Cyclooxygenases, Thromboxane, and Atherosclerosis
Plaque Destabilization by Cyclooxygenase-2 Inhibition Combined With Thromboxane Receptor Antagonism

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Background—Antagonism or deletion of the receptor (the TP) for the cyclooxygenase (COX) product thromboxane (Tx)A₂, retards atherogenesis in apolipoprotein E knockout (ApoE KO) mice. Although inhibition or deletion of COX-1 retards atherogenesis in ApoE and LDL receptor (LDLR) KOs, the role of COX-2 in atherogenesis remains controversial. Other products of COX-2, such as prostaglandin (PG) I₂ and PGE₂, may both promote inflammation and restrain the effects of TxA₂. Thus, combination with a TP antagonist might reveal an antiinflammatory effect of a COX-2 inhibitor in this disease. We addressed this issue and the role of TxA₂ in the promotion and regression of diffuse, established atherosclerosis in Apobec-1/LDLR double KOs (DKOs).

Methods and Results—TP antagonism with S18886, but not combined inhibition of COX-1 and COX-2 with indomethacin or selective inhibition of COX-2 with Merck Frosst (MF) tricyclic, retards significantly atherogenesis in DKO mice. Although indomethacin depressed urinary excretion of major metabolites of both TxA₂, 2,3-dinor TxB₂ (Tx-M), and PGI₂, 2,3-dinor 6-keto PGF₁α (PGI-M), only PGI-M was depressed by the COX-2 inhibitor. None of the treatments modified significantly the increase in lipid peroxidation during atherogenesis, reflected by urinary 8,12-iso-iPF₂α-VI. Combination with the COX-2 inhibitor failed to augment the impact of TP antagonism alone on lesion area. Rather, analysis of plaque morphology reflected changes consistent with destabilization of the lesion coincident with augmented formation of TxA₂. Despite a marked effect on disease progression, TP antagonism failed to induce regression of established atherosclerotic disease in this model.

Conclusions—TP antagonism is more effective than combined inhibition of COX-1 and COX-2 in retarding atherogenesis in Apobec-1/LDLR DKO mice, which perhaps reflects activation of the receptor by multiple ligands during disease initiation and early progression. Despite early intervention, selective inhibition of COX-2, alone or in combination with a TP antagonist, failed to modify disease progression but may undermine plaque stability when combined with the antagonist. TP antagonism failed to induce regression of established atherosclerotic disease. TP ligands, including COX-1 (but not COX-2)–derived TxA₂, promote initiation and early progression of atherogenesis in Apobec-1/LDLR DKOs but appear unimportant in the maintenance of established disease.

Key Words: thromboxane  atherosclerosis  lesion  prostaglandins  inflammation

Atherosclerosis bears many of the hallmarks of an inflammatory disease; however, there is scant evidence of a causal role of inflammation in disease progression in humans. Prostaglandins (PGs), particularly PGE₂ and PGI₂, are established mediators of inflammation, and inhibition of their biosynthetic enzymes, the cyclooxygenases (COXs), by nonsteroidal antinflammatory drugs (NSAIDs) affords relief of symptoms in the inflammatory arthritides. Expression of COX-1 and COX-2 is augmented in inflamed synovia and also in endothelium, vascular smooth muscle cells, and macrophages in human atherosclerotic lesions, which raises the prospect that COX inhibitors might retard plaque progression. Recently, a placebo-controlled trial of rofecoxib revealed a 2-fold increase of myocardial infarction and stroke that led to withdrawal of the drug from the market. This probably reflects a mechanism whereby depression of COX-2–derived prostacyclin (PGI₂) removes a constraint on platelet COX-1–derived thromboxane (Tx) A₂ and other agonists that elevate blood pressure, promote atherogenesis, and augment the thrombotic response to plaque rupture. Indeed, overview analysis of the experience with a structurally distinct inhibitor, valdecoxib, reveals a 3-fold increase in myocardial infarction and stroke, and a placebo-controlled clinical trial of a third, celecoxib, has been prematurely...
terminated because of an excess of cardiovascular events on the coxib. Despite these observations, a clinical trial designed to detect a cardioprotective effect of combining a third COX-2 inhibitor (celecoxib) has been announced. In this trial, celecoxib will be combined with low-dose aspirin to suppress COX-1–derived TxA2 in patients with osteoarthritis who are at high cardiovascular risk. It is assumed this strategy will reveal a beneficial effect of the COX-2 inhibitor.

COX-2–derived PGs mediate inflammation, a hallmark of atherosclerosis. Additionally, descriptive analysis of plaque morphology in case-control studies have implicated COX-2–derived PGE2 in the activation of matrix metalloproteinases and consequent destabilization of atherosclerotic plaques in humans. However, large-scale clinical trials of traditional NSAIDs have not been performed in cardiovascular disease.

Cardioprotection from aspirin may reflect inhibition of platelet COX-1–dependent TxA2 formation. Indeed, indirect comparisons across clinical trials fail to detect any difference in the degree of cardioprotection between low doses, which favor inhibition of COX-1 (75 to 150 mg/d), and higher (>325 mg/d) antiinflammatory doses, which inhibit both COX-1 and COX-2. A direct, controlled comparison of the effects of high- and low-dose aspirin on plaque burden or cardiovascular outcomes has not been performed.

These observations have prompted interest in mice as more tractable models of atherosclerosis in which the relative importance of the 2 COX enzymes and their products might be defined. Early evidence was obtained for the importance of COX-1. Thus, combined inhibition of COX-1 and COX-2, but not of COX-2 alone, retarded atherogenesis in LDL receptor (LDLRT) knockout (KOs) despite a similar depression (~60%) of the cytokines, soluble intercellular adhesion molecule and monocyte chemotactic protein-1, by the 2 regimens. Similarly, selective inhibition and genetic deletion of COX-1 both retard atherogenesis in apolipoprotein (Apo) E KOs. Given the evidence for platelet activation in human atherosclerosis and in mutant mice, these data seem congruent with evidence that antagonism or deletion of the TP, the TxA2 receptor, retards atherogenesis in ApoE KOs.

TxA2 is also a product of monocyte and macrophage COX-2. Furthermore, products of lipid peroxidation, such as the isoprostanes, which increase during atherogenesis, may activate the TP in a COX-independent manner. Thus, it is possible that selective antagonism of the TP might more effectively retard atherogenesis than COX-1 inhibition alone. Finally, it is unknown whether activation of the TP is relevant only to lesion initiation and early development or whether TP antagonism can induce regression of established atherosclerotic disease.

The role of COX-2 in atherogenesis is much more controversial. COX-2 inhibitors have been shown variably to retard, accelerate, fail to accelerate, or leave unaltered atherosclerosis in LDLR and ApoE KO mice. The contradictory nature of these results has been attributed to the timing of intervention, differences in the mouse models, and differences in the pharmacological probes used. The COX-2 products PGE2 and PGli may also act as restraints on the cardiovascular effects of TxA2 to offset their proinflammatory action. Thus, despite the cardiovascular hazard from selective COX-2 inhibition alone, TP antagonism or suppression of TxA2 by low-dose aspirin might theoretically reveal an antiinflammatory action of COX-2 inhibition, which might further augment its impact on atherogenesis.

We have addressed these outstanding issues in the Apobec-1/LDLR DKO. This model of atherosclerosis more faithfully replicates the human disease than either the LDLR KO or the ApoE KO in several respects, including a multifocal origin of disease, predominance of the elevated cholesterol in LDL, and a pronounced gender dependence of the phenotype. We have compared the impact of TP antagonism with coincidental inhibition of both COXs and selective inhibition of COX-2 in the initiation and early progression of disease. Furthermore, we have determined whether combination with a TP antagonist might reveal a beneficial effect of COX-2 inhibition attributable to suppression of inflammation. Finally, we addressed the possibility that TP antagonism might induce regression of established atherosclerotic disease.

**Methods**

**Animals**

The DKO mice were a gift from Drs Powell-Braxton and Bunting at Genentech Inc, South San Francisco, Calif. All animals were housed according to guidelines of the Institutional Animal Care and Usage Committee (IACUC) of the University of Pennsylvania. All procedures were considered and approved by the IACUC. Animals were housed in a controlled barrier environment that met university standards and US federal and statutory regulations. This includes climate, air-exchange and filtration, photo-light period, and caging specifications.

**Study Design**

Although the DKOs spontaneously develop atherosclerotic lesions on a regular chow diet, the “Western” high-fat diet (0.2% cholesterol, 21% saturated fat; formula TD 88137, Harlan Teklad) was chosen for the initial study. Extensive atherosclerotic lesions develop in mice fed this diet for 92 days. Male mice were randomized to each treatment regimen at 6 weeks and were euthanized at 19 weeks of age. Mice in this first study were randomized to receive the high-fat diet in combination with (1) S18886 (3-((6R)-6-(((4-chlorophenyl)sulfonyl)amino)-2-methyl-5,6,7,8 tetrahydro-1-naphthalenyl)propanoic acid, sodium salt), a nonprostanoid, highly selective TP antagonist, or (2) indomethacin at 6 mg/L in their drinking water. Similarly, we have compared the impact of TP antagonism with coincidental inhibition of both COXs and selective inhibition of COX-2 in the initiation and early progression of disease. Further, we have determined whether combination with a TP antagonist might reveal a beneficial effect of COX-2 inhibition attributable to suppression of inflammation. Finally, we addressed the possibility that TP antagonism might induce regression of established atherosclerotic disease.

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Male mice used for the second study, designed to assess the impact of COX-2 inhibition on TP antagonism in atherosclerosis, were randomized to (1) the TP antagonist alone (1% cholesterol chow and 5 mg/kg S18886 in their drinking water [n=14]) or (2) the combination regimen (1% cholesterol/COX-2 inhibitor [15 mg/kg] chow and 5 mg/kg S18886 in their drinking water [n=10]) at 6 weeks of age. Again, they were euthanized for analysis at 19 weeks of age.

The DKOs develop atherosclerotic lesions spontaneously on a regular chow diet. The third study was designed to assess the ability of TP antagonism to induce regression of established atherosclerotic disease. Male DKOs aged 16 months of age on a regular chow diet and were then randomized to (1) euthanasia and immediate harvest of their aortas as a baseline control (n=9), (2) initiation of treatment with the TP antagonist (n=7) S18886 (5 mg/kg/d) for a further 16 weeks, or (3) no treatment until they were killed (n=8) and vascular harvesting was performed after 16 weeks (to control for lesion progression without intervention).

**Lesion Analysis by the En Face Method**

The en face morphometric method quantifies the extent of atherosclerosis in the entire murine aorta. Significant correlation between the extent of lesions in the entire aorta calculated in this manner and lesion area measured at the aortic origin was demonstrated by Tangirala and colleagues in both LDLR KO and ApoE KO mice. Formalin-fixed aortas (from the heart to the iliac bifurcation) were cleaned of adventitial fat, opened longitudinally, and stained with Sudan IV (Sigma). Images were photographed and digitized with the Image Pro analysis system (Phase 3 Imaging Systems). The total area of the aorta and the atherosclerotic plaque area were measured. The percent lesion value was calculated as a ratio of the total aorta area to the lesion area for each aorta.

**Histological Examination of Lesion Morphology**

Sections (8 μm) of OCT embedded tissue were acetone fixed, and then incubated with 10 μg/mL rat anti-mouse CD16/CD32 to block Fc receptors (Pharmingen, USA). Serial sections were subjected to immunostaining with 5 μg/mL goat anti-rabbit antibody (Vector Laboratories Inc), and biotinylated goat anti-FITC secondary antibody (Vector Laboratories) followed by biotinylated goat anti-rabbit antibody (Vector Laboratories), respectively. This was followed by VIP (Vector Laboratories) and Vectastain ABC avidin-biotin amplification (Vector Laboratories). Images were photographed and digitized with the Image Pro analysis system (Phase 3 Imaging Systems). The total area of the aorta and the atherosclerotic plaque area were measured. The percent lesion value was calculated as a ratio of the total aorta area to the lesion area for each aorta.

**Urinary PGI-M**

Urinary PGI-M also increased during atherogenesis, as had been observed in the other mouse models. Similarly, indomethacin suppressed PGI-M excretion from 2.4±0.2 to 0.61±0.2 ng/mg (P<0.01) at 4 weeks of feeding (Figure 2C), an effect retained at 8 weeks (data not shown). Animals treated with S18886 failed to reduce PGI-M significantly (Figure 2B), and this effect was maintained at 8 weeks (data not shown). Although Tx-M tended to decline with the antagonist, this difference (Figure 2B) failed to attain statistical significance (P=0.08). Indomethacin alone maintained a significant suppression of Tx-M excretion at 92 days of treatment, immediately before animals were euthanized (Figure 3A). A tendency to a reduction of Tx-M with S18886 probably reflects an interruption of secondary formation of TxA₂ by activated platelets or the impact of the treatment on lesion burden and secondary platelet activation.

Inhibition of COX-2 Fails to Modulate Spontaneous Atherogenesis in DKOs

As expected, male mice developed atherosclerosis significantly faster than females (Figure 4A), attaining 11.0±0.6% coverage of the aorta of males versus 5.57±0.8% in females (Figure 4B) in 1% cholesterol-fed mice at 19 weeks of age (P<0.001). Selective inhibition of COX-2 significantly depressed PGI-M excretion from 4.3±0.4 to 2.6±0.5 ng/mg (P<0.05) but not Tx-M (80.2±15.2 versus 58.0±9.7 ng/mg).
The impact of combining S18886 with MF tricyclic was not associated with body weight, total cholesterol, HDL, or isoprostane levels. MF tricyclic did not significantly differ among treatment groups. 

Table 1. Urinary and Serum Levels of TxA2 and its Metabolites

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urinary 8,12-iso-iPF2(-)VI (ng/mg creatinine)</th>
<th>Serum Cholesterol (mg/dL)</th>
<th>Serum HDL (mg/dL)</th>
<th>Body weight (g)</th>
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<tr>
<td>Vehicle</td>
<td>4.2 ± 1.1</td>
<td>2699 ± 193</td>
<td>274 ± 33</td>
<td>29.4 ± 1</td>
</tr>
<tr>
<td>Indomethacin 5 mg/kg</td>
<td>2.1 ± 0.9</td>
<td>2454 ± 210</td>
<td>316 ± 12</td>
<td>30.4 ± 2</td>
</tr>
<tr>
<td>Indomethacin 10 mg/kg</td>
<td>3.6 ± 0.6</td>
<td>2657 ± 229</td>
<td>312 ± 30</td>
<td>30.0 ± 2</td>
</tr>
<tr>
<td>S18886</td>
<td>2.9 ± 0.5</td>
<td>2731 ± 193</td>
<td>328 ± 21</td>
<td>31.3 ± 1</td>
</tr>
</tbody>
</table>

**Figure 1.** TP antagonism is superior to COX inhibition in retarding atherogenesis in Apobec-1/LDLR DKO mice. A. En face quantification of percent % lesion area of aortas from male Apobec-1/LDLR DKO mice treated for 92 days on high-fat diet (1) alone or with (2) indomethacin (6 mg/L), (3) 5 mg/kg S18886, or (4) 10 mg/kg S18886. TP antagonism significantly reduced percent lesion area (8.1 ± 0.9; 1-way ANOVA P<0.001, Dunn’s multiple comparison test *P<0.05 and **P<0.01 vs vehicle). B. Representative en face aortas from each group. C. Urinary isoprostane (8,12-iso-iPF2\(-\)VI [ng/mg creatinine]) and serum and HDL cholesterol levels did not significantly differ among treatment groups.

yet failed to modulate atherogenesis. MF tricyclic did not alter body weight, total cholesterol, HDL, or isoprostane generation in either gender (Figure 4C).

**COX-2 Inhibition Fails to Augment the Beneficial Effect of TP Antagonism**

The impact of combining S18886 with MF tricyclic was assessed in a separate experiment. S18886, at 5 mg/kg, also retarded atherosclerosis on a 1% cholesterol diet for 92 days (Figure 5A; 11.03±0.6%, n=11 versus 7.9±0.8%, n=14, P<0.05), consistent with our earlier observations on the high-fat diet. Combination of the COX-2 inhibitor with S18886 failed to augment the impact of the TP antagonist alone on lesion area (Figure 5A; 5.8±0.8%, n=10 versus 7.9±0.8%, n=14; P>0.05). Similarly, body weight and urinary 8,12-iso-iPF2\(-\)VI were unaltered by addition of the COX-2 inhibitor. Although urinary TxA-M did not differ among the groups after 4 weeks of treatment, it was unexpectedly elevated (P<0.05) at the time of euthanasia in animals treated with the COX-2 inhibitor/TP antagonist combination (138.2±16.7 ng/mg) compared with those receiving placebo (80.2±15.2 ng/mg), the TP antagonist (76.2±5.8 ng/mg), or the COX-2 inhibitor alone (58.0±9.7 ng/mg).

**Coincidental TP Antagonism and COX-2 Inhibition Results in Morphological Changes Consistent With Lesion Destabilization**

Immunohistochemical analyses of sections of the aortic root were performed. Morphology was assessed in lesions of comparable size. Sections were immunostained with antilaminin (to delineate the extracellular matrix–rich/fibrotic regions of lesions while sparing regions rich in inflammatory cells and necrotic cores) or with anti-\(\alpha\)-smooth muscle (SM) actin. Laminin, a matrix component, was seen in association with endothelial cells and demarcated the extracellular matrix–rich areas of lesion (Figure 6, A, C, E, and G). Staining with anti-\(\alpha\)-SM-actin was used to identify differentiated smooth muscle cells in the media. In addition, fibrotic caps consist of \(\alpha\)-SM actin–positive cells. Formation of fibrotic caps has been associated with plaque stabilization. Actin-positive smooth muscle cells can be seen on the luminal aspect of the lesions in control mice and mice treated with either the TP antagonist (Figure 6D) or the COX-2 inhibitor alone (Figure 6F). However, \(\alpha\)-SM-actin–positive cells were conspicuously absent from the luminal aspect of lesions in animals treated with the COX-2 inhibitor in combination with the TP antagonist (Figure 6H). Furthermore, the depletion of medial \(\alpha\)-smooth muscle actin–positive cells appeared to be attenuated by the combined treatment compared with control mice or treatment with either the COX-2 inhibitor or TP antagonist alone.

**TP Antagonism Does Not Induce Regression of Established Atherosclerosis in DKOs**

Treatment of 16-month-old DKOs with an extensive burden of disease (45.7±2.5% of aortic area [n=9]) with the TP antagonist for 16 weeks failed to limit disease progression or to induce regression of established atherosclerosis (Table 1).

**Discussion**

The present studies confirm the efficacy of the TP antagonist S18886 in retarding murine atherosclerosis. Previously, Cayatte and colleagues reported this effect in ApoE KO mice, and it was confirmed by studies of TP deletion; we now record similar observations in Apobec-1/LDLR DKO mice, which even more faithfully represent the human disease.

This effect was dose related and was evident both when atherosclerosis was accelerated by provision of a high-fat diet and when it developed spontaneously on a 1% cholesterol chow diet. TxA\(_2\) and its PGH\(_2\) endoperoxide precursor represent the conventional ligands for the TP.
TxA₂ is the most abundant product of arachidonic acid metabolism in mature human platelets, which only express COX-1; however, TxA₂ is also a prominent product of monocyte/macrophage COX-2. The TP can also be activated by COX-independent ligands in vitro, among them the isoprostanes iPF₂α-III and iPE₂-III. Indeed, the effects of infusions of these compounds on blood pressure in vivo and platelet function ex vivo are abolished in TP KO mice; however, it is still unknown whether endogenous, COX-independent ligands attain sufficient concentrations to activate the TP in vivo. Isoprostane generation increases during atherogenesis in mice, and suppression of their formation with vitamin E retards atherogenesis. We confirmed that isoprostane generation increased during atherogenesis in the DKO mice by measurement of the abundant product 8,12-iso-iPF₂α-VI.

We have previously reported that combined inhibition of COX-1 and COX-2 with indomethacin retarded atherogenesis...
in LDLR KO mice. A similar indomethacin regimen here substantially depressed biosynthesis of both TxA2 and PGI2 to a degree similar to that observed in the previous studies; however, indomethacin failed significantly to retard atherogenesis in the DKO mice. The comparative efficacy of TP antagonism may reflect a role for COX-independent ligands. Alternatively, coincidental inhibition of PGI2 may have masked the benefit from TxA2 suppression by indomethacin. We have shown that PGI2 modulates the effects of TxA2 on the response to vascular injury in vivo. TXA2 acts as an amplifying signal in the process of platelet activation, and interruption of this feed-forward process explains the modest decline in TxA2 biosynthesis observed with TP antagonism in this and other studies. Platelet activation is recognized in human atherosclerosis and during atherogenesis in mice, and interruption of this process and its consequent effects on the vasculature may explain the effects of TP antagonism and deletion in atherosclerosis.

Controversy has surrounded the role of COX-2 in atherogenesis. Given its importance in inflammation, it has been suggested as a rational drug target for reduction of plaque burden and plaque stabilization. COX-2 is the dominant source of PGI2 biosynthesis in humans, and although PGI2 (and PGE2) may mediate inflammation, they also counter the effects of TxA2 on platelet activation, atherogenesis, vascular proliferation in response to injury, and vascular tone. Indeed, suppression of COX-2–dependent PGI2 formation, while leaving COX-1–dependent TxA2 intact, appears likely to explain the augmented risk of myocardial infarction and stroke observed with structurally distinct selective inhibitors of COX-2.

Although atherosclerotic mice rarely exhibit spontaneous plaque destabilization and subsequent thrombosis, as occurs in humans, they afford the opportunity to assess the impact of therapies on accumulation of plaque burden. However, COX-2 inhibitors have been reported variously not to influence, to accelerate, not to accelerate, or to retard atherogenesis. This conundrum may reflect differences in timing of the intervention, duration of treatment, or differ-
ences among mouse models and drug and dosing regimens (Table 2). Mice lacking COX-2 exhibit multiple reproductive defects, and abnormalities in renal and myocardial function are evident in survivors,46,47 which limits their usefulness as a mechanistic probe.

Selective inhibition of COX-2 failed to influence the extent of atherosclerosis in the present studies. Despite early intervention, a biochemically selective regimen of the inhibitor failed to influence the spontaneous atherogenesis that occurs in the DKOs on a chow diet. Furthermore, we addressed the hypothesis that antagonism of the TP might reveal the beneficial antiinflammatory consequences of COX-2 inhibi-

**Figure 5.** Coincident TP antagonism and COX-2 inhibition. A, En face quantification of percent lesion area of aortas from male Apobec-1/LDLR DKO mice fed 1% cholesterol diet (1) alone; (2) with MF tricyclic (15 mg/kg); (3) with 5 mg/kg S18886 in drinking water; or (4) supplemented with TP (S18886, 5 mg/kg), and COX-2 inhibitor (MF tricyclic, 15 mg/kg) shows reduction in lesion area with S18886 (as expected; 1-way ANOVA \(*P<0.001, \text{ Dunn's multiple comparison test * } P<0.05 \text{ vs vehicle}).\) Combination of S18886 (TP) and COX-2 inhibitor (COX-2) also significantly reduced lesion area (1-way ANOVA \(P<0.001, \text{ Dunn's multiple comparison test ** } P<0.01 \text{ vs vehicle})\), but this was not significantly different compared with S18886 alone (\(P>0.05)).\) B, Urinary isoprostane 8,12-iso-iPF\(_{206}\)-VI and body weight at time of euthanasia did not differ between treatment groups.

**TABLE 1.** Antagonism at the TP Does Not Retard Progression or Induce Regression of Established Atherosclerosis

<table>
<thead>
<tr>
<th>Sample size, n</th>
<th>16 Months of Age</th>
<th>20 Months of Age</th>
<th>16-Week Treatment With S18886 5 mg/kg (20 Months of Age)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion area, %</td>
<td>45.7 +/- 2.5</td>
<td>53.9 +/- 3.8</td>
<td>51.2 +/- 3.1</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>24.6 +/- 1.4</td>
<td>31.7 +/- 1.7</td>
<td>32.7 +/- 1.9</td>
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<tr>
<td>2,3-dinor Tx, ng/mg creatinine</td>
<td>132.3 +/- 25.7</td>
<td>147.9 +/- 31.8</td>
<td>117.8 +/- 28.4</td>
</tr>
</tbody>
</table>

En face quantification of percent lesion area of aortas from 16-month-old Apobec-1/LDLR DKO mice, 20-month-old mice, and 20-month-old mice that had been treated with 5 mg/kg S18886 from 16 months of age reveals S18886 had no effect on lesion area at this advanced stage of the disease.

**Figure 6.** Combination of COX 2 inhibitor with TP antagonist inhibits luminal \(\alpha\)-actin staining. Representative aortic root sections from each treatment group are shown. Lesions of similar size were chosen to compare morphological complexity from mice in vehicle (A and B), COX 2 (MF tricyclic, 15 mg/kg; C and D), TP (S18886, 5 mg/kg; E and F), and COX-2/TP (G and H) groups. Antilaminin staining demarcates endothelium (EC) and extracellular matrix-rich regions of lesions while sparing areas rich in inflammatory cells and necrotic cores (NC). Anti-\(\alpha\)-SM actin staining identifies medial (M) smooth muscle cells and luminal differentiated smooth muscle cells in fibrotic caps (FC), which are notably absent in combination regimen group (H). Also indicated are lumen (L), intima (I), and adventitia (Adv). Regions of media that have been largely depleted of \(\alpha\)-SM actin–positive cells are indicated by asterisks. Scale bar=200 \(\mu\)m.
platelet activation by suppression of PGI2.29 Second, although though little if any luminal lesions contained more necrotic cores (Figure 6G), and although plaque burden was not increased compared with treatment with the antagonist alone, lesion morphology was notably altered.

TP antagonism delayed spontaneous atherogenesis in the DKOs, as it had when lesion formation was accelerated by a high-fat diet; however, addition of the COX-2 inhibitor failed to augment the impact of TP antagonism on atherogenesis. Instead, it had 2 unexpected effects. First, biosynthesis of TxA2 was augmented, which perhaps reflects removal of a constraint on platelet activation by suppression of PGI2.29 Second, although plaque burden was not increased compared with treatment with the antagonist alone, lesion morphology was notably altered. Lesions contained more necrotic cores (Figure 6G), and although little if any luminal α-SM-actin staining (Figure 6H) was evident to suggest that “cap” formation was inhibited, the reduction in medial α-SM actin–positive cells appeared to be less severe. Fibrotic caps can presumably be formed by the migration of actin-positive smooth muscle cells from the media. Alternatively, cells in the fibrotic cap may be derived from the differentiation of intimal cells that lack actin expression or from the redifferentiation of smooth muscle cells initially derived from the media that downregulate expression of actin in association with their migration into the neointima. In either case, the lack of fibrotic caps suggests that the combined treatment with COX-2 inhibitor and TP antagonist results in plaque destabilization. In view of these results, it will be of interest further to investigate the role of COX-2 and the TP in regulating smooth muscle cell migration and phenotypic differentiation.

In the final study, we assessed the impact of TP antagonism on established atherosclerosis. Prolonged treatment of mice with extensive atherosclerosis that comprised ∼50% of aortic area for up to 16 weeks failed to delay progression of disease or to induce regression of atherosclerosis.

In summary, we have provided further evidence that activation of the TP promotes initiation and early development of atherosclerotic lesions in mice. This may involve receptor ligation both by conventional ligands, such as platelet COX-1–derived TxA2, and COX-independent ligands, such as the iso-
prostanes. Indeed, this may occur in an integrated fashion, because TP ligation may activate NADPH oxidase and consequent free radical generation.49 Experience in this model suggests that clinical use of TP antagonists would be expected to be useful in the earlier stages of disease, rather than in reversing accumulated plaque burden in patients with diffuse, established atherosclerosis. Even early intervention with a selective inhibitor of COX-2 failed to modulate the modest, spontaneous atherogenesis that develops on a chow diet in this model or to augment the beneficial impact of a TP antagonist. Indeed, because TP antagonism may mimic the effect of low-dose aspirin, these studies suggest that coincidental treatment with a COX-2 inhibitor may undermine the benefit of aspirin by predisposing atherosclerotic plaques to destabilization with subsequent vasoocclusive thrombosis. Given the clinical experience with rofeocoxib7 and valdecoxib,8 the impact of low-dose aspirin on gastroprotection in patients taking COX-2 inhibitors,50 and our present findings on plaque morphology, the wisdom of conducting trials to seek a cardiovascular benefit from selective inhibitors of COX-2 in atherothrombotic disease appears questionable.

Acknowledgments
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References

### Table 2: Divergent Effects of COX-2 Inhibitors in Mouse Atherosclerosis

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>COX-2 Inhibitor Initiation</th>
<th>Duration, wk</th>
<th>Diet</th>
<th>Phase of Disease</th>
<th>Effect on Lesion Area</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE KO</td>
<td>MF tricyclic at 8 weeks of age</td>
<td>3</td>
<td>Regular chow</td>
<td>Very early</td>
<td>Increase</td>
<td>26</td>
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<tr>
<td>LDLR KO</td>
<td>Rofecoxib at 10 weeks of age</td>
<td>6</td>
<td>High fat</td>
<td>Early</td>
<td>Decrease</td>
<td>25</td>
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<tr>
<td>LDLR KO</td>
<td>Nimesulide at 8 weeks of age</td>
<td>18</td>
<td>High fat</td>
<td>Intermediate</td>
<td>No increase</td>
<td>13</td>
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<td>ApoE KO</td>
<td>SC-236 at 7 weeks of age</td>
<td>8</td>
<td>1% Cholesterol</td>
<td>Intermediate-advanced</td>
<td>No effect</td>
<td>14</td>
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<tr>
<td>ApoE KO</td>
<td>MF tricyclic at 24 weeks of age</td>
<td>16</td>
<td>Regular chow</td>
<td>Advanced</td>
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<td>ApoE KO</td>
<td>Celecoxib at 26 weeks of age</td>
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<td>Regular chow</td>
<td>Advanced</td>
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<td>28</td>
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Cyclooxygenases, Thromboxane, and Atherosclerosis: Plaque Destabilization by Cyclooxygenase-2 Inhibition Combined With Thromboxane Receptor Antagonism
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An erratum has been published regarding this article. Please see the attached page for:
/content/111/18/2412.full.pdf
Correction

Because of an oversight, the name of Susanne Fries, MD, was excluded from the author list of the paper by Egan et al, “Cyclooxygenases, Thromboxane, and Atherosclerosis: Plaque Destabilization by Cyclooxygenase-2 Inhibition Combined With Thromboxane Receptor Antagonism,” that appeared in the January 25, 2005, issue of Circulation (Circulation. 2005;111:334–342).

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