Prostacyclin in Vascular Diseases – Recent Insights and Future Perspectives –

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Prostacyclin (PGI₂) is one of the important vascular prostanoids, the effects of which counteract those of thromboxane (TXA₂), and these 2 prostanoids provide an important balance in cardiovascular homeostasis. The clinical experience of COX-2 selective inhibitors having unexpected adverse effects in patients with cardiovascular risk has opened up a debate about the role of COX-2-derived prostanoids in vascular pathophysiology. PGI₂ is a major anti-atherogenic prostanoid produced by COX-2 in vascular cells, including endothelial and vascular smooth muscle cells. The balance between COX-2-derived PGI₂, COX-1-derived TXA₂, and other COX-2-mediated atherogenic prostanoids is a crucial factor in determining pathophysiological outcomes. Recent studies using stable PGI₂ analogs and genetically deficient mice have revealed novel effects of PGI₂ on its target cells, such as endothelial and endothelial progenitor cells. The role PGI₂ in the physiology and pathophysiology of vascular diseases is reviewed and the recent findings linking PGI₂, COX-2 and atherothrombosis are summarized. (*Circ J* 2010; **74**: 836–843)

Key Words: Atherosclerosis; Cyclooxygenase; Endothelial progenitor cells; Prostacyclin

Prostanoids are family of bioactive lipid mediators that are formed by cyclooxygenase (COX) from arachidonic acid (AA) contained in the cell membrane of all cells of the body. They are involved in numerous physiological activities, including platelet aggregation, vasorelaxation and vasoconstriction, local inflammatory response and leukocyte–endothelial cell adhesion.^{1,2} In this context, prostanoids modulate the pathogenesis of vascular diseases, such as thrombosis and atherosclerosis, in which inflammation has an important role in all the phases of progression, from the initial formation of the early plaque to the rupture of advanced plaques.³ It was believed that inflammatory-inducible COX-2 might be an ideal anti-inflammatory therapeutic target, but the clinical experience of some COX-2 selective inhibitors causing unexpected adverse effects in patients with potential cardiovascular risk has opened the debate about the role of COX-2-derived prostanoids in vascular pathophysiology and the benefits and risk of using COX-2 inhibitors in cardiovascular diseases.

The role of COX-2 in atherothrombosis appears to be quite complex, as it is an intermediate enzyme in the cascade of AA and generates not only pro-inflammatory but also anti-inflammatory prostanoids, according to the type of co-expressed downstream prostanoid synthases in each tissue or cell type.^{4,5} It is recognized that prostacyclin (PGI₂) is a key player in the complicated COX–prostanoids cascade in cardiovascular diseases, because PGI₂ is a major anti-atherogenic prostanoid produced by COX-2, and counteracts platelet-

derived thromboxane (TXA₂) and other COX-2 mediated atherogenic prostanoids.^{6,7} Recent studies using stable PGI₂ analogs and genetically deficient mice have revealed the novel effects of PGI₂ on target cells, such as endothelial cells and endothelial progenitor cells (EPCs), which have crucial functions in the pathogenesis of atherosclerosis. Accordingly, we review the role PGI₂ in the physiology and pathophysiology of vascular diseases, summarizing the recent findings linking PGI₂, COX-2, and atherothrombosis.

Effects of PGI₂ Through Dual Signal Pathways in the Cardiovascular System

PGI₂ is thought to be one of the most important prostanoid in regulating the homeostasis of the cardiovascular system.^{6,7} Platelet precursors, megakaryocytes, as well as vascular smooth muscle cells (VSMCs) are the major target cells of PGI₂. PGI₂ is a potent vasodilator, and an inhibitor of platelet aggregation, leukocyte adhesion, and VSMC proliferation.^{1,2} These actions of PGI₂ are mediated through specific cell surface receptors, known as IP. IP is a 7-membrane spanning G-protein coupled receptor that generates cyclic AMP (cAMP) to mediate its physiological effects.⁸ Peroxisome proliferator activated receptors (PPARs), including α , δ and γ , are members of the superfamily of nuclear receptors with activities relevant to broad aspects of cardiovascular biology.^{9,10} Accumulating evidence has revealed that the effects of PGI₂ on vasculature are mediated by the PPAR δ pathway, in addition

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to the classical IP–cAMP signal pathway.¹¹

The role of PPAR δ in VSMCs is somewhat controversial. It has been shown that the expression of PPAR δ is enhanced in proliferative rat VSMCs, and overexpression of PPAR δ in VSMCs increased proliferation.¹² In contrast, activation of PPAR δ has induced cell cycle arrest in VSMCs and inhibited their proliferation and migration.¹³ Recent works suggest that PPAR δ activation by PGI₂ may induce endothelial proliferation and angiogenesis.^{14,15} PGI₂ also protected endothelial cells from H₂O₂-induced apoptosis through inducing PPAR δ -mediated 14-3-3 protein expression, which prevents the action of the pro-apoptotic factor, Bad.¹⁶ It should be noted that most of these effects were observed using stable PGI₂ analogs. PGI₂ analogs such as iloprost and carba-prostacyclin, but not cicaprost, act not only on IP receptors, but also on PPAR δ to induce proliferation and angiogenesis.¹⁴ However, more recently, Tsai et al reported that contractile phenotype modulation (differentiation) of human VSMCs was induced through the activation of PPAR α/δ by endogenous endothelial PGI₂.¹⁷

Role of PGI₂ in Atherosclerosis

PGI₂ may have a protective effect in the atherogenic process by limiting platelet activation, leukocyte adhesion to the endothelium and VSMCs proliferation in plaque.^{18,19} In accordance with this idea, a PGI₂ stable analog, beraprost, or PGI₂ synthase (PGIS) gene transfer inhibit neointimal formation in animal models of arterial injury.^{20,21} In a clinical study, the IP variant (R212C), which was defective in cAMP production, was closely linked to disease severity and adverse car-

diovascular events in patients with cardiovascular diseases.²² The cardiovascular effects of PGI₂ contrast with those of TXA₂, which causes platelet aggregation, vasoconstriction, and vascular proliferation.²³ Accordingly, PGI₂ and TXA₂ are thought to be two of the most important prostanoids in regulating the homeostasis of the cardiovascular system.^{6,7}

Mice models of genetic prostanoid-receptor deficient have given valuable information about the function of prostanoids in cardiovascular diseases. Although IP-deficient mice had normal blood pressure and matured normally without suffering from spontaneous thrombosis, platelet aggregation and VSMC proliferation in response to vascular injury were enhanced compared with control littermates.^{6,24} Mice lacking IP also had aggravated atherogenesis with enhanced platelet activation and increased adhesion of leukocytes on the vessel walls in both the low-density lipoprotein (LDL)-receptor model and apoE knockout models.^{25,26} These findings suggest that PGI₂ plays a significant role in atherosclerosis only in response to endothelial damage and not in the basal systemic circulation. In contrast to IP-deficient mice, mice lacking the TXA₂ specific receptor, TP, exhibited an increase in bleeding tendency and decrease in platelet aggregation, confirming the role of TXA₂ in those activities.²⁷ Because TXA₂ is a mitogen of VSMCs, TP-knockout mice have decreased VSMCs proliferation and platelet activation in response to vascular injury,⁶ and a significant delay in atherogenesis is induced, compared with mice deficient in apoE alone.²⁶ Those results indicate that TXA₂ promotes, whereas PGI₂ prevents, the initiation and progression of atherogenesis. Simultaneous deletion of both TP and IP abrogated both of the augmented responses to vascular injury, and the mice did not show any

difference in vascular remodeling compared with wild-type controls.⁶ Because of this contradictory action of PGI₂ and TXA₂, many aspects of cardiovascular disease have been explained by alterations in the balance between PGI₂ and TXA₂ during the interactions between platelets and the vessel wall (**Figure 1**).^{28,29}

Lessons From Clinical Experience With COX-2 Selective Inhibitors

It has been traditionally recognized that inducible COX-2 is responsible for prostanoid production associated with inflammation, and COX-1 is important for the prostanoids with “housekeeping” functions, such as gastric cytoprotection.^{1,2} The ability of classical non-steroidal anti-inflammatory drugs (NSAIDs), the non-selective COX inhibitors, to inhibit COX-2 activity may explain their therapeutic anti-inflammatory effects, whereas inhibition of COX-1 may account for some of the adverse effects such as gastrointestinal and renal toxicity. According to this simple hypothesis, therefore, COX-2 selective inhibitors (coxibs) were expected to be ideal anti-inflammatory drugs, minimizing gastrointestinal toxicity.³⁰ Furthermore, based on the hypothesis that atherosclerosis is an inflammatory disease, it was proposed that selective inhibition of COX-2 might have anti-atherogenic effects. However, clinical studies have indicated that there is an increased cardiovascular risk in individuals taking some of the coxibs.^{31,32}

Balance Between COX-1-Derived TXA₂ and COX-2-Derived PGI₂

The undesirable effects of coxibs have been explained by the TXA₂ and PGI₂ imbalance theory by which selective inhibition of COX-2 leads to a reduction in the production of the anti-atherogenic PGI₂ while production of the atherogenic TXA₂, mostly COX-1-dependent, remains unaffected,³³ although some studies argue about this theory (**Figure 1**).³⁴ This theory is based on the finding that endothelial PGI₂ production is mediated through COX-2 activation. PGI₂ is the predominant COX-2 product of the vascular endothelium, and administration of coxibs to healthy subjects reduced PGI₂ metabolites in urine without affecting TXA₂ metabolites.³⁵ Although COX-1 is dominantly expressed in endothelial cells under static conditions,^{36,37} COX-2 expression and COX-2-dependent PGI₂ production are increased in vitro by laminar shear stress, thrombin, oxidized LDLs or hypoxia.^{38–41} COX-2 has been reported in low levels, or not detected, in normal human arteries, and COX-2 appears predominantly expressed in endothelial cells overlying vascular lesions in the carotid, aortic, or coronary artery districts.^{36,37,42} Notably, PGIS gene transfer modulated COX-2-derived PGI₂ synthesis and inhibited neointimal formation in injured rat vascular walls, indicating that COX-2 is functionally linked with PGIS to produce PGI₂ and regulate vascular remodeling in the injured artery.⁴³ These findings suggest that COX-2 is a key isoenzyme for PGI₂ production, especially in vascular injury, competing with anti-atherogenic TXA₂ (**Figure 1**).

COX-2-Derived PGI₂ and Other Atherogenic Prostanoids in Atherosclerosis

The PGI₂ and TXA₂ imbalance theory shows the clear involvement of endothelial COX-2-derived PGI₂ in cardiovascular diseases. Nevertheless, the net effect of COX-2 expression and COX-2-derived prostanoids in the different phases of atherogenesis remains controversial. Adhesion of circulating

monocytes and macrophages to activated endothelial cells appears to be a critical early event in atherosclerotic lesions.⁴⁴ COX-2 is localized predominantly in macrophages within the plaque lesions,^{37,45} and increased COX-2 expression is paralleled by increased levels of PGE₂.^{45,46} PGE₂ is considered to be one of the most atherogenic prostanoids, mediating the progression of atherogenesis through several mechanisms, such as induction of other pro-inflammatory mediators and adhesion molecules, facilitating migration of macrophages and other immune cells.³⁶ More recently, it was reported that COX-2/PGE₂ in macrophages mediates the susceptibility of plaques toward rupture by weakening the fibrous cap through the upregulation and activation of matrix metalloproteinase.⁴⁷ In LDL-receptor deficient mice, COX-2 promoted early atherosclerotic lesion formation, and COX-2 inhibition reduced lesion formation.⁴⁸ These observations suggest that selective inhibition of COX-2 might attenuate atherogenesis by inhibiting the production of pro-inflammatory prostanoids.

In addition to a pro-atherogenic role of inflammatory COX-2, an atheroprotective role of endothelial COX-2/PGI₂ production has been proposed. COX-2 related PGI₂ synthesis by endothelial cells is increased by inflammatory cytokines,^{39,40} which also potentially induce COX-2 and PGE₂ synthesis in inflammatory cells.^{49,50} Therefore, endothelial COX-2 expression may represent a negative feedback mechanism triggered in part by pro-atherogenic/thrombotic stimuli by monocytes and macrophages (**Figure 2**). In atherosclerotic lesions, COX-2-derived PGI₂ production is observed in not only endothelial cells, but also other cells, such as VSMCs.⁵¹ Sphingosine-1-phosphate (S1P) is a bioactive lysophospholipid present in plasma, and platelets are one of the main sources of S1P.⁵² S1P induces PGI₂ formation through COX-2 expression in endothelial and VSMCs.⁵³ Interestingly, plasma S1P is accumulated in high-density lipoprotein (HDL). Accordingly, it is suggested that HDL-associated S1P is in part responsible for the beneficial anti-atherogenic effects of these lipoproteins through the upregulation of PGI₂ in vascular cells.⁵⁴ As regards COX-2-derived prostanoids, the balance of pro-atherogenic prostanoids, such as PGE₂, from inflammatory cells and anti-atherogenic PGI₂ from vascular cells could be a determinant factor in the pathogenesis of atherosclerosis (**Figure 2**). Indeed, PGI₂ synthesis from the human aorta has been shown to be decreased as a function of progressing atherosclerotic lesions, and PGE₂ increases in parallel.⁵⁵ Accordingly, the beneficial or unfavorable effect of coxibs in atherogenesis is dependent upon the balance between these COX-2-derived prostanoids.

Novel Role of PGI₂ in the Vasculature

Because of the fragility of prostanoids, it has been difficult to clarify their effects, especially in vivo. Stable PGI₂ analogs such as beraprost, iloprost, and cicaprost have been developed and they mimic the biological properties of PGI₂, such as activation of adenyl cyclase and increasing the intracellular cAMP level through the activation of IP.⁵⁶ Cicaprost is a unique PGI₂ analog that acts on the IP receptor only, whereas other PGI₂ analogs also induce activation of the IP-independent PPAR δ pathway (see earlier).¹¹ By using PGI₂/IP signal system-stimulating chemicals or techniques of genetic manipulation, such as knockout animals and small RNA interference, various novel actions of PGI₂ have been revealed.

Angiogenesis and PGI₂

Angiogenesis, which is controlled by the balance between pro- and anti-angiogenic factors, has a role in the pathogenesis of several disorders, including cancer, chronic inflammatory diseases and ischemic diseases. Previous studies reported the anti-angiogenic function of NSAIDs, which were mediated mainly through COX-2 inhibition.⁵⁷ Recently, there has been increasing evidence of the relationship existing between PGI₂ and angiogenesis.^{57,58} PGI₂ is considered a key molecule linking the up- and downstream effects of pro-angiogenic factors. It has been reported that PGI₂ induces the production of vascular endothelial growth factor (VEGF) in several cell types, including vascular cells,^{59–61} and also enhances the biological effects of pro-angiogenic factors.⁶² Transfection of the PGIS gene enhanced the efficiency of gene-transfection of pro-angiogenic factors, such as heparin growth factor (HGF), in animal models of peripheral ischemia.^{63,64} On the other hand, the synthesis of PGI₂ in several cell types, including endothelial cells, is increased by pro-angiogenic factors such as VEGF.^{65–67}

Effects of PGI₂ on Endothelial Cells

It is known that IP is expressed on endothelial cells, but the effects of PGI₂ on endothelial cells were not able to be clarified until stable PGI₂ analog were developed. Recently, there have been studies indicating that PGI₂ analogs have protective functions against endothelial damage. Beraprost

restored endothelial dysfunction in diabetic rats, accompanied by the induction of vascular HGF.⁶⁸ Endothelial nitric oxide synthase (eNOS) is an important regulatory enzyme for endothelial functions, including vascular tone and cellular protection against oxidative stress.⁶⁹ Interestingly, Niwano et al reported that beraprost increased the expression of eNOS through the cAMP pathway, proposing the existence of cross-talk between eNOS and PGI₂ within endothelial cells.^{70,71} Thus, the protective effects of PGI₂ on endothelial cells might be mediated in part by the formation of NO. On the other hands, the vasorelaxant effects of PGI₂ are widely considered to be endothelium-independent, via IP receptor-mediated cAMP accumulation in VSMCs. It has also been proposed that complex interactions between NO and cAMP in endothelial cells are involved in the endothelial-dependent vasorelaxant effects of PGI₂.

New Target Cells for PGI₂; Endothelial Progenitor Cells (EPCs)

Since EPCs were identified in 1997,⁷² a growing body of evidence has outlined the key roles of circulating EPCs in vascular repair^{73,74} and angiogenesis.^{75,76} Once the vascular wall is injured or tissues suffer from ischemia, EPCs are mobilized to the peripheral blood and target on those sites, differentiating into endothelial cells and/or producing angiogenic growth factors, participating in their effects. However, the mechanisms underlying EPC-mediated functions are still

not clearly understood. Recent evidence suggests that bone-marrow-derived mononuclear cells (MNCs) or EPCs may contribute to the angiogenic or anti-atherogenic effects of PGI₂. Beraprost increased capillary density in ischemic myocardium of rats, and attenuated infarct size, effects that were well associated with the increased number of EPCs in both the plasma and within the ischemic myocardium.⁷⁷ Administration of beraprost enhanced the efficacy of therapeutic angiogenesis induced by autologous implantation of bone-marrow-derived MNCs in rabbit ischemic hindlimb tissues.⁷⁸ Iloprost, a PGI₂ analog, increased the number of circulating EPCs in patients with critical limb ischemia.⁷⁹ It is proposed that these PGI₂ effects are mediated partly through the production of EPC-mobilizing factors. Indeed, dibutyl cAMP (DBcAMP), which accelerates vascularization in wound sites, induces the expression of VEGF and stromal-cell-derived factor, leading to the mobilization of EPCs from bone marrow.⁸⁰

More recently, we and other group have reported that PGI₂ has a direct effect on EPCs functions in an autocrine or paracrine manner, to mediate the beneficial effects of PGI₂ in angiogenesis and repairing vascular walls.^{81,82} The EPCs were IP-expressing cells among bone-marrow-derived MNCs, and in vitro EPC functions (eg, adhesion to extracellular matrix and migration) were tightly regulated by the PGI₂/IP pathway.⁸² In bone-marrow-transplanted mice, which lack IP selectively in bone marrow, EPCs were normally mobilized

into peripheral blood in response to vascular injury. However, recruitment of EPCs to injured vascular walls was impaired, and subsequent neointimal formation was apparently enhanced. This worsened vascular remodeling was rescued by transplantation of exogenous intact wild-type EPCs, but not by IP-deleted EPCs. Thus, PGI₂ influences EPC functions through the IP pathway. In contrast, He et al reported that intracellular PGI₂ could stimulate the pro-angiogenic activities of EPCs through the PPAR δ pathway, but not through that of IP.⁸¹ Inactivation of either COX or PGIS decreased tube formation and cell proliferation of EPCs, which were rescued by treatment with iloprost or a selective PPAR δ agonist, but not by the selective PGI₂ receptor, IP agonist, cicaprost.

As regard the anti-atherogenic effects of EPCs, it would be worthwhile knowing the function of EPCs as PGI₂-supplying cells. In mouse EPCs isolated from bone marrow, or human EPCs outgrown from peripheral blood MNCs, a high level of COX-1 expression was observed in vitro under basal conditions, and PGI₂ biosynthesis was mediated through COX-1 activation.^{81,82} Expression of COX-2 protein was almost undetectable, but increased after treatment with tumor necrosis factor- α , and the level of COX-2-derived PGI₂ by EPCs was apparently greater than that of human endothelial cells.⁸¹ Thus, it is proposed that recruited EPCs may play role as a PGI₂-supplying cells, instead of endothelial cells in the injured vascular walls, in which endothelium is damaged

or denuded from vascular walls (Figure 2). Alternatively, cytoprotective PGI₂ released by EPCs may rescue damaged endothelial cells, and accelerate the regeneration of new endothelium in atherosclerotic lesions (Figure 3).^{82,83} In particular, paracrine effects of cytokines released by EPCs have also been proposed as a mechanism underlying the vasoprotective effects of EPCs.^{84,85}

Summary and Future Direction

The clinical experience with coxibs promotes understanding of the role of COX-2-derived prostanoids in cardiovascular diseases. COX-2 contributes to the synthesis of not only pro-inflammatory prostanoids but also potent anti-atherogenic PGI₂, and the balance between PGI₂ and other atherogenic prostanoids is crucial for determining atherothrombotic pathological outcomes. In addition to endothelial cells, other vascular cells, including VSMCs and recruited EPCs, could be important sources of PGI₂ within atherosclerotic lesions.

Experimental modifications of the PGI₂/IP signal by genetic IP knockout or pharmacological agonist/antagonism have revealed the novel effects of PGI₂ on target cells, such as endothelial cells and EPCs. This growing body of evidence outlines the mechanisms of the vasculoprotective role of PGI₂, and effective clinical applications of PGI₂ to vascular diseases could be further introduced. Particularly, EPCs provide a potential approach to vascular repair and angiogenesis. However, under pathological conditions, such as diabetes and aging, the functional capacities of EPCs appear to be impaired.⁸⁶ In this regard, the regulatory role of PGI₂ in EPC functions could be a new therapeutic strategy for cardiovascular diseases.

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