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Rosiglitazone, a peroxisome proliferator-activated receptor-γ agonist, attenuates acrolein-induced airway mucus hypersecretion in rats

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Abstract

Background: Peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of the ligand-activated nuclear receptor superfamily, has been shown to be implicated in anti-inflammatory and immunomodulatory responses, but its role in airway mucus hypersecretion remains not clear.

Objective: To investigate the role of PPAR-γ in airway mucus hypersecretion, we used an acrolein-exposed rat model treated with rosiglitazone, a peroxisome proliferator-activated receptor-γ agonist.

Methods: Rats were exposed to acrolein (3.0 ppm, 6h/day, 7 days/week) and orally administered with rosiglitazone (2, 4, 8 mg/kg) once daily for up to 2 weeks. The expressions of Muc5ac protein and mRNA, and infiltration of inflammatory cells and levels of inflammatory cytokines (interleukin (IL)-1β, tumor necrosis factor (TNF)-α) in bronchoalveolar lavage fluid (BALF) were detected with real-time PCR, Western blot, cell counting and ELISA. In addition, the role of nuclear factor (NF-κB) pathway in this process was also explored.

Results: Acrolein exposure significantly induced goblet cell hyperplasia in bronchial epithelium and Muc5ac mRNA and protein expressions in rat lungs, as well as the associated airway inflammation evidenced by the increased numbers of inflammatory cells and levels of inflammatory cytokines in BALF, which were attenuated with rosiglitazone treatment in a dose-dependent manner (P < 0.05). Simultaneously, the increased expression of NF-κB and decreased expression of cytoplasmic IκB in acrolein-exposed lungs were reversed by rosiglitazone treatment.

Conclusions: These findings suggest that PPAR-γ activation by its ligands can attenuate acrolein-induced airway mucus hypersecretion in rats, which may be involved in inhibition of NF-κB pathway.

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1. Introduction

Airway epithelium represents the first line of defense against airborne pollutants and pathogens. The normal respiratory epithelium is coated with mucus secreted by both goblet cells and submucosal gland mucous cells, which provides a variety of protective functions by trapping inhaled airborne pathogens and facilitating their removal from the airways via a mucociliary transport mechanism. Although mucus acts as an important component of host defense, mucus hypersecretion in the respiratory tract and goblet cell metaplasia in the airway epithelium contribute to the morbidity and mortality associated with airway inflammatory diseases (Williams et al., 2006; Kim, 1997; Lundgren and Shelhamer, 1990). Enhanced airway inflammation with mucus hypersecretion in response to inhaled noxious particles or gases from tobacco smoke or other environmental agents is considered to be a fundamental characteristic in chronic obstructive pulmonary disease (COPD) patients (Global Initiative, 2007).

Tobacco smoke exerts profound suppression of pulmonary immunity, resulting in an increased incidence and severity of respiratory tract infections. Acrolein is found in high concentrations in tobacco smoke and is absorbed primarily in the upper respiratory tract. Moreover, acrolein has been demonstrated to induce epithelial damage, mucous cell metaplasia, and mucus hypersecretion (Borchers et al., 1998; Costa et al., 1986). Muc5ac is considered to be a major mucin secreted from the goblet cells of the surface epithelium, and Muc5ac gene is most consistently reported to be upregulated by inflammatory cells (alveolar macrophages and
neutrophils) and inflammatory cytokines (tumor necrosis factor (TNF-α) and interleukin (IL)-1β) (Svanborg et al., 1999; Alimam et al., 2000; Hovenberg et al., 1996; Vovnov et al., 2004a, b; Song et al., 2003). Many literatures proved that acrolein can directly or indirectly (via inflammatory mediators) increase Muc5ac gene transcription in vivo and in vitro (Borcherds et al., 1998; 1999).

Peroxidase-proliferator-activated receptors (PPAR; isotypes α, β/δ, and γ) are ligand-inducible nuclear transcription factors and exhibit anti-inflammatory and immunomodulatory properties. PPAR-γ has been shown to broadly regulate inflammatory and reparative responses. PPAR-γ is expressed in both alveolar macrophages and neutrophils, and the ligand-dependent activation of this receptor results in regulating the expression of pro-inflammatory genes, and suppression of leukocyte effector responses, including cytokine production, the elaboration of reactive oxygen and nitrogen species, and migratory responses (Standiford et al., 2005). Furthermore, many documents have showed that treatment with PPAR agonists reduces inflammatory response and results in beneficial outcomes in animal models of asthma, COPD and pulmonary fibrosis (Milam et al., 2008; August et al., 2006; Lee et al., 2006a, b; Serhan and Devchand, 2001; Birrell et al., 2004), and thus PPARs may serve as novel targets in lung diseases (Belvisi and Hele, 2008).

Rosiglitazone, a PPAR-γ agonist, has been proved to reduce the airway neutrophil accumulation and inflammatory cytokines release (Lee et al., 2006a, b; Birrell et al., 2004), and also inhibits mucin production in vitro (Lee et al., 2006a, b). However, the effect of rosiglitazone on airway mucus hypersecretion has never been reported before. So, in the present study, we used an acrolein-induced rat model to investigate the effect of rosiglitazone on airway mucus hypersecretion and explore the role of PPAR-γ in this process.

2. Methods

2.1. Animal model preparation and treatment groups

The protocol was approved by the Animal Care and Use Committee of West China Hospital. Sprague–Dawley rats (male, 220–250 g) rats were randomly divided into 6 experimental groups (n = 10/group): control group (CT), rosiglitazone-only group (8 mg/kg, ROSI), acrolein group (2 mg/kg ACR), acrolein plus-rosiglitazone 4 mg/kg group, acrolein plus-rosiglitazone 8 mg/kg group. To induce airway mucus hypersecretion, rats were exposed to 3.0 ppm of acrolein for 6 h/day, 7 days/week, for up to 2 weeks. The exposure protocol was detailedly described (Chen et al., 2009).

2.2. Histopathology and immunohistochemistry

For histology, lung slices were fixed in 10% formalin for 24 h and then 70% ethanol before paraffin embedding, staining with alcian blue-periodic acid–Schiff (AB-PAS). Goblet cell count was performed by counting 500 bronchial epithelial cells as Ford et al. (2001). In brief, each lung was assigned a unit by computing the mean of the numerical scores. The numerical scores for the abundance of PAS-positive goblet cells in each airway were determined as follows: 0, <5% goblet cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; 4, 75% (Guingin et al., 1998). In addition, the relative area of PAS-positive airway epithelium was calculated by image software as previously described (Chen et al., 2009).

Immunohistochemical detection of Muc5ac performed on paraffin-embedded tissue sections using the avidin–biotin–peroxidase technique. After dewaxing and dehydration of sections, antigenic site retrieval was accomplished by microwaving each slide for 15 min in 0.01 mol/L citric acid buffer (pH 6.0). Endogenous peroxidase activity was blocked by incubation with 2% hydrogen peroxide for 20 min and non-specific binding was prevented by incubation with 1% BSA. Sections were subsequently incubated with monoclonal anti-Muc5ac antibody (1:100, Santa Cruz, CA, USA) and polyclonal anti-NF-κB p65 antibody (1:100, Santa Cruz, CA, USA) overnight at 4 °C. Antibody binding was detected using the SP kit (Vector Laboratories Ltd, Burlingame, CA, USA), which is based on the biotin–avidin system, according to the manufacturer’s protocol. The reaction was visualized using the diaminobenzidine kit (Vector Laboratories Ltd, Burlingame, CA, USA).

All measurements were performed with an Olympus light microscope using Image-Pro plus 4.5 software (Media Cybernetics Inc., Bethesda, MD, USA). To prevent observer bias, evaluating was done in a blinded fashion by two observers.

2.3. Bronchoalveolar lavage

Analysis of inflammatory cell profile of bronchoalveolar lavage fluid (BALF). Bronchoalveolar lavage was performed through a tracheal cannula with PBS (pH 7.4) as previously described (Fujita et al., 2003). This was repeated four times. Gold sterile PBS (2 ml) was used to inflate the lung, and the lavage fluid was recovered with ~80% of the original volume. The BALF was centrifuged (1000 × g, 10 min, 4 °C) and the cell-free supernatant was used for the biochemical measurements. For each rats, the cell pellet was then re-suspended in PBS. The total cells present in the lavage fluid were counted using a hemocytometer. Differential cells counts were performed on 200 cells stained with Wright-Giemsa.

Biochemical analyses. The supernatant of BALF was immediately stored at ~70 °C until the assay. BALF was analyzed for mucin (ELISA) and Muc5ac (ELISA) as previously described (Abdullah et al., 1996; Singer et al., 2002). The concentrations of IL-1β, IL-8 and TNF-α were measured using ELISA kits (Sensixion Biotechnology Co., Shanghai, China) according to the manufacturers’ instructions.

2.4. Real-time PCR

Total RNA was isolated from lung homogenates with Trizol (Invitrogen, Carlsbad, CA) and reverse transcribed. Each PCR was performed in a final volume of 25 μl (2 μl of cDNA, 0.3 μM of both forward and reverse primers, 2.5 mM Mg2+, 0.12 μM TaqMan probe, and 0.3 mM dNTP in 1 × PCR Gold buffer) under the following conditions: polymerase activation at 94 °C for 2 min followed by 40 cycles at 94 °C for 20 s, 50 °C for 30 s, and 72 °C for 40 s. The sequences of primers used in the present study are the following: Muc5ac (forward 5′-TCCCCCTGCAATGCTGTC-3′, reverse 5′-ACCAGTTGCAAGAATGTC-3′), GAPDH (forward 5′-CCTCAA GATTCGCAACT-3′, reverse 5′-CCTACACGTTTGCACTGAC-3′). Quantification of PCR products was carried out using the FTC-2000 (Funglyn Biotech Co., Ltd, Canada).

The fluorescence emitted by the reporter dye was detected in real-time, and the threshold cycle (CT) of each sample was recorded as a quantitative measure of the amount of PCR product in the sample. The Ct is the fractional cycle number at which the fluorescence generated by the reporter dye exceeds a fixed level above baseline. When indicated, the Muc5ac signal was normalized against the relative quantity of GAPDH and expressed as ΔΔCt = (CtMuc5ac − CtGAPDH). The changes in Muc5ac signal relative to the total amount of genomic DNA were expressed as ΔΔCt = Ctbaseline − CtCT. Relative changes in metastasis were then calculated as 2−ΔΔCt (Livak and Schmittgen, 2001).

2.5. Protein extraction

Cytosolic and nuclear proteins were prepared according to the method detailedly described before (Brassier et al., 2001; Han and Brassier, 1997). The Ilysis buffers were purchased from Biotek Corporation (Beijing, China). Lung tissues were homogenized in the Ilysis buffer A, containing 50 mM HEPES, pH 7.4, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml pepstatin A, 1 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml aprotinin, and 0.1% Nonidet P-40. After 10 min on ice, the lysates were centrifuged at 4000 × g for 4 min at 4 °C. After saving the supernatant (cytoplasmic extract), the nuclear pellet was suspended in Buffer A with 1.7 mM sucrose and centrifuged at 15,000 × g for 30 min at 4 °C. The purified nuclear pellet was then incubated in the Ilysis buffer B (10% glycerol, 50 mM HEPES, pH 7.4, 400 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.1 μg/ml PMSF, 1 μg/ml pepstatin A, 1 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, and 10 μg/ml aprotinin) with frequent vortexing for 30 min at 4 °C. After centrifugation at 15,000 × g for 5 min at 4 °C, the supernatant (nuclear extract) was frozen for assay.

2.6. Western blot

Cytosolic and nuclear proteins concentrations were measured using the BCA protein assay reagent kit (Pierce, Rockford, IL). Protein samples (20 μg) were separated on precasted 12% SDS-polyacrylamide gels (Bio-Rad, Hercules, CA) and semi-dry transferred to the polyvinylidene difluoride membranes. Non-specific binding to the membrane was blocked for 1 h at room temperature with 5% BSA in TBS-T. Membranes were then incubated at 4 °C overnight with the following antibodies (diluted 1:500 in 5% BSA in TBS-T, including primary anti-NF-κB (p65), anti-κB-α (Ser25), anti-phospho(κB)-κB-α (Ser25), anti-Lamin B1 and anti-β-actin antibodies (Santa Cruz, CA, USA). After incubation for 1 h at room temperature, membranes were washed with 1% BSA in TBS-T and incubated with HRP-conjugated secondary antibody for 1 h at room temperature in TBS-T (1:10,000), immunodetected proteins were visualized using ECL assay kit (Minipore, Bedford, MA) following the manufacturer’s recommended protocol.
2.7. Statistical analysis

All the data were expressed as mean ± SD. Statistical analysis was performed with SPSS 11.0 software. The differences between variables were analyzed by ANOVA followed by an appropriate post hoc test. Data were considered statistically significant at $P < 0.01$.

3. Results

3.1. Rosiglitazone attenuates acrolein-induced airway mucus hypersecretion and associated inflammatory response

3.1.1. Histological changes after acrolein exposure

To assess the effect of rosiglitazone on airway mucus hypersecretion, we studied the effect of rosiglitazone on goblet cell metaplasia and mucus glycoconjugate production in rats exposed to acrolein. Morphological changes from each airway tissue sections were measured by AB-PAS staining (Fig. 1A). The number of PAS-positive goblet cells and the area of PAS-positive cells served as the index of goblet cell metaplasia and mucus glycoconjugate production in respiratory tract. Histologic examination revealed that there were very few PAS-positive goblet cells in small airways of control rats. The number of PAS positive cells was markedly increased in acrolein-exposed rats ($P < 0.01$, Fig. 1). The increased number of PAS-positive goblet cells was then significantly decreased by administration of rosiglitazone (4 and 8 mg/kg) ($P < 0.01$). Moreover, higher magnification of stained airway epithelium found the abundant accumulation of mucous glycoconjugate material in the cytoplasm of epithelial cells of acrolein-exposed rats. Rosiglitazone (4 and 8 mg/kg) efficiently attenuated the increased area of PAS-positive cells in airway epithelium after acrolein exposure ($P < 0.01$, Fig. 1).

3.1.2. Muc5ac immunohistochemistry

Muc5ac immunohistochemical examination of airway tissue sections revealed the Muc5ac expression in airway epithelium (Fig. 1B). Acrolein exposure resulted in a marked increase in bronchial epithelial cells stained positively for Muc5ac compared with that in control rats. The positive staining for Muc5ac in bronchial epithelial cells was reduced in rats treated with rosiglitazone (4 and 8 mg/kg) ($P < 0.01$). The higher dose of rosiglitazone (8 mg/kg) was associated with a more significant reduction in Muc5ac positive staining ($P < 0.01$).

3.1.3. Mucin and Muc5ac release in BALF

Following acrolein exposure, the levels of mucin and Muc5ac in BALF were markedly increased compared with that in control rats (Fig. 2), which were significantly attenuated with administration of rosiglitazone (4 and 8 mg/kg) ($P < 0.01$). The higher dose of rosiglitazone was associated with a more significant reduction in mucin and Muc5ac release in BALF ($P < 0.01$).

Fig. 1. Photomicrographs of rat airway epithelium stained with AB-PAS and stained with Muc5ac protein. (A) Representative photomicrographs of lung sections stained with AB-PAS in (a) control group, (b) acrolein group, (c) acrolein-plus-rosiglitazone 4 mg/kg group, and (d) acrolein-plus-ROSI 8 mg/kg group. The numerical scores for the abundance of PAS-positive goblet cells and the relative area of PAS-positive cells in airway epithelium were calculated by image analysis. (B) Representative photomicrographs showing immunohistochemical staining for Muc5ac. Optical density of Muc5ac staining was assessed by image analysis. In the control state, AB-PAS and Muc5ac staining were minimal. Acrolein increased AB-PAS and Muc5ac staining. Rosiglitazone administration significantly prevented the acrolein-induced effects. Photomicrographs are shown at ×400 magnification. Bars indicate mean ± SD. *$P < 0.01$ versus control rats; **$P < 0.01$ versus acrolein rats; ***$P < 0.01$ versus acrolein-plus-rosiglitazone 4 mg/kg rats.
3.1.4. Muc5ac mRNA expression in rat lungs

In order to evaluate the expression of mucin gene in the lungs of acrolein-exposed and control rats, Muc5ac mRNA levels were examined by real-time PCR (Fig. 3). Marked expression of Muc5ac mRNA was observed in acrolein-exposed lungs compared with that in controls. Moreover, the increased expression of Muc5ac mRNA induced by acrolein was significantly attenuated by administration of rosiglitazone (4 and 8 mg/kg). The higher dose of rosiglitazone was associated with a more significant reduction in Muc5ac mRNA expression (P<0.01).

Fig. 3. Expression of Muc5ac mRNA in rat lungs. (A) Representative agarose gel showing real-time PCR analysis for Muc5ac mRNA. (B) Relative expression of Muc5ac mRNA as measured by 2^−ΔΔCT method. All data presented as the fold-change over control in Muc5ac gene expression. The expression of Muc5ac mRNA was significantly increased in acrolein-exposed rats, and the upregulation of Muc5ac mRNA expression was inhibited by rosiglitazone (4 and 8 mg/kg). The higher dose of rosiglitazone was associated with a more significant reduction in Muc5ac mRNA expression. *P<0.01 versus control rats; **P<0.01 versus acrolein rats; ***P<0.01 versus acrolein-plus-rosiglitazone 4 mg/kg rats.

3.1.5. Analysis of the inflammatory cells and cytokines in BALF

Inflammatory cells infiltration and increased inflammatory cytokines production are believed to play a critical role in the pathogenesis of airway mucus hypersecretion. To determine whether rosiglitazone could influence inflammation process in airway mucus hypersecretion, acrolein-exposed rats were administrated with rosiglitazone at different doses. The numbers of macrophages and neutrophils in BALF were markedly increased after acrolein exposure (P<0.01, Fig. 4). Rosiglitazone (4 and 8 mg/kg) administration significantly decreased the numbers of macrophages and neutrophils in acrolein-exposed rats (P<0.01). In addition, exposure to acrolein for 12 days resulted in the increase of IL-1β, IL-8 and TNF-α concentration in BALF (P<0.01, Fig. 4). The increased concentrations of IL-1β, IL-8 and TNF-α were then inhibited by rosiglitazone (4 and 8 mg/kg) administration (P<0.01), but not affected by 2 mg/kg rosiglitazone (P>0.01). The higher dose of rosiglitazone was associated with a more significant reduction in the numbers of inflammatory cells and the concentration of inflammatory cytokines in BALF (P<0.01).

3.2. Rosiglitazone inhibits NF-κB activation in rat lungs after acrolein exposure

3.2.1. Immunohistochemistry analysis of NF-κB

Activation of the transcription factor NF-κB has been suggested to be a major mechanism of airway inflammation and mucus hypersecretion. In the present study, immunohistochemical analysis revealed that the rat bronchiolar epithelium was stained with NF-κB p65 (Fig. 5). Acrolein exposure resulted in an increased expression of NF-κB p65 in airway epithelium compared with that in control rats. The positive staining of NF-κB p65 was observed in both cytoplasm and nucleus of bronchial epithelial cells.

3.2.2. Inhibition of NF-κB activation by rosiglitazone

NF-κB activation was assessed by detecting NF-κB subunit p65 nuclear translocation and IkκB phosphorylation. Western blot was used to analyze the expressions of NF-κB p65, IkκB and phospho-IκB in cytoplasm and nucleus. Cytosolic and nuclear NF-κB p65 expressions after rosiglitazone treatment were showed in Fig. 6A. A marked increase in nuclear NF-κB p65 was observed following exposure to acrolein (P<0.01). However, rosiglitazone (4 and 8 mg/kg) treatment significantly inhibited acrolein-induced nuclear NF-κB p65 expression in a dose-dependent style. In contrast, NF-κB p65 protein in the cytoplasmic extract modestly
Fig. 4. Inflammatory cell numbers and inflammatory cytokines in BALF. The numbers of total cells, neutrophils and macrophages in BALF were significantly increased in acrolein-exposed rats. The administration of rosiglitazone (4 and 8 mg/kg) significantly decreased the numbers of total cells, neutrophils and macrophages in BALF of acrolein-exposed rats. The levels of IL-1β, IL-8 and TNF-α in BALF were significantly increased in acrolein-exposed rats. Rosiglitazone (4 and 8 mg/kg) treatment significantly decreased the concentration of IL-1β, IL-8 and TNF-α in BALF of acrolein-exposed rats. Bars indicate mean ± SD. *P < 0.01 versus control rats; **P < 0.01 versus acrolein rats; ***P < 0.01 versus acrolein-plus-rosiglitazone 4 mg/kg rats.

increased following treatment with rosiglitazone (4 and 8 mg/kg) (P<0.01, Fig. 6C). Simultaneously, Western blot analysis revealed that acrolein exposure induced a marked decrease in cytosolic IkB level (P<0.01), which was reversed after rosiglitazone (4 and 8 mg/kg) treatment (P<0.01, Fig. 6D and E). In contrast, compared with that in control lungs, the phospho-IkBα levels were enhanced in acrolein-exposed lungs (P<0.01), which was dose-dependently decreased with rosiglitazone (4 and 8 mg/kg) treatment (P<0.01, Fig. 6D and F). These findings indicate that rosiglitazone can prevent not only translocation of NF-κB, but also IkB phosphorylation.

4. Discussion

In the present study, we demonstrated that rosiglitazone inhibited acrolein-induced airway mucus hypersecretion, as indicated by decreased goblet cell metaplasia and level of Muc5ac expression in rat airways. In addition, rosiglitazone treatment abrogated NF-κB translocation via IkB-dependent pathway. These findings suggest that PPAR-γ can attenuate acrolein-induced airway mucus hypersecretion and NF-κB pathway may play a role in this process.

Airway mucus hypersecretion is a key pathological feature in airway diseases, such as asthma and COPD (Rogers and Barnes, 2006;
Fig. 6. NF-κB, IκB and p-IκB protein expressions in rat lungs. Lung tissue extracts were electrophoresed on 12% SDS-PAGE gel, followed by Western blot with primary antibodies against rat p65, phosphor (p)-IκB, IκB (A, D). Lamin B1 and β-actin served as the loading controls. Western blot analysis and quantification of NF-κB protein expressions from cytoplasmic (C) and nuclear (B) fraction showed rosiglitazone treatment significantly attenuated NF-κB translocation. Meanwhile, cytosolic IκB (E) and p-IκB (F) expressions analyzed by Western blot showed the effect of rosiglitazone on inhibitory protein IκB phosphorylation from NF-κB complex. Bars indicate mean ± SD. *P<0.01 versus control rats; **P<0.01 versus acrolein rats; ***P<0.01 versus acrolein-plus-rosiglitazone 4 mg/kg rats.

Perez-Vilar et al., 2003). Muc5ac is considered to be the marker of goblet cell metaplasia that is one of the most important pathological events in the process of airway mucus hypersecretion (Alimam et al., 2000; Rogers and Barnes, 2006). In an in vitro study, Lee and his colleagues have examined the inhibitory effect of rosiglitazone on cigarette smoke-induced Muc5ac synthesis in NCI-H292 cells (a human pulmonary mucoepidermoid carcinoma cell line), which suggests PPAR-γ may be a regulator of cigarette smoke-induced airway inflammation and mucus hypersecretion (Lee et al., 2006a, b). In our in vivo study, we further determined acrolein, an important component of cigarette smoke, significantly induced airway mucus hypersecretion in rats, and treatment with rosiglitazone markedly alleviated goblet cells metaplasia and Muc5ac immunostain in bronchiolar epithelium, attenuated mucin and Muc5ac glycoprotein release in BALF, and decreased the upregulation of Muc5ac mRNA expression in rat lungs after acrolein exposure. In summary, these results suggest that rosiglitazone efficiently attenuated acrolein-stimulated mucus hypersecretion in rat airway.

A link between inflammatory reactions and mucus hypersecretion in airway diseases has been described. Acrolein, as an important stimuli of airway inflammation, triggers inflammatory cells such as neutrophils and macrophages into the airways (Kutzman et al., 1985; Leikauf et al., 1989; Lundgren and Shelhamer, 1990), and mediates alveolar macrophage activation (Facchinetti et al., 2007). Moreover, cytokines and mediators, such as IL-1, TNF-α,
IL-8, released by the inflammatory cells, are capable of inducing goblet cell metaplasia, mucin production, and thus mucus hypersecretion (Voyoov et al., 2004a, b; Cohen et al., 1991; Dabbagh et al., 1995; Kuwahara et al., 2006; Nakamura et al., 1992). PPAR-γ ligands could inhibit the release of proinflammatory cytokines from activated macrophages and airway epithelial cells (Jiang et al., 1998; Wang et al., 2001). Noticeably, rosiglitazone (a ligand of PPAR-γ) has been shown to reduce the airway neutrophil accumulation and cytokine release induced by lipopolysaccharide (LPS), attenuate inflammatory mediator production in bleomycin-induced lung injury, and modulate inflammatory response in Toluene Diisocyanate-induced asthma (Lee et al., 2006a, b; Genovese et al., 2005; Birrell et al., 2004). In the present study, rosiglitazone attenuated cellular stimulation, IκBα nuclear translocation, IκBα phosphorylation by IκB kinase and degradation by the 26S proteasome complex (Rémier et al., 1997; DiDonato et al., 1997; Beg et al., 1993) and removal of IκBα protein from the NF-κB/IKK complex enables NF-κB to translocate to the nucleus where it controls the transcription of many cytokines (Baldwin, 1996; Wang et al., 1999). It was widely proved that NF-κB signaling pathway played an important role in tobacco smoke-induced airway inflammation and mucus hypersecretion (Vlahos et al., 2006; Yang et al., 2006, 2007; Baginski et al., 2006; Yoshida and Tudor, 2007). We have shown here that the translocation of NF-κB increased in acrolein-exposed lungs, and then inhibited by rosiglitazone treatment. Simultaneously, the level of NF-κB activation was positively correlated with the concentration of inflammatory cytokines (IL-1β, IL-8 and TNF-α) in BALF, as well as the expression of Muc5ac mRNA in rat lungs. PPAR-γ ligands have been shown to stabilize the IκB/NF-κB interaction, blocking NF-κB entry to the nucleus, thereby inhibiting pro-inflammatory gene expression (Birrell et al., 2004; Perez et al., 2008). Taking together, our data suggest that NF-κB pathway may contribute to the inhibitory effect of rosiglitazone on acrolein-exposed airway mucus hypersecretion.

The synthetic PPAR-γ ligands are already clinically used for their potent antiinflammatory effects, and the anti-inflammatory effects of these agents have been demonstrated in animal models of lung injury, pulmonary fibrosis and airway remodeling (Milam et al., 2008; Lee et al., 2006a, b; Birrell et al., 2004). The present study demonstrates that PPAR-γ activation by its ligands may be a novel strategy of anti-mucus hypersecretion in airway diseases, such as asthma and COPD.

Conflict of interest statement

None.

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