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Review Article

Phophodiesterase-4 enzyme inhibitors as potential anti-inflammatory agents

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Abstract

The Phosphodiesterases (PDEs) are a diverse family of enzymes that hydrolyze cyclic nucleotides such as cAMP & cGMP to the inactive 5'-AMP and 5'-GMP, respectively and thus play a key role in regulating intracellular levels of these second messengers and hence modulate cell functions which regulate a number of cellular processes such as metabolism, cell proliferation and differentiation, secretion, vascular and airway smooth muscle relaxation & the release of inflammatory mediators. The PDE superfamily contains 11 PDE isoenzymes (PDE1 to PDE11), which differ in primary structures, affinities for cAMP and cGMP, responses to specific effectors, sensitivities to specific inhibitors and mechanism of regulation. Each isoenzyme possesses at least one subtype. PDE4 has mainly four subtypes i.e. PDE4a, PDE4b, PDE4c, and PDE4d. The development of selective PDE4b inhibitors has not gained success till yet because of the structural similarity between the two isoforms of PDE4 i.e. PDE4b & PDE4d. Inhibition of PDE4b is responsible for the therapeutic effect while that of PDE4d for the side effects. The present review will be focused on the selective PDE4 enzyme inhibitors & their potential therapeutic utility.

Keywords: Phosphodiesterases (PDE), anti-inflammatory, cAMP and cGMP.

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1. Introduction

The PDE4 family

The PDE4 family in humans is composed of four subtypes (PDE4a, PDE4b, PDE4c and PDE4d) [1-5]. More than 20 PDE4 subtypes are present in cells arising from alternative mRNA splicing or the use of different transcriptional units [6, 7]. These PDE4 isozymes share a highly conserved catalytic domain of approximately 320-350 amino acids with more than 80% sequence identity between the members of the four isotypes [8-10]. The sequences of the N-terminus regulatory domains among the four subfamilies are divergent except for those in the two upstream conserved regions (UCR1 and UCR2), which are unique to the PDE4 proteins [11]. The four subfamily isozymes can be sub grouped into three forms: the 'long' forms which contain both UCR1 and UCR2, the 'short' forms which lack UCR1, and the 'super-short' forms which contain only the C-terminal portion of UCR2 [12]. The C-terminal sequence of the PDE4 enzymes is divergent and their functional significance remains to be defined. UCR1 and UCR2 are functional modules of approximately 60 and 80 residues, respectively [8, 11]. UCR2 bears an auto inhibitory nature, a property inferred from observations that removal of a portion of this domain causes an increase in the catalytic activity of the enzyme [8, 9, 13]. UCR1 contains a protein kinase A (PKA) phosphorylation site. Elevation of cAMP levels in cells induces phosphorylation of its serine residue by PKA, which then leads to a rapid activation of PDE4 as well as increases in the sensitivity of the enzyme to the PDE4 inhibitor rolipram, as demonstrated in PDE4d3 [14, 15]. Experiments have further indicated that PDE4 activation led by PKA phosphorylation in UCR1 may be the

consequence of relieving the inhibitory constraint of UCR2 on the catalytic domain [13]. Moreover, a potentially electrostatic intramolecular interaction between UCR1 and UCR2 has been described in PDE4d3. Phosphorylation of UCR1 appears to disrupt this interaction [16]. Evidence also indicates that UCR1 and UCR2 in PDE4 long forms can interact intermolecularly, thus leading to PDE4 dimerization, whereas short forms lacking UCR1 are monomers [17, 18]. PKA phosphorylation of UCR1 does not interfere with this interaction. The dimerization takes place between the C-terminal region of UCR1 and the N-terminal region of UCR2 as deletion of either part leads to failure of PDE4 dimerization. Disruption of dimerization abrogates the activation of PDE4d3 by PKA phosphorylation as well as reduces the sensitivity of the enzyme to rolipram [17, 18].

Expression of PDE4 in inflammatory cells

The PDE4 isozymes are widely distributed in human cells and tissues, implicating the diverse biological function of these proteins. PDE4s are the predominant cAMP-degrading isozymes in most, if not all, immune and inflammatory cells, including T cells, B cells, eosinophils, neutrophils, dendritic cells, monocytes, and macrophages (19]. Three PDE4 subtypes, PDE4a, PDE4b and PDE4d, are expressed in these cells, while PDE4C is minimal or absent [4, 20]. PDE3 and PDE7 are also detected in most of the inflammatory cells [4, 20]. The expression levels of these PDE isozymes are differentially regulated by a variety of inflammatory stimuli. As demonstrated in Jurkat T-cells and human peripheral blood T-cells, 8-Bromo-cAMP or prostaglandin E2 evidently induces PDE3 and PDE4 enzyme activity, and this effect is associated with increased PDE3b, PDE4a4, PDE4a1, 4d2, and 4d3 mRNA expressions [21]. Stimulation of human peripheral blood cluster of differentiation (CD4+ T) cells with anti-CD3 and anti-CD28 antibodies also regulates the expression of PDE4 subtypes differentially, with PDE4a and PDE4d mRNAs up regulated along with enzyme activity within 5 days but PDE4b mRNA up regulated transiently with highest levels at 24 h after stimulation [22]. In addition, lipopolysaccharide (LPS) has been shown to selectively induce PDE4b2 mRNA expression in human circulating monocytes [23]. This regulation of PDE4b expression has been confirmed in monocytes and peritoneal macrophages of PDE4 knockout mice [24, 25]. Among 12 PDE4 isozymes tested, PDE4a4 and PDE4b2 were detected at higher levels in peripheral blood monocytes of smokers compared with nonsmokers [26]. Moreover, PDE4a4 transcripts were found significantly up regulated in alveolar macrophages from smokers with COPD compared with smokers without COPD [26]. Although the functional consequences of the PDE4 regulation remain to be determined; these PDE4 isozymes altered in pathophysiological processes may serve as potential therapeutic targets for a variety of inflammatory conditions.

PDE4 and inflammation

To date, our understanding of the cellular functions of PDE4 has been mostly derived from experiments involving PDE4 inhibitors. These small molecule compounds, including the prototype inhibitor rolipram and second-generation compounds such as roflumilast and cilomilast have been shown to produce a wide range of pharmacological effects *in vitro* and *in vivo*. These include anti-inflammatory and immunomodulatory effects, [19, 20, 27-30]. antidepressant and antischizophrenia actions, [31-33] and cognitive enhancement, [34, 35] clearly demonstrating a broad, critical role of PDE4 in cellular and physiological functions. Among these effects, the inflammatory aspect of PDE4 functions has been explored most extensively. In fact, PDE4 is the major family of PDE enzymes expressed in immune and inflammatory cells. Inhibition of PDE4 has been shown to suppress a diverse spectrum of inflammatory responses *in vitro* and *in vivo* [4, 19, 20]. More importantly, many PDE4 inhibitors in development are efficacious in animal models of various inflammatory disorders, such as asthma, COPD, psoriasis, inflammatory bowel diseases, and rheumatoid arthritis, [20, 36] as well as in clinical trials for asthma and COPD [37-39]. These data thus provide strong evidence that PDE4 is a valid, promising drug target for different inflammatory conditions.

Function of cyclic adenosine monophosphate (cAMP)

Cyclic AMP is a second messenger important in many biological processes. It is derived from adenosine triphosphate (ATP) and metabolized to 5'AMP. In the lung it is involved in the regulation

of many functions related to inflammatory cells, mucociliary clearance, and fibrotic and pulmonary vascular remodeling. It suppresses immune and inflammatory cell activity (in inflammatory cells such as neutrophils, T-lymphocytes and macrophages), relaxes airway smooth muscle and modulates pulmonary nerve activity. An increase in the intracellular concentration of cAMP interferes with the expression of pro-inflammatory mediators such as TNF-α and inhibits the activity of inflammatory cells [103].

PDE inhibitors

The non-selective PDE inhibitor theophylline

The nonselective PDE inhibitor theophylline, a methylxanthine drug, has been used in therapy for respiratory diseases such as asthma and COPD for almost 90 years [40-42]. Although initially recognized as a PDE inhibitor, theophylline is also known as a potent adenosine receptor antagonist [43, 44] and an activator of histone deacetylase 2 (HDAC2) [45]. It is thought that the beneficial effects of theophylline on asthma and COPD are largely due to its anti-inflammatory properties. Several mechanisms have been proposed for such effects, which include [46] increasing intracellular cAMP concentrations via inhibition of PDE (mainly PDE4), [19, 47] decreasing inflammatory gene expression through inducing HDAC activity, [45] and reversal of corticosteroid resistance by inhibiting oxidative stress dependent phosphoinositide 3 kinase δ [48, 49]. In clinical practice, theophylline is known to interact with a number of drugs, such as cimetidine and phenytoin, and have a narrow therapeutic window. It causes a myriad of side effects at higher doses including nausea, diarrhea, headache, insomnia, increased heart rate, and arrhythmias [50, 51]. These disadvantages together with its relatively low efficacy compared with inhaled glucocorticoids or β2-agonists have limited its usage in asthmatic patients. Because of its nonselectivity towards most of the PDEs expressed in body cells, the pharmaceutical industry has devoted massive efforts in developing inhibitors selective for PDE4s, the isozymes expressed predominantly in proinflammatory cells [52, 53]. In fact; PDE4 inhibitors are considered promising therapeutic agents because of their prominent anti-inflammatory effects.

PDE4 inhibitors

Numerous PDE4 selective inhibitors have been patented in the past two decades, and some of them have been evaluated in clinical trials for several inflammatory conditions, such as asthma, COPD, atopic dermatitis, multiple sclerosis, and rheumatoid arthritis. The development of most of these compounds, however, has been discontinued because of narrow therapeutic windows. A major reason for their poor clinical results is the consequence of dosing limitation caused by side effects such as nausea and emesis. The PDE4 inhibitors explored in clinical trials have been mostly for asthma, likely because of the high prevalence and increasing morbidity of the disease. However, no compounds have yet reached the market as asthma treatments. Nevertheless, the first orally active PDE4 inhibitor roflumilast was approved in June 2010 by the European Medicines Agency Committee for severe COPD associated with chronic bronchitis in adult patients. In March 2011, the United States Food and Drug Administration approved the drug for reducing COPD exacerbations [54, 55]. Clinical studies have shown that roflumilast improves lung function and reduces the frequency of COPD exacerbations in patients with symptoms of chronic bronchitis [55-58]. Although the side effects were generally mild to moderate, nausea, diarrhea, headache, and weight loss are noted with roflumilast [54]. In view of the side effect profile of second-generation PDE4 inhibitors, new strategies for the design of active and nonemetic compounds have been attempted to hopefully overcome the problems and to improve therapeutic ratios. It has been hypothesized that the side effects of the PDE4 inhibitors are probably a result of their nonselectivity to all four PDE4 subtypes, and thus generation of new PDE4 inhibitors with subtype selectivity may provide clinical benefits by maintaining therapeutic efficacy while decreasing the side effects [59]. This notion is supported by a series of studies where PDE4 gene-targeted mice were used to define the function of individual PDE4 subtypes [60]. For example, the data have shown that ablation of PDE4b, but not PDE4a or PDE4d, significantly suppresses LPS-induced tumor necrosis factor (TNF)-α production in circulating monocytes and peritoneal macrophages [61, 62]. In addition, in a murine model of allergic asthma, Th2 cell functions including proliferation and cytokine production were also disrupted in mice

deficient in PDE4b, but not PDE4a or PDE4d [63, 64]. In a separate study, reversing α2-adrenoceptor- mediated anesthesia, a behavioral correlate of emesis in nonvomiting species such as rodents, was evaluated in xylazine/ketamine-treated mice and the results indicated that inhibition of PDE4d but not PDE4b may be responsible for the emetic effects of non-selective PDE4 inhibitors [65]. Taken together, these findings in PDE4 knockout mice suggest that an inhibitor with PDE4b selectivity should retain many beneficial anti-inflammatory effects without the emetic effects. In spite of the significant challenges of synthesizing PDE4 subtype-selective inhibitors due to the highly conserved catalytic domain of PDE4 isozymes, generation of inhibitors with PDE4 subtype selectivity has recently been described [66, 67]. A series of potent PDE4b inhibitors with more than 100-fold selectivity over PDE4d have been synthesized from lead 2-arylpyrimidine derivatives [66]. Biological evaluation of a selective PDE4b inhibitor revealed its potent anti-inflammatory effects *in vitro* and *in vivo*. Investigation in ferrets also showed a significantly less emesis with the compound compared with the non-selective PDE4 inhibitor cilomilast [66]. In a separate report, small-molecule allosteric modulators of PDE4d have been generated using a nontraditional approach [67]. These modulators do not completely inhibit enzyme activity, yet show potent cognition enhancement in animal models. Interestingly, the results from the rodent model of a behavioral correlate of emesis indicated that PDE4d allosteric modulators have reduced potential to cause emesis whereas PDE4d full inhibitors are highly emetic [67]. The reason for this different emetic effect is probably because PDE4d allosteric modulators have less effect on cAMP levels, because of their lower potency of PDE4 inhibition compared with full inhibitors of PDE4, and thus are able to better maintain cAMP signaling spatially and temporally while reducing target-based toxicity [67]. To avoid the problem of systemic side effects caused by oral administration, development of PDE4 inhibitors with alternative routes of delivery has been reported [68]. In general, when delivered by inhalation, the drug may have reduced systemic exposure and focused delivery to lung tissues, thus minimizing the potential of systemic side effects. GSK256066 is an inhaled PDE4 inhibitor which shows a protective effect on both early and late asthmatic responses to inhaled allergen in atopic asthmatics [68]. The drug was well tolerated with low systemic exposure, but larger studies are needed to establish the safety profile. Topical application of PDE4 inhibitors is another potential means to minimize systemic side effects. Benzylamine-substituted phthalazinones have recently been developed as potent topically active PDE4 inhibitors, and have shown anti-inflammatory effects in a mouse model of dermatitis [69]. Additional studies are required to evaluate the therapeutic index of the compounds. Although the majority of orally administered PDE4 inhibitors face the issue of side effects, a number of oral compounds currently in development, such as apremilast for psoriasis [70] and the PDE4d allosteric modulators as described above, [67] are reported to be less emetic and have wider therapeutic ratios. The molecular mechanism of this tolerability has not been reported.

PDE4 inhibition in COPD

The inhibition of PDE4 as a novel approach in the treatment of COPD has been discussed and investigated by the medical community for many years. The PDE4 inhibitor roflumilast, is now indicated for maintenance treatment of severe COPD (FEV_1 post-bronchodilator less than 50% predicted) associated with chronic bronchitis in adult patients with a history of frequent exacerbations as add on to bronchodilator treatment [71-75]. In this patient population, roflumilast 500 μg once-daily reduces exacerbation rate and improves lung function [73-75].

Effect of PDE4 inhibition on cAMP levels

Inhibition of PDE4 can increase the intracellular concentration of cAMP by inhibiting its degradation leading to reduced inflammatory cell activity [76].

Physiological effects of PDE4 inhibition in the lung

A large body of pre-clinical research has shown that PDE4 inhibition has the potential to target the three main components of COPD: bronchoconstriction, mucus hypersecretion and airway re-modeling [77-79]. As PDE4 is the major cAMP-metabolizing enzyme, inhibition of PDE4 suppresses the inflammatory response [78]. Also, by suppressing epidermal growth factor receptor-

induced Mucin 5AC over-expression, PDE4 inhibition directly inhibits mucus production [79]. Inhibition of PDE4 may also lead to minimization of airway remodeling by suppressing the release of TNF-α [80].

Proposed beneficial actions of PDE4 inhibition in COPD

A number of PDE4b inhibitors, and also short interfering RNAs (siRNAs) to specific PDE4b subtype [81], have been used to investigate the effect of inhibition of this enzyme on aspects of cellular function. In general, PDE4b inhibition potently prevents the release of pro-inflammatory mediators from a range of cell types. PDE4b inhibitors have been shown to inhibit adhesion molecule expression, chemotaxis, prolifration, migration and differentiation, and the release of re-modelling factors. They also inhibit or promote apoptosis, the latter appearing to be selective effect on lymphotic leukemia cells. In addition, PDE4b inhibitors relax inherent airway smooth muscle tone and can activate cystis fibrosis transmembrane conductance regulator (CFTR) - mediated chloride ion secretion. This diverse spectrum of biological effects has thus implicated PDE4b as a potential therapeutic target for a range of disease indications. The consequence of PDE4b inhibition in individual cell types is described below.

Eiosinophils:

Eiosinophils are thought to play an important role in asthma and also in exacerbations of chronic obstructive pulmonary disease (COPD) through their ability to release a plethora of pro-inflammatory mediators, which cause tissue injury, re-modeling and contractions of smooth muscles. PDE4a, b & d have been detected in human eosinophils [82]. A range of PDE4b inhibitos have been shown to inhibit N- formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated release of reactive oxygen species from human eosinophils [83] as well as inhibiting complement 5a (C5a) and platelet activating factor (PAF)-stimulated leukotrine LTC_4 synthesis [84]. In addition, PDE4b inhibitors can supress immunoglobulin (Ig)- induced degranulation [85]. PDE4b inhibitors can also inhibit PAF-induced CD11b expression & L-selectin shedding by 50% [30], and both C5a- and PAF-stimulated eosinophil chemotaxis [84]. In addition, they have also been shown to delay spontaneous human eosinophil apoptosis.

Neutrophils: Neutrophils are thought to play a vital role in the chronic lung inflammation and tissue destruction present in COPD, severe asthma and cystic fibrosis, through their ability to release many toxic substances such as proteases and oxygen radicals which cause tissue injury and re-modeling [82, 86]. PDE4a, b and d are expressed in human neutrophils [87-89], with evidence that PDE4b2 is the predominant PDE4 isoform in human neutrophils [87]. PDE4a is exclusively located within a subset of myeloper- oxidase (MPO) containing neutrophil granules [88]. PDE4 inhibitors have been shown to inhibit the release of a range of pro-inflammatory mediators from human neutrophils. For example, they inhibit fMLP-stimulated release of LTB4 and reactive oxygen species [90-92], together with the fMLP/ tumor necrosis factor-α (TNF-α)-stimulated release of matrix metalloproteinase-9 (MMP-9) and neutrophil degranulation products such as neutrophil elastase and MPO. PDE4b inhibitors can also inhibit PAF- induced CD11b expression and L-selectin shedding by 50% [93] and both TNF-α- and fMLP-mediated neutrophil adhesion to human umbilicalve in endothelial cells [94, 95]. PDE4b inhibitors have also been shown to delay spontaneous human neutrophil apoptosis [96].

Monocytes and macrophages:

Tissue macrophages differentiate from a common precursor, the circulating monocyte and play a major role in defence. Alveolar macrophages have, however, been implicated in tissue injury associated with inflammatory disease of the lung including asthma, acute respiratory distress syndrome & COPD [97]. PDE4 a, b & d are expressed in human neutrophils [87-89], with evidence that isoform in human monocytes [87], and is selectively induced by lipopolysaccharide (LPS). This induction is inhibited by interleukin-4(IL-4) and IL-10. PDE4b inhibitors are capable of completely abolishing LPS stimulated TNF-α release from peripheral blood monocots [98, 99]. Interestingly, in PDE4b deficient mice (but not PDE4d deficient mice), there is a mark reduction in the ability of LPS to stimulate TNF-α release from peripheral blood leukocytes [100], suggesting a key role for PDE4b in this response. Further support for this role can be derived from a separate study demonstrating that mean IC_{50}

values for inhibition of LPS stimulated TNF-α release are significantly correlated with compound potency against the catalytic activity of recombinant human PDE4b, but not the catalytic activity o recombinant PDE4d [4].

***Lymphocytes*:**

Hyperactive Th_1-mediated immune responses are thought to be involved in the pathogenesis of many autoimmune diseases, including multiple sclerosis, rheumatoid arthritis and Crohn's disease, whereas agents targeting Th_2 cells are sought for diseases such as bronchial asthma and ulcerative colitis. Knockdown of PDE4b or PDE4d (but not PDE4a) inhibited IL-2 release, whereas knockdown of PDE4d showed the most predominant inhibitory effect on interferon-γ (IFN-γ) and IL-5 release. PDE4 inhibitors have also been shown to partially inhibit IL-4 and IL-5 gene expression in Th_2 cells together with IL-4 and IL-5 release from human $CD4^+$ T cells. In contrast, a separate 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). PDE4 inhibitors have also been shown to partially inhibit phytohaemagglutinin and anti-CD3/anti-CD28-stimulated proliferation of $CD4^+$ and $CD8^+$ T cells. In a separate study, dual PDE4a/b and PDE4d selective inhibitors inhibited antigen-stimulated human T cell proliferation. Mean IC50 values significantly correlated with compound potency against the catalytic activity of recombinant PDE4a or b, but not with catalytic activity of recombinant PDE4d. In contrast, a PDE4d siRNA (but not PDE4 a or b siRNAs) significantly inhibited anti-CD3/CD28-stimulated $CD4^+$ proliferation. The reason for the apparent difference in PDE4 subtype involvement in this proliferative response is unclear, but could be related to either the fact that different T cell populations were used, or that different stimuli were used to elicit proliferation. Lastly, inhibition of PDE4 results in a potent induction of apoptosis in chronic lymphocytic leukemia cells, suggesting that PDE4 inhibitors may be of benefit in lymphoid malignancies. Importantly, this effect appears to be relatively specific, as comparable treatment of human peripheral blood T cells does not induce apoptosis [4].

Airway epithelial cells:

Airway epithelial cells are not only important barrier cells, but also play an integral role in the pathophysiology of airway diseases through their ability to release multiple pro-inflammatory and pro- re-modelling mediators. In addition, CFTR is the primary cAMP-activated chloride channel on the apical membrane of airway epithelia, there by playing an integral role in controlling the electrolyte/fluid balance and mucociliary clearance. CFTR activation has the potential to enhance mucociliary clearance, which may be of benefit in diseases such as COPD, asthma and cystic fibrosis. PDE4a, c and d are expressed in human airway epithelial cells. The PDE4b inhibitor, rolipram, partially inhibited granulocyte-macrophage colony-stimulating factor release from IL-1b-stimulated human airway epithelial cells; an effect which was maximally inhibited by dual inhibition of PDE3 and PDE4. In addition, rolipram inhibited LPS-stimulated IL-6 release from human airway epithelial cells, although relatively high concentrations were required to see an inhibitory effect, with an IC50 value of 24 mM, suggesting the effect could have been mediated through other PDE enzymes. Inhibition of PDE4 (in particular the D isoform) has been shown to activate CFTR-mediated chloride secretion in an epithelial cell line. More recently, it has been demonstrated that this effect is likely to be due to an interaction of PDE4b with AMP-activated kinase [4].

***Endothelial cells*:**

The endothelium acts a major permeability barrier of the blood vessel wall, and facilitates the transmigration of blood cells to tissue, through expression of adhesion molecules. During inflammation, however, leukocytes may damage endothelial cells, resulting in increased vascular permeability. Endothelial cells also play an important physiological role in angigenesis. Excessive angiogenesis, however, allows vascularisation of solid tumours and provides routes through which cancer cells may metastasise. Thus agents, which can limit processes involved in angiogenesis (e.g. cell migration), may represent novel therapies for pathologies such as cancer. 42 Human aortic, umbilical vein and microvascular endothelial cells express PDE4a, b and d. Inhibition of PDE4b in combination with appropriate activation of adenylate cyclase inhibits TNF-α-induced E

selectin expression on human lung microvascular endothelial cells. In addition, rolipram potently blocked H2O2-induced endothelial permeability when combined with prostaglandin E1. Inhibition of PDE4 decreased vascular endothelial growth factor induced migration of endothelial cells [4].

Mast cells and basophils:

Anti-IgE stimulation of mast cells and basophils is a central event in acute allergic disorders such as anaphylactic shock, asthma, allergic rhinitis, and some skin conditions including urticaria and atopic dermatitis. PDE4b has been detected in human basophils, although no data is published on which isoforms are present. There does not appear to be any published data describing the nature of PDE4b subtypes present in human mast cells, but the PDE4b inhibitor rolipram inhibits cAMP hydrolysis in mast cells by 50%. PDE4b inhibitors have been shown to suppress IgE and IL-3-dependent generation of IL-4, IL-13and histamine and also anti-IgE-induced histamine and LTC4 release from basophils. PDE4b inhibitors, however, have no effect on anti-IgE-stimulated histamine or LTC_4 release from human lung mast cells [4].

Smooth muscle cells:

Airway smooth muscle: Airway smooth muscle cells may contribute to airway re-modeling observed in lung diseases such as asthma and COPD, through the release of growth factors, cytokines and extracellular matrix proteins. PDE4b and d are expressed in human airway smooth muscle cells. Roflumilast has been shown to be capable of inhibiting transforming growth factor-b (TGF-β)-induced fibronectin deposition in human airway smooth muscle cells and also TGF-β-induced connective tissue growth factor, collagen I and fibronectin expression in human bronchial tissue rings. PDE4b inhibitors have also been shown to relax inherent tone in isolated human bronchial muscle; but a combination of PDE3 and PDE4 inhibitors is necessary to inhibit allergen or LTC4-induced contraction. Interestingly, in a study using siRNA targeted to PDE4D5, this PDE4 splice variant was shown to be the key physiological regulator of β2-adrenoceptor-induced cAMP turnover with in human airway smooth muscle [4].

Pulmonary artery smooth muscle cells: Aberrant regulation of smooth muscle cell proliferation and apoptosis in distal pulmonary arteries is a characteristic feature of pulmonary artery hypertension. Pulmonary artery smooth muscle cells have been shown to express all PDE4 isoforms. Interestingly several PDE4 splice variants (PDE4a10, a11, b2 and d5) have been shown to be up regulated following hypoxia. PDE4b inhibitors have been shown to inhibit the proliferation of TNF-α/ phorbol 12-myristate-13-acetate (PMA)-stimulated increase in MMP-2and MMP-9 activity of distal human pulmonary artery smooth muscle cells.

Vascular smooth muscle cells: Vascular smooth muscle cells (VSMCs) alter their phenotype in response to vascular injury displaying a reduced contractile capacity and increased proliferative, migratory and synthetic capabilities. PDE4d is the dominant PDE4 isoform in VSMCs and PDE4 inhibitors have been shown to inhibit VSCM proliferation. Given the effects of PDE4 inhibitors on VSMC and the selective expression of the PDE4d isoform in this cell type, the potential utility of selective PDE4d inhibitors in adjunctive pharmacotherapy after percutaneous coronary interventions has been suggested, and is reviewed by Houslay [4].

Fibroblasts: Therapies that mitigate the fibrotic process may be able to slow progressive loss of airways function in many lung diseases. Pulmonary fibroblast to myofibroblast conversion is a pathophysiological feature of idiopathic pulmonary fibrosis and COPD. PDE4 is expressed in human fibroblasts; although the subtype(s) have not yet been defined. Lung fibroblast to myofibroblast differentiation (induced by TGF-β) is inhibited by the PDE4 inhibitor piclamilast. PDE4 inhibitors can promote inhibition of TNF-α-stimulated pro-MMP1 and pro-MMP2 release from human lung fibroblasts. Cilomilast and rolipram inhibit chemotaxis of foetal lung fibroblasts towards fibronectin and inhibit contraction of three-dimensional collagen gels [4].

Therapeutic importance of PDE4b inhibitors:

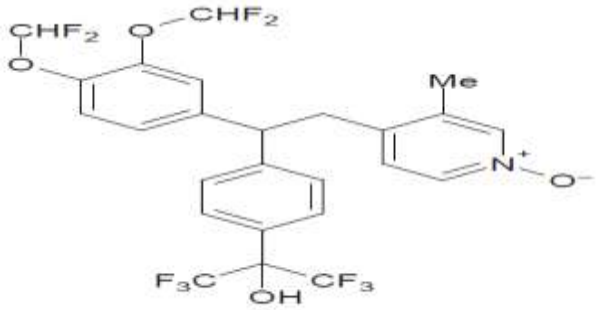
Asthma: Exposure of animals to bronchoconstrictor agents (e.g. histamine and methacholine) or exposure of previously sensitized animals to an allergic stimulus, e.g. ovalbumin (OVA) is widely used models to mimic characteristic features of asthma. Several studies have demonstrated that both oral and inhaled administration of PDE4 inhibitors can inhibit

allergen-induced pulmonary eosinophilia in guinea pig, mouse and rat. Local administration of PDE4 inhibitors such as roflumilast, AWD12-281[10] or UK-500, 001inhibits allergen, histamine, methacholine and LTD4-induced bronchoconstriction in guinea pigs, allergen-induced bronchial hyper-reactivity in mice and antigen-induced reductions in forced vital capacity. PDE4 inhibitors have also been shown to inhibit OVA-induced mucus production and goblet cell hyperplasia in allergic rat and mouse models and sub epithelial collagenisation and airway wall thickening in a murine chronic asthma model. PDE4d appears to be the subtype in the airways mediating the response to cholinergic and antigenic responses, at least in the mouse, as airway so PDE4d-deficient mouse are no longer responsive to cholinergic stimulation or antigen-induced airway hyper-reactivity [4].

COPD: Various pre-clinical species (most commonly rats and mice) have been exposed to LPS or cigarette smoke in order to try and induce some of the pathological features observed in COPD, specifically inflammatory cell infiltration and emphysematous changes. In acute cigarette smoke exposure studies in mice, oral treatment with cilomilast inhibited recruitment of neutrophils to the lung together with increases in BAL macrophage inflammatory protein-1a, and roflumilast partially inhibited neutrophil influx to the lung. In more chronic smoke exposure studies, roflumilast (oral treatment for 7 months) has been shown to fully prevent emphysema in mice, and the PDE4 inhibitor, GPD-1116 (oral treatment for 8 weeks) has also been shown to markedly attenuate the development of cigarette smoke-induced emphysema in senescence-accelerated P1 mice. Local administration of PDE4 inhibitors directly to the lung has also been shown to be effective in inhibiting LPS-induced neutrophil recruitment to the lung in a range of species: rats, ferrets and pigs. In a lung injury model utilizing PDE4a, b and d knockout mice, PDE4b and PDE4d (but not PDE4a) appear to be important in mediating LPS- induced neutrophil transepithelial migration; an effect which is mediated in part by up regulation of neutrophil CD18 expression [4].

Cough: The allergen-induced increase of cough response to inhaled capsaicin in sensitized animals, and normal cough response in unsensitised animals, have both been shown to be reduced by a PDE4 inhibitor [4].

Multiple sclerosis: In an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), a non-brain penetrant PDE4 inhibitor (L-826,141) reduced the severity of EAE and also delayed disease onset. Furthermore, in a relapsing-remitting EAE model of the SJL mouse, rolipram reduced the clinical signs of EAE during both the initial episode of the disease and also during subsequent relapses. Rolipram also markedly reduced demyelination, central nervous system (CNS) inflammation, and secretion of Th1 cytokines [4].



(1) L-826,141

Inflammatory bowel disease: A number of studies have demonstrated that PDE4b inhibitors can prevent colitis and reverse established colitis in a range of pre-clinical disease models. Dextran sodium sulphate, trinitrobenzene sulphonic acid [TNBS] and indomethacin-induced colitis mimic aspects of Crohn's disease and ulcerative colitis, the two major forms of human Inflammatory bowel disease (IBD) (as reviewed by Banner and Trevethick). In addition to the well described anti-inflammatory effects of PDE4 inhibitors in these disease models, one rat TNBS-induced colitis study demonstrated that treatment with rolipram could prevent intestinal collagen deposition, suggesting that PDE4b inhibitors may also be able to inhibit tissue re-modelling in this setting [4].

Rheumatoid arthritis: Administration of PDE4b inhibitors suppressed the pannus-like inflammation by inhibition of cytokine production from macrophages and synovial fibroblast proliferation in a mouse model of rheumatoid arthritis [4].

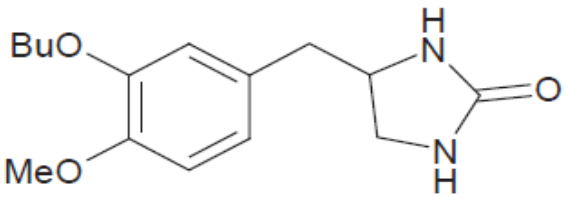
Memory: Rolipram is effective in animal models of memory. Specifically, it facilitates the establishment of long-lasting long-term potentiation and improves memory and

ameliorates experimentally induced impairments of learning and memory in rodents [4].

Depression: PDE4b inhibitors, including rolipram, produce anti-depressant-like effects in several pre-clinical models. Specifically, they have been shown to reduce the time of immobility in the forced-swim test; decrease response rate and increase reinforcement rate under a differential-reinforcement-of-low-rate schedule; reverse the effects of chronic, mild stress; normalize the behavioral deficits observed in Flinders sensitive-line and olfactory-bulbectomized rats ;antagonize the effects of reserpine and potentiate yohimbine-induced toxicity. Utilizing PDE4 subtype deficient mice, it appears that PDE4d is an essential mediator of the anti- depressant effects PDE4 inhibitors [4].

Pulmonary artery hypertension: Rolipram prevented hypoxia-induced PDE4 and PDE1 gene up regulation and interfered with the development of pulmonary artery hypertension in transgenic sickle cell mice, most likely through modulation of vascular tone and inflammatory factors [4].

Renal diseases: The PDE4b inhibitor RO-20-1724 alone has been shown to be effective in both the preventative and therapeutic setting in endotoxin-induced acute renal failure in rats [4].



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Adverse effects of PDE4 inhibitors

The therapeutic window of orally administered selective PDE4b inhibitors in clinical trials is limited by gastro intestinal side effects of nausea, vomiting, diarrhoea, abdominal pain and dyspepsia, although some of these appear to resolve with continued treatment. Regulatory agencies are, however, particularly concerned by the development of mesenteric vasculitis in laboratory animals. Mesenteric vasculitis has, however, never been seen in man, and generally has not been seen in non-human primates. Indeed mesenteric vasculitis has never been seen in human patients treated for many years with bronchodilator doses of theophylline, a regime which produces medial necrosis of mesenteric vessels in rats. Rats and dogs may have an increased susceptibility to drug-induced vascular legions as arteriopathies commonly occur in these species, and species differences have been shown to exist for both PDE4 expression and functional effects of PDE4b inhibitors. For example, a recent study demonstrated that levels of PDE4 enzyme activity are much higher in rats than humans in multiple tissues, making rats more susceptible to PDE4 inhibitor-induced toxicities. In addition, thePDE4b inhibitor IC542 (structure not available) markedly enhanced LPS-induced IL-6 release from rat whole blood, but not from human or non-human primate blood. Nevertheless vasculitis requires careful monitoring in man, and indeed current research is focused on identifying potential predictive biomarkers. To this end, tissue inhibitor of metalloproteinase-1appears to be an early and sensitive predictive biomarker of PDE4 inhibitor-induced vascular injury in rats [4].

Dual PDE3/4 and PDE4 inhibitors

Phosphodiesterase (PDE)4, and to a lesser extent, PDE3/4 inhibitors have attracted considerable interest as potential therapeutic agents for diseases including chronic obstructive pulmonary disease. Indeed, ibudilast and theophylline are utilized clinically, and roflumilast is in late-stage clinical development. Unfortunately, however many PDE4 and dual PDE3/4 inhibitors have failed in early development due to low therapeutic ratios. The majority of these compounds are however orally administered and non-selective for either PDE3 (a, b) or PDE4 (a, b, c, d) subtypes. Developing an inhaled dual PDE3/4 inhibitor with subtype specificity may represent one strategy to improve the therapeutic index. Indeed combined inhibition of PDE3 and PDE4 inhibitor has additive and synergistic anti-inflammatory and bronchodilatory effects versus inhibition of either PDE3 or PDE4 alone. Given that synergy has been seen in terms of efficacy end points, an obvious concern is that synergy may also be observed in side effects. Interestingly, however, no synergy or additive effects with a combination of a PDE3 and PDE4 inhibitor in a cardiomyocyte assay were observed. This review will summarize the rationale for developing an

inhaled dual PDE3/4 inhibitor, as a treatment for chronic obstructive pulmonary disease together with recent advances in trying to understand the pathogenesis of PDE inhibitor-induced mesenteric vasculitis (a key potential dose-limiting side effect of these agents), highlighting potential early and sensitive predictive biomarkers [101].

Selective PDE4 and dual PDE3/4 inhibitors have attracted considerable interest as potential therapeutic agents for the treatment of respiratory diseases, largely by virtue of their anti-inflammatory (PDE4) and bifunctional bronchodilator/anti-inflammatory (PDE3/4) effects. Many of these agents have, however, failed in early development for various reasons, including dose-limiting side effects when administered orally and lack of sufficient activity when inhaled. Indeed, only one selective PDE4 inhibitor, the orally active roflumilast-n-oxide, has to date received marketing authorization. The majority of the compounds that have failed were, however, orally administered and non-selective for either PDE3 (a, b) or PDE4 (a, b, c, d) subtypes. Developing an inhaled dual PDE3/4 inhibitor that is rapidly cleared from the systemic circulation, potentially with subtype specificity, may represent one strategy to improve the therapeutic index and also exhibit enhanced efficacy versus inhibition of either PDE3 or PDE4 alone, given the potential positive interactions with regard to anti-inflammatory and bronchodilator effects that have been observed pre-clinically with dual inhibition of PDE3 and PDE4 compared with inhibition of either isozyme alone. The study summarizes recent clinical data obtained with PDE inhibitors and the potential for these drugs to treat COPD and other inflammatory airways diseases such as asthma and cystic fibrosis [102].

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) and IPL512602, 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 or UK-500001, 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, 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 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, however, the activity of these cells is only inhibited to a small degree by PDE4 inhibitors 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. 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 [104].

Table No 01: Current PDE4 inhibitors in clinical trials

Drug (Institution/University)	Disease Condition	Phase	Allotted no
Rolipram (US National Institutes of Health)	Depression	Phase I	NCT00369798
Rolipram (GlaxoSmithKline)	Huntington's disease	Phase I	NCT01602900
GSK356278 (GlaxoSmithKline)	Huntington's disease	Phase I	NCT01602900
ASP9831 (Astellas Pharma)	Non-alcoholic steatohepatitis	Phase II	NCT00668070
GSK256066 (GlaxoSmithKline)	Rhinitis	Phase II	NCT00464568
CHF6001 (Chiesi Farmaceutici)	Asthma; COPD	Phase II	NCT01730404
Apremilast (Celgene)	Ankylosing spondyloarthritis	Phase III	NCT01583374
Apremilast (Celgene)	Acne	Phase II	NCT01074502
MK0952 (Merck Sharp & Dohme)	Alzheimer's disease	Phase II	NCT00362024
CHF6001 (Chiesi Farmaceutici)	COPD	Phase II	NCT01730404
Roflumilast (Takeda)	Atopic dermatitis	Phase II	NCT01856764
Roflumilast (Takeda)	Dementia	Phase II	NCT01433666
Roflumilast (The National Heart, Lung and Blood Institute)	Obesity	Phase II	NCT01862029
Sildenafil (University of Milan)	Heart failure	Phase III	NCT00407446
Sildenafil, tadalafil (Cedars-Sinai Medical Center)	Duchenne muscular dystrophy	Phase I	NCT01580501; NCT01359670
Sildenafil (IRCCS, San Raffaele)	Endocrine function in patients with diabetes	Phase III	NCT00420901
Sildenafil (Vanderbilt University)	Impaired glucose tolerance	Phase III	NCT01812434
Sildenafil (University of Minnesota)	Cardiac vasculopathy	Phase II	NCT01812434
Sildenafil (Massachusetts General Hospital)	Schizophrenia	Phase IV	NCT00455715
Tadalafil (Västra Götaland Region)	diabetes	Phase II	NCT01238224
Tadalafil (University of Roma La Sapienza)	Diabetic cardiomyopathy	Phase IV	NCT01803828
Tadalafil (Cedars-Sinai Medical Center)	Becker muscular dystrophy	Phase IV	NCT01070511
Tadalafil (Sidney Kimmel Comprehensive Cancer Center)	Multiple myeloma	Phase II	NCT01374217
Tadalafil (Washington University School of Medicine)	Aortic stenosis	Phase IV	NCT01275339
Tadalafil (Sidney Kimmel Comprehensive Cancer Center)	Head and neck cancer	Phase IV	NCT01697800
Tadalafil (Sanjay Gandhi Institute of Medical Sciences)	Lung diseases	Phase III	NCT01553981
Udenafil (Seoul National University Hospital)	Raynaud's phenomenon	Phase III	NCT01280266
PF-00489791 (Pfizer)	Diabetes nephropathy	Phase III	NCT01200394
PF-04447943 (Pfizer)	Alzheimer's disease	Phase II	NCT00930059
PF-04447943 (Pfizer)	Alzheimer's disease	Phase II	NCT00988598
PF-02545920 (Pfizer)	Schizophrenia	Phase I	NCT01244880
PF-02545920 (Pfizer)	Huntington's disease	Phase I	NCT01806896
RO5545965 (Hoffmann-La Roche)	Unknown	Phase I	NCT01923025
AMG 579 (Amgen)	Schizophrenia	Phase I	NCT01568203
TAK- 063 (Takeda)	Schizophrenia	Phase I	NCT01879722
AN2898 and AN2728 (Anacor Pharmaceuticals)	Atopic dermatitis	Phase II	NCT01301508

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