

EP Receptors and Coxibs Seeing the Light at the End of the Tunnel

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Few chemical compounds have demonstrated a controversial association with a specific disease such as arachidonic acid with atherothrombosis. Arachidonic acid metabolism is deeply linked to atherothrombosis because compounds generated by this cascade, the eicosanoids, are key regulators of several pathophysiological processes critically involved in vessel homeostasis and blood clotting. After its release from membrane-bound phospholipids, arachidonic acid is metabolized by 4 main pathways: (1) prostaglandin (PG) endoperoxidase synthase, usually referred as cyclooxygenase (COX); (2) lipoxygenase; (3) P450 epoxygenase; and (4) nonenzymatic isoprostane biosynthesis¹ (Figure 1). Among these pathways, cardiovascular research has mainly focused on COX-derived eicosanoids, usually called PGs. There are 2 main isoforms of COX, named COX-1 and COX-2, which are encoded by 2 separate genes. Whereas COX-1 is constitutively expressed in many tissues, the expression of COX-2 is induced by several proinflammatory cytokines and growth factors. In the vascular tree, COX-1 is expressed in endothelial cells and in vascular smooth muscle cells (VSMCs) both in healthy vessels and in atheromatous areas. On the contrary, COX-2 expression is evident in atherosclerotic regions, and several proatherosclerotic stimuli (ie, oxidized low-density lipoprotein, angiotensin II, advanced glycation elements)² as well as vascular injury³ may increase its expression. Notably, COX-2 is highly expressed in macrophages located in the shoulder region of atherosclerotic plaques, both in mice⁴ and in humans.⁵ In macrophages, the expression of COX-2 has predominantly proinflammatory and proatherosclerotic effects, and selective deletion of COX-2 in macrophages significantly hampers the progression of atherosclerosis in mice.^{4,6}

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Both COX isoforms catalyze the formation of PGH₂, an unstable molecule that is further metabolized by other synthases

and isomerases to produce different series of PGs (D, E, F, I) and thromboxane. Despite the first enzymatic step leading to PGH₂ production being showed by every cell type, the expression of downstream enzymes displays considerable cell-type specificity. In particular, the combined action of COX and PGE synthase (PGES) leads to the production of PGE₂ in macrophages. Three different isoforms of PGES have been identified, called cytosolic PGES and type 1/type 2 microsomal PGES (mPGES¹; Figure 1). Although cytosolic PGES is constitutively expressed, mPGES-1 is induced in response to inflammatory stimuli and frequently colocalizes with COX-2 in cell perinuclear membrane.⁷ It is noteworthy that evidence supports the view that the proatherogenic effects of COX-2 are attributable to PGE₂ production. Deletion of mPGES-1 in myeloid cells leads to a reduction of early atherosclerosis in mice.⁸ More interestingly, COX-2 and mPGES-1 colocalize in macrophages in the shoulder of atherosclerotic plaques, their expression is increased in atherosclerotic plaques from patients with recent ischemic stroke, and this promotes the release of proteinases involved in plaque destabilization⁹ (Figure 2).

Nevertheless, COX-2 also is expressed in VSMCs,⁹ together with the receptors for the main PGs, and may contribute to angiotensin II–induced hyperplasia and hypertrophy.¹⁰ Remarkably, VSMCs play a key role in the first steps of atherosclerosis, and their proliferation is a hallmark of neointimal hyperplasia and restenosis after stent implantation. Involvement of COX-2 and mPGES-1 in these processes has been confirmed by the observation that selective inhibition of both these enzymes results in reduced hyperplasia after vascular injury.^{3,8,11} Notably, these findings are in agreement with results from clinical trials in which treatment with selective COX-2 inhibitors reduced neointima proliferation after percutaneous coronary angioplasty and drug-eluting stent placement.^{12,13} Nevertheless, despite this beneficial effect on vessel restenosis, no effects (or perhaps an increase¹² in cardiovascular events, ie, myocardial infarction) have been found in these patients. Furthermore, several clinical and epidemiological studies have raised doubts about the cardiovascular safety of COX-2 inhibition in humans.^{2,14} One potential explanation for this may be the presence of off-target effects of COX-2 inhibition in other cells. In particular, COX-2 expression in endothelial cells and VSMCs is responsible for the production of prostacyclin (PGI₂),^{9,15} a key regulator of vascular homeostasis working as an inhibitor of leukocyte adhesion, platelet aggregation, and VSMC proliferation^{2,9} (Figure 2). Thus, the widespread inhibition of COX-2 may realize a dangerous disequilibrium in which the potential benefit of PGE₂ reduction is counterbalanced by reduction in PGI₂ synthesis. In this

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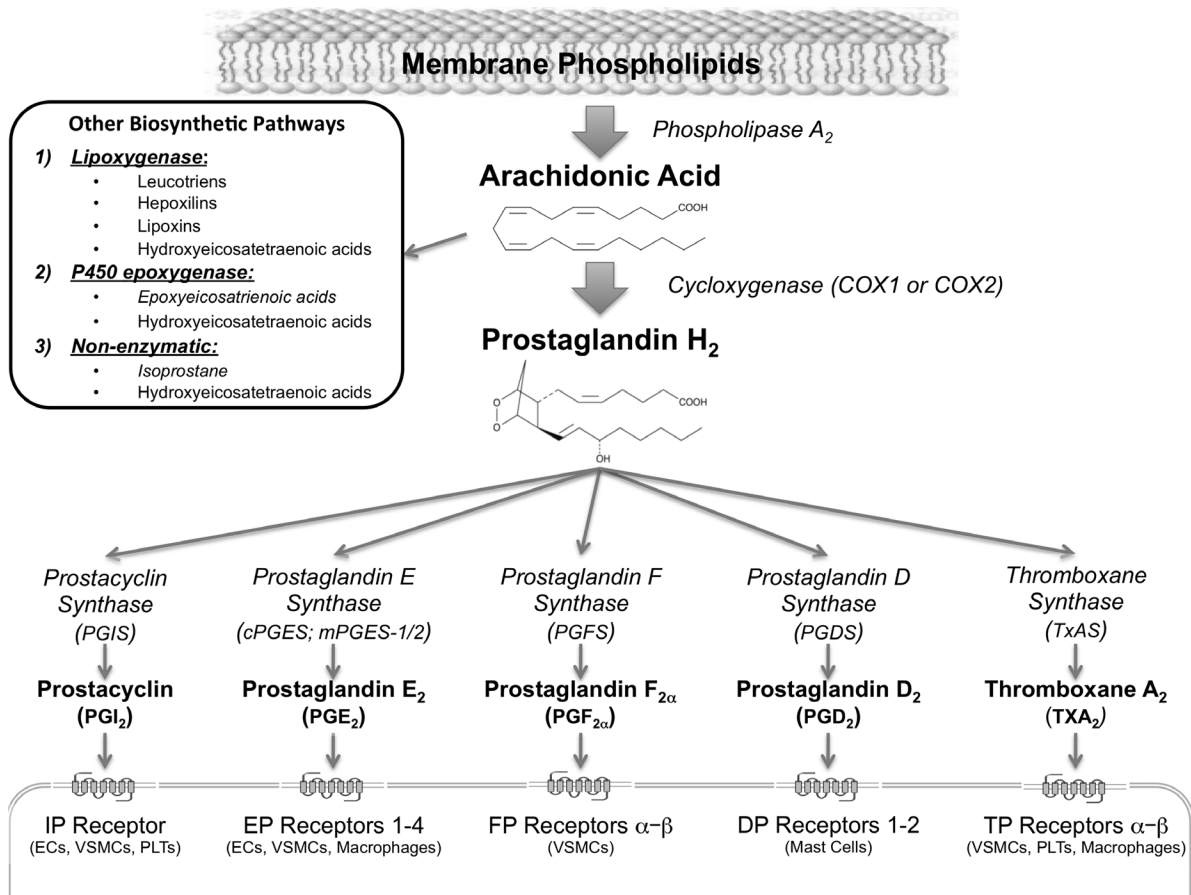


Figure 1. The cyclooxygenases pathway. ECs indicates endothelial cells; EP, PGE₂ receptor; DP, PGD₂ receptor; FP, PGF_{2α} receptor; IP, prostacyclin receptor; PLTs, platelets; TP, thromboxane receptor; and VSMCs, vascular smooth muscle cells.

light, the comprehension of the intimate mechanism(s) linking COX-2, PGE₂, and arterial restenosis clearly represents a priority to overcome this limitation and improve the selectivity of therapeutic modulation.

In this issue of *Circulation Research*, Zhang et al¹⁶ have carefully investigated the downstream pathway of COX-2 to better-define the molecular regulators of VSMC proliferation. First, they have genetically silenced COX-2 expression, confirming that its inhibition has beneficial effects on neointima

formation as reflected by lower luminal narrowing and reduction in intima-to-media ratio after vascular injury in COX-2 knockout mice. Then, to confirm that these beneficial effects were not merely a consequence of PGI₂ reduction (which is known to be involved in neointima formation)¹⁷ but in contrast were attributable to PGE₂ deficiency, they also have realized transgenic mice in which COX-2 is substituted by COX-1 but is under the transcriptional control of COX-2 regulatory elements. In these mice, the production of PGE₂ was restored

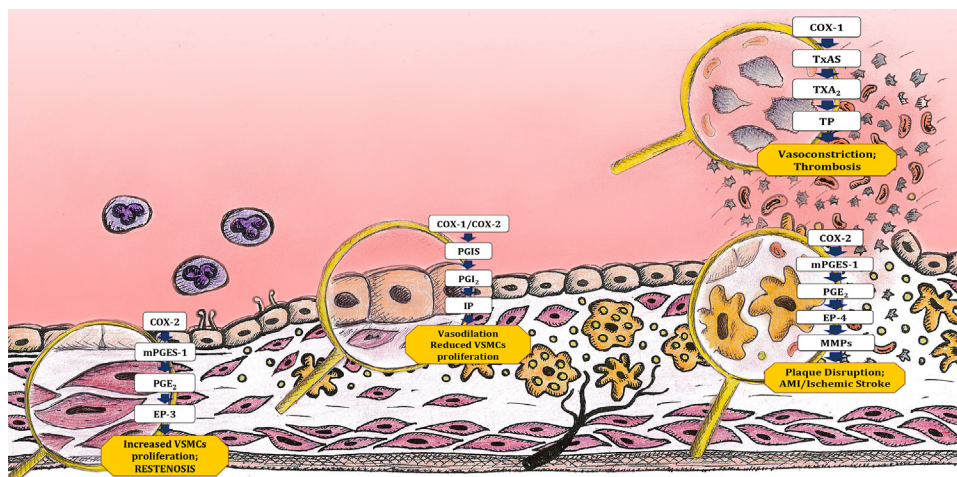


Figure 2. Role of cyclooxygenases and prostaglandins in the different cell types in atherosclerosis development and progression. AMI indicates acute myocardial infarction; COX, cyclooxygenase; EP, PGE₂ receptor; MMP, matrix metalloproteinases; mPGES, microsomal PGE₂ synthase; PG, prostaglandin; PGI₂, prostacyclin; PGIS, prostacyclin synthase; TxAS, thromboxane synthase; and VSMC, vascular smooth muscle cell.

as compared with COX-2 knockout mice, whereas no differences were found in PGI₂ metabolites (because their production was mainly COX-2-dependent).^{9,15} Interestingly, these mice showed an exaggerate hyperplastic neointimal response when compared with both COX-2 knockout and wild-type mice. Taken together, these results suggest that during COX-2 suppression, the beneficial effects of reducing PGE₂ synthesis in VSMCs and macrophages (recruitment is considerably increased after vascular injury) may greatly overlook the detrimental effects of PGI₂ inhibition and may lead to reduction of neointima. After confirming the important role of PGE₂ in restenosis, the authors also have examined in depth the tiny mechanism(s) leading to these effects. Now, it is well-known that PGE₂ (as well as the other PGs) is an autacoid that activates membrane receptors close to its site of synthesis, and its complex final effect depends on the activation of 4 different EP receptors.^{1,2} All these EP receptors are members of the G-protein-coupled receptor family and are expressed in VSMCs, where they mediate both vasodilation (EP2/EP4) and vasoconstriction (EP1/EP3).¹⁸ Thus, correctly, the authors have focused on the downstream signaling pathway of PGE₂ in VSMCs. To do that, they have selectively inhibited each PGE₂ receptor by both pharmacological tools and the RNA interference approach, thus demonstrating that only the blockage of EP3 receptor, in particular EP3 α and EP3 β subtypes, may result in loss of VSMCs polarization (with formation of randomly oriented lamellipodia) and migration. These in vitro findings also were confirmed by in vivo experiments, showing that EP3 knockout mice have a reduced rate of restenosis after wire injury, a condition that was reverted by lentiviral re-expression of EP3 α and EP3 β . Finally, the authors performed additional studies to demonstrate that activation of EP3 α and EP3 β leads to these effects by activation of small GTPases through both G α i/cAMP/PKA and G β γ /PI3K/Akt/GSK3 β pathways.

In conclusion, results from the study by Zhang et al¹⁶ may contribute to improving our knowledge about involvement of the COX-2 pathway in arterial restenosis and complete the puzzle of the coxibs saga. Furthermore, by identifying a new actor in the EP3 receptor for preventing in-stent restenosis, they point out a new target for future and more selective pharmacological therapies.

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Disclosures

None.

References

1. Buczynski MW, Dumlaio DS, Dennis EA. Thematic review series: proteomics. An integrated omics analysis of eicosanoid biology. *J Lipid Res*. 2009;50:1015–1038.

2. Santovito D, Mezzetti A, Cipollone F. Cyclooxygenase and prostaglandin synthases: roles in plaque stability and instability in humans. *Curr Opin Lipidol*. 2009;20:402–408.
3. Ogawa M, Suzuki J, Hirata Y, Nagai R, Isobe M. A critical role of COX-2 in the progression of neointimal formation after wire injury in mice. *Expert Opin Ther Targets*. 2009;13:505–511.
4. Burleigh ME, Babaev VR, Yancey PG, Major AS, McCaleb JL, Oates JA, Morrow JD, Fazio S, Linton MF. Cyclooxygenase-2 promotes early atherosclerotic lesion formation in ApoE-deficient and C57BL/6 mice. *J Mol Cell Cardiol*. 2005;39:443–452.
5. Cipollone F, Prontera C, Pini B, Marini M, Fazio M, De Cesare D, Iezzi A, Uccchino S, Boccoli G, Saba V, Chiarelli F, Cucurullo F, Mezzetti A. Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaque instability. *Circulation*. 2001;104:921–927.
6. Burleigh ME, Babaev VR, Oates JA, Harris RC, Gautam S, Riendeau D, Marnett LJ, Morrow JD, Fazio S, Linton MF. Cyclooxygenase-2 promotes early atherosclerotic lesion formation in LDL receptor-deficient mice. *Circulation*. 2002;105:1816–1823.
7. Matsumoto H, Naraba H, Murakami M, Kudo I, Yamaki K, Ueno A, Oh-ishi S. Concomitant induction of prostaglandin E₂ synthase with cyclooxygenase-2 leads to preferred production of prostaglandin E₂ over thromboxane and prostaglandin D₂ in lipopolysaccharide-stimulated rat peritoneal macrophages. *Biochem Biophys Res Commun*. 1997;230:110–114.
8. Chen L, Yang G, Xu X, Grant G, Lawson JA, Bohlooly-Y M, FitzGerald GA. Cell selective cardiovascular biology of microsomal prostaglandin E synthase-1. *Circulation*. 2013;127:233–243.
9. Yu Y, Ricciotti E, Scalia R, Tang SY, Grant G, Yu Z, Landesberg G, Crichton I, Wu W, Puré E, Funk CD, FitzGerald GA. Vascular COX-2 modulates blood pressure and thrombosis in mice. *Sci Transl Med*. 2012;4:132ra54.
10. Young W, Mahboubi K, Haider A, Li I, Ferreri NR. Cyclooxygenase-2 is required for tumor necrosis factor- α - and angiotensin II-mediated proliferation of vascular smooth muscle cells. *Circ Res*. 2000;86:906–914.
11. Yang HM, Kim HS, Park KW, You HJ, Jeon SI, Youn SW, Kim SH, Oh BH, Lee MM, Park YB, Walsh K. Celecoxib, a cyclooxygenase-2 inhibitor, reduces neointimal hyperplasia through inhibition of Akt signaling. *Circulation*. 2004;110:301–308.
12. Kang HJ, Oh IY, Chung JW, et al. Effects of celecoxib on restenosis after coronary intervention and evolution of atherosclerosis (Mini-COREA) trial: celecoxib, a double-edged sword for patients with angina. *Eur Heart J*. 2012;33:2653–2661.
13. Koo BK, Kim YS, Park KW, et al. Effect of celecoxib on restenosis after coronary angioplasty with a Taxus stent (COREA-TAXUS trial): an open-label randomised controlled study. *Lancet*. 2007;370:567–574.
14. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. *JAMA*. 2006;296:1633–1644.
15. Barbieri SS, Amadio P, Gianellini S, Tarantino E, Zacchi E, Veglia F, Howe LR, Weksler BB, Mussoni L, Tremoli E. Cyclooxygenase-2-derived prostacyclin regulates arterial thrombus formation by suppressing tissue factor in a sirtuin-1-dependent-manner. *Circulation*. 2012;126:1373–1384.
16. Zhang J, Zou F, Tang J, Zhang Q, Gong Y, Wang Q, Shen Y, Xiong L, Breyer RM, Lazarus M, Funk CD, Yu Y. Cyclooxygenase-2-derived prostaglandin E₂ promotes injury-induced vascular neointimal hyperplasia through the E-prostanoid 3 receptor. *Circ Res*. 2013;113:104–114.
17. Todaka T, Yokoyama C, Yanamoto H, Hashimoto N, Nagata I, Tsukahara T, Hara S, Hatae T, Morishita R, Aoki M, Ogihara T, Kaneda Y, Tanabe T. Gene transfer of human prostacyclin synthase prevents neointimal formation after carotid balloon injury in rats. *Stroke*. 1999;30:419–426.
18. Foudi N, Gomez I, Benyahia C, Longrois D, Norel X. Prostaglandin E₂ receptor subtypes in human blood and vascular cells. *Eur J Pharmacol*. 2012;695:1–6.

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