

Phosphodiesterase Inhibitors for the Treatment of Asthma and Chronic Obstructive Pulmonary Disease

Clive P. Page

Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London, London, UK

Key Words

Phosphodiesterase · Chronic obstructive pulmonary disease · Asthma · Anti-inflammatory activity · Bronchodilation

Abstract

Xanthines like theophylline have long been recognised as being effective drugs for the treatment of asthma and chronic obstructive pulmonary disease (COPD). They are of interest as they possess both anti-inflammatory and bronchodilator activity in the same molecule. Since the discovery of phosphodiesterases (PDEs) in the late 1950s, it has been suggested that xanthines work, in part, by acting as non-selective PDE inhibitors. However, it has also been suggested that the ability of xanthines to non-selectively inhibit PDEs contributes to their many unwanted side effects, thus limiting their use since the arrival of inhaled drugs with more favourable safety profiles. As our understanding of PDEs has improved over the last 30 years, and with the recognition that the distribution of different PDEs varies across different cell types, this family of enzymes has been widely investigated as targets for novel drugs. In particular, PDE3 in airway smooth muscle and PDE4 and PDE7 in inflammatory cells have been targeted to provide new bronchodilators and anti-inflammatory agents, respectively. This review discusses

the progress made in this field over the last decade in the development of selective PDE inhibitors to treat COPD and asthma.

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From Xanthines to Selective PDE Inhibitors

Xanthines have been widely used for many years to treat respiratory diseases and are now recognised as having both bronchodilatory and anti-inflammatory activity in patients with asthma [1] and chronic obstructive pulmonary disease (COPD) [2]. However, with the introduction of inhaled β_2 agonists, muscarinic receptor antagonists and topically active corticosteroids, xanthines have fallen increasingly out of favour, largely because of their narrow therapeutic window and their propensity for drug/drug interactions. Phosphodiesterases (PDEs) were first discovered in 1958 as being the enzymes involved in the intracellular metabolism of the cyclic nucleotides, limiting the action of these nucleotides as signalling molecules in a variety of cells [3]. Xanthines were originally described as non-selective PDE inhibitors as a mechanism of action explaining their clinical effectiveness [4], but also their side effects, as PDEs were shown to be present in many different cell types. The discovery that

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Correspondence to: Prof. Clive P. Page
Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science
King's College London, Franklin Wilkins Building
150 Stamford Street, London SE1 9NH (UK)
E-Mail clive.page@kcl.ac.uk

Table 1. Family of PDEs and isoforms

PDE family	Genes	Splice variants	Selectivity for cGMP and cAMP	Selective inhibitors
PDE1	3	16	both	8MM-IMBX
PDE2	1	4	both	Bay 607550
PDE3	2	4	both	cilostamide
PDE4	4	27	cAMP	roflumilast
PDE5	1	3	cGMP	sildenafil
PDE6			cAMP	?
PDE7	2	7	cAMP	ASB 16165
PDE8	2	11	cAMP	PF0 4957325
PDE9	1	20	cGMP	Bay 736691
PDE10	1	6	both	?
PDE11	1	4	both	?

Modified from Giembycz and Maurice [127].

the family of PDEs is very large and that different PDE isoforms are present in different cell types opened up the possibility of finding selective PDE inhibitors for targeting different cell types, with a potentially better safety profile than xanthines. However, it must be appreciated that, in parallel with this research into PDEs, a considerable amount of work has also taken place postulating the main mechanism of action of xanthines to not be via PDE inhibition but other mechanisms including the stimulation of histone deacetylase enzymes, adenosine receptor antagonism and the inhibition of intracellular kinases [5]. This has been reinforced by the observations that some clinically effective xanthines, such as doxophylline, do not induce significant PDE inhibition or adenosine receptor antagonism [6].

It is now known that there are 11 major classes of PDE. Some have subtypes and splice variants of the subtypes, making this a very large family of enzymes ripe for targeting with selective drugs (table 1) [7]. To date, a number of selective PDE inhibitors have been developed and clinically approved (table 2). The most notable are milrinone as a vasodilator for acute treatment of patients with chronic heart failure and cilostazol for the treatment of intermittent claudication [7] (possibly via its anti-platelet effects; this property has also led to a bid for the wider use of this drug in the prevention of stroke). There are also selective PDE5 inhibitors for the treatment of erectile dysfunction and pulmonary hypertension. The most recent are roflumilast N-oxide for the treatment of severe COPD and apremilast for the treatment of psoriasis [7], with others under development.

Cellular Distribution of PDEs

The cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) regulate a variety of cellular processes including the relaxation of airway smooth muscle and the release of inflammatory mediators [7]. PDEs hydrolyse cAMP and cGMP to inactive 5'AMP and 5'GMP, respectively, and thus the inhibition of PDEs represents a potential mechanism by which cell functions such as airway smooth-muscle contraction and inflammatory-mediator release can be modulated [7]. Eleven PDE gene families have been identified, denoted as PDE1–PDE11. They differ with regard to their primary structure, their affinities for cAMP and cGMP, their responses to specific effectors, their sensitivities to specific inhibitors and their mechanisms of regulation (table 1) [7–9]. Each family contains at least one member, and in some cases the members are products of more than one gene. The PDE subtypes of interest that have been identified to date and are of relevance in inflammatory airway diseases are PDE3, PDE4 and PDE7 [7].

PDE4 is a low K_m (approx. 1–10 μM) cAMP-specific PDE that has only a weak affinity for cGMP ($K_m > 50 \mu\text{M}$). The PDE4 family is comprised of four genes, i.e. PDE4A–D, each of which has multiple splice variants. PDE4 gene products have a broad tissue distribution including the brain, gastrointestinal (GI) tract, spleen, lung, heart, testis and kidney [9]. In addition, PDE4 is expressed in almost all inflammatory cell types except for platelets containing PDE3 and PDE5 [7].

PDE3 hydrolyses both cAMP and cGMP with relatively high affinities (K_m cAMP $< 0.4 \mu\text{M}$; K_m cGMP $< 0.3 \mu\text{M}$), although the V_{max} for cAMP hydrolysis is nearly 10-fold higher than for cGMP. Two genes have been identified for PDE3, known as PDE3A and PDE3B, which have $> 80\%$ amino acid identity for the catalytic region. Splice variants have only conclusively been demonstrated for the 3A isoform. PDE3A is expressed in the platelets, vascular smooth-muscle cells, cardiac myocytes, oocytes [10], B lymphocytes and dendritic cells [11] whereas PDE3B is relatively highly expressed in adipocytes, hepatocytes and spermatocytes and has also been detected in vascular smooth-muscle cells, the pancreas, T lymphocytes and macrophages [10]. No inhibitors have been described that clearly distinguish between PDE3A and PDE3B, although there are 2 reports of compounds showing some selectivity [12, 13].

Data are now available demonstrating PDE7 in some inflammatory cell types, where it is thought to be involved

Table 2. PDE inhibitors under development for the treatment of respiratory diseases

Examples of PDE inhibitors	Company	Indication	Phase of development
PDE4 inhibitors			
Roflumilast (oral)	Takeda	severe COPD	approved
Apremilast (oral)	Celgene	?	already approved for psoriasis
GSK 256006 (inhaled)	GSK	asthma/COPD	phase 2
CHF 6001 (inhaled)	Chiesi	asthma/COPD	phase 2
PDE3/4 inhibitors			
RPL 554 (inhaled)	Verona Pharma	asthma/COPD	phase 2
PDE4/7 inhibitors			
ASB 16165	Asubio	immune responses	preclinical

with the metabolism of the so-called rolipram-resistant pool of cAMP [7]. This has led to the development of a number of PDE7 inhibitors capable of inhibitory activity on a range of inflammatory cells [7, 14].

Effects of Selective PDE Inhibitors on Airway Smooth Muscle, Inflammatory Cells and the Mucociliary Escalator

Whilst PDE4 inhibitors are very efficacious at inhibiting the activation and release of inflammatory mediators from certain cells (e.g. neutrophils and eosinophils) [15–19], there is little evidence of selective PDE3 inhibitors inhibiting inflammatory cell function. However, evidence is growing that suggests that dual inhibition of PDE3 and PDE4 can be additive or even synergistic at suppressing inflammatory-mediator release from some cell types that also express PDE3 and are thought to play a role in asthma and COPD (e.g. macrophages, dendritic cells, epithelial cells, lymphocytes, airway smooth-muscle cells and endothelial cells) [20–23].

PDE3 inhibitors cause airway smooth-muscle relaxation in vitro [24–26] and in vivo [27], with PDE3 found to be the primary PDE in airway smooth muscle [4, 25, 28]. Furthermore, recent data has suggested that PDE3 is up-regulated in the airway smooth muscle obtained from patients with asthma [29]. This effect has now been translated into drugs that cause bronchodilation [30] (and see below). PDE4 is also found in airway smooth muscle [25] and, whilst PDE4 inhibitors appear not to have acute bronchodilatory activity clinically [31, 32], there is evidence from preclinical studies to suggest that combined inhibition of PDE3 and PDE4 suppresses the spasmogen-induced contraction of human and bovine airway smooth

muscle in a synergistic fashion [24, 25]. PDE3 and PDE4 inhibitors have also been shown to activate cystic fibrosis transmembrane conductance regulator (CFTR)-mediated chloride secretion, which suggests that they may be able to stimulate mucociliary clearance [33].

Effects on Airway Smooth Muscle

PDE3, together with PDE4B and PDE4D, is expressed in human airway smooth-muscle cells [24, 25, 34, 35]. Some studies have demonstrated that PDE4 inhibitors can relax inherent tone in isolated human bronchial muscle [25, 26]. In 1 study using siRNA targeted to PDE4D5, this splice variant was shown to be the key physiological regulator of β_2 -adrenoceptor-induced cAMP turnover within human airway smooth muscle [34, 35]. Further evidence to support a key role for the D isoform in the contractile response of airway smooth muscle can be derived from a study on PDE4D^{-/-} mice, in which a significant disruption in airway smooth-muscle contractility was observed in the isolated trachea, highlighted by a marked reduction in maximal tension and reduced sensitivity to muscarinic cholinergic agonists [36]. PDE3 has also been shown to play an important role in airway smooth-muscle contraction, as the PDE3 inhibitor olprinone, caused a dose-dependent antagonism methacholine-induced bronchoconstriction in the dog [27].

Effects on Inflammatory Cells

In vitro

A number of PDE4 isoenzymes (PDE4A, PDE4B and PDE4D) are expressed in human neutrophils [37–39]. Some evidence suggests that PDE4B2 is the predominant PDE4 isoform [39] and that PDE4A is exclusively located within a subset of myeloperoxidase-containing neutrophil granules [38]. PDE4 inhibitors can inhibit the release

of a range of pro-inflammatory mediators from human neutrophils including LTB₄, reactive oxygen species [16, 18], matrix metalloproteinase (MMP)9 and neutrophil degranulation products such as neutrophil elastase and myeloperoxidase [17]. PDE4 inhibitors also inhibit PAF-induced CD11b expression, L-selectin shedding [40] and TNF- α - and fMLP-mediated neutrophil adhesion to human umbilical vein endothelial cells [17, 41].

The PDE4 isoenzyme subtypes (A, B and D) are also present in human eosinophils, with evidence to suggest that PDE4A is exclusively located in eosinophil granules [38]. PDE4 inhibitors inhibit the release of reactive oxygen species from human eosinophils [16] as well as C5a- and PAF-stimulated LTC₄ synthesis [42]. PDE4 inhibitors can also inhibit PAF-induced CD11b expression and L-selectin shedding [40] as well as C5a- and PAF-stimulated eosinophil chemotaxis [42].

PDE4 isoenzyme subtypes (A–D) are also present in human lung macrophages and peripheral blood monocytes [37, 43], and PDE3B has been detected in macrophages [10]. PDE4A4 is found to be up-regulated in the lung macrophages from smokers with COPD when compared with control smokers [37], and PDE4A4 and PDE4B2 are increased in the peripheral blood monocytes from smokers as opposed to non-smokers [37]. PDE4 inhibitors can inhibit LPS-stimulated TNF- α release from peripheral blood monocytes [43, 44]. Interestingly, in PDE4B^{-/-} mice (but not in PDE4D^{-/-} mice), there is a marked reduction in the ability of LPS to stimulate TNF- α release from peripheral blood leukocytes [45], suggesting a key role for PDE4B in this response. Further support for a key role of PDE4B in LPS-induced TNF- α release from monocytes came from a separate study demonstrating that mean IC₅₀ values for the inhibition of LPS-stimulated TNF- α release significantly correlated with compound potency against the catalytic activity of recombinant human PDE4B (and PDE4A), but not the catalytic activity of recombinant human PDE4D [43].

PDE3 and PDE4 (A, B and D) are present in monocyte-derived dendritic cells [11] and PDE4A appears to be the predominant PDE4 isoform [46]. The PDE4 inhibitor rolipram partially inhibits LPS-stimulated TNF- α release from dendritic cells [11].

PDE4A, B and D and PDE3 have been detected in human T and B lymphocytes [20, 21, 47–49] with PDE3A and PDE3B detected in B lymphocytes [20] and T lymphocytes [10], respectively. Knockdown of PDE4B or PDE4D (but not PDE4A) using siRNA, inhibited IL-2 release whereas knockdown of PDE4D showed the most predominant inhibitory effect on IFN- γ and IL-5 release

[50]. In one study, PDE4 inhibitors were described as partially inhibiting IL-4 and IL-5 gene expression in Th2 cells [51], together with IL-4 and IL-5 release from human CD4+ T cells [52]. Another study demonstrated that specific inhibition of PDE4 had no significant effect on Th2 cell-mediated IL-4 or IL-13 generation, but preferentially inhibited Th1 cell cytokine generation (IFN- γ) [52]. PDE4 inhibitors also partially inhibit phytohemagglutinin and anti-CD3/anti-CD28 stimulates the proliferation of CD4+ and CD8+ T cells [21, 53]. A separate study demonstrated that dual PDE4A/B and PDE4D inhibitors inhibited antigen-stimulated human T cell proliferation, with mean IC₅₀ values significantly correlating with compound potency against the catalytic activity of recombinant PDE4A or PDE4B, but not with the catalytic activity of recombinant PDE4D [43]. In contrast, a PDE4D siRNA (but not PDE4A or PDE4B siRNAs) also significantly inhibited anti-CD3/CD28-stimulated CD4+ proliferation [50]. The reason for this apparent difference in PDE4 subtype involvement in this proliferative response is unclear, but could be related to the fact that different T cell populations or different stimuli were employed to elicit proliferation.

PDE4 (A, C and D) and PDE3 are both expressed in human airway epithelial cells [22, 54, 55]. The PDE4 inhibitor rolipram has been found to partially inhibits IL-1 β -stimulated GM-CSF release from human airway epithelial cells and A549 cells [22]. Another study demonstrated that rolipram inhibited LPS-stimulated IL-6 release from human airway epithelial cells, but relatively high concentrations were required to see an inhibitory effect, with an IC₅₀ of 24 μ M, suggesting the effect could have been mediated through other PDEs such as PDE3 [56]. In a more recent study, roflumilast N-oxide was found to inhibit respiratory syncytial virus infection in human bronchial epithelial cells, preventing the loss of ciliated cells and markers, reducing the increase of CLCA1 and inhibiting the increase of IL-13, IL-6, IL-8, TNF- α and ICAM-1. In addition, roflumilast N-oxide reversed the reduction of Nrf2, HO-1 and GPx mRNA levels and consequently restored the total antioxidant capacity and reduced H₂O₂ formation [53].

Endothelial cells express PDE4 (A, B and D) [57–62] and PDE3 [60–62]. PDE4 inhibition in combination with appropriate activation of adenylate cyclase inhibits TNF- α -induced E-selectin expression on human lung microvascular endothelial cells [23]. Rolipram has also been shown to potently block H₂O₂-induced endothelial permeability when combined with PGE₁ [60], suggesting that this inhibition of enhanced vascular

permeability may be another beneficial effect of PDE4 inhibition.

PDE7 has been found as two isoforms, 7A and 7B, known to be cAMP-specific enzymes. PDE7 has been found in a broad range of cells and tissues including the spleen, brain, lung and kidney and also in lymphoid and myeloid cells, particularly T lymphocytes. PDE7A is thought to be involved in normal T-cell activation [7, 21] and PDE7 inhibitors (e.g. ASB 16165 and BRL 50481) have been shown to be able to influence lymphocyte function and certain types of inflammatory response [7, 14].

In vivo

The orally administered PDE4 inhibitors, cilomilast and roflumilast N-oxide, are effective at suppressing neutrophil recruitment to the lung in mice that have been acutely exposed to cigarette smoke [63, 64]. In other chronic cigarette smoke exposure studies, roflumilast N-oxide (oral treatment for 7 months) fully prevented emphysema in mice [64], and the PDE4 inhibitor GPD-1116 (oral treatment for 8 weeks) also markedly attenuated the development of cigarette smoke-induced emphysema in the senescence-accelerated mouse P1 strain [65]. PDE4 inhibitors have also been shown to reduce MMP9 and TGF- β release during LPS-induced lung injury in mice [66].

Local administration of PDE4 and dual PDE3/4 inhibitors directly to the lung also inhibits LPS-induced neutrophil recruitment to the lung in a range of species including rats [66–68], ferrets [66] and pigs [66]. In addition, local administration of an inhaled PDE3/4 inhibitor SDZ ISQ 844 in dogs decreased airways responsiveness to inhaled methacholine at a dose which did not affect baseline respiratory resistance [69].

Studies on PDE4 subtype-deficient mice suggest that PDE4B and PDE4D (but not PDE4A) are important in mediating LPS-induced neutrophil recruitment to the lung, as neutrophil migration was inhibited by 31 and 48% in PDE4B^{-/-} and PDE4D^{-/-} mice, respectively. These effects were associated with a reduction in the expression of CD18, but not CD11 [70], suggesting that the PDE4B and PDE4D inhibition of adhesion molecule expression may contribute to the reduced neutrophil recruitment. PDE4 inhibitors have also more recently been shown to promote the PKA-dependent resolution of established neutrophilic inflammation in vivo (LPS-induced neutrophil recruitment to the pleural cavity in mice) by inducing apoptosis of neutrophils mediated by activation of caspase-3, which correlated with the inhibition of the PI3K/AktMcl-1 pathway in the mouse [71].

PDE7 inhibitors have been reported to inhibit skin inflammation and T-lymphocyte activation in vivo [7]. However, there are very limited data in the context of airway disease to date, although there is no difference in the expression of PDE7 in cells obtained from patients with asthma or COPD compared with healthy subjects [72].

Effects on the Mucociliary Escalator

CFTR is the primary cAMP-activated chloride channel on the apical membrane of airway epithelia, and a number of studies have shown that acquired CFTR dysfunction may cause delayed mucociliary clearance, even in the absence of the congenital CFTR dysfunction seen in cystic fibrosis patients [33]. Cigarette smoke causes CFTR dysfunction in vitro and in vivo, an effect which has been suggested to contribute to COPD pathogenesis by causing a predisposition to epithelial dysfunction, mucus retention and chronic bronchitis. Inhibition of PDE4 (in particular the D isoform) and PDE3 has been found to activate CFTR-mediated chloride secretion in an epithelial cell line [33, 73], suggesting that PDE3/4 inhibitors may have additional benefit in COPD by being able to enhance mucociliary clearance.

Interestingly, roflumilast N-oxide was recently shown to partially restore CFTR activity in human bronchial epithelial cells exposed to cigarette smoke, an effect which was additive with ivacaftor (the recently approved CFTR corrector compound) [74]. In addition, roflumilast N-oxide improved the depleted airway surface liquid depth of human bronchial epithelial monolayers exposed to smoke. CFTR activation by roflumilast N-oxide also induced CFTR-dependent fluid secretion in the murine intestine, increasing the wet/dry ratio and diameter of ligated murine segments [74], an effect which has been suggested to likely be responsible for the diarrhea that has been seen clinically in some patients treated with roflumilast N-oxide. It has also recently been shown that roflumilast N-oxide inhibits a respiratory syncytial virus-induced increase in MUC5AC [75], and that PDE3 and PDE4 inhibitors accelerate ciliary beat frequency in upper and lower airway tissue in vitro [76].

Effects on Structural Cells in the Airways

Fibroblasts and airway smooth-muscle cells are thought to contribute to the airway remodelling that has been observed in COPD and other obstructive lung diseases, through the release of growth factors, cytokines and extracellular matrix proteins [77]. The PDE4 inhibitor

piclamilast inhibits the differentiation of lung fibroblasts to myofibroblasts (induced by TGF- β) [78]. In addition, PDE4 inhibitors can inhibit the TNF- α -stimulated pro-MMP1 and pro-MMP2 release from human lung fibroblasts [79] and the chemotaxis of fetal lung fibroblasts towards fibronectin [80]. Roflumilast N-oxide has also been shown to inhibit TGF- β -induced fibronectin deposition in human airway smooth-muscle cells and TGF- β -induced CTGF, collagen I and fibronectin expression in human bronchial-tissue rings [77].

Combined PDE Inhibitors Can Have Additive or Synergistic Effects

Anti-Inflammatory Effects

Whilst PDE4 inhibitors can completely suppress LPS-stimulated TNF- α release from monocytes, they have only a partial inhibitory effect on LPS-stimulated TNF- α release from human alveolar macrophages [19] and dendritic cells [11]. This appears to be due to the presence and importance of PDE3B [10], as the combined inhibition of PDE3 and PDE4 can completely suppress this effect [11, 19]. In a separate study concerning human alveolar macrophages, whilst a PDE3 or PDE4 inhibitor alone caused only about 20% inhibition of LPS-induced cytokine release in the presence of PGE₂, the combined inhibition of PDE3/PDE4 effectively inhibited the LPS-induced cytokine secretion by about 90%. Interestingly, this inhibitory effect was sustained in the presence of oxidative stress. This was in contrast to the inhibitory effect of the corticosteroids, budesonide and dexamethasone, which was reduced from 90 to 30% under conditions of oxidative stress [43].

There is also evidence that combined inhibition of PDE3 and PDE4 can have synergistic anti-inflammatory effects in CD4⁺ and CD8⁺ human T lymphocytes. The PDE3 inhibitor SK&F 95654 alone had no effect on mitogen-stimulated IL-2 release, but it potentiated the inhibitory effect of the PDE4 inhibitor rolipram [21, 52]. In addition, rolipram only partially inhibited IL-1 β -stimulated GM-CSF release from human airway epithelial cells and A549 cells whereas the dual PDE3/4 inhibitor (ORG-9935) completely suppressed this effect [22].

Combined inhibition of PDE3 and PDE4 has also been shown to have a synergistic inhibitory effect on VCAM-1 expression and eosinophil adhesion to activated human lung microvascular endothelial cells [23]. Combined inhibition of PDE4 and PDE7 has also been demonstrated to have an effect on T lymphocyte function [14].

Bronchodilatory Effects

Whilst some studies have demonstrated that PDE4 inhibitors can relax inherent tone in isolated human bronchial muscle [24, 26], others have found that PDE3 or PDE4 inhibitors alone are ineffective but that, in combination, they can cause the relaxation of inherent tone [25]. In addition, PDE3 or PDE4 inhibition alone had no effect on allergen- or LTC₄-induced contraction of human airway smooth muscle, but, in combination, acted synergistically to inhibit contraction [24]. Furthermore, we recently showed that the dual PDE3/4 inhibitor RPL 554 is very effective in relaxing human bronchial smooth muscle precontracted by a range of contractile agents [80]. It has also been found to interact synergistically with muscarinic receptor antagonists and, to a lesser extent, with β_2 agonists in relaxing human bronchial smooth muscle [80] and small airways [81], as well as showing a similar positive interaction with anti-cholinergics and β_2 agonists in the lungs of guinea pigs in vivo [82].

Clinical Data

PDE4 Inhibitors

Roflumilast N-Oxide

Roflumilast N-oxide was first identified in 1993 and was shown to have subnanomolar potency for PDE4, with high selectivity for PDE4 against other PDEs. It was initially developed as an orally active drug for the treatment of patients with asthma because early clinical work had demonstrated that it inhibits exercise-induced bronchospasm in patients with mild asthma [83] and has a modest effect on the late-onset allergic response in allergic asthmatics [84]. This confirmed earlier reports on the selective PDE4 inhibitor CDP 840 [31] and, more recently, GSK 2190915 [85]. It has been shown that roflumilast is as effective as inhaled beclomethasone dipropionate in the treatment of patients with asthma [86]; however, despite a number of studies reporting this effectiveness, so far it has only been approved as a treatment for patients with severe COPD (summarised in [87]).

Two pivotal, 1-year, phase 3 clinical trials with COPD patients have shown that roflumilast N-oxide reduces exacerbations and produces a modest improvement in lung function [88, 89]. Roflumilast has also demonstrated anti-inflammatory effects in patients with COPD, thought to contribute to its clinical effectiveness. Specifically, in a cross-over study in which 38 patients with COPD received 500 μ g roflumilast N-oxide or placebo once daily for 4 weeks, roflumilast N-oxide significantly reduced the ab-

solute number of neutrophils and eosinophils per gram of sputum as well as the levels of soluble IL-8, neutrophil elastase, eosinophil cationic protein and α_2 -macroglobulin in the sputum, and the release of TNF- α from blood cells. This study also showed that post-bronchodilator FEV₁ improved significantly during treatment with roflumilast N-oxide compared with placebo, with a mean difference between treatments of 68.7 ml [90]. Another selective PDE4 inhibitor cilomilast has also been shown to reduce the number of inflammatory cells in biopsies obtained from patients with COPD when compared with placebo-treated patients [91].

In the European Union in 2010 and in the USA in 2011, roflumilast N-oxide was licensed for the maintenance treatment of severe COPD (GOLD stages 3 and 4, i.e. post-bronchodilator FEV₁ <50%) associated with chronic bronchitis and a history of frequent exacerbations as an add-on to standard therapy. Two phase 3 clinical trials over 6 months demonstrated the beneficial effects of roflumilast N-oxide on patients already receiving treatment with the long-acting β_2 agonist salmeterol or the anti-cholinergic tiotropium bromide (reviewed in [87]). To date, it remains the only PDE4 inhibitor approved for treating patients with COPD, in the most severe cases only as an add-on therapy. It has not yet been widely adopted, however, due to its dose-limiting side effects in a significant number of patients which effectively means it cannot be optimally dosed.

The development of a number of other orally active PDE4 inhibitors has been stopped because of unacceptable adverse effects [7], and this has led to alternative strategies to find PDE4 inhibitors with improved therapeutic ratios. A number of highly potent inhaled PDE4 inhibitors have thus been developed, including RP 73401 [26], AWD 12–281 [66], GSK 256066 [85] and, more recently, a novel class of PDE4 inhibitors [92]. Unfortunately, a number of clinical trials have not shown these to have any major therapeutic effect, either in patients with asthma or COPD [66, 85]. It is not known why these drugs have failed clinically when administered by inhalation but have been effective when administered orally [93]. It is important to understand this if we are to make further progress in this area.

Dual PDE3/4 Inhibitors

It is now clear from a wide range of clinical studies that selective PDE4 inhibitors do not induce acute bronchodilation [92–94], but, as described above, it is evident that selective PDE3 inhibitors are indeed able to induce bronchodilation in humans. Given the success of the combination of β_2 agonists as bronchodilatory drugs and inhaled

glucocorticosteroids as anti-inflammatory drugs in the treatment of patients with asthma and in some COPD patients, a number of research groups have attempted to develop dual PDE3/4 inhibitors as single molecules that possess bifunctional bronchodilatory and anti-inflammatory activity. Researchers at the Leiden/Amsterdam Center for Drug Research have reported the synthesis and structure activity relationship of a series of potent dual PDE3/4 inhibitors which are able to inhibit arachidonic acid-induced ear edema in the mouse, achieving 44% inhibition at an oral dose of 16 mg/kg [95].

Altana Pharmaceuticals (Wesel, Germany, now part of Takeda) have a number of patents for benzonaphthyridine derivatives as dual PDE3/4 inhibitors, some of which have reached the clinic (see below), but there are limited data available on these compounds beyond the investigation of pumafentrine [96].

Kyorin Pharmaceuticals (Tokyo, Japan) have also recently reported a series of hybrid PDE3/4 dual inhibitors which potently suppress histamine-induced bronchoconstriction following intravenous (i.v.) administration and exhibit anti-inflammatory activity in preclinical animal models when administered by the inhaled route [97]. However, most of these agents have not been developed beyond the preclinical stage due to causing unwanted effects in the GI system.

To date, there have been at least 5 dual PDE3/4 inhibitors which have reached clinical development (zardaverine, benzafentrine, pumafentrine, tolafentrine and RPL 554). All of these appear to have been discontinued except for RPL 554.

In animal models, zardaverine [98] has shown bronchodilatory [98, 99] and anti-inflammatory [98, 100] effects. In the first clinical study [101], it was reported to have a modest and short-lasting bronchodilatory activity when given by inhalation to 12 patients with reversible bronchial obstruction; 4 puffs of either zardaverine (total dose 6 mg) or placebo were inhaled at 15-min intervals. Compared to placebo, specific airway conductance and FEV₁ increased significantly during the first hour of the repeated inhalations. In 7 of the patients, FEV₁ increased by >15%, but the duration of action varied considerably between patients. Three patients experienced side effects (headache, drowsiness, vertigo or nausea), and 1 dropped out of the study due to vomiting. In 1995, a second study was published in which zardaverine was administered with a metered-dose inhaler at single doses of 1.5, 3.0 or 6.0 mg, and compared with salbutamol (0.3 mg) and placebo (administered on separate days) to 10 patients with partially reversible airway obstruction. In this trial, zar-

daverine did not improve airway function whilst salbutamol did [102]. No further studies on zardaverine have been published and it appears to have been discontinued.

Benzafentrine [103] has been administered to healthy volunteers by the oral, i.v. and inhaled routes. When given at doses of up to 90 mg orally, it failed to protect against methacholine-induced bronchoconstriction. However, when given by inhalation, it produced a dose-dependent bronchoprotection to methacholine challenge, with an ED₅₀ of approximately 9.2 mg. Interestingly, when it was given by i.v. infusion (20 or 40 mg), a short-lived bronchodilatory response was also observed without the blood pressure or heart rate being affected [103]. This pilot study does imply that an inhaled approach which delivers a PDE3/4 inhibitor directly to the lung may provide a good therapeutic window. It also demonstrates that even after i.v. administration, drugs with PDE3/4 activity may be able to provide improvements in lung function without causing significant effects on the cardiovascular system. However, despite these somewhat encouraging early clinical results, the development of benzafentrine appears to have been stopped.

Pumafentrine [104], also known as BY 343, was used in phase 2 clinical trials for the treatment of asthma, but was discontinued in 2002, reportedly due to a short duration of action. An active metabolite of pumafentrine, hydroxypumafentrine, has also been described, although there have been no published clinical data on this compound [105].

Tolafentrine is cited as a dual PDE3/4 inhibitor, although there is a distinct lack of published information regarding the ability of this drug to inhibit PDE3 and PDE4. The compound has been found to be effective by inhaled delivery in rodent models of pulmonary hypertension [106, 107]. In 2002, it was reported to be in phase 1 clinical trials for the treatment of primary pulmonary hypertension [108] (having previously been described as synergistically prolonging the vasodilating properties of prostanoids in the treatment of secondary pulmonary hypertension) [109]. However, to date, no clinical studies on tolafentrine administration to patients with asthma or COPD have been reported.

More recently, another dual PDE3 and PDE4 inhibitor RPL 554 has been described that is derived from a series of analogues of trequinsin, which exhibits both bronchodilatory and anti-inflammatory activities in preclinical *in vitro* and *in vivo* model systems [110]. RPL 554 also produces a rapid, significant and sustained bronchodilatory effect in patients with mild-to-moderate COPD and in asthma patients, when administered by the inhaled route, and is also

bronchoprotective against methacholine challenge [111]. Importantly, RPL 554 appears to be at least as effective as the β_2 agonist salbutamol as a bronchodilator in the same patients and achieves these beneficial effects on lung function without significant effects on the cardiovascular system. At the same dose that elicits bronchodilation, RPL 554 also exhibits highly significant anti-inflammatory effects in humans, as it is able to reduce the ability of LPS to induce recruitment of inflammatory cells into the airways, particularly the absolute numbers of neutrophils, eosinophils, lymphocytes and macrophages [111], again without significant effects on the cardiovascular system. RPL 554 has limited systemic bioavailability with rapid plasma clearance and no evidence of drug accumulation. It has been administered in clinical trials to more than 100 people to date (healthy volunteers and patients with asthma or COPD), with adverse events being generally mild and of equal frequency to placebo-treated groups [111]. RPL 554 is an effective and well-tolerated bronchodilatory, bronchoprotective and anti-inflammatory drug that shows promise for the treatment of COPD and asthma [112].

The development of many selective PDE4 inhibitors for oral administration has been curtailed because the therapeutic window is too narrow, with significant GI side effects such as nausea, vomiting, diarrhea, abdominal pain and dyspepsia, although some of these do appear to resolve over time [7]. These side effects have also been observed with some of the inhaled PDE4 inhibitors [101], presumably due to the oral absorption of the swallowed portion of the drug. The mechanism of why some PDE4 inhibitors have significant GI side effects, going back to the original observations with rolipram which was initially developed as an anti-depressant, is not understood, though many hypotheses have been put forward to explain it [7, 113]. Despite such side effects, roflumilast N-oxide was recently still approved for the treatment of severe COPD in a number of countries including the USA, but the unwanted effects are clearly limiting its wider use. It is encouraging, therefore, to see that both a number of orally active PDE4 inhibitors like apremilast [114], approved recently for the treatment of psoriatic arthritis, and some inhaled compounds appear to not have significant GI side effects when under development [7].

Of particular concern to regulatory agencies is the observation that mesenteric vasculitis develops in some laboratory animal species with some systemically administered PDE4 inhibitors. However, this has also been seen with theophylline, a drug that has been clinically widely used for almost a century [115, 116], which throws into question the clinical relevance of these preclinical toxicol-

ogy findings. To date, there have been no reports of mesenteric vasculitis in patients treated with roflumilast N-oxide or another orally active PDE4 inhibitor cilomilast, widely used in patients with asthma and COPD in phase 3 clinical trials (FDA website). It is therefore plausible that rats and dogs have an increased susceptibility to drug-induced vascular lesions because such arteriopathies commonly occur in these species (used regularly in preclinical toxicology studies [117, 118]), with differences across species in terms of both PDE4 expression and the functional effects of PDE4 inhibitors. For example, a recent study demonstrated that levels of PDE4 activity in many tissues are much higher in rats than in humans, which would explain why rats are more susceptible to PDE4 inhibitor-induced toxicities [119]. In addition, the PDE4 inhibitor IC 542 significantly enhances LPS-induced IL-6 release from rat whole blood [120], but has no potentiating effect on LPS-induced IL-6 release from human or non-human primate blood. The potential for developing vasculitis with systemically active PDE inhibitors does, however, require careful monitoring in humans, and there have been attempts to identify potential predictive biomarkers for this unwanted effect. Indeed, the tissue inhibitor of MMP1 appears to be an early and sensitive predictive biomarker of the inflammatory and tissue remodelling components of PDE4 inhibitor-induced vascular injury in rats [121].

Some insight into the potential isoform subtypes responsible for mediating the side effects of PDE4 inhibition can be gleaned from studies with subtype knockout mice, although potential species differences need to be considered here. PDE4A^{-/-} and B^{-/-} mice have normal neonatal growth survival and fertility, but this is impaired in PDE4D^{-/-} mice [122]. Furthermore, PDE4D^{-/-} mice have been shown to suffer from various cardiovascular complications [122]. However, PDE4D^{-/-} (but not PDE4B^{-/-}) mice have shortened α_2 adrenoceptor-mediated anesthesia, which has been suggested to be a behavioural correlate of emesis [113], and it has thus been hypothesized that the D isoform is responsible for the emesis observed with orally administered PDE4 inhibitors. However, some inhaled PDE inhibitors have been shown to elicit GI side effects in patients (e.g. zardaverine), suggesting that the inhaled route is not sufficient alone to reduce this troublesome side effect profile [102]. Rather, there is a need to develop non-emetic pharmacophores such as RPL 554 [111].

PDE3 inhibitors were developed in the 1980s as 'safer' alternatives to cardiac glycosides for the treatment of dilated cardiomyopathy, and beneficial effects on the force of myocardial contraction and vascular smooth muscle tone were reported in the short term. However, chronic treat-

ment (>28 days of systemic treatment) resulted in a significant increase in mortality [123]; this has somewhat sensitized the field to avoiding the development of drugs with significant PDE3 inhibitory activity [7]. However, the PDE3 inhibitor milrinone, implicated in these early clinical trials, is still in regular clinical use for the short-term therapy of severe congestive heart failure, and 4 other PDE3 inhibitors (amrinone, cilostazol, pimobendane and enoximone) are administered for a number of indications like stroke prevention and anti-platelet activity. Importantly, however, in the discussion about the potential liability of the side effects of PDE3 inhibitors, it needs to be appreciated that some of these drugs are not just PDE3 inhibitors but exhibit a whole range of other pharmacological actions which may also contribute to the adverse effects seen with these compounds in clinical practice, particularly with long-term systemic administration. Milrinone, for example, is an adenosine 1 receptor antagonist [124]. So the contribution of inhibiting PDE3A and PDE3B relative to these adverse effects is unclear, not least because these inhibitors can inhibit both PDE3 isoforms. However, some insight into the potential roles of PDE3A and PDE3B can be derived from knockout mouse studies, since PDE3A^{-/-} and PDE3B^{-/-} mice have normal neonatal growth and survival. PDE3A^{-/-} mice have an increased heart rate and are infertile [124, 125]. PDE3B^{-/-} mice do not share these characteristics, but metabolic dysregulation including systemic insulin resistance has been observed in this mouse strain [126].

Given that the additive and synergistic effects of dual PDE3/4 inhibition have been observed in terms of important efficacy end points, a clear concern is that these effects could also be seen with respect to potential adverse events. In this regard, it would clearly be of interest to investigate the phenotype of dual PDE3^{-/-} and PDE4^{-/-} mice, but this is not yet available. Of interest, however, is that whilst selective inhibition of PDE3 or PDE4 in wild-type cardiomyocytes causes elevated calcium transients, sarcoplasmic reticulum Ca²⁺ content and phospholamban phosphorylation, combined PDE3 and PDE4 inhibition causes no further increases in sarcoplasmic reticulum function. The reason for this perhaps unexpected finding is unclear, but it could be related to the compartmentalization of pools of cAMP. Nevertheless, no pre-clinical findings were identified in the toxicology studies which have thus far prevented 5 inhaled dual PDE3/4 inhibitors progressing to early clinical trials. RPL 554 is currently being used in phase 2 clinical trials, and, to date, has not been associated with any clinically significant side effects [111]. We recently demonstrated in guinea pigs in vivo that at certain doses, RPL 554 interacts positively

with either β_2 agonists or muscarinic receptor antagonists to produce further bronchodilation, with no potentiation observed on cardiovascular parameters when these drugs were administered in combination [82].

Conclusion

Combining inhibition of PDE3 and PDE4 is an attractive strategy for treating COPD and other inflammatory airway diseases, given the broad anti-inflammatory and bronchodilatory effects of the inhibitory agents, together with their potential to stimulate mucociliary clearance. It is clear that such dual inhibition is required in order to optimally inhibit the activity of certain key inflammatory cell types (e.g. macrophages, lymphocytes, epithelial cells and airway smooth-muscle cells) that are involved in the pathogenesis of COPD. The inhaled route of administration of the PDE3/4 inhibitors would seem to be a sensible strategy, in order to maximise the therapeutic ratio and reduce the adverse effects that have been previously seen

with systemic administration (which has limited their therapeutic use). Designing an agent with subtype selectivity could also potentially offer an advantage, based on the data generated by studies on knockout mice, which, together with the expression profile of PDE3B, suggest that a compound which could selectively inhibit PDE3B rather than PDE3A could be beneficial to potentially mitigate cardiovascular risk. Selective inhibitors of PDE3A and PDE3B would clearly be required to confirm this. It appears that PDE4A, PDE4B and PDE4D play an important role in mediating the anti-inflammatory effects of PDE4 inhibitors, and that PDE4D may contribute to the effects on airway smooth muscle, even though no PDE4 inhibitor has been found to exhibit any meaningful selectivity against the various PDE4 isoforms (and, interestingly, does not elicit any acute bronchodilatory activity). The availability of an inhaled dual PDE3/4 inhibitor, RPL 554, that has both bronchodilatory and anti-inflammatory activity and is rapidly cleared from the systemic circulation, may provide a novel approach to treating COPD and other inflammatory airway diseases.

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