Mechanisms of disease

Role of prostacyclin in pulmonary hypertension

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Abstract

Prostacyclin is a powerful cardioprotective hormone released by the endothelium of all blood vessels. Prostacyclin exists in equilibrium with other vasoactive hormones and a disturbance in the balance of these factors leads to cardiovascular disease including pulmonary arterial hypertension. Since it’s discovery in the 1980s concerted efforts have been made to make the best therapeutic utility of prostacyclin, particularly in the treatment of pulmonary arterial hypertension. This has centred on working out the detailed pharmacology of prostacyclin and then synthesising new molecules based on its structure that are more stable or more easily tolerated. In addition, newer molecules have been developed that are not analogues of prostacyclin but that target the receptors that prostacyclin activates. Prostacyclin and related drugs have without doubt revolutionised the treatment and management of pulmonary arterial hypertension but are seriously limited by side effects within the systemic circulation. With the dawn of nanomedicine and targeted drug or stem cell delivery systems it will, in the very near future, be possible to make new formulations of prostacyclin that can evade the systemic circulation allowing for safe delivery to the pulmonary vessels. In this way, the full therapeutic potential of prostacyclin can be realised opening the possibility that pulmonary arterial hypertension will become, if not curable, a chronic manageable disease that is no longer fatal. This review discusses these and other issues relating to prostacyclin and its use in pulmonary arterial hypertension.
Introduction

Prostacyclin was discovered in 1976 by a group led by Salvador Moncada and John Vane\(^1\). Initially called prostaglandin (PG)\(X\), prostacyclin was identified as an unknown lipid mediator formed by microsomes prepared from rabbit or pig aortas that inhibited human platelet aggregation and relaxed some preparations of isolated blood vessels. Early studies showed that PG\(X\) is the major metabolite of arachidonic acid in the arterial walls of a number of species, including man\(^2\). PG\(X\) was later identified as 5\(\alpha\)-5,6-didehydro-9-deoxy-6,9a-epoxyprostaglandin F\(_{1}\) and renamed as prostacyclin\(^3\). Early studies attributed prostacyclin release as the mechanism mediating the anti-thrombotic properties of the endothelium\(^4\) and its place as a fundamental mediator in cardiovascular health was set. A current (2014) PubMed search of the term ‘prostacyclin’ generates 17958 hits with 1992 hits for the terms ‘prostacyclin’ and ‘pulmonary hypertension’. Pulmonary hypertension is a devastating, progressive and ultimately fatal condition with few treatment options, which, at best slow progression but do not cure the disease. Traditionally drugs have been designed to target the pulmonary vasculature as either vasodilators or inhibitors of smooth muscle remodeling. Most recently the right heart, which fails under the burden of extra work exerted on it by increased pulmonary pressures, has become a viable therapeutic target in the search for new drugs to treat pulmonary hypertension.

This review will cover what is known about the synthetic and receptor pathways associated with prostacyclin and how this knowledge has been applied and translated to produce treatments. Specifically the review will discuss how the known actions of prostacyclin provide a compelling case for its utility for treatment of both pulmonary vessels and the right heart. The review will also identify the limitations of prostacyclin therapies and speculate upon how modern medical technologies might be applied to improve its utility in this disease. Finally, with the idea that pulmonary arterial hypertension may, in the future, be treated with stem cell therapies to supplement organ regeneration and/or transplant, the potential role of prostacyclin in these approaches will be highlighted.

Synthesis of prostacyclin

Endothelial cells are the predominant source of prostacyclin in the body and prostacyclin is the main eicosanoid made by endothelial cells. Prostacyclin is synthesised from the 20 carbon fatty acid (20:4) arachidonic acid by the concerted actions of cyclo-oxygenase (COX) and prostacyclin synthase\(^5\) (Figure 1). Arachidonic acid is not normally free in cells but acetylated
in membrane phospholipids. The first step in prostacyclin release is activation of enzymes that liberate arachidonic acid from membranes. The best-studied pathway for arachidonic acid liberation involves phospholipase A\textsubscript{2} (Figure 1). There are multiple forms of phospholipase A\textsubscript{2} but cytosolic forms (cPLA\textsubscript{2}) and, in some circumstances, calcium-independent PLA\textsubscript{2} (iPLA\textsubscript{2}) are thought to drive arachidonic acid liberation in endothelial cells. Arachidonic acid can also be liberated through a second pathway after phospholipase C cleaves an inositol triphosphate group, giving diacylglycerol (DAG), which can then be hydrolyzed by lipases to monoacylglycerol and then to free arachidonic acid and glycerol.

Once free inside the cell arachidonic acid is metabolized by various enzymatic and non-enzymatic routes to eicosanoids (or icosanoids; lipid mediators derived from 20 carbon fatty acids). Prostacyclin is a prostanoid member of the eicosanoid family produced by a two-step reaction metabolized by COX. In the first step arachidonic acid is converted to prostaglandin (PG)G\textsubscript{2} via an oxygenase reaction and then in the second step, to PGH\textsubscript{2} by a peroxidase reaction. PGH\textsubscript{2} is relatively unstable but within cells provides the substrate for a range of synthase enzymes downstream of COX (Figure 1). It is the relative expression of these PG synthase enzymes that critically dictate the profile of prostanoids released by a given cell type under different conditions. For example, endothelial cells and platelets both express the isoform COX-1 but prostacyclin synthase is highly expressed in endothelial cells with little or no thromboxane synthase. In contrast, in platelets thromboxane synthase is highly expressed whilst there are negligible levels of prostacyclin synthase. As a result, despite both tissue types being high expressers of COX-1, the prostanoid products they produce are highly polarized and, in this way, perform diametrically opposed functions within the cardiovascular system. Like COX, prostacyclin synthase is a P450 enzyme, expression of which in endothelial cells, is regulated by shear stress and growth factors.

Pulmonary arterial hypertension is classically associated with reduced vasodilators (including prostacyclin) and increased vasoconstrictors, which is why current therapies rely so heavily on manipulation of vasoactive pathways. Specifically, in terms of eicosanoids, pulmonary arterial hypertension is associated with reduced urinary markers of prostacyclin and increased markers of thromboxane\textsuperscript{6}. This is in line with reduced prostacyclin synthase in lungs of patients with pulmonary arterial hypertension\textsuperscript{7}. Further, transgenic mice overexpressing prostacyclin synthase or mice inoculated with prostacyclin synthase gene\textsuperscript{8}\textsuperscript{9} are protected from development of disease symptoms\textsuperscript{10}. Prostacyclin synthase gene delivery in the form of
genetically modified stem cells has also been reported to protect against development of experimental pulmonary arterial hypertension\textsuperscript{11,12}.

Once formed by endothelial cells prostacyclin doesn’t simply diffuse out of cells but is exported by highly regulated transporter systems, most likely of the ABC\textsuperscript{13} class with the likely member of this class most used for prostanoids, including prostacyclin, being multidrug resistance protein 4 (MRP4/ABCC4)\textsuperscript{14}. Once released from cells prostacyclin is then free to act on receptors to mediate its actions. The idea that pulmonary arterial hypertension may be associated with reduced secretion of prostacyclin at the level of a transporter has not been addressed and, where tested, inhibition of MRCP4 leads to protection in animal models attributed to an action on cGMP/cGMP transport\textsuperscript{15}. Nevertheless, the lack of literature in this area suggests that elucidation of precise mechanisms of prostacyclin flux in pulmonary vessels during disease may provide insight and new drug targets.

**Receptor pathways utilized by prostacyclin and implications for treatments in pulmonary arterial hypertension**

Prostanoid signaling is mediated by selective cell surface receptors and in some tissues by activation of cytosolic PPAR receptors. For prostacyclin, the cell surface receptor is the G-protein coupled ‘IP’ receptor (Figure 2). IP receptors are coupled to Gs leading to activation of adenylate cyclase and subsequent conversion of ATP to the second messenger cAMP (Figure 2). The biological effects of cAMP in a given tissue, which are diverse, are mediated by activation of cAMP-dependent protein kinases (also known as protein kinase A) and Exchange protein activated by cAMP (Epac; Figure 2). In vascular smooth muscle cAMP mediates relaxation and reduces proliferation and in platelets reduces thrombosis via regulation of calcium levels and associated pathways. Protein kinase A and Epac act synergistically to inhibit vascular smooth muscle cell proliferation\textsuperscript{16} and whilst pulmonary artery smooth muscle cells express both of these pathways, preliminary studies suggest that Epac is down regulated in pulmonary hypertension\textsuperscript{17}.

As with other prostanoids, the complicating pharmacological feature of prostacyclin is that, whilst its acts preferentially on its designated subtype (i.e. IP) receptors, it can cross over and activate any of the other prostanoid receptors in particular circumstances (Figure 2). This means that, for example, where IP receptors are limiting, prostacyclin can activate
thromboxane (TP) receptors. As mentioned above, the opposing properties of thromboxane and prostacyclin in the cardiovascular system are critical to the maintenance of vascular health. This balance is broken when thromboxane is produced in excess, or, similarly where IP receptors are saturated. In these settings prostacyclin becomes a mimetic for thromboxane inducing vasoconstriction. Prostacyclin and related drugs can also cross over onto constrictor EP and FP receptors, which, as with TP, can limit the dilator actions of prostacyclin, as well as dilator EP and DP receptors which may have a beneficial effect. This issue of specificity is of relevance to the use of prostacyclin drugs to treat pulmonary arterial hypertension since there is, as with all pharmaceutical preparations, the danger of overriding local sensing pathways.

The existence of multiple IP receptor subtypes has been suggested in some tissues but these observations are based on pharmacological studies and have not been validated at the gene level. Nonetheless, the authors of a recent study claim to have conclusively identified two IP receptor subtypes using a human airway epithelial cell line exposed to a host of IP agonists in the presence or absence of a selective IP antagonist. Whilst this observation is potentially very important, it remains to be seen whether the distinct IP receptor subtypes can be identified in other human cells.

The potential for GPCRs to homo- and hetero-dimerize is well-established. IP receptors can form homodimers via the interactions of disulphide bonds. Importantly IP receptors may also form heterodimerize with thromboxane TP receptors. The IP-TPα complex has been suggested to have a protective role in promoting a “PGI2-like” response from TPα activation by TP ligands. After activation, IP receptors are desensitized by PKC-dependent phosphorylation and receptor internalization, which constitute endogenous pathways to regulate and limit prostacyclin signalling. As is common for drugs acting on natural receptor pathways the prospect of IP desensitization/internalization may be a confounding factor in utilizing prostacyclin analogues as therapeutic interventions and, as discussed can shunt biological responses away from dilator to constrictor pathways. Evidence of desensitisation of IP receptors and/or their down stream pathways has been noted in clinical studies. In line with this continuously infused epoprostenol is associated with tolerance in patients with severe pulmonary hypertension, and dose adjustments have to be made to maintain clinical effects. Indeed, in patients with pulmonary arterial hypertension secondary to COPD the
dilator effects of epoprostenol on pulmonary pressures were subject to tachyphylaxis within 24 hours\textsuperscript{25}.

Prostacyclin is also a ligand for the cytosolic nuclear receptor PPAR\textsubscript{β}\textsuperscript{26} (Figure 2). PPAR\textsubscript{β} is considered to be anti-inflammatory in a number of settings where it acts by genomic and non-genomic mechanisms\textsuperscript{26} (Figure 2). Importantly for the treatment of pulmonary arterial hypertension, the prostacyclin drug, treprostinil, activates PPAR\textsubscript{β} in platelets\textsuperscript{27}, lung fibroblasts\textsuperscript{28} and blood vessels\textsuperscript{29}. Work from our group and others has also shown that selective, non-IP, PPAR\textsubscript{β} agonists relax pulmonary artery smooth muscle cells\textsuperscript{30} and prevent hypertension in an hypoxic rat model\textsuperscript{29}. In animal models we found that whilst the PPAR\textsubscript{β} agonist GW0742 prevented pulmonary arterial hypertension, reducing right heart hypertrophy, it did not reduce muscularization of vessels in the lung\textsuperscript{29}. This suggested to us that PPAR\textsubscript{β} agonists might have a protective action directly on the right heart in pulmonary arterial hypertension. Recently we, with collaborators, tested this idea using a pulmonary artery banding model where workload is applied to the right heart mechanically without any contribution from pulmonary pressure \textit{per se}\textsuperscript{31}. Of direct relevance, others have shown that PPAR\textsubscript{β} activation in adult hearts facilitates mitochondrial function and improves cardiac performance under pressure-overload conditions\textsuperscript{32}. In our study, GW0742 prevented right heart remodeling and transcripomic profiling of heart tissue suggested that the mechanism was classically genomic involving the PPAR target gene \textit{Angptl4}\textsuperscript{31}. Angptl4 is a member of the angiopoitin-like family and regulates angiogenesis and lipid metabolism. While no data currently exist relating Angptl4 to idiopathic pulmonary arterial hypertension, it has recently been associated with high-altitude adaptation in Tibet\textsuperscript{33} and Angptl4 is associated with left heart failure where it protects against myocardial infarction and no reflow through preservation of vascular integrity\textsuperscript{34}. These observations support the idea that Angptl4 may be a viable mechanism by which PPAR\textsubscript{β} activation leads to cardioprotection and suggest that this pathway may be therapeutically important in other forms of heart failure, such as seen in pulmonary arterial hypertension. This is an interesting notion since our work shows this is independent of actions on vessels which means that activation of PPAR\textsubscript{β} could be a good adjunct therapy to current drugs acting on vasodilator pathways. In our work we have suggested that the time could be right for a clinical study to assess the effects of PPAR\textsubscript{β} in pulmonary arterial hypertension, since there are orally active drugs available that have already been used in man\textsuperscript{35}. However, this needs to be treated with extreme caution for two key reasons. Firstly, PPAR\textsubscript{β} drugs may negatively interact with current drugs\textsuperscript{29} and secondly
PPARβ drugs are associated with increased risk of cancer\textsuperscript{36, 37} and warnings have been issued for their use, particularly directed at sports performance dosing where illicit procurement of drug maybe considered by athletes.

**Role of COX-1 and COX-2 in prostacyclin generation and implications for pulmonary arterial hypertension**

As described above, prostacyclin is formed from PGH\textsubscript{2} produced by the enzyme COX. COX has two isoforms COX-1, which is constitutively expressed and COX-2 that is induced at the site of inflammation\textsuperscript{38}. COX-1 is the predominate enzyme present in endothelial cells and its loss in vessels virtually abolishes release of prostacyclin\textsuperscript{39-41}. This is also true in conditions of inflammation associated with atherosclerosis\textsuperscript{42}.

However, COX-2 takes over from COX-1 as the driver for prostacyclin release under conditions of gross systemic inflammation such as that associated with sepsis\textsuperscript{43}. Outside large vessels COX-2 is expressed in some key tissues, including in the lung\textsuperscript{40}. It is now accepted that pulmonary arterial hypertension is, at least in part, driven by inflammatory cytokines\textsuperscript{44-47} and interferon\textsuperscript{48, 49}. With this in mind our group was the first to suggest that induction of COX-2 by cytokines may be implicated in pulmonary arterial hypertension\textsuperscript{50}. Others showed similar data in cells relevant to pulmonary arterial hypertension\textsuperscript{48, 51-53}. If prostacyclin is the main product of cells expressing COX-2 in the lungs in pulmonary arterial hypertension it is likely to form a protective responses. This idea is supported by data showing a detrimental effect of COX-2 gene deletion in mouse models of pulmonary arterial hypertension\textsuperscript{54, 55}. However, if prostacyclin synthase is overwhelmed and/or if the IP receptor population is saturated, COX-2 will drive a constrictor response and this may explain a protective effect of COX-2 inhibitors in other experimental models\textsuperscript{56}. It should be noted however, that there is no evidence to suggest that this phenomenon predominates and the role of COX-2 and associated prostacyclin release in human pulmonary arterial hypertension remains the subject of investigation.

**Prostacyclin as a drug to treat pulmonary arterial hypertension**

Pulmonary arterial hypertension is rare, but fatal, with mean survival rates without therapy of less than 2 years. The introduction of prostacyclin therapies in the early 1990s has led to increased survival rates to around 5-7 years with some patients living with pulmonary arterial hypertension on prostacyclin therapy for more than 10 years. The features of pulmonary
arterial hypertension include reduced prostacyclin/thromboxane balance, constriction, remodeling and thrombosis. With these features in mind the therapeutic utility of prostacyclin (also known as epoprostenol) was assumed very early in the field and in 1984 a placebo controlled trial was conducted where prostacyclin was continuously infused in patients with peripheral vascular disease. This paved the way for a landmark trial in 1996 where prostacyclin, was infused intravenously for 12 weeks in patients with pulmonary arterial hypertension. 41 patients received prostacyclin and 40 the conventional treatment at the time, which consisted of anticoagulants, oral vasodilators, diuretic agents, cardiac glycosides, and supplemental oxygen. Exercise capacity, measured using the 6 minute walk test, was improved in all 41 patients treated with prostacyclin but was reduced in the 40 patients treated with conventional therapy. Importantly, mortality was improved in the patients administered prostacyclin. However, serious side effects were noted in the prostacyclin arm, which included catheter associated sepsis. Epoprostenol (FLOLAN®) remains a therapeutic option in the treatment of pulmonary arterial hypertension but is seriously limited by its very short half-life at room temperature and side effects associated with the need for continuous infusion requiring permanent intravenous catheter and pump (Figure 3). To address some of these limitations a number of more stable prostacyclin analogues have been developed for the treatment of pulmonary arterial hypertension (Figure 4). These include iloprost (Ventavis®) and treprostinil (Remodulin®), which together with epoprostenol constitute the current prostacyclin therapies in patients with pulmonary arterial hypertension (Figure 3). Treprostinil, which has similar pharmacodynamics to epoprostenol is more stable and can be administered subcutaneously and intravenously. Iloprost is administered as an inhaled preparation using a nebulizer 6-9 times a day. Treprostinil is also available in and inhaled formulation (TYVASO®; Figure 3) given approximately each 4 hours.

**Prostacyclin and combination therapy in pulmonary arterial hypertension**

Despite their effectiveness and because of their limitations and side effects, prostacyclin drugs are generally restricted to patients with pulmonary arterial hypertension and who are in functional class III or IV. Intravenous epoprostenol is often the preferred drug with intravenous treprostinil given as an alternative. Inhaled iloprost is generally reserved for patients for whom intravenous therapy is not acceptable or appropriate. As prostacyclin drugs are reserved for patients with severe pulmonary arterial hypertension in most cases they will be given in combination with either a phosphodiesterase type 5 (PDE5) inhibitor and/or an endothelin receptor antagonist (ETRA). The utility and mechanism of action of PDE5
inhibitors\textsuperscript{59, 60} and ETRAs\textsuperscript{61} are reviewed in detail elsewhere. However, in brief, PDE5 inhibitors work by increasing the bioactivity of endogenously released NO. NO, like prostacyclin, is a vasodilator, but acts on a parallel signaling pathway via activation of soluble guanylate cyclase leading to increases in the second messenger cGMP. PDE5 removes cGMP, thus blocking PDE5 potentiates NO signaling. The effects of NO and prostacyclin are additive in blood vessels\textsuperscript{60} and work in powerful synergy in platelets\textsuperscript{60, 62}. ETRA drugs, on the other hand, work independently of the NO or prostacyclin pathways by blocking the actions of the powerful constrictor peptide endothelin-1. It is not clear how the pharmacology of these three pathways affects particular combinations of drugs in pulmonary arterial hypertension and there are no validated biomarkers that can predict which drugs will work together optimally. However, this is an area of research that our group and others are investigating using endothelial cells grown from blood progenitors allowing insights into vascular function in patients with pulmonary arterial hypertension\textsuperscript{63, 64}.

**Limitations of prostacyclin drugs in pulmonary arterial hypertension**

As with other treatments for pulmonary arterial hypertension prostacyclin drugs are very expensive with estimated annual costs of $30,000 to more than $200,000 per patient per year in the United States. However, the main limitations of prostacyclin drugs for those that require the drug to be infused are infection and pain at the site of injection. In addition all forms of prostacyclin drugs are associated with side effects such as systemic hypotension, flushing, jaw pain and nausea. Intense research efforts are ongoing to address these limitations and include the development of small molecule, non-prostacyclin, selective IP receptor agonists most notably selexipag (Figure 4). Selexipag is a potent, orally active a pro-drug whose active metabolite, MRE269 (ACT-333679), is a selective prostacyclin IP receptor agonist. Unlike prostacyclin analogue drugs, because selexipag is specific for the IP receptor, it has little or no effect on other prostanoid receptors. This means that where drugs based on prostacyclins structures may be limited by underlying constrictor actions on EP, TP or FP receptors, selexipag targets dilator IP pathways only. However, this high specificity for IP receptors means selexipag will also fail to activate DP receptors and PPAR\textbeta that may contribute to the efficacy of other prostacyclin drugs. Nonetheless, a phase II proof of concept study showed favorable results\textsuperscript{65} and in 2009 the GRIPHON\textsuperscript{66}, (Prostacyclin (\textit{PGI}_2) Receptor agonist In Pulmonary arterial HypertensioN) trial was initiated by Actelion to test the utility of selexipag in a randomized, multicenter, double-blind, placebo-controlled trial in patients with pulmonary arterial hypertension. In June 2014 Actelion announced that initial
analysis of the GRIPHON study showed that selexipag decreased the risk of a morbidity/mortality and that the overall tolerability profile of selexipag in GRIPHON was consistent with existing prostacyclin therapies. According to the Actelion website in December 2014 marketing authorizations will be submitted to the European Medicines Agency (EMA) for selexipag (Uptravi®) in the treatment of pulmonary arterial hypertension with similar applications pending to the US Food and Drug Administration (FDA). However, even if oral dosing with selexipag proves to be as efficacious as prostacyclin drugs dosed by infusion or inhalation, it is still limited by side effects common to prostacyclin therapy due to its actions on the systemic circulation. In the wake of success with orally active IP-selective selexipag, most recently the FDA approved the first orally active formulation of a prostacyclin drug, treprostinil (Orenitram™). Orenitram™ is treprostinil in an extended-release tablet formulation for the treatment of patients with pulmonary arterial hypertension. The approval comes after the FREEDOM studies. Whilst current data in patients not previously taking prostacyclin drugs are disappointing, studies show that in some patients oral treprostinil may successfully replace existing use of continuously infused drug. However, as with selexipag, oral dosing does not prevent side effects and future studies and development in formulations will be needed to improve prostacyclin drugs in all their guises.

**Future of prostacyclin drugs in pulmonary arterial hypertension**

Clearly prostacyclin drugs in all their forms have proven utility in pulmonary arterial hypertension but are severely limited by route of delivery and effects of on the systemic circulation. Attempts to circumvent the need for drug infusion have been successful with drugs such as inhaled treprostinil and iloprost and orally active selexipag, but the systemic side effects remain the limitation in realizing the full potential of this class of drugs. One approach being adopted in other human diseases is nanomedicine, where targeted drug delivery can improve efficacy and overcome side effects (Figure 5). The use of nanomedicine technology has, in some cases, revolutionized drug formulations for treatment of cancer. Nanomedicine is a relatively young science and can be defined as the medical application of nanotechnology, in the case of drug delivery systems this equates to the use of formulations in the nanometer range. As the field grows the types of potential formulations suitable to encapsulate drugs increases. The idea that this technology can be applied to drugs for pulmonary arterial hypertension was recently reviewed but the idea remains relatively novel and untested. Nevertheless, we suggest that the following approaches may solve the current limitations of prostacyclin drugs. Firstly a safe and effective encapsulation of
prostacyclin drug within a suitable nanoparticle to evade the systemic circulation is required. This may be enough to allow specific targeting of pulmonary vessels if similar characteristics of local tissue environment exist to those in tumors. In tumors some nanomedicines can accumulate because of increased vascular leak and reduced lymphatic drainage. However, in the case of specific delivery of a prostacyclin drug to affected pulmonary vessels additional molecular engineering may be required. One approach to this would be to use an antibody-drug conjugate (Figure 5). Here it would first be necessary to identify a specific antigen expressed locally within pulmonary vessels, manufacture and humanize the antibody. This may be possible by using comparative systems approaches such as proteomics, recently used to identify translationally controlled tumor protein (TCTP) as a marker of pulmonary arterial hypertension. These, of course, are not trivial tasks and would require the concerted efforts of chemists, bioengineers, pharmacologists and clinicians.

Future application for the prostacyclin pathway in stem cell and organ regeneration therapies

Current therapies have had dramatic effects at increasing the life expectancy of patients with pulmonary arterial hypertension. However, ultimately, in most cases, these fail and in some patients having a lung transplant is the only therapeutic option. Needless to say this is not a perfect solution nor is it one that can benefit most patients. With this in mind there are increasing efforts in the use of stem cell therapy to treat pulmonary arterial hypertension. This may be either at the level of giving stem cells in an attempt to repopulate the diseased vessels in the pulmonary vasculature or, at the most ambitious end of the spectrum, to grow lung tissue in bio incubators for transplant.

Prostacyclin pathways play a potentially important role in these approaches. Any stem cell therapy in pulmonary arterial hypertension would require a fully functioning COX/prostacyclin synthase pathway and would similarly require fully functioning prostacyclin receptors to be present. This type of approach in stem cell and gene therapy has been reviewed elsewhere but remains very much at the theoretical and experimental stage.

Summary and conclusions

Prostacyclin is a multifaceted cardioprotective hormone released by the endothelium. Since its discovery in the 1980s prostacyclin has been the subject of thousands of publications yet we are still discovering new insights into its biology and pharmacology. Prostacyclin remains
arguably the most effective therapy for patients with pulmonary arterial hypertension but current drugs based on its pharmacology have serious limitations. It is hoped that in the future specific targeting of prostacyclin drugs alone, or in combinations with other medications can resolve these limitations and allow for less frequent but more effective administration of high doses of drug, that will, if not cure this disease, at least convert it to an effectively managed non-fatal condition.
**Figure 1: Synthesis of prostacyclin.** Prostacyclin (PGI₂) is synthesized from arachidonic acid (AA) by the concerted actions of the enzymes cyclo-oxygenase (COX) and prostacyclin synthase. AA is liberated by from plasma phospholipids by phospholipase enzymes where it is metabolized to prostaglandin (PG)H₂ by COX. PGH₂ is then further metabolized by prostacyclin synthase to PGI₂, by PGE₂ synthases/isomerases to PGE₂ or thromboxane synthase to thromboxane (TXA₂).
Figure 2: Signaling pathways utilized by prostacyclin (PGI$_2$). PGI$_2$ acts preferentially on cell surface IP receptors that activate adenylate cyclase to convert ATP to cAMP. cAMP activates protein kinase A and Exchange protein activated by cAMP (Epac). In blood vessels and platelets this results in calcium sequestration and inhibition of activation equating to vessel relaxation, reduced remodeling and reduced thrombosis respectively. These signaling events also reduce inflammation. In the cytosol, PGI$_2$ activates PPAR$\beta$ receptors which work by three discreet pathways. Firstly, PPAR$\beta$ binds to RXR to drive transcription of target genes. Secondly it represses BCL6 and thirdly, when activated it can bind and repress PKC$\alpha$. Activation of PPAR$\beta$ can lead to similar functional effects to activation of IP receptors, although by these very different pathways. When PGI$_2$ is present in excess and these pathways are overwhelmed it can activate other prostanoid receptors leading to IP-type signaling in the case of EP$_2$, EP$_4$ and DP or to functionally opposing effects in the case of TP, FP, EP$_1$ or EP$_3$ receptors.
Figure 3: Pulmonary arterial hypertension drugs acting on prostacyclin pathways. Synthetic prostacyclin (epoprostenol), injected treprostinil, inhaled treprostinil (ita treprostinil), oral treprostinil (otreprostinil) or iloprost are drugs based on the structure of prostacyclin, which activate the IP receptor, but may also activate other prostaglandin receptors (PGRs). Selexipag is a non-prostacyclin drug given orally which selectivity activates the IP receptor. GW0742 is a non-prostanoid, non-IP small molecule drug that activates PPARβ in experimental models of pulmonary hypertension.
Figure 4: 2D (a-d) and 3D (e-f) structures of prostacyclin drugs epoprostenol (a,e), remodulin (b,f) and iloprost (c,g) and the non-prostacyclin IP agonist pro-drug selexipag (d,h). The active metabolite of selexipag is MRE269 where the terminal nitrogen is replaced with oxygen (see insert; panel d). Each of the listed drugs has similar molecular weights.

- **Epoprostenol**
  - $\text{C}_{20}\text{H}_{32}\text{O}_5$
  - $M_r$ 352.4

- **Treprostinil**
  - $\text{C}_{23}\text{H}_{34}\text{O}_5$
  - $M_r$ 390.5

- **Iloprost**
  - $\text{C}_{22}\text{H}_{32}\text{O}_4$
  - $M_r$ 360.5

- **Selexipag**
  - $\text{C}_{26}\text{H}_{32}\text{N}_4\text{O}_4\text{S}$
  - $M_r$ 496.6
  - **MRE 269**
    - $\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_3$
    - $M_r$ 419.5

Figure 5: Targeted delivery of prostacyclin to affect pulmonary vessels in disease. Prostacyclin could be encapsulated to form a nanomedicine formulation to protect it from metabolism and the systemic circulation. Attachment of an antibody directed at a specific antigen within pulmonary arteries would allow for targeted delivery and evasion of the systemic circulation.
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