The mechanistic basis for prostacyclin action in pulmonary hypertension

Pulmonary arterial hypertension (PAH) is a progressive disease of the small pulmonary arteries in which patients suffer from elevated pulmonary arterial pressure, right ventricular failure and a reduction in gas exchange. Left untreated the median survival from diagnosis is ~2.8 years, though outcome is significantly worse if patients have underlying pulmonary fibrosis or scleroderma. Injury to the endothelium probably initiates the disease process, with increased production of vasoconstrictors (endothelin and thromboxane) and growth factors accompanying the loss of vasodilator and anti-platelet agents, prostacyclin and nitric oxide, which results in vascular remodelling. To date prostacyclin therapy still remains the most efficacious treatment for PAH, although its short half-life and cumbersome delivery (continuous infusion) meant analogues with improved stability and alternative routes of delivery were developed. Classically, prostacyclin agents are thought to produce haemodynamic and anti-proliferative effects through prostacyclin (IP) receptors coupled to cyclic AMP generation, though other prostanoid receptors may contribute (EP_2, EP_4) or counterbalance (EP_1, EP_3) these responses. Increasing evidence suggests peroxisome proliferator-activated receptors (PPARs) are also cellular targets for prostacyclin agonists, regulating cell growth, inflammation and apoptosis through these transcription factors. Activation involves ligand binding and/or membrane receptors but probably not cyclic AMP. Here we discuss recent advances in our understanding of PPARs and how they may represent an important therapeutic target in PAH.

Prostacyclin (PGI_2) is a 20-carbon prostanoid derivative formed within vascular endothelial and smooth muscle cells by cyclooxygenase (mainly COX-2) mediated oxidation of arachidonic acid.[1] Back in 1976, Sir John Vane and co-workers reported that arteries (but not platelets) contained an enzyme (PGI_2 synthase) which transformed prostaglandin intermediates (endoperoxides) to an unstable substance (PGI_2) that inhibited platelet aggregation (reviewed in Gryglewski[1]). This prostanoid was subsequently found to be a potent dilator of both the systemic and pulmonary circulation and an inhibitor of smooth muscle cell proliferation.[1] In the early 1980s, Rubin and colleagues were the first to give prostacyclin to adult patients with idiopathic pulmonary arterial hypertension (IPAH), showing it could acutely reduce mean pulmonary arterial pressure and pulmonary vascular resistance and increase cardiac output.[2]

Subsequently, a number of randomised controlled trials of intravenous prostacyclin (epoprostenol) have described improvements in pulmonary haemodynamics, exercise tolerance and clinical symptoms.[3] Alongside epoprostenol, other therapies have been developed, including endothelial antagonists and phosphodiesterase (PDE) type five inhibitors.[4] These improve clinical symptoms in PAH, though to date only prostacyclin has been shown to have a significant impact on long-term survival.[3,4] A key thing to note is that <20% of PAH patients respond to vasodilator therapy, and many have improvement exceeding that pre-

dicted from vasodilator testing.[3] This strongly suggests additional benefits of PGI_2, probably relating to anti-proliferative, anti-thrombotic and anti-inflammatory properties of this agent in the pulmonary vasculature. It is assumed this translates into a regression of the vascular remodelling process,[5] though evidence so far is lacking in IPAH patients.[6] Indeed lesion formation was actually reported to be more extensive in IPAH patients on combined prostacyclin and bosantan (mixed endothelin antagonist) therapy.[7]

In this article, we will discuss the recent advances in our understanding of the mechanistic basis for prostacyclin therapy, highlighting differences in the pharmacological action of prostacyclin agonists, the impact of PAH on prostacyclin signalling through prostacyclin (IP) receptors and peroxisome proliferator-activated receptors (PPARs), and the complex effects of prostacyclin on vascular remodelling, which in the long term may cause these agents to fail.

PROSTACYCLIN ANALOGUES

Prostacyclin is clinically very hard to work with and has to be refrigerated if not used immediately or in hotter climates, the *i.v.* delivery system requires an ice pack. The half-life is approximately 3 minutes when kept in buffer at a physiological temperature, the vinyl-ether linkage making it susceptible to hydrolysis to 6-keto-$PGF_{1\alpha}$ (Figure 1), a chemically stable product but weak inhibitor of platelet function and vascular tone.[8] The metabolism is complex, but 6-keto-$PGF_{1\alpha}$ can be further metabolised in the kidney to the 2,3-dinor derivative.[8] Therefore, prostacyclin is typically monitored by measurement of 6-keto-$PGF_{1\alpha}$ in plasma or its derivative in urine, 2,3-dinor-6-keto-$PGF_{1\alpha}$.

Given this inherent instability, chemists made modifications in the lower and upper side chains, giving rise to ana-

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Figure 1. Structures of prostacyclin and its analogues. Prostacyclin is a chemically unstable prostaglandin which is readily hydrolsed in a physiological buffer to 6-keto-$PGF_{1\alpha}$. It is also subject to metabolism by 15 hydroxyl prostaglandin dehydrogenase and β-oxidation yielding the urinary metabolite 2,3-dinor-6-keto-$PGF_{1\alpha}$. To increase stability, a series of modifications in the structure of prostacyclin were made, giving rise to chemically stable analogues with longer plasma half-lives (given as terminal). Stability comes from a hindered alcohol group at carbon 15 as well as an ether moiety replacing the methyl group at carbon 3 of the carboxylic acid side chain.
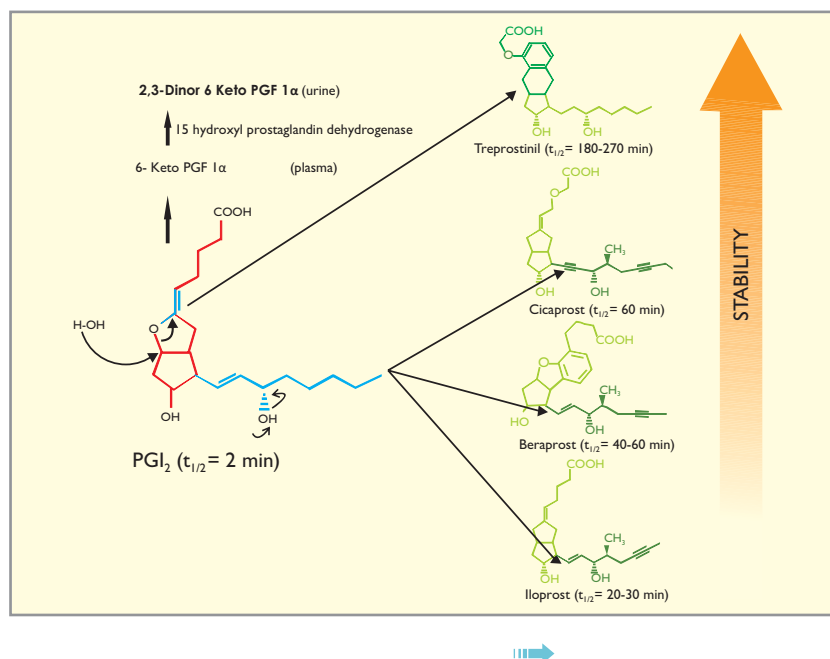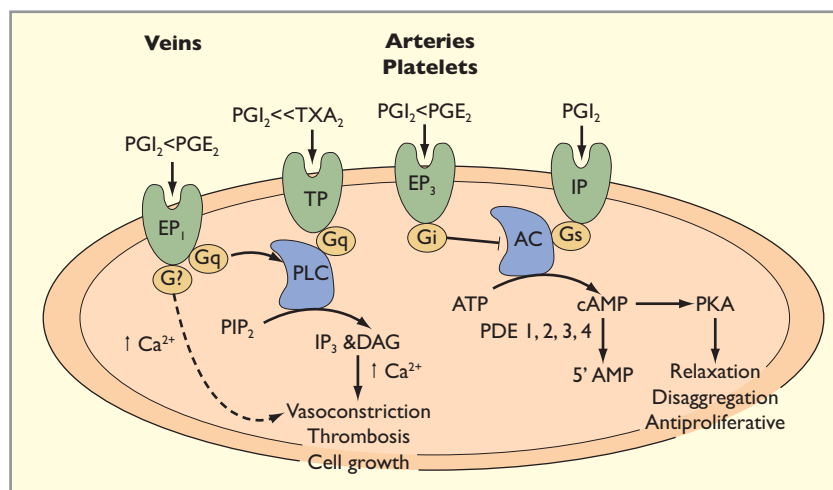
Figure 2. Prostacyclin poorly discriminates between prostanoid receptors. Prostacyclin binds to the IP receptor, which is coupled via Gs protein to adenylyl cyclase and cyclic AMP production. Prostacyclin can also bind to and activate other prostanoid receptors, including EP_3 and TP in human pulmonary arteries, and platelets and EP_1 and TP in pulmonary veins. These receptors counteract IP receptor signalling by elevating intracellular Ca^{2+} and/or lowering cyclic AMP though Gq, Gi and an unknown G protein pathway. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; G, G protein; IP_3, inositol triphosphate; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; PKA, protein kinase A; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; TXA_2, thromboxane.

logues not readily hydrolysable in solution (Figure 1). For example, the ability of treprostinil (formally UT-15) to inhibit platelet aggregation is not compromised if incubated in human plasma for 4 hours at 37°C prior to transfer to platelet-enriched plasma.[8] Iloprost, which shares the same pentanoic side chain to prostacyclin, will, however, very slowly degrade at room temperature and is most susceptible to β-oxidation, making its terminal plasma half-life less (20–30 minutes) than either beraprost (40–60 minutes) or treprostinil (180–270 minutes).[9–11] Several drug formulations now exist for clinical use including intravenous (iloprost, treprostinil), oral (beraprost, treprostinil), inhaled (iloprost and treprostinil) and subcutaneous (treprostinil).

CELLULAR TARGETS OF PROSTACYCLIN AND ANALOGUES
IP receptors versus PPARs
The classical way by which PGI_2 (or its stable analogues) is thought to exert its biological effects locally is through activation of plasma membrane prostacyclin (IP) receptors coupled via Gs protein to adenylyl cyclase and cyclic AMP (cAMP) production.[12] Once elevated, cAMP is rapidly broken down by specific phosphodiesterases (PDE). In the lung PDE 1, 3, 4 appear responsible for regulating basal levels and analogue-induced elevation.[13,14] However, PGI_2 synthase is also heavily expressed in the perinuclear regions of cells, including in smooth muscle.[15] Once formed, prostacyclin can then directly interact with the ligand-binding domain of a family of transcription factors called peroxisome proliferator-activated receptors (PPARs). Three main isoforms exist, PPARα, PPARβ(δ) and PPARγ, regulating diverse cellular processes including lipid and glucose metabolism, insulin sensitivity, cell growth and inflammation.[15–17] There is the potential for cross talk between IP receptors and PPARs since cAMP-dependent protein kinase A (PKA) is known to phosphorylate and activate PPARs, although many other kinases can regulate activity both in the absence and presence of ligands,[17] suggesting regulation by other receptor pathways.

PGI_2 signalling through PPARβ is thought to have important roles in embryonic development, tumourigenesis, apoptosis and endothelial cell survival.[15,17] PGI_2 is probably an endogenous ligand for PPARα and PPARγ, though complex regulation by other prostaglandin metabolites (e.g. 15-Deoxy-$\Delta^{12,14}$-PGJ_2) make its hard to assess the precise role of these two isoforms.[15,18] Nonetheless, PPARα is a very

important therapeutic target for the treatment of hyperlipidaemia (fibrates) and PPARγ for type 2 diabetes (glitazones), with increasing evidence suggesting they may also be novel targets in inflammatory and remodelling lung diseases.[16,19,20]

Some analogues, including beraprost and iloprost, but not cicaprost, bind to and transcriptionally activate PPARs,[15,21] though cicaprost is still capable of activating PPARγ in HEK293 cells.[22] The mechanism by which analogues activate PPARs has not been extensively investigated. However, with respect to PPARγ, both IP receptor and IP receptor-independent mechanisms have been reported, though this does not appear to necessitate cAMP elevation but could involve phosphorylation of the ligand-binding domain.[22,23]

Signalling through other prostanoid receptors
In trying to understand the cellular effects of prostacyclin, one has to remember that it has poor selectivity for prostanoid receptors, binding to and activating EP_1, EP_3 and TP receptors, albeit at higher concentrations (15–45-fold for EP_1 and EP_3 and <100-fold for TP) compared with the corresponding natural ligand.[24–26] Activation of these receptors would tend to elevate Ca^{2+} and/or lower cAMP through different G protein pathways,[12,18] leading to vasoconstriction, thrombosis and cell proliferation (Figure 2). In human lung and platelets, EP_3 and TP receptors are the main prostanoid receptor to oppose signalling through IP receptors, the exception being pulmonary veins where EP_1 and TP receptors appear to counteract prostanoid-induced relaxation.[18,27] Thus prostacyclin has the potential for deleterious effects if IP receptor expression is compromised and/or if TP/EP receptor signalling is enhanced. Indeed, in mice genetically deficient in the IP receptor, vascular proliferation and platelet activation are increased in response to tissue injury, whereas the opposite occurs in TP receptor knockout mice, suggesting the balance between these two receptor pathways is crucial in maintaining vascular homoeostasis.[28] Moreover, thromboxane levels rise when the IP receptor gene is deleted and the vasodilator response to cicaprost is converted to vasoconstriction.[28]

As shown, all analogues bind potently to the IP receptor with a similar affinity (Table 1). However, like prostacyclin, iloprost has poor selectivity for prostanoid receptors, being essentially equipotent at activating IP, EP_1 and EP_3 receptors.[12,29] Thus, it is not surprising that iloprost-induced vasorelaxation can be enhanced by EP_1 receptor blockade in the isolated rabbit perfused lungs[30] or in guinea pig aorta.[31] On the other hand, cicaprost responses in rat tail artery are enhanced by blocking Gi/Go coupling, presumed to relate to the EP_3 receptor, which is negatively coupled to adenylyl cyclase via Gi.[32] In addition, cicaprost has significant activity at the EP_4 receptor, as does iloprost for the human and rat but little for the mouse receptor (Table 1). Despite the expression of EP_4 receptors in the human lung,[33] they do not appear to mediate prostanoid-induced relaxation of human pulmonary arteries; in veins they may, though DP and IP receptors still assume the greater role.[18,27] However, in situations where the IP receptor is downregulated (in PAH or high cell passage number), EP_4 receptors may well generate sufficient cAMP for a functional response on vascular tone or cell proliferation.[33]

Surprisingly little is known about the pharmacology of treprostinil, though enhanced and more prolonged cAMP generation compared to other analogues was reported in

Table 1. Receptor binding affinities (Ki) of prostacyclin analogues to human and mouse prostanoid receptors							
Ligands		IP	DP	TP	EP$_1$	EP$_2$	EP$_3$	EP$_4$

Ligands		IP	DP	TP	EP$_1$	EP$_2$	EP$_3$	EP$_4$
Carbacyclin	Human	282	132		23	942	14	352
	Mouse	110				1600	31	2300
Cicaprost	Human	17	>1340	>1340	>1340	>1340	255	44
	Mouse	10			1300		170	
Iloprost	Human	11	1035		11	1870	56	284
	Mouse	11			21	1600	27	2300
Beraprost	Human	39					680	
	Mouse	16					110	
Treprostinil	Human	8**	ND	ND	ND	ND	ND	ND
	Mouse	YES	ND	ND	ND	YES	ND	NO
PGE$_2$	Human	YES	307		9.1	4.9	0.33	0.79
	Mouse				20	12	0.85	1.9

*Radioligand binding data (Ki in nM) has been taken from references.[12,29,80] Blank means Ki value >3μM, ND means not done, YES indicates evidence for functional activity and NO means the opposite. **Ki value taken from cAMP generation in cultured human pulmonary smooth muscle cells.[31]*

both human pulmonary smooth muscle cells (PASMCs) and mouse alveolar macrophages, suggesting signalling through additional Gs protein-coupled receptors,[34,35] largely accounted for in macrophages by activation of EP$_2$ but not EP$_4$ receptors. Thus prostacyclin agonists can act upon different cellular targets, meaning a similar spectrum of clinical effects cannot be readily assumed with this class of agents.

IMPACT OF PAH ON PROSTACYCLIN SIGNALLING

Several studies point to defects in prostacyclin signalling in PAH which may either contribute to disease pathology and/or explain why prostacyclin therapy wanes as the disease progresses, necessitating doses to be increased. Decreased urinary levels of 2,3-dinor-6-keto-PGF$_{1\alpha}$ are found in patients with IPAH[36] or pulmonary hypertension linked with congenital heart disease.[37] In IPAH this is associated with a progressive loss of PGI$_2$ synthase expression from large to small pulmonary arterial vessels, with virtually no expression in plexiform lesions.[38] Conversely overexpression of PGI$_2$ synthase is protective in monocrotaline- and hypoxic-induced models of PAH, reducing both medial thickening and pulmonary pressure.[39,40] A reduction in IP receptor expression has recently been reported in IPAH lungs or in rats following monocrotaline treatment.[33] Such a loss is, however, unlikely to cause PAH, as mice lacking the IP receptor gene do not spontaneously develop pulmonary hypertension, although they are more susceptible to the hypertensive and remodelling effects of hypoxia.[41] Thus, PGI$_2$ synthase and the IP receptor both appear protective in the context of PAH.

For a number of reasons, IP receptor desensitisation is likely to be a problem with the long-term use of prostacyclin. Analogues are known to induce rapid time- and concentration-dependent phosphorylation and internalisation of the IP receptor,[42] with desensitisation lasting for several hours after agonist removal.[43] The mechanism of receptor desensitisation is mutifactorial and involves both PKA and protein kinase C (PKC). Cross-desensitisation can also occur via EP$_1$[30] or thromboxane A$_2$[44] receptors being activated either by prostacyclin agonists themselves or by increased production of their respective endogenous ligands, PGE$_2$ or thromboxane A$_2$, both of which can increase in lung disease.[36,45]

Heightened activity of PDE1 (PDE1A and PDE1C)[14,46] and to a lesser extent PDE3 has been reported in IPAH,[14] suggesting this may lead to reduced effectiveness of prostacyclin and its analogues. Indeed, blockade of PDE1 and PDE3 restored the ability of beraprost to increase cAMP levels in pulmonary smooth muscle cells derived from IPAH patients.[14] In addition, only a combination of iloprost with a PDE1 or PDE3 inhibitor could reverse pulmonary hypertension and distal pulmonary artery muscularisation induced by chronic hypoxia.[13] Likewise, in a chronic monocrotaline model, combined administration of iloprost and a mixed PDE3/4 PDE inhibitor was required to fully normalise haemodynamic and right heart changes when administered after full establishment of pulmonary hypertension.[5] Taken together, this suggests that a PDE1 inhibitor and/or a PDE3 inhibitor might be useful in potentiating analogue effects in the clinical setting. The role of PDE4 is less clear.

The extent to which IPAH impacts on PPAR isoform expression is relatively unexplored. Ameshima and colleagues found reduced staining of PPARγ in IPAH lungs and none in the proliferating cells of plexiform lesions.[47] Similarly, PPARγ expression was also significantly reduced in the vascular lesions in a rat model of severe PAH caused by hypoxia in the presence of a vascular endothelial growth factor (VEGF) blocker. Moreover, PPARγ knockdown in endothelial cells leads to an abnormal, proliferating, apoptosis-resistant phenotype,[47] while targeted deletion of PPARγ in smooth muscle causes pulmonary hypertension and muscularisation of distal pulmonary arteries.[48] Thus loss of PPARγ is likely to impact on disease severity. In contrast, PPARγ ligands (e.g. rosiglitazone) can protect against the pulmonary effects of monocrotaline and hypoxia (see Nisbet et al.[16]). Therefore, depletion of two co-existing prostacyclin targets (IP receptor and PPARγ) in proliferating intimal cells may contribute both to uncontrolled vascular remodelling and, in conjunction with receptor desensitisation, to lack of responsiveness of prostacyclin agents in advanced disease.

PROSTACYCLIN AND VASCULAR TONE

A large body of evidence suggest that PGI_2 analogues inhibit vascular tone almost exclusively through plasma membrane potassium channels, activation of which will inhibit calcium influx through voltage-dependent calcium channels.[32,49,50] Potassium channels are able to influence vascular tone because of the very steep relationship between membrane potential and calcium influx, with a small change (<5 mV) capable of enhancing calcium entry twofold.[50] Depending on the vascular bed studied, ATP-sensitive (K_{ATP}), large conductance Ca^{2+}-activated (BK_{Ca}) and inward rectifier potassium channels have been implicated in the relaxation induced by iloprost, beraprost and treprostinil.[31,32,49] This can occur through both cAMP-dependent activation of PKA or by direct Gs protein coupling of the IP receptor to the channel.[49] In some blood vessels cAMP does not appear to mediate relaxation at all, suggesting IP receptors probably couple through other G protein signalling pathways, the nature of which remain unknown.[32]

In the lung, K_{ATP} and BK_{Ca} channels contribute to the reversal by iloprost of hypoxic-induced increases in perfusion pressure in the rat,[51] while treprostinil activates a PKA-sensitive background potassium (TASK-1) current which is turned off by hypoxia and ET-1 in human PASMCs.[52] In addition, depressed activity and expression of voltage-gated (Kv) potassium channels (in particular Kv1.5) is consistently reported in response to hypoxia, serum, anorexic agents, ET-1 and in IPAH.[50,53,54] Moreover, serum and ET-1 will also inhibit K_{ATP} channel function, though proliferating human PASMCs are still able to hyperpolarise to pharmacological openers. The combined loss of potassium function will lead to PASM depolarisation and a sustained rise in intracellular calcium, triggering not only vasoconstriction but cell proliferation and a reduction in apoptotic signals (reviewed in Clapp and Tennant,[50] Morrell *et al.*[54] and Hassoun *et al.*[55]). Thus, potassium channel activation is likely to underlie some of the beneficial effects of PGI_2 therapy, possibly counteracting potassium channel dysfunction induced as a consequence of PAH.

IP RECEPTOR AND PPARS AS REGULATORS OF CELL PROLIFERATION

Previous studies in normal human PASMCs have shown that prostacyclin analogues can inhibit the mitogenic responses to PDGF and serum in a largely cAMP-dependent manner, with adenylyl cyclase inhibitors blocking around 75% of the analogue responses in these cells.[34,56] The downstream mechanisms are not well understood, but prostacyclin analogues appear to inhibit smooth muscle cell proliferation by blocking progression from G1 to S phase.[57,58] This may occur through phosphorylation of the cAMP response element-binding protein (CREB) inhibiting cyclin A expression[57] and upregulating the inducible cAMP early repressor;[58] the latter pathway is thought to promote apoptosis as well.

There are other mechanisms by which cAMP cascades can impede cell growth. Treprostinil inhibits serum-induced cell growth in human PASMCs in part through cAMP-dependent activation of ATP-sensitive potassium (K^+) channels.[50] In aortic smooth muscle cells, cAMP elevating agents inhibit platelet-derived growth factor (PDGF) proliferation through inhibition of the calcineurin and NFAT (nuclear factor of activated T-cells) pathway.[59] The exact mechanism was not fully explored, but PKA is known to promote the nuclear export of the calcineurin/NFAT complex, while a lowering of intra-

cellular calcium levels would inhibit the phosphatase activity of calcineurin, thereby maintaining NFAT in the phosphorylated state and preventing its translocation into the nucleus (see Jabr *et al.*[59]). Such a mechanism may counteract the elevated NFAT activity and expression reported in IPAH or in remodelled pulmonary arteries.[54] Furthermore, this transcription factor appears largely responsible for the downregulation of voltage-gated (Kv) current and Kv1.5 expression reported in human PASMCs from these patients,[53] as well as the expression of pro-inflammatory cytokines and growth-promoting genes (including endothelin-1) in a number of different cell types.[54,60] Prostacyclin analogues have been reported to reduce ET-1 synthesis stimulated by mitogens in PASMCs[61,62] and to reduced elevated plasma levels in patients with systemic sclerosis.[63] Whether this relates to inhibition of NFAT activity, which itself can be suppressed by a direct interaction of PPARγ,[19] remains to be determined.

Activation of PPARs is also likely to contribute to the antiproliferative effects of PGI_2 agents in a variety of cell types. Recent studies have shown that PPARγ contributes to the IP receptor-dependent antiproliferative effects of treprostinil in HEK293 cells[22] and to iloprost-induced inhibition of lung tumourigenesis.[23] In the latter study, pulmonary-specific overexpression of either PGI_2 synthase or PPARγ resulted in suppression of tumour incidence and multiplicity in these lung models,[23] while gene deletion of the IP receptor had no effect on tumour growth. Furthermore, non-small cell lung cancer cells overexpressing PPARγ exhibit significantly less invasiveness and metastases.[23] Taken together, this suggests a key role for both PGI_2 and PPARγ in regulating cancer progression. A contribution from PPARβ was ruled out on the basis that iloprost could not activate a PPARβ-specific response element in lung epithelial cells.[23] Furthermore, PPARβ activation is consistently associated with human lung carcinoma cell growth (e.g. Han *et al.*[64]). This contrasts with other studies where PPARβ appears to mediate the antiproliferative effects of treprostinil in human lung fibroblasts[65] or beraprost in cultured rat aortic smooth muscle.[21] However, PPAR activation occurred in the micromolar range, suggesting these effects may lie outside the therapeutic concentration range. That aside, the role of PPARβ in the cardiovascular system is complex since overexpression of PPARβ actually promotes vascular smooth muscle proliferation and atherosclerosis, while PPARβ ligands appear to do the opposite and also cause vasorelaxtion.[17]

VASCULAR WALL REMODELLING

The aggressive pulmonary vascular obliterative disease characteristic of IPAH involves all cell types within the vessel wall. Smooth muscle cells are initially hyperplastic and hypertrophied, and then become atrophied as intimal proliferation develops. Adventitial fibroblasts proliferate and migrate. Endothelial damage is marked and plexiform lesions are thought to consist of proliferating abnormal endothelial cells, which may sometimes consist of monoclonal endothelial cell expansion.[54,66] Thus, it comes as no surprise that patients with IPAH have elevated levels of several growth factors, including PDGF, VEGF, epidermal growth factor and angiopoietin.[54,55,67] These agents induce proliferation and migration of smooth muscle, endothelial cells and fibroblasts, while VEGF and angiopoietin (1 and 2) are also key markers of angiogenic remodelling. Metalloproteinases (MMPs), which are also elevated in PAH[67] and other inflammatory lung diseases,[19] contribute to structural remodelling

by growth factor activation, degradation of extracellular matrix proteins (collagens, gelatins and proteoglycans) and disruption of the internal elastic lamina. Furthermore, in monocrotaline-treated animals, increases in MMP-9 and MMP-2 proteins levels and activity were somewhat suppressed by iloprost treatment, but co-treatment was required with a PDE3/4 inhibitor to have significant effects and return levels to those seen in control animals.[5] Thus, the mechanism of suppression is likely to involve cAMP, though PPARα and PPARγ ligands are also potent suppressors of MMP-9 and MMP-2 activity.[19]

The effects of PGI_2 on remodelling markers in patients is largely unknown though clinical improvement with treprostinil treatment for 12 weeks in a placebo-controlled trial was correlated with a reduction in angiopoietin-2 and MMP-9 plasma levels, with a trend towards lower PDGF levels.[67] In contrast, circulating VEGF levels were actually found to be significantly enhanced by treatment and patients were clinically worse (as assessed by the 6-minute walk test) the higher the change from baseline.[67] Indeed many studies have shown increased VEGF production with prostacyclin agents (e.g. Eddahibi et al.[68] and Biscetti et al.[69]). Moreover, iloprost can induce angiogenesis in vivo, an effect ameliorated by sequestering VEGF with soluble Flt-1 or by gene deletion of PPARα.[69] Whether this highlights a potential unwanted side-effect of prostacyclin agents is far from clear and warrants further investigation.

On the one hand, it is becoming increasingly accepted that the success of any therapy, at least for severe PAH, requires endothelial cell growth to be controlled or perhaps reversed.[54,66] Thus therapies utilised in cancer are currently being trialled in PAH.[55,66] On the other hand, VEGF stimulates nitric oxide and PGI_2 production in endothelial cells and is critical for maintaining endothelial integrity.[54,66] Indeed, this latter concept is supported by studies showing that VEGF receptor blockade causes mild pulmonary hypertension and muscularisation of pulmonary arteries associated with endothelial cell death and also exacerbates the effects of chronic hypoxia, with animals presenting with severe PAH and occlusive vascular lesions (see Stenmark et al.[66]). Thus it might well be argued that aggressive prostacyclin treatment early on in the disease, before the onset of extensive intimal proliferation and lesion formation, may offer the best chance of reversing or slowing disease progression with this therapy.

Perhaps another thing worth considering is that PGI_2 analogues may only become angiogenic agents in cells expressing low levels of IP receptor, PGI_2 synthase and PPARγ, as appears the case in cells contained within plexiform lesions (see comments above). Finally, one should consider the possibility that there may be scenarios where new blood vessel growth might be advantageous, perhaps in critical limb ischaemia or in sclerodema patients with Raynaud's syndrome for whom ischaemic digital ulcers are a major clinical problem.

Interestingly, in an experimental model of flow-mediated pulmonary hypertension, improvement in right ventricular function with iloprost was associated with a restoration of capillary to myocyte ratio, with no detectible change in vascular remodelling or pulmonary arterial pressure, suggesting new capillary growth may improve a failing heart.[70] It should be noted that this study failed to detect any changes in message levels for VEGF or angiopoietin-2 after iloprost treatment for 28 days. Given that markers were measured at the same time point as histological changes
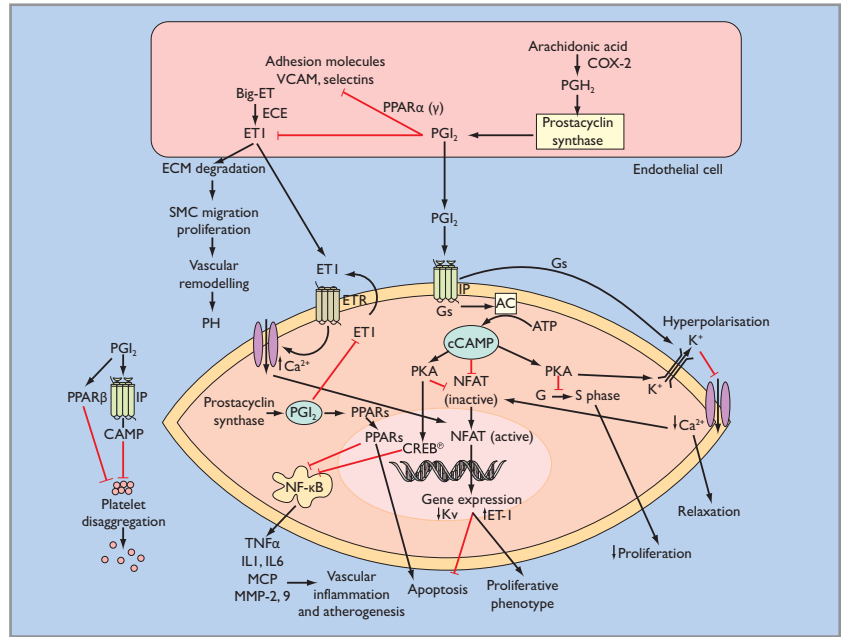
were recorded, this may indicate that this was too late to pick up changes associated with new vessel growth.

ANTI-INFLAMMATORY ACTIONS OF PROSTACYCLIN

Endothelial dysfunction plays a key role in the development of PAH and this in turn causes the expression of adhesion molecules (e.g. P-selectin, ICAM-1) and the subsequent adherence of platelets and leukocytes to the injured endothelium.[7,55,71] Extensive infiltration of T-lymphocytes, macrophages and dendritic cells occurs in the distal arteries and plexiform lesions of children and adults with IPAH,[7,55,66] culminating in an environment where pro-inflammatory cytokines, particularly interleukins 1 (IL-1) and 6 (IL-6) and chemokines (e.g. CCL2, also known as monocyte chemotactic protein, MCP-1) are upregulated.[55] It is becoming increasingly recognised that the anti-inflammatory actions of prostacyclin may contribute to the beneficial effects of these agents in PAH as well as in critical limb ischaemia or sclerodema. Prostacyclin and in particular iloprost are capable of inhibiting the expression of selectins (P and E) and the adhesion molecules ICAM and VCAM in endothelial or inflammatory cells of patients with PAH, systemic sclerosis and peripheral vascular disease.[63,72,73] Furthermore, analogues can downregulate pro-inflammatory cytokine (TNF-α, IL-1 IL-6 and interferon-γ) and chemokine production (MCP-1 and granulocyte macrophage colony-stimulating factor) in response to bacterial products in alveolar macrophages,[74] dendritic cells[75] and T-lymphocytes.[76] NF-κB plays a critical role in the expression of proinflammatory cytokines and chemokines in these inflammatory cell types, and analogues suppress NF-κB activity[74-76] in a largely, though not exclusively, IP receptor-driven manner, in part involving PKA.[75,76] Studies in patients are limited, though iloprost-inhibited plasma TNF-α levels in critical limb ischaemia.[77] In IPAH, epoprostenol treatment reduced elevated circulating levels of MCP-1,[78] and in combination with bostantan, significantly reduced human leukocyte antigen–DR expression, a marker of endothelial cell activation.[7] Whether PPARs are involved in analogue suppression of inflammatory mediators remains to be determined, though in the lung, PPARα and to a lesser extent PPARγ are regulators of adhesion molecule expression,

Figure 3. Schematic representation of the cellular pathways by which prostacyclin inhibits vascular tone, cell proliferation, platelet activation and inflammation. AC, adenylyl cyclase; CREB, cAMP response element binding protein; cAMP, cyclic adenosine monophosphate; COX-2, cyclooxygenase-2; ECM, extracellular matrix; ET, endothelin, ETR, endothelin receptor; ICAM, intercellular adhesion molecule; IFγ, interferon-γ; IL-1, interleukin-1; IL-6, interleukin-6; IP, prostacyclin receptor; NFAT, nuclear factor of activating T-cells; NF-κB, nuclear factor kappa B; MMP, metalloproteinase; MCP, monocyte chemoattractant protein; PGI₂, prostacyclin; PGH₂, prostaglandin H₂; PKA, protein kinase A; PPARs, peroxisome proliferator-activated receptors; SMC, smooth muscle cell; TNF-α, tumour necrosis factor α; VCAM, vascular cell adhesion molecule-1.

while both PPARα and PPARγ are major inhibitors of pro-inflammatory cytokine production via transrepression of NF-κB.[17,20] By comparison, little is known about the role of PPARβ, but it has been implicated in the antithrombotic effects of treprostinil in human platelets.[79]

CONCLUDING REMARKS

Prostacyclin can no longer be considered a hormone that just produces its biological effects through activation of the IP receptor. The family of transcription factors knows as PPARs must now be considered a target through which prostacyclin or its stable analogues can modulate cell growth, endothelial cell activation, inflammation and apoptosis and produce beneficial effects in PAH (Figure 3). While PPARs can be activated independently of the IP receptor, few studies have considered the role membrane receptors (or for that matter cAMP) either in inducing activation or modulating ligand binding. The future challenge will be in identifying the role of specific PPAR isoforms not only the aetiology of PAH, but also in response to analogue activation.

The role of VEGF in PAH (vascular protective vs. angiogenic) is clearly an area that needs further investigation, in particular whether upregulation of this growth factor by prostacyclin analogues is detrimental in PAH patients or contributes to lack of efficacy in end-stage disease. Given that the aim of any treatment is to reverse the remodelling process, then the possibility of earlier and aggressive intervention with prostacyclin agonists should be considered.

Finally, prostacyclin agonists differ in their pharmacological profile, meaning that the clinician should not readily assume they are dealing with a homogenous class of agents when it comes to clinical treatment or side-effect profile. It may be that such differences can be exploited in future therapies or be utilised to tease out the role of different prostanoid receptor subtypes.

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