6 Phosphodiesterase-5 inhibitors in pulmonary arterial hypertension

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6.1 The origins of phosphodiesterase-5 inhibitor development

Nitrate drugs are an exogenous source of nitric oxide (NO), a labile gas that can diffuse across cell membranes into vascular smooth muscle cells where it stimulates the action of soluble guanylate cyclase to convert guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) (Moncada, Palmer and Higgs, 1991). The formation of cGMP initiates a cascade of reactions that decreases intracellular calcium levels, thereby promoting vascular smooth muscle relaxation (Pfeifer et al., 1999; Lucas et al., 2000). Thus nitrates act as mixed dilators of arteries and veins. The resulting decrease in peripheral vascular resistance and cardiac preload, coupled with improved perfusion of ischemic myocardium, led to its clinical development for angina. However, the therapeutic potential of nitrates is limited by the rapid induction of tachyphylaxis with prolonged administration (Parker and Parker, 1998). Although the precise mechanism of tolerance to nitrates is not clear,
treatment that does not directly increase NO levels might circumvent this problem. Thus, it was hypothesized that a downstream target in the NO/cGMP pathway could be modulated. Cyclic nucleotides (cAMP and cGMP) are degraded by intracellular phosphodiesterases (PDEs) (Plate 4). To date, eleven PDE subtypes have been recognized. PDE3 and PDE4 catalyze the breakdown of cAMP, while PDE1 and PDE2 catalyze the breakdown of both cAMP and cGMP. The fifth member of this group, PDE5, catalyzes the breakdown of cGMP (Tables 6.1 and 6.2). PDE5 is present in the smooth muscle of the systemic vasculature and in platelets. Studies by Corbin and coworkers demonstrated that the regulatory domain in the amino-terminal portion of PDE5 contains the phosphorylation site (Ser-92), the two allosteric cGMP-binding sites, and at least a portion of the dimerization domain. The catalytic domain in the carboxyl-terminal portion of the protein contains the two Zn$^{2+}$-binding motifs A and B, and a cGMP substrate-binding site (Plate 5) (Corbin and Francis, 1999).

The introduction of sildenafil to the market revolutionized the treatment of erectile dysfunction (ED), and within a few weeks of approval, over one million patients in the US had received prescriptions for sildenafil. The first-line treatment of ED began to move from specialists such as urologists and psychiatrists to a general practice setting and in 1998, case reports of myocardial infarction (MI), stroke and sudden death were reported in patients taking sildenafil for ED. However, subsequent clinical trials and epidemiological studies have not demonstrated that sildenafil provokes MI or stroke when used in accordance with the prescribing instructions (Herrmann et al., 2000; Arruda-Olsen et al., 2002; DeBusk et al., 2004; Wysowski, Farinas and Swartz, 2002; Boshier, Wilton and Shakir, 2004). Indeed there are now multiple scientific papers suggesting a potential utility of sildenafil.

### Table 6.1 PDE Nomenclature and families

<table>
<thead>
<tr>
<th>PDE Family</th>
<th>(number of splice variants)</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A (4), B (1), C (5)</td>
<td>cAMP/cGMP</td>
</tr>
<tr>
<td>2</td>
<td>A (3)</td>
<td>cAMP/cGMP</td>
</tr>
<tr>
<td>3</td>
<td>A (1), B (1)</td>
<td>cAMP/cGMP</td>
</tr>
<tr>
<td>4</td>
<td>A (8), B (3), C (4), D (5)</td>
<td>cAMP</td>
</tr>
<tr>
<td>5</td>
<td>A (3)</td>
<td>cGMP</td>
</tr>
<tr>
<td>6</td>
<td>A (1), B (1), C (1)</td>
<td>cGMP</td>
</tr>
<tr>
<td>7</td>
<td>A (3), B (1)</td>
<td>cAMP</td>
</tr>
<tr>
<td>8</td>
<td>A (5), B (1)</td>
<td>cAMP</td>
</tr>
<tr>
<td>9</td>
<td>A (6)</td>
<td>cGMP</td>
</tr>
<tr>
<td>10</td>
<td>A (2)</td>
<td>cAMP/cGMP</td>
</tr>
<tr>
<td>11</td>
<td>A (4)</td>
<td>cAMP/cGMP</td>
</tr>
</tbody>
</table>

PDE: phosphodiesterase; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate.
<table>
<thead>
<tr>
<th>PDE family</th>
<th>Role(s)</th>
<th>Evidence&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vascular smooth muscle proliferation; Ca&lt;sup&gt;2+&lt;/sup&gt; modulation of olfaction</td>
<td>Broad distribution, but highest levels in proliferating vascular smooth muscle cells, testes, heart, and neural tissues (e.g., olfactory epithelial cells); binding and inactivation by Ca&lt;sup&gt;2+&lt;/sup&gt;/calmodulin</td>
</tr>
<tr>
<td>2</td>
<td>Regulation of Ca&lt;sup&gt;2+&lt;/sup&gt; channels, olfaction, platelet aggregation, and aldosterone secretion</td>
<td>Broad distribution, but highest levels in brain and adrenal cortex&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Cardiac contractility, insulin secretion, and lipolysis</td>
<td>Broad distribution, but particular abundance in adipose tissue, liver, cardiac muscle, vascular smooth muscle, and platelets; inhibited by drugs with cardiotonic, vasodilatory, thrombolytic, and antiplatelet aggregation properties; stimulated by insulin, leptin, and insulin-like growth factor</td>
</tr>
<tr>
<td>4</td>
<td>Immunological and inflammatory signaling processes; smooth muscle tone; depression</td>
<td>Broad distribution, highest levels in neural and endocrine tissue; inflammatory cells thought to participate in the pathogenesis of inflammatory diseases (i.e., asthma and chronic obstructive pulmonary disease), preferentially express PDE4</td>
</tr>
<tr>
<td>5</td>
<td>Penile erection; smooth muscle tone of vasculature, airways, and gastrointestinal tract</td>
<td>Abundant distribution in smooth muscle; clinical efficacy of the PDE5-specific inhibitor, sildenafil, for treatment of erectile dysfunction</td>
</tr>
<tr>
<td>6</td>
<td>Vision</td>
<td>Distribution in rod and cone photoreceptor cells; some visual defects related to PDE6 mutations</td>
</tr>
<tr>
<td>7</td>
<td>T-lymphocyte activation and proliferation; skeletal muscle metabolism</td>
<td>Distribution is predominantly in T-lymphocytes (PDE7A1); PDE7 mRNA is abundant in skeletal muscle tissue, T-lymphocytes, and B-lymphocytes, but protein and activity are readily measurable only in T-lymphocytes</td>
</tr>
<tr>
<td>8</td>
<td>T-cell activation</td>
<td>PDE8A mRNA widely expressed (highest in testis); PDE8B is unique to thyroid gland</td>
</tr>
<tr>
<td>9</td>
<td>Possibly maintains basal intracellular cGMP levels or natriuresis and vascular tone</td>
<td>mRNA widely expressed, particularly in spleen, intestine, kidney, heart, and brain</td>
</tr>
<tr>
<td>10</td>
<td>Unknown</td>
<td>Human PDE10 widely distributed</td>
</tr>
<tr>
<td>11</td>
<td>Sperm capacitation; other functions unknown</td>
<td>mRNA occurs at highest levels in skeletal muscle, prostate, kidney, liver, pituitary and salivary glands, and testis; protein localised to vascular smooth muscle cells, cardiac myocytes, corpus cavernosum of the penis, prostate, and skeletal muscle</td>
</tr>
</tbody>
</table>

PDE: phosphodiesterase; cGMP: cyclic guanosine monophosphate.
<sup>a</sup>Francis, Turko and Corbin, 2001; Fawcett et al., 2000; Beavo, 1995; Dousa, 1999; Hayashi et al., 1998; Soderling, Bayuga and Beavo (1998).
<sup>b</sup>Yang et al., 1994.
<sup>c</sup>Pyne and Furman, 2003.
<sup>d</sup>Glavas et al., 2001.
Adapted from Ghofreni et al., 2006.
in protecting the ischemic myocardium and in treating stroke (Fox et al., 2003; Halcox et al., 2002; Bocchi et al., 2002; Mahmud, Hennessy and Feely, 2001; Katz et al., 2000; Desouza et al., 2002; Ockaili et al., 2002; Zhang et al., 2002).

Since 2000, there have been occasional case reports (Egan and Pomeranz, 2000; Cunningham and Smith, 2001; Pomeranz et al., 2002; Pomeranz and Bhavsar, 2005) of non-anterior ischemic optic neuropathy (NAION) in patients taking sildenafil. Although NAION is the most common acute optic neuropathy in people over 50 years of age, it is a relatively rare event, causing partial visual loss in one eye, and is associated with various risk factors including cardiovascular disease and a small cup : disk ratio. In a published review of clinical trial data (Gorkin et al., 2006), Gorkin and coworkers estimated an incidence of 2.8 cases of NAION per 100,000 patient-years of sildenafil exposure, which is similar to estimates reported in men aged > 50 years in the general US population (2.5–11.8 cases per 100,000) (Hattenhauer et al., 2006; Johnson and Arnold, 1994). Recently, the original authors of many of the case reports published a further review (Fraunfelder, Pomeranz and Egan, 2006) and concluded that most of the case reports of NAION may be an expected coincidence, as sildenafil is frequently used by patients who are older, vasculopathic and already at risk of NAION, and conclude that the only patients who should avoid PDE5 inhibitors for visual reasons are those who have previously suffered NAION in one eye.

### 6.2 Pulmonary hypertension as a new indication for phosphodiesterase-5 inhibitor treatment

After the approval of sildenafil for the treatment of ED, thoughts started to turn to other potential indications. Sanchez and coworkers observed up-regulation of phosphodiesterase (PDE5) gene expression in pulmonary hypertensive lungs (Sanchez et al., 1998). Furthermore, it was observed that zaprinast (M&B 22948), E4021 and dipyridamole (a relatively non-selective PDE inhibitor with PDE5-inhibitory activity) appeared to play a role in ameliorating the increased pulmonary arterial pressure (PAP) in experimental pulmonary hypertension (PH) models (Ziegler et al., 1998; Ichinose et al., 1998; Ichinose et al., 1995; Nagamine, Hill and Pearl, 2000; Thusu et al., 1995). With the availability of the more potent and selective PDE5 inhibitor, sildenafil, a series of preclinical and clinical investigations were conducted to investigate its potential role as a therapeutic agent in pulmonary vascular diseases. The first placebo-controlled study (Pfizer study 1024) evaluated the dose–response of intravenous sildenafil. This study, conducted between 1998 and 2000, showed that sildenafil selectively reduced pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) in more than 80 patients with pulmonary arterial hypertension (PAH), pulmonary venous hypertension and/or hypoxic PH. It was also observed that the effect reached a plateau at a plasma concentration of ~100 ng/ml of sildenafil. During this period, interest in the role of sildenafil in PH gained significant momentum.
6.3 Role of phosphodiesterase-5 in the pulmonary vasculature

Nitric oxide is constitutively produced in the lung by NO synthases (NOS). The main cellular sources of lung NO production are the vascular endothelium and the airway epithelium (Bohle et al., 2000; German et al., 2000). Adaptation of the perfusion distribution to well-ventilated areas of the lung (ventilation/perfusion (V/Q) matching) is regulated primarily by local NO production (Ide et al., 1999; Grimminger et al., 1995), since the most prominent stimulus for local NO production in the lung is alveolar distension during inspiration (Grimminger et al., 1995; Ghofrani et al., 2004a; Weissmann et al., 2000; Schulz et al., 2000; Spriestersbach et al., 1995). Thus, local NO release results in redirection of blood flow to well-ventilated areas of the lung (V/Q matching) (Plate 6). NOS is regulated at the transcriptional and post-translational levels (Michelakis, 2003). The most important cyclic GMP degrading phosphodiesterase – PDE-5 – is abundantly expressed in lung tissue (Ahn et al., 1991; Fink et al., 1999; Giordano et al., 2001; Wharton et al., 2005). When compared with the expression of PDE5 in other tissues such as the myocardium, the expression and activity of PDE5 is considerably higher in lung tissue (Corbin et al., 2005). PDE5 is thus an ideal target for treatment of pulmonary vascular disorders, including PAH, and PH associated with underlying lung disorders. Moreover, sildenafil is the first oral drug with the potential to augment NO-related vasodilatation in regions of perfusion demand and, in the case of the lung, prevent wasted perfusion (venous admixture) and wasted ventilation (dead space ventilation).

In 1991, Haynes and coworkers demonstrated that the PDE5 inhibitor zaprinast decreased the vasoconstrictor response of isolated rat lungs to acute alveolar hypoxia (Haynes et al., 1991). Zaprinast was also shown to induce selective pulmonary vasodilatation when compared to its effects on the systemic circulation in intact anesthetized newborn lambs exposed to acute hypoxia (Braner et al., 1993), and in chronically hypoxic rats (Cohen et al., 1996). However, in the latter study, the PDE5 inhibitor E4021 turned out to be more selective for the pulmonary vascular bed, without any dilating effects in the systemic circulation. Inhibition of hypoxic pulmonary vasoconstriction (HPV) was also achieved in isolated rabbit lungs by zaprinast (Weissmann et al., 2000). Investigations with the PDE5 inhibitor sildenafil in isolated perfused rodent lungs demonstrated a marked inhibition of HPV (Zhao et al., 2001; Zhao et al., 2003), thereby confirming that PDE5 inhibitors act as potent pulmonary vasodilators. Oral treatment of chronically hypoxic mice with sildenafil prevented the development of PH (Zhao et al., 2001). In these studies, Zhao and coworkers also demonstrated that it was not only NO derived from endothelial nitric oxide synthase (eNOS) that contributed to these effects of the PDE5 inhibitor (Zhao et al., 2001). These observations are consistent with their more recent study demonstrating that in natriuretic peptide (NPR-A) knockout mice, sildenafil decreased the PH and right ventricular hypertrophy, suggesting that the natriuretic peptide pathway may play a major role in the effects of sildenafil (Zhao et al., 2003). While all of the
above investigations initiated the sildenafil treatment at the onset of hypoxia, Sebkhi and coworkers demonstrated that starting sildenafil after established hypoxic PH also reduced pulmonary artery pressure and pulmonary vascular muscularization in lungs from chronically hypoxic rats (Sebkhi et al., 2003). These investigations therefore demonstrated that PDE5 inhibition appears to have remodeling effects, with selective effects on the pulmonary vascular resistance. Thus, the selective pulmonary effects of PDE5 inhibitors appear attributable to a high level of PDE5 in the pulmonary circulation compared with the systemic circulation (Ahn et al., 1991, Giordano et al., 2001; Corbin et al., 2005; Hanson et al., 2003), and that NO production in the lung is high, akin to the situation in the corpus cavernosum (Grimminger et al., 1995; Spriestersbach et al., 1995; Nangle, Cotter and Cameron, 2003; Bloch et al., 1998). Itoh and others recently reported that sildenafil alone, and in combination with the prostacyclin analogue beraprost, decreased right ventricular systolic pressure (RVSP), right heart hypertrophy and pulmonary vascular medial wall thickness in the MCT-induced rat PH model (Itoh et al., 2004). The beneficial effects of sildenafil after the development of PH yielded similar results (Schermuly et al., 2004), i.e. sildenafil reduced pulmonary artery pressure and vascular muscularization in the MCT-PH model rat lungs, and reduced the expression of matrix metalloproteinases (MMP) 2 and 9 after development of PH. In addition, the degree of fully muscularized small (< 50 µm) pulmonary arteries was decreased.

### 6.4 Clinical experience with sildenafil for the treatment of chronic pulmonary hypertension

The vasodilatory effects of inhaled NO appear to be restricted to the pulmonary vasculature. Nitric oxide, with its very short half-life, i.e. ~20 s, is used as a screening agent for acute pulmonary vasoreactivity (Sitbon et al., 1998), and is effective in improving gas exchange in selected patients with adult respiratory distress syndrome (ARDS) (Rossaint et al., 1993). Weaning from chronic NO treatment in patients with ARDS has been facilitated by oral sildenafil (Atz and Wessel 1999). The first case report of an adult patient with PAH treated chronically with high doses of oral sildenafil indicated that this approach could be effective (Prasad, Wilkinson and Gatzoulis, 2000). An early report using oral sildenafil in a child with severe PH also appeared efficacious, raising interest not only within the medical community but also in the media (Abrams, Schulze-Neick and Magee, 2000; Patole and Travadi, 2002; Oliver and Webb, 2002). Sildenafil is frequently given if a patient cannot be easily weaned off inhaled NO, e.g. after ‘corrective’ open heart surgery in children with systemic-to-pulmonary shunts. The sildenafil is then weaned or continued based on whether the patient has persistent PH post-operatively.

Trials addressing the characterization of the acute effects of sildenafil on pulmonary and systemic hemodynamics in a larger number of patients with PAH showed that sildenafil effectively reduces pulmonary vascular resistance in a
dose-dependent manner (Ghofrani et al., 2002a). Notably, the vasodilator effects are predominantly restricted to the pulmonary circulation and appear to be greater than the effects seen with inhaled NO. In combination with inhaled iloprost, augmentation of the pulmonary vasodilator effect of each single agent was observed (Ghofrani et al., 2002a; Wilkens et al., 2001). Long-term oral sildenafil treatment in PAH patients has been investigated in a number of single-center studies, all suggesting its high efficacy and safety (Kothari and Duggal, 2002; Sastry et al., 2002; Sastry et al., 2004).

Interestingly, sildenafil appears to be effective also for treating patients with PH with etiologies other than idiopathic PAH. In patients with human immunodeficiency virus (HIV)-related PH, sildenafil was similarly effective in reducing pulmonary vascular resistance as it was in idiopathic PAH (Schumacher et al., 2001; Carlsen, Kjeldsen and Gerstoft, 2002). Recent data also suggest that long-term oral sildenafil treatment in patients with inoperable chronic thromboembolic PH is beneficial (Ghofrani et al., 2003a; Reichenberger et al., 2007).

6.5 Pivotal trial and approval of sildenafil for the treatment of pulmonary arterial hypertension (SUPER-1 study)

Based on studies between 1998 and 2001 suggesting the efficacy of sildenafil for the treatment of PAH, a large randomized, double-blind, placebo-controlled international trial was carried out to confirm that treatment with sildenafil is safe and efficacious for the treatment of PAH. The SUPER-1 (Sildenafil Use in Pulmonary HypERTension) study (started in 2002) included 278 patients with symptomatic PAH treated either with placebo or sildenafil (20, 40, or 80 mg) orally three times daily (TID) for 12 weeks. The primary end-point – as in many previous PAH trials – was the change from baseline to week 12 in the 6-minute walk distance (6MWD). Sildenafil, in all three applied doses, improved exercise capacity (~45–50 m; placebo corrected value), functional class and hemodynamics, as compared to placebo-treated patients, and was well tolerated (Galie et al., 2005). In addition, patients completing the double-blind phase were eligible to enter a long-term extension trial, conducted over a two-year period with 80 mg sildenafil TID. The increase in the 6MWD achieved after three months in the placebo-controlled phase was maintained after one year of therapy in the patients who continued on sildenafil, as were the improvements in functional class, both indicative of maintenance of the effect; however, these long-term data were uncontrolled and observational; in addition, patients could have had additional PAH therapies added at the discretion of the investigator.

Based on the favorable effects of this new oral treatment, sildenafil was approved by the FDA and the EMEA in 2005 for the treatment of patients with symptomatic PAH. Both agencies approved only the 20 mg TID dose, as a non-significant dose–effect relationship between 20 and 80 mg TID was observed with
regard to the primary endpoint of the study, the change in 6MWD over 12 weeks of treatment, in the overall patient population. However, there were significant
dose responses demonstrated in some of the secondary endpoints, e.g. mean pulmo-

nary arterial pressure (mPAP) and PVR in the overall population, as well as in
some subgroup analyses, e.g. idiopathic PAH, with respect to improvement in
6MWD. Moreover, in the majority of prior short- and long-term studies, daily
doses of 100 up to 300 mg were investigated and reported to be efficacious and
well tolerated (Ghofrani et al., 2002; Wilkens et al., 2001; Kothari and Duggal,
2002; Sastry et al., 2004; Michelakis et al., 2003). Thus, future studies are war-
ranted addressing the long-term efficacy of 20 mg TID, higher doses, or perhaps
lower doses, of sildenafil for the treatment of PAH. However, the duration of
action of sildenafil reported from some clinical and experimental settings may not
be accurately reflected by plasma levels and the applied dosage (Moncada et al.,
2004). One possible explanation is that intracellular rebinding of sildenafil to the
catalytic site of PDE5 could occur repeatedly and may retard clearance of the
inhibitor from the cells; it has also been shown that the affinity of sildenafil to
PDE5 increases after intracellular phosphorylation of the enzyme (Mullershausen
et al., 2003). This led to the hypothesis that sildenafil may saturate the PDE5
enzyme intracellularly with higher affinity than previously estimated. However,
whether the conformational changes of the PDE5 and the slow dissociation rate of
sildenafil from the enzyme reflect dose effects remains speculative (Francis et al.,
1998; Gopal, Francis and Corbin, 2001; Corbin and Francis, 2002; Huai et al.,
2004).

6.6 Other phosphodiesterase-5 inhibitors

In a comparative clinical trial, 60 consecutive PAH patients (NYHA classification II–IV)
undergoing right heart catheterization for acute pulmonary vasoreactivity testing
received initial short-term NO inhalation and were subsequently 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 (Ghofrani et al., 2004b) (Figure 6.1).
Hemodynamics and gas exchange responses were assessed over a subsequent 120 min
observation period. All three PDE-5 inhibitors caused significant pulmonary vasorelax-
ation, accompanied by an increase in cardiac output, with maximum effects obtained
after 40–45 min (vardenafil), 60 min (sildenafil) and 75–90 min (tadalafil). Sildenafil and
tadalafil, but not vardenafil, caused a significant reduction of the pulmonary-to-systemic vas-
cular resistance ratio. Significant improvement in systemic arterial oxygenation, corre-
sponding to that observed during NO inhalation, was noted only with sildenafil. Thus,
the three PDE-5 inhibitors (Table 6.3) appeared to differ in kinetics of pulmonary vasore-
relaxation (most rapid effect by vardenafil), selectivity for the pulmonary circulation (silde-
nafil and tadalafil, but not vardenafil) and impact on systemic arterial oxygenation
(improvement only after sildenafil). Tadalafil has also been shown to be safe and effica-
cious for the treatment of symptomatic pulmonary arterial hypertension as monotherapy
6.6 OTHER PHOSPHODIESTERASE-5 INHIBITORS

and as add on therapy to bosentan in the randomized, double-blind, placebo-controlled PHIRST trial (Pulmonary arterial Hypertension and ResponSe to Tadalafil). Compared with placebo treated patients, patients treated with tadalafil had increased exercise capacity, improved hemodynamics, and less clinical worsening.

Table 6.3 PDE inhibition and selectivity

<table>
<thead>
<tr>
<th>Compound</th>
<th>PDE1 (µM)</th>
<th>PDE2 (µM)</th>
<th>PDE3 (µM)</th>
<th>PDE4 (µM)</th>
<th>PDE5 (µM)</th>
<th>PDE6 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sildenafil</td>
<td>0.281</td>
<td>&gt;30</td>
<td>16.2</td>
<td>7.68</td>
<td>0.00350</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>(80)</td>
<td>(&gt;8570)</td>
<td>(4630)</td>
<td>(2190)</td>
<td></td>
<td>(11)</td>
</tr>
<tr>
<td>Tadalafil</td>
<td>&gt;30</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.00674</td>
<td>1.26</td>
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<tr>
<td></td>
<td>(&gt;4450)</td>
<td>(&gt;14,800)</td>
<td>(&gt;14,800)</td>
<td>(&gt;14,800)</td>
<td></td>
<td>(187)</td>
</tr>
<tr>
<td>Vardenafil</td>
<td>0.070</td>
<td>6.20</td>
<td>&gt;1.0</td>
<td>6.10</td>
<td>0.00014</td>
<td>0.0035</td>
</tr>
<tr>
<td></td>
<td>(500)</td>
<td>(44,290)</td>
<td>(&gt;7140)</td>
<td>(43,570)</td>
<td></td>
<td>(25)</td>
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</table>

<table>
<thead>
<tr>
<th>PDE6 (cone)</th>
<th>PDE7A (µM)</th>
<th>PDE8A (µM)</th>
<th>PDE9A (µM)</th>
<th>PDE10A (µM)</th>
<th>PDE11A (µM)</th>
</tr>
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<tbody>
<tr>
<td>Sildenafil</td>
<td>0.034</td>
<td>21.3</td>
<td>29.8</td>
<td>2.61</td>
<td>9.80</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(6090)</td>
<td>(8510)</td>
<td>(750)</td>
<td>(2800)</td>
</tr>
<tr>
<td>Tadalafil</td>
<td>1.30</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td>(193)</td>
<td>(&gt;14,800)</td>
<td>(&gt;14,800)</td>
<td>(&gt;14,800)</td>
<td>(&gt;14,800)</td>
</tr>
<tr>
<td>Vardenafil</td>
<td>0.0006</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>0.581</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(&gt;214,000)</td>
<td>(&gt;214,000)</td>
<td>(4150)</td>
<td>(21,200)</td>
</tr>
</tbody>
</table>

IC$_{50}$ were determined using either native enzyme purified from human tissue (PDE1, heart; PDEs 2, 3, and 5, corpus cavernosum; PDE4, skeletal muscle; PDE6, retina) or using recombinant human enzymes expressed in $S/9$ cells (PDEs 7–11) and purified by anion-exchange chromatography. PDE: phosphodiesterase; IC$_{50}$: drug concentration necessary to inhibit 50% of enzyme activity. Adapted from Gbekor et al., 2002.
6.7 Combination therapy

As in many other progressive diseases, patients with PAH may experience clinical and hemodynamic deterioration despite ongoing initial effective monotherapy with the currently available disease-specific PAH drugs (i.e. PDE5i, prostanoids, endothelin receptor antagonists). It may thus be reasonable to consider combination therapy from another complementary group of drugs. However, because of unknown risks, costs, drug interactions, etc., combination therapy is not generally recommended until randomized, controlled trial data is available that demonstrates safety and efficacy with the various possible combinations and in the various PAH subgroups.

Experience to date with combination of phosphodiesterase-5 inhibitors and prostanoids

The combination of prostanoids and sildenafil has potential as synergistic treatment for PAH. The combination of sildenafil plus inhaled iloprost has to date been studied in three small trials. During a randomized, controlled acute trial, 30 patients (17 PAH, 13 chronic thromboembolic PH) in NYHA functional class III–IV were given either sildenafil in one of two doses (12.5 mg or 50 mg) or sildenafil plus inhaled iloprost (Ghofrani et al., 2002). Before randomization, the pulmonary hemodynamic effects of NO alone and of inhaled iloprost alone were tested. Inhaled iloprost showed a greater and longer-lasting effect than did NO (60–90 min vs 5 min). The efficacy of sildenafil was dose-dependent. The best results were obtained with high-dose sildenafil (50 mg) plus inhaled iloprost. Using this combination, the PVR decreased ~50% with a concomitant ~50% increase in cardiac index, with maintenance of effect for more than 3 h. Another pilot study involving five idiopathic PAH patients reported similar results. Inhaled iloprost decreased mPAP more than sildenafil (reduction by 9.4 vs 6.4 mm Hg; \( p < 0.05 \)). Receiving a combination of the two drugs led to a greater decrease in mPAP than with inhaled iloprost alone (13.8 vs 9.4 mm Hg; \( p < 0.009 \)). Systemic blood pressure remained unaffected (Wilkens et al., 2001).

The third trial was a long-term observational study examining the effects of adding sildenafil in 14 patients whose condition deteriorated during the course of long-term treatment with inhaled iloprost after an initial improvement (Ghofrani et al., 2003b). Before the start of iloprost monotherapy, the patients had an average 6MWD of 217 m. With inhaled iloprost, the distance walked initially improved to 305 m before deteriorating over the course of 18 months to 256 m. At this point sildenafil was added, leading to an improvement in the 6MWD to 346 m. The combination therapy thus resulted in a walk distance (346 m) that was above the distance attained with the initial monotherapy (305 m) – even though more than 18 months had passed. The effect of the combination was
retained throughout the 12-month observation period (349 m at the end of the trial; \( p = 0.002 \)).

**The PACES-1 trial**

The addition of sildenafil to long-term intravenous epoprostenol (EPO) treatment was investigated in the PACES-1 (Pulmonary Arterial hypertension Combination study of Epoprostenol and Sildenafil) trial (Simonneau et al., 2007). In this 16-week international, double-blind, placebo-controlled, parallel group study, 267 patients with PAH on stable dose EPO were randomized to sildenafil or placebo. Patients randomized to sildenafil received 20 mg orally TID, uptitrated to 40 mg and to 80 mg TID, as tolerated, at four-week intervals. The primary endpoint was change from baseline in exercise capacity measured by the 6MWD. Secondary endpoints included change from baseline in mPAP, time to clinical worsening, and Borg dyspnea score. There was a treatment-adjusted increase of 26.0 m (\( p < 0.001 \)) in 6MWD with subjects in the sildenafil-EPO group. The combination therapy also decreased mPAP to a greater extent than EPO alone (–3.9 mm Hg, \( p < 0.0001 \)). Time to clinical worsening was also longer in patients on combination therapy compared with EPO monotherapy (\( p < 0.012 \)). No patients died when treated with combination therapy compared with seven deaths in the EPO alone group. Thus, sildenafil-EPO combination therapy was more effective than EPO alone in improving exercise capacity, mPAP and time to clinical worsening over 16 weeks.

**Combination of phosphodiesterase-5 inhibitors and endothelin receptor antagonists**

The first open label study adding sildenafil to the endothelin receptor antagonist bosentan after transient improvement with bosentan demonstrated an increase in exercise capacity with the addition of sildenafil (Hoeper et al., 2004). The 6MWD at baseline was 346 ± 66 m and improved to 403 ± 80 m three months after starting bosentan treatment. However, this effect was not sustained and, after 11 ± 5 months, the walk distance had declined to 277 ± 80 m. At this point, sildenafil was added to bosentan. Three months later, the 6MWD had increased to 392 ± 61 m and the patients remained stable throughout the median follow-up of 9 months (range 6–12 months); increases in maximum oxygen uptake during cardiopulmonary exercise testing were consistent with the improvements observed in 6MWD. The combination of bosentan and sildenafil appeared well tolerated. These preliminary data suggest that combining bosentan and sildenafil may be safe and effective in patients with idiopathic PAH, and support further investigation.
6.8 Potential new indications for phosphodiesterase-5 inhibitors outside pulmonary arterial hypertension

Raynaud’s phenomenon and digital ulcers in connective tissue diseases (CTD)

Patients with PAH often complain about intermittent, temperature-dependent peripheral vasospasms resulting in perfusion deficiencies in their fingers and toes, i.e. Raynaud’s phenomenon, even in the absence of a defined CTD (Celoria, Friedell and Sommers, 1960; Smith and Kroop, 1957; D’Alonzo et al., 1991). When treated with vasodilators, these symptoms may improve in parallel with, but also independent of, improvements in hemodynamics. Raynaud’s phenomenon and digital ulcers are of even higher prevalence and clinically more important in patients with systemic sclerosis, limited sclerosis or systemic lupus erythematosus (SLE) (Kallenberg, 1995). Treatment currently includes calcium channel blockers, infused prostanoids, and alpha-2 blockade (Pope et al., 2000; Belch and Ho, 1996; Boin and Wigley, 2005).

However, the clinical efficacy of these therapies is often modest at best. A growing number of uncontrolled trials suggest efficacy with sildenafil for the treatment of digital ulcerations and Raynaud’s phenomenon in patients with scleroderma with or without PH (Kamata et al., 2005; Gore and Silver, 2005; Rosenkranz et al., 2003; Lichtenstein, 2003). In a pilot randomized, controlled trial, Fries and coworkers investigated the effects of sildenafil (50 mg bid) on symptoms and capillary perfusion in patients with Raynaud’s phenomenon (Fries et al., 2005); only patients who showed insufficient improvement when previously treated with other vasodilators were studied. In contrast to the effects of placebo, chronic sildenafil treatment for four weeks reduced the frequency and duration of Raynaud attacks and lowered the Raynaud’s condition score. Moreover, capillary blood flow velocity increased in all patients, and the capillary flow velocity of all patients more than quadrupled after sildenafil treatment (Fries et al., 2005). Interestingly, while sildenafil had clear effects in the diseased vascular areas, significant reductions in systemic blood pressure were not reported. This is consistent with the notion of selectivity of sildenafil for certain vascular beds (e.g. pulmonary circulation, corpus cavernosum) and indicates that PDE5 may be differentially expressed in the affected vasculature of digital ulcers as opposed to non-affected regions of the systemic circulation (Maurice et al., 2003). Taken together, there is rationale to further evaluate sildenafil as treatment for Raynaud’s phenomenon and digital ulcerations.

Pulmonary hypertension associated with ventilatory disorders

When PH is associated with interstitial lung disease, systemic administration of vasodilators increases blood flow to low- or non-ventilated areas of the lung by
interfering with the physiological hypoxic vasoconstrictor mechanism. This worsens pre-existent V/Q mismatch and increases shunt flow (Agusti and Rodriguez-Roisin, 1993). The decrease in systemic arterial oxygenation and wasting of the small ventilatory reserve of these patients are important negative consequences of this effect. Oral sildenafil, however, has been shown to cause pulmonary vasodilatation in patients with lung fibrosis and PH, with an overall vasodilatory potency corresponding to that of intravenous prostacyclin. In contrast to an infused prostanoid, selectivity for well-ventilated lung areas was demonstrated with sildenafil, resulting in improvement, rather than deterioration, in gas exchange (Ghofrani et al., 2002b). PH impairs right ventricular performance due to increased right heart afterload. However, it is still unclear to what extent exercise capacity is limited by this mechanism.

In a recent investigation, this issue was addressed under conditions of acute hypoxia at sea level, and with prolonged hypoxia at the altitude of Mount Everest Base Camp (Ghofrani et al., 2004c). These investigations were performed in healthy volunteers to exclude other confounding factors that might have added to the limitation of exercise capacity in patients with chronic hypoxia (e.g. muscle wasting, chronic immobilization, etc.). Both acute and prolonged hypoxia induced significant PH in the study subjects. As expected, exercise capacity was reduced as a consequence of severe hypoxemia and significant PH. Sildenafil reduced PH under resting conditions as well as during exercise. An interesting finding was that the reversal of PH resulted in an immediate improvement in exercise capacity, regardless of improvements in systemic arterial oxygenation. Further studies investigating the effects of acute and chronic sildenafil administration in hypoxic PH confirmed the anti-pulmonary hypertensive potential, and the beneficial effects of sildenafil on exercise performance, under these conditions (Richalet et al., 2005; Hsu et al., 2006). The results of these studies stimulated further investigations addressing the therapeutic potential of sildenafil in patients with chronic hypoxic PH as it occurs in various chronic diseases (e.g. chronic obstructive lung disease (COPD), interstitial lung disease, and obstructive sleep apnea, etc.) (Naeije, 2005; Higenbottam, 2005; Voelkel and Cool, 2003; Barbera, Peinado and Santos, 2003). In fact, there is work that supports the possibility of effective treatment of PH in patients with advanced COPD (Alp et al., 2005). Based on the significant impact of COPD on public health, further studies in this field are warranted.

**Sildenafil in heart failure**

Chronic heart load leads to ventricular hypertrophy as an initial process of adaptation and may ultimately result in ventricular dilatation and failure if not treated (Jessup and Brozena, 1995). Although cardiac hypertrophy applies to disorders that lead to
both left ventricular and to right ventricular loading, there are important differences with respect to the reversibility of muscular hypertrophy of the two ventricles. While the right ventricle – even at advanced stages of dilatation and decompensation – can virtually normalize structure and function once the load is reduced effectively, left ventricular hypertrophy is only partly reversible once a certain degree of hypertrophy has been exceeded (Anversa et al., 1995; Anversa et al., 1992). In chronic PH, right ventricular dysfunction is the most common cause of death; however, effective reduction of PVR, e.g. after lung transplantation, can reverse right ventricular hypertrophy in addition to PAP and PVR (Kasimir et al., 2004). Wilkins and coworkers suggest that treatment of PAH with sildenafil not only improves functional capacity, but also reduces right ventricular mass in these patients, as assessed by magnetic resonance imaging (Wilkins et al., 2005). To date, reduction of right ventricular hypertrophy in patients with chronic PH has been attributed to treatment-related reductions in right ventricular load (Kentera and Susic, 1980; Rich and Brundage, 1987; Pelouch et al., 1997; O’Blenes et al., 2001). However, the observations of Wilkins and others raise the possibility of a direct anti-hypertrophic effect of sildenafil on cardiomyocytes (Takimoto et al., 2005). In another study, Takimoto and others reported that sildenafil reduced ventricular hypertrophy and improved myocardial function in a mouse model of chronic left ventricular pressure load (induced by transaortic constriction) in a protein kinase G-1-dependent manner (Takimoto et al., 2005). In addition, cGMP levels were shown to inversely correlate with the cardiac hypertrophy in an isoproterenol-induced cardiac hypertrophy model in rats (Hassan and Ketat, 2005).

However, the potential benefit of sildenafil in chronic heart failure may result from a variety of actions in addition to its effect on ventricular hypertrophy. Multiple investigations have indicated that sildenafil should be cautiously administered only to selected patients with left heart failure (Katz et al., 2005; Lepore et al., 2005; Hirata et al., 2005; Freitas et al., 2006; Fisher et al., 2005; Webster et al., 2004; Alaeddini et al., 2004; Guazzi et al., 2004; Mickley and Poulsen, 2004; Mikhail, 2004). Endothelial function (the ability of arterioles to increase regional blood flow in response to appropriate stimuli such as ischemia) is limited in chronic congestive heart failure, and in an elegant study, Katz and coworkers showed that sildenafil could improve endothelial function in such patients (Katz et al., 2005). Further studies investigating the long-term effects of PDE5 inhibitors in patients with these diseases appear warranted. In addition, by virtue of its effects on vasodilatation, arterial stiffness and wave reflection, sildenafil may reduce aortic pressure and augmentation index, and thus could have a role in systemic hypertension (Mahmud, Hennessy and Feely, 2001).

6.9 Conclusions

PDE5 inhibitors, e.g. sildenafil, were initially developed for the treatment of systemic hypertension and angina. However, sildenafil subsequently evolved into a novel treatment for erectile dysfunction. Sildenafil was then further developed as
an oral treatment for PAH, and also appears effective in treating Raynaud’s phenomenon associated with systemic sclerosis and digital ulceration. The PDE5 inhibitor tadalafil is also being developed as an oral treatment for PAH. In later investigative studies, PDE5 inhibitors also appear to have promise in the treatment of respiratory disorders with ventilation/perfusion mismatch, congestive cardiac failure, systemic hypertension and even stroke. Although these findings might appear quite disparate and unrelated, in fact all of the above disorders are characterized by regional deficiencies in blood supply. The successful application of PDE5 inhibitors (as opposed to non-selective vasodilators) to treat these conditions can be understood in terms of the ability of these drugs to reverse endothelial dysfunction, and to selectively improve regional blood flow in areas of greatest need. Many patients with erectile dysfunction or PAH are now benefiting from the advances in our understanding of vascular biology and pathophysiology, and the advent of selective inhibitors of PDE5. In addition, sildenafil and other PDE5 inhibitors may have increased efficacy when used in combination with other disease-specific targeted PAH drugs, e.g. endothelin receptor antagonists and/or prostanoids. It is hoped that the clinical potential of these mechanisms to treat other serious medical conditions such as those described above will soon be realized, so that more patients may benefit from these breakthroughs in science, technology and medicine.

References


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