Phosphodiesterase Type 4 Inhibitors Cause Proinflammatory Effects in Vivo

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ABSTRACT

Phosphodiesterase type 4 (PDE4) inhibitors are currently being evaluated as potential therapies for inflammatory airway diseases. However, this class of compounds has been shown to cause an arteritis/vasculitis of unknown etiology in rats and cynomolgus monkeys. Studies in rodents have demonstrated the anti-inflammatory effects of PDE4 inhibitors on lipopolysaccharide (LPS)-induced airway inflammation. The aim of this work was to assess the direct effects of PDE4 inhibitors on inflammatory cells and cytokine levels in the lung in relation to therapeutic effects. The effects of the PDE4 inhibitors 3-cyclopentyl-4-difluoromethoxy-5-(3,5-di-chloropyrid-4-yl)benzamide (roflumilast) and 3-(cyclopentyloxy)-4-difluoromethoxy-N-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide (piclamilast) were assessed in vivo, using BALB/c mice, and in vitro, in unstimulated human endothelial and epithelial cell lines. In BALB/c mice, LPS challenge caused an increase in neutrophils in bronchoalveolar lavage (BAL) and lung tissue and BAL tumor necrosis factor-α levels, which were inhibited by treatment with either roflumilast or piclamilast (30–100 mg/kg subcutaneously). However, roflumilast and piclamilast alone (100 mg/kg) caused a significant increase in plasma and lung tissue keratinocyte-derived chemokine (KC) levels, and lung tissue neutrophils. In vitro, both piclamilast and roflumilast caused an increase in interleukin (IL)-8 release from human umbilical vein endothelial cells but not BEAS-2B cells, suggesting that one source of the increased KC may be endothelial cells. At doses that antagonized an LPS-induced inflammatory response, the PDE4 inhibitors possessed proinflammatory activities in the lung that may limit their therapeutic potential. The proinflammatory cytokines KC and IL-8 therefore may provide surrogate biomarkers, both in preclinical animal models and in the clinic, to assess potential proinflammatory effects of this class of compounds.

Phosphodiesterase type 4 (PDE4) inhibitors are currently being evaluated as potential therapies for inflammatory airway diseases such as chronic obstructive pulmonary disease and asthma. Indeed, roflumilast and cilomilast are currently in phase III clinical trials for one or both diseases. However, this class of compounds has been associated with some toxicity issues (for recent review, see Giembycz, 2005). PDE4 inhibitors have been shown to cause arteritis and vasculitis (inflammation of the arteries and vessels) in rats (Langle et al., 1994; Larson et al., 1996; http://www.fda.gov/ohrms/dockets/ac/03/transcripts/3976T1.doc; Mecklenburg et al., 2006) and minipigs (Vogel et al., 1999). This finding was demonstrated more recently in cynomolgus monkeys, which was the first observation using this class of compounds in nonhuman primates (Losco et al., 2004). Recent data provided further evidence for inflammatory implications of PDE4 inhibitors. Evaluations of the ryanodine-receptor complex in knockout mice and in human cells suggest that reducing PDE4D activity causes defective RyR2-channel function that is associated with heart failure and arrhythmias (Lehnart et al., 2005). To date, there does not seem to be published data reporting any kind of proinflammatory effect in the lung in response to treatment with single or multiple doses of this class of compounds.

The effects of PDE4 inhibitors in animal models of LPS-induced airway inflammation have been described in the literature. In mice, PDE4 inhibitors have been shown to inhibit neutrophil numbers, tumor necrosis factor (TNF)-α,
transforming growth factor-β levels, and matrix metalloproteinase-9 activity in the bronchoalveolar lavage (BAL) (Corbel et al., 2002). In other experiments, PDE4 inhibitors demonstrated suppression of lung myeloperoxidase activity, serum TNF-α levels (Miotla et al., 1998), BAL neutrophilia, and lymphocyte numbers (Trifilieff et al., 2002). Several PDE4 inhibitors have also been demonstrated to be effective in rat models. Studies have shown inhibition of neutrophil numbers (Spindler et al., 2001; Billah et al., 2002; Trifilieff et al., 2002; Kuss et al., 2003) and neutrophil activation in BAL (Trifilieff et al., 2002). In guinea pigs, rolipram treatment was shown to completely reverse the bronchoconstriction produced by chronic treatment with LPS (Toward and Broadley, 2002).

However, the effects of these inhibitors on both BAL and lung tissue inflammation have not been extensively investigated, and assessment of biomarker levels seems to have been limited mainly, but not exclusively, to measurement of TNF-α. More importantly, the direct effects of this class of compounds on inflammatory cells and biomarkers in the lung have not yet been elucidated.

The objective of the experiments described here was to determine the effects of two PDE4 inhibitors, 3-cyclo-propylmethoxy-4-difluoromethoxy-N-[3,5-di-chloropyrid-4-y1]-benzamide (roflumilast) and 3-(cyclopentolxy)-N-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide (piclamilast), either alone or against LPS-induced airway inflammation in BALB/c mice, to determine whether there were inflammatory effects additional to those already extensively described in the literature. Cellular inflammation, TNF-α levels, and the levels of the neutrophilic chemokines keratinocyte-derived chemokine (KC) and macrophage inhibitory protein (MIP)-2, shown to be established markers of inflammation in response to LPS (Schwartz et al., 1994; Ulich et al., 1995; Korsgren et al., 2000; Haddad et al., 2001; Birrell et al., 2004), were measured in the airway lumen as well as in lung tissue. The effects on these parameters in the circulation were also determined. In addition, to assess the direct effects of PDE4 inhibitors on structural cells, concentration response experiments were performed using primary human umbilical vein endothelial cells (HUVECs) and a human bronchial epithelial cell line (BEAS-2B).

Materials and Methods

Animals

Male BALB/c mice (20–25 g) were purchased from Harlan (Indianapolis, IN) and housed for 5 days before initiating experiments. Food and water were supplied ad libitum. All experiments were conducted in accordance with Institutional Animal Care and Use Committee guidelines, in a program approved by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Supplemental Methods

Effect of Roflumilast, Piclamilast, and Dexamethasone on LPS-Induced Cellular Inflammation in the BALB/c Mouse. BALB/c mice were challenged with an aerosol of endotoxin-free sterile water or LPS (0.3 mg/ml for 10 min). Vehicle (polysorbate 80 suspension, 5 ml/kg), roflumilast or piclamilast (3, 10, 30, and 100 mg/kg) was administered subcutaneously 30 min before challenge. Three hours after challenge, white cell numbers in the circulation were determined. As described above, the effect of PDE4 inhibitor or steroid alone was determined at a single dose.

Effect of Roflumilast, Piclamilast, and Dexamethasone on Basal Cellular Inflammation in the Circulation. BALB/c mice were challenged with an aerosol of endotoxin-free sterile water (10 min). Vehicle (polysorbate 80 suspension, 5 ml/kg), roflumilast or piclamilast (100 mg/kg) was administered subcutaneously 30 min before challenge. Dexamethasone (1 mg/kg s.c.) was included as a control and dosed 1 h before water challenge. One vehicle group was challenged with LPS (0.3 mg/ml for 10 min). Three hours after challenge, white cell numbers in the circulation were determined. As described above, the effect of PDE4 inhibitor or steroid alone was determined at a single dose.

Effect of Roflumilast and Piclamilast on Basal IL-8 Release from Primary HUVECs and Transformed BEAS-2B Cells. Primary HUVECs and the transformed human bronchial epithelial cell line BEAS-2B were obtained from Cambrex Bio Science Walkersville (Walkersville, MD) and the American Type Culture Collection (Manassas, VA), respectively. HUVECs were grown to confluence in tissue culture flasks (75 cm²) containing 15 ml of endothelial growth medium-2 complete medium and then seeded onto 96-well plates at an initial density of 3200 cells per well. Cells were treated upon reaching confluence and were used at passages 3 to 4. BEAS-2B cells were grown to near confluence in 75-cm² tissue culture flasks containing 15 ml of LHC9 medium, which was changed to LHC8 medium 18 h before assay. Cells were seeded into 96-well plates at a density of 50,000 cells per well and allowed to adhere for approximately 2 h before treatments.

To determine whether the PDE4 inhibitors caused an increase in basal levels of IL-8, cells were treated with roflumilast, piclamilast (10⁻¹⁰–10⁻⁴ M) or 0.5% DMSO vehicle. IL-1β (10 ng/ml) and LPS (100 ng/ml) treatments were included as stimulus controls to compare compound effects. Cells were incubated at 37°C in a thermostatically controlled incubator at a 5% CO₂ atmosphere. After 24 h, the amount of IL-8 released into the cell culture supernatant was measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

Quantification of Airway Inflammation following LPS Challenge. Three hours after water or LPS challenge, animals were bled from the tail vein and then euthanized with sodium 50 mg/kg pentobarbital. The trachea was cannulated. BAL cells were recovered from the airway lumen by flushing the airways with RPMI 1640 medium (three 0.3-ml washes, pooled; Invitrogen, Carlsbad, CA) delivered through the tracheal cannula and removed after a 30-s interval. The lungs were then perfused with RPMI 1640 medium to remove the blood pool of cells, removed, and finely chopped. The inflammatory cells were extracted from the lung tissue by collagenase digest as described by Underwood et al. (1997) using 6 ml of digest fluid (RPMI 1640 medium, 10% fetal bovine serum containing 1 mg/ml collagenase, and 25 μg/ml DNase).
Total white cell counts were determined on the EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA) using an FITC-conjugated antibody to CD45. In brief, in 100-mm flow tubes, 100 μl of sample was stained with 10 μl of CD45 FITC and incubated in the dark at room temperature for 10 min. Samples were then lysed/ fixed with 500 μl of Optilyse C and incubated for a further 10 min under the same conditions. Then, 500 μl of phosphate-buffered saline was added to the samples and vortexed. Immediately before reading, 100 μl of flow count beads was added to each sample to function as a calibrator. Samples were read for 30 s using Expo 32 software (Beckman Coulter), and the amount of total white blood cells was counted.

Cytospins of the BAL and lung tissue digest samples were prepared by centrifugation of 100-μl aliquots in a cytospin (Thermo Electron Corporation, Waltham, MA) at 700 rpm for 5 min, low acceleration at room temperature. Slides were fixed and stained on a Hema-Tek 2000 (Spectron Corp., Kirkland, WA) with modified Wright’s-Giemsa stain. Differential counts on 200 cells per slide were performed following standard morphological criteria, and the percentage of neutrophils was determined.

**Assessment of Cytokine Protein Expression.** BAL and blood samples were centrifuged at 1900 and 3500 rpm, respectively, for 10 min at 4°C, and the resultant supernatant and plasma were removed and used to measure cytokine levels. For the determination of cytokine protein levels in the lung tissue, the lungs were homogenized in 2 ml of ice-cold saline using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The samples were then centrifuged at 5000 rpm for 15 min, and the resultant supernatants were used for cytokine protein quantification.

In the BAL and lung tissue protein levels for TNF-α, KC, and MIP-2 were determined by ELISA using mouse Duosets according to the manufacturer's instructions (R&D Systems). For lung tissue, cytokine levels were further corrected for total protein content, which was measured using the Bradford assay (Bio-Rad, Hercules, CA). Blood samples were assessed for KC only, because previous
in-house experiments had determined that there was no increase in TNF-α or MIP-2 levels after LPS challenge (data not shown).

Assessment of Circulating Cellular Inflammation. Total white cell counts were determined in whole blood samples as described above. Blood smears were prepared on microscope slides using 10 μl of sample, and then slides were stained and fixed on the Hema-Tek 2000 as described above. Differential counts on 100 cells per slide were performed following standard morphological criteria, and the percentage of neutrophils was determined.

Materials

Sodium pentobarbital (50 mg/kg) was obtained from Abbott Laboratories (Abbott Park, IL). RPMI 1640 medium and fetal bovine serum were from Invitrogen. Roche Diagnostics (Plaisant, CA) supplied the collagenase, DNase, and penicillin streptomycin solution. Roflumilast and piclamilast were synthesized by the Department of Medicinal Chemistry of Theravance Inc. (South San Francisco, CA). Polysorbate 80 suspension was prepared by the Department of Pharmacology at Theravance Inc. (South San Francisco, CA). LPS, dexamethasone, DMSO, and Wright’s-Giemsa stain were purchased from Sigma-Aldrich (St. Louis, MO). CD45 FITC and reagents for the flow cytometer were purchased from Beckman Coulter. LHCS and LHCG media were purchased from BioSource International (Camarillo, CA). Endothelial growth medium-2 complete medium was obtained from Cambrex Bio Science Walkersville. All ELISA kits and Duoset kits were obtained from R&D Systems.

Results

Effect of Roflumilast and Piclamilast on LPS-Induced Cytokine Release in the BAL. Challenge with an aerosol of LPS resulted in a significant increase in BAL TNF-α, MIP-2, and KC levels (Figs. 1 and 2, A–C). Treatment with roflumilast or piclamilast caused a dose-dependent inhibition of TNF-α, which was significant at 30 and 100 mg/kg (Figs. 1 and 2A). However, there was no inhibition of LPS-induced elevations in MIP-2 or KC levels following treatment with either compound (Figs. 1, B and C, and 2, B and C). The synthetic glucocorticoid dexamethasone caused a significant inhibition of all three biomarkers. Neither roflumilast, nor dexamethasone had any effect on basal BAL inhibition of all three biomarkers. Neither roflumilast, nor dexamethasone caused a significant increase in neutrophil numbers in the lung tissue. Both TNF-α and MIP-2 levels were increased in the lung in response to LPS challenge (895 ± 68.9 to 1872.7 ± 164.8 and 1656 ± 76.9 to 2272.3 ± 166.4 pg/mg tissue, respectively). Treatment with roflumilast caused a significant inhibition of TNF-α at 100 mg/kg, but no inhibition of MIP-2 protein levels (838.8 ± 54.3 and 2167.4 ± 124.6 pg/mg tissue). Treatment with piclamilast resulted in significant inhibition of TNF-α at 30 mg/kg and MIP-2 at 100 mg/kg (892.4 ± 45.6 and 1715.4 ± 96.9 mg/kg tissue). Dexamethasone caused significant inhibition of both biomarkers. Neither PDE4 inhibitor caused any increase in levels of these mediators (data not shown).

Effect of Roflumilast and Piclamilast on LPS-Induced Cytokine Release in the Plasma and Lung Tissue. Treatment with roflumilast and piclamilast had no inhibitory effect on the increase in plasma KC levels caused by challenge with inhaled LPS (Fig. 3, A and B). However, both compounds caused an increase in basal levels of KC, compared with vehicle control. These stimulatory effects were greater than those observed following LPS challenge. In addition, treatment with roflumilast at 100 mg/kg also caused a significant increase in KC levels in the LPS challenged group. This may reflect the sum of the LPS response plus the increase in basal KC described for roflumilast. Dexamethasone significantly attenuated the LPS-induced elevation in plasma KC levels. In a separate experiment, both inhibitors were also shown to cause a significant increase in basal levels of KC when tested at a lower dose of 30 mg/kg, compared with vehicle control (from 187.5 ± 17.9 to 1410 ± 382.1 and 1982 ± 629.5 pg/ml for roflumilast and piclamilast, respectively; p < 0.05). Although this was a separate experiment, the increase in KC observed was lower than that produced by the PDE4 inhibitors tested at 100 mg/kg in the present study.

Neither roflumilast nor piclamilast treatment caused any increase in the levels of either TNF-α or MIP-2 protein levels in the plasma. As with vehicle control, levels remained below the limit of detection of the assay (data not shown).

Neither PDE4 inhibitor caused any inhibition of the LPS-induced increase in lung tissue KC levels (Fig. 3C); however, treatment with both roflumilast and piclamilast alone caused a significant increase in levels of this chemokine compared with vehicle control. Dexamethasone treatment caused a significant inhibition of KC levels in the lung tissue. Both TNF-α and MIP-2 levels were increased in the lung in response to LPS challenge (895 ± 68.9 to 1872.7 ± 164.8 and 1656 ± 76.9 to 2272.3 ± 166.4 pg/mg tissue, respectively). Treatment with roflumilast caused a significant inhibition of TNF-α at 100 mg/kg, but no inhibition of MIP-2 protein levels (838.8 ± 54.3 and 2167.4 ± 124.6 pg/mg tissue). Treatment with piclamilast resulted in significant inhibition of TNF-α at 30 mg/kg and MIP-2 at 100 mg/kg (892.4 ± 45.6 and 1715.4 ± 96.9 mg/kg tissue). Dexamethasone caused significant inhibition of both biomarkers. Neither PDE4 inhibitor caused any increase in levels of these mediators (data not shown).

Effect of Roflumilast and Piclamilast on Circulating Cellular Inflammation. LPS challenge caused a significant increase in neutrophils in the BAL, which was inhibited in a dose-dependent manner by roflumilast, reaching significance at 30 and 100 mg/kg inhibitor (Fig. 4A). Although not statistically significant, the lowest dose of roflumilast used (3 mg/kg) produced 62% inhibition (data assessed using nonparametric one-way analysis of variance with appropriate post-test). LPS challenge also resulted in a significant increase in lung tissue neutrophils that was unaffected at any dose of roflumilast tested (Fig. 4B). Treatment with dexamethasone resulted in a significant inhibition of neutrophils in both compartments. Interestingly, roflumilast seemed to have a proinflammatory effect in the lung tissue, where basal neutrophil numbers were increased (Fig. 4B). Indeed, this apparent pro-inflammatory effect of roflumilast in the lung tissue may account for the lack of inhibition of inflammation produced upon LPS challenge. Even at the highest dose tested (100 mg/kg), cell numbers were similar to the levels produced by treatment with roflumilast alone.

LPS challenge caused a significant increase in neutrophils in the BAL and lung tissue, which was inhibited in a dose-dependent manner by piclamilast, reaching significance at 100 and 30 mg/kg, respectively (Fig. 5, A and B). Treatment with dexamethasone caused a significant inhibition of neutrophil number in both compartments. Piclamilast also seemed to have a proinflammatory effect in the lung tissue (Fig. 5B). Basal neutrophil numbers were increased following treatment with piclamilast, although this did not reach statistical significance (p = 0.06).

Effect of Roflumilast and Piclamilast on Basal Cell Burden in the Blood. Treatment with piclamilast caused a significant increase in neutrophil numbers in the circulation (from 433.7 ± 52 to 1199.3 ± 167.3 cells × 10⁹/ml; p < 0.05). Indeed, this was of a similar magnitude to the increase in
neutrophils produced by LPS challenge (1254.7 ± 119.7 cells × 10^3/ml). This effect is very similar to that observed in the lung tissue, in that there was no increase in total white cell numbers but an increase in neutrophil numbers. Treatment with dexamethasone had no effect on either neutrophils or eosinophils. In contrast treatment with roflumilast had no effect on neutrophil numbers (234.7 ± 43.9 to 371.9 ± 94.9 cells × 10^3/ml).

**Effect of Roflumilast and Piclamilast on Basal IL-8 Release in Vitro.** In HUVECs, roflumilast caused a significant increase in basal IL-8 levels at both 10^{-5} and 10^{-4} M (from 244.4 ± 26.9 to 898.2 ± 36.2 pg/ml, 10^{-4} M; Fig. 6A). Piclamilast caused a significant increase in basal IL-8 levels at 10^{-4} M (from 214.9 ± 15.6 to 472.3 ± 83.7 pg/ml; Fig. 6B). The increases in response to either compound were appreciable and for roflumilast, were comparable to that elicited by LPS treatment (1250.7 ± 32.5 pg/ml). In the bronchial epithelial cell line BEAS-2B, neither PDE4 inhibitor caused any significant increase in release of IL-8 compared with vehicle control, even at 10^{-4} M (roflumilast, from 36.6 ± 2.8 to 47.7 ± 10 pg/ml; piclamilast, from 36.6 ± 2.8 to 51.6 ± 13.4 pg/ml). For comparison, the positive control IL-1β caused an increase in IL-8 levels up to 6701 ± 258.8 pg/ml. This suggests that, in vivo, the source of the KC may be endothelial and not epithelial cells.

**Discussion**

The objective of the experiments described here was to determine the direct effects of two PDE4 inhibitors, roflumi-
control group, with nonstimulated control group; was observed using cilomilast, SCH 351591, NVP-ABE171, NVP-ABE171 (Trifilieff et al., 2002) reduced BAL neutrophil treatment with piclamilast (assessed from 1 to 30 mg/kg) or agrees with published data. In mice (Corbel et al., 2002), reduction of neutrophil numbers in the BAL, a finding that 2001). Treatment with roflumilast and piclamilast caused a information in mice (Schwartz et al., 1994; Korsgren et al., 2000; Birrell et al., 2004) and rats (Ulich et al., 1995; Haddad et al., 2004; McCluskie et al., 2004). Our data show that levels of both mediators were increased, in response to challenge with LPS, in BAL and lung tissue. However, there was no reduction in the levels of either chemokine following treatment with the PDE4 inhibitors, suggesting that these compounds only partially inhibit the LPS-mediated inflammatory response. Although there does not seem to be any published data that addresses the effects of PDE4 inhibitors on these chemokines in aerosolized LPS models, the effect of rolipram on the increased neutrophilia and chemokine release in response to Klebsiella pneumoniae infection has been investigated (Soares et al., 2003). Although neutrophil numbers were decreased, this was not due to inhibition of KC, but rather of TNF-α, a finding that is in agreement with our data. However, in human peripheral blood monocytes stimulated with LPS, treatment with PDE4 inhibitors was found to have no effect on IL-8 but effectively inhibited TNF-α levels (Yoshimura et al., 1997). Our data show that levels of KC were increased in the plasma in response to aerosolized LPS challenge; however, there were no increases in the levels of either TNF-α or MIP-2. This is possibly an indication that KC is very sensitive to LPS stimulation. Alternatively, KC release could be secondary to the release of other proinflammatory mediators.

Both PDE4 inhibitors demonstrated proinflammatory properties in vivo in non-LPS stimulated mice at the single dose tested. KC levels were significantly increased in both the lung tissue and plasma and seem to be associated with an increase in lung tissue and plasma neutrophil numbers. This may account for the attenuated inhibition of LPS-induced lung tissue neutrophil numbers by these compounds. BAL neutrophil numbers were substantially reduced by PDE4 inhibitors (100 mg/kg). This effect was greater than that observed after treatment with the positive control dexamethasone. However, in the lung tissue, the inhibition produced by rolipram and piclamilast at the highest dose (100 mg/kg)
was similar to the proinflammatory effect produced by the compounds at the same dose. Indeed, roflumilast did not produce a significant inhibition of lung tissue neutrophils at any dose tested. It is noteworthy that the current results do not preclude the possibility that residual blood neutrophils may affect the observations made in lung tissue. The increase in basal levels of KC in the lung tissue may also explain the lack of inhibition by these compounds on LPS-induced KC levels. In addition, there was no increase in basal KC levels in the BAL. Because KC is generated in the vasculature, the time course of the experiment may allow for extravasation into the interstitial fluid, but not into the BAL. It should be
considered that using the current markers the first indications of a pulmonary and systemic proinflammatory effect were seen at 100 mg/kg. It is conceivable that other metabolic mechanisms that result in an underlying proinflammatory effect could be present at lower doses.

The mechanisms by which PDE4 inhibitors increase levels of KC are not known. Further experiments are required to assess compound effects on transcription factors and biomarker mRNA levels. In vitro data suggested that the mechanism of action by which roflumilast inhibits inflammatory mediators was through inhibition of nuclear factor-κB, p38 mitogen-activated protein kinase and c-Jun NH₂-terminal kinase activation in macrophages (Kwak et al., 2005). Investigation of these transcription factors in vivo may elucidate the mechanism of action of these compounds.

To determine the cellular source of KC, experiments were conducted in human endothelial and epithelial cells, which are known to release IL-8 (Khair et al., 1996; Striz et al., 1998; Mul et al., 2000). Roflumilast and piclamilast treatment led to a significant increase in IL-8 release from HUVECs, but not from BEAS-2B cells. These data would suggest that the source of KC observed in our in vivo experiments may be endothelial rather than epithelial cells. However, the possibility of other cellular sources cannot be excluded. Because IL-8 was released from only one of the two cell types tested in vitro, it also suggests that this effect is specific, rather than simply a general cytotoxic effect due to a high concentration (10⁻⁴ M) of compound used. The airway diseases to which these inhibitors are being targeted require chronic therapy; therefore, any observed proinflammatory effect of these compounds could be a cause for concern.

Fig. 6. Effect of roflumilast (A) and piclamilast (B) on basal IL-8 release from primary HUVECs. Cells were treated with roflumilast, piclamilast (10⁻¹⁰–10⁻⁴ M) or 0.5% DMSO vehicle for 24 h. LPS (100 ng/ml) and IL-1β (10 ng/ml) were included as stimulus controls. After 24 h, cell culture supernatants were removed and assessed for IL-8 release by ELISA. Values are expressed as mean ± S.E.M. Statistical analysis was made using a Mann-Whitney U test for unpaired data, or, for multiple comparisons, a Kruskal-Wallis test followed by a Dunn’s post-test (*, p < 0.05 compared with non-stimulated vehicle, n = 9 from three separate experiments).

Agents that elevate cyclic adenosine monophosphate (cAMP) levels have shown an increase in IL-8 release from different cell types. This has been demonstrated in granulosa cells using dibutyryl cAMP (Zeineh et al., 2003) and in HeLa cells using forskolin (Iourgenko et al., 2003). However, the effects of these agents on the release of IL-8 from epithelial cells are controversial. β₂-agonists have been shown to increase IL-8 levels released from human bronchial epithelial cells (Linden, 1996; Korn et al., 2001); an effect not shared by either forskolin, or the specific PDE4 inhibitor, rolipram (Fuhrmann et al., 1999). It seems that the effects of elevating cAMP levels on IL-8 release in this particular cell type remain unclear.

The toxicity issues of this class of compounds is associated with arteritis in several species (Langle et al., 1994; Larson et al., 1996; Vogel et al., 1999; Mecklenburg et al., 2006), including cynomolgus monkeys (Losco et al., 2004). Recent data also highlighted a role in heart failure and arrhythmias (Lehnart et al., 2005). The data presented here now demonstrate further proinflammatory activities of selective PDE₄ inhibitors. This is the first time such properties have been observed in the lung. Although the doses at which proinflammatory effects were observed in this animal model are higher than those used in clinical studies, they are within the range of efficacious doses published previously for similar animal models (Corbel et al., 2002). It should be considered that
animal models may be less sensitive to the anti-inflammatory effects of PDE4 inhibition and that the clinical doses may be submaximal for efficacy to maintain a therapeutic window, yet side effects remained a problem even at those lower doses (Storton and Fitzgerald, 2002).

In summary, the data presented here show for the first time that the PDE4 inhibitors exhibit proinflammatory activities in the lung that may limit their therapeutic potential in the chronic treatment of inflammatory respiratory diseases. In addition, these data suggest that KC and IL-8 may provide surrogate biomarkers, in both preclinical animal models and in the clinic, with which to measure potential proinflammatory effects of this class of compounds. This may, in turn, aid in the discovery of safer PDE4 inhibitors that can be used in the treatment of inflammatory airway diseases such as asthma and chronic obstructive pulmonary disease.

References


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