



Prostacyclin: an inflammatory paradox

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Prostacyclin (PGI₂) is a member of the prostaglandin family of bioactive lipids. Its best-characterized role is in the cardiovascular system, where it is released by vascular endothelial cells, serving as a potent vasodilator and inhibitor of platelet aggregation. In recent years, prostacyclin (PGI₂) has also been shown to promote differentiation and inhibit proliferation in vascular smooth muscle cells. In addition to these well-described homeostatic roles within the cardiovascular system, prostacyclin (PGI₂) also plays an important role as an inflammatory mediator. In this review, we focus on the contribution of prostacyclin (PGI₂) as both a pathophysiological mediator and therapeutic agent in three major inflammatory-mediated disease processes, namely rheumatoid arthritis, where it promotes disease progression (“pro-inflammatory”), along with pulmonary vascular disease and atherosclerosis, where it inhibits disease progression (“anti-inflammatory”). The emerging role of prostacyclin (PGI₂) in this context provides new opportunities for understanding the complex molecular basis for inflammatory-related diseases, and insights into the development of current and future anti-inflammatory treatments.

Keywords: prostacyclin, IP receptor, inflammation, atherosclerosis, rheumatoid arthritis, pulmonary fibrosis

PHARMACOLOGY OF PROSTACYCLIN AND ITS RECEPTOR

Prostacyclin (PGI₂) is a member of the prostaglandin family of bioactive lipids, and is a derivative of the 20-carbon, omega-6 fatty acid, arachidonic acid (AA or 5,8,11,14-eicosatetraenoic acid). Both cyclooxygenase enzymes (COX-1 and COX-2) convert AA into the prostaglandin precursor PGH₂, which is subsequently synthesized into prostacyclin (PGI₂) via prostacyclin synthase (PGIS; **Figure 1**). However, the majority of PGI₂ produced *in vivo*, particularly within the systemic and pulmonary vasculature (Moncada et al., 1977; Catella-Lawson et al., 1999; McAdam et al., 1999), and other regions like the synovium (Brodie et al., 1980; Crofford et al., 1994), appears to be derived from COX-2. PGI₂ is unstable at physiological pH and, thus, has a very short half-life *in vivo* (<2 min), rapidly forming the inactive hydration product 6-keto-prostaglandin F1 α (6-keto-PGF_{1 α} ; Lewis and Dollery, 1983; Smyth and FitzGerald, 2002). The actions of PGI₂ are mediated through a seven-transmembrane-spanning G-protein coupled receptor (GPCR), referred to as the IP receptor (International Union of Pharmacology nomenclature). The IP receptor is a Class A rhodopsin-like GPCR that couples predominantly to the Gs subunit of the heterotrimeric G-protein and mediates intracellular signaling via adenylyl cyclase (AC) activation and cyclic AMP (cAMP) production (Boie et al., 1994). Animal studies have also shown that PGI₂ may also signal through alternate Gq- and Gi-related pathways (Lawler et al., 2001), as well as nuclear receptor-mediated pathways, such as the peroxisome proliferator activated receptor gamma (PPAR δ) pathway (Lim and Dey, 2002). Stitham et al. (2003) have elucidated the putative binding pocket for the human IP receptor, which has been reported to also accommodate type E prostanoids (i.e., PGE₁ and PGE₂) in addition to its native ligand PGI₂ and its analogs (Boie et al., 1994; Nakagawa et al., 1994). The physiological effects of PGI₂ are vast with much remaining to be uncovered. Within the vasculature, PGI₂ serves as a potent vasodilator and is the major inhibitory prostanoid in platelet aggregation (Smyth et al., 2009), and has also been shown

to inhibit vascular smooth muscle cell (VSMC) proliferation and de-differentiation (Fetalvero et al., 2006, 2007). Within the lungs, PGI₂ reduces pulmonary blood pressure as well as bronchial hyper-responsiveness (Idzko et al., 2007). Within the kidneys, PGI₂ serves to regulate renal blood flow and glomerular filtration rate, as well as mediates the release of renin (Komhoff et al., 1998). In the nervous system, PGI₂ has been shown to elicit nociceptive pain response (Murata et al., 1997).

PROSTACYCLIN AS AN INFLAMMATORY MEDIATOR

As described, prostacyclin (PGI₂) is best known for its regulatory role within the cardiovascular system, where it promotes VSMC relaxation (vasodilatation) and inhibits platelet aggregation (anti-thrombotic). However, it is also an important inflammatory mediator. The seminal work by Vane (1971) demonstrating the inhibition of prostaglandin biosynthesis as the mechanism of action for aspirin (acetylsalicylic acid) and other aspirin-like drugs first highlighted the importance of the prostaglandin family of molecules, and set the stage for the development of many pharmacologic agents, such as traditional, non-selective non-steroidal anti-inflammatory drugs (tNSAIDs) and the newer selective COX-2 inhibitors. Further work by Davies et al. (1984) pinpointed particular prostaglandins, principally prostaglandin E₂ (PGE₂) and prostacyclin (PGI₂), in the mediation of vascular permeability associated with the hyperemia and edema seen with acute inflammation. Murata et al. (1997) demonstrated the involvement of prostacyclin (PGI₂)-mediated inflammatory swelling *in vivo*, using prostacyclin receptor deficient (IP^{-/-}) mice. In these critical experiments, it was shown that mice lacking the prostacyclin receptor had a reduced inflammatory response, as measured by percent change in vascular permeability using a carrageenan-induced paw-edema model (Murata et al., 1997). Limb edema was decreased by approximately 50% in IP-deficient mice, similar to levels seen in mice pre-treated with the non-steroidal anti-inflammatory agent indomethacin. Moreover, a significant

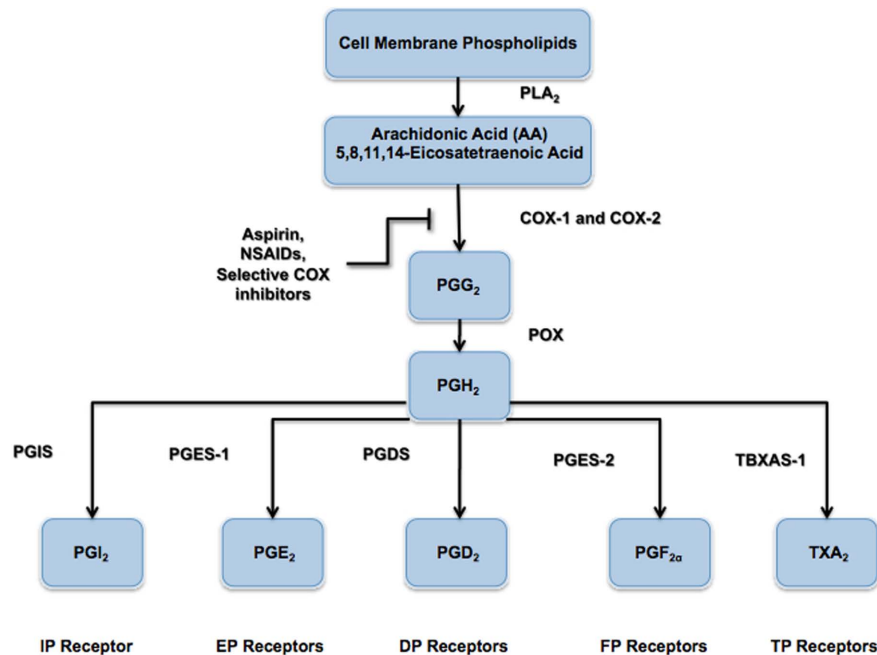


FIGURE 1 | Prostanoid biosynthesis pathway. The enzyme phospholipase A2 (PLA2) hydrolyzes arachidonic acid (AA) from the phospholipids of the extracellular membrane. Arachidonic acid is modified by the cyclooxygenase (COX) enzymes (COX-1 and COX-2) to form the intermediate precursor prostaglandin G₂ (PGG₂) via the addition of two oxygen (O₂) molecules. Prostaglandin H₂ (PGH₂) is subsequently formed by the actions of peroxidase

enzyme, which releases a single oxygen (O₂) molecule. As shown, all prostanoids are derived from the parent compound PGH₂ and are formed via their respective synthase enzymes, namely prostaglandin I₂ synthase (PGIS), prostaglandin E₂ synthase (PGES-1), prostaglandin D₂ synthase (PGDS), prostaglandin F_{2α} synthase (PGES-2), and thromboxane A₂ synthase (TBXAS-1).

reduction in lung exudate volume, using a carrageenan-induced pleurisy model, was also observed (although data not shown) for IP-deficient mice as well (Murata et al., 1997). In contrast, a study by Takahashi et al. (2002) demonstrated the IP-deficient mice showed higher skin and airway immune responses (relating to increased capillary permeability in these tissues) in antigen-sensitized inflammation, suggesting a protective role for PGI₂ in allergic inflammation. These studies (among others) have highlighted prostacyclin as a major endogenous mediator of inflammation – both pro-inflammatory and anti-inflammatory, depending upon the tissue and pathological model being studied (Figure 2).

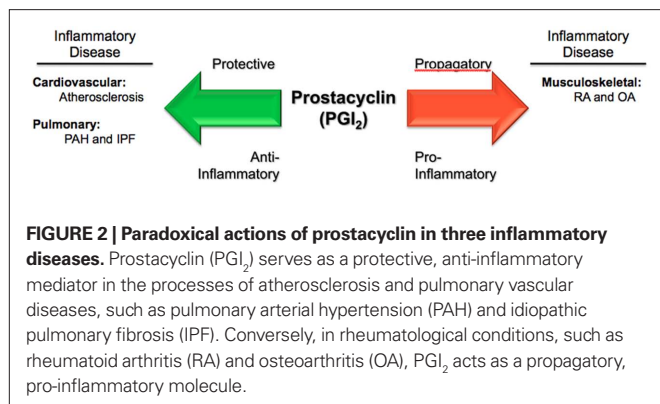
ROLE OF PROSTACYCLIN IN ARTHRITIS

While the majority of focus has centered around the role of prostaglandin E₂ (PGE₂) in rheumatoid arthritis (RA), some studies have shown that the predominate prostaglandin detected within the synovial fluid of patients with RA is in fact prostacyclin (PGI₂; Brodie et al., 1980). Moreover, using both collagen-induced arthritis and collagen-antibody-induced arthritis models, Honda et al. (2006) showed that prostacyclin receptor knockout (IP^{-/-}) mice exhibited significantly reduced clinical and histological arthritic scores versus control mice, in both arthritis models, placing further emphasis on receptor-mediated prostacyclin activity in the pathogenesis of RA. Using a K/BxN serum-transfer arthritis model, Chen et al. (2008) administered serum from arthritic K/BxN mice to induce an IgG-mediated autoantibody-induced inflammatory arthritis to recipient mice lacking either prostaglandin E synthase-1 (mPGES-1^{-/-})

or the prostacyclin receptor (IP^{-/-}), in order to determine the relative importance of PGE₂ and PGI₂, respectively. Findings revealed that mice deficient in prostaglandin E synthase-1 (mPGES-1) (and therefore unable to produce PGE₂), developed arthritis in normal fashion, whereas mice lacking the receptor for PGI₂ demonstrated a significant decrease (31% versus wild-type) in clinical arthritis. Furthermore, using COX-1 and COX-2 knockout animals, it was shown that mice lacking COX-1 isoform were resistant to the development of arthritic disease, while those lacking the COX-2 isoform remained vulnerable (Wang et al., 2008).

These results convey two important points: (1) a substantial proportion of the prostanoid contribution to joint inflammation (at least in the K/BxN serum-transfer arthritis model) can be accounted for by PGI₂ and its interaction with the IP receptor, and (2) selective COX-1 inhibition through genetic knockout prevented the development of disease, suggesting that COX-1-derived PGI₂ is the major inflammatory mediator within this arthritis model (Wang et al., 2008). More importantly, such studies lend weight to the involvement of prostacyclin in chronic inflammatory disease processes, as well as being an acute mediator, and also call into question the paradigm regarding COX-1- and COX-2-derived prostaglandin functions *in vivo* (i.e., regulatory “housekeeping” versus inflammatory induction).

Interestingly, from the perspective of clinical therapies, there does not seem to be a difference in efficacy according to COX selectivity. As Chen et al. (2008) showed, meta-analysis from a systematic review of 145 randomized controlled trials, examining the clinical



effectiveness of a variety of COX-2 inhibitors (including etodolac, meloxicam, celecoxib, rofecoxib, etoricoxib, valdecoxib, and lumiracoxib), showed similar efficacy compared to non-selective NSAIDs (including naproxen, diclofenac, ibuprofen, loxoprofen, nabumetone, piroxicam, indomethacin, tenoxicam, and nimesulide) in the symptomatic relief of both RA and osteoarthritis (OA), but with superior gastrointestinal tolerability and protection against complicated upper gastrointestinal events (e.g., ulcers, bleeding, perforations) – the majority of evidence coming from OA populations. However, the amount of evidence for this gastro-protective effect varied considerably across individual drugs. Moreover, an increased risk of myocardial infarction (MI) was also observed among those drugs with greater volume of evidence in terms of exposure in patient-years (Chen et al., 2008), presumably as a by-product of the discriminating suppression of COX-2-derived PGI₂, which has been shown to be cardioprotective (Murata et al., 1997; Cheng et al., 2002; Egan et al., 2004). Along these same lines, as chronic inflammation has been linked to enhanced development of atherogenesis (Libby et al., 2002), individuals with RA may already be at increased risk for cardiovascular disease. In fact, a recent comparative study involving disease-duration-matched RA and diabetes mellitus (DM) patients found that RA was a substantial and independent cardiovascular risk factor, with similar severity to DM, with significantly worsened preclinical atherosclerotic markers, increased intima-to-media thickness, as well as lower flow-mediated dilatation (measure of endothelial function; Stamatelopoulos et al., 2009). Thus, the combination of chronic RA-mediated inflammation, perpetuating an increased atherosclerotic burden, along with COX inhibition therapy – either selective (COX-2 inhibitor) or non-selective (NSAIDs) – would undoubtedly increase cardiovascular risk drastically, given what we now know about PGI₂. Thus, PGI₂ is emerging as an important intermediary in inflammatory conditions such as rheumatoid and OA, with the dualistic purpose of pro-inflammatory mediator on the one hand – involved in disease pathophysiology – and cardioprotective factor on the other – both of which are central in the consideration of pharmacologic therapies and adverse effects.

ROLE OF PROSTACYCLIN IN PULMONARY FIBROSIS AND PULMONARY HYPERTENSION

While pulmonary fibrosis and pulmonary hypertension are distinct pathophysiological entities, they do share some commonalities, some of which involve PGI₂. Pulmonary arterial hypertension

(PAH) is in fact a heterogeneous group of diseases sharing similarities in pathophysiological mechanisms, clinical presentation, and therapeutic approaches (Simonneau et al., 2009). The pathogenesis of PAH is complex and incompletely understood, with both genetic and environmental factors contributing to altered vascular structure and function (Badesch et al., 2007). The main vascular changes in PAH are vasoconstriction, VSMC proliferation, endothelial loss or dysfunction, and thrombosis (Farber and Loscalzo, 2004), which implicates a disruption of vascular hemostasis and its principle mediators, particularly PGI₂ and TxA₂, among others. This is evidenced by findings in patients with PAH, whereby both the production of prostacyclin synthase (PGIS) within small-to-medium pulmonary arteries, as well as urinary metabolites (6-keto-prostacyclin F2α) of prostacyclin, were shown to be decreased, while levels of thromboxane metabolites (thromboxane B2) were increased (Christman et al., 1992; Tuder et al., 1999). Interestingly, this imbalance of PGI₂ and TxA₂ within the pulmonary vasculature, leading to increased mean pulmonary artery pressure, mimics that of the cardio-systemic vasculature system. In fact, many of the pathophysiological mechanisms identified in PAH overlap with those involved in atherogenesis, including vascular smooth muscle and endothelial cell dysfunction, enhanced platelet activity and thrombosis, inflammation, and cellular chemotaxis (Essop, 2010). Owing to its potent vasodilatory, anti-thrombotic, and anti-proliferative effects, PGI₂ has secured a central role in the treatment of PAH. Continuous intravenous epoprostenol (synthetic PGI₂) is the best-studied advanced therapy for PAH and remains a first-line agent, particularly for those with severe disease (WHO functional class IV), as it has been shown to improve overall symptoms, exercise capacity, and hemodynamic status in controlled clinical trials (Barst et al., 2009), as well as confer a survival benefit in both idiopathic and heritable forms of PAH (IPAH and HPAH; Barst et al., 2009). There are limitations to treatment with epoprostenol based upon its pharmacology (plasma half-life = 3–5 min) and long-term use requires a permanent central venous catheter and a portable infusion pump. Analogs of PGI₂ have also been used clinically, and are administered by a variety of routes, including intravenously (e.g., treprostinil and iloprost), subcutaneously (e.g., treprostinil), inhalation (e.g., iloprost), and orally (e.g., beraprost). These medications are generally more stable with longer half-lives, but have variable safety and efficacy equivalencies compared to epoprostenol, and clinical trials with these (and other) alternative agents are limited with respect to severe disease classification (Barst et al., 1996). Other vasoactive therapies for PAH include the dual endothelin receptor (ET_A and ET_B) antagonist (e.g., bosentan) and phosphodiesterase type-5 (PDE-5) inhibitors, which have been proven effective, alone or in combination therapy, in milder forms of PAH (WHO functional class II and III; Rubin et al., 2002; Sitbon et al., 2003; Galie et al., 2005; McLaughlin et al., 2005; Pepke-Zaba et al., 2008). Again, the central role of PGI₂, as both an inflammatory and hemodynamic mediator, puts it at the forefront in understanding the pathophysiology and pharmacological treatment of pulmonary vascular diseases, particularly PAH.

Interestingly, PGI₂ seems to have a similar safeguarding effect in the chronic inflammatory condition of idiopathic pulmonary fibrosis (IPF), as recent studies have shown that COX-2-derived PGI₂ serves a protective role against bleomycin-induced pulmonary fibrosis – a

major animal model for IPF that mimics the progressive fibrosis and interstitial inflammation of sub-pleural lung tissue in humans. In a study by Lovgren et al. (2006), IP-deficient mice were more susceptible to bleomycin-induced pulmonary fibrosis, demonstrating increased collagen deposition and cellularity after bleomycin administration compared with the wild-type mice. These observations correlated with increases in quantitative measurements of histological lung scores and hydroxyproline levels within the lung parenchyma. Similar results were found using COX-2-deficient mice, but were not supported using knockouts for either the EP2 or EP4 receptors, which bind COX-2-derived prostaglandin E₂ (PGE₂; Lovgren et al., 2006). Such findings put direct focus on the loss of COX-2-derived prostacyclin as a protective factor. While COX-2-derived prostacyclin is now well known for its protective effects within the cardiovascular system, such results provide compelling evidence for PGI₂-mediated protection against fibrotic pathologies as well.

A more recent animal study by Zhu et al. (2010) also confirms these findings, pharmacologically, and identifies PGI₂ as a potential new therapeutic agent for pulmonary fibrotic disease. Using intra-peritoneal injections of iloprost, a stable PGI₂ analog, it was demonstrated that a single dose of iloprost (200 µg/kg; prior to bleomycin injection) could preclude pulmonary inflammation and fibrosis in mice (Zhu et al., 2010). Pre-treatment with iloprost seemed to significantly reduce both inflammatory infiltration and collagen deposition with the pulmonary interstitium, as well as improve lung mechanics (reduced static compliance and elevated tissue elastance; Zhu et al., 2010). However, the specific inflammatory cell subtype being suppressed could not be delineated in this current study. Iloprost pre-treatment decreased production of pro-inflammatory and fibrotic cytokines, such as TNF-α, IL-6, and TGF-β-1, and increased release of anti-fibrotic mediators, including IFN-γ and chemokine CXCL10/IP-10, as measured by mRNA expression levels or ELISA. Moreover, the cumulative mortality in iloprost-treated mice was 10% at day 21 versus 60% in the non-iloprost-treated cohort (Zhu et al., 2010).

In human studies, inhaled iloprost has been proven efficacious in the treatment of various forms of pulmonary hypertension, including pulmonary hypertension secondary to pulmonary fibrosis (Olschewski et al., 1999). In fact, it has been suggested that the majority of vascular resistance in fibrotic lung disease is due to persistent vasoconstriction (Olschewski et al., 1999), which may explain the effectiveness of PGI₂ analog therapy. Certain studies also suggest a long-term clinical benefit from continued therapy with inhaled iloprost, which was well tolerated and required no substantial dose increase over a 2-year trial (Olschewski et al., 2010). In the United States, its use has been approved for PAH New York Heart Association (NYHA) functional class III and IV; for individuals with marked limitations or inability to carry on physical activity (Gomberg-Maitland and Olschewski, 2008). Thus, PGI₂ seems to be an important effector in both these fibro-proliferative disorders of the lung, playing both a protective role against disease development, as well as a therapeutic role in symptom management.

ROLE OF PROSTACYCLIN IN ATHEROSCLEROSIS

Atherosclerosis is now known as an inflammatory disease, with the same complex cellular interactions involving monocytes, macrophages, lymphocytes, extracellular matrix (ECM) components, and

connective tissue cells as seen in other chronic inflammatory and fibro-proliferative diseases (e.g., RA, pulmonary fibrosis, glomerulosclerosis; Ross, 1999). The critical role of PGI₂ in atherosclerosis is quickly emerging, with evidence spanning from molecular and cell biology to human clinical trials. Mounting data has demonstrated the protective effect of prostacyclin activity against the development of atherothrombotic cardiovascular disease through the inhibition of various cellular processes, including platelet activation, leukocyte adhesion, as well as VSMC modulation. As such, PGI₂ analogs (e.g., iloprost) are able to down-regulate lymphocyte adhesion to endothelial cells, which suggests an ability to block the early events in atherosclerosis (Della Bella et al., 2001). Furthermore, interactions within the realm of lipid metabolism has lent further support toward the atheroprotective properties of PGI₂ (Thiemermann, 1991). Specifically, HDL has been shown to induce COX-2 expression and PGI₂ production in both endothelial and VSMCs (Pomerantz et al., 1985; Vinals et al., 1997, 1999) while, conversely, PGI₂ has been shown to induce cholesterol ester hydrolase activity, which catalyzes the first step in the removal of cholesterol from foam cells, critical components in atherogenesis (Hajjar and Weksler, 1983; Weksler et al., 1983; Hajjar et al., 1989). The effects of PGI₂ on VSMCs are becoming an important target for understanding both the pathophysiology of atherothrombosis and the atheroprotective effects of prostacyclin. In mature blood vessels, VSMCs are quiescent and exhibit a differentiated, contractile phenotype. However, in response to vascular injury, these cells have been shown to re-enter the cell cycle, proliferate, migrate toward attractants, down-regulate expression of contractile proteins, and up-regulate synthesis of proteins, particularly ECM (Campbell et al., 1988). PGI₂ has been shown to exert both anti-proliferative (Grosser et al., 1995; Zucker et al., 1998) and anti-migratory (Blindt et al., 2002) effects on smooth muscle cells. In advanced atherosclerotic lesions (as well as restenotic lesions), expression levels of smooth-muscle-specific differentiation markers are markedly reduced (Wilcox, 1992; O'Brien et al., 1993), and as Fetalvero et al. (2006) have shown, treatment with the stable PGI₂ analog, iloprost, induces VSMC differentiation via a cAMP-PKA-mediated signaling pathway. In similar fashion, Kasza et al. (2009) went on to further show that, in addition to increased contractile protein expression and contractile morphology, iloprost-treated VSMCs up-regulate COX-2 expression, mediated not only by cAMP-PKA, but also novel pathways involving ERK-1/2 activation and Akt-1 inhibition. In turn, the up-regulated COX-2 expression lead to subsequent PGI₂ release (i.e., prostacyclin-induced prostacyclin release), which was shown to have a paracrine, positive-feedback effect on neighboring VSMCs (not exposed to iloprost), inducing similar cellular responses (Kasza et al., 2009). Thus, there appears to be a clear link between the major atheroprotective effects of prostacyclin and VSMC modulation. As such, the phenotypic change in VSMCs toward a proliferative and de-differentiated state, which is a hallmark occurrence in the progression of atherosclerosis and restenosis, necessitates a clear understanding of this regulatory process and is an extremely important area of research.

Mouse models have provided valuable insight into the role of prostacyclin in cardiovascular homeostasis and pathogenesis. IP-deficient (IP^{-/-}) mice display increased propensities toward thrombosis (Murata et al., 1997), intimal hyperplasia

and restenosis (Cheng et al., 2002), and reperfusion injury (Xiao et al., 2001). Moreover, in both atherogenic apolipoprotein E (apo-E)- and low-density lipoprotein receptor (LDL-R)-deficient backgrounds, mice lacking the IP receptor demonstrated greater atherosclerotic burden with higher platelet reactivity and leukocyte adhesion to endothelial cells (Egan et al., 2004; Kobayashi et al., 2004). Moreover, Egan et al. (2004) went on to further demonstrate that, in female pre-menopausal LDL-R knockout mice, the atheroprotective effect of estrogen is significantly reduced in the absence of the IP receptor (i.e., double LDL-R and IP knockout). Moreover, as long-term estrogen exposure was shown to increase COX-2 expression, as well as the formation of the PGI₂ metabolite 6-keto-PGF_{1α} in mouse aortic smooth muscle cells (mASMCs), this study suggests a significant contribution of estrogen-mediated, COX-2-derived PGI₂ in the protection against atherogenesis (Egan et al., 2004). Such *in vivo* findings highlight the important functional presence of prostacyclin activity in the maintenance of cardiovascular homeostasis and, in turn, implicate receptor-ligand (hIP-PGI₂) dysfunction in the acceleration of atherogenesis and the subsequent development of related disorders, including stroke, MI, and hypertension. In humans, the importance of PGI₂ in atherogenesis has also been shown. Arehart et al. (2008) demonstrated that patients harboring a dysfunctional human prostacyclin receptor variant (R212C) exhibited an enhanced atherothrombotic phenotype, with a higher incidence of triple-vessel coronary artery disease (CAD) and greater number of clinical cardiovascular events (including MI, stroke, PTCA, CABG, PVD, and unstable angina) in high-risk patients versus age- and risk-factor-matched normal-allele patients. Another study by Patrignani et al. (2008) correlated the R212C prostacyclin receptor polymorphism, as well as two other synonymous variants, with intimal hyperplasia and progressive deep venous thrombosis, respectively. Biochemical analyses have revealed potential mechanistic explanations for these R212C disease association studies. Ibrahim et al. (2010) demonstrated that co-expression of the R212C variant, together with wild-type hIP, resulted in dominant-negative inhibition of signaling through receptor homo-dimerization, with enhanced wild-type IP localization to the endoplasmic reticulum. Interestingly, a similar effect was observed with co-expression of the R212C variant and the wild-type thromboxane receptor α (TPα), which revealed R212C hetero-dimerization, with subsequent inhibition of TPα receptor activity (Ibrahim et al., 2010). Since this time, Stitham, Arehart, and colleagues have identified other functionally deficient prostacyclin receptor polymorphisms (i.e., R215C and L104R) associated with increased CAD (Ibrahim et al., 2010). While such mutations are rare, it is notable that single, heterozygous, point mutations within the prostacyclin receptor gene (*PTGIR*) are associated with clinically significant disease changes.

Similarly, mutations and polymorphisms in the prostacyclin synthase (*PGIS*) gene have been associated with essential hypertension, MI, and cerebral infarction (Iwai et al., 1999; Nakayama, 2005). The sum of these results correspond not only with the aforementioned IP knockout mice studies, but also parallel findings from the world-wide withdrawal of the selective COX-2 inhibitors (e.g., rofecoxib and valdecoxib) whose discriminating suppression of COX-2-derived prostacyclin (PGI₂) resulted in increased risk of cardiovascular events (e.g., MI and thrombotic stroke), particularly in

predisposed patients (Fitzgerald, 2004; White et al., 2004; Bresalier et al., 2005). These latter findings relating adverse cardiovascular events to unmatched suppression of COX-2-derived PGI₂ have been widely discussed and demonstrate that a disrupted balance between PGI₂ and TxA₂ – favoring unopposed COX-1-derived TxA₂ production – is responsible, at least in fair part, for pro-thrombotic and perhaps pro-atherogenic effects (McAdam et al., 1999; Vane, 2002). In a recent opinion article, Rovati et al. (2010) proposed that concomitant TP receptor antagonism, along with selective COX-2 inhibition (dual COXIB-TP antagonists), may abrogate such adverse cardiovascular events (caused by the imbalance between PGI₂ and TxA₂) and improve the safety profile of selective COX-2 inhibitors. Other groups have proposed this concept as well, but clinical trials have yet to be pursued. As another approach, Capone et al. (2010) suggest assessment of COX-2 activity in whole blood *ex vivo*, perhaps in combination with biomarkers – such as biochemical (urinary levels of 6-keto-PGF_{1α}) and genetic (IP receptor and other prostacyclin-related polymorphisms) – as potential surrogate endpoints to assess for prostacyclin synthesis *in vivo* as a predictor of cardiovascular risk.

NOVEL PROSTACYCLIN-RELATED THERAPIES IN INFLAMMATION

As the role of PGI₂ is becoming more defined in inflammatory-related diseases, the development of novel agonists and antagonists for the IP receptor is at the forefront of research. As previously stated, the role of PGI₂ in arthritic diseases (RA and OA) is one of a pro-inflammatory mediator, and the use of wide-ranging inhibitors of prostaglandin synthesis (NSAIDs and selective COX-2 inhibitors) has remained a mainstay of therapy. However, targeted antagonism of PGI₂ activity has proven effective in reducing pain and inflammation in preclinical trials. Using a mono-iodoacetate (MIA)-induced rodent model of chronic OA, Pulichino et al. (2006) have shown that a novel arylamide compound (Keitz et al., 2004) with specific IP receptor antagonism significantly reduced joint discomfort in a dose-dependent manner, and with similar efficacy to diclofenac as well as an MF-tricyclic COX-2 inhibitor. Furthermore, in a collagen-antibody-induced model of RA, the same IP antagonist reduced mean scores for all arthritic parameters by 93% (AUC of the clinical scores) when given in prophylactic mode 1 day prior to collagen antibody injection, in IP+/- mice (Pulichino et al., 2006). Comparably, a 91% reduction in arthritic scores was observed for IP-/- mice and a 98% reduction for COX-2-treated mice. Interestingly, treatment in therapeutic mode (6 days post-injection) had no effect on clinical scores (Pulichino et al., 2006). However, as discussed earlier, targeted PGI₂ antagonism has the potential for increased risk of adverse cardiovascular effects, which may be even more pronounced than with broader-spectrum NSAIDs or selective COX-2 inhibitor therapies.

For the treatment of pulmonary vascular disease such as PAH and IPF, the vasoactive PGI₂ analog formulations (e.g., epoprostenol, treprostinil, and iloprost) are all in clinical use, but are reserved for advanced therapy for persistent disease (Barst et al., 2009). Moreover, these agents have limitations, including short half-lives, parenteral (non-oral) routes of administration, and heterogeneous therapeutic response. Thus, the quest for novel therapies for these diseases is ongoing. In preclinical studies of

both PAH and IPF, a novel, non-prostanoid prostacyclin receptor agonist (ONO-1301, Toray Industries) that also has potent inhibitory activity against thromboxane synthase (TBXAS1) has shown promise. Murakami et al. (2006) have demonstrated attenuation of bleomycin-induced pulmonary fibrosis, as well as improved survival in mice, using ONO-1301. Kataoka et al. (2005) demonstrated similar protective results in a monocrotaline-induced PAH model, whereby rats treated with ONO-1301 showed improved pulmonary hemodynamics and survival, along with a reduction of vascular remodeling (media hypertrophy) and plasma TxA₂ metabolites. Moreover, the pharmacokinetic profile (half-life approximately 5.6 h) of ONO-1301 appears to be better than some of the PGI₂ analogs in current therapeutic use (Antoniu, 2006) and, thus, if the same clinical efficacy can be proved in humans, ONO-1301 could be a new therapy for PAH and IPF. In lieu of this, several new orally available, non-prostanoid, selective IP receptor agonists have reached clinical trial status for PAH, including APD811 (Arena Pharmaceuticals), which is in Phase I and NS-304, a.k.a., Selexipag (Actelion Pharmaceuticals; Kuwano et al., 2007, 2008), which is currently in Phase III.

With the imbalance between PGI₂ and TxA₂ playing an important role in atherogenesis and atherothrombosis, the development of dual-acting compounds seems like a promising direction for the development of novel therapeutic agents. In a similar fashion to the aforementioned dual COXIB-TP antagonists, both Miyamoto and Yamada have demonstrated the potential for TRA-418 (Toray Industries), a novel compound with both PGI₂ (IP receptor) agonistic and TxA₂ (TP receptor) antagonistic activities (Miyamoto et al., 2003; Yamada et al., 2003). *In vitro* studies showed that TRA-418 inhibited platelet aggregation through impedance of both glycoprotein IIb/IIIa (GPIIb/IIIa) activation and P-selectin expression, which are key markers of platelet activation (Miyamoto et al., 2003). Moreover, the TRA-814 compound was also shown to inhibit platelet-leukocyte complex formation in a dose-dependent manner (Miyamoto et al., 2010). These results suggest that this compound may be useful as both an anti-thrombotic and anti-atherogenic agent. Similarly, Ohno et al. (2005) have developed a dual-acting benzofuran compound (Toray Industries) that

possesses similar PGI₂ agonism and TxA₂ antagonism, which maintains potent anti-platelet activity with minimal effect on blood vessel dilation.

Thus, while the current pool of PGI₂-centered compounds is primarily investigational, and clinical safety and efficacy in human disease still largely unproven, their development emphasizes the centrality of PGI₂ activity in various inflammatory-mediated pathologies, and provides exciting new directions to steer drug development.

CONCLUSION

The culmination of data presented in this review reinforces the notion that Bunting et al. (1983) put forth almost 30 years ago – that a dynamic balance between the prostaglandins prostacyclin (PGI₂) and thromboxane A₂ (TxA₂; in addition to many other mediators) is crucial in maintaining cardiovascular homeostasis, and has critical pathophysiological and therapeutic implications. However, we are just now realizing the potential breadth and scope of this seminal proposition with PGI₂ playing a central role. Our current knowledge of PGI₂, as both a physiological–pathophysiological mediator and therapeutic agent, in a host of inflammatory-related diseases, is growing rapidly. As demonstrated, PGI₂ has been shown to play protective roles in atherogenesis – relating to CAD, MI, stroke, and other cardiovascular abnormalities. It has also been shown to be involved in certain fibro-proliferative and pulmonary vascular diseases, such as IPF and PAH, where it serves as both a protective factor and first-line pharmacotherapy. Furthermore, in the setting of RA, PGI₂ seems to play a pro-inflammatory role and, as evidence increases, perhaps may one day be considered a therapeutic target in RA and related disorders (Figure 2). Further study of this important prostaglandin, in both the realms of basic science and clinical medicine, is needed and will undoubtedly yield new insights into inflammatory disorders and pharmacological treatments.

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