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SERIES "PULMONARY HYPERTENSION: BASIC CONCEPTS FOR PRACTICAL MANAGEMENT"
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Phosphodiesterase inhibitors for the treatment of pulmonary hypertension

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ABSTRACT: The pulmonary vascular bed is both a source of and target for a number of vasoactive factors. Among the most important for pulmonary vascular homeostasis are factors that utilise cyclic guanosine monophosphate (cGMP) as an intracellular second messenger. These include nitric oxide and the natriuretic peptide family (atrial, brain and C-type natriuretic peptides). In the search for therapeutic strategies that engage the cGMP signalling pathway for the treatment of pulmonary arterial hypertension (PAH), inhibition of cGMP metabolism by phosphodiesterase type 5 (PDE5)-targeted compounds has proven most successful to date. One PDE5 inhibitor, sildenafil, has been shown to improve pulmonary haemodynamics and exercise capacity in patients with PAH and is now an approved treatment. Others are under investigation.

An interesting, although still tentative, observation is the potential of sildenafil to reduce pulmonary vascular resistance without adversely affecting ventilation–perfusion matching. Another is the expression of phosphodiesterase type 5 in the hypertrophied right ventricle. These data suggest that phosphodiesterase type 5 inhibitors may have effects that distinguish them from other treatments for pulmonary hypertension and merit further study.

KEYWORDS: Cyclic guanosine monophosphate, hypertension, phosphodiesterase inhibition, pulmonary

THE CYCLIC GUANOSINE MONOPHOSPHATE SIGNALLING PATHWAY

Nitric oxide (NO) is a highly reactive molecule synthesised from L-arginine by NO synthases (NOS): endothelial (eNOS), inducible (iNOS) and neuronal. Studies in knockout mice indicate that eNOS-derived NO is the principle mediator of endothelium-dependent vasodilation in the pulmonary circulation, but both eNOS and iNOS play a role in chronic modulation of basal tone [1]. NO diffuses into vascular smooth muscle cells, where it stimulates cyclic guanosine monophosphate (cGMP) production from soluble guanylyl cyclase (fig. 1).

The natriuretic peptides interact with specific cell membrane receptors [2]. Two subtypes of natriuretic peptide receptor (NPR), NPR-A and NPR-B, are transmembrane guanylyl cyclases (particulate guanylyl cyclases), where the extracellular domain binds atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) or C-type natriuretic peptide and the intracellular domain hydrolyses guanosine triphosphate (GTP) to cGMP.

Increasing evidence suggests that the intracellular distribution of cGMP is not uniform, but rather is compartmentalised within cells, and this may underlie the differential effects of cGMP generated by soluble and particulate guanylyl cyclases [3]. The diverse effects of stimulating cGMP production in cardiovascular tissues are dependent on its binding to at least three groups of proteins: cGMP-dependent protein kinase (or protein kinase-G; PKG), cGMP-regulated phosphodiesterases (PDEs) and cyclic nucleotide-gated ion channels

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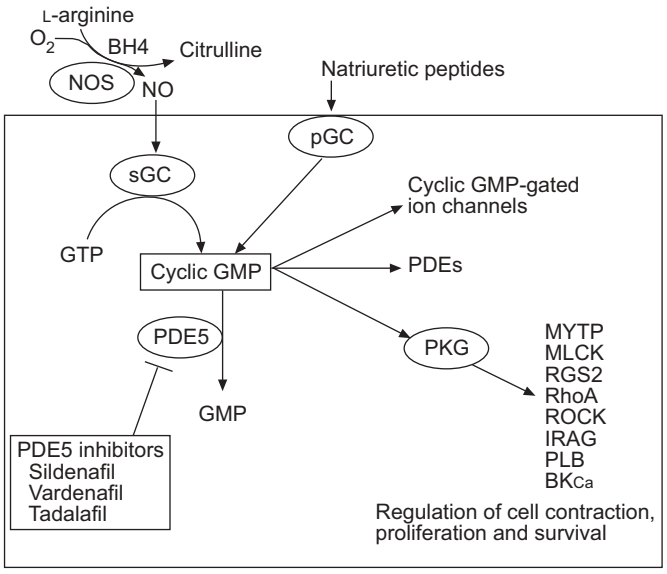

FIGURE 1. Schematic diagram of the nitric oxide (NO)–cyclic guanosine monophosphate (GMP) signalling pathway. NO is synthesised from L-arginine by NO synthases (NOS) with the cofactor tetrahydrobiopterin (BH4). In vascular smooth muscle cells, NO interacts with soluble guanylyl cyclase (sGC) to convert guanosine triphosphate (GTP) to cyclic GMP, which is hydrolysed and inactivated by phosphodiesterase type 5 (PDE5). Functions of cyclic GMP include the regulation of cation channels and PDEs and activation of cyclic GMP-dependent protein kinase (PKG). Activation of PKG results in the regulation of several downstream targets, including myosin phosphatase targeting subunit (MYPT), myosin light chain kinase (MLCK), regulator of G-protein signalling (RGS)2, RhoA, Rho kinase (ROCK), inositol 1,4,5-triphosphate receptor-associated PKG substrate (IRAG), phospholamban (PLB) and calcium-sensitive potassium channels (BK_{Ca}). PGC: particulate guanylyl cyclases.

[4]. PKG is the most important of these intracellular mediators, whereas the cGMP-regulated PDE type 5 (PDE5) is mainly responsible for modulating intracellular cGMP levels and PKG-dependent signalling produced by NO and natriuretic peptides [5–7]. In addition to being hydrolysed by PDE5, cGMP and its downstream mediator PKG also modulate the activity of this enzyme. Thus, cGMP binding affinity and hydrolysis are increased by PKG-induced phosphorylation of PDE5 [8]. Binding of cGMP to the so-called GAF-A (cGMP-activated PDEs, adenocyclase and Fh1A) domain also promotes phosphorylation of PDE5 and increases catalytic activity [9, 10]. In addition to these feedback mechanisms, there is the potential for gene regulation, as the promoter region of human PDE5 contains sites responsive to cyclic nucleotides [11, 12].

cGMP AS A REGULATOR OF PULMONARY VASCULAR HOMEOSTASIS

The pivotal role of the cGMP pathway in regulating pulmonary vascular tone is evident from pharmacological and genetic manipulation of this pathway *in vitro* and *in vivo*. For example, mice deficient in eNOS, GTP cyclohydrase-1 (the rate-limiting enzyme in tetrahydrobiopterin synthesis, a cofactor for NO production) or dimethylarginine dimethylaminohydrolase (the enzyme responsible for metabolising endogenous NOS inhibitors) have all been reported to exhibit pulmonary hypertension (PH) in a normally oxygenated atmosphere [13–15], while mice lacking NPR-A showed an exaggerated response to hypoxia [16]. There have been occasional conflicting reports [17]. Significantly, inhibition of NO production in humans using N^G-monomethyl-L-arginine, a competitive antagonist of NOS, resulted in an increase in pulmonary vascular resistance [18, 19].

In addition to regulating pulmonary vascular tone, it is also evident that cGMP levels influence pulmonary vascular structure directly, through effects on vascular smooth muscle proliferation and survival. Studies with human distal pulmonary vascular smooth muscle cells in culture have shown that elevation of cGMP, through stimulation of soluble guanylyl cyclase or by inhibition of its breakdown, inhibits serum-stimulated proliferation and promotes apoptosis [20].

Studies in humans have indicated that PH is associated with reduced endogenous NO production. Measurements of NO in exhaled breath were lower in patients with idiopathic pulmonary arterial hypertension (IPAH) than in healthy controls [21]. Histological examination of human lung samples has shown reduced expression of eNOS in pulmonary vessels from IPAH patients compared with healthy lung, although eNOS is well represented in plexiform lesions [22]. Studies in subjects at altitude have suggested that increasing or maintaining NO production in the lung is important for attenuating hypoxia-induced PH; those less able to do so are at most risk of developing PH [23]. In contrast, circulating ANP and BNP levels are elevated in all forms of PH, a consequence of pressure overload on the right ventricle and atrium stimulating the synthesis and release of these peptides [24–26]. This is a compensatory response, an attempt to reduce workload on the right heart by reducing pulmonary vascular resistance, but the magnitude of this endogenous response is insufficient to counteract the disease process. Finally, increased levels and reduced catabolism of asymmetric and symmetric dimethylarginines, known to be potent endogenous inhibitors of NOS, may contribute to PH in patients suffering from pulmonary vascular disorders [27, 28].

PDE EXPRESSION IN THE LUNG

In total, 11 PDE families have been identified, which vary in substrate affinity, selectivity and regulatory mechanisms [29]. Of these enzymes, PDE5, -6 and -9 are highly selective for cGMP, PDE1, -2, and -11 bind cGMP and cyclic adenosine monophosphate (cAMP) with equal affinity, and PDE3 and -10 are cGMP-sensitive but cAMP-selective. In the pulmonary circulation, PDE5 and -1 have attracted the most interest by virtue of their tissue distribution.

PDE5 mRNA is widely expressed in human tissues, but is most abundant in the lung and in pulmonary vascular smooth muscle cells [30]. Three splice variants have been identified (PDE5A1, PDE5A2 and PDE5A3), which encode proteins with similar cGMP catalytic activities and sensitivities to sildenafil, but distinct N-terminal sequences, and while PDE5A1 and A2 variants occur in most tissues, expression of the smallest splice variant, PDE5A3, seems to be limited to smooth muscle cells [31]. For all three molecular forms of PDE5, immunoreactivity has been demonstrated in lung homogenates and isolated pulmonary vascular smooth muscle cells [6, 31]. Expression of PDE5 and α-smooth muscle actin (SMA) proteins was found to be greater in lung tissues from patients with PH compared

with controls [20]. Specifically, expression of the predominant 98 kDa (PDE5A1) and 77 kDa PDE5 isoforms was significantly increased and PDE5 immunoreactivity colocalised with α-SMA in cells in intimal lesions and neomuscularised distal vessels, as well as in smooth muscle cells in the medial layer of the diseased pulmonary vasculature. These findings are consistent with an increase in PDE5 expression, as well as muscle mass, in the hypertensive pulmonary vasculature, and suggest that PDE5 may represent a marker of vascular remodelling, as well as a potential therapeutic target.

A recent report has extended these findings by demonstrating induction of PDE5 expression in the right ventricles of patients with PH [32]. PDE5 is normally expressed in the coronary vasculature but not in myocytes. Pressure overload on the myocardium leads to the expression of PDE5 in myocytes. Furthermore, studies *in vitro* have indicated that PDE5 inhibition enhances contractility of myocardium that expresses the enzyme, suggesting that PDE5 inhibition might directly improve right function in PH.

PDE1 is expressed at low levels in pulmonary tissues under normal conditions [33]. However, recent studies have shown significant upregulation of this enzyme in proliferating pulmonary vasculature, indicating a pathogenic role under disease conditions [33–35]. Interestingly, when analysing PDE1 expression in whole lung tissue homogenates, no significant increase was observed in PH *versus* normal control tissue. However, immunohistochemistry, as well as expression analyses from micro-dissected tissue, revealed strong upregulation of PDE1A and PDE1C in pre-capillary pulmonary arterial resistance vessels [33]. In the same study, inhibition of the PDE1 with 8-methoxymethyl 3-isobutyl-1-methylxanthine in animal models of PH improved haemodynamics, right heart hypertrophy and vascular remodelling [33]. Finally, the PDE1-selective inhibitor PI79 inhibited DNA synthesis in proliferating human pulmonary arterial smooth muscle cells in culture. Taken together, these findings indicate that upregulation of PDE1C plays a role in the structural remodelling process underlying severe PH and, thus, offers a novel therapeutic target. Interestingly, sildenafil, which is the approved PDE5 inhibitor for the treatment of pulmonary arterial hypertension (PAH), in addition to its PDE5 inhibitory capacity also has appreciable effects on PDE1 in clinically relevant dosages.

PDE5 INHIBITORS

Pharmacology

A number of drugs, including dipyridamole and theophylline, inhibit PDE5 as part of their spectrum of pharmacological activity. Three selective PDE5 inhibitors, sildenafil, tadalafil and vardenafil, have been licenced for use in humans and investigated for their effects on the pulmonary circulation. These drugs differ significantly in chemical structure but all inhibit with median inhibitory concentration (IC_{50}) values in the low nM range: sildenafil 1–9 nM [36–39]; tadalafil 1–7 nM [37, 39, 40]; and vardenafil 0.09–0.8 nM [37, 40–42]. The effect of sildenafil, tadalafil and vardenafil on other PDEs is shown in table 1. In brief, sildenafil also inhibits retinal PDE6 at ~10-fold higher concentrations and shows 80-fold selectivity over PDE1. Vardenafil also inhibits PDE6 (with a four- to 25-fold difference in selectivity) but has no significant effect on other PDEs. Tadalafil has little effect on PDE6 but inhibits PDE11 with a five-fold difference in IC_{50}. Tadalafil, in contrast to sildenafil, has no effect on PDE1c.

Pharmacokinetics of selective PDE5 inhibitors

Sildenafil absorption is rapid and ~95% following a single oral dose, although absolute bioavailability is reduced to ~40% because of first-pass metabolism [43]. The time to maximum concentration (T_{max}) in the plasma is ~1 h, but this increases further, by ~60 min, if taken with a high-fat meal, and peak plasma concentration falls by 29% (with no effect on area under curve). This is most likely a result of delayed gastric emptying. The plasma half-life is ~4 h but, taking into account the IC_{50} for inhibition of PDE5 *in vitro* and the high protein binding of the parent drug, the plasma concentration of sildenafil is maintained above the IC_{50} for 12–18 h following a 100-mg dose. The main route of clearance is by hepatic metabolism, as expected for a relatively lipophilic drug. The major metabolite is *N*-desmethylsildenafil. This metabolite has the same PDE specificity as sildenafil but about half the potency, and plasma levels are ~40–50% of the parent drug [43, 44].

Studies with human liver microsomes and microsomes expressing individual human cytochrome P (CYP) enzymes have indicated that 75% of the metabolism of sildenafil is due to CYP3A4 and the remainder to CYP2C9 [45–47]. These studies predict a significant drug–drug interaction through CYP3A4, and this has been found in practice. Co-administration of potent CYP3A4 inhibitors, such as erythromycin, ketoconazole and saquinavir, results in significant increases

TABLE 1 Comparison of inhibitory compound selectivity for phosphodiesterase (PDE) family members

Compound	PDE1	PDE5	PDE6 (rod)	PDE6 (cone)	PDE7	PDE9	PDE11
Sildenafil	281 (80)	3.5	37 (11)	34 (10)	21300 (6100)	2610 (750)	2730 (780)
Vardenafil	70 (500)	0.14	3.5 (25)	0.6 (4)	>30000 (>214000)	580 (4150)	162 (1160)
Tadalafil	>30000 (>4450)	6.74	1260 (187)	1300 (193)	>100000 (>14800)	>100000 (>14800)	37 (5)

Data are presented as median inhibitory concentration values in nM (fold selectivity *versus* PDE5).

in the peak plasma concentration and area under curve of sildenafil, with no effect on plasma half-life; this is consistent with inhibition of pre-systemic metabolism without effect on elimination. Sitaxentan, a weak CYP3A4 inhibitor *in vitro*, has little effect on sildenafil levels. Conversely, co-treatment with bosentan, a CYP3A4 inducer, significantly reduces plasma sildenafil levels (by ~50%) at therapeutic bosentan doses. Sildenafil is a weak inhibitor of several major drug-metabolising enzymes, but the plasma concentrations reached, even following maximum recommended dosing (100 mg), are unlikely to be clinically important. Nonetheless, competition with co-administered bosentan, through CYP3A4 metabolism, leads to a significant increase in bosentan levels. Modestly increased plasma sildenafil levels are seen in elderly patients (aged >65 yrs) and patients with hepatic or severe renal impairment, as a result of reduced drug clearance.

Oral absorption of tadalafil is rapid, with a T_{max} in plasma of ~2 h. Bioavailability is 36%, protein binding (principally α1-acid glycoprotein and albumin) is 94% and the plasma half-life is 17.5 h. Systemic exposure increases linearly over the daily dose range of 2.5–20 mg and, at steady state (~5 days), plasma levels are 1.6-fold higher than at the start of treatment.

Tadalafil is cleared by oxidative metabolism, mainly by CYP3A4, to a catechol, which then undergoes methylation and glucuronidation. The methyl catechol glucuronide is 13,000-fold less potent for PDE5 than the parent molecule. Plasma tadalafil T_{max} and half-life are increased slightly in renal failure. The main metabolite, which is renally excreted, shows a significant increase in half-life with progressive renal impairment (36 h in health to 54–77 h in end-stage disease). CYP3A4 inhibitors reduce tadalafil clearance; for example, therapeutic doses of ketoconazole increase tadalafil area under curve by 4.1-fold and maximum concentration (C_{max}) by 1.2-fold. Studies *in vitro* predict that tadalafil would not reversibly inhibit the metabolism of other drugs metabolised by the major human CYPs, but has the potential to be a weak mechanism-based inhibitor and an inducer of CYP3A4. However, in clinical studies, tadalafil at 10 and 20 mg had little effect on the clearance of the other CYP3A4 substrates, midazolam and lovastatin [48].

Vardenafil is rapidly absorbed (T_{max} ~40 min) and metabolised, with a plasma half-life of ~4 h and an absolute bioavailability of 14.5% (compared with 40% for sildenafil and vardenafil). Systemic exposure is linear over the dose range 5–20 mg. A high-fat (>55% fat calories) breakfast modestly reduces C_{max} by 18% and prolongs the T_{max} of vardenafil by ~1 h, while a moderate-fat meal (30% fat calories) has no significant effects on vardenafil pharmacokinetics. As with other selective PDE5 inhibitors, the major route of metabolism is *via* hepatic enzymes, including CYP3A4. The major metabolite, an *N*-desethyl derivative, and other minor metabolites are removed principally by biliary excretion. Co-administration of CYP3A4 inhibitors, such as ritonavir, can affect hepatic metabolism. The *N*-desethyl derivative is a four-fold less potent inhibitor of PDE5 than its parent compound, contributing ~7% to the overall efficacy of vardenafil.

PRE-CLINICAL STUDIES

PDE5 inhibitors inhibit the pressor response to acute hypoxia and the thromboxane antagonist, U46619, in isolated rodent lung preparations [49–52]. Further support for PDE5 as a therapeutic target in PH is evident from chronic dosing studies in animal models of the condition. The most commonly used are chronic hypoxia-induced and monocrotaline-induced rodent models. These studies show that PDE5 inhibition attenuates the rise in pulmonary artery pressure and vascular remodelling if given before exposure to hypoxia or monocrotaline (preventative strategy) and partially reverses the pathology if given up to 2 weeks after inducing PH (treatment strategy) [53, 54]. Right ventricular hypertrophy is also reduced and survival improved. Studies in genetically manipulated mice reveal that both eNOS and natriuretic peptides contribute to the therapeutic effects of PDE5 inhibition [16, 52].

PDE5 inhibition has also been examined in combination with other treatments. Sildenafil has been combined with beraprost, a prostacyclin analogue [55]. The two drugs were given by oral gavage singly or in combination twice daily for 3 weeks, commencing from the time of administration of monocrotaline. Both drugs attenuated the development of PAH, but the combination of the two drugs had additive effects on plasma cAMP and cGMP, pulmonary haemodynamics and vascular remodelling. In a study of the effect on survival, again combination treatment appeared to be superior, with 100% survival at 6 weeks, compared with 30% survival on monocrotaline alone, 90% when monocrotaline- and sildenafil-treated and 80% when monocrotaline- and beraprost-treated. The combination of sildenafil and bosentan has also been reported to be superior to either treatment alone in the monocrotaline-treated rat model [56].

CLINICAL PHARMACOLOGY OF SELECTIVE PDE5 INHIBITORS

Consistent with the vascular distribution of PDE5, systemic administration of PDE5 inhibitors produces measurable effects on the cardiovascular system, coupled with increases in plasma and urinary cGMP levels.

Studies in healthy males have shown that sildenafil, in single doses of 100–200 mg, produces a modest fall in both systolic and diastolic blood pressure; the mean maximum decrease in systolic/diastolic blood pressure was 10/7 mmHg at 3 h, returning to baseline by 6 h, with no dose–response relationship [57]. There were no accompanying changes in heart rate or cardiac index. The plasma cGMP area under curve increased by ~50% from baseline. Reductions in mean systolic blood pressure of 1.4-6.6 mmHg and in mean diastolic blood pressure of 2-4.8 mmHg have been reported with vardenafil in males with erectile dysfunction [58]. In a cross-over study in males with erectile dysfunction, both sildenafil 50 mg and vardenafil 10 mg produced significant reductions in systemic blood pressure. Greater variability in response was noted with vardenafil, with three patients reporting fainting following the first dose [59]. Little change in systemic blood pressure was observed in healthy volunteers treated with tadalafil 20 mg or in males aged >45 yrs with erectile dysfunction treated daily for 26 weeks [60]. Co-treatment with other vasodilator agents (nitrates in particular but also doxazosin) augmented the fall in blood pressure [60, 61]. The effect of nitrates is most likely mediated by enhancing the NO–cGMP pathway [60, 61].

In patients with stable ischaemic heart disease, intravenous infusion of sildenafil (up to 40 mg over 60 min) produced small reductions in right atrial pressure, pulmonary arterial occluded pressure and systolic and diastolic systemic arterial pressures at rest, but no change in heart rate [57]. The per cent changes from baseline were -28% for right atrial pressure, -27% for pulmonary arterial pressure, -20% for pulmonary arterial occluded pressure, -6% for systolic systemic arterial pressure and -10% for diastolic systemic arterial pressure. There was also a small (7%) reduction from baseline in cardiac output. Small, but significant, dose-related reductions in standing blood pressure were reported in males with ischaemic heart disease, 2 h (*i.e.* at T_{max}) after single oral doses (5 or 10 mg) of tadalafil [60].

During an exercise test performed after a sildenafil infusion (up to 40 mg over 60 min), maximum pulmonary arterial occluded pressure and mean pulmonary arterial pressure were reduced by 23 and 19%, respectively, from the measurements during exercise pre-infusion. Systolic and diastolic systemic arterial pressures both decreased by 6%, cardiac output decreased by 11% and heart rate was not affected by the sildenafil treatment. In similar patient populations, vardenafil 10 mg and tadalafil 10 mg did not alter exercise treadmill time or time to first awareness of angina, and did not alter blood pressure or heart rate achieved [62, 63].

Single 50-mg oral doses of sildenafil have been reported to prevent endothelial dysfunction induced by ischaemia and reperfusion in healthy volunteers [64] and in patients with chronic heart failure [65, 66], when measured using a flow-mediated dilation protocol. Administration of 20 mg tadalafil on alternate days for 4 weeks has been reported to improve endothelial function in males with increased cardiovascular risk (10-yr cardiovascular risk >20%) [67]. The effect of sildenafil was blocked by glibenclamide, suggesting that the effect of sildenafil was a result of opening adenosine triphosphate–sensitive potassium channels [64].

The effects of single oral therapeutic and supratherapeutic doses of sildenafil (50 and 400 mg) and vardenafil (10 and 80 mg) on the Q–T interval corrected for heart rate (QT_c) have been examined in a well-designed study in healthy males [68]. Placebo-corrected mean changes in QT_c were calculated by two methods. Mean (range) Fridericia-corrected QT_c durations at 1 h after dose were 8 (6–9) ms for vardenafil 10 mg and 6 (5–8) ms for sildenafil 50 mg. Individualised QT_c values yielded similar trends: 4 (3–6) ms for vardenafil 10 mg and 4 (2–5) ms for sildenafil 50 mg. Small increases were noted at the higher dose but the dose–response relationship was very shallow and it was concluded that the small increases in QT_c with these PDE5 inhibitors were clinically insignificant.

PDE5 is abundant in platelets and PDE5 inhibition abbrogates platelet aggregation [69, 70]. An effect with sildenafil is detectable at higher doses. Studies in platelet-rich plasma from healthy volunteers have detected prolongation of platelet aggregation time in response to collagen at 1 h but not at 4 h following a single 100-mg oral dose [71]. No effect was seen with 50 mg. Sildenafil did not inhibit adenosine diphosphate-induced aggregation at either dose.

STUDIES IN PATIENTS WITH PH

Typical of pharmacological studies in PH, the majority of studies have involved patients with IPAH or PAH associated with connective tissue disease and in World Health Organization (WHO) class III.

Acute effects

Sildenafil reduces pulmonary vascular resistance in a dose-dependent manner when given as a single oral dose [72]. Notably, the vasodilator effects are most pronounced in the pulmonary circulation, compared with the systemic circulation, and at least as strong as with inhaled NO at clinically relevant doses [73, 74]. Moreover, sildenafil increases and prolongs the effects of inhaled iloprost [72, 75].

A comparison of the acute responses to sildenafil, tadalafil and vardenafil suggests that each agent has to be assessed individually, despite their common classification as PDE5 inhibitors [76]. In total, 60 consecutive PAH patients (New York Heart Association functional class II–IV) were assigned to oral intake of 50 mg sildenafil (n=19), 10 mg (n=7) or 20 mg (n=9) vardenafil, or 20 mg (n=9), 40 mg (n=8), or 60 mg (n=8) tadalafil. Haemodynamics and changes in oxygenation were assessed over a subsequent 120-min observation period. All three PDE5 inhibitors caused significant pulmonary vasorelaxation, with maximum effects being obtained after 40–45 min (vardenafil), 60 min (sildenafil) and 75–90 min (tadalafil). Sildenafil and tadalafil, but not vardenafil, caused a significant reduction in the pulmonary to systemic vascular resistance ratio. Significant improvement in arterial oxygenation (equal to that achieved by NO inhalation) was only noted with sildenafil. It was concluded that the three PDE5 inhibitors differ markedly in their kinetics of pulmonary vasorelaxation (the most rapid effect being achieved by vardenafil), their selectivity for the pulmonary circulation (sildenafil and tadalafil being selective, but not vardenafil), and their impact on arterial oxygenation (improvement with sildenafil only). At the time when the present review was drafted, a large randomised placebo controlled trial assessing the effects of chronic tadalafil treatment in patients with PAH had just completed recruitment (ClinicalTrials.gov Identifier NCT00125918) [77].

Chronic treatment

Several small single-centre studies have reported beneficial effects from long-term treatment of patients with PAH with sildenafil [78–81] (tables 2, 3 and 4). One study compared sildenafil treatment with bosentan therapy using cardiac magnetic resonance-based measurements of right ventricular mass as an end-point [80]. There were no significant differences between the two treatments (table 3). Both improved 6-min walk distance but only sildenafil significantly reduced right ventricular mass over the 4-month period of study, an effect accompanied by a reduction in circulating BNP levels. These data are of particular interest in the light of recent reports of the induction of PDE5 expression in the right ventricle in PH [32] and the finding that sildenafil improves right ventricle function in patients with heart failure complicated by secondary PH [82].

The landmark study for sildenafil in PH was the Sildenafil Use in Pulmonary Arterial Hypertension (SUPER-1) study [81] (table 4). This study examined 278 patients with symptomatic

TABLE 2 Phosphodiesterase type 5 inhibitors; summary of the randomised study by SASTRY *et al.* [79]

	Study outline	Patients	Main outcome measures		Side-effects	Comments
			Placebo-corrected change	p-value		
	Prospective, randomised, double blind crossover design. Oral sildenafil (weight-based regimen 25–100 mg *t.i.d.*) *versus* placebo. Duration 12 weeks.	22 patients (10 males, 12 females). 22 IPAH.	After 12 weeks (6 week crossover period): 1 death, 1 withdrawal.		Backache, headache, constipation and numbness reported more frequently but no significant differences.	Systolic PAP measured using echocardiography. Exercise capacity measured by treadmill. QoL measured using chronic heart failure questionnaire.
Age yrs		16–55				
Functional class II/III		18/4				
Mean exercise time s		440	+211	<0.0001		
QoL score						
Dyspnoea		22	+4	0.009		
Fatigue		20	+2	0.04		
Emotional		34	+3	0.06		
Mean systolic PAP mmHg		107	-7	0.09		
Mean cardiac index L·min^{-1}·m^{-2}		2.8	+0.65	<0.0001		

IPAH: idiopathic pulmonary arterial hypertension; PAP: pulmonary artery pressure; QoL: quality of life.

PAH treated either with placebo or oral sildenafil (20, 40 or 80 mg *t.i.d.*) for 12 weeks. The primary end-point was the change from baseline to week 12 in the 6-min walk test. All three doses of sildenafil improved exercise capacity (up to 50 m in the 80 mg group, after correction for placebo), functional class and haemodynamics, compared with placebo-treated patients, and the drug was very well tolerated. The increase in the 6-min walk distance achieved after 3 months in the placebo-controlled phase was maintained in those patients who continued on open-label treatment (80 mg *t.i.d.*). Based on these data, sildenafil 20 mg *t.i.d.* was approved by the US Food and Drug Administration and the European Agency for the Evaluation of Medicinal Products in 2005 for the treatment of patients suffering from PAH. The approval of the lowest study dose was based on the flat (nonsignificant) dose–effect relationship between 20–80 mg *t.i.d.* for the primary end-point of the study.

The decision to license only the 20 mg *t.i.d.* dose for PH has caused some difficulties for physicians managing these patients. First, the interaction with bosentan means that, in combination, the effective dose of sildenafil is halved and, therefore, below the approved treatment dose. The regulatory authorities have requested a further study of sildenafil using a lower dose, 10 mg *t.i.d.*, and a combination study with bosentan. Secondly, many physicians feel that, over time, with progression of the disease, the dose of sildenafil needs to be increased and a target dose of 50 mg *t.i.d.* has been proposed [83]. In support of this, the 80 mg *t.i.d.* dose did have a greater effect on pulmonary vascular resistance [81]. In addition, there remains an outstanding question as to whether *t.i.d.* dosing is required. This was based on plasma half-life of the drug, but there is evidence from some clinical and experimental settings that the duration of action of sildenafil might not be accurately reflected by plasma levels [84]. The increased affinity of sildenafil for PDE5 after intracellular phosphorylation of the enzyme, and the slow dissociation rate of sildenafil from the enzyme, could contribute to the flat dose–effect relationship [40, 85]. Clearly, there is more to be done to define the best dose regimen for patients with PH, as well as to determine the effect on survival.

Studies to establish the effect of sildenafil in combination with other drugs have been encouraging but are unblinded. The combination of sildenafil with bosentan is being used with increasing frequency, with reports of benefit (*i.e.* rescuing patients from deterioration) in the short term despite the concerns over pharmacokinetic interactions [86, 87]. The combination may be used without increasing hepatotoxicity from increasing plasma bosentan levels. Combination of sildenafil with sitaxentan or ambrisentan would have more predictable pharmacokinetics (as there is no clinically significant interaction) but no pharmacodynamic data from such combinations are currently available. Clinically, sildenafil has been combined with inhaled iloprost both acutely [72] and as long-term therapy [88], showing beneficial additive effects without increasing the side-effects of either agent. In the future, the combination of a PDE5 inhibitor and an agent that augments cGMP production may be therapeutically advantageous [73, 74].

Interestingly, sildenafil also seems to be effective for treating patients with PH associated with some other diseases. In patients suffering from HIV-related PH, sildenafil was effective in reducing pulmonary vascular resistance [89, 90]. Recent

TABLE 3 Phosphodiesterase type 5 inhibitors; summary of the randomised study by WILKINS *et al*. [80]

	Study outline	Patients		Main outcome measures			Side-effects	Comments
	Prospective, randomised, double blind. Oral sildenafil (50 mg *t.i.d.*) *versus* bosentan (125 mg *b.i.d.*). Duration 16 weeks.	26 patients (5 males, 21 females). 22 IPAH, 3 CTD. 14 received sildenafil; 12 received bosentan.		After 16 weeks: 1 death in sildenafil group; per protocol analysis.			3 patients on bosentan had admissions to hospital, 2 with fluid retention.	First head-to-head study. Used right ventricle mass (measured by cardiac magnetic resonance) as primary end-point. No difference between treatments based on intention-to-treat analysis, but sildenafil produced a significantly greater reduction in right ventricle mass on per protocol analysis. Kansas Cardiomyopathy QoL assessment used.
				Change from baseline		Treatment effect		
		Bos	Sil	Bos	Sil			
Mean age yrs		41	44					
Functional class		III	III					
Mean systolic PAP mmHg		91	96					
Mean 6MWD m		304	290	+59	+114	+55*		
Mean right atrial volume mL		87	83	+4	-4	-8#		
Mean right ventricle mass g		134	160	-3	-8.8	-5.8#		
Mean cardiac index $L \cdot min^{-1} \cdot m^{-2}$		2.2	2.3	+0.3	+0.3	0#		
Mean Borg score		3.7	4.8	+0.2	-1.5	-1.7#		
Mean BNP $fmol \cdot mL^{-1}$		50	67	-6	-19	-13#		
QoL score		44	36	+6	+27	+22¶		

Bos: bosentan; Sil: sildenafil; IPAH: idiopathic pulmonary arterial hypertension; CTD: connective tissue disease; QoL: quality of life; 6MWD: 6-min walking distance; PAP: pulmonary arterial pressure; BNP: brain natriuretic peptide. #: nonsignificant; ¶: $p<0.002$; * $p<0.05$.

data also suggest that long-term oral sildenafil treatment is beneficial in patients with non-operable chronic thromboembolic PH [91, 92].

HYPOXIA-ASSOCIATED PH

There is interest in the use of PDE5 inhibitors for the treatment of PH associated with hypoxia. An early study showed that a single oral dose of sildenafil (50 mg) inhibited the acute pressor response to hypoxia (11% inspired oxygen) in healthy volunteers [60]. To address this further, several studies have been conducted in subjects at altitude [93–95].

In one such study, systolic pulmonary artery pressure, cardiac output and peripheral arterial oxygen saturation were measured in 14 healthy mountaineers and trekkers at rest and during assessment of maximum exercise capacity on cycle ergometry, first while breathing a hypoxic gas mixture with 10% inspired oxygen at low altitude and secondly at high altitude (the Mount Everest base camp) [93]. Subjects were assigned sildenafil 50 mg or placebo. Sildenafil treatment significantly reduced systolic pulmonary artery pressure at rest and during exercise in both scenarios, while increasing cardiac output and maximum workload.

Another study compared the effect of sildenafil 40 mg *t.i.d.* for 6 days with placebo in 12 subjects housed at 4,350 m [94]. Treatment was started 6–8 h after arrival from sea level to high altitude. Systolic pulmonary artery pressure (measured by ECG) increased at high altitude before treatment (+29% *versus* sea level) but normalised on sildenafil, while remaining elevated in the placebo group. Arterial oxygen tension was higher and alveolar–arterial difference in oxygen tension was lower on sildenafil than on placebo at rest and during exercise, and the altitude-induced decrease in maximal oxygen consumption was smaller on sildenafil. These data suggest that sildenafil protects against the development of altitude-induced PH and improves gas exchange, limiting the altitude-induced hypoxaemia and decrease in exercise performance.

One study has examined the effect of sildenafil in patients with PH living 2,500 m above sea level in the Kyrgyz Republic [96]. At 3 months, patients on sildenafil 25 mg *t.i.d.* had a significantly lower mean pulmonary artery pressure (6.9 mmHg lower) at the end of the dosing interval than those on placebo. A similar effect was seen with sildenafil 100 mg *t.i.d*. Both doses improved 6-min walk distance, the lower dose by 45.4 m and the higher dose by 40.0 m.

It would be inappropriate to extrapolate from these studies to the treatment of PH associated with chronic obstructive pulmonary disease (COPD) and interstitial fibrosis. Nonetheless, there is interest in the use of PDE5 inhibitors in the management of these conditions. One concern with vasodilators in the presence of pulmonary disease is perturbation of ventilation–perfusion (V'/Q') matching. Nonspecific vasodilators can cause vasodilatation and increase perfusion of poorly ventilated alveoli, thereby reducing arterial oxygen saturation. There is some evidence that PDE5 inhibitors may preserve V'/Q' matching [97]. Alveolar oxygenation is an important determinant of local NO production (and so of cGMP levels). Poorly ventilated areas of lung would be expected to have lower NO and cGMP levels and so the local effect of PDE5 inhibition would be less than in well-ventilated lung. In a randomised, controlled, open-label trial, in 16

TABLE 4 Summary of Sildenafil Use in Pulmonary Arterial Hypertension (SUPER-1) study [81]

Study outline	Patients		Main outcome measures				Side-effects	Comments
Prospective, randomised, double blind. Oral sildenafil (20, 40 or 80 mg *t.i.d.*) *versus* placebo. Duration 12 weeks. Open-label extension (sildenafil 80 mg *t.i.d.*).	278 patients (69 males, 209 females). 175 IPAH, 84 CTD, 18 CHD.		After 12 weeks: 4 deaths, 9 withdrawals				Main side-effects were headache, flushing and dyspepsia. Visual disturbance dose dependent; not reported at 20 mg. Only 2 serious events considered attributable to Sil: 1 left-heart dysfunction; 1 postural hypotension.	No difference noted between sub-groups. No significant difference in 6MWD between doses, but significant difference in improvement in functional capacity and haemodynamics. Extension study (median follow-up 589 days) with 259 patients: 14 deaths, 15 withdrawals at 1 yr; 8 received additional therapy. Mean change in 6MWD 51 m at 1 yr with overall 1-yr survival 96%.
	Placebo	**Sil**	**Placebo**	**Sil 20**	**Sil 40**	**Sil 80**		
Subjects n	70	207	70	69	67	71		
Mean age yrs	49	51						
FC I:II:III:IV	1:32:34:3	0:75:126:6						
			Placebo-corrected change					
Mean 6MWD m	344	344		+45#	+46#	+50#		
			Change from baseline					
Mean RAP mmHg	9	9	+0.3	-0.8	-1.1	-1.0		
Mean PAP mmHg	56	52	+0.6	-2.1*	-2.6*	-4.7#		
Mean PVR dyn·s·cm^{-5}	1051	925	+49	-122*	-143*	-261#		
Mean cardiac index L·min^{-1}·m^{-2}	2.2	2.4	0	+0.21	+0.24*	+0.37#		
Improvement in FC %			7	28#	36#	42#		

Sil: sildenafil; IPAH: idiopathic pulmonary arterial hypertension; CTD: connective tissue disease; CHD: congenital heart disease; 6MWD: 6-min walking distance; FC: functional class; RAP: right atrial pressure; PAP: pulmonary arterial pressure; PVR: pulmonary vascular resistance. #: $p \leqslant 0.001$; *: $p < 0.05$ *versus* placebo. No significant differences noted between dosage groups.

individuals with PH secondary to lung fibrosis, a single oral dose of sildenafil 50 mg reduced the pulmonary vascular resistance index and improved V'/Q' matching, as measured using multiple inert gas elimination. Shunt flow and perfusion of low V'/Q' areas decreased under this agent, resulting in a significant increase in partial arterial pressure of oxygen [97].

The results of these studies have stimulated further investigations to address the therapeutic potential of sildenafil in patients suffering from chronic hypoxic pulmonary hypertension associated with COPD, interstitial lung disease and obstructive sleep apnoea [98–102]. In fact, very recent work supports the possibility of effective treatment of pulmonary hypertension in patients suffering from advanced COPD [102]. Based on the significant impact of COPD on public health, further definitive studies in this field are warranted.

CONCLUSION

The availability of orally active PDE5 inhibitors has added another dimension to the treatment of PH. The data for sildenafil demonstrate clinically significant improvements in haemodynamic and exercise capacity over several months in patients with IPAH and PH associated with connective tissue disease and life at high altitude. The evidence base indicates that sildenafil is a first- or second-line treatment option for patients who present with IPAH in WHO class II and III. It may have a role in the treatment of other presentations of PH and as an add-on therapy, but patients who present in WHO class IV are best treated with a prostanoid.

Of great interest is the idea that PDE5 inhibition may differ from other available treatments for PH in two respects. One is the potential to reduce pulmonary vascular resistance without adversely affecting V'/Q' matching, a property which would lend itself to the treatment of PH associated with lung disease. The second is the finding of PDE5 in the hypertrophied right ventricle of patients with PH. A direct effect on the right ventricle which enhances contractility and cardiac output is a potentially valuable attribute, given the importance of right ventricular function as a determinant of prognosis in PH.

However, care must be taken to avoid over-interpretation of existing data. Further studies are needed in order to examine these possibilities, and there is an urgent need for more information on long-term survival on sildenafil. A final word of caution: given that there are differences between phosphodiesterase type 5 inhibitors in their acute effects on the

pulmonary circulation that may be related to their phosphodiesterase specificity, careful examination of the clinical effects of chronic treatment with each phosphodiesterase type 5 inhibitor is warranted.

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