Why do cyclooxygenase-2 (COX2) inhibitors increase the risk of heart attack and stroke? And can such adverse effects be avoided while retaining anti-inflammatory efficacy? Potential answers to both questions are suggested by a recent study from Garret FitzGerald and colleagues published online in the *Journal of Clinical Investigation*.

The development of selective COX2 inhibitors was initially stimulated by the hypothesis that COX1 was a constitutively expressed isoform whose inhibition could cause the gastrointestinal side effects characteristic of traditional non-steroidal anti-inflammatory drugs (NSAIDs), whereas COX2 was an inducible isoform whose inhibition results in the anti-inflammatory effects of these drugs. However, the hypothesis seems over-simple in the light of the problems with COX2 inhibitors, and considerable attention is now being focused on gaining a deeper understanding of the mechanisms underlying the beneficial and adverse effects of COX inhibition.

A key to understanding these effects seems to be the relative influence of COX1 and COX2 inhibition on the levels of the various prostanoids that result from further enzymatic modification of the product of both COX enzymes, prostaglandin H₂. For example, suppression of the production of the prostanoid prostaglandin E₂, which has prothrombotic effects, and prostacyclin, which has antithrombotic effects — might cause cardiovascular side effects.

To investigate the importance of the levels of various prostanoids, and the effects of COX inhibition on them, FitzGerald et al. used several mouse models with knockout, knockdown or mutation of one or both of the COX enzymes, and also selective COX2 inhibitors. These experiments provided evidence that COX2 is the dominant source of prostacyclin in vivo, as is COX1 for thromboxane A₂, supporting the proposal that disruption of the balance between prostacyclin and thromboxane A₂ could underlie the cardiovascular problems associated with selective COX2 inhibition. Indeed, inhibition, deletion or inactivation of COX2 augmented the response to thrombogenic stimuli and also elevated blood pressure, and these responses were attenuated by knockdown of COX1, which mimics the known antithrombotic effects of low-dose aspirin.

So, is there any way to obtain the beneficial anti-inflammatory effects of COX2 inhibition without increasing the risk of serious cardiovascular events? Intriguingly, a previous study had shown that deletion of microsomal PGE synthase 1 (mPGES1), which synthesizes prostaglandin E₂ from prostaglandin H₂, is as effective as traditional NSAIDs in models of pain and inflammation, and so the authors investigated the effects of mPGES1 deletion further. Prostaglandin E₂ was depressed, prostacyclin was augmented, there was no effect on thromboxane A₂ and, most importantly, mPGES1 deletion did not affect either thrombogenesis or blood pressure. Taken together, these observations suggest that inhibitors of mPGES1 could retain the anti-inflammatory effects of COX2 inhibitors while being less prone to their adverse cardiovascular consequences, and so might represent a promising focus for future drug development efforts.

**New clues in the COX2 mystery**


Cyclooxygenase-2 in Endothelial and Vascular Smooth Muscle Cells Restrains Atherogenesis in Hyperlipidemic Mice

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Methods and Results—In the present study, selective depletion of COX-2 in vascular smooth muscle cells and endothelial cells depressed biosynthesis of prostaglandin I2 and prostaglandin E2, elevated blood pressure, and accelerated atherogenesis in Ldrl knockout mice. Deletion of COX-2 in vascular smooth muscle cells and endothelial cells coincided with an increase in COX-2 expression in lesional macrophages and increased biosynthesis of thromboxane. Increased accumulation of less organized intimal collagen, laminin, α-smooth muscle actin, and matrix-rich fibrosis was also apparent in lesions of the mutants.

Conclusions—Although atherogenesis is accelerated in global COX-2 knockouts, consistent with evidence of risk transformation during chronic nonsteroidal anti-inflammatory drug administration, this masks the contrasting effects of enzyme depletion in macrophages versus vascular smooth muscle cells and endothelial cells. Targeting delivery of COX-2 inhibitors to macrophages may conserve their efficacy while limiting cardiovascular risk. (Circulation. 2014;129:1761-1769.)

Key Words: atherogenesis ■ cyclooxygenase ■ endothelial cell ■ prostaglandin ■ vascular smooth muscle

Nonsteroidal anti-inflammatory drugs (NSAIDs) designed specifically to inhibit cyclooxygenase-2 (COX-2) relieve pain and inflammation but expose patients to a cardiovascular hazard comprising myocardial infarction and stroke, hypertension, heart failure, arrhythmogenesis, and sudden cardiac death. These effects are attributable to suppression of COX-2–derived cardioprotective prostaglandins, particularly prostacyclin (prostaglandin I2 [PGI2]) in the vasculature and in cardiomyocytes. More controversial has been the potential impact of COX-2 inhibition on atherosclerosis. Extended dosing with COX-2 inhibitors in 3 placebo-controlled trials was associated with emergence of a detectable increase in cardiovascular events in patients initially selected to be at low risk of heart disease. Consistent with this observation, deletion of the PGI2 receptor fosters initiation and early development of atherogenesis in hyperlipidemic mice, offering a potential mechanism for risk transformation during chronic drug exposure. However, experiments with inhibitors of COX-2 and conventional COX-2 knockout (KO) mice showed accelerated, delayed, or no effect on atherosclerosis. Because this confusion may have reflected the failure to characterize the pharmacological specificity of enzyme inhibition and the diverse consequences of missing COX-2 during development, we induced global deletion of COX-2 postnatally. In these mice, atherogenesis is accelerated, consistent with the result in prostacyclin receptor KOs.

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Cells tend to make 1 or 2 dominant COX products, often with contrasting biological effects, such as the divergent effects of platelet COX-1–derived thromboxane A2 (TxA2) and COX-2–derived endothelial PGI2 on platelet activation. Activated macrophages make predominantly TxA2 and prostaglandin E2 (PGE2), and ligation of the TxA2 receptor or the E prostanooid receptor-3 fosters atherogenesis. Consistent with these observations, deletion of myeloid cell COX-2 restrains atherogenesis, an effect most likely attributable to macrophage gene deficiency because COX-2 is not expressed in mature platelets and is expressed minimally in dendritic cells and neutrophils. Given the contrasting effects of global
and macrophage COX-2 deletion on disease evolution and the causative implication of enzyme inhibition in vascular cells in other aspects of the NSAID-related cardiovascular hazard, we sought to elucidate the impact of COX-2 in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) on atherosclerosis.

Methods

Materials
All reagents used were purchased from Sigma-Aldrich (St Louis, MO) unless otherwise stated.

Generation of Vascular COX-2 KO

Hyperlipidemic Mice

Endothelial cell COX-2 knockout (Tie2Cre/COX-2−/−; EC KO), vascular smooth muscle cell COX-2 knockout (SM22Cre/COX-2−/−; VSMC KO), and endothelial/vascular smooth muscle cell double COX-2 knockout (Tie2Cre/SM22Cre/COX-2−/−; E/V DKO) mouse lines were generated as described.4-6 These mouse lines were crossed with Ldlr−/− mice fully backcrossed onto a C57BL/6 background (The Jackson Laboratory, Bar Harbor, ME). All KOs were genotyped with tail DNA to confirm the presence of genotype (The Jackson Laboratory, Bar Harbor, ME). All KOs were compared with appropriate strain-, age-, and sex-matched control animals. Single nucleotide polymorphism analyses showed that EC KO, VSMC KO, and E/V DKO mouse lines for the atherosclerosis study were normalized with creatinine.

Animals

In all experiments, COX-2-deficient transgenic mice were compared with appropriate strain-, age-, and sex-matched control animals. Single nucleotide polymorphism analyses showed that EC KO, VSMC KO, and E/V DKO mouse lines for the atherosclerosis study achieved at least 90.5% to 92.5% purity on the C57BL/6 background. Mice of both sexes were fed a high-fat diet (HFD; 21.2% fat, 0.2% cholesterol; TD.88137, Harlan Teklad, Madison, WI) from 8 weeks of age for 3 and 6 months. Mice were weighed before and after the HFD feeding. All animals in this study were housed according to the guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania. All experimental protocols were approved by the Institutional Animal Care and Use Committee.

Preparation of Mouse Aortas and En Face

Quantification of Atherosclerosis

Mice were transferred after HFD feeding to new cages without food from 8 am to noon. Water was provided ad libitum. All mice were euthanized between noon and 4 pm by CO2 exsuffusion. The extent of atherosclerosis (Phase 3 Imaging Systems, Glen Mills, PA) was determined by the en face method and by assessment of aortic root lesion burden, as described previously.7

Blood Pressure Measurement

Systolic blood pressure was measured in conscious mice with the use of a computerized noninvasive tail-cuff system (Visitech Systems, Apex, NC), as described.18 Blood pressure was recorded once each day from 8 am to 11 am for 5 to 7 consecutive days after 3 days of training. Average systolic blood pressure was reported.

Mass Spectrometric Analysis of Prostanoids

Urinary prostanoid metabolites were measured by liquid chromatography/mass spectrometry as described.19 Such measurements provide a noninvasive, time-integrated measurement of systemic prostanoid biosynthesis,20 reflective of short-term minor alterations in product formation21 and of vascular stimulation.22 Briefly, mouse urine samples were collected with the use of metabolic cages over a 15-hour period (6 pm to 9 am). Systemic production of PGI2, PGE2, PGD2, and TxA2, was determined by quantifying their major urinary metabolites: 2,3-dinor 6-keto PGF1α (PGI-M); 7-hydroxy-5, 11-diketotetranorpropane-1, 16-dioic acid (PGE-M); 11, 15-dioxo-9, hydroxy-2, 3, 4, 5-tetranorprostan-1, 20-dioic acid (tetranor PGD-M); and 2, 3-dinor TxB2 (Tx-M), respectively. Results were normalized with creatinine.

Immunohistochemical Examination of Lesion Morphology

Mouse hearts were embedded in OCT, and 10-μm serial sections of the aortic root were cut and mounted on SuperFrost Plus slides (Fisher Scientific) for analysis of lesion morphology. Samples were fixed in acetone for 15 minutes at -20°C. Before treatment with the first antibody, sections were consecutively treated to block endogenous peroxidase (3% H2O2 for 15 minutes) with 10% normal serum blocking solution (dependent on host of secondary antibody, in 1% BSA/PBS for 15 minutes) and endogenous biotin (streptavidin-biotin blocking kit, SP-2002, Vector Laboratories). Sections were then incubated with the desired primary antibody in blocking solution overnight at 4°C. Samples were individually stained for collagen type I (1 μg/mL, 1310-01, Southern Biotech), laminin (2 μg/mL, L9393, Sigma), α-smooth muscle actin (α-SMA; 12.3 μg/mL, F3777, Sigma), vascular cell adhesion molecule-1 (VCAM-1; 10 μg/mL, 553331, BD Bioscience), COX-1 (1 μg/mL, 160109, Cayman Chemicals), COX-2 (1 μg/mL, 160106, Cayman Chemicals), CD11b (5 μg/mL, 557395, BD Bioscience), and CD11c (5 μg/mL, 553800, BD Bioscience), all with isotype-matched controls. Where required, sections were then incubated with biotinylated-IgG secondary antibody (specific to host of primary antibody, all 1 μg/mL, Vector Laboratories) diluted in 1% bovine serum albumin-phosphate-buffered saline (BSA/PBS) for 1 hour at room temperature. Sections were then incubated with streptavidin/horseradish peroxidase (1 μg/mL, 016-030-084, Jackson Immunoresearch) diluted in 1% BSA/PBS for 30 minutes at room temperature. Slides were equilibrated in sterile H2O for 5 minutes at room temperature, then developed with the use of the DAB substrate kit (K3468, Dako) according to the manufacturer’s protocol. Samples were counterstained with hematoxylin, dehydrated, and mounted in Cytoseal–60 (12-547, Fisher Scientific). Isotype-matched controls were performed in parallel and showed negligible staining in all cases.

COX-2 CD11b Stepwise Double Staining

Ten-micrometer frozen sections were left to air-dry for 5 minutes at room temperature. Samples were then fixed in acetone for 15 minutes at -20°C. Before treatment with the first antibody, samples were consecutively treated to block endogenous peroxidase (3% H2O2 for 15 minutes) with 10% normal goat serum blocking solution (in 1% BSA/PBS for 15 minutes) and endogenous biotin (streptavidin/biotin blocking kit, SP-2002, Vector Laboratories). The COX-2 primary antibody (1 μg/mL, 160106, Cayman Chemicals) or COX-2 IgG control (1 μg/mL, 011-000-003, Jackson Immunoresearch) diluted in blocking solution was then added, and samples were incubated overnight at 4°C. After three 5-minute washes with 0.05% Tween-20/PBS, samples were incubated with biotinylated goat anti-rabbit IgG secondary antibody (1 μg/mL, BA-1000, Vector Laboratories) diluted in 1% BSA/PBS for 1 hour at room temperature. Three more washes were performed before samples were next incubated with streptavidin/alkaline phosphatase (1 μg/mL, 016-050-084, Jackson Immunoresearch) diluted in 1% BSA/PBS for 30 minutes at room temperature. Samples were again washed 3 times before being equilibrated in 0.1 mol/L Tris-HCl (pH 8.2) for 5 minutes at room temperature, then developed with the use of the Vector Red substrate kit (SK-5100, Vector Laboratories) according to the manufacturer’s protocol. Before double staining with CD11b, samples were once again treated with the streptavidin/biotin blocking kit. Biotinylated CD11b primary antibody (5 μg/mL, 557395, BD Pharmingen) or biotinylated IgG control (5 μg/mL, 13-4031, eBioscience) diluted in 1% BSA/PBS was then added, and samples were incubated overnight at 4°C. After 3 washes, samples were incubated with streptavidin/horseradish...
peroxidase (1 μg/mL, 016-030-084, Jackson Immunoresearch) diluted in 1% BSA/PBS for 1 hour at room temperature. After 3 more washes, samples were equilibrated in sterile H2O for 5 minutes at room temperature and developed with the use of the DAB substrate kit (SK-4100, Vector Laboratories) according to the manufacturer’s protocol. Samples were then counterstained with hematoxylin, dehydrated, and mounted in Cytoseal-60 (12-547, Fisher Scientific).

Second Harmonic Generation Analysis of Fibrillar Collagen Structure
Ten-micrometer aortic root frozen sections were left to air-dry for 5 minutes at room temperature before second harmonic generation (SHG) microscopy analysis of fibrillar collagen structure was performed. Samples were then fixed in acetone for 15 minutes at -20°C and submerged in PBS in a tissue culture dish. SHG images of the fibrillar collagen and background tissue autofluorescence images were captured at x10 magnification with the use of a Prairie Technologies Ultima 2-Photon Microscope system (Middleton, WI). Images were taken with an excitation wavelength of 910 nm and captured through emission filters of 457 to 487 nm (SHG signal) and 525 to 570 nm (autofluorescence). The SHG and autofluorescence signals were pseudocolored in green and red, respectively, for observation of structure and morphology. Quantification of fibrillar collagen content, intensity, and organization was calculated with the use of Fiji Image Analysis software. First, the fibrillar collagen signal was isolated by subtracting the background autofluorescence signal from the original SHG image. Collagen content (area and intensity) was then quantified as percentage of total lesion area and total integrated density of the collagen signal, respectively. To measure fibrillar collagen organization, the Directionality macro (within Fiji) was first used to generate a fast Fourier transform powerplot of the fibrillar collagen signal. An ellipse was then superimposed over the positive signal generated within each powerplot, and the aspect ratio of the ellipse for each lesion was calculated. An aspect ratio with a value closer to 0 indicated random orientation, and that closer to 1 indicated orientated collagen fibers. For each lesion, 6 sections (equally spaced over the entire aortic root, ≈350 μm) were analyzed for fibrillar collagen content, intensity, and organization.

Statistical Analysis
For data analyzed by ANOVA, the Holm-Sidak or Dunnett posttest was used to compare the differences between the means only if the ANOVA returned a P < 0.05. Sample sizes were based on variation of the test measurement and the desire to detect a minimal 10% difference in the variables assessed with (α=0.05 and power (1-β)=0.8.

Results
Aortic COX-2 Expression Is Modulated by Diet and Gene Deletion
Expression of COX-2 in the aortic arch and thoracic aorta of WT mice increased with the HFD-fed compared with normal chow–fed animals (Figure 1A in the online-only Data Supplement). Vascular tissue–specific COX-2 KOs on a chow diet revealed significant reductions in aortic COX-2 expression compared with WT mice on both a chow diet (Figure 1B and IC in the online-only Data Supplement) and after HFD feeding for both 3 and 6 months (Figure 1F and IG in the online-only Data Supplement). Although data from female mice at 6 months of HFD are illustrated, regardless of sex or study duration, COX-1 expression in aortic arch or thoracic aorta was not significantly altered in the mutants on a chow diet (Figure 1D and IE in the online-only Data Supplement) or a HFD (Figure 1H and II in the online-only Data Supplement).

Deletion of COX-2 in Vascular Cells Modulates Prostaglandin Biosynthesis in Mice on a HFD
There were no significant effects of genotype or sex on plasma cholesterol, triglycerides, glucose, or weight gain at different times in mice on a HFD. Thereafter, we focused our analysis on males at 3 months and on females at 6 months of a HFD because the extent of their atherosclerotic lesions was similar and their lesional morphology was not advanced to the point at which genotype-dependent effects are often undetectable. Deletion of COX-2 in vascular cells generally depressed biosynthesis of PGI2, (Figure 1A and IE) and PGE2, (Figure 1B and IF), as reflected by their urinary metabolites. However, this was not observed with EC deletion alone in female mice. This may reflect the imperfect matching of lesional development between males at 3 months and females at 6 months on a HFD. Biosynthesis of PGD2, was depressed by COX-2 deletion only in males (Figure 1C). By contrast, thromboxane biosynthesis was increased in the single vascular mutants in both sexes and in the female compound mutants (Figure 1D and IH). However, it was depressed in the compound male mutants.

Vascular COX-2 Depletion Elevates Systolic Blood Pressure and Restrains Atherogenesis
Although systolic blood pressure was not altered in single EC or VSMC KO male mice after 3 months on a HFD, a significant elevation was observed in the compound E/V mutants compared with WT mice (WT versus E/V DKO, 114±1 versus 124±2 mmHg; P=0.0002; Figure 2A). For female mice fed a HFD for 6 months, systolic blood pressure was also significantly elevated in both the compound mutants and those lacking COX-2 in VSMCs (WT versus VSMC KO and E/V DKO, 111±2 versus 121±2 versus 120±3 mmHg, respectively; P=0.0001; Figure 2B). Atherosclerotic lesion burden was increased by deletion of COX-2 in vascular cells in both males at 3 months (WT versus EC, VSMC, and E/V DKOs, 3.72±0.4% versus 7.17±0.7%, 5.19±0.3%, and 4.73±0.6%, respectively; P=0.0001; Figure 3A) and females at 6 months (10.15±0.7% versus 13.36±0.6%, 11.76±0.8%, and 13.19±0.6%, respectively; P=0.003; Figure 3B) on a HFD. As lesions became advanced in males at 6 and 11 months on a HFD (Figure II in the online-only Data Supplement), these genotype-dependent changes were lost.
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in the DKO, as revealed by the fast Fourier transform–based analyses (Figure 4B). Representative SHG-collagen images of WT and E/V DKO are shown (Figure 4C and 4D).

α-SMA staining, a marker of differentiated VSMCs, was increased in both single and compound COX-2 KO lesions (Figure 5C). Necrotic cores in COX-2 KOs did not differ significantly in size compared with those in WTs (Figure 5A through 5C). However, only single COX-2 KOs showed an upregulation of VCAM-1 for activated VSMCs (Figure 5D).

COX-2–positive lesional cells in the vascular mutants (Figure IVD in the online-only Data Supplement) costained with the inflammatory macrophage marker CD11b (Figure 6) in compound vascular mutants, whereas COX-1 expression was unaltered (Figure IVC in the online-only Data Supplement).

Discussion

Nine placebo-controlled trials of COX-2–selective NSAIDs (rofecoxib, celecoxib, and valdecoxib) have revealed a cardiovascular risk of myocardial infarction, hypertension, stroke, and heart failure.23,24 These risks are explicable in terms of the suppression of cardioprotective products of COX-2, particularly PGI₂. In mice, inhibition or deletion of COX-2–dependent PGI₂ formation augments the response to thrombogenic stimuli, elevates systemic and pulmonary blood pressure, disrupts vascular remodeling, and predisposes the animals to cardiac failure and arrhythmogenesis.12,14,18,25

Three of these placebo-controlled trials were performed in patients selected to be at low demographic risk of cardiovascular disease.3–5 Despite this, an increase in cardiovascular
events became detectable with extended dosing with either celecoxib or rofecoxib for >1 year. The time course of emergent risk would be consistent with a drug effect on atherogenesis, such as was observed in prostacyclin receptor–deficient mice. However, the results of studies of COX-2 deletion or inhibition in hyperlipidemic mice have been conflicting. This may reflect the systemic consequences of COX-2 deficiency in utero in the case of the conventional knockouts and of a failure to characterize the actual biochemical selectivity for COX-2 inhibition of the pharmacological regimens used. This is an important point because COX-1 inhibition or deletion attenuates atherogenesis,26,27 More recently, we generated mice in which global COX-2 deletion was accomplished postnatally, and in this case atherogenesis was accelerated in both sexes when they were crossed into apolipoprotein E–deficient mice.9
We have reported previously that both EC and VSMC COX-2 contribute substantially to systemic PGI2 formation under physiological conditions in normolipidemic mice, as reflected by urinary PGI-M.10 In the present study, we extended these observations, showing that on a hyperlipidemic background, biosynthesis of both PGI2 and PGE2 was derived in both sexes substantially from COX-2 in vascular cells and that the enzyme contributed to PGD2 biosynthesis in males. Expression of COX-2 was upregulated in both the aortic arch and thoracic aorta of littermate control mice fed a HFD, consistent with the increase in urinary PGI-M in apolipoprotein E–deficient and LdlR-deficient mice.28

Figure 3. Vascular cyclooxygenase-2 restrains atherogenesis. Aortic atherosclerotic lesion burden, represented by the percentage of lesion area to total aortic area, was quantified by en face analysis of aortas from male mice fed a high-fat diet (HFD) for 3 months and from female mice at 6 months. Representative en face preparations are shown (bottom). Lesion area tended to increase in male or female cyclooxygenase-2 mutants fed a HFD for 3 or 6 months, respectively (A and B). One-way ANOVA revealed a significant effect of genotype (male, P=0.0001; female, P=0.003) on lesion progression. Holm-Sidak multiple comparison tests were used to test significant differences between wild-type (WT) and cyclooxygenase-2 knockouts (KOs). Data are mean±SEM. **P<0.01, ***P<0.001; n=18 to 22 per genotype. EC indicates endothelial cell; E/V DKO, endothelial/vascular smooth muscle cell double cyclooxygenase-2 knockout; and VSMC, vascular smooth muscle cell.

Figure 4. Vascular cyclooxygenase-2 restrains aortic root lesion burden and orientation of structural collagen in lesions. A, Quantification of cross-sectional analysis of aortic root samples from female mice fed a 6-month high-fat diet was performed by measuring total lesion area across the aortic root, as detailed in Methods. One-way ANOVA (Kruskal-Wallis test) revealed a significant effect of genotype (P=0.048) on lesion progression. Dunnett multiple comparison tests were used to test significant differences between wild-type (WT) and cyclooxygenase-2 knockouts (KOs). *P<0.05. Data are mean±SEM; n=5 per group; 7 to 9 sections from each animal were averaged. B, Second harmonic generation detection of structural collagen in WT (n=22) and endothelial/vascular smooth muscle cell double cyclooxygenase-2 knockout mice (E/V DKO; n=21) lesions from female mice on a high-fat diet for 6 months. Fast Fourier transform (FFT)–based analyses revealed a more random orientation of collagen fibers in E/V DKO lesions. Data are mean±SEM. **P<0.01 (Mann-Whitney test, 2-tailed). C and D, Representative WT and E/V DKO second harmonic generation images are shown. VSMC indicates vascular smooth muscle cell.
Platelet- and neutrophil–vessel wall interactions during the development of atherosclerosis are reflected by the urinary thromboxane metabolite TxA_2. TxA_2 is also a dominant product of activated macrophages. Theoretically, cells outside the vascular compartment (glial cells and glomeruli, for example) have the capacity to generate thromboxane and may contribute to urinary Tx-M. Mice lacking the prostacyclin receptor fed a HFD excrete higher levels of Tx-M in both sexes. Moreover, previous studies have shown that COX-2–dependent formation of PGI_2 in the vasculature restrains TxA_2 biosynthesis. In the present study, urinary Tx-M was increased in COX-2 mutants fed a HFD, which perhaps reflects removal of a regulatory constraint on myeloid cell activation by suppression of the biosynthesis of PGI_2. This phenomenon was not observed in male E/V DKOs, in which urinary Tx-M was depressed. Whether this reflects a feedback response to the differential impact of male sex hormones on thromboxane receptor expression that would be more exaggerated in the compound mutant or some other mechanism is unknown.

Previous studies in which COX-2 is deleted in a cell-specific fashion have highlighted the importance of prostaglandins in regulation of expression of their biosynthetic enzymes. Thus, deletion of COX-2 in cardiomyocytes results in COX-2 upregulation in cardiac fibroblasts, with a shift in the dominant enzyme product from PGI_2 to PGF_2α in the latter. Similarly, COX-2 deletion in plaque macrophages was associated with enzyme upregulation in VSMCs, implying a shift from TxA_2 and PGE_2 as the dominant product in the former to PGI_2 in the latter. In both cases, the shift in product formation may have contributed to the resultant phenotype: cardiac fibrosis and arrhythmogenesis in the heart and attenuated atherogenesis in the vasculature. In the present study, we observed increased expression of COX-2 in lesional...
vascular cells. Mature differentiated VSMCs retain the capacity for phenotypic plasticity. As an example, in a rat model of balloon injury of carotid artery, medial VSMCs dedifferentiate after endothelial injury and migrate to the intima, where they proliferate and secrete extracellular matrix components, such as collagen fibrils and elastin. In the present study, the proliferative VSMCs in mature neointima (after 14 days of endothelial injury) gradually redifferentiate and attain a contractile-like phenotype, as observed in the media.

Whereas immunohistochemical analysis clearly revealed an increase in lesional collagen content, SHG 2-photon microscopy analyses indicated that lesion size, fibrillar collagen content, and intensity were apparently unaltered between WT and E/V DKO. This disparity could be explained by the limitation of the SHG microscope in detecting the finer fibrillar collagen of lower-order structures. In addition to higher-order collagen structures, the antibody recognized the epitopes of fine collagen I structures, thus giving the higher signal. Despite this, we observed changes in the orientation of fibrillar collagens that accumulated in the atherosclerotic lesions. Specifically, fibrillar collagens in COX-2 E/V DKO lesions were significantly more random or less organized than those in WT lesions.

Figure 6. Vascular cyclooxygenase-2 (COX-2) deletion increases COX-2 expression in lesional macrophages. Lesion morphology in aortic roots from female mice fed a high-fat diet for 6 months was analyzed. Quantification of immunohistochemistry staining of COX-2 (A) and CD11b (B) from wild-type (WT) and endothelial/vascular smooth muscle cell double COX-2 knockout (E/V DKO) mice is shown. A Mann-Whitney test (2-tailed) revealed a significant increase in COX-2–positive cells in DKO compared with WT. **P<0.01; n=8 to 12 per genotype. Data are mean±SEM. C, Representative aortic root sections from COX-2 staining (left) and CD11b (right) from E/V DKO are shown. Arrows indicate lesional cells that are stained positive for both COX-2 and CD11b.
in placebo-controlled, randomized trials of NSAIDs specific for inhibition of COX-2.27 However, this masks divergent effects of macrophage COX-2-derived TxA2, fostering disease and PGII derived from COX-2 in ECs and VSMCs acting as restraint. These observations and others28–31 suggest that targeting inhibitors of COX-2 selectively to the macrophage may fundamentally alter the balance of cardiovascular efficacy and risk for NSAIDs.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Placebo-controlled trials of cyclooxygenase-2 (COX-2)–selective nonsteroidal anti-inflammatory drugs have revealed higher cardiovascular risks associated with myocardial infarction, hypertension, heart failure, and stroke consequent to the suppression of cardioprotective prostanooids, particularly prostacyclin. We have reported previously that global postnatal deletion of COX-2 accelerates atherogenesis and that deletion of the receptor for prostaglandin I(2) leads to initiation and early development of atherosclerosis in mice. Because of the contrasting biological impact of products of COX-2 and their varied predominance in cells during disease evolution, we have attempted to address the role of COX-2 in a tissue-specific manner. We have reported that deletion of COX-2 in myeloid cells retarded atherogenesis and that deletion in T cells had a minimal effect on lesion burden. In the present study, when mice were placed on a high-fat diet, deletion of COX-2 in endothelial or vascular smooth muscle cells or both increased systolic blood pressure and accelerated atherogenesis coincident with suppression of prostaglandin I(2) biosynthesis. Suppression of COX-2 removes a constraint on enzyme expression in lesional macrophages, reflecting the interplay of the enzyme in cells relevant to the disease. Having a better understanding of the cell-specific biology of COX-2 deletion, we may advance the prospect for cell-targeted nonsteroidal anti-inflammatory drug delivery and minimize the cardiovascular adverse effects of these drugs.