

Phosphodiesterase Inhibitors in the Treatment of Inflammatory Diseases

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Abstract Phosphodiesterase 4 (PDE4) belongs to a family of enzymes which catalyzes the breakdown of 3, 5'-adenosine cyclic monophosphate (cAMP) and is ubiquitously expressed in inflammatory cells. There is little evidence that inflammatory diseases are caused by increased expression of this isoenzyme, although human inflammatory cell activity can be suppressed by selective PDE4 inhibitors. Consequently, there is intense interest in the development of selective PDE4 inhibitors for the treatment of a range of inflammatory diseases, including asthma, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease, and psoriasis. Recent clinical trials with roflumilast in COPD have confirmed the therapeutic potential of targeting PDE4 and recently roflumilast has been approved for marketing in Europe and the USA, although side effects such as gastrointestinal disturbances, particularly nausea and emesis as well as headache and weight loss, may limit the use of this drug class, at least when administered by the oral route. However, a number of strategies are currently being pursued in attempts to improve clinical efficacy and reduce side effects of PDE4 inhibitors, including delivery via

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the inhaled route, development of nonemetic PDE4 inhibitors, mixed PDE inhibitors, and/or antisense biologicals targeted toward PDE4.

Keywords Asthma · Cilomilast · COPD · Emesis · Inflammation · PDE4 · Roflumilast

Abbreviations

COPD Chronic obstructive pulmonary disease

PDE Phosphodiesterase

1 Introduction

Theophylline has been used in the treatment of asthma and chronic obstructive pulmonary disease (COPD) since the 1930s, although its popularity has declined due to the introduction of β_2 -adrenoceptor agonists and glucocorticosteroids. Several mechanisms have been proposed to explain the potential beneficial action of theophylline in respiratory disease. Theophylline has been shown to inhibit the activity of a cyclic 3', 5' nucleotide PDE with a K_i of 100 μM (Butcher and Sutherland 1962), which has been suggested to contribute to its ability to promote suppressor cell activity in lymphocytes (Shore et al. 1978; Zocchi et al. 1985; O'Shaughnessy et al. 2007) and its beneficial actions in patients with asthma (Sullivan et al. 1994), COPD (Rennard 2004), or psoriasis (Papakostantinou et al. 2005). However, theophylline has been relegated to second- or third-line therapy for the treatment of respiratory disease and is not routinely used in the treatment of other inflammatory conditions such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease, in part because it is not recognized as an anti-inflammatory drug, but more importantly because of the very significant drug interactions and narrow therapeutic window exhibited by theophylline; the side effect profile of theophylline includes nausea, emesis, gastrointestinal disturbances, and arrhythmias. These problems, in part, promoted an interest in understanding how the PDE family of enzymes regulated cAMP levels within cells and the potential for developing drugs inhibiting such enzymes, in a selective way as a way of improving the therapeutic window of theophylline.

There are presently 11 known families of PDEs and at least 21 isoforms with numerous splice variants that are characterized by differences in structure, substrate specificity, inhibitor selectivity, tissue and cell distribution, regulation by kinases, protein-protein interaction, and subcellular distribution (Conti and Beavo 2007; McCahill et al. 2008). However, targeting PDE4, an enzyme family that exclusively metabolizes cAMP (Houslay et al. 2005, 2007), has been the focus for the development of drugs that could prove beneficial in the treatment of depression, schizophrenia (Millar et al. 2007), respiratory diseases such as asthma and COPD

(Barnes 2008), allergic skin diseases and psoriasis (Baumer et al. 2007), and inflammatory bowel disease (Keshavarzian et al. 2007).

It is therefore of interest that plasma levels achieved with a dose of theophylline that demonstrated significant anti-inflammatory activity (Sullivan et al. 1994) are well below the K_i for PDE inhibition and suggested that PDE4 inhibition alone does not completely explain the clinical effectiveness of this drug (Barnes et al. 2005). Nevertheless, highly potent and selective PDE4 inhibitors have been developed to target a range of inflammatory diseases. However, targeting PDE enzymes is not unique to inflammatory diseases as exemplified with the development and clinical success of a number of PDE5 inhibitors for the treatment of erectile dysfunction as exemplified by sildenafil (Boolell et al. 1996).

2 Phosphodiesterase 4

PDE4 is highly selective for catalyzing the hydrolysis of cAMP, which terminates the downstream signalling of this second messenger (Houslay et al. 2007; Houslay 2010). There are 4 gene families (A, B, C, and D), where alternative mRNA splicing and the use of distinct promoters generates more than 20 splice variants (Houslay et al. 2007). While hydrolysis of cAMP is a common feature of this family, it is clear that these isoforms can be targeted to different domains and signalling complexes within the intracellular compartment, where they play a pivotal role in underpinning compartmentalized cAMP signaling (Houslay 2010). Furthermore, their activities can be differentially regulated by phosphorylation by various protein kinases, suggesting that these isoforms have specific functions in the control of cellular activity (McCahill et al. 2008).

X-ray crystallography has resolved the catalytic domain of these enzymes, which comprise three important domains: a bivalent metal binding pocket (Zn^{2+} , Mg^{2+}), which is thought to form a complex with the phosphate moiety of cAMP, a pocket containing an invariant glutamine (Q pocket), which forms hydrogen bonds with the nucleotide (purine) moiety of cAMP, and a solvent pocket. PDE4 inhibitors occupy this active site through a number of important interactions and prevent cAMP metabolism. These include indirect binding to the metal ions via the formation of hydrogen bonding to water while hydrophobic interactions between the planar ring structure of these inhibitors and hydrophobic amino acid residues such as phenylalanine and isoleucine serve to “clamp” the inhibitor within the active site. There are also hydrogen bond interactions between the aromatic ring structure of these inhibitors and the invariant glutamine residue in the Q pocket, the site which is normally occupied by the nucleotide moiety of cAMP (Xu et al. 2000; Card et al. 2004; Wang et al. 2007).

There are considerable challenges to the synthesis of subtype selective inhibitors due to the high degree of sequence and structural homology within the catalytic domains of the PDE4 subtypes (Xu et al. 2000; Card et al. 2004;

Houslay et al. 2005; Wang et al. 2007). One possibility might be to exploit subtle differences between the interaction of these inhibitors with the catalytic active site. Alternatively, compounds that inhibit catalysis by targeting regions outside the catalytic site may be possible. Compounds that selectively interact with the N terminal regions of the respective PDE4, which contain phosphorylation sites and/or protein binding sequences, might indirectly interfere with PDE4 activity (Houslay et al. 2005). Moreover, inhibitors may act through a combined interaction with residues in regions that fold over or near the catalytic site, e.g., UCR2 which provides a regulatory region unique to PDE4 enzymes as well as some residues in the catalytic domain (Kranz et al. 2009; Burgin et al. 2010).

PDE4 is expressed in a number of cell types that are considered important in the pathogenesis of inflammatory disease (Table 1). It might reasonably be argued that targeting the inhibition of PDE4 could potentially suppress the function of numerous cell types. However, it is well known that other PDE enzymes are also expressed in these cells and the contribution of other PDEs to cell function (e.g., PDE3, PDE7) in the context of regulating inflammatory cell function is also being explored (Smith et al. 2004; Boswell-Smith et al. 2006a; Banner and Press 2009). Although it would seem prudent to develop subtype selective PDE4 inhibitors in attempts to maximize therapeutic benefit at the expense of adverse effects, there is also the possibility that nonselective PDE inhibitors might offer a better approach in targeting multiple target cells in the disease process. Indeed, it has been suggested that clozapine is a better antipsychotic than newer generation atypical antipsychotics because this drug targets numerous receptors and as such, has been described as a “magic shotgun” for the treatment of schizophrenia (Roth et al. 2004).

3 Asthma and PDE4

The underlying pathophysiological features of asthma are a consequence of the contribution of numerous cells including the epithelium, dendritic cells, T-lymphocytes, eosinophils, mast cells, and airway smooth muscle. Thus, there is a complex web of interconnecting interactions between different cell types and inflammatory mediators. Whilst glucocorticosteroids remain the mainstay treatment of this disease, there still remains a group of asthmatics with poor symptom control despite treatment with these drugs (Barnes 2008). It is therefore of interest that many of the cell types illustrated in Table 1 express PDE4 and inhibition of this enzyme family can suppress the function of these cells.

A number of studies have investigated whether the pathogenesis of asthma might be a consequence of increased PDE4 protein expression and activity. This hypothesis had some currency in view of studies in the field of dermatology, which purported to demonstrate the expression of a novel and distinct monocyte-derived cAMP-PDE obtained from individuals with atopic dermatitis. Monocytes from these individuals had increased functionality and this corresponded with the presence of protein with increased PDE activity. This was thought to be

Table 1 PDE distribution within human cells of interest for the treatment of respiratory diseases such as asthma and COPD

Cell type	PDE4 subtype ^a	Other PDE's	Biological consequence of PDE4 inhibition	Reference
T-lymphocytes CD4 CD8 Th1, Th2 Th17	A, B, D ^b	3, 7	Inhibition of proliferation and cytokine release	Essayan et al. (1997), Gantner et al. (1997b), Hatzelmann and Schudt (2001), Smith et al. (2003), Peter et al. (2007), Ma et al. (2008)
B cells	A, B, D	7	Increased proliferation	Gantner et al. (1998), Smith et al. (2003)
Eosinophils	A, B, D	7	Inhibition of superoxide anion generation; delayed apoptosis	Hatzelmann and Schudt (2001), Smith et al. (2003), Parkkonen et al. (2007)
Neutrophils	A, B, D	7	Inhibition of superoxide anion and neutrophil elastase release	Hatzelmann and Schudt (2001), Smith et al. (2003), Jones et al. (2005)
Monocyte	A, B, D	7	Inhibition of TNF α release	Hatzelmann and Schudt (2001), Smith et al. (2003), Heystek et al. (2003), Jones et al. (2005)
Macrophages	A, B, D	1, 3, 7	Inhibition of TNF α release ^c	Gantner et al. (1997a), Hatzelmann and Schudt (2001), Smith et al. (2003), Barber et al. (2004)
Dendritic cells	A, B, D	1, 3	Inhibition of TNF α release	Hatzelmann and Schudt (2001), Heystek et al. (2003)
Osteoblast	A, B, D	3	Stimulates RANKL-induced osteoclast formation	Noh et al. (2009)
Chondrocytes	A, B, D	1	Inhibition of IL-1 β stimulated production of nitric oxide	Tenor et al. (2002)
Mast cells			Little if any mast cell stabilization	Weston et al. (1997), Shichijo et al. (1998)
Airway epithelial cells		1-3, 4, 5, 7, 8	Increased production of PGE ₂ ; inhibition of IL-6 production	Fuhrmann et al. (1999), Haddad et al. (2002)
Endothelial cells		2, 3, 4, 5	Inhibition of adhesion molecule expression	Jones et al. (2005), Sanz et al. (2007)
Fibroblasts	A, B, D	1, 4, 5, 7	Inhibition of fibroblast chemotaxis; inhibition of pro-MMP1,2 release Differentiation into myofibroblasts	Kohyama et al. (2002), Smith et al. (2003), Martin-Chouly et al. (2004), Dunkern et al. (2007)
^d Sensory nerves	D	1, 3	Inhibition of neuropeptide release	Spina et al. (1995)

^aPDE4 subtype mRNA expression illustrating relative abundance in cells^bPDE4D absent in Th1 cells^cIn the presence of a PDE3 inhibitor^dGuinea-pig sensory nerves

the underlying basis for the pathology associated with atopic dermatitis (Chan et al. 1993). However, soluble PDE4 activity was not increased in a range of peripheral blood leukocytes from atopic subjects of either mild or severe severity (Gantner et al. 1997b). Similarly, while increased total PDE catalytic activity was observed in peripheral blood monocytes from individuals with mild asthma, this was associated, paradoxically, with reduced PDE4 activity (Landells et al. 2001), and the expression of PDE4A, B, and D was not increased in peripheral blood CD4 positive T-lymphocytes in patients with mild asthma (Jones et al. 2007). Together these studies indicate that the underlying pathogenesis of mild asthma cannot be attributed to enhanced PDE4 expression or activity. A genome-wide association search has demonstrated single nucleotide polymorphisms on chromosome 5q12 for PDE4D (Himes et al. 2009).

The significance of these findings remains to be established given that this isoform is purportedly linked with emesis and targeting of PDE4A and PDE4B is seen as a rationale approach to develop nonemetic anti-inflammatory PDE4 inhibitors (Manning et al. 1999; Kranz et al. 2009). If this assertion is correct, then the challenge remains to develop a PDE4 subtype selective inhibitor that differs by at least three orders of magnitude, but this has yet to be achieved after more than a decade of work in this field. However, this hypothesis has been questioned following the discovery of PDE4D selective allosteric inhibitors, which promote anti-inflammatory activity and are largely devoid of emesis in animal models (Burgin et al. 2010). The implication of these novel findings is that selectivity for different PDE4 subtypes is not a critical determinant in avoiding side effects such as nausea, but whether partial inhibition can be achieved, as in this example using allosteric inhibitors which interact with upstream conserved regions (UCRs), and the catalytic domain of PDE4D (Burgin et al. 2010).

Numerous preclinical studies in models of allergic pulmonary inflammation have repeatedly documented the ability of PDE4 inhibitors to inhibit two important characteristic features of asthma, namely, the recruitment of eosinophils to the airways and bronchial hyperresponsiveness (Spina 2003). One disadvantage of these studies is the inability to ascertain the role of PDE4 isoforms because of the nonselective nature of the PDE4 inhibitors currently under development. However, the use of genetically modified mice has revealed some interesting findings. Airway inflammation characterized by recruitment of eosinophils to the airways of mice deficient in PDE4D was not different from that of wild-type controls (Hansen et al. 2000). This indicated that other PDE4 subtypes contribute in the metabolism of intracellular cAMP since cell recruitment to the airways was inhibited when animals were treated with nonselective PDE4 inhibitors (Kung et al. 2000; Kanehiro et al. 2001). However, airway obstruction caused by methacholine was enhanced in wild-type allergic mice, but abolished in PDE4D gene-deficient mice. These mice were *hyporesponsive* to methacholine, even in the absence of allergic sensitization, and this feature appeared to be related to an increase in the production of a dilator prostaglandin in the airways of these PDE4D gene-deficient mice (Hansen et al. 2000; Mehats et al. 2003). However, this effect was specific for methacholine because the enhanced airway obstruction in response to serotonin

was unaffected by the removal of PDE4D (Hansen et al. 2000). This highlights the potential complementary role of PDE4 isoforms in regulating allergic airway inflammation, and the need to target more than one PDE4 isoform since the inflammatory response, bronchial hyperresponsiveness, and airway remodeling in allergic wild-type mice could be inhibited by nonselective PDE4 inhibitors such as rolipram or roflumilast (Kung et al. 2000; Kanehiro et al. 2001; Kumar et al. 2003). Whilst the use of gene knockout studies can offer insights into the function of proteins, it is likely that compensatory mechanisms at birth and the mixed background of the generated knockouts could confound attempts to define the biological role of a particular protein. The use of conditional PDE4 knockouts or a biological approach (e.g., siRNA) may be required to understand the biological role(s) of PDE4 subtypes completely (Huston et al. 2008).

The numerous preclinical studies reporting the anti-inflammatory potential of PDE4 inhibitors in models of allergic inflammation and in human cells *in vitro* have to some degree been corroborated in clinical trials in asthmatic subjects. Twice-daily treatment for 9.5 days with the PDE4 inhibitor CDP840 inhibited the development of the late phase response in asthmatic subjects by 30% (Harbinson et al. 1997). A similar degree of inhibition of the late phase response was observed following once daily treatment for 7- to 10-day with roflumilast (van Schalkwyk et al. 2005), while 2-week treatment with MK-0359 improved baseline FEV1 and reduced symptom scores in chronic asthmatic subjects (Lu et al. 2009). This late phase response is used by clinicians to model the inflammatory component following an allergic insult to the airways. In both allergen challenge studies, the effect of drug treatment on the acute allergen bronchoconstriction was modest and consistent with the lack of demonstrable action of PDE4 inhibition on both mast cell and airway smooth muscle function. This highlights the role of other PDE enzymes, namely PDE3 in the regulation of the function of these cell types in the airway (Table 1). Bronchial hyperresponsiveness was not reduced by these drugs, with only one study purporting to show modest protection against allergen-induced bronchial hyperresponsiveness (Louw et al. 2007). This suggests that PDE4 may not be a suitable target for this particular phenomenon, or that higher doses are required to provide complementary and persistent inhibition of the enzyme and hence attenuation of bronchial hyperresponsiveness. It is of interest that roflumilast has a plasma half-life of 16 h following a single oral administration and is metabolized by CYP3A4 to the active N-oxide metabolite, which has considerably greater bioavailability with a half-life of 20 h that would favor prolonged enzyme exposure (David et al. 2004). This favorable pharmacokinetic profile would be anticipated to produce longer periods of PDE4 inhibition. However, whilst there was a significant reduction in the activity of circulating monocytes in subjects maintained on roflumilast for 4 weeks, the magnitude of this change was small, resulting in only an approximately 1.3-fold reduction in TNF α production by monocytes in response to endotoxin challenge *in vitro* (Timmer et al. 2002). One could argue that only a partial inhibition of PDE4 activity was achieved at the dose employed in clinical studies and consequently suppression of inflammatory cell function within the airway tissue compartment is not maximal, a hypothesis borne out in several clinical studies (Gamble et al. 2003; Grootendorst et al. 2007).

Unfortunately, the doses that can be administered clinically with most orally active PDE4 inhibitors are limited by side effects, the most commonly reported being headache, nausea, and diarrhea of a mild-to-moderate severity (van Schalkwyk et al. 2005; Lu et al. 2009). The availability of other nonemetic drugs such as CDP840 (Harbinson et al. 1997) might provide hope that such PDE4 inhibitors can be developed for the treatment of asthma, a worthy goal to pursue in light of at least one clinical study reporting comparable clinical efficacy between roflumilast and the inhaled glucocorticosteroids beclomethasone dipropionate in persistent asthma (Bousquet et al. 2006). Such systemic side effects may also be eliminated by using the inhaled route such that relatively higher doses of PDE4 inhibitor can be delivered to the intrapulmonary compartment whilst reducing systemic bioavailability as recently reported with GSK256066 (Singh et al. 2010). However, another inhaled PDE inhibitor, UK 50,000 failed to show any clinical benefit (Phillips et al. 2007) and an early mixed PDE 3/4 inhibitor zardaverine actively elicited frank emesis at inhaled doses that induced bronchodilation (Brunnee et al. 1992). Mesenteric arteritis, characterized by inflammation of the arterioles is observed in rodents and primates following doses of some PDE4 inhibitors far exceeding those that would be used clinically; however, this condition has not been observed in subjects in clinical trials with roflumilast and therefore is unlikely to be a major issue and even less so if inhaled formulations of this drug class are developed (Spina 2008).

4 COPD and PDE4

Unlike asthma, COPD is primarily caused by cigarette smoking, although in developing countries smoke derived from burning biomass fuels is also a predisposing factor (Salvi and Barnes 2009). COPD is also an inflammatory disease but the nature of the inflammatory response is distinct from asthma. The inflammatory response in COPD is characterized by the activation of macrophages and airway epithelial cells, which in turn, secrete a range of chemokines and lipid mediators resulting in the recruitment of neutrophils and CD8+ T-lymphocytes to the lung. The secretion of a range of proteases from neutrophils (elastase, MMP9, cathepsins) and macrophages (MMP12) is thought to contribute toward fibrosis of the small airways and increased mucus secretion and destruction of the alveolar wall (Barnes 2008) These pathological changes give rise to the symptoms of cough, mucus secretion, dyspnea, and emphysema. Many of the cell types implicated in the COPD inflammatory response express PDE4 (Table 1).

The expression of PDE4A-D mRNA transcripts in peripheral blood neutrophils and CD8 T cells is not altered in subjects with mild COPD (Jones et al. 2007). However, the expression of PDE4A4 mRNA transcript was significantly increased in macrophages purified from bronchoalveolar lavage fluid from subjects with mild-to-moderate COPD compared with healthy subjects or smokers who did not present with COPD (Barber et al. 2004). Of the 12 PDE4 variants analyzed, only the

activity of PDE4A4 was increased and suggested that local events/processes within the lung of subjects with COPD specifically upregulated this variant (Barber et al. 2004). However, the functional consequence of this change remains to be established in light of findings showing that PDE4 inhibition has only a modest effect in suppressing TNF α production from human macrophages derived from cultured monocytes. Moreover, the contribution of other PDEs (e.g., PDE3, PDE7) and other PDE4 isoforms in regulating function in this cell type clearly need to be explored (Gantner et al. 1997a; Smith et al. 2004). Genotyping studies have also revealed the presence of single nucleotide polymorphisms within the PDE4D gene in COPD subjects compared with smokers within the Japanese population that was in linkage disequilibrium with the IL-13 gene. However, the significance of these findings remains to be established since this relationship was absent in an Egyptian population (Homma et al. 2006).

In vivo models of COPD are very limited. However, the recruitment of neutrophils to the airways can be readily induced using the bacterial wall component, endotoxin, although it is widely appreciated that this stimulus can only model neutrophil recruitment to the airways (Leclerc et al. 2006). Neutrophil recruitment into the airways of wild-type mice was inhibited by around 50% in PDE4B and PDE4D-deficient mice and a greater degree of inhibition was observed when wild type mice were treated with roflumilast (Ariga et al. 2004). This once again highlights the complementary roles of PDE4 isoforms in regulating neutrophil recruitment to the airways. Similarly, smoking-induced neutrophil recruitment to the airways, release of chemokines, and emphysematous changes to the lung were attenuated by PDE4 inhibitors (Martorana et al. 2005; Leclerc et al. 2006). Together, these studies highlight the utility of inhibiting PDE4 in cell types implicated in COPD.

A number of phase III clinical trials have assessed the potential utility of PDE4 inhibitors in the treatment of COPD (Rabe et al. 2005; Rennard et al. 2006; Calverley et al. 2007). All three studies report modest, but nevertheless significant improvements in spirometry over placebo, quality of life scores, and reduction in the number of exacerbations in the severest group of COPD subjects. Indeed, recent phase III clinical trials evaluating the effect of roflumilast in symptomatic moderate-to-severe asthmatics have reported more promising findings (Fabbri et al. 2009; Calverley et al. 2009). Roflumilast caused a significant 48-ml improvement in FEV1 compared with placebo and reduced exacerbation rates of moderate-to-severe intensity by 17% over a 52-week period (Calverley et al. 2009). Similarly, roflumilast consistently improved baseline FEV1 in moderate-to-severe COPD patients already taking either a long-acting β_2 -agonist (salmeterol) or a long-acting muscarinic antagonist (tiotropium bromide) by 49 and 80 ml, respectively, during 24 weeks of treatment (Fabbri et al. 2009).

The mechanism of the improvement in spirometry is unlikely to be due to relaxation of airway smooth muscle because this drug class has weak bronchodilator activity. It is possible that this improvement is due to an anti-inflammatory action of the drugs (Table 1), although no biomarker of inflammation was measured in these studies. However, separate studies have addressed whether PDE4

inhibitors are anti-inflammatory in patients with COPD. Both roflumilast (Grootendorst et al. 2007) and cilomilast (Gamble et al. 2003) reduced both the number of inflammatory cells, such as, neutrophils and lymphocytes recruited to the airways and the levels of two biochemical markers of this disease, namely IL-8 and neutrophil elastase. The magnitude of the change in the number of these inflammatory cells and concentration of mediators was between 30 and 50%, which might contribute to the beneficial action of these two PDE4 selective in the clinic trials. However, to date the substantial inhibition of PDE4 with an orally active inhibitor has not been achieved because of dose-limiting side effects, which may also explain the modest clinical benefit shown with these drugs. As discussed for asthma, it is plausible that other PDE species (e.g., PDE3, 7; see Table 1) may require targeting for a full anti-inflammatory therapeutic action to be attained in treating this disease (Banner and Press 2009).

The most common side effect reported with roflumilast included diarrhea (9%), headache (5%), and nausea (5%) (Rabe et al. 2005; Calverley et al. 2007, 2009; Fabbri et al. 2009). This was of the same order of magnitude as reported with cilomilast, although abdominal pain and vomiting were also reported for this drug (Rennard et al. 2006). The adverse effects seemed to disappear with continued use; nonetheless, these side effects were a major reason why patients did not continue with the study during the first 3–4 weeks of treatment. No cardiovascular liabilities were noted. One unanticipated side effect that was noted following prolonged treatment with roflumilast was a significant reduction in body weight in subjects. This might be attributed to gastrointestinal discomfort in susceptible patients, although it was interesting to note that weight loss was also reported in patients not complaining of gastrointestinal side effects and might be an effect worthy of further study (Fabbri et al. 2009; Calverley et al. 2009).

One might anticipate that the risk/benefit ratio would be improved by inhaled delivery of a PDE4 inhibitor directly to the lung. Unfortunately, to date only one study has evaluated the effect of an inhaled PDE4 nonselective subtype inhibitor. This was used to treat moderate-to-severe COPD and no significant improvement in baseline FEV1 was observed after 6 weeks of treatment (Vestbo et al. 2009). As no inflammatory biomarker was measured in this study, it is unclear whether a sufficient level of sustained PDE4 inhibition was achieved. This seems likely because adverse events such as headache and improvements in lung function that were noted at 2 weeks were absent after 6 weeks of treatment with the high dose. Whether inhaled PDE4 inhibitors will be of utility in COPD remains to be determined but there may be a cause for optimism in view of the amelioration of the allergen-induced late phase response in asthma with the inhaled PDE4 inhibitor GSK256066 (Singh et al. 2010). This demonstrates that in principle one can achieve inhibition of PDE4 following topical delivery to the lung that could lead to clinical benefit, but the relevant clinical trials need to be undertaken with GSK256066 to ascertain whether this drug will be of any clinical benefit to patients with COPD.

5 Other Inflammatory Diseases and PDE4

Numerous inflammatory conditions such as psoriasis (Nestle et al. 2009), rheumatoid arthritis (Taylor and Feldmann 2009), and inflammatory bowel disease (Abraham and Cho 2009) are characterized by a complex interplay between cells resulting in their activation and recruitment, release of proinflammatory cytokines, and tissue damage. Biologicals directed against cytokines are one of the most important therapies to emerge in the past decade and have revolutionized the treatment of inflammatory diseases as exemplified by those targeting TNF α (Nestle et al. 2009; Taylor and Feldmann 2009; Abraham and Cho 2009). It is therefore of interest that PDE4 inhibitors can suppress TNF α production by various inflammatory cell types, and inhibit the activity of immune cells so critical in these diseases (Table 1). It is therefore no surprise that PDE4 inhibitors are also being considered for the treatment of a number of nonrespiratory inflammatory diseases.

Earlier observations suggested the targeting of cAMP PDE might prove beneficial in the treatment of skin inflammatory disease. For example, potential deficits in adenylyl cyclase signaling in psoriasis (Wright et al. 1973) and expression of a novel monocyte-derived cAMP PDE in atopic dermatitis (Chan et al. 1993) were reported. Consequently, several selective PDE4 inhibitors have been evaluated in clinical trials. Patients with psoriasis and treated with apremilast, an orally active PDE4 inhibitor demonstrated an improvement in clinical scores and reduced T-cell number in the dermis in an open-label clinical trial (Gottlieb et al. 2008). Similarly, the systemic administration of apremilast reduced pathological features of psoriatic human skin transplanted onto immunocompromised mice (Schafer et al. 2010). One of the advantages of inflammatory dermatoses is that topical administration could limit the systemic liability associated with orally administered PDE4 inhibitors and this strategy has been adopted for the development of AN2728 for the treatment of psoriasis and atopic dermatitis with promising results in phase II clinical trials (Nazarian and Weinberg 2009; Akama et al. 2009).

Inflammatory bowel diseases might also be amenable to treatment with PDE4 inhibitors since Th1 and Th17 cells are implicated in this disease and the activity of these cell types can be suppressed by PDE4 inhibitors (Table 1). The PDE4 inhibitor, tetomilast, appeared to reduce various indices of inflammation in IL10-deficient mice that spontaneously develop chronic gastrointestinal inflammation characterized by mucus secretion, rectal prolapse, and diarrhea (Ichikawa et al. 2008). Tetomilast has also been evaluated in a phase II clinical trial in active ulcerative colitis (Schreiber et al. 2007). Improvement in disease as defined by a disease activity index of ≥ 3 was achieved in 53% (25 mg dose) and 39% (50 mg dose) of patients after 8 weeks of treatment, although this was not statistically different from the placebo group. However, post-hoc analysis revealed that patients with more severe disease showed significant improvement when treated with tetomilast. One of the potential issues with this study is the reporting of nausea and emesis, particularly with the higher dose of tetomilast which will be of concern when treating patients with gastrointestinal inflammation.

Rheumatoid arthritis is amenable to anti-TNF α monoclonal antibodies, which highlights an important role this cytokine plays in disease pathology. Similarly, T cells (e.g., Th1, Th17), macrophages, chondrocytes, and osteoblasts all play a role in this disease (Nestle et al. 2009). All these cell types express PDE4 and inhibition of this enzyme can lead to a reduction in cell activity that might be conducive to a beneficial action in this disease (Table 1).

Preclinical studies have shown that PDE4 inhibitors can suppress pannus-like inflammation in animals injected with methylated bovine serum albumin. Furthermore, PDE4 inhibitors suppressed cytokine production from peritoneal macrophages and inhibited synovial fibroblast proliferation in culture (Kobayashi et al. 2007). The recent finding that PDE4 inhibitors suppressed IL-17 production from human peripheral blood mononuclear cells and CD4+ T lymphocytes and inhibited proliferation of human memory Th17 (Ma et al. 2008) could explain the preclinical findings. Indeed, the expression of IL-23 from human peripheral blood mononuclear cells, a cytokine which supports the expansion of Th17 cells was suppressed by PDE4 inhibition (Schafer et al. 2010). Similarly, roflumilast partially attenuated nitric oxide release from human chondrocytes in culture, these cells are involved in articular cartilage destruction in rheumatoid arthritis (Tenor et al. 2002). Loss of bone is also a feature of rheumatoid arthritis, and PDE3 and PDE4 inhibitors have been shown to augment the ability of prostaglandin E2 to promote murine osteoclast differentiation in co-cultures of calvarial osteoclasts and bone marrow cells (Noh et al. 2009). However, it is well established that PGE2 promotes bone formation in vivo, and this anabolic effect is also observed with PDE4 inhibitors in ovariectomized rats (Yao et al. 2007). PDE4 inhibitors appear to suppress inflammation, cartilage destruction, and bone loss in rheumatoid arthritis, but it remains to be established whether this drug class will be significantly better than current biological agents.

6 Unwanted Side Effects

Nausea and other gastrointestinal side effects are commonly reported side effects associated with many PDE4 inhibitors and the mechanism responsible for these side effects has been investigated in an attempt to discover nonemetic PDE4 inhibitors. The direct recording of neuronal activity within the area postrema of dogs conclusively demonstrated that substances known to cause nausea (e.g., apomorphine) caused the excitation of neurons within this anatomical location (Carpenter et al. 1988). Neuronal activity within the area postrema was also increased following the systemic administration of 8-bromo cAMP or following elevation of endogenous levels of cAMP within neurones by forskolin, an activator of adenylyl cyclase (Carpenter et al. 1988). Elevated levels of cAMP within the area postrema enhanced the emetogenic response. Dogs treated with theophylline and the PDE4 selective inhibitor, 4-(3-Butoxy-4-methoxyphenyl)methyl-2-imidazolidone (Ro 20-1724)

reduced the emetic threshold of the D2 agonist, apomorphine (Carpenter et al. 1988). One consequence of elevated levels of cAMP is the transcriptional activation of the early response gene *c-fos*, following upstream activation of the transcription factor, cAMP response element binding (CREB) by protein kinase A. This method was employed to demonstrate increased *c-fos* immunoreactivity in neurons within area postrema and nucleus tractus solitarius following systemic administration of a PDE4 inhibitor and providing conclusive proof that this drug class can lead to the activation of neurons within the emetic centers of the central nervous system (CNS) (Bureau et al. 2006). Direct application of a highly potent PDE4 inhibitor via intracerebroventricular administration in order to limit systemic bioavailability provide a means of directly activating neurons within the CNS, consequently resulting in emesis in the ferret (Robichaud et al. 1999). This ability of PDE4 inhibitors to induce emesis in the ferret was inhibited by the alpha₂-selective agonist, clonidine (Robichaud et al. 2001) and suggested that raising cAMP within central noradrenergic terminals by PDE4 inhibitors promoted emesis, and this could be attenuated via alpha₂-adrenoceptor mediated inhibition of adenylyl cyclase.

The emetic response to systemically administered PDE4 inhibitors is reduced by anti-emetic agents including the 5HT₃-antagonist, ondansetron, and the NK₁ antagonist, (+)-(2*S*,3*S*)-3-(2-[¹¹C]Methoxybenzylamino)-2-phenylpiperidine (CP-99,994) (Robichaud et al. 1999, 2001). Similarly, the increased expression of *c-fos* within the emetic centers of the brain was also reduced following treatment with the NK₁ antagonist, RP67580, thus implicating substance P in this response (Bureau et al. 2006).

Many studies have documented the expression of PDE4D within the area postrema, nucleus tractus solitarius, and nodose ganglion neurons in various species including man and implicated this isoform in nausea and vomiting (Cherry and Davis 1999; Takahashi et al. 1999; Perez-Torres et al. 2000; Lamontagne et al. 2001). However, it should also be recognized that detectable transcripts for PDE4B were also found within the nucleus tractus solitarius and area postrema in humans and rodents, respectively, and could just as well be involved in the emetic response (Perez-Torres et al. 2000). Consequently, there is a general consensus that inhibition of PDE4D is responsible for side effects such as nausea and emesis. Since rodents lack an emetic reflex, it is not possible to directly investigate the role of different isoforms of PDE4 in emesis in this model. However, a surrogate biological response, which measures the reversal of anesthesia induced by alpha₂-adrenoceptor agonists (e.g., clonidine, xylazine), has been used to study the role of PDE4 subtypes in emesis (Robichaud et al. 2001, 2002a). Deletion of PDE4D and not PDE4B reduced the duration of anesthesia induced by xylazine, compared with wild-type mice, and the ability of PDE4 inhibitors to shorten xylazine-induced anesthesia was impaired in PDE4D but not PDE4B knockout mice (Robichaud et al. 2002b). Together these studies suggested that PDE4 inhibitors with low affinity for PDE4D should have reduced emetic potential.

However, the validity of this hypothesis has been questioned in light of the development of PDE4 selective inhibitors which preferentially distribute to the brain and are relatively free from emesis in a range of animal models (Burgin et al. 2010).

Furthermore, there are examples of nonselective PDE4 inhibitors that have *in vivo* anti-inflammatory activity but are not emetogenic (Aoki et al. 2000, 2001; Gale et al. 2002). Similarly, the emetic profile of various PDE4 inhibitors (PMNPQ > R-rolipram > CT-2450) could not be explained by a preferred selectivity for PDE4D over PDE4A or PDE4B (Robichaud et al. 1999, 2002b). Some PDE4 inhibitors may preferentially partition within the area postrema in the CNS and thereby enhancing the inhibition of PDE4D in area postrema neurons and explaining the differences in the emetic potential of these drugs (Aoki et al. 2001; Robichaud et al. 2002a). Indeed, brain penetration by PMNPQ was 46-fold greater than CT-2450; however, the concentration of the “low emetic” PDE4 inhibitor, CT-2450, within the CNS was still 475-fold greater than the PDE4D inhibitory potency for CT-2450, and presumably still in sufficient concentrations to inhibit this enzyme. Moreover, the area postrema is not completely behind the blood–brain barrier and therefore accessible to free drug within the circulation (Gross et al. 1990). Whether differential partitioning of these inhibitors within the area postrema accounts for why some PDE4 inhibitors have a reduced emetic profile remains to be established. Alternatively, partial inhibition of PDE4D within the emetic center of the brain may not be sufficient to promote an emetogenic signal, although this degree of inhibition within inflammatory cells is sufficient enough to exert an *in vitro* anti-inflammatory effect (Burgin et al. 2010).

A number of preclinical studies have highlighted a number of other potential disadvantages to targeting PDE4, and these include the development of mesenteric vasculitis (Spina 2004), immunosuppression (Spina 2004), heart failure, and arrhythmia (Lehnart et al. 2005). However, none of these events appear to be realized in phase II and phase III clinical trials undertaken to date, at least with cilomilast and roflumilast. Similarly, slow release theophylline has been used for decades in the treatment of asthma and COPD and has not been associated with a number of these potentially adverse events despite being shown to cause mesenteric vasculitis preclinically in some models (Nyska et al. 1998; Ohta et al. 2004). It has also been suggested that PDE4 inhibitors may have pro-inflammatory properties, which is based on the finding that at very high doses, roflumilast (100 mg/kg) promoted the recruitment of neutrophils to the airways and this correlated with the release of IL-8 from cultured endothelial cells *in vitro* (McCluskie et al. 2006). This mechanism might explain why animals that have been chronically treated with high doses of PDE4 inhibitors document vasculitis. However, the concentrations required to achieve these untoward effects are at least 1,000 times greater than the ED₅₀ and EC₅₀ values reported for roflumilast against several *in vivo* biomarkers of inflammation and cell function *in vitro*, respectively (Bundschuh et al. 2001; Hatzelmann and Schudt 2001). It is unlikely that the plasma concentrations required to produce this purported pro-inflammatory effect could be achieved even with chronic dosing. Similarly, another study has shown that PDE4 inhibitors, at concentrations that are pharmacologically relevant, delay apoptosis of neutrophils and eosinophils, an effect that increased when combined with β_2 -agonists (Parkkonen et al. 2007). However, the extent to which these findings translate into the clinic is unclear, particularly as the beneficial effect of roflumilast in moderate-to-severe COPD

subjects was not compromised in patients taking long-acting β_2 -agonists (Fabbri et al. 2009). The clinical evidence suggests that PDE4 inhibitors suppress rather than exacerbate inflammation in the airways (Gamble et al. 2003; Grootendorst et al. 2007). Furthermore, there was no evidence of an increased incidence of pneumonia in patients with COPD clinically treated with roflumilast, which might be anticipated if PDE4 inhibitors interfere with host defense (Fabbri et al. 2009; Calverley et al. 2009). Interestingly, weight loss was an unexpected effect in subjects independent of any reports of gastrointestinal discomfort (Fabbri et al. 2009; Calverley et al. 2009) and could be a result of the well-recognized effect of elevating cAMP in adipocytes in promoting lipolysis since it has been previously reported that β_2 -agonists have a well-known lipolytic action. Mice deficient in PDE4D but not PDE4B have abnormal growth development as demonstrated by reduced fertility and litter size by female mice, reduced growth rates, and body weight in both sexes, the latter a consequence of a significant reduction in the weight of bone, muscle, and body organs (Jin et al. 1999; Jin and Conti 2002). Although the effect of these gene deficiencies were not studied on lipolysis per se, both PDE4B and PDE4D are present in rat adipocytes and inhibition of these enzymes promotes basal lipolysis (Wang and Edens 2007).

7 PDE4 Inhibition and the Future

Strategies at improving the risk/benefit ratio for PDE4 inhibition will be important if this drug class is to be widely used in the treatment of inflammatory diseases. The therapeutic window between the anti-inflammatory action of these drugs and side effects such as nausea and emesis is probably not wide enough for cilomilast, and may limit the use of roflumilast. It is of interest that there are a number of PDE4 inhibitors currently in development that appear to lack significant emetic action; these include oglemilast and IPL512602 (Boswell-Smith et al. 2006b), apremilast (Schafer et al. 2010), and the recently reported compounds produced by the Biotech company Decode (Burgin et al. 2010), but the molecular basis for this lack of emesis has not been published.

Most PDE4 inhibitors under development are designed for oral administration; however, the inhaled route would deliver a PDE4 inhibitor directly to target cells within the lung and thereby minimize systemic absorption and of course this is a widely accepted route of administration in pulmonary medicine as a way of minimizing systemic side effects with other drug classes. Both AWD 12-281 (*N*-(3,5-dichloropyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic acid amide) (Kuss et al. 2003) and UK-500,001 (Phillips et al. 2007; Vestbo et al. 2009) are examples of inhaled PDE4 inhibitors. Indeed, a 7-day treatment with the inhaled PDE4 inhibitor GSK256066 produced modest attenuation of both the early and the late asthmatic response to antigen challenge (Singh et al. 2010). It clearly should be possible to obtain anti-inflammatory activity by direct delivery of this drug class to the lung, although this does not always mean a complete loss of

emesis and nausea, as exemplified by the mixed PDE3/4 inhibitor zardaverine, which although inducing bronchodilation clinically also induced nausea (Brunnee et al. 1992).

Another approach might be the use of antisense oligodeoxynucleotides targeting PDE4, which could be delivered by the inhaled route. The positive results obtained in the successful targeting of the adenosine A1 receptor in a rabbit model of allergic inflammation (Nyce and Metzger 1997) illustrates the potential of this approach. A preclinical study has demonstrated that antisense oligonucleotides against mRNA for PDE4B/4D and PDE7 delivered topically to the lung by the endotracheal route of administration suppressed inflammation following 2 weeks of cigarette smoke exposure in mice (Fortin et al. 2009). The advantage of this technique is that side effects such as nausea and emesis are likely to be avoided. Alternatively, the recently identified allosteric modulators of PDE4 are proposed to confer lower emetic potential because despite only partially inhibiting PDE4 activity, the effect is nonetheless sufficient to inhibit inflammatory cell activity completely (Burgin et al. 2010). This is an important possibility that could be exploited in the discovery of nonemetic PDE4 inhibitors for the treatment of inflammatory diseases.

Another reason that targeting PDE4 alone may not fully resolve airway inflammation is the fact that there are other PDE types exist in structural and inflammatory cells in the lung (Table 1). Therefore, targeting multiple PDEs may be required for optimal anti-inflammatory action. For example, the macrophage is viewed as a critical cell type in the pathogenesis of COPD (Barnes 2008); however, the ability of these cells to release TNF α in response to endotoxin was only inhibited to a small degree by PDE4 inhibitors (Hatzelmann and Schudt 2001) and the potential functional involvement of PDE3 and PDE7 in these cells cannot be completely ignored. The inhibitory action of PDE4 inhibitors on the cellular activity of CD8⁺ T-lymphocytes and macrophages was significantly increased in the presence of PDE7 selective inhibitors (Smith et al. 2004). Similarly, combined PDE3 and PDE4 inhibitor in a single molecule offers the advantage of delivering a bronchodilator and anti-inflammatory substance. Moreover, it is likely that retention of the inhibitor within the lung may be required in order to maintain anti-inflammatory activity with the airways (Boswell-Smith et al. 2006a).

8 Conclusion

A number of clinical trials assessing the efficacy of PDE4 inhibitors for the treatment of inflammatory disease including asthma, COPD, atopic dermatitis, and psoriasis have demonstrated moderate success. However, the dose-limiting side effects such as nausea, emesis, and headache potentially limit the utility of these drugs. Importantly, there are examples of PDE4 inhibitors that have low emetogenic potential, although the molecular basis of this phenomenon remains to be established. Other strategies include topical delivery (e.g., inhalation for administration to the lung; direct application to the skin), development of subtype

selective PDE4 inhibitors, use of mixed PDE inhibitors, interference with PDE4 activation, targeting proteins that are involved in locating PDE4 to specific micro-cellular domains and finally the potential for use of antisense oligonucleotides may offer another solution to the problem of targeting PDE4 in inflammation is a cause for optimism.

Conflict of Interest. The authors are consultants for Veronapharma plc who are developing RPL554 as a novel inhaled PDE3/4 inhibitor for the treatment of inflammatory airways disease. CP is also a founder and has equity in Veronapharma plc.

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