p38α-Selective Mitogen-Activated Protein Kinase Inhibitor SD-282 Reduces Inflammation in a Subchronic Model of Tobacco Smoke-Induced Airway Inflammation

Satyanarayana Medicherla, Mary F. Fitzgerald, Dianne Spicer, Paul Woodman, Jing Y. Ma, Ann M. Kapoun, Sarvajit Chakravarty, Sundeep Dugar, Andrew A. Protter, and Linda S. Higgins


Received June 28, 2007; accepted November 28, 2007

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is characterized by pulmonary inflammation, which is relatively insensitive to inhaled corticosteroids. The extent of the pulmonary inflammation in COPD correlates with disease severity, and it is thought to play a significant role in disease progression. We have evaluated a selective p38α-selective mitogen-activated protein kinase (MAPK) inhibitor, indole-5-carboxamide (ATP-competitive inhibitor of p38 kinase) (SD-282), in an 11-day model of tobacco smoke (TS)-induced pulmonary inflammation in A/J mice, by using dexamethasone as a reference steroid. Two oral treatment paradigms were evaluated in this TS model: prophylactic with daily pretreatment before each daily exposure, and therapeutic with daily treatment for 6 days commencing after 5 days of smoke exposure. Bronchoalveolar lavage and histological evaluation of lung sections taken after exposure to TS revealed an inflammatory response composed of increased numbers of macrophages and neutrophils and enhanced mucin staining. Phospho-p38 staining in macrophages and type II epithelial cells after TS exposure was also observed. Given prophylactically or therapeutically, dexamethasone failed to inhibit any of the TS-induced inflammatory changes. By contrast, SD-282 inhibited TS-induced increases in macrophages. Furthermore, SD 282 reduced TS-induced increases in cyclooxygenase-2 and interleukin-6 levels, and phospho-p38 expression in the lungs. In conclusion, SD-282 markedly reduced TS-induced inflammatory responses when given prophylactically or therapeutically whereas dexamethasone was ineffective. This is the first evidence that a p38α-selective MAPK inhibitor can exert pulmonary anti-inflammatory activity in a TS exposure model when given in a therapeutic mode, establishing the potential of p38 MAPK inhibitors as a therapy for COPD.

Chronic obstructive pulmonary disease (COPD) is the sixth leading cause of death in the world, and the prevalence of physiologically defined COPD in adults aged ≥40 years is ~9 to 10% (Halbert et al., 2006). COPD is characterized by airway obstruction and progressive lung inflammation that is associated with the influx of inflammatory cells (O’byrne and Postma, 1999; Loppow et al., 2001; Culpitt et al., 2003). The primary cause of COPD is smoking, with up to 50% of smokers developing disease normally in cities, with additional identifiable risk factors of increasing age and continued smoking (Lundbäck et al., 2003; Eisner et al., 2006). Inflammation in COPD lungs is present in both small and large airways, and it is thought to be critical in the development of the pathology of the disease. Indeed, the severity of inflammation is associated with disease severity as measured by spirometry (Saetta et al., 2001). In small airways inflammation, fibrosis, smooth muscle hypertrophy, and goblet cell hyperplasia are present, and these features increase in intensity as the disease progresses. Inflammatory cell involvement in the small airways consists of CD4 and CD8 T lymphocytes, B cell follicles, macrophages, and in more severe disease it also involves neutrophils (Saetta, 1999; Hogg et al., 2004). Inflammation is also present in large airways,
and it consists of macrophages, CD8+ lymphocytes, and plasma cells (Saetta, 1999). Key contributors to the progression of airway obstruction in COPD have been shown to be the increase in the volume of tissue in the small airway wall, the accumulation of mucous exudates, and the infiltration of the airway wall with cells of the innate and adaptive immune response (Turato et al., 2002; Hogg et al., 2004).

The anti-inflammatory efficacy of inhaled corticosteroids, which are widely used in COPD, predominantly in combination with inhaled β2 receptor agonists, has been explored in several studies (Loppow et al., 2001; Culpitt et al., 2003). However, the data are variable, and whether inhaled corticosteroids are effective as anti-inflammatory agents in COPD remains contentious (Highland, 2004; Sutherland and Cherniack, 2004), with stronger evidence for effectiveness in treating or preventing exacerbations, and weaker evidence for effects on disease progression. Therefore, the development of more effective anti-inflammatory drugs for the treatment of COPD has become a key objective for drug discovery companies (Barnes, 2005a).

New anti-inflammatory approaches are currently in clinical trials, and, if effective, are anticipated to modify the progression of the disease. In this context, the PDE4 inhibitor roflumilast, which is in phase III clinical trials for COPD, fully prevented the development of emphysema in mice chronically exposed to cigarette smoke. This prevention of lung destruction was associated with a reduction in pulmonary accumulation of macrophages, although effects on other cell types were not investigated (Martorana et al., 2005). However, PDE4 inhibition may be associated with side effects that could limit use in COPD (Fan Chung, 2006). There is significant evidence from animal models linking p38 MAP kinase to chronic inflammation in arthritis and type-1 diabetes (Medicherla et al., 2006a,b) and inhibitors of this kinase are in preclinical and clinical development for lung diseases, including COPD (Tigani et al., 2003; Tsai et al., 2006). However, there is currently little preclinical data related to the potential role for p38 MAP kinase inhibition in COPD. The use of short-term TS exposure models allows the evaluation of potential anti-inflammatory mechanisms and the comparison of their efficacy to other treatments. The studies presented here first compare the pulmonary cell influx induced in the mouse by five daily exposures to tobacco smoke (TS) to that seen after 11 days of exposure to TS. In addition, the anti-inflammatory actions of a p38α selective MAP kinase inhibitor, SD-282, are compared with the effects of a corticosteroid, dexamethasone, on the inflammation induced by an 11-day smoke exposure period in a prophylactic and a therapeutic treatment regimen. These data were originally presented in abstract form (Fitzgerald et al., 2006).

Materials and Methods

Chemical Description, Potency, and Selection of Doses of SD-282. SD-282, an indole-5-carboxamide ATP-competitive inhibitor of p38α kinase, and its structure were reported previously (Kapoun et al., 2006). It is a small-molecule orally active inhibitor of p38α MAPK. As reported previously (Lim et al., 2004; Sweitzer et al., 2004; Medicherla et al., 2006a), SD-282 demonstrates 14.3-fold selectivity for p38α MAPK (IC₅₀ value of 0.0011 μM) compared with p38β MAPK (IC₅₀ value of 0.022 μM), whereas inhibition of p38γ MAP kinase and p38δ MAP kinase was less than 50%, even at concentrations of 10 μM. To compare the selectivity of SD-282 against p38α, a second structurally different p38 inhibitor, SB-202190, was used as a control in these specificity studies and found SD-282 but not SB-202190 is highly specific to p38α (Medicherla et al., 2006a). Sequence alignment shows 95% identity between murine and human p38α at the amino acid level, and SD-282 has been shown to have efficacy in other murine models of disease and to suppress pharmacokinetically active enzymes (Nath et al., 2006; S. Medicherla, unpublished observations). Therefore, SD-282 has a suitable selectivity profile to use in the evaluation of the role of p38 MAP kinase in an animal model of COPD. When tested in vitro at a concentration of 10 μM, SD-282 demonstrated no inhibitory activity against a panel of other kinases, including extracellular signal-regulated kinase-2, c-Jun NH₂-terminal kinase-1, and MAPK activated protein kinase 2. In addition, SD-282 demonstrates no effect on the activity of purified COX-1 or COX-2 enzymes (Medicherla et al., 2006a). Pharmacokinetics studies in female A/J mice revealed, SD-282 at 30 and 90 mg/kg resulted 3 and 8 μM circulating concentrations, respectively, at 30 min after its administration and its trough levels at those doses are trace amounts at 8 h. Hence, mice in the studies reported here were treated twice daily by oral gavage with 30 and 90 mg/kg SD-282.

Animals. Six-week-old female A/J mice (Harlan UK Limited, Bicester, Oxon, UK) were used. Animal experimental protocols were approved by the Preclinical Ethical Committee of Argenta Discovery, UK.

LPS Studies. Mice were dosed with vehicle or dexamethasone (0.3 mg/kg/two times) p.o. 1 h before intranasal (i.n.) instillation of lipopolysaccharide (LPS) /Escherichia coli/; 0.5 μg per mouse) and 6 h after instillation (n = 10 per group). Sham animals received PBS i.n., and they received vehicle 1 h before i.n. instillation of PBS (50 μl per mouse) and 6 h after instillation (n = 5 per group). Animals were euthanized by anesthetic overdose (pentobarbitone sodium; 100 mg/kg i.p.) 24 h after i.n. LPS challenge. Bronchoalveolar lavage (BAL) was performed, and total and differential cell counts were performed as described in the section below.

Exposure to Cigarette Smoke. In prophylactic and therapeutic studies, groups of five mice were exposed daily to air or TS (generated using 1R1 cigarettes purchased from the Institute of Tobacco Research, University of Kentucky, Lexington, KY) for 5 (baseline group) or 11 consecutive days. All animals received oral vehicle or drug treatment daily 1 h before and 6 h after air or TS exposure. In all studies, the vehicle used was 0.5% methyl cellulose. The tobacco smoke exposure system is similar to that described by Shapiro and coworkers (Hautamaki et al., 1997). Smoke exposure was gradually increased from 15 min on day 1 to 50 min from day 5 to day 11. Sham-exposed control mice were exposed to air for an equivalent time period using a similar exposure system but without the use of cigarettes. This was to ensure that sham controls were subjected to conditions as similar as possible to the TS-exposed animals. Mice were euthanized either 24 h after the fifth air or TS exposure or 24 h after the final exposure of 11 consecutive daily exposures to air or TS.

Prophylactic Studies. Two separate experiments were performed in which drug treatment was given before each of the 11 daily TS exposures (Fig. 1). In the first study, mice were treated orally twice daily with SD-282 or vehicle in prophylactic (before exposure) mode from day 1 through day 11 TS exposure. In this study, 10 mice per group were treated orally with 90 mg/kg or vehicle 1 h before and 6 h after every exposure to TS. A control group of mice was exposed to air daily for an equivalent length of time, and they received vehicle orally at 1 h before and 6 h after exposure each day for 11 days. In the second study, mice were treated orally twice daily with 0.3 mg/kg dexamethasone or vehicle (n = 10 in each group) 1 h before and 6 h after every exposure to TS. This study was performed alongside the first therapeutic study described below. This enabled us to directly compare prophylactic and therapeutic treatment with SD-282.

Therapeutic Studies. Two separate experiments were conducted to evaluate the effect of SD-282 on the progression of inflam-
mation in animals with an ongoing pulmonary inflammation (Fig. 1). The first experiment examined the effect of SD-282 at 30 mg/kg (low dose) and 90 mg/kg (high dose), and the second experiment studied the comparative effects of SD-282 at 90 mg/kg and dexamethasone at 0.3 mg/kg. In this series of experiments, 10 mice per group in the first study and 15 mice per group in the second study were treated orally twice daily either with SD-282 or dexamethasone from the sixth TS exposure onward, i.e., on day 6 to 11. In addition, two extra groups of mice (n = 10) were included in the second study to obtain baseline data on day 6. This baseline group was exposed either to air or TS for 5 days, and these mice received no treatment. In both studies, BAL and RT-PCR were performed after the protocols described for the prophylactic studies. In the second study, the lungs from five animals of each group were fixed in situ with 10% neutral-buffered formalin, and the lungs were gently inflated to a pressure of 25 cm H2O for a minimum of 30 min. Lungs were fixed, processed, and stained with hematoxylin and eosin for histological assessment of lung inflammation, edema, mucin, and alveolar wall thickening and also by periodic acid Schiff staining. Phospho-p38 immunohistochemistry was also performed; phospho-p38 was evaluated by the number of the positively stained cells in the lungs and scored as 0, indicating no positively stained cells in the section; 1, indicating intense staining of one to five cells in the section; 2, indicating intense staining of 5 to 10 cells in the section; 3, indicating intense staining of 10 to 15 cells in the section; and 4, indicating intense staining of more than 15 cells in the section. Using semi-quantitative scale, alveolar wall thickness was scored.

**Sample Analysis.** In the studies described, BAL was performed using phosphate-buffered saline. BAL fluid was centrifuged to separate cells from supernatant. BAL cells were identified as neutrophils and macrophages using standard morphological techniques, and the identity of macrophages was confirmed by using coexpression of CD45 and F4/80. After cytospin preparation, standard cell morphometry was used for identification of neutrophils and macrophages. BAL KC (an ortholog of IL-8) was quantified by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN), with total BAL protein being determined by the bicinchoninic acid assay as supplied in kit form from Pierce Chemical (Rockford, IL). Lungs from the SD-282 study were harvested for RT-PCR for evaluation of COX-2 and IL-6 transcript levels.

**Determination of mRNA Levels (Real-Time RT-PCR).** Real-time RT-PCR was performed in a two-step manner. cDNA synthesis and real-time detection were carried out in a PTC-100 Thermal Cycler (MJ Research, Waltham, MA) and an ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA), respectively. Random hexamers (QIAGEN, Valencia, CA) were used to generate cDNA from 200 ng of RNA as described in Applied Biosystems User Bulletin 2. TaqMan Universal PCR Master Mix (Applied Biosystems) was used in subsequent PCR reactions according to the manufacturer’s protocols. Relative quantitation of gene expression was performed using the relative standard curve method. All real-time RT-PCR reactions were performed in triplicate. Expression levels were normalized to 18S rRNA. Sequence-specific primers and probes were designed using Primer Express Version 2 software (Applied Biosystems) for COX-2 forward, 5’-CCACCATCTCACCACATGT-3’; reverse 5’-GTCCACTCTCATGGCCGAGT-3’, probe 5’-6FAM-AGATCATAGCCGAGGCTGGTCTACC-TAMRA-3’; for IL-6, forward 5’-CTGGGAAACCTATGTGCTGA-3’, reverse 5’-ACAGGATATTTTCTGACGACAGTGA-3’, probe 5’-6FAM-ACCGGCGCAAGACGGACCAG-TAMRA-3’; and for 18S forward, 5’-AAATGCTTTCG-3’; and for 18S reverse, 5’-GCCGCTAGAGGTGAAATTCTTG-3’, probe 5’-6FAM-AGATCATAGCCGAGGCTGGTCTACC-TAMRA-3’.

**Statistical Analysis.** Student’s t test or a nonparametric Mann-Whitney, or analysis of variance with Bonferroni multiple comparison test were used, where applicable, to determine a significant difference between groups. Differences were considered statistically significant when p < 0.05. All statistical analyses were done using Prism version 3.02 (GraphPad Software Inc., San Diego, CA).

**Results**

**LPS Studies**

Dexamethasone Inhibits Pulmonary Neutrophilia Induced by LPS Challenge. To confirm the oral anti-
inflammatory efficacy of dexamethasone at 0.3 mg/kg in a pulmonary inflammation model, this dose was evaluated in the industry standard mouse LPS model. Twenty-four hours after an i.n. challenge with LPS, a marked pulmonary neutrophilia response was induced in bronchoalveolar lavage. This neutrophilia was significantly inhibited by oral dexamethasone at 0.3 mg/kg (Fig. 2), confirming that this dose is appropriate for use in the TS studies. Lower doses did have a significant inhibitory effect versus the LPS response, but they were not used in the TS studies reported, because they were less than the effects seen at 0.3 mg/kg (data not shown).

**Prophylactic Studies**

**SD-282 but Not Dexamethasone Prevented Pulmonary Inflammation in Prophylactic Treatment Mode.** Exposure of mice to TS for 11 consecutive days resulted in pulmonary inflammation measured 24 h after the final smoke exposure, as evidenced by an increase in neutrophils (147-fold) and macrophages (9-fold) in the bronchoalveolar lavage. Tobacco smoke also induced small but statistically significant increases in the levels of protein and KC recovered in BAL. Oral pretreatment with SD-282 (90 mg/kg b.i.d.) at 1 h before and 6 h after TS exposure (−1 and +6 h) on each of the 11 days effectively reduced all indices of pulmonary inflammation induced by smoke exposure (>50% reduction compared with vehicle-treated animals; \( p < 0.01 \)) (Table 1). SD-282 at 90 mg/kg treatment was also associated with a significant reduction in mRNA levels compared with the smoke-exposed vehicle for the proinflammatory gene IL-6 (IL-6/18S: air, 1.0 ± 0.41; vehicle, 1.16 ± 0.47; and SD-282, 0.69 ± 0.22; \( p < 0.01 \)). Expression levels of COX-2 were reduced compared with the smoke-exposed vehicle, but the change was not statistically significant (COX-2/18S: air, 1.00 ± 0.41; vehicle, 1.16 ± 0.41; and SD-282, 0.90 ± 0.31). In contrast to the inhibition seen with prophylactic treatment with SD-282 (Table 1), oral treatment with dexamethasone (0.3 mg/kg b.i.d.) failed to significantly inhibit any of the inflammatory markers measured (Table 1). Higher doses of dexamethasone could not be investigated, because doses greater than 0.3 mg/kg administered twice daily for 11 days caused a significant weight loss.

**Therapeutic Studies**

Exposure to TS for 5 or 11 days resulted in an inflammatory response, measured 24 h after the final exposure. The pulmonary inflammatory response to smoke was significantly greater after 11 days than after 5 days exposure with respect to the increase in both macrophage and neutrophil number in BAL (Fig. 3; Table 3), suggesting that there is a progression in the extent of the inflammatory response with increased exposure to TS that is achieved by increasing the duration of the daily exposure and by increasing the number of days that the animals are exposed. TS exposure for 11 days also induced an increase in the levels of BAL KC and a small but statistically significant increase in protein (albeit not statistically different from those seen with 5 days of exposure). Phospho-p38 staining in macrophages and type II epithelial cells was also observed in the TS-exposed/vehicle-treated group compared with the air exposed control group (data shown in Fig. 7).

**SD-282 Dose-Dependently Reversed Pulmonary Inflammation in Therapeutic Treatment Mode.** Therapeutic treatment of mice with SD-282 at 30 or 90 mg/kg from days 6 to 11 dose-dependently reversed all inflammatory parameters measured, including BAL protein and KC induced by exposure to tobacco smoke for 11 days (Table 2). In addition, SD-282 significantly reduced COX-2 and IL-6 transcript levels compared with the smoke-exposed vehicle control (Fig. 4).

**SD-282 but Not Dexamethasone Reversed Pulmonary Inflammation in Therapeutic Treatment Mode.** TS-induced increases in macrophages seen after 11 days were inhibited by 59% in the SD-282 treatment group compared with vehicle group, and it is of note that the numbers of macrophages in the SD-282-treated group was similar to the number of macrophages in the BAL of animals exposed to smoke for 5 days (baseline). This suggests that SD-282 treatment reduces the numbers of macrophages to the numbers

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>Air</th>
<th>Vehicle</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells</td>
<td>0.61 ± 0.26</td>
<td>3.86 ± 1.32</td>
<td>2.23 ± 0.91*</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.61 ± 0.28</td>
<td>3.79 ± 1.39</td>
<td>2.22 ± 0.96*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0 ± 0</td>
<td>0.08 ± 0.03</td>
<td>0.014 ± 0.15**</td>
</tr>
<tr>
<td>BAL protein</td>
<td>0.20 ± 0.04</td>
<td>0.38 ± 0.05</td>
<td>0.24 ± 0.08**</td>
</tr>
<tr>
<td>BAL KC</td>
<td>16 ± 3.3</td>
<td>40.5 ± 12</td>
<td>24.9 ± 6*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.02 ± 0.09</td>
<td>1.63 ± 0.34</td>
<td>1.84 ± 0.54*</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.02 ± 0.09</td>
<td>1.33 ± 0.25</td>
<td>1.40 ± 0.50*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0 ± 0</td>
<td>0.19 ± 0.12</td>
<td>0.26 ± 0.17*</td>
</tr>
</tbody>
</table>

For SD-282, * \( p < 0.01 \) and ** \( p < 0.001 \) compared with the vehicle group. Dexamethasone failed to significantly inhibit TS-induced increases in BAL cells; * \( p \) is not significant compared with the vehicle group.
Fig. 3. Smoke but not air significantly increases macrophage and neutrophil influx into the lungs of A/J mice. Mice were exposed daily to air or tobacco smoke for 11 consecutive days, and they received vehicle p.o. twice daily (−1 to +6 h) from days 1 to 11. Mice were euthanized 24 h after the final exposure to air/smoke, and lungs were subjected for BAL analysis. Total number of macrophages (A) and neutrophils (B) recovered in BAL of A/J mice 24 h after exposure to air or smoke. Smoke significantly and time-dependently increased total numbers of macrophages and neutrophils by day 5 and day 11 compared with the air groups on day 5 and day 11, respectively. *p < 0.01 and **p < 0.001 versus air/control group. Values are reported as the mean ± S.D. (n = 10).

TABLE 2
SD-282 in the therapeutic mode dose-dependently reverses inflammatory responses in A/J mice induced by 11 daily consecutive tobacco smoke exposures
Data are presented as mean ± S.D. (10⁵ cells) and subjected to ANOVA, followed by Bonferroni’s correction for multiple comparisons.

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>Total cells</th>
<th>Macrophages</th>
<th>Neutrophils</th>
<th>BAL Protein</th>
<th>BAL KC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Air</strong></td>
<td>0.61 ± 0.26</td>
<td>3.79 ± 1.39</td>
<td>0 ± 0</td>
<td>0.20 ± 0.04</td>
<td>16 ± 3.3</td>
</tr>
<tr>
<td><strong>Tobacco Smoke</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.86 ± 1.32</td>
<td>8.61 ± 3.12</td>
<td>0.08 ± 0.03</td>
<td>0.38 ± 0.05</td>
<td>40.5 ± 12</td>
</tr>
<tr>
<td>SD-282 (30 mg/kg b.i.d. from Day 6 to Day 11)</td>
<td>2.92 ± 0.97</td>
<td>2.86 ± 1.00</td>
<td>0.03 ± 0.02*</td>
<td>0.19 ± 0.06**</td>
<td>19.6 ± 6.3**</td>
</tr>
<tr>
<td>SD-282 (90 mg/kg b.i.d. from Day 6 to Day 11)</td>
<td>1.45 ± 0.50*</td>
<td>1.45 ± 0.53*</td>
<td>0.007 ± 0.009*</td>
<td>0.23 ± 0.08**</td>
<td>24.5 ± 12*</td>
</tr>
</tbody>
</table>

* p < 0.01 and ** p < 0.001 compared with the vehicle group.

Fig. 4. Therapeutic treatment with SD-282 reduces COX-2 and IL-6 transcript levels in the lungs of A/J mice. Mice were exposed daily to air or tobacco smoke for 11 consecutive days, and they received SD-282 p.o. twice daily (−1 to +6 h) from days 6 to 11. Mice were euthanized 24 h after the final exposure to air/smoke, and lungs were subjected to real-time RT-PCR analysis. SD-282 at 30 and 90 mg/kg reduced both COX-2 and IL-6 transcripts in the 6-day therapeutic treatment mode. *, p < 0.01 and **, p < 0.001 versus vehicle group. Significance was tested by one-tailed analysis of variance followed by Bonferroni’s correction using Prism 4 software (GraphPad Software Inc.). Values are reported as the mean ± S.D. (n = 10 for air, 30 mg/kg SD-282, and 90 mg/kg SD-282 and n = 9 for vehicle).

seen at the start of drug treatment. Data are shown for SD-282 treatment at 90 mg/kg and dexamethasone at 0.3 mg/kg in Table 3. TS induced increases in neutrophils seen after 11 days were inhibited by 90% in the SD-282 treatment group compared with vehicle group and to less than that seen at baseline after five TS exposures. Thus, therapeutic treatment with SD-282 reduced both macrophage and neutrophil number back to the baseline inflammatory response seen after 5-day exposure to TS. By contrast, therapeutic treatment with dexamethasone failed to reduce any of the in-
flamatory parameters in BAL in a statistically significant manner (Table 3). Dexamethasone but not SD-282 caused significant loss in body weight compared with the vehicle group. This effect of steroids in animals has been described previously, is not unexpected (Ma et al., 2003) (Fig. 5), and indicates that at plasma exposures sufficient to have side effects no efficacy is observed. Histological evaluation of lung sections revealed an inflammatory response composed of increased numbers of macrophages and neutrophils and enhanced bronchiolar wall thickening and mucin staining, and these features were markedly reduced by therapeutic dosing with SD-282 (Fig. 6). Phospho-p38 was located in the lung type-II epithelial cells and infiltrated macrophages. SD-282 at 90 mg/kg significantly reduced phospho-p38 MAPK expression in epithelial cells and macrophages (Fig. 7).

**Discussion**

p38α MAPK is a member of the MAPK family of structurally related kinases that are expressed in a variety of cell types, including inflammatory cells such as neutrophils, monocytes, macrophages, B and CD4+ T cells, and endothelial cells. p38α MAPK is activated by oxidative injury, ischemia, and other inducers of cell stress (Lee et al., 1994; Badger et al., 1996; New and Han, 1998). As an integral part of leukocyte activation in acute and chronic inflammatory states, p38α MAPK mediates the production of proinflammatory cytokines (Lee et al., 1994; Badger et al., 1996; New and Han, 1998; Medicherla et al., 2006a). As a selective inhibitor of p38α MAPK, SD-282 blocks the synthesis in vitro of tumor necrosis factor-α, vascular endothelial growth factor, IL-6, COX-2, and IL-1β as well as reducing tumor necrosis factor-α activity (Lim et al., 2004). All of these mediators are thought to play essential roles in lung inflammation. Treatment with SD-282 at the dose used in this study has previously been shown to significantly reduce disease progression in a cytokine-driven animal model for chronic arthritis (Medicherla et al., 2006a).

In a standard LPS-induced pulmonary neutrophilia model, oral dexamethasone at 0.3 mg/kg, as would be expected from the literature, reduced the BAL inflammation by 73%. The dose used was the lowest oral dose that had a greater than 50% inhibitory effect, and it was therefore deemed suitable for use in the TS studies.

In the present murine inflammatory model of COPD, BAL and histological evaluation of lung sections taken after exposure to TS for 11 days revealed inflammatory response comprised of increased numbers of macrophages and neutrophils and enhanced mucin staining. Phospho-p38 staining in macrophages and type II epithelial cells was also observed. In the prophylactic treatment mode, anti-inflammatory activity of SD-282 was demonstrated by the inhibition of macrophage and neutrophil influx recruitment, and it was associated with a decrease in BAL levels of protein and KC. In contrast, dexamethasone given prophylactically failed to significantly inhibit any of the TS-induced increases in inflammatory markers. Therapeutic treatment with SD-282 reduced macrophage and neutrophil numbers back to baseline levels. In addition, SD-282 dose-dependently reduced smoke-induced increases in COX-2 and IL-6 transcript levels when given in either prophylactic or therapeutic modes. The anti-inflammatory efficacy of SD-282 seen in this model is similar to the effects of other p38 inhibitors in models of acute lung injury (Arcaroli et al., 2001; Xu et al., 2005). However, it has not been specifically established how SD-282 exerts its anti-inflammatory effects on cellular recruitment in this model. These effects could be directly mediated by effects on cell chemotaxis or by reducing the release of inflammatory chemokines from activated cells. The latter is supported by the reduction of BAL KC levels by SD-282, which could play a role in the reduction in BAL neutrophil numbers. The lack of dose dependence seen with the two doses of SD-282 probably reflects the 24-h time point after the last TS exposure used in

**TABLE 3**

SD-282 in the therapeutic mode but not dexamethasone reverses inflammatory responses in A/J mice induced by 11 daily consecutive tobacco smoke exposures

Data are presented as mean ± S.D. (105 cells) and subjected to ANOVA, followed by Bonferroni’s correction for multiple comparisons.

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>Air (Vehicle, Baseline after Five TS Exposures)</th>
<th>Tobacco Smoke (Vehicle, SD-282 (90 mg/kg b.i.d from Days 6 to 11)</th>
<th>Dexamethasone (0.3 mg/kg b.i.d from Days 6 to 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells</td>
<td>0.14 ± 0.07</td>
<td>0.83 ± 0.28</td>
<td>2.67 ± 0.52</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.14 ± 0.08</td>
<td>0.77 ± 0.28</td>
<td>1.11 ± 0.40**</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.05 ± 0.04</td>
<td>0.11 ± 0.62</td>
<td>0.03 ± 0.03**</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0 ± 0</td>
<td>0.05 ± 0.04</td>
<td>0.28 ± 2.67</td>
</tr>
</tbody>
</table>

*p < 0.01 and **p < 0.001 compared with the vehicle group.

**Fig. 5.** Therapeutic treatment with dexamethasone but not SD-282 causes body weight loss in A/J mice. Mice were exposed daily to air or tobacco smoke for 11 consecutive days, and they received either SD-282 or dexamethasone (Dex) p.o. twice daily (~1 to +6 h) from days 6 to 11. Mice were euthanized 24 h after the final exposure to air/tobacco smoke. After 6 or 11 consecutive days of tobacco smoke exposure, body weights were measured. Dexamethasone but not SD-282 caused significant reduction (+, p < 0.001) in body weight compared with the vehicle group. Values are reported as the mean ± S.D. (n = 10).
this study. The peak of KC release occurs between 4 and 6 h after TS exposure, whereas cell numbers are at a maximum at 24 h after exposure. Further studies would be required to investigate the dose dependence of the KC response to p38 MAP kinase inhibition. However, it is probable that the anti-inflammatory activity observed in this model is due to a
combination of outcomes are the result of p38 MAP kinase inhibition. In contrast, therapeutic treatment with dexamethasone failed to reduce any of the inflammatory parameters in BAL in a statistically significant manner. The potentiation of BAL neutrophilia by dexamethasone is an interesting observation, and it is similar to that seen in the lung biopsies of COPD patients treated with inhaled fluticasone (Cox, 1995; Meagher et al., 1996; Gizycki et al., 2002). This effect of dexamethasone in both these studies may reflect delayed apoptosis of neutrophils by dexamethasone, an effect that has been observed in vitro (Zhang et al., 2001).

The relative insensitivity of this model to steroids is similar to that reported in patients where anti-inflammatory effects seem limited and dependent on dose and duration of therapy (Gan et al., 2005). In addition, the relative lack of efficacy of a steroid in the study reported here is similar to that reported in other animal models of COPD (Birrell et al., 2005; Leclerc et al., 2006), and it may reflect an effect of TS exposure on histone deacetylase activity (Barnes, 2005b). In mice, a very high dose of dexamethasone (10 mg/kg) moderately attenuated neutrophilia induced by TS smoke exposure, whereas in a PDE4 inhibitor, cilomilast, significantly reduced this response at a range of doses (Grootendorst et al., 2005).

The most widely studied anti-inflammatory mechanism in animal models of COPD is PDE4 inhibition. Roflumilast, currently in phase III for COPD, has been shown to inhibit TS-induced inflammation in both mice and guinea pigs and to prevent emphysema development in mice (Martorana et al., 2005). This efficacy is similar to that reported for SD-282, and it is supportive of evaluating p38 MAP kinase inhibitors clinically. In COPD patients, roflumilast has also been shown to reduce sputum inflammatory biomarkers, including neutrophils, after therapy for 4 weeks, suggesting that the inflammation in COPD is not refractory to all therapies (Leclerc et al., 2006). Despite the promising data with PDE4 inhibitors, no drugs with this mechanism of action have yet been approved for COPD, and the search for an effective anti-inflammatory therapy for COPD remains a key research objective for the drug discovery industry (Halbert et al., 2006).

The effects of SD-282 on cell accumulation in this inflammatory model contrast with the data seen in mouse asthma models where p38 MAP kinase inhibitors fail to inhibit antigen-induced eosinophilia (Chialda et al., 2005; Nath et al., 2006), suggesting that inhibition of this kinase may not be an effective approach to targeting the inflammation in asthma. The reasons for this difference are not clear and warrant further study.

In summary, we report that a p38 α-selective MAP kinase inhibitor, SD-282, markedly reduces TS-induced inflammatory responses when given prophylactically or therapeutically in a model where dexamethasone was ineffective. This is the first evidence that p38 MAP kinase inhibitors can exert anti-inflammatory activity in the lung when given in a therapeutic setting, establishing the therapeutic potential of p38 MAP kinase inhibitors in COPD.

References


Address correspondence to: Dr. Satyanarayana Medicherla, Fellow, Schering Plough Biopharma, 901 California Ave., Palo Alto, CA 94304. E-mail: satya.medicherla@spcorp.com