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SERIES "PULMONARY HYPERTENSION: BASIC CONCEPTS FOR PRACTICAL MANAGEMENT"
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Prostacyclin therapies for the treatment of pulmonary arterial hypertension

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ABSTRACT: Prostacyclin and its analogues (prostanoids) are potent vasodilators and possess antithrombotic, antiproliferative and anti-inflammatory properties. Pulmonary hypertension (PH) is associated with vasoconstriction, thrombosis and proliferation, and the lack of endogenous prostacyclin may considerably contribute to this condition. This supports a strong rationale for prostanoid use as therapy for this disease. The first experiences of prostanoid therapy in PH patients were published in 1980.

Epoprostenol, a synthetic analogue of prostacyclin, and the chemically stable analogues iloprost, beraprost and treprostinil were tested in randomised controlled trials. The biological actions are mainly mediated by activation of specific receptors of the target cells; however, new data suggest effects on additional intracellular pathways. In the USA and some European countries, intravenous infusion of epoprostenol and treprostinil, as well as subcutaneous infusion of treprostinil and inhalation of iloprost, have been approved for therapy of pulmonary arterial hypertension. Iloprost infusion and beraprost tablets have been approved in few other countries. Ongoing clinical studies investigate oral treprostinil, inhaled treprostinil and the combination of inhaled iloprost and sildenafil in pulmonary arterial hypertension. Combination of other targeted therapies with prostanoids appears to be effective and safe.

After 25 yrs of continued knowledge, prostanoids remain a mainstay in the treatment of pulmonary arterial hypertension.

KEYWORDS: Cor pulmonale, inhaled drugs, 6-min walk distance, prostacyclin, pulmonary circulation, vascular remodelling

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Prostacyclin and its analogues (prostanoids) are potent vasodilators and possess antithrombotic, antiproliferative and anti-inflammatory properties. Pulmonary hypertension is associated with vasoconstriction, thrombosis and proliferation, and this may be partly due to a lack of endogenous prostacyclin [1] secondary to prostacyclin synthase downregulation [2]. This supports a strong rationale for prostanoid use as therapy for the disease, with the first experiences in pulmonary hypertension patients published in 1980 [3], and the first patient with severe idiopathic pulmonary arterial hypertension (iPAH) to receive long-term therapy in 1984 [4]. After 25 yrs of continued knowledge, prostanoids remain a mainstay in the treatment of these patients.

PROPERTIES OF PROSTANOIDS

Before the discovery of its chemical structure, prostacyclin was characterised as vasodilatory and anti-aggregative. This was related to its action on vascular smooth muscle cells (SMC) and platelets. Later, investigations demonstrated its antiproliferative actions and the reduction of matrix secretion in SMC, endothelial cells and fibroblasts, as well as an anti-inflammatory profile in leukocytes (fig. 1) [5]. Because endothelial cells are the major source of endogenous prostacyclin, the action of this mediator is

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directed on both the local vascular wall and blood cells, particularly those that adhere to the endothelium.

Prostacyclin was discovered in 1976 [6] and, in the same year, a chemical analogue, epoprostenol, was synthesised and tested for its biological actions [7]. Several chemically stable analogues were synthesised in the following years. Clinical studies for patients with pulmonary arterial hypertension (PAH) were performed with epoprostenol, iloprost, beraprost and treprostinil, while cicaprost, the most specific prostaglandin I (IP) receptor agonist, was not clinically developed. The main target of prostanoids is the IP receptor, which is abundantly expressed in blood vessels, leukocytes and thrombocytes, and is rapidly activated by prostanoids. The IP receptor is coupled with Gs proteins and activates adenylate cyclase, leading to increased cyclic adenosine monophosphate levels in the target cells, which explains most of the biological effects. Principally, it also couples with Gq proteins and might activate vasoconstrictive pathways under certain circumstances [8, 9]. However, prostacyclin is not highly specific to the IP receptor. It also activates prostaglandin E (EP) receptors [10], which are located on the cell surface as well as in the nucleus [11, 12], and peroxisome proliferator activated receptor (PPAR)δ, which is located in the nucleus [13]. Both PPARα and PPARδ may also be activated *via* IP receptor-dependent protein kinase (PK)A activation, but the intracellular prostaglandin (PG)I_2 from the endogenous PGI synthase seems to specifically activate the apoptosis pathway by activation of PPARδ (fig. 2) [14–17].

DIFFERENCES BETWEEN PROSTANOIDS

Epoprostenol, like endogenous prostacyclin, is a chemically unstable compound with a plasma half-life <3–5 min. After mixing the drug powder with the solvent, a highly basic glycine buffer, the solution has to be used within 12–24 h due to spontaneous degradation of the compound. When administered *via* peripheral veins, there will be painful vein irritation after a short time. Therefore, epoprostenol therapy can only be used as continuous intravenous infusion through a central venous catheter, with freshly dissolved drug filled into the pump system. All other prostanoids are chemically stable in solution and their plasma half-life is much longer: ~30 min with iloprost and beraprost, and ⩽4.5 h with treprostinil. Vein irritation is common to all prostanoids. All stable prostanoids have been provided as oral preparations; however, as detailed hereafter, only beraprost has received approval outside of the USA and oral treprostinil is currently being investigated. Iloprost has been approved as inhalative therapy and inhaled treprostinil is currently being investigated in a phase III clinical trial.

Apart from differences in pharmacokinetics and pharmacodynamics, there may be additional effects of prostanoids that are specific to each compound. The dose–response curves of the antiproliferative effects of the prostanoids were comparable but treprostinil appeared to be more potent than iloprost and beraprost [18], suggesting additional specific effects on other intracellular pathways. Non-IP-receptor effects of prostanoids have attracted some interest in tumour biology [19] and might also be interesting for pulmonary hypertension. Iloprost and cicaprost were found to have different effects in the murine corneal model of angiogenesis. While iloprost caused significant angiogenesis, comparable with vascular endothelial growth factor (VEGF), cicaprost had no such effects [20]. The explanation may be that iloprost and carbacyclin, but not cicaprost, activate PPARs [21], which results in VEGF secretion [22, 23]. VEGF increase may antagonise endothelial dysfunction and represents a potential beneficial effect on its own [24, 25].

There are some biological effects that have been described in only one of the compounds, but can probably be applied to several if not all prostanoids. Treprostinil, for example, was found to augment the positive inotropic effects of catecholamines in isolated ventricular myocytes [26], although it had no positive inotropic effects of its own. This effect may have clinical relevance because during right heart failure there is an increased catecholamine drive and, from the clinical perspective, it has long been speculated that prostanoids might have positive inotropic effects that explain their instantaneous beneficial clinical effects in right heart failure patients. These effects may add on indirect effects that originate from systemic vasodilation and subsequent baroreflex activation (fig. 3), and improved ventriculoarterial coupling [27].

Iloprost suppresses neutrophil adhesion, respiratory burst and elastase secretion [28]. This may be important because inflammation appears to play a role among the pathological mechanisms of PAH [29]. Recently, a number of beneficial changes in gene

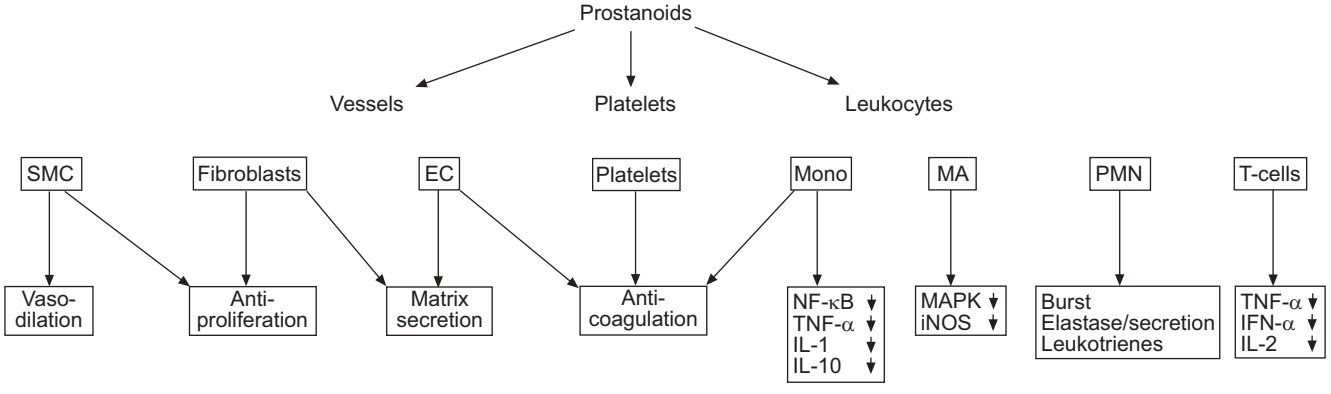

FIGURE 1. Effects of prostanoids on the vessel wall and adherent blood cells. SMC: smooth muscle cells; EC: endothelial cells: Mono: mononuclear cells; MA: macrophages; PMN: polymorphonuclear neutrophils; T-cells: T-lymphocytes; NF: nuclear factor; TNF: transforming nuclear factor; IL: interleukin; MAPK: mitogen-activated protein kinase; iNOS: inducible nitric oxide synthase; burst: generation of reactive oxygen species; IFN: interferon. Reproduced from [5] with permission from the publisher.

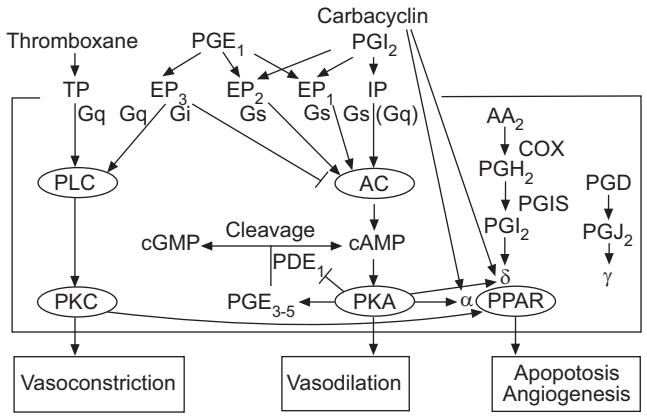

FIGURE 2. Intracellular targets of prostacyclin. Extracellular prostaglandin $(PG)I_2$ mainly, but not specifically, acts on the prostaglandin I (IP) receptor and the postaglandin E $(EP)_1$ receptor, while PGE_1 is more specific to EP receptors. TP: prostaglandin T; Gs, Gq and Gi: G proteins; PLC: phospholipase C; AC: adenylyl-cyclase; PKC: protein kinase C; cGMP: cyclic guanosine monophosphate; PDE: phosphodiesterases; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; AA_2: arachidonic acid; COX: cyclooxygenases; PGIS: prostacyclin synthase; PPAR: peroxisome proliferator activated receptor.

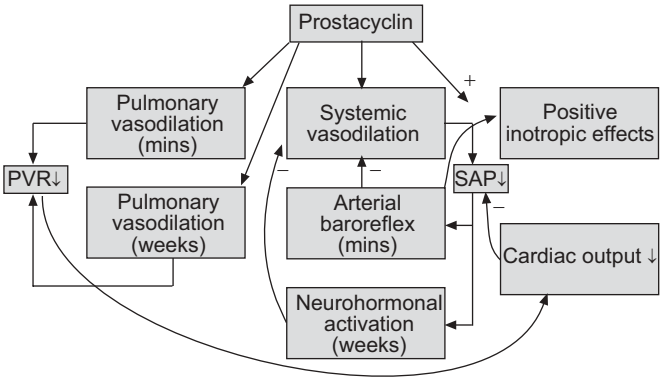

FIGURE 3. Haemodynamic effects of systemically applied prostanoids. Prostanoids cause a pulmonary and systemic vasodilatation within minutes, which is more prominent in the systemic vessels. This activates the arterial baroreflex, which reduces systemic vasodilatation and increases cardiac output. Within weeks, the neurohormonal activation increases systemic tone, while the slow vasorelaxing effects in the pulmonary arteries cause additional decrease in pulmonary vascular resistance (PVR). Indirect positive inotropic effects may ameliorate systemic hypotension. SAP: systemic arterial pressure as measured with the method of Riva Rocci. +: positive effect; –: negative effect.

expression (hyaluronidonic acid, cyclooxygenase 2 and VEGF upregulation, and monocyte chemotactic protein and plasminogen activator inhibitor-1 downregulation) were seen in SMC after incubation with iloprost [30, 31]. If it is considered that endothelial dysfunction or damage may play a major role in the pathological development of pulmonary artery remodelling, this translates into a number of beneficial effects of iloprost. Whether this translates to all prostanoids remains speculative (fig. 4).

HAEMODYNAMIC EFFECTS

The haemodynamic effects, including the side-effects of the different prostanoids, are similar; however, the mode of application and the applied doses, as well as the long-term adaptive changes of patients, may have an impact on pulmonary effects and tolerability (fig. 3). If the dose of a continuous *i.v.* infusion is gradually increased over several weeks, there will be beneficial haemodynamic effects in the vast majority of patients, with a significant decrease in pulmonary vascular resistance (PVR) and a minor decrease in systemic pressure. However, if the dose is rapidly increased, systemic vasodilatation and its side-effects, as well as other side-effects, may cause intolerable symptoms and a systemic pressure drop. If a prostanoid is infused at a constant dose, there may be IP receptor desensitisation with a complete loss of vasodilatory effect. This has recently been shown using iloprost [32]. This desensitisation is PKC dependent. It is possible that other effects of the compounds, *e.g.* on the EP1 receptor, become more and more important during constant infusion, as suggested by SCHERMULY *et al.* [32]. In clinical practice, there is no loss of pharmacological effect during constant infusion, but the dose has to be gradually increased (from 4 $ng \cdot kg^{-1} \cdot min^{-1}$ as an average tolerated dose of epoprostenol to ~20-60 $ng \cdot kg^{-1} \cdot min^{-1}$ after 1 yr, and from ~0.5 $ng \cdot kg^{-1} \cdot min^{-1}$ of iloprost to ~2.5 $ng \cdot kg^{-1} \cdot min^{-1}$ after 1 yr) to keep the same level of pulmonary vasodilatation and systemic side-effects. If the IP-receptor downregulation is also present in humans, this would suggest that most, if not all, prostanoid effects are mediated by their effects on EP receptors and PPARs. If the prostanoid drug is applied by inhalation every 3 h, there is no loss of effect [33–35] and there is a need for dose increase in only a few patients (~16% per yr; unpublished data), suggesting that this mode of application may cause less receptor desensitisation. The reasons for this difference between infusion and inhalation remain speculative; it may be due to the intermittent application and/or to different local drug concentration gradients in the different lung compartments.

CLINICAL STUDIES

Intravenous epoprostenol

The emergence of the first approved therapy for PAH, continuous *i.v.* prostacyclin (epoprostenol), transformed the field of pulmonary hypertension as the first therapy for an orphan disease. Early individual experience and small-scale registries [4, 36, 37] demonstrating improvement in symptoms and haemodynamics, led to the design of the first prospective, randomised open-label controlled trial.

Investigators enrolled 81 subjects with primary pulmonary hypertension, classified as New York Heart Association (NYHA) functional class (FC) III or IV patients, and randomised them to epoprostenol in addition to conventional therapy or conventional therapy alone (warfarin, digoxin, oxygen and oral vasodilators) [37] for 12 weeks. Subjects on active therapy had improvement in exercise capacity, as the mean 6-min walk distance (6MWD) improved by 32 m compared with a decrease of 15 m in the conventional therapy group ($p<0.003$). Haemodynamic improvement was evidenced by a decrease in pulmonary artery pressure of 8% in the epoprostenol group *versus* an increase of 3% in the conventional group (difference in mean change (95% confidence interval (CI)) -6.7 (10.7– -2.6) mmHg; $p<0.002$). The mean PVR decreased by 21% in the epoprostenol group *versus* an increase of 9% in the conventional group (difference in means (95% CI) -4.9 (-7.6– -2.3) $mmHg \cdot L^{-1} \cdot min^{-1}$; $p<0.001$).

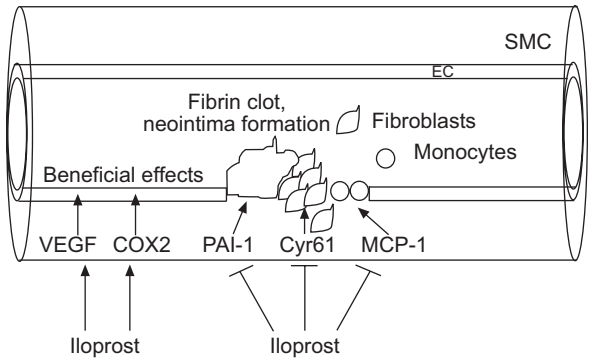

FIGURE 4. Hypothesis for neointimal formation and possible role of prostanoids. After endothelial damage, monocytes, fibroblasts and the coagulation system will form a neointima, which leads to intima fibrosis. Iloprost has been shown to inhibit the mRNA expression of important mediators of these processes. SMC: smooth muscle cells; EC: endothelial cells; VEGF: vascular endothelial growth factor; COX: cyclooxygenase; PAI: plasminogen activator inhibitor; Cyr61: cystein-rich angiogenic protein, MCP: monocyte chemotactic protein.

Eight patients died during the 12-week trial, all in the conventional therapy group (p=0.003; table 1). Importantly, this is the only short-term study (12 weeks) to date that demonstrates a positive effect on survival.

Observational cohort analyses in the USA and Europe provide demonstrable evidence of the long-term benefits of epoprostenol therapy. McLaughlin *et al.* [44] reported clinical and haemodynamic improvements in 162 subjects classified as NYHA FC III and IV in the USA. Patients had improved survival in comparison with expected survival based on historical data (National Institutes of Health registry equation). The 1-, 2- and 3-yr survival rates of 87.8, 76.3 and 62.8% were significantly better than the expected survival rates of 58.9, 46.3 and 35.4% [39]. Prognostic factors included FC, exercise tolerance, cardiac index and mean pulmonary pressure after 1 yr of epoprostenol therapy. Sitbon *et al.* [45] evaluated a similar cohort of 178 subjects in France after 1 yr of therapy. Compared with a historical cohort with the same severity, survival rates improved from 58, 43, 33 and 28% to 85, 70, 63 and 55% at 1, 2, 3 and 5 yrs, respectively. Univariate analysis found that a history of right-sided heart failure, NYHA FC IV, 6MWD $\leqslant$250 m, right atrial pressure $\geqslant$12 mmHg and mean pulmonary artery pressure <65 mmHg predicted poor outcome. However, when both baseline variables and those measured after 3 months on epoprostenol were included on multivariate analysis, only a history of right-sided heart failure, persistence of NYHA FC III or IV at 3 months and the absence of a fall in total pulmonary resistance >30% relative to baseline, were associated with poor survival. Although both studies were observational, they demonstrated long-term efficacy and those patients who presented with severe disease had a worse prognosis (fig. 5).

Evidence currently supports epoprostenol use in PAH from associated aetiologies. A multicentre randomised study demonstrated improved exercise capacity in scleroderma PAH subjects [43]. Subjects with scleroderma treated with epoprostenol appear to have a worse prognosis than subjects with primary pulmonary hypertension in uncontrolled

TABLE 1 Overview of published randomised controlled trials with prostanoids

Drug	[Ref.]	Application	Study conduct	Dose ng·kg^{-1}·min^{-1}	Disease	Patients n	Functional class	Primary end-point	6MWD change#			Deaths *verum*/ control
									All¶	iPAH¶	p-value+	
Beraprost	[38]	Oral *q.i.d.*	3 months, DB	3.0	iPAH+SS+SH+other	63+13+24+30	II–III	6MWD change#	25.1	46.1	0.035	1/1
Treprostinil	[39]	Continuous s.c.	3 months, DB	9.3	iPAH+SS+SH	270+90+109	II–IV	6MWD change#	16	m.d.	0.006	7/7
Iloprost	[40]	Inhaled 6–9 times per day	3 months, DB	0.31	iPAH+SS+CTE	111+35+57	III–IV	Comb. clin.	36.4	58.8	0.007	1/4
Iloprost	[41]	Inhaled 6–9 times per day	3 months, DB	0.31	iPAH^f+SS^f	37+30	III–IV	Safety	26	m.d.	0.051	0/0
Epoprostenol	[42]	Continuous *i.v.*	3 months, OL	9.2	iPAH	81	III–IV	6MWD change#	47	47	0.003	0/8
Epoprostenol	[43]	Continuous *i.v.*	3 months, OL	11.2	SS	111	(II), III–IV	6MWD change#	108	NA	<0.001	4/5

6MWD: 6-min walk distance; iPAH: idiopathic pulmonary arterial hypertension (PAH); s.c.: subcutaneous infusion; *i.v.*: intravenous infusion; DB: double blind; OL: open label; SS; scleroderma-associated PAH; SH: left-to-right shunt-associated PAH; CTE: chronic thromboembolic pulmonary hypertension; comb. clin.: combined clinical; m.d.: missing data; NA: not available; p-value: probability of error for rejection of the null hypothesis (no difference between verum and control). #: with respect to baseline; ¶: including data imputation (last observation carried forward and death set to 0 m); +: *versus* control; f: only patients on stable therapy with bosentan. Data obtained and modified from [38–43].

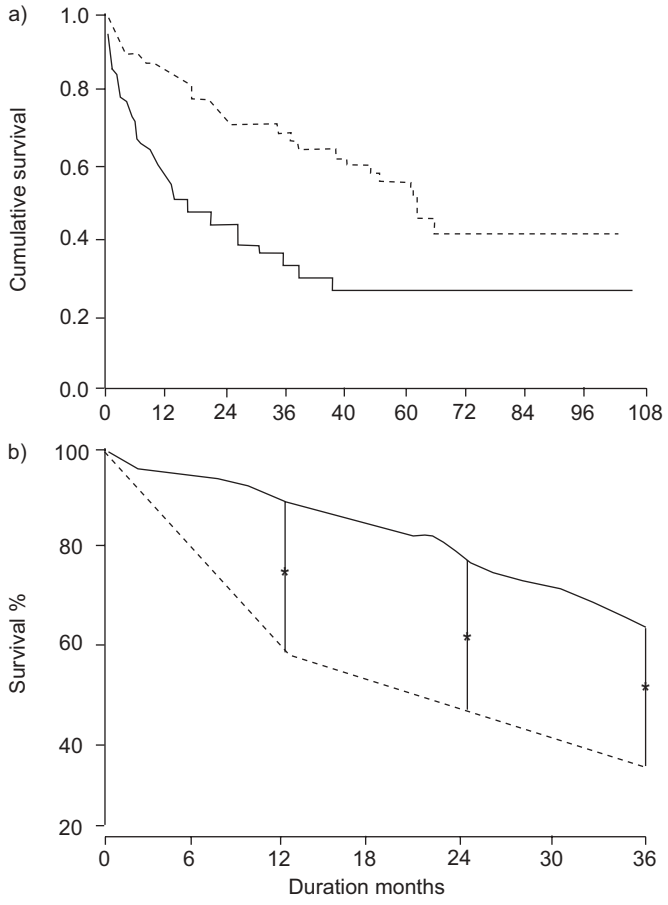

FIGURE 5. a) Cumulative survival and b) survival in idiopathic pulmonary arterial hypertension (iPAH) patients with epoprostenol. a) The number of patients at risk in the functional class IV epoprostenol group (·····) at 0, 12, 24, 36, 48, 60, 72, 84 and 96 months of treatment were 178, 129, 85, 57, 36, 21, 7, 3 and 1, respectively. The number of patients at risk in the historical control group (——) at 0, 12, 24, 36, 48, 60, 72, 84 and 96 months of treatment were 135, 59, 34, 20, 11, 4, 2, 2, and 1, respectively. b) Observed (n=162; ——) and expected (------) survival in iPAH patients. a) $p<0.0001$; b) $p<0.001$. Reproduced and modified from [44] and [45] with permission from the publisher (a and b, respectively).

analyses [46, 47]. Similar improvements in exercise functional capacity and FC have been observed in subjects with congenital left-to-right cardiac shunts [48] and infection with HIV [49, 50], while results are ambiguous in portopulmonary hypertension [51] and show unfavourable effects in pulmonary hypertension secondary to left heart failure [52].

Over the past decade, increasing use of epoprostenol has delayed the use of lung transplantation in subjects with severe disease at presentation. A USA survey [53] indicated that treatment with epoprostenol allowed two thirds of subjects to deactivate from the transplant list. It is unclear how long the disease will remain stable and, therefore, frequent clinical follow-up with examination, exercise and haemodynamic measures is recommended [54, 55].

Treatment with epoprostenol has limitations based on its pharmacology, which requires initiation by experienced physicians at designated centres. Long-term administration of

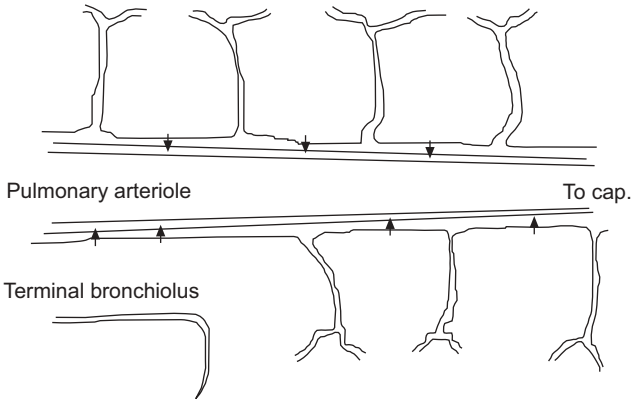

FIGURE 6. Inhaled route of application for prostanoids. The black arrows indicate the areas where locally deposited drug can penetrate the airway wall and directly diffuse into the pulmonary artery wall. Note that the terminal arterioles, carrying most of the resistance, are completely surrounded by alveolar surfaces. Cap.: pulmonary capillaries.

epoprostenol requires a permanent central venous catheter and a portable infusion pump. Medication needs to be prepared daily and to be kept cold, requiring ice packs to be worn with medication in 24-h cassettes. Once patients are at a stable dose they are able to use 8-h cassettes without ice packs; unfortunately, many insurance companies in the USA do not reimburse for this method of administration. Patients need educating in of sterile technique, operation of the pump and care of the catheter, and a strong support system to help ensure a safe environment prior to initiation. A ''mixing partner'', a family member or friend who lives in close proximity to the patient, is strongly recommended to obviate problems if the subject is too ill or unable to prepare medication on a given day. Serious complications include infection and thrombosis of the catheter and temporary interruption of the infusion due to: disconnection of the infusion by switching the line to an alternative line without priming the new line; inadvertent disconnection; or pump malfunction [47]. The incidence of catheter-related sepsis ranges 0.1–0.6 cases per patient-yr [45, 47].

The side-effect profile of epoprostenol is predominantly related to its ability to vasodilate the vasculature. Side-effects are usually well tolerated, may be dose related and vary in intensity and number between individuals. The most common side-effects include flushing, headache, nausea, loose stool, jaw discomfort with ''first bite'' and foot pain [42, 44, 45]. The foot pain is described as pain in the sole of the foot and the ankle with prolonged standing or walking.

Inhaled iloprost

Direct dilatation of the pulmonary vasculature by inhalation is a potential therapeutic option. A targeted approach, with inhalation of prostacyclin, allows for more intrapulmonary selectivity, avoidance of right-to-left shunt blood flow, less systemic side-effects [56, 57] and avoidance of a cumbersome *i.v.* infusion [33]. This approach uses the anatomical properties of the lung with their proximity of the airways to the small pulmonary arteries (fig. 6). From the haemodynamic effects and arterial blood concentrations [34] it is concluded that inhaled prostanoids directly act on the pulmonary artery wall

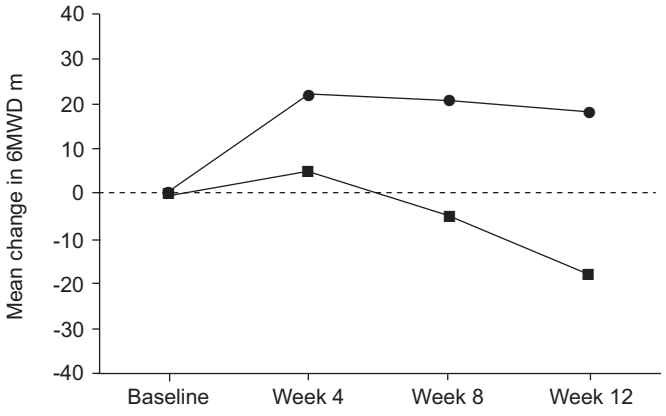

FIGURE 7. Change in 6-min walk distance (6MWD) with inhaled iloprost treatment (●) compared with placebo (■). p=0.004. Reproduced and modified from [40] with permission from the publisher.

(from the adventitial side) and not by drug recirculation from the pulmonary or bronchial arteries. The inhalative approach has been used with epoprostenol since the early 1990s, with iloprost from 1994 and with treprostinil from 2003.

Iloprost is a chemically stable prostacyclin analogue that can be delivered by suitable nebulisers [34]. To ensure alveolar deposition, the delivery system produces small aerosolised particles (median diameter ~3.0 μm) [58]. Iloprost must be inhaled 6–9 times per day to achieve good clinical effects. The inhaled doses for significant clinical effects were much smaller with inhaled iloprost (~0.31 $ng \cdot kg^{-1} \cdot min^{-1}$) [40] compared with the *i.v.* infusion of iloprost for PAH.

Inhaled iloprost is an effective agent in PAH subjects with significant limitation in functional capacity. It was approved for: PAH NYHA FC III and IV in the USA; PAH NYHA FC III and IV, as well as inoperable chronic thromboembolic pulmonary hypertension (CTEPH), in Australia; and iPAH NYHA FC III in Europe. Inhaled iloprost was investigated in a double-blind randomised controlled study and was approved for PAH NYHA FC III and IV in the USA and FC III in Europe. A total of 203 subjects with iPAH/familial PAH, PAH associated with connective tissue diseases or inoperable CTEPH with FC III or IV were enrolled in a 12-week multicentre placebo-controlled trial [40]. The primary end-point was a combined clinical end-point, where four criteria had to be met to be counted as a responder: 1) a 10% increase in 6MWD; 2) an improvement in NYHA FC; 3) no deterioration; and 4) no death. This end-point was reached by 17% of patients on iloprost compared with 4% in the placebo group (p=0.007). The mean increase in 6MWD was 36.5 m (p=0.004) and 58.8 m among subjects with primary pulmonary hypertension (fig. 7). Subjects' well-being improved as evidenced by quality-of-life scores and the Mahler dyspnoea index. When pre-inhalation values were considered, haemodynamic measures at 12 weeks improved slightly but significantly in the treatment group compared with placebo (p<0.001). When post-inhalation values were considered, a major improvement was observed. Side-effects consisted of symptoms related to systemic vasodilation. More syncopal episodes (eight *versus* five; p=NS) occurred in the iloprost group, although they were not associated with clinical deterioration. Iloprost also improved haemodynamics and physical capacity in a small series of lung fibrosis patients [59], and decompensated right heart failure [35] and HIV patients [60]. A long-term observational study in Germany [61] described patients who were started on treatment with inhaled iloprost in the period 1996–2002 as compassionate treatment, a time were no approved therapies were available in that country. After 1, 2 and 3 yrs, survival rates were 79, 70 and 59%, respectively; compared with these, event-free survival rates, *i.e.* survival without transplantation on iloprost monotherapy, were only 53, 29 and 20%, respectively. When these results are considered, it should be remembered that alternative options (in particular oral beraprost and bosentan) became available during the observation period and that financial restrictions may have biased the result.

Intravenous iloprost

Iloprost *i.v.* has been approved for pulmonary hypertension in New Zealand but it has also been used in other countries, such as Germany, Switzerland, UK, Australia, Thailand, Israel, Argentina and Brazil. It has similar acute haemodynamic effects as epoprostenol [62]. The practical advantages are that it does not need cooling and, due to its longer half-life, it is less risky in case of accidental therapy disruption. In Europe, the typical long-term dose of infused iloprost is $\leqslant 3\ ng \cdot kg^{-1} \cdot min^{-1}$ [63]. It has not been formally tested whether infused iloprost has the same clinical efficacy as infused epoprostenol or treprostinil.

Oral beraprost

Beraprost tablets are absorbed rapidly after the administration of an oral dose under fasting conditions; it reaches a peak concentration within 30 min and has an elimination half-life of 35–40 min. In Europe, 130 NYHA FC II and III subjects were enrolled in a 12-week randomised double-blind placebo-controlled trial of beraprost [38]. Subjects had PAH caused by iPAH, connective tissue diseases, congenital left-to-right shunts, portal hypertension and HIV. At a median dose of 80 μg four times daily, subjects had a mean increase of 25 m on 6MWD (p=0.04). Subjects with iPAH had a mean increase of 46 m (p=0.04), whereas subjects with PAH from other causes experienced no significant improvement. In the USA, a similar trial was designed but it included NYHA FC II and III patients and had a study duration of 12 months [64]. This study demonstrated improved 6MWD at 3 and 6 months compared with placebo, which was not sustained at 9 and 12 months [64]. Beraprost is not approved in the USA and Europe but is an approved therapy in Japan and several other countries in the Far East.

Subcutaneous treprostinil

Treprostinil is a tricyclic benzene prostacyclin analogue that, by virtue of its longer elimination half-life, pH neutrality and stability at room temperature, may be delivered by either subcutaneous (half-life 4.6 h) or *i.v.* infusion (half-life 4.4 h) [65]. A permanent central venous catheter can be avoided with *s.c.* therapy. Pre-mixed medication is infused *via* a small self-inserted *s.c.* catheter with continuous injection by syringe through an ambulatory pump. The *s.c.* infusion of treprostinil does not require daily mixing and preparation of the infusion.

The infusion site is instructed to be changed every 3–4 days according to the package insert. While this is based on the initial studies, in clinical practice patients often leave sites in for 2–4 weeks if a ''good site'' has been found [66]. The drug comes in a pre-mixed syringe, there is no need for ice packs or *i.v.* catheter care. The most common side-effect occurring in ~85% of the patients is pain [39]. Patients are treated with variable response with a combination of local anaesthetic solutions, nonsteroidal anti-inflammatory agents, neuropathic agents, such as gabapentin and pregabalin, or low-dose narcotics. The pain does not appear to be dose related, it is unclear why it occurs and it is unknown which patients will develop severe pain.

The application of *s.c.* treprostinil was Food and Drug Administration (FDA) approved for the treatment of NYHA FC II–IV PAH patients in 2002. A 12-week multicentre, randomised, double-blind trial that enrolled patients with iPAH, PAH related to congenital heart defects or connective tissue disease, compared treprostinil with placebo in a total of 470 patients (fig. 8) [39]. Treprostinil improved dyspnoea, fatigue, signs, symptoms of pulmonary hypertension and quality of life, and caused a modest but significant improvement in 6MWD. NYHA FC IV PAH patients and patients receiving higher doses of treprostinil had a more significant improvement. Notably, the maximum dose allowed at week 12 was 22.5 $ng \cdot kg^{-1} \cdot min^{-1}$, which is relatively low compared with current practice patterns. At 12 weeks, there was also an improvement in haemodynamic parameters, including right atrial pressure, mean pulmonary artery pressure, PVR and cardiac output. In subset analyses from the *s.c.* trials, patients who had PAH related to connective tissue disease experienced improvement in exercise capacity, symptoms of PAH and haemodynamics [67].

Recent retrospective and observational studies of *s.c.* treprostinil suggest long-term clinical improvement and survival benefits. LANG *et al.* [66] evaluated clinical outcomes in 99 PAH patients and 23 patients with pulmonary hypertension secondary to inoperable chronic thromboembolic disease on *s.c.* treprostinil for a mean follow-up of 26.2±17.2 months in the open-label phase of a randomised clinical trial. The mean (range) dose was 40±2.6 (16–84) $ng \cdot kg^{-1} \cdot min^{-1}$. Patients maintained their improved exercise tolerance (6MWD) and FC at 3 yrs. Event-free survival (no hospitalisation for clinical worsening, transition to *i.v.* epoprostenol, or need for combination therapy or atrial septostomy) was 83.2% at 1 yr and 69% at 3 yrs, and overall survival was 88.6% at 1 yr and 70.6% at 3 yrs; similar rates to those observed on *i.v.* epoprostenol.

BARST *et al.* [68] evaluated 860 patients classified as NYHA FC II (15%), III (76%) and IV (9%) and treated for ⩽4 yrs in randomised controlled trials or newly started on therapy (*de novo*). The mean (range) age was 46 (5–84) yrs and 76% were female. The aetiologies of the patients enrolled were iPAH (48%), PAH associated with congenital heart disease (21%), PAH associated with connective tissue disease (19%), chronic thromboembolic disease (6%) or portal pulmonary hypertension (5%). Survival in patients on monotherapy, censoring patients with addition of targeted PAH therapy (130 patients) or premature discontinuation due to adverse events (199 patients), was 88, 79, 73, and 70% at 1, 2, 3 and 4 yrs, respectively. Similarly to reported epoprostenol observational studies, patients with better FC at study entry lived longer. The most frequently reported side-effect in both studies was site pain, which occurred in 75 and 92% of the patients in the studies by BARST and co-workers [64, 68], respectively. Discontinuation due to site pain occurred predominantly in the first year, with a plateau at 2–3 yrs.

Intravenous treprostinil

Most recently, the FDA approved the use of *i.v.* treprostinil based on bioequivalence to *s.c.* therapy. The advantage over epoprostenol is that the cassette is changed every other day and it does not require ice packs. The longer half-life of *i.v.* treprostinil may also decrease the risk of cardiovascular collapse in case of inadvertent interruption of the infusion. In the first reported open-label trial of *i.v.* treprostinil in untreated PAH patients, 16 NYHA FC III or IV patients improved their 6MWD by a mean±SE of 82 m, from 319±22 m to 400±26 m ($p=0.001$) [69]. Haemodynamics also improved on therapy: mean pulmonary artery pressure (-4.2 mmHg; $p=0.03$); cardiac index (0.47 $L \cdot min^{-1} \cdot m^{-2}$; $p=0.002$); and PVR index (-9.4 $U \cdot m^{-2}$; $p=0.001$). One death, thought to be unrelated to the study drug, occurred in a patient who received 3 days of therapy and died 2 weeks after discontinuation. Patients can successfully transition in the hospital from *i.v.* epoprostenol to *i.v.* treprostinil [70]. A total of 27 NYHA FC II and III PAH patients completed the study, there were no deaths and four patients transitioned back to epoprostenol, three due to leg pain and one with worsening PAH symptoms in the setting of pneumonia. Transition maintained exercise capacity (438±16 m at baseline and 439±16 m at week 12) and FC, but with an increase in mean pulmonary artery pressure (4±1 mmHg; $p<0.01$) and reduction in cardiac index (-0.4±0.1 $L \cdot min^{-1} \cdot m^{-2}$; $p=0.01$). Patients finished on more than twice the dose of treprostinil compared with epoprostenol, with a mean value of 83±38 $ng \cdot kg^{-1} min^{-1}$ *versus*

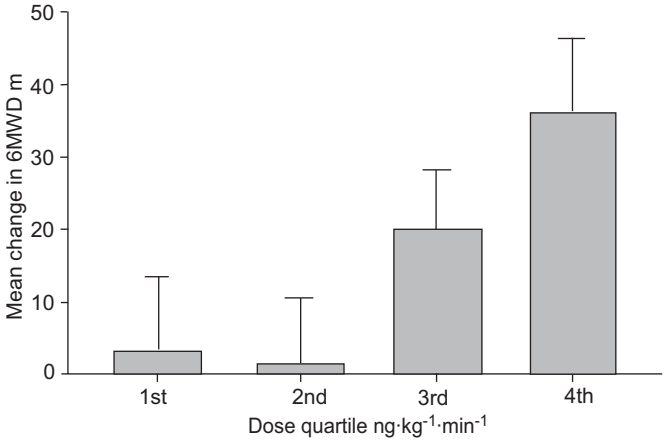

FIGURE 8. Change from baseline in 6-min walk distance (6MWD) with subcutaneous treprostinil according to dose quartile. The first quartile (n=45; mean±SD 3.3±10) corresponds to a dose of <5 $ng \cdot kg^{-1} \cdot min^{-1}$; the second quartile (n=49; mean±SD 1.4±9) to 5–<8.2 $ng \cdot kg^{-1} \cdot min^{-1}$; the third quartile (n=55; mean±SD 20±8) to 8.2–<13.8 $ng \cdot kg^{-1} \cdot min^{-1}$; and the fourth quartile (n=53; mean±SD 36.1±10) to ⩾13.8 $ng \cdot kg^{-1} \cdot min^{-1}$. Reproduced and modified from [39] with permission from the publisher.

40 ± 4 ng·kg^{-1}·min^{-1}. It is unclear whether the haemodynamic differences between treatments would have been less with further treprostinil up-titration and whether the haemodynamic changes after 12 weeks are clinically important long-term.

Inhaled treprostinil

VOSWINCKEL *et al.* [71] conducted three different studies in 123 patients to assess the pulmonary haemodynamics and gas exchange of inhaled treprostinil. The first study compared the haemodynamic and systemic side-effects of inhaled treprostinil with inhaled iloprost in an open-label randomised single-blind crossover study. Drug effects were monitored for 60 min after each inhalation session: iloprost at 4 μg·mL^{-1} (6-min inhalation time) and treprostinil at 4 μg·mL^{-1} (6-min inhalation time), 8 μg·mL^{-1} (6-min inhalation time) or 16 μg·mL^{-1} (3-min inhalation time). The second study estimated the pharmacodynamic and pharmacokinetic effects of inhaled treprostinil at 180 min and determined the maximally tolerated dose, with half the cohort receiving 30 μg treprostinil (16 μg·mL^{-1}) or placebo, and subsequent patients receiving 60 μg (32 μg·mL^{-1}) or 120 μg treprostinil (64 μg·mL^{-1}) for an inhalation time of 6 min. In the final study, the investigators sought to determine the shortest inhalation time for a 15-μg dose by a randomised, open-label, single-blind study.

Inhaled treprostinil had maximal effects occurring later than iloprost at mean$\pm$SEM 18 ± 2 min compared with 8 ± 1 min ($p<0.001$), and the effects lasted longer ($p<0.0001$): while the haemodynamic effects of iloprost had vanished within 1 h, they persisted for >2 h after treprostinil inhalation. A transient drop in systemic arterial pressure occurred after iloprost inhalation but not with treprostinil. The maximally tolerated dose with a near maximal acute decrease in PVR occurred with the 30-μg dose. After inhalation with a pulsed ultrasonic nebuliser with 18, 9, 3, 2 or 1 pulse (*i.e.* one breath), each mode achieved comparable, sustained vasodilation without significant side-effects. These studies demonstrated its ability to lower PVR with few inhalations and over a short period of time [71].

Comparison of clinical effects

It is difficult to directly compare the clinical effects and the side-effects of different prostanoids. This is due to differences in the enrolled patient populations, the mode of application and dosing, and trial design; double-blind *versus* open-label. Table 1 summarises the randomised controlled trials that have been performed. Dosing may be different in clinical practice compared with these studies. This is of particular interest for *i.v.* epoprostenol, and *s.c.* and *i.v.* treprostinil.

COMBINATION THERAPIES

Treatment of PAH by inhibiting multiple pathways concurrently may produce additive benefit. As prostacyclin therapy is neither curative, nor does it normalise pulmonary haemodynamics in the majority of cases. Investigators have examined combining a prostanoid with agents that act on the endothelin pathway and agents that increase cyclic guanosine monophosphate.

Prostacyclin and endothelin antagonists

In the Bosentan Randomized Trial of Endothelin Antagonist Therapy (BREATHE)-2 study [72], 33 subjects with PAH were started on epoprostenol with up-titration for 16 weeks and randomised in a 2:1 ratio to bosentan (62.5 mg twice daily for 4 weeks and then 125 mg twice daily) or placebo. There was a trend but no significant benefit in clinical or haemodynamic measurements. HOEPER *et al.* [73] studied 20 patients with iPAH that were on either inhaled iloprost or oral beraprost. Therapy of 3 months' duration with bosentan added to the prostacyclin resulted in improvement in exercise capacity (*i.e.* 6MWD) and maximal oxygen consumption.

The reverse order, addition of inhaled prostacylin to oral endothelin antagonist was used in the STEP trial (iloprost inhalation solution safety and pilot efficacy trial in combination with bosentan for evaluation in PAH), a double-blind placebo-controlled trial of 67 NYHA FC III and IV PAH patients on a stable dose of bosentan for 3 months [41]. Subjects were randomised to either iloprost 5 μg 6–9 times daily or placebo with 6MWD distance post-inhalation as the primary end-point. The mean improvement of 26 m *versus* placebo did not meet statistical significance ($p=0.051$), but improvements in secondary end-points, including FC and time to clinical worsening, favoured inhaled iloprost. Obtaining the 6MWD data pre-inhalation may have affected the outcome.

HOEPER *et al.* [74], in their recent 12-week Combination Therapy of Bosentan and Aerosolized Iloprost in Idiopathic Pulmonary Arterial Hypertension (COMBI) study, randomised stable iPAH patients NYHA FC III, currently on bosentan (125 mg twice daily) to either inhaled iloprost 5 μg six times daily or no additional therapy. The investigators powered the study with an assumption that addition of iloprost to bosentan would yield the same improvement as iloprost monotherapy compared with placebo, an increase in the mean$\pm$SD 6MWD distance of 45 ± 75 m. According to the interim analysis (36 completed of the 40 enrolled patients), a larger study could not demonstrate a statistically significant improvement in 6MWD for combination therapy leading to early trial termination.

Inhaled treprostinil in combination with oral therapies may be an additional theurapeutic option. Addition of investigational inhaled treprostinil at either 30 μg ($n=6$) or 45 μg ($n=6$) to oral bosentan in a small cohort found significant improvements in 6MWD, predominantly at the higher dose with nearly all patients (nine out of 11) improving FC [75]. Further studies with larger cohorts and longer follow-up are necessary in order to assess all these combination therapy approaches.

Prostacyclin and prostaglandin-5 enzyme inhibitors

Sildenafil, a prostaglandin-5 enzyme inhibitor, in combination with prostacyclin may be better than either agent alone. True synergistic effects are suggested [76]. GHOFRANI *et al.* [77] investigated the effect of adjunct therapy with sildenafil in patients on inhaled iloprost in 14 out of 73 PAH patients who were on monotherapy with inhaled iloprost. Over 9–12 months follow-up exercise capacity and haemodynamics improved. Open uncontrolled studies of addition of sildenafil to epoprostenol improved haemodynamics [78], and addition to treprostinil improved exercise capacity (*i.e.* exercise metabolic equivalents/treadmill time) and functional class [79]. A study by SIMONNEAU *et al.* [80] reported the results of a 16-week multinational, double-blind, placebo-controlled trial assessing the safety and efficacy of sildenafil in addition to epoprostenol (PACES). Patients had improvements in exercise capacity

(adjusted increase 26 m; $p<0.001$), haemodynamics (mean pulmonary artery pressure -3.9 mmHg; $p<0.0001$) and time to clinical worsening ($p=0.012$). The long-term open-label extension at 1 yr maintained this benefit [81].

Currently, two international randomised double-blind controlled trials are ongoing. The TRIUMPH study (Treprostinil Inhaled Used in Pulmonary Hypertension) investigates the effects of inhaled treprostinil in PAH patients on a stable therapy with bosentan. According to a press release (United Therapeutics, November 2007) this study showed highly significant, but modest effects in favour of treprostinil on 6MWD, the primary end-point of the study. The VISION study (Ventavis Inhalation with Sildenafil to Improve and Optimize pulmonary arterial hypertension) investigates the effects of inhaled iloprost in patients who have been on sildenafil therapy or sildenafil in combination with bosentan.

CONCLUSION

Prostanoid therapy is an efficacious therapy for pulmonary hypertension patients. With over 25 years of usage, the prostanoids remain critical agents in the therapeutic algorithm for the care of pulmonary arterial hypertension patients. Continued research will determine if combination therapy is more efficacious and, with new agents on the horizon, the need for continuous infusions may be obviated. The present authors are confident that experts and research will continue to determine the best therapies and approaches to treat pulmonary arterial hypertension patients.

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