Microsomal Prostaglandin E Synthase-1 Deletion Suppresses Oxidative Stress and Angiotensin II–Induced Abdominal Aortic Aneurysm Formation

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Background—Microsomal prostaglandin (PG) E₂ synthase-1 (mPGES-1) catalyzes isomerization of the cyclooxygenase product PGH₂ into PGE₂. Deletion of mPGES-1 modulates experimentally evoked pain and inflammation and retards atherogenesis. The role of mPGES-1 in abdominal aortic aneurysm is unknown.

Methods and Results—The impact of mPGES-1 deletion on formation of angiotensin II–induced abdominal aortic aneurysm was studied in mice lacking low-density lipoprotein receptor (LDLR⁻/⁻). Male mice deficient in both mPGES-1 and LDLR (mPGES-1⁻/⁻/⁻LDLR⁻/⁻) and littermate LDLR⁻/⁻ mice were initiated on a high-fat diet at 6 months of age, followed 1 week later by continuous infusion of angiotensin II (1 μg/kg per minute) for an additional 4 weeks. Angiotensin II infusion upregulated aortic expression of cyclooxygenase-2 and mPGES-1, increased aortic macrophage recruitment and vascular nitrotyrosine staining (which reflects local oxidative stress), and augmented urinary excretion of the isoprostane 8,12-iso-iPF₂α-VI (which reflects lipid peroxidation in vivo) and the major metabolite of PGE₂ (PGE-M). Deletion of mPGES-1 decreased both the incidence (87.5% versus 27.3%; \( P = 0.02 \)) and the severity of abdominal aortic aneurysm and depressed the aortic and systemic indices of oxidative stress. Deletion of mPGES-1 also depressed urinary PGE-M, whereas it augmented excretion of PGD₂ and PGI₂ metabolites, reflecting rediversion of the accumulated PGH₁ substrate in the double knockouts.

Conclusions—Deletion of mPGES-1 protects against abdominal aortic aneurysm formation induced by angiotensin II in hyperlipidemic mice, coincident with a reduction in oxidative stress. The potential efficacy of selective inhibition of mPGES-1 in preventing or retarding aneurysm formation warrants further investigation. (Circulation. 2008;117:1302-1309.)

Key Words: aneurysm • aorta • drugs • prostaglandins
lated at the individual level by the patient’s underlying cardiovascular risk, the extent of drug exposure, and concomitant therapies.8

Microsomal prostaglandin E synthase-1 (mPGES-1),9,10 catalyzes the isomerization of PGE₂ into PGI₂ and is a member of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) superfamily; it has been suggested as an alternative antiinflammatory drug target.9 Two other PGE synthases have been identified: mPGES-211 and cytosolic PGES.12,13 However, mPGES-1 is the dominant source of PGE₂ biosynthesis, at least in mice.14 mPGES-1 is often coregulated with COX-215 but has been colocalized with both COX isoforms in some settings.12,16 mPGES-1 deletion in mice has been reported to modulate experimentally evoked pain and inflammation to a degree indistinguishable from treatment with traditional NSAIDs.17,18 It has also been implicated in immune-induced pyresis.19 However, in contrast to the effects of inhibition or deletion of COX-2, mice deficient in mPGES-1 exhibit a favorable cardiovascular profile. Deletion of mPGES-1 does not enhance responsiveness to a thrombogenic stimulus in vivo20 and retards atherogenesis in hyperlipidemic mice.21 These properties may reflect redirection of the mPGES-1 substrate, PGH₂, to PGI synthase, as biosynthesis of PGI₂ is augmented in these mice.14,20 So a cardiovascular profile may render indirect aorta was dissected out, immediately put into PBS, and cleaned of adventitial fat. The maximal abdominal aortic diameter was measured with the use of a caliper under a dissection microscope, while the aorta rested in PBS buffer without any physical stretching. A >50% increase in external diameter of the abdominal aorta was used to define the occurrence of an AAA. AAA severity was classified visually as described previously22 and also assessed by the external diameter and the wet weight of the abdominal aorta.

Analyses in Plasma
Blood was drawn from the vena cava of CO2-euthanized mice, and EDTA (final concentration, 10 mmol/L) was added immediately. Plasma glucose, total cholesterol, and triglyceride levels were measured enzymatically on a Cobas Fara II autoanalyzer (Roche Diagnostics Systems Inc, Nutley, NJ) with reagents from Wako Chemicals (Richmond, Va).

Real-Time Polymerase Chain Reaction Analysis of Gene Expression in Mouse Aorta
TagMan gene expression assays (Applied Biosystems, Foster City, Calif; catalog No. 4331182) for mPGES1 (Mm00452105_m1), COX-1 (Mm04772141_m1), COX-2 (Mm04783741_m1), and Ang II receptors (AT₁_a; Mm01957722_s1; AT₁_b; Mm02620738_s1; AT₂; Mm03413733_m1) were performed on an ABI Prism 7900 Sequence Detection System. Results were normalized with 18S rRNA (Hs99999901_s1).

Histological Examination of Vascular Morphology
Suprarenal abdominal aortae were harvested on day 8 of the Ang II infusion. Peroxidase-quenched sections (8 μm) of OCT-embedded abdominal aorta were blocked with 3% BSA (in PBS) containing 20 μg/mL goat IgG (Jackson ImmunoResearch, West Grove, Pa) followed by incubation with primary antibodies: FITC-conjugated mouse anti-α-smooth muscle actin clone I4A (Sigma-Aldrich, St Louis, Mo), biotinylated rat anti-CD11b (BD Biosciences, San Jose, Calif), rabbit anti-nitrotyrosine IgG (Millipore, Billerica, Mass), rabbit anti-mouse mPGES-1 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif), and rabbit anti-mouse COX-1 and COX-2 antibodies (Cayman Chemicals, Ann Arbor, Mich). Sections were then incubated with either biotinylated goat anti-rabbit IgG, mouse anti-FITC IgG (Jackson ImmunoResearch, West Grove, Pa), followed by Vectastain ABC avidin-biotin (Vector Laboratories, Burlingame, Calif) or directly with Vectastain ABC avidin-biotin, and developed with DAB (DAKO, Carpinteria, Calif). All sections were counterstained with Gill’s Formulation No. 1 hematoxylin (Fisher Scientific, Fair Lawn, NJ), and isotype controls were run in parallel, with negligible staining observed in all cases. CD11b, nitrotyrosine, mPGES-1, COX-1, and COX-2 staining was performed on nonfixed OCT-embedded samples, and smooth muscle cell staining was performed on Prefer (Anatech, Battle Creek, Mich) fixed and OCT-embedded samples.

Analysis of Prostanoids
Urine was collected for 24 hours at baseline and 4 weeks after Ang II infusion. Systemic production of PGE₂, thromboxane A₂ (TXA₂), PGI₂, and PGD₂ was determined by stable isotope dilution, tandem mass spectrometric quantification of their major urinary metabolites23: 9,15-dioxo-11-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid (PGE₂-M), 2,3-dinor-thromboxane B₂ (Tx-M), and 11,15-dioxo-9a-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid (PGD₂-M), respectively. The nonenzymatic lipid peroxidation product, 8,12-iso-PGF₂α-VI, was measured as previously published.23 Metabolite levels were corrected for urinary creatinine (Oxford Biomedical Research, Oxford, Mich).

Statistical Analysis
Data are expressed as mean±SEM. Comparisons of multiple groups were performed by ANOVA and a Bonferroni post-ANOVA multiple comparison test when the ANOVA was significant. When only 2 mean values were compared, the 2-tailed Mann-Whitney test was used. Differences were considered statistically significant at P<0.05.
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## Results

### mPGES-1 Deletion Attenuates Ang II–Induced Aortic Aneurysm

Ang II infusion induces formation of aneurysms predominantly of the abdominal aortae in hyperlipidemic mice.\(^2\)\(^1\)\(^\,\)\(^\,\)\(^2\)\(^4\) mPGES-1 deletion decreased both the incidence (LDLR\(^{-/-}\)/H11002/H11002/H11002: 87.5\% versus mPGES-1\(^{-/-}\)/H11002/H11002/H11002: 27.3\%) of AAA formation and its severity, as reflected by the maximal diameter and the wet weight of the abdominal aorta (Figure 1A to 1E). Sudden death due to rupture of the abdominal aorta was both delayed and reduced in the mPGES-1\(^{-/-}\)/H11002/H11002/H11002 mice (Figure 1F and 1G). Ang II infusion increased blood pressure, but a divergence was not observed between LDLR\(^{-/-}\)/H11002/H11002/H11002 and mPGES-1\(^{-/-}\)/H11002/H11002/H11002 mice throughout the study (Figure 1H). There was no impact of mPGES-1 deletion on fasting plasma total cholesterol, free cholesterol, high-density lipoprotein cholesterol, triglycerides, or glucose in LDLR\(^{-/-}\)/H11002/H11002/H11002 mice (Table).

### Ang II Upregulates mPGES-1 and Increases Oxidative Stress

Considering the advanced stage of most of the AAAs formed after 4 weeks of Ang II infusion (Figure 1D), we sought evidence for pathogenesis in samples harvested at an earlier time point: day 8 of Ang II treatment. The most striking morphological change at this time was that Ang II caused...
macrophage recruitment to the adventitia and endothelium; however, this was not affected by deletion of mPGES-1 (Figure 2). CD11b was used in this study as a macrophage marker, although it may also identify granulocytes. The number of CD11b-positive cells (Figure 2B) was not altered by mPGES-1 deletion. Ang II increased aortic expression of both COX-2 and mPGES-1, but not COX-1, as detected by real-time polymerase chain reaction, and deletion of mPGES-1 abolished detection of its transcript, whereas expressions of COX-2 and COX-1 were not altered significantly (Figure 3A). Immunohistochemistry confirmed upregulation of mPGES-1 and COX-2 but not COX-1 in Ang II–treated LDLR–/– mice. mPGES-1 staining was apparent in smooth muscle cells and endothelial cells in Ang II–treated mice (Figure I in the online-only Data Supplement), whereas Ang II–induced COX-2 expression was most striking in endothelial cells in both single and double knockout mice with some staining of adventitial cells in LDLR–/– mice (Figure II in the online-only Data Supplement). mPGES-1 deletion significantly attenuated Ang II–induced indices of both aortic and systemic oxidative stress (Figure 3B and 3C). Peroxynitrite, the product of the reaction of O2−· and nitric oxide, reacts with and nitrates tyrosine groups of proteins. Thus, the relative level of immunoreactive nitrotyrosine in a tissue is a widely recognized marker of general oxidative stress. Ang II treatment potently augmented immunoreactive nitrotyrosine in aortic endothelium, adventitia tunica, and tunica media (Figure 3B, top and middle panels), which is indicated by the staining of smooth muscle actin (Figure 3B, bottom panel). This effect on tissue nitrotyrosine was evident even before aneurysm formation was detected. mPGES-1 deletion significantly attenuated this index of aortic oxidative stress. The pro-oxidant effect of Ang II was also reflected by increased urinary 8,12-iso-IP2-VI, a sensitive index of lipid peroxidation in vivo.25 Deletion of mPGES-1 depressed significantly Ang II–induced augmentation of urinary 8,12-iso-IP2-VI (Figure 3C). Differential Impact of mPGES-1 Deletion on Systemic Prostanoid Generation Coincident with increased aortic expression of mPGES-1, Ang II infusion augmented systemic biosynthesis of its product, PGE2, as reflected by urinary excretion of its major metabolite, PGE-M. This effect was abolished in mice lacking mPGES-1 (Figure 4A). Ang II increased excretion of metabolites of PGD2, PGI2, and TxA2. mPGES-1 deletion augmented further the production of PGD2 and PGI2, but not of TxA2, at baseline or after Ang II treatment (Figure 4B to 4D). Ang II–induced AAA formation in mice is mediated by its receptors.22 Deletion of mPGES-1 did not apparently affect the aortic expression of any of the angiotensin receptors: AT1A, AT1B, or AT2 (Figure III in the online-only Data Supplement). An ≈60% reduction in aortic activity of matrix metalloproteinase 2 (MMP-2) was associated with mPGES-1 deficiency in the Ang II–treated mice (Figure IV in the online-only Data Supplement). Activity of aortic MMP-9 was much lower than that of MMP-2 in the same zymography study, and comparative analysis of the impact of mPGES-1 deletion on MMP-9 versus MMP-2 activity was unsuccessful.

**Discussion**

Studies in mice and humans have implicated COX-2 and its products in the pathogenesis of aortic aneurysm. Because this is a common but probably underdiagnosed and potentially fatal condition, the possibility of a chemopreventative strat-
egy has considerable appeal. However, the appreciation that NSAIDs selective for COX-2 (which include several of the older traditional NSAIDs) themselves confer a cardiovascular hazard\(^7\) limits their utility in a population often characterized by diffuse atherosclerotic disease, a condition associated with AAA.\(^26\) Recently, mPGES-1 has emerged as a potentially attractive alternative drug target to COX-2. Although other sources of formation of PGE\(_2\) have been identified\(^11\)–\(^13\) and prostanoids other than PGE\(_2\), such as PGI\(_2\),\(^27\)\(^,\)^\(^28\) can mediate pain and inflammation, mPGES-1 deletion has been as effective as traditional NSAIDs in alleviating some paradigms of pain and inflammation in mice.\(^17\),\(^18\)

In the present study, we report that mPGES-1 deletion has a marked impact on the development and extent of Ang II–induced AAA formation in mice. It is unclear to what extent this commonly used model\(^29\) simulates the human condition. However, there is increased expression of COX-2 both in the lesions of this model and in human aneurysmal tissue,\(^3\) and there is some preliminary evidence that prostaglandins might contribute to lesion progression in humans.\(^3\) Patients presenting with aortic aneurysm are often characterized by extensive atherosclerotic disease. Because placebo-controlled trials\(^7\) have shown that NSAIDs selective for inhibition of COX-2 confer a cardiovascular hazard, they are not an attractive therapeutic option despite data in mice and humans implicating COX-2 in the pathogenesis of AAA.

Initial studies in mice raise the possibility that selective inhibitors of mPGES-1 may have a more favorable cardiovascular profile than COX-2 inhibitors. We have reported previously that mPGES-1 deletion, unlike deletion or inhibition of COX-2 or deletion of the PGI\(_2\) receptor, does not enhance the response to a thrombogenic stimulus in vivo.\(^14\) Furthermore, unlike inhibition and deletion of COX-2, which have been reported to variably influence atherogenesis,\(^30\) mPGES-1 deletion markedly retards lesion progression in LDLR\(^{−/−}\) mice.\(^20\) Finally, we and others have reported that
mPGES-1 deletion failed to elevate blood pressure, even in mice on a high-salt diet. However, Jia et al have reported a hypertensive response to mPGES-1 deletion. Potential explanations for these divergent results include differences in genetic background, a more intense salt-loading regimen, and the use of wild-type but not littermate controls. mPGES-1 deletion did not augment the hypertensive response to Ang II in hyperlipidemic mice in the present study. Although the extent to which these early observations extend to treating humans with selective mPGES-1 inhibitors remains to be established, they raise the prospect of an attractive approach to limiting inflammatory diseases in patients with established cardiovascular disease. Selective inhibitors of mPGES-1 are under development. Experiments with knockout mice suggest that they may be less likely to confer cardiovascular hazard than NSAIDs selective for inhibition of COX-2, perhaps because of substrate rediversion to cardioprotective prostanoids.

Aneurysmal lesions in both humans and mice exhibit the hallmarks of inflammation, and the attendant oxidant stress has been implicated in the pathogenesis of the disease. In the present study, we show that infusion of Ang II results in early recruitment of macrophages and extensive evidence of oxidative stress in the vasculature, as reflected by medial staining for nitrotyrosine, especially proximal to the endothelium and the adventitia. Deletion of mPGES-1 has a marked impact on such staining as well as on a systemic index of oxidant stress: urinary excretion of the most abundant F₂ isoprostane, 8,12-iso-PF₂ᵥ-VI. Although macrophage recruitment was unaltered by mPGES-1 deletion, the impact on oxidant stress may have reflected an alteration in their production of prostaglandins. Thus, concomitant with suppression of PGE₂, biosynthesis of both PGI₂ and PGD₂ is augmented in mice lacking mPGES-1. Both PGI₂ and PGD₂ have been implicated previously in restraining oxidant stress via their induction of antioxidant enzymes. Although Ang II increased formation of TxA₂, which has pro-oxidant properties, TxA₂, unlike PGI₂ and PGD₂, was not increased further consequent to mPGES-1 deletion during AAA formation.

Prostaglandin intermediates that accumulate after inhibition or deletion of synthases downstream of the COXs may be shuttled between cells to form transcellular products of functional importance. An example is the diversion of platelet PGH₂ substrate to endothelial PGI₂ synthase, which contributed to the functional effects of thromboxane synthase inhibitors. Evidence for substrate rediversion within cells after mPGES-1 deletion has been shown previously. The predominant products of rediversion vary according to cell type; thus, PGI₂ is by far the most abundant product in vascular smooth muscle cells, whereas both TxA₂ and PGI₂ may result from mPGES-1 deletion in macrophages. Rediversion of substrate to endothelial PGI₂ might also be relevant given the upregulation of COX-2 in both endothelial cells and smooth muscle cells by Ang II infusion in the present studies. Measurement of urinary metabolites reflects alterations in
systemic biosynthesis of prostanoids and does not permit direct assignation of cellular origin to a particular product. PGD₂ is a product of macrophage COXs, and this may account for its augmented formation in the present study. However, if so, it is surprising that thromboxane biosynthesis is not altered. A more intriguing possibility is that it derives from mast cells. PGD₂ is the predominant COX product in mast cells, and mast cells have previously been implicated in atherosclerotic plaque destabilization. Future studies will address the origin and the functional relevance of increased formation of PGD₂ in the attenuation of AAA consequent to mPGES-1 deletion.

MMPs are thought to be involved in AAA pathogenesis. Deletion of mPGES-1 impaired aortic MMP-2 activity (Figure IV in the online-only Data Supplement). This may be due to a direct effect of mPGES-1–derived PGE₂ and/or mast cells, and mast cells have previously been implicated in atherosclerotic plaque destabilization. Future studies will address the origin and the functional relevance of increased formation of PGD₂ in the attenuation of AAA consequent to mPGES-1 deletion.

In the present study, we provide evidence that deletion of mPGES-1 retards AAA formation and severity. This suggests the potential utility of mPGES-1 inhibitors in a condition presently not amenable to chemoprevention. Given that mPGES-1 deletion has a concomitant beneficial impact on atherogenesis, mPGES-1 inhibition may have particular value in the treatment of inflammatory syndromes in patients with established cardiovascular disease.

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Disclosures

Dr FitzGerald has served as a consultant to AstraZeneca, Biolipox, Daiichi, the Genome Institute of the Novartis Foundation, Lilly, Novartis, and Merck. The other authors report no conflicts.

References

Abdominal aortic aneurysm is an inflammatory disorder characterized by localized connective tissue degradation and smooth muscle cell apoptosis, leading to aortic dilatation and rupture. Abdominal aortic aneurysms represent a major cause of morbidity and mortality in humans. Many cases are undiagnosed until they declare clinically by leakage or rupture. Nonsurgical treatments that retard aneurysm development or induce its regression remain to be identified. Human aortic aneurysm biopsies stain strongly for cyclooxygenase-2 ex vivo, and some earlier studies provided evidence for the potential efficacy of nonsteroidal anti-inflammatory drugs, particularly those selective for inhibition of cyclooxygenase-2. However, cyclooxygenase-2-selective inhibitors themselves increase the risk of myocardial infarction, heart failure, and stroke. In the present study, we explored the potential utility of targeting microsomal prostaglandin (PG) E2 synthase-1 (mPGES-1), an anti-inflammatory drug target downstream of cyclooxygenase-2 and a major source of PGE2. We have shown previously that mPGES-1 reverts atherosclerosis; here gene deletion retarded formation of abdominal aortic aneurysm induced by an angiotensin II infusion in mice lacking low-density lipoprotein receptor. This occurred concomitant with suppression of aortic and systemic indices of oxidative stress, previously implicated in the pathogenesis of abdominal aortic aneurysm. Deletion of mPGES-1 inhibited production of PGE2 but also resulted in substrate redirevision to augment production of PGF2 and PDGβ2, both of which upregulate antioxidant enzymes and restrain oxidant stress. This study raises the possibility that inhibition of mPGES-1 might have therapeutic potential in this potentially fatal disease.
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