15-Deoxy-prostaglandin J2 anti-inflammation in a rat model of chronic obstructive pulmonary disease and human bronchial epithelial cells via Nrf2 activation

X.Y. Li[1,2], B.L. Luo[1], L.J. Wang[1], W.D. Zhang[2] and Z.G. Liu[2]

[1]Department of Respiratory Medicine, Xiangya Hospital of Central South University, Changsha, Hunan, China
[2]Department of Respiratory Medicine, Hunan People's Hospital, Changsha, Hunan, China

Corresponding author: B.L. Luo
E-mail: bllo181@163.com

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ABSTRACT. Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates antioxidant and anti-inflammatory genes, and it plays a crucial role in the pathogenesis of chronic obstructive pulmonary disease (COPD). Moreover, 15-deoxy-delta12,14-prostaglandin J2 (15d-PGJ2) plays a protective role against oxidative stress and inflammation both *in vivo* and *in vitro*. In a previous study, we found that 15d-PGJ2 increased the expression of Nrf2 in a COPD rat model. This study aims to elucidate the role of 15d-PGJ2 in COPD pathogenesis and the relationship between Nrf2 and human bronchial epithelial (HBE) cells. Normal HBE (HBE) cells were cultured. Following cigarette smoke extract (CSE) stimulation, pre-incubation with or without small interfering RNA

(siRNA) Nrf2, and stimulation with or without 15d-PGJ2, the expression levels of Nrf2, NF-κBp65, and IL-8 were detected by reverse transcription-polymerase chain reaction and western blot, respectively. The expression of NF-κBp65 and IL-8 in CSE-stimulated normal HBE cells was inhibited by 15d-PGJ2 at both the mRNA level and the protein level. Moreover, the expression of Nrf2 in normal HBE cells was improved by 15d-PGJ2 at both the mRNA level and the protein level. However, the inhibitory or improving effects of 15d-PGJ2 were disengaged by siRNA Nrf2 at both the mRNA level and the protein level. 15d-PGJ2 possesses anti-inflammatory properties in the pathogenesis of COPD, and HBE cells stimulated by CSE via Nrf2 activation.

Key words: Chronic obstructive pulmonary disease; Nrf2; NF-κB; 15-Deoxy-delta12,14-prostaglandin J2

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality, and it is characterized by irreversible airflow limitation and chronic inflammation of the respiratory tracts. It was recognized as the fifth greatest worldwide disease burden of 2012 according to the World Bank/World Health Organization (Vestbo et al., 2012). Therefore, the significance of COPD makes the discovery of new ways of preventing and treating the disease imperative.

Recently, studies have indicated that nuclear factor-erythroid 2-related factor 2 (Nrf2) is a key factor in the regulation of antioxidant and anti-inflammatory genes, and plays a crucial role in the pathogenesis of COPD (Rahman and MacNee, 2000; MacNee, 2001). It has also been reported that Nrf2 can protect against the development of emphysema by regulating oxidant/anti-oxidant balance, inflammation, and the protease/anti-protease balance (Iizuka et al., 2005).

More importantly, Nrf2 deficiency influences susceptibility to steroid resistance (Adenuga et al., 2010; Malhotra et al., 2011), and can improve antibacterial defenses and prevent exacerbations of COPD caused by bacterial infection (Harvey et al., 2011), so Nrf2 is considered to be a promising therapeutic target in COPD (Boutten et al., 2011). However, another study showed that the expression of Nrf2 decreased in COPD patients (Suzuki et al., 2008), but nuclear factor-kappa B (NF-κB), a transcription factor involved in the regulation of inflammatory cytokines such as interleukin-8 (IL-8), increased in the airway epithelial cells of COPD patients and patients who smoked, especially in patients with acute exacerbations of COPD (Maziak et al., 1998; Corradi et al., 1999; Rahman et al., 2002; Block and Hong, 2005).

Prostaglandin D2 (PGD2), a type of cyclopentenone prostaglandin, is formed by the cyclooxygenation of arachidonic acid, and 15-deoxy-delta12,14-prostaglandin J2 (15d-PGJ2) is the ultimate metabolite of PGD2. 15d-PGJ2 possesses anti-inflammatory properties, and plays a protective role against oxidative stress both *in vitro* and *in vivo* (Giri et al., 2004; Itoh et al., 2004; Qin et al., 2006). Several studies have shown that 15d-PGJ2 affects the Nrf2-mediated transcription pathway. These encouraging findings have inspired the present study, the objectives of which were to determine whether 15d-PGJ2 could inhibit inflammation via the activation of Nrf2 in the pathogenesis of COPD.

MATERIAL AND METHODS

Cell culture

Normal human bronchial epithelial (HBE) cells were purchased from the Cell Culture Collection Center of Central South University. They were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum, and were grown in tissue culture flasks in a humidified gas environment with 95% air and 5% carbon dioxide at 37°C. For subculture, the cells were detached with 0.125% trypsin. After 80% confluence had been reached, the cells were seeded onto six-well plates at a density of 2 x 10^5 cells/well to grow again to 80% confluence. After 24 h in serum-free medium, the cultures were grouped as follows: 1) normal HBE (Group A); 2) cells stimulated with cigarette smoke extract (CSE, 5%, 24 h; Group B); 3) cells stimulated with CSE, then pre-incubated with 15d-PGJ2 (10 μM, 30 min) (Group C); 4) cells subjected to small interfering RNA (siRNA) against Nrf2 (Nrf2 siRNA (human), NFE2L2-1579 sense: 5'-AGAUUUAGAUCAUUUGAAATT-3', anti-sense: 5'-UUUCAAAUGAUCUAAAUCUTG-3' (GenePharma, Shanghai, China) 2 μL, 6 h), and then stimulated with CSE (Group D).

Reverse transcription-polymerase chain reaction (RT-PCR)

The mRNA expression levels of Nrf2, IKKα/β, and NF-κBp65 in the bronchi and lung tissues of rats and HBE cells were determined using RT-PCR. Total RNAs were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNAs were synthesized according to the manufacturer instructions (Invitrogen). The amplification reaction was carried out in a volume of 25 μL containing 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 3 mM $MgCl_2$, 250 μM of each dNTP, 0.2 μM of each primer, 100-200 ng template cDNA, and 0.25 U LA Taq polymerase (TaKaRa, Dalian, China). The primer sequences (all the primers were synthesized by Invitrogen) are listed in Tables 1 and 2. PCR products were separated by electrophoresis on 1% agarose gel, photographed using a gel documentation system (UVP GelDoc-It™ Imaging System, Cambridge, UK), and quantitatively analyzed by the Labworks 4.5 analysis software.

Table 1. Rat gene primer and reaction conditions.

Target gene	Primer sequence	Annealing temperature (°C)	Products (bp)	Cycles
Nrf2	S 5-3: CCATTTACGGAGACCCAC A 5-3: TCTGAGCGGCAACTTTAT	55	450	26
NF-κBp65	S 5-3: CAACCCAGGGAACGAAGA A 5-3: GCGACTGTGAACCACGATG	58	463	28
β-actin	S 5-3: GTTGTCCCTGTATGCCTCT A 5-3: ATGTCACGCACGATTTCC		220	28

Table 2. Human gene primer and reaction conditions.

Target gene	Primer sequence	Annealing temperature (°C)	Products (bp)	Cycles
Nrf2	S 5-3: CCCTTGTCACCATCTCAG A 5-3: CAGCCACTTTATTCTTACCC	52	316	30
NF-κBp65	S 5-3: TGCCGAGTGAACCGAAAC A 5-3: GCTCAGGGATGACGTAAAGG	50	480	28
β-actin	S 5-3: CGCACCACTGGCATTGTCAT A 5-3: TTCTCCTTGATGTCACGCAC		285	32

Western blotting

Cells were harvested using lysis buffer, and the lysate was removed by centrifugation at 12,000 *g* for 10 min at 4°C. The proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes, then probed with primary antibodies (dilution ratio shown in Table 3) against human target protein. The primary antibodies that bound to the target proteins were detected using horseradish peroxidase-conjugated anti-rabbit/anti-mouse secondary antibody (dilution ratio shown in Table 3). The antibodies were visualized by enhanced chemiluminescence detection (Pierce Biotechnology, Rockford, IL, USA). Densitometric analysis was used to assess the relative change in the expression of Nrf2, NF-κBp65, and IL-8 protein, the optical density of the target protein bands was corrected by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) calculated with an automatic image analysis system, the FluorChem 8900 software system (18, 19). In this analysis, GAPDH was analyzed and used as a loading control, and Nrf2, NF-κBp65, and IL-8 protein levels are reported as the ratio of band optical intensity of Nrf2, NF-κBp65, and IL-8 to GAPDH.

Table 3. List of the western blot antibodies.

Primary antibody	Molecular weight of target protein (kDa)	Dilution ratio	Company/code No.	Secondary antibody	Dilution ratio
Rabbit Nrf2 antibody	about 57	1:500	SANTA, SC-722	Goat anti-rabbit IgG/HRP	1:40,000
Rabbit IKKα/β antibody	about 85-87	1:500	SANTA, SC-7607	Goat anti-rabbit IgG/HRP	1:40,000
Mouse NF-κBp65 antibody	about 65	1:8000	Cell Science, MSC8008	Goat anti-mouse IgG/HRP	1:80,000
Mouse GAPDH antibody	about 37	1:8000	ProMab, Mab-2005079	Goat anti-mouse IgG/HRP	1:80,000

Statistical analysis

The data are generally reported as means ± SD, and the SPSS version 11.0 software was used for statistical analyses. Statistical significance among mean values was evaluated with one-way analysis of variance. The least significant difference *t*-test was used for comparison between groups and differences were considered significant when the P value was less than 0.05.

RESULTS

The RT-PCR results are shown in Table 4. We found that the expression levels of Nrf2 and NF-κBp65 increased significantly in contrast to the control Group A ($P < 0.01$). After stimulation with 15d-PGJ2 (Group C), the expression levels of Nrf2 and NF-κBp65 increased significantly in contrast to Group B, but the expression of Nrf2 and NF-κBp65 was significantly inhibited both at the mRNA level and the protein level associated with Nrf2 siRNA (Group D) in contrast to Group C ($P < 0.01$).

Table 4. Reverse transcription-polymerase chain reaction of the target factors in human bronchial epithelial cells.

	A	B	C	D
Nrf2	0.51 ± 0.032	$0.60 \pm 0.011^{\Delta}$	$0.87 \pm 0.023^{\Delta\Delta}$	$0.17 \pm 0.025^{\Delta\Delta}$
NF-κBp65	0.38 ± 0.010	$0.61 \pm 0.025^{\Delta}$	$0.52 \pm 0.089^{\Delta\Delta}$	$0.75 \pm 0.010^{\Delta\Delta}$

$^{\Delta}$*vs* A group: $^{\Delta}P < 0.05$; $^{\Delta\Delta}$*vs* B group: $^{\Delta}P < 0.05$.

The results of western blotting are shown in Table 5. It was similar with that of RT-PCR results.

Table 5. Western blot of the target factors in human bronchial epithelial cells.

	A	B	C	D
Nrf2	0.17 ± 0.0039	0.29 ± 0.012$^{\Delta}$	0.46 ± 0.023$^{\Delta\Delta}$	0.17 ± 0.0037$^{\Delta\Delta}$
NF-κBp65	0.18 ± 0.0062	0.38 ± 0.016$^{\Delta}$	0.29 ± 0.0090$^{\Delta\Delta}$	0.45 ± 0.0085$^{\Delta\Delta}$
IL-8	0.18 ± 0.0070	0.37 ± 0.013$^{\Delta}$	0.28 ± 0.0054$^{\Delta\Delta}$	0.45 ± 0.0072$^{\Delta\Delta}$

$^{\Delta}$*vs* A group: $P < 0.05$; $^{\Delta\Delta}$*vs* B group: $P < 0.05$.

DISCUSSION

In our previous study, we found that NF-κBp65 and IL-8 in our established animal model of COPD increased significantly, suggesting an activated or increased inflammation in COPD. However, with the 15d-PGJ2 intervention, NF-κBp65 and IL-8 decreased significantly, but the levels of Nrf2 increased. Therefore, we speculated that 15d-PGJ2 had delayed the progression of COPD and reduced inflammation, as in the previous reports (Rahman et al., 1997), suggesting that this feature is related to the increase of Nrf2, and that Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema (Prasad et al., 2008). We designed and performed the present study to uncover and explore this hypothesis.

In this study, we have found that CSE can stimulate the inflammatory response, with increased expression of NF-κBp65 and IL-8. However, after treatment with 15d-PGJ2, the expression of NF-κBp65 and IL-8 decreased, suggesting that 15d-PGJ2 can reduce inflammation in HBE cells with increased levels of Nrf2. Moreover, in previous studies, it has been speculated that 15d-PGJ2 reduces inflammation by increasing the levels of Nrf2 (Ishii et al., 2005; Mochizuki et al., 2005). In this study, in keeping with this speculation, we demonstrated that pretreatment with Nrf2 siRNA and CSE can also stimulate the inflammatory response, and the levels of NF-κBp65 and IL-8 did not decrease even following treatment with 15d-PGJ2. Therefore, our study demonstrates that 15d-PGJ2 can reduce inflammation via increased activation of Nrf2, which provides a new target to delay the progression of COPD.

Conflicts of interest

The authors declare no conflict of interest.

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