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ORIGINAL ARTICLE

Comparison of Anti-inflammatory Efficacy between Dexamethasone and a Standardized Herbal Formula, PM014, in a Cigarette Smoke-induced Subacute Mouse Model of Chronic Obstructive Pulmonary Disease

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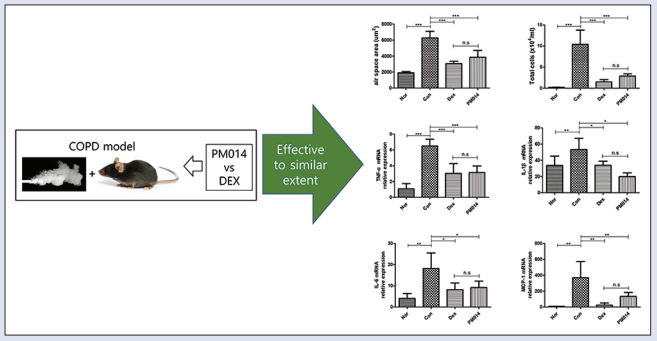
ABSTRACT

Objective: A standardized herbal formula, PM014, originated from *Chungsangboha*-tang, which has been used to treat various respiratory diseases, including bronchitis, asthma, and emphysema. Several previous studies have reported that the therapeutic mechanism of PM014 was mediated by an anti-inflammatory effect. Therefore, we compared anti-inflammatory efficacy between PM014 and dexamethasone (DEX) using a mouse model of cigarette smoke (CS)-induced subacute pulmonary inflammation. **Materials and Methods:** Female C57BL/6 mice were revealed to CS for 2 h/day, three cigarettes per day, 5 days a week for 3 weeks; the control group got no other treatment, while the DEX and PM014 groups received 1 mg/kg of DEX and 100 mg/kg of PM014, respectively (both were orally administered). The histological morphology and average alveolar size were determined by lung histology. The inflammatory cell profiles and protein expressions of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1 β, IL-6, and one pro-inflammatory chemokine (monocyte chemoattractant protein 1 [MCP-1]), were measured in bronchoalveolar lavage fluid (BALF). The mRNA expressions of the pro-inflammatory cytokines and chemokine were also measured in lung tissues. **Results:** Both PM014 and DEX attenuated histological injury and air space enlargement in lung tissue and decreased the number of inflammatory cells in BALF. Both also decreased the mRNA expressions of TNF-α, IL-6, and IL-1 β in lung tissue and reduced the protein expressions of TNF-α, IL-1 β, IL-6, and MCP-1 in BALF. **Conclusion:** Our results showed that the anti-inflammatory efficacy of PM014 and DEX was equivalent in a subacute mouse model of CS-induced chronic obstructive pulmonary disease.

Key words: Anti-inflammation, chronic obstructive pulmonary disease, cigarette smoke, corticosteroid, PM014

SUMMARY

- PM014, originated from *Chungsangboha*-tang, which has been used to treat various respiratory diseases, demonstrated anti-inflammatory efficacy similar to that of dexamethasone in a mouse model of subacute cigarette smoke-induced chronic obstructive pulmonary disease (COPD). The results of the present study suggest that PM014 could be used as an adjunctive agent or substitute for oral steroids in COPD patients.



Abbreviations used: BALF; bronchoalveolar lavage fluid, CS; cigarette smoke, DEX; dexamethasone, IL; interleukin, MCP; monocyte chemoattractant protein, TNF-α; tumor necrosis factor-alpha

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INTRODUCTION

Cigarette smoking is one of the major global health problems, which has been the cause of more than 1 in 10 deaths.[1] It mainly causes respiratory diseases, cardiovascular diseases, and cancers.[2,3] Cigarette smoke (CS) is a complex mixture of various noxious chemical compounds, such as nicotine, tar, and benzopyrene; this mixture can rapidly induce inflammation and injury of the tissues, common pathologic features, in the CS-associated diseases.[4] When CS enters the lungs, it activates the immune-inflammatory pathway, thus increasing the activity of macrophages, neutrophils, and lymphocytes, as well as the production of related mediators (e.g., pro-inflammatory cytokines such as tumor necrosis factor-alpha [TNF-α], interleukin (IL)-1 β and IL-6 and chemokines such as monocyte chemoattractant protein 1 [MCP-1]). This inflammation can induce chronic obstructive pulmonary disease (COPD)-like lung damage, as well as the destruction and obstruction of various parts of the airways.[4-6]

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Glucocorticoids have been commonly used for the treatment of many airway inflammatory diseases and are known as typical anti-inflammatory therapeutic agents.[7,8] Notably, glucocorticoid was shown to have a preventive effect against COPD in the TORCH study.[9] However, glucocorticoid can cause a variety of side effects, including weight gain, Cushing syndrome, muscle breakdown, insulin resistance, osteoporosis, mood changes, and glaucoma.[10] Therefore, as alternatives to glucocorticoids, other agents with anti-inflammatory effects have been developed for the various respiratory diseases.

A standardized herbal formula, PM014, originated from *Chungsangboha*-tang, which has been used to treat chronic respiratory disease (e.g., asthma and COPD) in Korea for centuries.[11] The original herbal formula contained 18 species of medical plants, leading to difficulties in standardization; thus, it was simplified to contain seven herbs and renamed PM014. Previous studies have shown the therapeutic effects of PM014 on various respiratory diseases through its anti-inflammatory effects.[12-15] We aimed to confirm the preventive effect of PM014 against inflammation and to compare anti-inflammatory efficacy between PM014 and dexamethasone (DEX), through histological analysis and measuring the expression of cytokines and chemokines in lung tissues and bronchoalveolar lavage fluid (BALF) using a mouse model of subacute CS-induced COPD.

MATERIALS AND METHODS

Reagents

The methods of extraction and standardization of PM014 were described in a previous report.[12] Seven medicinal herbs were used to prepare PM014 [Table 1]. Each extract granule was purchased from Sun Ten Pharmaceutical (Taipei, Taiwan), and mixtures of the herbal extract granules were dissolved in 0.9% saline to achieve a final concentration of 10% (w/v). A supernatant was then obtained by centrifugation at 27,000 ×*g* for 20 min at 4°C (Eppendorf, Hamburg, Germany) and sterilized by passage through a 0.22-μm syringe filter. The stock of the PM014 extract was then diluted and stored at 4°C. DEX (Sigma Aldrich, St. Louis, MO, USA), which was used as a positive control, was also dissolved in saline.

Animal models

Female C57BL/6 mice, 6–7 weeks old and weighing 16–17 g, were obtained from Orient Bio Inc., (Seongnam, South Korea). Mice were housed in sterile microisolator cages and kept at 20°C under a 12-h day–night cycle and fed a standard sterile diet with free access to water. Bodyweight of each mouse was measured individually every 2 days using a digital scale with 0.1 g accuracy and adjusting animal movement function (Ohaus Corporation, Parsippany, NJ, USA). All experiments were conducted in compliance with the requirements of the Animal Care and Ethics Committee of Kyung Hee University. Twenty mice were divided into four groups. In the normal group (NOR), mice were freely disclosed to room air within a chamber and treated with distilled water (DW). The other three groups were the control (CON), DEX, and PM014 groups; all mice in these groups were exposed to CS. CS was made from the standard reference cigarettes 3R4F (University of Kentucky, Lexington, KY, USA), using a smoking apparatus. Mice were exposed to CS from three cigarettes for 2 h, 5 days/week for 3 weeks. The 2 h of exposure were composed of 30 min exposure to fresh air between 1-h exposure to CS. The CON was treated with DW, the DEX group with 1 mg/kg DEX, and the PM014 group with 100 mg/kg PM014. DW, DEX, and PM014 were administered orally once per day for 3 weeks following the first CS exposure. All mice were sacrificed on day 22 and their lung tissues were extracted [Figure 1].

Table 1: Prescription of PM014

Herb	Pharmaceutical name	Amount (g/56 g)
Suckjihwang	Root of *Rehmannia glutinosa*	16
Mockdanpi	Cortex of *Paeonia suffruticosa*	8
Omija	Fruit of *Schisandra chinensis*	8
Chunmundong	Root of *Asparagus cochinchinensis*	8
Hwangkum	Root of *Scutellaria baicalensis*	6
Hengin	Seed of *Prunus armeniaca*	6
Baekbukuen	Root of *Stemona sessilifolia*	4

Collection of bronchoalveolar lavage fluid

Bronchoalveolar lavage was performed by cannulating the trachea and washing the lungs three times with phosphate-buffered saline (PBS; pH, 7.2; room temperature), using a volume of 700 mL/wash. The retrieved fluid was stored on ice. After washing three times, the BALF was centrifuged for 10 min at 1,300 rpm. The concentrations of cells were measured using a hemocytometer. After cytocentrifugation and Diff-Quick staining, differential cell counts were conducted. A total of approximately 500 cells were counted.

The cell-free supernatant was stored at −80°C until further use.

RNA extraction and real-time polymerase chain reaction

To measure the gene expression of TNF-α, IL-1 β, IL-6, and MCP-1, total RNA from whole lung tissues was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's protocol. 2 μg of RNA were reverse-transcribed using random hexamer primers and M-MuLV reverse transcriptase (Fermentas, Pittsburgh, PA, USA). The synthesized complementary DNA was used for polymerase chain reaction (PCR) amplification, and real-time PCR was performed. The Thermal Cycler Dice Real-Time PCR System (Takara Bio Inc., Shiga, Japan) was used according to the protocol. The forward and the reverse primers (Cosmogenetech, Seoul, Korea) were as follows: The primers of TNF-α were 5'-CAAGGGACAAGGCTGCCCG-3' and 5'-TAGACCTGCCCGGACTCCGC-3'; The primers of IL-1 β were 5'-TCATGGGATGATGATGATAACCTGCT-3' and 5'-CCCATACTTTAGGAAGACACGGATT-3'; The primers of IL-6 were 5'-TGCTCCTGACAACCACGGCCT-3' and 5'-ACAGGTCTG TTGGGAGTGGTATCCT-3'; The primers of MCP-1 were

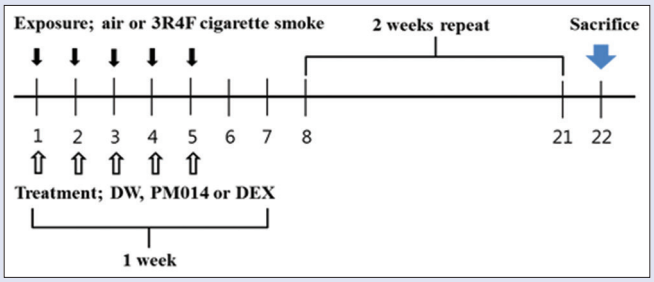

Figure 1: Schedule for cigarette smoke exposure. Twenty female C57BL/6 mice were divided into four groups (n = 5 per group): normal group (air + distilled water), control (cigarette smoke + distilled water), dexamethasone (cigarette smoke + dexamethasone), and PM014 (cigarette smoke + PM014). The mice were exposed to the smoke from three cigarettes for 2 h/day, 5 days/week for 3 weeks

5'-TCACAGTTGCCGGCTGGAGC-3' and 5'-CAGCAGGTGAGT GGGGCGTT-3'; The primers of GAPDH were 5'-TCTGACG TGCCGCCTGGAGA-3' and 5'-TGGGCCCTCAGAYGCCTGCT-3'. A single peak for each reaction for TNF-α, IL-1 β, IL-6, MCP-1, and GAPDH was confirmed by dissociation curve analyses, and 40 cycles of PCR were performed under the following cycle conditions, which were denaturation for 10 s at 95°C, annealing for 10 s at 60°C, and elongation for 12 s at 72°C. After the threshold of the gene of interest was measured three times, the mean cycle threshold was calculated and normalized with the mean cycle threshold of the internal reference gene, GAPDH.

Enzyme-linked immunosorbent assay

According to the manufacturer's instructions, TNF-α, IL-1 β, IL-6, and MCP-1 were measured by an enzyme-linked immunosorbent assay (ELISA) kit (OptELA™ Kits; BD Biosciences, San Diego, CA, USA). In brief, 96-well plates were prepared and coated for 24 h at 4°C with a coating buffer. The buffer contained the antibodies of TNF-α, IL-1 β, IL-6, or MCP-1 as capture antibodies. The plates were washed three times using PBS with Tween-20 (PBS-T20), respectively. They were blocked for 1 h at room temperature using PBS with 10% fetal bovine serum and then washed with PBS-T20. Following to add the standard to the wells with diluted culture media and the plates were incubated for 2 h. After washing, the detection antibodies of TNF-α, IL-1 β, IL-6, and MCP-1 were added to the wells. Subsequently, the plates were incubated for 1 h and washed seven times. Then, the TMB Substrate Reagent (BD Biosciences, San Diego, CA, USA) was added and incubated for 30 min at room temperature. After the reaction was induced, the stop solution (2N H_2SO_4) was added for fixation. The optical density of each well was read on an ELISA plate reader using 450- and 570-nm filters.

Morphometric and histological analysis

The lung was harvested, and the left lung was inflated with fixatives followed by paraffin embedding. For histological examination, the lung was cut in 4-μm thick slices using a rotary microtome (Leica, Nussloch, Germany) and stained with hematoxylin and eosin (H and E). For the quantitating airspace enlargement, the maximum cross-section of parenchyma was selected and the morphometric assessment was conducted using digital image tool.[16] The mean alveolar airspace area was calculated from the sum of the lumen divided by the number of alveoli by Image Pro-plus 5.1 software (Media Cybernetics, Silver Spring, MD, USA).

Statistical analysis

All data were statistically analyzed using Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and are expressed as mean ± standard error of the mean. One-way ANOVA followed by Newman–Keuls *post hoc* test was used for multiple comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of PM014 and dexamethasone on histological damage and air space enlargement in lung tissue

To determine the effects of PM014 and DEX on lung tissue damage caused by CS exposure, we performed a histological examination using H and E staining. Airway space area enlargement meaning the destruction of the lung parenchyma was measured as the mean alveolar air spaces area (μm²). The CON group showed significantly greater airspace enlargement compared with the NOR group, while PM014 and DEX groups showed significantly less airspace enlargement – to a similar extent – compared with the CON group [Figure 2]. These results mean that PM014 and DEX inhibited CS-induced pulmonary alveolar destruction to a similar degree.

Effects of PM014 and dexamethasone on the inflammatory cell count in bronchoalveolar lavage fluid

Because macrophages and neutrophils play an important role in smoking-induced COPD,[17] we investigated their counts in BALF. We found that the total cell, neutrophil, and macrophage counts were significantly higher in the CS group than in the CON group, whereas they were significantly lower in the PM014 and DEX groups than in the CS group [Figure 3a-c]. These results indicated that PM014 exerted anti-inflammatory effects in CS-induced COPD-like lung inflammation, which were similar to those observed in the DEX group.

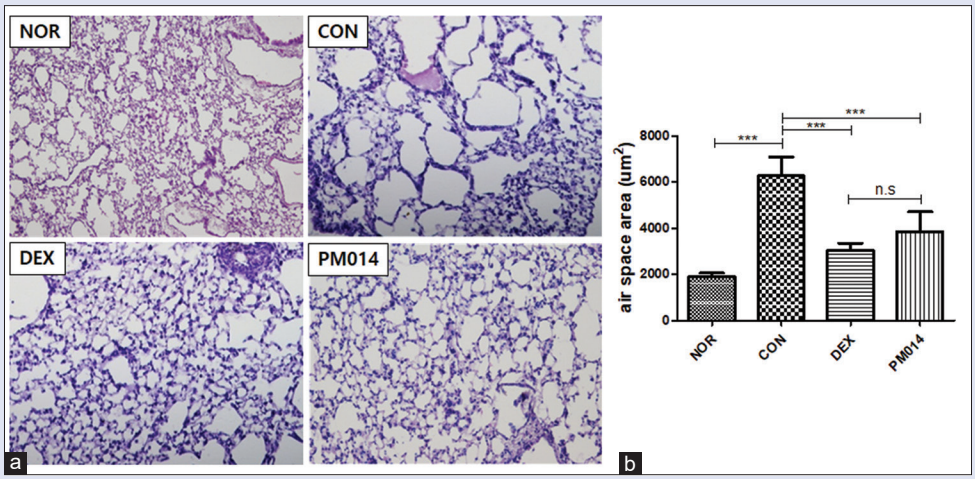

Figure 2: Effects of PM014 and dexamethasone on the histological morphology of lung tissue. Lung tissues were fixed, sectioned at a thickness of 4 μM, and stained with hematoxylin and eosin (H and E solution; magnification, ×100) (a). The evaluation of airspace changes by digital image analysis of H and E stained sections (b). Values are expressed as mean ± standard error of the mean. Statistical analysis was performed using one-way ANOVA followed by Newman–Keuls *post hoc* test. $^{**}P < 0.01$, $^{***}P < 0.001$

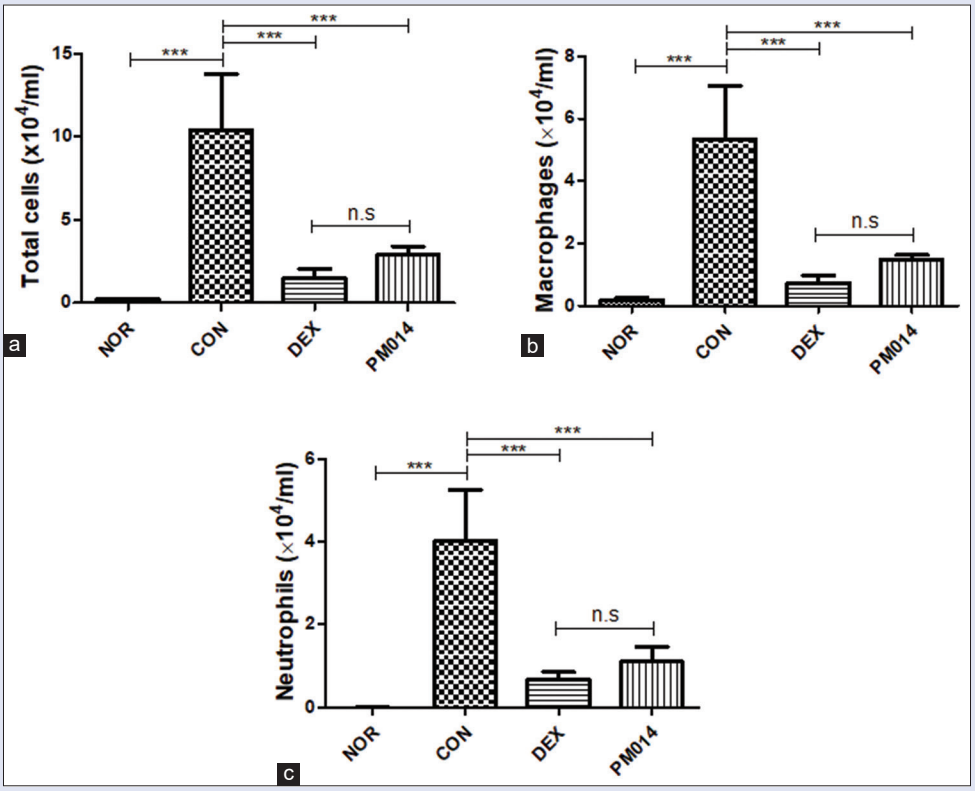

Figure 3: Effects of PM014 and dexamethasone on the inflammatory cell profiles in bronchoalveolar lavage fluid. The numbers of inflammatory cells in bronchoalveolar lavage fluid were measured after 3 weeks of cigarette smoke exposure. The total cell (a), macrophage (b), and neutrophil (c) counts were determined in bronchoalveolar lavage fluid using light microscopy after Diff-Quick staining. Values are expressed as mean ± standard error of the mean. All statistical analyses were performed using one-way ANOVA followed by Newman–Keuls *post hoc* test. ***$P < 0.001$

Effects of PM014 and dexamethasone on mRNA expression of pro-inflammatory cytokines and monocyte chemoattractant protein 1 in lung tissues

To confirm the anti-inflammatory effects of PM014 and DEX, we evaluated mRNA expression in whole lung tissues using real-time PCR. Because TNF-α, IL-1 β, IL-6, and MCP-1 are common inflammatory mediators in the lung, the mRNA expression levels of these mediators were evaluated the degree of pulmonary inflammation. Notably, the mRNA expression levels of TNF-α, IL-1 β, IL-6, and MCP-1 increased significantly in lung tissues from the CS group compared with tissues from the CON group. In contrast, the tissues from the PM014 group showed significantly lower levels of TNF-α, IL-6, and IL-1 β relative to those in the CS group. Tissues from the DEX group were used as positive controls; these showed significant reductions in the inflammatory mediators, similar to the PM014 group [Figure 4].

Effects of PM014 and dexamethasone on the release of pro-inflammatory cytokines and monocyte chemoattractant protein 1 in bronchoalveolar lavage fluid

To determine the anti-inflammatory effects of PM014 and DEX at the protein level, ELISA analysis was performed on BALF from CS-exposed mice. This analysis revealed that significantly higher levels of TNF-α, IL-1 β, IL-6, and MCP-1 protein were released in the CS group than in the CON group. A number of pro-inflammatory mediator proteins showed reductions in the PM014 group. In addition, PM014 decreased significantly the protein expressions of TNF-α, IL-1 β, IL-6, and MCP-1 compared with CS. However, the degree of reduction was similar between PM014 and DEX [Figure 5].

DISCUSSION

In the current subacute mouse model of CS-induced COPD, anti-inflammatory effects in groups treated with 100 mg/kg of PM014 were similar to those observed in groups treated with 1 mg/kg of DEX. PM014 reduced the total cell, macrophage and neutrophil numbers in BALF, inhibited lung injury and reduced mRNA levels and protein levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and a pro-inflammatory chemokine (MCP-1), similar to DEX.

CS exposure causes an inflammatory response in the lungs, which results in a variety of chronic lung disorders, including COPD.[4] The most important mechanism in the inflammatory response is the collection of numerous innate and adaptive immune cells from the blood; these include leukocytes, lymphocytes (neutrophils), macrophages, and dendritic cells. The inflammatory cells infiltrate the airways and lung parenchyma, eventually inducing emphysematous destruction of the structure and function of alveoli.[18] In the present study, 3 weeks of CS exposure-induced COPD-like lung injuries, including airway destruction and increased inflammatory cells, as in previous studies.[19-21]

Since COPD was first identified approximately 10 years ago, oral corticosteroids have been widely used to control it.[22,23] However, long-term oral steroid treatment for stable COPD has been reported to cause a deterioration of respiratory and peripheral muscle strength and pulmonary function, as well as comorbidities, such as diabetes,

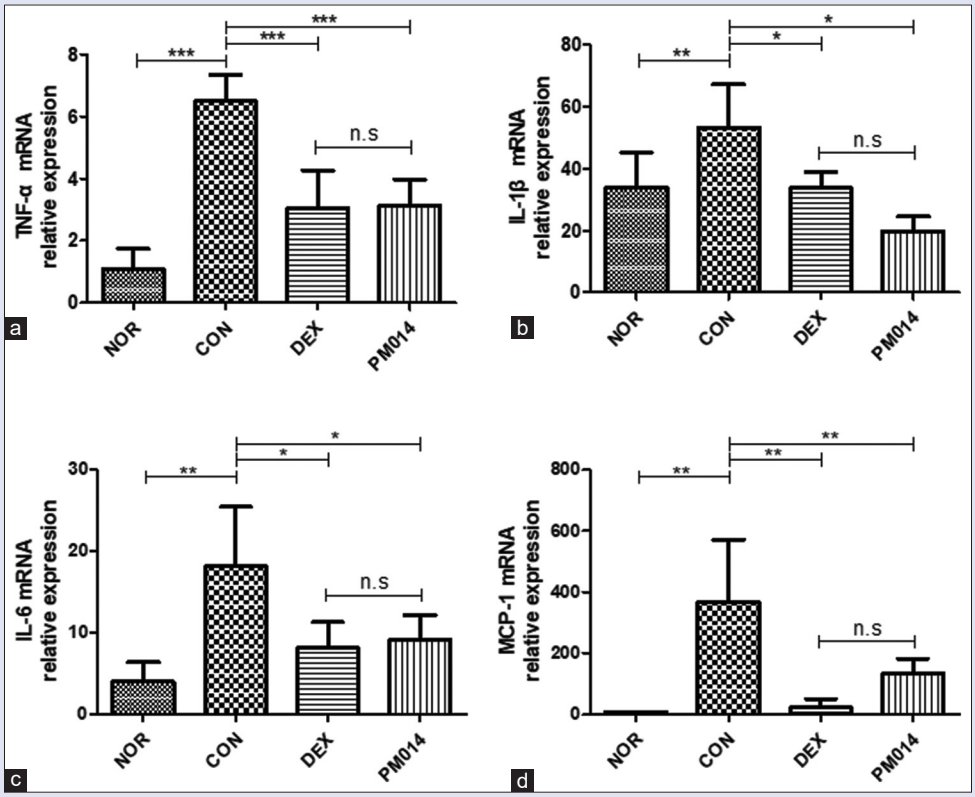

Figure 4: Effects of PM014 and dexamethasone on the mRNA expression of pro-inflammatory cytokines and monocyte chemoattractant protein 1 in lung tissues. mRNA levels of tumor necrosis factor-alpha (a), interleukin-6 (b), interleukin-1 β (c), and monocyte chemoattractant protein 1 (d) were measured by a real-time polymerase chain reaction in the lung tissues. The results are shown as fold changes in expression relative to GAPDH, a reference gene. Values are expressed as mean ± standard error of the mean. Statistical analysis was performed using one-way ANOVA followed by Newman–Keuls *post hoc* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

hypertension, and osteoporosis,[24] and increased mortality;[25] thus, the oral corticosteroid is recommended for use only in patients with an acute exacerbation of COPD.[26] Accordingly, there has been a demand for the development of other anti-inflammatory medicines that could substitute for, or enhance treatment with, inhaled steroid. The anti-leukotriene agent, montelukast, as a representative example, is widely used[27] and has shown anti-inflammatory efficacy similar to that of DEX.[28] Notably, DEX is a synthetic glucocorticoid that has shown an anti-inflammatory effect. It was reported to inhibit release of granulocyte-macrophage colony-stimulating factor from monocytes,[29] U937 monocytic cells,[30] and porcine alveolar macrophages;[31] it was also reported to decrease *in vitro* release of IL-8, a chemokine for attraction and activation of neutrophils, from human airway smooth muscle cells[32] and human airway epithelial cells.[33] Furthermore, DEX has been shown to induce transcription of IκBα, the cytoplasmic inhibitor of NF-κB,[34,35] thereby suppressing the expression of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-8,[36] and cell adhesion molecules involved in the migration of monocytes into the extravascular space, including MCP-1.[37] Based on these mechanisms, DEX has been used as a positive control in various anti-inflammatory studies involving CS exposure.[38-40]

The anti-inflammatory effect of PM014 has been reported in various lung inflammation models.[12-15] A standardized herbal formula, PM014, contains seven species of medicinal plants, some of which have been studied for their anti-inflammatory effects. Hong *et al.*[41] Showed that *Paeonia suffruticosa* inhibited inflammatory responses by regulating the nuclear factor-κB/IκB signaling pathway. The roots of *Stemonae sessilifolia* have long been used to relieve cough, tuberculosis, and bronchitis; the main alkaloids of this plant have shown antitussive activity in a guinea pig cough model.[42] *Rehmannia glutinosa* has an effect on nitric oxide inhibitory activities.[43] The major components of *Scutellaria baicalensis* include baicalin, baicalein, and wogonin, which are bioactive flavones. Baicalin has been reported to ease inflammation in a model of CS-induced inflammation.[44,45] The effects of PM014 and its individual constituent herbs on lung inflammation were investigated in a previous study, in which PM014 elicited the reduction of immune cell recruitments in the lungs compared with individual herbs.[12]

Histological changes associated with COPD include enlargement of alveolar airspaces due to destruction of alveolar septa, as well as an increase in the number of airspaces. Thus, airspace enlargement is used as a criterion for determining the severity of emphysematous changes.[46,47] In the present study, PM014 attenuated CS-induced airspace enlargement to a similar extent compared to DEX, which suggests that both agents prevented alveolar destruction in a similar manner.

Macrophages and neutrophils play important roles in CS-induced lung inflammation.[48] Macrophages, the first line of defense of the lung against foreign bodies, mediate the inflammatory process through the release of chemokines that attract various immune cells, including neutrophils.[49,50] Neutrophils migrate to the airway and are activated by chemotactic factors.[51,52] Then activated neutrophils and macrophages cause lung destruction by secretion of various proteolytic enzymes, such as neutrophil elastase, matrix metalloproteinases (MMPs), including MMP-8, MMP-9, and MMP-12 (macrophage elastase), and numerous lung-damaging pro-inflammatory cytokines and chemokines.[5,53,54]

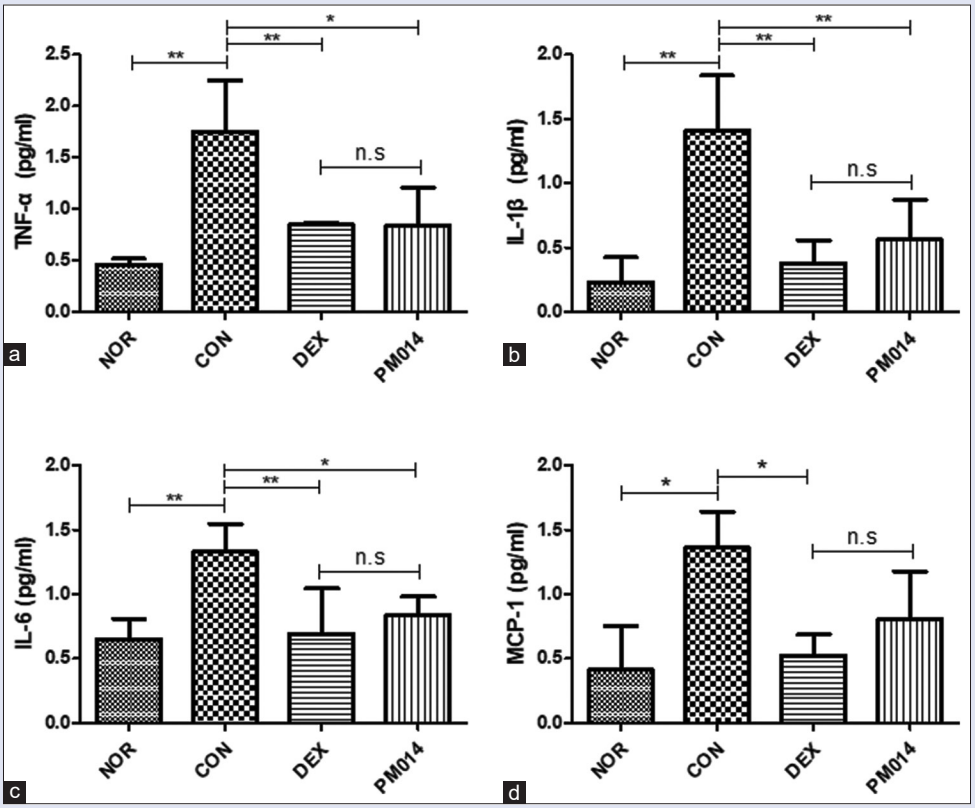

Figure 5: Effects of PM014 and dexamethasone on protein levels of pro-inflammatory cytokines and monocyte chemoattractant protein 1 in bronchoalveolar lavage fluid. Protein levels of tumor necrosis factor-alpha (a), interleukin-6 (b), interleukin-1 β (c), and monocyte chemoattractant protein 1 (d) in bronchoalveolar lavage fluid were measured by enzyme-linked immunosorbent assay. Values are expressed as mean ± standard error of the mean. Statistical analysis was performed using one-way ANOVA followed by Newman–Keuls *post hoc* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

There have been several reports showing that increasing macrophages and neutrophils in BALF or sputum were correlated with COPD severity in human and murine models.[20,55-57] In the present study, PM014 and DEX showed similar effects in reducing macrophages and neutrophils in BALF.

Various cytokines play crucial roles in the pathological progression of COPD. TNF-α, IL-1β, IL-6, and MCP-1 have been suggested as underlying causes of lung inflammation and COPD development.[48] With respect to the relationships between these cytokines and CS, the levels of TNF-α, IL-1 β, IL-6, IL-8, and MCP-1 are known to be increased in the BALF of chronic smokers.[58] TNF-α is an important chemotactic protein for neutrophils;[59] it may activate airway inflammatory cells to release inflammatory mediators through the upregulation of adhesion-molecule expression, thus enhancing the migration of inflammatory cells into the lower airways and activating profibrotic mechanisms involved in airway remodeling.[60] TNF-α is also believed to play a key role in CS-induced lung inflammation;[61] notably, CS exposure induces TNF-α overexpression in lung tissue, which stimulates the secretion of MMPs from macrophages, resulting in an emphysematous change in the alveoli.[62] Clinical analyses of COPD patients have shown that TNF-α levels are increased in sputum and blood.[63] IL-1 β is another important pro-inflammatory cytokine in COPD, and CS-induced emphysematous changes[64] increased IL-1 β has been observed in the BALF and sputum of smokers[65,66] and is inversely correlated with pulmonary function in COPD patients,[67] but directly correlated with the severity of COPD.[68] IL-6 is a pleiotropic cytokine that regulates the immune system and has been associated with the progression of COPD severity;[69] increased serum IL-6 has been reported in COPD patients[70] and was a predictive factor of mortality in COPD.[71,72] Monocyte recruitment to the lungs is an important step in the progression of COPD: monocytes release many macromolecules that mediate inflammation.[53] In addition, MCP-1 is an important chemokine that regulates migration and infiltration of monocytes and macrophages;[73] levels of MCP-1 were found to be increased in the BALF of COPD patients and the sputum of smokers.[74] The above pro-inflammatory cytokines influence each other during the progression of inflammation in COPD.[75,76] In particular, it is well-known that IL-1 β and TNF-α promote the production of each other; this is because their signaling pathways are similar.[77] Therefore, IL-1 β induces the production of TNF-α from alveolar macrophages[78] and the CS-induced increase in TNF-α is blocked by the inhibition of IL-1 β.[79] In addition, pro-inflammatory agonists induce increases in IL-1 β and IL-6,[80] and IL-6 and MCP-1 are released concomitantly from the monocytes of COPD patients on stimulation with lipopolysaccharide.[81] Therefore, blockade of various inflammatory pathways and mediators, including inhibition of the cytokines and chemokines assessed in the present study, may constitute an ideal method to prevent COPD.[82] In the present study, PM014 and DEX induced similar reductions in the protein and mRNA expression levels of pro-inflammatory cytokines and chemokines.

CONCLUSION

In the present study, PM014 demonstrated anti-inflammatory efficacy similar to that of DEX in a mouse model of subacute CS-induced COPD. Previous studies have shown that PM014 is effective in various lung inflammation models; the results of the present study suggest that PM014

could be used as an adjunctive agent or substitute for oral steroids in COPD patients. Future studies should include assessments of underlying molecular mechanisms; furthermore, clinical studies of side effects and potential interactions with inhaled steroids are needed.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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