Anti-inflammatory effects of budesonide in human lung fibroblasts are independent of histone deacetylase 2

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Objective and design: Reduced expression of histone deacetylase 2 (HDAC2) in alveolar macrophages and epithelial cells may account for reduced response of chronic obstructive pulmonary disease (COPD) patients to glucocorticoids. HDAC2 expression and its role in mediating glucocorticoid effects on fibroblast functions, however, has not been fully studied. This study was designed to investigate whether HDAC2 mediates glucocorticoid effects on release of inflammatory cytokines and matrix metalloproteinases (MMPs) from human lung fibroblasts.

Methods: Human lung fibroblasts (HFL-1 cells) were stimulated with interleukin (IL)-1β plus tumor necrosis factor (TNF)-α in the presence or absence of the glucocorticoid budesonide. Cytokines (IL-6 and IL-8) were quantified by enzyme linked immunosorbent assay (ELISA) and MMPs (MMP-1 and MMP-3) by immunoblotting in culture medium. The role of HDAC2 was investigated using a pharmacologic inhibitor as well as a small interfering ribonucleic acid (siRNA) targeting HDAC2.

Results: We have demonstrated that budesonide concentration-dependently (10⁻⁶–10⁻⁷ M) inhibited IL-6, IL-8, MMP-1, and MMP-3 release by HFL-1 cells in response to IL-1β plus TNF-α. While an HDAC inhibitor significantly blocked the inhibitory effect of budesonide on human bronchial epithelial cells (HBECs) and monocytes (THP-1 cells), it did not block the inhibitory effect of budesonide on release of cytokines and MMPs from HFL-1 cells. Similarly, an HDAC2-siRNA blocked budesonide inhibition of cytokine release in HBECs, but it did not block the inhibitory effect of budesonide on HFL-1 cytokine and MMP release. Furthermore, budesonide significantly blocked release of cytokines and MMPs to a similar degree in normal and COPD lung fibroblasts as well as in HFL-1 cells exposed or not exposed to cigarette smoke extract.

Conclusion: These findings suggest that, in contrast to airway epithelial cells and monocytes/macrophages, HDAC2 is not required for budesonide to inhibit MMP and cytokine release by lung fibroblasts and this inhibitory pathway appears to be intact in cultured fibroblasts from COPD patients. These results also suggest that budesonide has the potential to modulate fibroblast-mediated tissue remodeling following airway inflammation in COPD, which is mediated via an HDAC2 independent pathway.

Keywords: budesonide, fibroblasts, HDAC2

Introduction

Chronic obstructive pulmonary disease (COPD) is an increasing global health problem. Airway inflammation and tissue remodeling that leads to peribronchial fibrosis are characteristic of COPD, and contribute to the relentless progress of the disease.1,2 Inhaled glucocorticoids are very effective anti-inflammatory agents in asthma, but have limited benefits in COPD. Reduced expression of histone deacetylase 2 (HDAC2) in alveolar macrophages and airway epithelial cells in COPD has been suggested to contribute to the
limited ability of glucocorticoids to inhibit the inflammation present in COPD. However, HDAC2 expression in lung fibroblasts and its role in mediating the effect of glucocorticoids on lung fibroblast functions are unclear.

Histone acetyltransferases and histone deacetylases (HDACs) play important roles in regulating gene expression. Acetylation of histone core structure results in opening up of the chromatin allowing transcription factors and ribonucleic acid (RNA) polymerase to bind to DNA and initiate gene transcription. Histone acetylation can be reversed by HDACs. There are eleven HDAC isoenzymes that deacetylate histones within the nucleus and specific HDACs may regulate different groups of genes. Expression of inflammatory genes is regulated by acetylation of histone 4 and HDAC2. In COPD alveolar macrophages and airway biopsy specimens, histones that are associated with the promoter region of inflammatory genes are highly acetylated and the degree of acetylation increases with disease severity. This increased acetylation of inflammatory genes seems to be not due to increased activity of acetyltransferase but rather due to the reduced expression of HDAC2 in human alveolar macrophages and lung tissue.

Lung fibroblasts play a key role in airway remodeling and tissue repair following inflammatory injury of small airways. The expression of HDAC2 and its role in mediating glucocorticoid anti-inflammatory effect in fibroblasts has not been investigated. The current study, therefore, was designed to investigate HDAC2 expression in human lung fibroblasts from control and COPD subjects and to determine the role of HDAC2 in regulating the effect of budesonide on the release of inflammatory mediators by lung fibroblasts. To accomplish this, human lung fibroblasts were treated with interleukin (IL)-1β and tumor necrosis factor (TNF)-α, the two well-known pro-inflammatory cytokines that are believed to play a role in COPD and that stimulate human lung fibroblasts to synthesize and release several inflammatory mediators including IL-6 and IL-8, matrix metalloproteinase (MMP)-1 and MMP-3. The role of HDAC2 in mediating budesonide inhibition of the release of IL-6, IL-8, MMP-1, and MMP-3 was then assessed using a pharmacologic inhibitor and RNA interference.

**Material and methods**

**Cell culture**

Human fetal lung fibroblasts (HFL-1), obtained from the American Type Culture Collection (Manassas, VA, USA), were cultured with Dulbecco’s Modified Eagle’s Medium (DMEM; Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (FCS) (Life Technologies), 50 U/mL penicillin, 50 µg/mL streptomycin (Life Technologies) and 1 µg/mL amphotericin B (X-Gen, Big Flats, NY, USA). The cells were cultured in 100-mm tissue culture dishes. Fibroblasts were trypsinized (0.05% trypsin, 0.481 mM ethylene diamine tetraacetic acid [EDTA]; Life Technologies), plated into tissue culture plates, grown to near confluence and used for assays.

Normal human bronchial epithelial cells (HBEcs) were acquired from bronchial biopsies under a research protocol approved by the University of Nebraska Institutional Review Board using a previously published method with slight modifications. Briefly, the biopsy tissues were allowed to attach to the type I collagen-coated 60 mm tissue culture dishes under serum-free conditions using a 1:1 mixture of LHC-9/RPMI-1640 (Laboratory of Human Carcinogenesis-9/ Roswell Park Memorial Institute-1640) medium (Life Technologies) without addition of cortisol. Medium was changed every other day. After 7–10 days culture, HBEcs were trypsinized and plated on collagen (PureCol; Advanced BioMatrix, San Diego, CA, USA) coated tissue culture dishes (Falcon; Becton-Dickinson Labware, Lincoln Park, NJ, USA) at 37°C in a humidified, 5% CO₂ atmosphere. Cells were then passaged once a week at a 1:3 ratio. Cells between the 3rd and 10th passage were characterized by staining for pancytokeratin and vimentin and used for experiments.

Monocytic cell line cells (THP-1), obtained from the American Type Culture Collection, were cultured in RPMI-1640 supplemented with 10% FCS (Life Technologies).

Primary human lung fibroblasts were cultured from lung tissues obtained from subjects with or without clinical and functional signs of COPD as described earlier. All subjects were undergoing surgery and acquisition of samples was approved by the Human Studies Committee of the Medical Board of the State of Schleswig-Holstein (Germany). All subjects also provided written informed consent for research. Fibroblasts from six normal subjects (forced expiratory volume in 1 second [FEV1]: 89.4%±5.7% predicted [mean ± standard error of the mean], range 75.6%–115.4%) and six COPD subjects (FEV1: 57.0%±1.6% predicted [mean ± standard error of the mean], range 53.2%–63.1%) were used in the current study.

**Cytokine quantification by ELISA**

IL-6 was quantified by enzyme linked immunosorbent assay (ELISA) as described previously. Briefly, 96-well plates were coated with capturing antibodies specific for IL-6 (R&D Systems, Minneapolis, MN, USA) at 4°C overnight. Samples and standard (R&D Systems) were added and allowed to react for 1 hour. After washing, the second antibody (Calbiochem, EMD Millipore, Billerica, MA, USA) was
applied and incubated for 1 hour at room temperature. After washing, horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (Rockland Immunochemicals, Gilbertsville, PA, USA) was applied for 1 hour at room temperature. O-phenylenediame (OPD) substrate was applied and absorbance read at 495 nm wavelength.

IL-8 was quantified by using Human IL-8 DuoSet Kit (R&D Systems) following the manufacturer's instruction.

**Immunoblotting**

To evaluate MMP-1 and -3, conditioned media (2 mL) collected from the monolayer culture of the fibroblasts were concentrated by ethanol precipitation and re-suspended in distilled H$_2$O (50 µL). The samples (15 µL) were then added to the same volume of 2x concentrated electrophoresis sample buffer (0.5 M Tris pH 6.8, 2% sodium dodecyl sulfate [SDS], 20% glycerol, 0.1% bromophenol blue) and heated for 5 minutes at 95°C. As MMPs were evaluated in culture medium in which there are no reference proteins, care was taken to load equal volumes and quantitative results are expressed relative to control cells treated only with cytokines (IL-1β and TNF-α).

Thirty microliters of each sample was loaded into each lane of a 10% SDS-polyacrylamide gel electrophoresis (PAGE) gel and electrophoresed. The proteins were transferred to a polyvinylidene difluoride (PVDF) membrane with a semi-dry transfer apparatus. The membrane was blocked in 5% milk in phosphate buffered saline (PBS)-Tween at room temperature for 1 hour, then exposed to primary antibodies (mouse anti-human MMP-1 or MMP-3 from R&D Systems), which were subsequently detected using HRP-conjugated rabbit anti-mouse immunoglobulin G (IgG) in conjunction with an enhanced chemiluminescence (ECL) detection system.

For HDAC, whole cell lysate proteins were extracted with 100 µL per 60 mm dish of cell lysis buffer (35 mM Tris-HCl, pH 7.4, 0.1% Triton X-100, 0.4 mM ethylene glycol tetraacetic acid [EGTA], 10 mM MgCl$_2$) containing a protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO, USA). Cells were briefly sonicated followed by centrifugation at 10,000 g for 10 minutes at 4°C and the supernatant was subjected to immunoblotting for HDAC2 (1:200 dilution; Abcam, Cambridge, MA, USA), with β-actin (1:4000 dilution; Sigma-Aldrich) as loading control. SDS-PAGE electrophoresis, protein transfer and immunoblotting were conducted as described above.

**HDAC inhibition**

To inhibit HDAC, a non-selective HDAC inhibitor, trichostatin A (TSA; Cell Signaling, Danvers, MA, USA) was used.

After incubation of HFL-1, HBEcs and THP-1 cells with various concentrations of TSA for 30 minutes, varying concentrations of budesonide (AstraZeneca, Lund, Sweden) and IL-1β plus TNF-α (R&D Systems) were added to the final concentrations of IL-1β plus TNF-α, 1 ng/mL each. After 24 hours, media were harvested for quantification of IL-6 and IL-8 by ELISA, as well as MMP-1 and MMP-3 by immunoblotting. Cells were trypsinized and counted with a Coulter Counter (Beckman Coulter, Brea, CA, USA). Levels of the cytokines were normalized by cell number for each experiment.

**Selective inhibition of HDAC2 by RNA interference**

To selectively silence HDAC2, RNA interference was performed. Briefly, cells were seeded in 6-well dishes at a cell density of 2 × 10$^5$ cells per well. The next day, cells were transfected with small interfering (si)RNA targeting HDAC2 or nontargeting control siRNA (final concentration of siRNA was 50 nM; Santa Cruz Biotecntology Inc, Santa Cruz, CA, USA) in Opti-MEM (Invitrogen, Carlsbad, CA, USA) using Lipofectamine 2000 (Invitrogen). After 16 hours transfection, media were changed to 10% FCS-DMEM for HFL-1 cells or LHC-9/RPMI for HBECs. After 24 hours, the cells were treated with cytokines (IL-1β and TNF-α) and/or budesonide (AstraZeneca). Media were harvested and the cell lysates were extracted on day 4 and the efficacy of RNA interference was assessed by immunoblotting.

**Preparation of cigarette smoke extract (CSE)**

CSE was prepared with a modification of the method reported previously. Briefly, the smoke from one 84 mm cigarette (research cigarette 3R4F; University of Kentucky, Lexington, KY, USA) without filter was bubbled through 15 mL of deionized water at a speed of 50 cc/minute. The filtered solution with a 0.22 µm pore filter (Lida Manufacturing, Kenosha, WI, USA) was considered to be 100% CSE and applied to fibroblast cultures within 30 minutes of preparation. In the current study, fibroblasts were exposed to 5% CSE diluted with serum free DMEM.

**Statistical analysis**

Results were always confirmed by repeating each experiment on separate occasions at least three times. Statistical comparisons were based on separate experiments. Group data were analyzed by one-way analysis of variances (ANOVA) followed by Tukey’s test or two-way ANOVA followed.
Results

HDAC inhibitor blocked budesonide effect in HBECs but not in HFL-1 cells

In order to study the effect of budesonide on release of cytokines and MMPs by fibroblasts, HFL-1 cells were treated with IL-1β plus TNF-α (1 ng/mL, each) in monolayer culture to assess IL-6 and IL-8 as well as MMP-1 and MMP-3. As expected, IL-1β plus TNF-α significantly stimulated IL-6, IL-8, MMP-1, and MMP-3 release by HFL-1 cells (Figure 1A–D). Budesonide (from $10^{-10}$ to $10^{-7}$ M) significantly inhibited IL-1β plus TNF-α-induced release of all four mediators (Figure 1A–D, $P < 0.01$). TSA ($10^{-9}$ to $10^{-7}$ M), a nonselective HDAC inhibitor, did not block budesonide inhibition of cytokine or MMP release (Figure 2A–D) in human lung fibroblasts. Interestingly, in the presence of IL-1β plus TNF-α, TSA ($10^{-7}$ M) significantly inhibited MMP-1 release (Figure 2C), but significantly stimulated MMP-3 release (Figure 2D).

In contrast to the lack of effect of TSA on budesonide inhibition of mediator release in fibroblasts, TSA significantly blocked the inhibitory effect of budesonide ($10^{-7}$ M) on IL-8 release in HBECs (Figure 3A, TSA at $10^{-7}$ M) and in the THP-1 cell line (Figure 3B, TSA at $10^{-8}$ M). Similarly, TSA also significantly blocked the inhibitory effect of budesonide on cytokine-induced IL-6 release by HBECs; budesonide ($10^{-7}$ M) reduced release to $59.0\% \pm 9.5\%$ (mean ± standard deviation).

![Figure 1](https://www.dovepress.com/)

**Figure 1** Budesonide inhibits release of cytokines and MMPs by human lung fibroblasts.

**Notes:** HFL-1 cells were cultured until sub-confluence and treated with varying concentrations of budesonide for 30 minutes followed by stimulation with cytokines (IL-1β plus TNF-α, 1 ng/mL each). After 24 hours, medium was harvested for quantification of IL-6 (A) and IL-8 (B) by ELISA, and MMP-1 (C) and MMP-3 (D) by immunoblotting. (A and B): Effect on IL-6 and IL-8 release. Cell number was counted with a Coulter counter and the cytokine level was normalized to the cell number. Vertical axis: amount of IL-6 or IL-8 expressed as percentage of response in cytokine-stimulated cells; horizontal axis: treatment with cytokines and varying concentrations of budesonide. Data presented as the mean ± SEM of three separate experiments. Vertical axis: density of the image expressed as percentage of density in cytokine-treated cells; horizontal axis: treatment with cytokines and varying concentrations of budesonide. *$P < 0.05$; **$P < 0.01$ compared to cytokines alone by one way analysis of variance followed by Tukey’s test.

**Abbreviations:** ELISA, enzyme linked immunosorbent assay; IL, interleukin; MMP, matrix metalloproteinase; SEM, standard error of the mean; TNF, tumor necrosis factor.
error of the mean) of cytokine-stimulated cells (100%) and TSA blocked this effect resulting in 98.2% ± 10.2% release (mean ± standard error of the mean; control non-stimulated cells release was 45.7% ± 2.9% of cytokine-stimulated cells). These results demonstrate that budesonide can inhibit fibroblast mediator release via an HDAC independent mechanism, while the effect on other cells requires HDAC.

**HDAC2 suppression by siRNA blocked the effect of budesonide in HBECs but not in HFL-1 cells**

In order to further investigate the role of HDAC in mediating budesonide inhibition of cytokine and MMP release by fibroblasts, HFL-1 cells were transfected with an siRNA specifically targeting HDAC2, followed by treatment with budesonide. HDAC2 was significantly suppressed by the siRNA (Figure 4A; \( P < 0.01 \)), however, despite suppression of HDAC2, budesonide (\( 10^{-7} \) M) still significantly inhibited cytokine-stimulated IL-6, IL-8, MMP-1, and MMP-3 (Figure 4B–D, F and G, \( P < 0.01 \)). In order to ensure that the siRNA was active, the effect of siRNA suppression of HDAC2 was investigated on budesonide (\( 5 \times 10^{-7} \) M) inhibition of IL-6 release by HBECs. The siRNA targeting HDAC2 was found to significantly abolish the effect of the high concentration of budesonide in HBECs (Figure 4E and H, \( P < 0.05 \)).

**Budesonide effect was not impaired in COPD lung fibroblasts and in HFL-1 cells exposed to cigarette smoke**

Finally, since HDAC2 expression has been reported to be reduced in alveolar macrophages from COPD patients, HDAC2 protein expression was examined in lung fibroblasts
Discussion

The current study demonstrates that budesonide inhibits release of cytokines (IL-6 and IL-8) and MMPs (MMP-1 and MMP-3) from IL-1β plus TNF-α-stimulated human lung fibroblasts (HFL-1 and primary cells), HBECs, and cells from a human monocytic cell line (THP-1). Human lung fibroblasts express HDAC2, but neither inhibition of HDAC by a pharmacologic inhibitor TSA nor suppression with HDAC2 specific siRNA affected budesonide inhibition of mediator release from fibroblasts. This contrasts with HBECs and THP-1 cells, in which the inhibitory effect of budesonide was significantly blocked by HDAC inhibition. HDAC2 protein expression in lung fibroblasts from COPD patients was slightly but not significantly lower than that in normal lung fibroblasts whereas it was significantly decreased by nearly 50% in the lung fibroblasts (HFL-1) exposed to CSE compared to nonexposed cells. Nevertheless, budesonide inhibited production of IL-6, IL-8, MMP-1, and MMP-3 in both normal and COPD lung fibroblasts or CSE-exposed and nonexposed cells similarly. Taken together, these results demonstrate that budesonide can inhibit fibroblast mediator release via an HDAC independent mechanism and that fibroblasts from COPD patients or CSE-exposed fibroblasts are sensitive to this inhibition.

Histone acetylation and deacetylation play an important role in gene expression. Acetylation of core histones by histone acetyltransferase results in opening the chromatin structure to allow transcription factors and RNA polymerase binding to DNA thus, initiating gene transcription. Histone acetylation is reversed by HDACs, and by this mechanism gene expression is turned off. There are 11 HDAC isoenzymes described and, of these, HDAC2 has been reported to be critically important in mediating glucocorticoid suppression of airway inflammation. Budesonide is a glucocorticoid
with potent anti-inflammatory effects and limited systemic bioactivity due to extensive (90%) first-pass hepatic metabolism by cytochrome P450 enzymes. Inhalation of glucocorticoids has been shown to be effective in suppressing airway inflammation in asthmatics but is generally less effective in COPD. Reduction of HDAC2 expression in airway macrophages and epithelial cells in COPD patients is believed to account for the inflammation in COPD being less responsive to glucocorticoid therapy. This resistance poses a paradox, as glucocorticoids have well-demonstrated therapeutic benefits in COPD, including improvement in airflow and reduction in the frequency of exacerbations. Because the clinical benefits develop slowly over a matter of weeks, an anti-inflammatory action has been suggested, despite the reports that macrophages and epithelial cells are resistant to the anti-inflammatory effects of glucocorticoids in COPD.\(^3\,4\,8\)

Consistent with previous reports, the current study demonstrated that budesonide inhibited IL-6 and IL-8 release by human bronchial epithelial cells, lung fibroblasts, and monocytic cells. Moreover, also consistent with previous reports, inhibition of HDAC activity by TSA blocked the inhibitory effect of budesonide on IL-8 and IL-6 release by HBEcs and THP-1 cells. In contrast, the inhibitory effect of budesonide on the lung fibroblasts was not sensitive to TSA. Interestingly, in the presence of IL-1β plus TNF-α, TSA alone inhibited MMP-1 release but stimulated MMP-3 release indicating that it had an effect on the fibroblasts, although it did...
was reduced by 75% in lung fibroblasts and was similarly inhibited in HBECs, but had no effect in monocytes/macrophages, and demonstrates that glucocorticoids may still modulate the inflammatory response by inhibiting the release of mediators from lung fibroblasts, which is not HDAC dependent.

TSA is not specific for HDAC2 and a specific HDAC2 inhibitor was not available. However, we also used siRNA knockdown to specifically target HDAC2. This approach had a clear effect in blocking budesonide-mediated inhibition of mediator release in HBECs, but had no effect in lung fibroblasts. siRNA knockdown was confirmed by western blotting. As is usually the case, knockdown was not complete in either cell type, although HDAC2 expression was reduced by 75% in lung fibroblasts and was similarly reduced in HBECs when normalized to β-actin. Our experiments cannot exclude the possibility of an effect of residual HDAC2 in fibroblasts, and it is possible that the amount of HDAC2 activity required may vary among cell types. Importantly, however, the results with the siRNA and the TSA consistently support no role for HDAC2 in mediating the effect of budesonide in lung fibroblasts.

Fibroblasts are one of the major cell types present in the lung. They are also the major cell type present in the subepithelial tissue in the airways and comprise up to 25% of alveolar cells, although the markedly attenuated structure of the alveolus makes them difficult to recognize on routine histology. In both locations, fibroblasts are believed to be the major source of extracellular matrix macromolecules. Fibroblasts are also potent sources of other mediators. These include growth factors for epithelial and endothelial cells, and enzymes that can mediate the degradation of connective tissue. Fibroblasts also produce both inflammatory mediators and enzymes that can modulate the inflammatory response.

The relative importance of various cell types in driving inflammation is not delineated. However, fibroblasts, because...
of their presence in large numbers in normal tissues, may be particularly important in mediating chronic inflammatory processes. In this context, fibroblasts respond vigorously to the early inflammatory response mediators IL-1β and TNF-α.28,29 These mediators induce a number of responses in fibroblasts, including release of the cytokines IL-6,10 IL-8,10 and MMPs.16 Thus, fibroblasts may participate in the amplification of IL-1β and TNF-α-induced inflammation. The quantitative production of mediators by fibroblasts may be less than that of monocytes/macrophages or epithelial cells, however, this does not exclude a role for fibroblasts in amplifying and sustaining an inflammatory response. The current study provides evidence that the mechanism of glucocorticoid inhibition of lung fibroblast-mediated inflammation differs from that of monocytes/macrophages and airway epithelial cells, which may help to explain the clinical benefits of inhaled glucocorticoids in COPD.

We also examined expression of HDAC2 in the lung fibroblasts from normal and COPD subjects as well as in HFL-1 cells exposed to 5% CSE for 72 hours. In contrast to the reduced HDAC2 expression reported in alveolar macrophages from COPD patients,3 there was no statistically significant difference in HDAC2 expression between normal and COPD lung fibroblasts. Nevertheless, the slight numerical difference observed (24% reduction in COPD lung fibroblasts, N = 6) in the current study does not exclude the possibility that evaluation of a larger number of samples, or samples from more severely affected COPD patients, might show a statistically significant difference. Based on the data obtained, a study of 25 subjects per group would be required to have an 80% chance of showing a statistically significant difference of the size observed in the current study. Thus, a modest inhibition of HDAC2 in lung fibroblasts from COPD patients cannot be excluded. In support of this concept, CSE exposure resulted in significant decline of HDAC2 expression in HFL-1 cells. However, in both the normal and COPD fibroblasts as well as in HFL-1 cells exposed or not exposed to CSE, the release of IL-6, IL-8, MMP-1, and MMP-3 in response to IL-1β and TNF-α was significantly inhibited by budesonide to a similar degree. Thus, the reduction of HDAC activity appeared to have no effect on glucocorticoid inhibition of mediator release from lung

**Notes:**

- **Fig 6** CSE effect on HDAC expression by human lung fibroblasts and its role in mediating budesonide effect.

**Abbreviations:** Bud, budesonide; CSE, cigarette smoke extract; Cyto, cytokines; hDAC, histone deacetylase; hFl, human lung fibroblast; Il, interleukin; MMP, matrix metalloproteinase; SEM, standard error of the mean; TNF, tumor necrosis factor.
fibroblasts, further supporting our finding that in human lung fibroblasts budesonide inhibition of inflammatory mediator release is independent of HDAC2 level.

**Conclusion**

HDAC2 is expressed in human lung fibroblasts. Budesonide inhibits release of IL-6, IL-8, MMP-1, and MMP-3 from human lung fibroblasts stimulated by IL-1β plus TNFα. Suppression of HDAC2 by either a pharmacologic inhibitor (TSA) or an siRNA targeting HDAC2 does not block the inhibitory effects of budesonide in fibroblasts, which contrasts with HDAC-dependent inhibition in HBECs and monocytic cells. The demonstration that the anti-inflammatory effects of budesonide in lung fibroblasts are HDAC-independent suggests that budesonide has the potential to modulate fibroblast-mediated tissue remodeling following airway inflammation in COPD and provides a mechanism for the therapeutic benefits of glucocorticoids in COPD.

**Acknowledgment**

The authors are grateful for the secretarial support of Ms Lillian Richards. This work was supported by AstraZeneca and by the Larson Endowment, University of Nebraska Medical Center.

**Disclosure**

AM-L is an employee of AstraZeneca. EW was an employee of AstraZeneca at the time when the study was performed and the manuscript written. The authors report no other conflicts of interest.

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