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REVIEW

PDE4 inhibitors: current status

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Phosphodiesterase4 inhibitors are currently under development for the treatment of respiratory diseases including asthma and chronic obstructive pulmonary disease. The rationale for the development of this drug class stems from our understanding of the role of PDE4 in suppressing the function of a range of inflammatory and resident cells thought to contribute toward the pathogenesis of these diseases. Similarly, numerous preclinical *in vivo* studies have shown that PDE4 inhibitors suppress characteristic features of these diseases, namely, cell recruitment, activation of inflammatory cells and physiological changes in lung function in response to a range of insults to the airways. These potentially beneficial actions of PDE4 inhibitors have been successfully translated in phase II and III clinical trials with roflumilast and cilomilast. However, dose limiting side effects of nausea, diarrhoea and headache have tempered the enthusiasm of this drug class for the treatment of these respiratory diseases. A number of strategies are currently being pursued in attempts to improve clinical efficacy and reduce side effects, including delivery via the inhaled route, and/or development of non-emetic PDE4 inhibitors and mixed PDE inhibitors.
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Keywords: asthma; COPD; cilomilast; roflumilast; PDE4; inflammation; emesis

Abbreviations: CD, cluster of differentiation; COPD, chronic obstructive pulmonary disease

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 long acting β_2-adrenoceptor agonists and glucocorticosteroids, either alone or in combination. Theophylline is often used with glucocorticosteroids as second- or third-line therapy where it has proven anti-asthmatic activity in asthma (Sullivan *et al.*, 1994; Weinberger and Hendeles, 1996; Lim *et al.*, 2000) and in combination with long acting bronchodilator drugs in COPD (Rennard, 2004). As an p.o formulation, this drug offers the advantage of improved compliance; however, its perceived lack of efficacy, the necessity to monitor plasma levels, coupled with numerous side effects, known drug interactions, and the effect of smoking on plasma clearance (Boswell-Smith *et al.*, 2006a) have provided an impetus to discover a better theophylline. Moreover, whereas glucocorticosteroids are of clinical utility in asthma (Barnes, 2006a), they are of limited use in COPD (Rennard, 2004). There is clearly an unmet clinical need for the development of disease-modifying drugs in COPD because other than cigarette smoke cessation, current drug therapy does not prevent the accelerated decline in lung function.

The mechanism of action of theophylline, which explains its clinical effect is not entirely certain, but several have been proposed. It was originally shown that theophylline inhibited the activity of a cyclic 3′, 5′ nucleotide PDE with a K*i* of 100 μM (Butcher and Sutherland, 1962). This might account for its beneficial effects clinically, as an increase in the intracellular levels of cyclic AMP can reduce the activation of a wide range of inflammatory and lung resident cells. There are presently 11 known families of PDE 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 (Houslay *et al.*, 2005; Bender and Beavo, 2006). However, targeting PDE4, the enzyme responsible for metabolizing cyclic AMP has been the focus for the development of drugs that could prove beneficial in the treatment of respiratory diseases such as asthma (Torphy, 1998; Houslay *et al.*, 2005). It is therefore of interest that plasma levels achieved with a dose of theophylline that demonstrated significant anti-inflammatory activity (Sullivan *et al.*, 1994) was well below the K*i* for PDE inhibition and suggested that PDE4 inhibition alone does not completely explain this drugs clinical effectiveness (Barnes *et al.*, 2005).

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Nevertheless, highly potent and selective PDE4 inhibitors have been developed to target inflammatory airway disease. This strategy is not unique and is exemplified with the development and clinical success of a PDE5 inhibitor, sildenafil, for the treatment of erectile dysfunction (Boolell *et al*., 1996).

PDE4

PDE4 catalyses the hydrolysis of cyclic AMP, which terminates the downstream signalling of this second messenger. There are four gene families (A–D), although there is added complexity with over 20 splice variants (Houslay *et al*., 2007). Hydrolysis of cyclic AMP is a common feature of this family, and it is clear that these isoforms can be targeted to different domains within the extracellular compartment and their activity differentially regulated by kinases, suggesting that these isoforms have specific functions in the control of cellular activity (Houslay *et al*., 2007). X-ray crystallography has resolved the catalytic domain of these enzymes, which are comprised of three important domains, consisting of a bivalent metal-binding pocket (Zn^{2+}, Mg^{2+}), which forms a complex with the phosphate moiety of cyclic AMP, a pocket containing glutamine (Q pocket), which forms hydrogen bonds with the nucleotide (purine) moiety of cyclic AMP, and a solvent pocket. PDE4 inhibitors occupy this active site through a number of important interactions and prevent cyclic AMP metabolism. These include indirect binding to the metal ions by the formation of hydrogen bonding to water, whereas 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 and hydrogen bond interaction 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 cyclic AMP (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; Wang *et al*., 2007). One possibility might be to exploit subtle differences between the interaction of these inhibitors to the catalytic active site, or alternatively, by non-active site inhibition by targeting the N-terminal region of the enzyme, which contain phosphorylation sites and/or protein-binding sequences and so indirectly interfere with PDE4 activity (Card *et al*., 2004; Wang *et al*., 2007).

With well over 100 mediators including prostaglandins, leukotrienes, chemokines, cytokines, proteases and growth factors and numerous cell types including mast cells, neutrophils, eosinophils, macrophages, DCs and lymphocytes implicated in the pathogenesis of asthma and COPD (Barnes, 2006b; Holgate, 2007), suggests that a chemical strategy designed to target a single mediator or cell type is unlikely to be successful particularly as many of these mediators and cell types have overlapping and complementary roles in disease pathology. PDE4 is expressed in a number of cell types that are considered suitable drug targets for the treatment of respiratory diseases such as asthma and COPD (Table 1). It might reasonably be argued that targeting PDE4 could potentially suppress the function of numerous cell types; however, it is well known that other PDE enzymes

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	B > A	3, 7	Inhibition of proliferation and cytokine release	(Gantner *et al*., 1997b; Hatzelmann and Schudt, 2001; Smith *et al*., 2003; Peter *et al*., 2007)
B cells	B, D > A	7	Increased proliferation	(Gantner *et al*., 1998; Smith *et al*., 2003)
Eosinophils	A, D > B	7	Inhibition of superoxide anion generation; delayed apoptosis	(Hatzelmann and Schudt, 2001; Smith *et al*., 2003; Parkkonen *et al*., 2007)
Neutrophils	A, D > B	7	Inhibition of superoxide anion and neutrophil elastase release	(Hatzelmann and Schudt, 2001; Smith *et al*., 2003; Jones *et al*., 2005)
Monocyte	B > A, 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[b]	(Gantner *et al*., 1997a; Hatzelmann and Schudt, 2001; Smith *et al*., 2003; Barber *et al*., 2004)
DCs	A > B, D	1, 3	Inhibition of TNF-α release	(Hatzelmann and Schudt, 2001; Heystek *et al*., 2003)
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	(Kohyama *et al*., 2002; Smith *et al*., 2003; Martin-Chouly *et al*., 2004; Dunkern *et al*., 2007)
Sensory nerves[c]	D	1, 3	Inhibition of neuropeptide release	(Spina *et al*., 1995)

Abbreviations: CD, cluster of differentiation; COPD, chronic obstructive pulmonary disease; IL, interleukin; TNF, tumour necrosis factor.
[a]PDE4 subtype mRNA expression illustrating relative abundance in cells.
[b]In the presence of a PDE3 inhibitor.
[c]Guinea-pig sensory nerves.

are also expressed in these cells and the contribution of other PDEs to cell function (for example, PDE3, PDE7) in the context of benefits in respiratory diseases is being explored (Smith *et al.*, 2004; Boswell-Smith *et al.*, 2006b). It would seem prudent to develop subtype selective PDE4 inhibitors in attempts to maximize therapeutic benefit at the expense of adverse effects, whereas 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 anti-psychotic than newer generation atypical anti-psychotics 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).

Asthma and PDE4

Increased PDE4 function due to either increased protein expression or activity might provide a plausible mechanism to account for the pathogenesis of asthma. The expression of a novel and distinct cyclic AMP-PDE was isolated from monocytes obtained from individuals with atopic dermatitis. This enzyme had increased PDE activity and as a consequence monocyte function was increased and thought to be 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 patients of either mild or severe severity (Gantner *et al.*, 1997b). Similarly, increased total PDE catalytic activity was observed in peripheral blood monocytes from individuals with mild asthma, whereas this was associated, paradoxically, with reduced PDE4 activity (Landells *et al.*, 2000) and the expression of PDE4A–D was not increased in peripheral blood cluster of differentiation (CD)4 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.

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 (Torphy, 1998; 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. 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 no different to wild-type controls (Hansen *et al.*, 2000). This indicated that other PDE4 subtypes contributed in the metabolism of intracellular cyclic AMP, as 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 was abolished in PDE4D gene-deficient mice. These mice were hyporesponsive to this stimulus, even in the absence of allergic sensitization and appeared to be related to an increase in dilator prostaglandin production in the airways of these 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 study highlighted the potential complimentary role of PDE4 isoforms in regulating allergic airway inflammation, and the need to target more than one PDE4 isoform because inhibition of the inflammatory response, hyperresponsiveness and airway remodelling in allergic wild-type mice was observed following exposure to PDE4 inhibitors such as rolipram and roflumilast (Kung *et al.*, 2000; Kanehiro *et al.*, 2001; Kumar *et al.*, 2003).

The numerous preclinical studies reporting the anti-inflammatory potential of PDE4 inhibitors in models of allergic inflammation and in human cells *in vitro* have been, to some degree, corroborated in clinical trials in asthmatic patients. Twice daily treatment for 9.5 days with the PDE4 inhibitor CDP840 inhibited the development of the late phase response in asthmatic patients by 30% (Harbinson *et al.*, 1997). A similar degree of inhibition of the late phase response was observed following once daily treatment for 7–10 days with roflumilast (van Schalkwyk *et al.*, 2005). This late phase response is used by clinicians to model the inflammatory component following an allergic insult to the airways. In both studies, the effects of drug treatment on the acute allergen bronchoconstriction was modest and is consistent with the lack of demonstrable action of PDE4 inhibition on mast cell function (Table 1) and highlight the role of other PDE enzymes, namely PDE3 in the context of airway smooth muscle relaxation (Boswell-Smith *et al.*, 2006b). Bronchial hyperresponsiveness was not reduced by these drugs, although a later study purported to show modest protection against allergen-induced bronchial hyperresponsiveness (Louw *et al.*, 2007), which might suggest 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 p.o 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 favour prolonged enzyme exposure (David *et al.*, 2004). This favourable pharmacokinetic profile would be anticipated to produce long periods of PDE4 inhibition. There was a significant reduction in the activity of circulating monocytes in patients maintained on roflumilast for 4 weeks, whereas the magnitude of this change was small, resulting in approximately 1.3-fold reduction in tumour necrosis factor-α production by monocytes in response to endotoxin challenge *in vitro* (Timmer *et al.*, 2002). Therefore, it is questionable whether total PDE4 inhibition can be achieved in cells within the airway tissue compartment at the dose used in clinical studies.

Side effects most commonly reported were headache, nausea and diarrhoea of a mild-to-moderate severity (Harbinson *et al.*, 1997; van Schalkwyk *et al.*, 2005) and suggest that unless the risk/benefit ratio can be improved, then this may well hamper the use of this drug in asthma.

This is a worthy aim to pursue in the light of a clinical study reporting comparable clinical efficacy between roflumilast and beclomethasone diproprionate in persistent asthma (Bousquet *et al*., 2006).

COPD and PDE4

Chronic obstructive pulmonary disease unlike asthma is caused by cigarette smoking, although in developing countries smoke derived from burning biomass fuels is also a predisposing factor. The nature of the inflammatory response is distinct from asthma and is characterized by the activation of macrophages, airway epithelial cells, which in turn, secrete a range of chemokines and lipid mediators resulting in the recruitment of neutrophils and $CD8^+$ T lymphocytes. The secretion of a range of proteases from neutrophils (elastase, MMP9, cathepsins) and macrophages (MMP12) is thought to contribute towards airway fibrosis of the small airways, increased mucus secretion and destruction of the alveolar wall (Barnes, 2006b) These pathological changes give rise to the symptoms of cough, mucus secretion, difficult breathing and emphysema. Many of the cell types implicated in this disease process express PDE4 (Table 1).

The expression of PDE4A–D in peripheral blood neutrophils and CD8T cells is not altered in patients with mild COPD (Jones *et al*., 2007). However, the expression of PDE4A4 and total cyclic AMP PDE activity was significantly increased in macrophages purified from bronchoalveolar lavage fluid from patients with mild-to-moderate COPD compared with healthy patients or smokers who did not present with COPD (Barber *et al*., 2004). Of the 12 PDE4 variants analysed, only the activity of PDE4A4 was increased and suggested that local events/processes within the lung of patients 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 modest effect in suppressing tumour necrosis factor-α production from human macrophages derived from cultured monocytes, and the contribution of PDE3 and PDE7 in regulating function in this cell type cannot be ignored (Gantner *et al*., 1997a; Smith *et al*., 2004).

There are a limited number of preclinical *in vivo* models of COPD. 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. The recruitment of these cells to 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 rolipram (Ariga *et al*., 2004). This once again highlighted the complimentary 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 this disease.

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 significant improvements in spirometry over placebo, quality of life scores and reduction in the number of exacerbations in the severest group of COPD patients. 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 COPD. Both roflumilast (Grootendorst *et al*., 2007) and cilomilast (Gamble *et al*., 2003) reduced 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 interleukin-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% and might underlie their beneficial action in the phase III clinical trials. However, the biomarker study also highlights a recurring theme that complementary inhibition of PDE4 was not achievable because of dose-limiting side effects; or alternatively, other PDE isoforms (for example, PDE3, 7, see Table 1) in these same inflammatory processes may also require targeting for a full anti-inflammatory action to be revealed in this disease.

The most common side effect reported with roflumilast included diarrhoea (9%), headache (6%) and nausea (5%) (Rabe *et al*., 2005; Calverley *et al*., 2007), which was of the same order of magnitude as that reported with cilomilast, although abdominal pain and vomiting were also reported for this drug (Rennard *et al*., 2006). The adverse effects appeared to disappear with continued use but were a major reason why patients discontinued with the study during the first 3–4 weeks of treatment. No cardiovascular liabilities were noted.

PDE4 inhibitors: unwanted effects

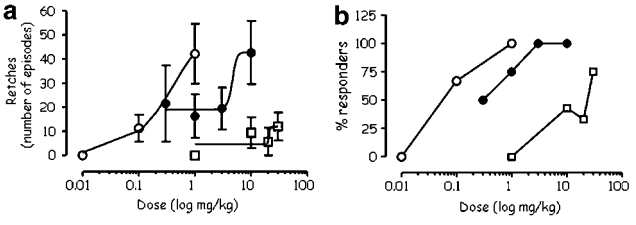
Nausea is a commonly reported side effect associated with theophylline and therefore not surprisingly, PDE4 inhibitors also produce a similar constellation of adverse events and are a major drawback for the therapeutic use of these drugs. The mechanism responsible for this side effect has been investigated in an attempt to discover non-emetic PDE4 inhibitors.

The direct recording of neuronal activity within the area postrema conclusively demonstrated that substances known to cause nausea (for example, apomorphine) caused the excitation of neurones within the area postrema of dogs (Carpenter *et al*., 1988). Neuronal activity within the area postrema was also increased following the systemic administration of 8-bromo cyclic AMP or following elevation of endogenous levels of cyclic AMP within neurones by forskolin, an activator of AC (Carpenter *et al*., 1988). Elevated levels of cyclic AMP 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). Similarly, the i.c.v. administration of highly potent PDE4 inhibitors also induced emesis in the ferret (Robichaud *et al*., 1999), and the emetic response to systemically administered PDE4 inhibitors is reduced by anti-emetic agents including the 5HT3-antagonist, ondansetron, and the neurokinin 1 antagonist, (+)-(2*S*,3*S*)-3-(2-[^{11}C]methoxybenzylamino)-2-phenylpiperidine (CP-99994) (Robichaud *et al*., 1999, 2001).

Many studies have documented the expression of PDE4D within the area postrema, nucleus tractus solitaris and nodose ganglion neurones 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 transcript for PDE4B was also found within the nucleus tractus solitaris and area postrema in humans and rodents, respectively, and could be involved in the emetic response (Perez-Torres *et al*., 2000). As rodents do not possess an emetic reflex, it is not possible to directly investigate the role of different isoforms of PDE4 in emesis. However, a surrogate biological response that measures the reversal of anaesthesia induced by α2-adrenoceptor agonists has been used to study the role of PDE4 subtypes in emesis (Robichaud *et al*., 2001, 2002a). It was previously shown that the ability of PDE4 inhibitors to induce emesis in the ferret was inhibited by the α2-selective agonist, clonidine (Robichaud *et al*., 2001) and suggested that raising cyclic AMP within central noradrenergic terminals by PDE4 inhibitors promoted emesis, and this could be attenuated by α2-adrenoceptor-mediated inhibition of AC. The hypnotic action of xylazine was reversed in rodents treated with PDE4 inhibitors (Robichaud *et al*., 2001) and therefore used as a surrogate for emesis. Deletion of PDE4D and not PDE4B reduced the duration of anaesthesia induced by xylazine, compared with wild-type mice, and second, the ability of PDE4 inhibitors to shorten xylazine-induced anaesthesia 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, it is not entirely clear whether PDE4D inhibition alone is the sole basis of emesis as there are examples of PDE4 inhibitors that document *in vivo* anti-inflammatory activity but are not emetogenic (Gale *et al*., 2002) and there are examples of PDE4 inhibitors that have little emetogenic activity but potent anti-inflammatory activity in preclinical studies (Aoki *et al*., 2000, 2001). Similarly, the emetic profile of various PDE4 inhibitors in the ferret cannot be explained by their selectivity for PDE4D or to differences in PDE4D inhibitor potency (Figure 1). One possible explanation might be that some PDE4 inhibitors preferentially partition within the CNS and hence the degree of PDE4D inhibition in area postrema neurones might explain the differences in the emetic potential of these drugs (Aoki *et al*., 2001; Robichaud *et al*., 2002a). However, the concentration of the 'low emetic' PDE4 inhibitor, CT-2450 within the CNS was comparable to



	PDE4A	PDE4B	PDE4C	PDE4D (IC50 nM)[1]	Dose (mg/kg, s.c)	[Brain][2] (μM)	[Plasma][2] (μM)	TNFα (IC50)[3]
PMNPQ	0.07	0.05	0.14	0.08	1	0.42	0.11	0.05
R-rolipram	3.5	5.5	32	4	1	0.43	0.24	2.2
CT-2450	0.9	0.7	1.6	0.5	30	0.38	4.65	2.5

Figure 1 Line graph drawn from data presented in Table 2 of Robichaud *et al*. (1999) showing the number of retches (**a**) and the percentage of animals who retch (**b**) in response to increasing p.o doses of PDE4 inhibitors, PMNPQ (open circles), R-rolipram (closed circles) and CT-2450. [1]Inhibitory potency (IC_{50}) for these inhibitors against human cloned PDE4 subtypes. [2]Concentration of PDE4 inhibitors measured in homogenates of whole brain and in plasma 1 h following systemic administration of drug. [3]IC_{50} values against whole blood tumour necrosis factor-α (references cited in Robichaud *et al*. 2002b). CT-2450, (R)-*N*-(4-[1-3-cyclopentyloxy-4-methoxyphenyl)-2-(4-pyridyl)ethyl]phenyl)-*N*′-ethylurea; PMNPQ; 6-(4-pyridylmethyl)-8-(3-nitrophenyl)quinoline.

that achieved by the most potent emetic PDE4 inhibitor, PMNPQ (Figure 1). Second, the area postrema is not completely behind the blood–brain barrier (Gross *et al*., 1990), and therefore accessible to free drug within the circulation. It would seem unlikely that differential partitioning of these inhibitors within the CNS is a likely explanation for their ability to induce emesis. However, it remains to be established whether the magnitude of PDE4 inhibition within the area postrema differs between the various PDE4 inhibitors.

A number of preclinical studies have highlighted a number of disadvantages to targeting PDE4, include the development of mesenteric vasculitis, 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 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 (Ohta *et al*., 2004). It has been suggested that PDE4 inhibitors may have proinflammatory properties (McCluskie *et al*., 2006). This conclusion was based on the finding that roflumilast at very high doses (100 mg/kg) promoted the recruitment of neutrophils to the airways and this correlated with the release of interleukin-8 from cultured endothelial cells *in vitro*, although the concentrations required to achieve these effects were at least 1000 times greater than the ED_{50} and EC_{50} 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 proinflammatory 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 β2-adrenocep-

tor agonists (Parkkonen *et al*., 2007). However, to what extent these findings translate into the clinic, particularly, if combined with bronchodilator drugs, remains to be established. The clinical evidence suggests that PDE4 inhibitors suppress and not exacerbate inflammation in the airways (Gamble *et al*., 2003; Grootendorst *et al*., 2007).

PDE4 inhibitors and the future

Although there is cause for optimism concerning the potential therapeutic utility of PDE4 inhibitors for the treatment of respiratory diseases such as asthma and COPD, it is clear that further improvements are required. Strategies at improving the risk to benefit ratio will be important, if this drug class is to be widely used. The therapeutic window for 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 in asthma. There are PDE4 inhibitors currently in development, which appear to lack significant emetic action (for example, oglemilast) (Glenmark Pharmaceuticals, 2005) and IPL512602 (Inflazyme pharmaceuticals, 2005), although the molecular basis for this has not been published.

Most PDE4 inhibitors under development are designed for p.o administration, however, the inhaled route would deliver PDE4 inhibitor directly to target cells within the lung and thereby minimize systemic absorption as in the case of AWD 12-281 (N-(3,5-dichloropyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic acid amide (Kuss *et al*., 2003) or UK-500001 (Phillips *et al*., 2007; Vestbo *et al*., 2007), although clinical trials in respiratory disease have thus far been disappointing. Nonetheless, the development of a potent, long acting PDE4 inhibitor through the inhaled route would offer a solution to the issues of emesis and nausea. Another approach might be the use of antisense oligodeoxynucleotides targeting PDE4, which could be delivered by the inhaled route, and in view of 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.

Another reason why targeting PDE4 alone may not fully resolve airway inflammation is the fact that other PDE types exist in structural and inflammatory cells in the lung (Table 1) and therefore, targeting multiple PDE enzymes 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, 2006b); however, the activity of these cells is 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 to maintain anti-inflammatory activity within the airways (Boswell-Smith *et al*., 2006b).

Conclusion

A number of clinical trials assessing the efficacy of PDE4 inhibitors for the treatment of respiratory diseases such as asthma and COPD have been moderately successful. The dose limiting side effects of nausea, emesis and headache potentially limit the utility of these drugs. However, there are examples of PDE4 inhibitors that have low emetogenic potential, although the molecular basis of this phenomenon remains to be established. Other strategies including delivery through the inhaled route, 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 microcellular domains and finally the potential of antisense oligonucleotides may offer another solution to the problem of targeting PDE4 in the context of respiratory diseases, is a cause for optimism.

Conflict of interest

The author is a consultant for Veronapharma plc.

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