMP-2 was expressed more diffusely such that meaningful quantification was not possible, although qualitatively, probucol also seemed to decrease the expression of MMP-2 (data not shown). Similarly, probucol inhibited endogenous activities of MMP-2 and MMP-9 (Fig. 6B) and the ex vivo production of MMP-2 and MMP-9 by arterial segments (Fig. 6C), although the drug did not affect the pro-MMP-2/MMP-2 ratio (Fig. 6C). Because plasmin is a relevant activator of pro-MMPs in ApoE−/− mice (Carmeliet et al., 1997), we also assessed plasmin and PAI-1 activity in carotid arteries (Reuning et al., 2003), where we observed no difference in the activity of each enzyme between control and probucol-fed animals (Fig. 7).

**Discussion**

This study shows that in ApoE−/− mice, compensatory remodeling of the common carotid artery occurs simultaneously with and is consistently proportional to increasing plaque size. This reinforces the compensatory role of outward remodeling and provides a platform for future intervention studies to define the basic mechanisms of de novo atherosclerotic remodeling. We also show that despite decreasing the size of atherosclerotic lesions by approximately 50%, probucol retards compensatory remodeling by decreasing intimal macrophages influx and MMPs-2 and -9, resulting in effective loss of lumen of the common carotid artery.

The occurrence of remodeling in the aorta and carotid arteries of ApoE−/− mice has been reported previously (Lutgens et al., 2001; Choudhury et al., 2002). The present study confirms this earlier observation and, for the first time, provides information on the temporal relationship between atherosclerosis and remodeling. We show that remodeling occurs in consistent proportion to plaque growth, independent of time and plaque size, indicating that remodeling does not occur before atherosclerosis, at least in this model of disease. The near perfect compensation for plaque growth during the time period examined also adds weight to the suggestion that inward remodeling may be due to healing of plaque rupture, an event that does not occur in the carotid arteries of ApoE−/− mice.

Previous studies reported probucol to increase lesion size in the aortic root of ApoE−/− mice (Zhang et al., 1997; Moghadamian et al., 1999), although subsequent studies showed probucol to decrease disease distal to the aortic root (Witting et al., 2000; Choy et al., 2005). The observed inhibition by probucol of atherosclerosis at the prebifurcational common carotid artery is consistent with these latter studies. Despite this significant reduction in plaque growth, however, there was an even greater inhibition of vessel enlargement such that overall, there was gradual lumen loss with increasing time and lesion size. If probucol had similar opposing effects on atherosclerosis and remodeling in humans, it could help explain why no benefit of probucol on de novo atherosclerosis was reported in the PQRST study, where disease progression...
was estimated indirectly as a change in lumen volume (Wall-diussi et al., 1994). Thus, our results question the basis upon which probucol was withdrawn from the United States market, although we recognize that in human femoral arteries, atherosclerosis can result in vessel enlargement or shrinkage (Pasterkamp et al., 1997). In addition, it is well known that probucol has other side effects (lowering of high density lipoprotein-cholesterol and QT prolongation). Indeed, in the present study, probucol was used simply as a model compound for novel probucol analogs such as AGI-1067 that do not share these unwanted side effects (Tardif et al., 2003; Wu et al., 2006).

It has been reported that probucol retards constrictive remodeling in humans after angioplasty (Côté et al., 1999). However, the present study is the first to examine the role of probucol in de novo atherosclerotic compensatory remodeling. Although remodeling after angioplasty and remodeling during de novo atherosclerosis are quite different processes, it is possible that key components of both forms of remodeling are shared and may be affected by probucol. For example, collagen deposition (Lafont et al., 1999; Burke et al., 2002) and inflammatory cells (Pasterkamp et al., 1998; Okamoto et al., 2001) play key roles in both forms of remodeling and have been reported previously to be affected by probucol in ApoE−/− mice (Zhang et al., 1997; Choy et al., 2005). Similarly, MMPs seem to be important in both forms of remodeling (de Smet et al., 2000; Pasterkamp et al., 2000).

In light of the importance of inflammatory cells and MMPs in compensatory remodeling, our results suggest that the inhibitory activity of probucol was due to its ability to inhibit the accumulation of macrophages (Fig. 5) and MMP-2 and MMP-9 protein in carotid plaques and time-dependent changes to MMP-9 content in control (open circles) and probucol-treated (filled circles) mice after different duration of HFD (n = 3–4). Note the presence of MMP-2 in the media (arrows) and diffused throughout the plaque (×400). B, MMP-9 and MMP-2 activity in control (open bars) and probucol-treated (filled bars) mice at the time points indicated; MMP activity gel shown was obtained from a pool of n = 8 arteries per treatment and time point, with data for control mice expressed relative to that of probucol-treated animals. C, ex vivo secretion of MMP-9 and MMP-2 activity and ratio of pro-MMP-2/MMP-2 by carotid arterial tissue obtained from control (open bars) and probucol-treated (filled bars) mice after 60 days HFD (n = 4). *P < 0.05; **P < 0.01 compared with respective control value.
MMP-9 (Fig. 6). Inhibition of MMP activity probably resulted from decreased secretion rather than inhibition of activation of the latent forms of the enzymes, as indicated by the unaltered pro-MMP-2/MMP-2 ratio in probucol-treated versus control animals (Fig. 6C). Because oxidative processes, including oxygenation of the cysteine switch domain, can contribute to the activation of MMPs (Fu et al., 2001), the unaltered pro-MMP-2/MMP-2 ratio suggests that probucol did not act as an antioxidant. This interpretation is consistent with recent findings indicating that antioxidant activities may not be responsible for probucol’s protection against experimental atherosclerotic vascular disease (Choy et al., 2005; Wu et al., 2006).

The situation in the de novo atherosclerotic remodeling seems similar to that in experimental heart failure, where probucol has been reported to inhibit both filtration of the myocardium by monocytes and tissue MMP activity (Nakamura et al., 2002). In that study, probucol also decreased the expression of monocyte chemoattractant protein-1 (Nakamura et al., 2002), although such activity was not seen in experimental atherosclerosis (Fruebis et al., 1997). As reported in low-density lipoprotein receptor-deficient rabbits (Fruebis et al., 1997), we observed probucol to reduce VCAM-1 expression (Fig. 5A). We also show that probucol inhibited macropage proliferation in the vessel wall (Fig. 5P). Both activities probably contribute to the anti-inflammatory action of probucol on de novo atherosclerosis. Because probucol can induce heme oxygenase-1 in vascular cells (Deng et al., 2004), and this is associated with decreased cell proliferation (Duckers et al., 2001; Deng et al., 2004), it will be interesting to examine whether, and if so to what extent, the biologically distinct anti-inflammatory activities induced by probucol (i.e., inhibition of adhesion molecule expression and macrophage accumulation and proliferation) are mediated by induction of heme oxygenase-1.

In summary, our study shows that early in the development of atherosclerosis in ApoE/−/− mice, there is simultaneously almost complete compensatory remodeling, and this process is significantly retarded by probucol. Because compensatory remodeling has been associated with subsequent plaque rupture and acute vascular syndromes (Filardo et al., 2000; Yamagishi et al., 2000; Newby, 2005) that usually have devastating consequences, inhibition of compensatory remodeling by probucol may be seen as beneficial. In this context, it will be interesting to examine directly whether probucol can inhibit plaque rupture. Further studies are also required to determine whether the activities reported here for probucol extend to AGI-1067 and/or other probucol analogs currently undergoing evaluation as novel anti-atherosclerotic agents. In any case, our results clearly indicate that lumen volume is not a suitable measurement to assess the antiatherosclerotic activity of probucol.

References
of MMP-2 deficiency on atherosclerotic lesion formation in apoE-deficient mice. 


Address correspondence to: Roland Stocker, Centre for Vascular Research, Department of Pathology, University of Sydney, Medical Foundation Building K25, 92-94 Parramatta Road, Camperdown NSW 2006, Australia. E-mail: rstocker@med.usyd.edu.au