Effects of Chung-Pae Inhalation Therapy on a Mouse Model of Chronic Obstructive Pulmonary Disease

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Chung-pae (CP) inhalation therapy is a method frequently used in Korea to treat lung disease, especially chronic obstructive pulmonary disease (COPD). This study investigated the effects of CP inhalation on a COPD animal model. C57BL/6 mice received porcine pancreatic elastase (PPE) and lipopolysaccharide (LPS) alternately three times for 3 weeks to induce COPD. Then, CP (5 or 20 mg/kg) was administered every 2 h after the final LPS administration. The effect of CP was evaluated by bronchoalveolar lavage (BAL) fluid analysis, histological analysis of lung tissue, and reverse transcription polymerase chain reaction analysis of mRNA of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-6, and tumor growth factor-β (TGF-β). Intratracheal CP administration reduced the number of leukocytes and neutrophils in BAL fluid, inhibited the histological appearance of lung damage, and decreased the mRNA levels of the proinflammatory cytokines IL-1β, TNF-α, IL-6, and TGF-β. Intratracheal CP administration effectively decreased the chronic inflammation and pathological changes in a PPE- and LPS-induced COPD mouse model. Therefore, we suggest that CP is a promising strategy for COPD.

1. Introduction

Inhalation therapy is a treatment technique for administering a variety of inhalable drugs to target lung tissue, airway secretion components, and microorganisms in the upper, central, and/or peripheral airways [1]. Such therapy is used widely to treat chronic obstructive pulmonary disease (COPD) in the respiratory tract [2]. Inhalation administration has an advantage over oral administration for treating respiratory disease in that it allows rapid and substantial drug absorption and has fewer side effects [3]. Typically, herbal medicines are administered orally in the form of decoction or granular extract; however, several studies have reported the direct delivery of herbal medicine to the airway via inhalation [4–7]. The current study employed the MicroSprayer, which generates a plume of liquid aerosol (mass median diameter (MMD) of 16–22 μm), enabling the administration of drugs directly to the lung via the trachea [8].

Chung-pae (CP), composed of Ephedrae Herba, Caryophylli Flos, Pogostemonis (Agastachis) Herba, and Zingiberis Rhizoma Crudus, is a representative aerosol agent used in the respiratory clinic at Kyung Hee Oriental Medicine Hospital, Seoul, Korea, for relieving the symptoms of patients with dyspnea and cough. Previously, we investigated the effect of intratracheal (i.t.) CP administration on lipopolysaccharide-(LPS-) induced acute lung injury (ALI) in a mouse model. We found that CP suppressed neutrophil infiltration to the lung and reduced the production of proinflammatory cytokines via decreased expression of the proinflammatory transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and activation of the anti-inflammatory factor, nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2) [7].

The current study investigated the activities of CP on chronic lung injuries including COPD. COPD was selected for the study because it is a prevalent chronic respiratory
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disease that has become a major public health problem [9].
LPS and porcine pancreatic elastase (PPE) were used to
induce COPD in a mouse model. Long-term administration
of LPS by inhalation induces emphysema [10–12], and PPE
augments the emphysematous changes that are critical char-
acteristics of COPD [13, 14].
In the present study, the effect of CP on chronic lung
injury was evaluated in a mouse model of COPD generated
using LPS and PPE. The effect of CP was assessed by analyz-
ing bronchoalveolar lavage (BAL) fluid, lung histology, and
mRNA levels of proinflammatory cytokines.

2. Materials and Methods

2.1. Preparation of Chung-Pae Water Extract (CP). CP was
prepared as described previously [7]. Briefly, 20.0 g Ephed-
rae, 20.0 g Ephedrae Herba, (Agastachis) Herba, 10.0 g Cary-
ophylli Flos, and 10.0 g Zingiberis Rhizoma Crudus were
boiled in 1L distilled water for 2h. The mixture was
centrated to 50 mL with a low-pressure evaporator and then
freeze-dried to yield 6.0 g of powder.

2.2. Animals. Male C57BL/6 mice were supplied by Orient
Bio Inc. (Seongnam, Korea) and were bred in a pathogen-free
facility at Pusan National University, Yangsan, Korea. Ani-
mals were housed in certified standard laboratory cages and
fed with food and water ad libitum prior to the experiments.
All experimental procedures were approved by the Guidelines
of the Institutional Animal Care and Use Committee of Pusan
National University, Busan, Republic of Korea (protocol
number: PNU-2010-00028).

2.3. COPD Mouse Model and Treatment. COPD was induced
in mice using the method reported previously with some
modifications [15]. A MicroSprayer (syringe assembly, MSA-
250-m, the PennCentury Inc., PA, USA) was used to deliver
all materials to the lungs via i.t. Mice (20–30 g) were exposed
to 0.25 U of PPE (on days 1, 7, and 14) and 70 μg of LPS (on
days 4, 11, and 18) for three consecutive weeks. In this manner,
the treated mice received PPE and LPS alternately. Two doses
of CP (low dose of 5 mg/kg and high dose of 20 mg/kg)
in 25 μL of PBS were administered 2h after every LPS
administration. The vehicle-treated group was treated with
25 μL of PBS using the same method and treatment schedule
as the CP-treated group. Normal, untreated non-COPD mice
were included as a control in the analyses.

2.4. BAL Fluid Analysis. BAL fluid analysis was conducted on
day 21. BAL was obtained using two consecutive instillations
of PBS (1.0 mL) using a 24-gauge intravascular catheter.
The total cell number was determined using a hemocytometer.
Macrophages, lymphocytes, and neutrophils were counted by
Hemacolor (Merck, Darmstadt, Germany) after centrifuga-
tion and staining; 100 cells were counted for each microscopic
field, and the mean number of cells per field was reported.

2.5. Lung Histological Analysis. Mice were perfused with
saline and the whole lung was inflated with fixatives. After
paraffin embedding, lung tissue were cut in 5-μm thick slices
and stained with hematoxylin and eosin (H&E). Three
separate H&E-stained sections were evaluated in each mouse
under a microscope using 100x magnification.

2.6. Isolation of Total RNA from Tissue and Reverse-Trans-
scription-Polymerase Chain Reaction (RT-PCR). Total RNA
was isolated with the QIAGEN RNeasy mini kit (Qiagen,
Hilden, Germany) according to the manufacturer’s instruc-
tions. Two micrograms of total RNA were reverse-transcribed
by M-MLV reverse transcriptase (Promega, Madison, WI,
USA), and single-stranded cDNA was amplified by PCR
using specific primers. The forward and the reverse primers
for interleukin- (IL-) 1β were 5’-TCATGGGATGATGAT-
GATAACCTGCT-3’ and 5’-CCCATCTTTAGGAAGAC-
ACGGATT-3’, respectively; the primers for tumor necrosis
factor- (TNF-) α were 5’-GGCAGGTCTACTTTGGAG-
TCATTGC-3’ and 5’-ACATTGAGGCTCCAGTGAT-
TGG-3’, respectively; the primers for IL-6 were 5’-CTG-
GTGCAACACGGGCCCTTCTA-3’ and 5’-ATGCTT-
AGGCATACGCACTAGGT-3’, respectively; the primers
for tumor growth factor- (TGF-) β were 5’-GGCGAGCT-
GTACATCGACT-3’ and 5’-ACTGTGTGTCAGGCTCC-
AA-3’, respectively; and the primers for glyceraldehyde-
3-phosphate dehydrogenase (GAPDH) were 5’-GGAGCC-
AAAAGGTATCAT-3’ and 5’-GTATGGCATGACT-
GTTGTT-3’, respectively. For PCR amplification, TaqPCRx
DNA polymerase recombinant (Invitrogen, Carlsbad, CA,
USA) was used according to the manufacturer’s protocol. The
reaction conditions were as follows: initial denaturation at
95°C for 5 min followed by 22–30 cycles of denaturation for
40 sec at 95°C, annealing for 40 sec at 57°C, and extension
for 50 sec at 72°C with a final extension for 7 min at 72°C. Ampli-
cons were separated in 1.2% agarose gels in boric acid buffer
at 100 V for 30 min, stained with ethidium bromide, and
visualized under UV light. GAPDH was used as an internal
control to evaluate the relative expressions of IL-1β, TNF-α,
IL-6, and TGF-β.

2.7. Statistical Analysis. Group comparisons were performed
using one-way analysis of variance (ANOVA) with Duncan’s
post hoc test. The analysis was conducted using SPSS 18.0
for Windows (SPSS, Chicago, IL, USA). p values < 0.05
were considered to indicate significant differences. All experiments
were performed independently at least three times.

3. Results

3.1. Effect of CP on the Total Cell Count and Inflammatory Cell
Numbers in the BAL Fluid of PPE- and LPS-Induced COPD
Mice. The total cell and neutrophil counts in the BAL fluid
of PPE- and LPS-induced COPD mice increased significantly
compared to those in the normal group (p < 0.01, Figures
I(a) and I(b)). CP treatment significantly decreased the total
cell and neutrophil counts in the BAL fluid compared to
the vehicle-treated group (p < 0.05, Figures I(a) and I(b)).
However, no difference was detected between groups treated
with 5 or 20 mg/kg CP.
The macrophage population in the vehicle-treated group
increased significantly compared to that in the normal group
Figure 1: Effect of CP on the total cell number (a), number of neutrophils (b), and number of macrophages (c) in the BAL fluid of PPE- and LPS-induced COPD mice. Data are presented as means ± SEM (n = 5). Letters (A–C) indicate different levels of significance (95% level, Duncan’s test).

4. Discussion

In the current study, i.t. administration of CP to PPE- and LPS-induced COPD mice reduced the number of leukocytes and neutrophils in the BAL fluid, inhibited lung injury, and decreased the mRNA levels of the proinflammatory cytokines IL-1β, TNF-α, IL-6, and TGF-β.

In our clinic, patients usually received CP at a daily dose of 5 mg/kg; however, long-term administration of 20 mg/kg of CP for 3 weeks showed no adverse effect on vital organs, including the liver and kidney (data not shown). Therefore, in this study, we administered CP at doses of 5 or 20 mg/kg in the PPE- and LPS-induced COPD mice. Infiltration of inflammatory cells in the BAL fluid was observed in the PPE- and LPS-induced COPD mice. Subsequent i.t. administration of CP reduced the total number of infiltrating cells, especially neutrophils, suggesting that CP could inhibit neutrophils, the most deleterious inflammatory mediator in COPD. However, CP did not significantly decrease the macrophage number compared to the vehicle-treated group (42.3% versus 37.6%); this result was consistent with a previous study [7].
COPD is characterized mainly by increased levels of activated neutrophils, macrophages, and T-lymphocytes [16]. Macrophages mediate inflammation in COPD through the release of chemokines that attract neutrophils, monocytes, and T-cells [17]. Neutrophils are key mediators of COPD, as they migrate to the airway under the control of chemotactic factors and become activated [18, 19]. Activated neutrophils secrete proteolytic enzymes that can induce emphysema as well as numerous lung-damaging, proinflammatory cytokines and chemokines (e.g., matrix metalloproteinase (MMP-) 8, 9, and 12) [20–22]. Moreover, increased numbers of neutrophils in the airway lumen and BAL fluid in individuals with COPD are correlated with disease severity [23, 24].

The COPD model used in this study involved the inhalation of LPS and elastase to induce emphysematous change [14, 25]. Generally, emphysema is induced by a proteolytic-antiproteolytic imbalance. Proteolytic enzymes may augment the inflammatory cell influx into airspaces, which causes destruction of alveolar septa and increased airspaces [10, 26]. Thus, air space enlargement is a criterion used for measuring the severity of emphysematous change [10, 27, 28]. In the current study, CP reduced the vacuole size compared to that in the vehicle group, suggesting that it prevented alveolar destruction. Histological analysis of lung tissue showed increased cell and neutrophil numbers in the BAL fluid.

Numerous cytokines play important roles in the pathological processes of COPD through the recruitment, activation, and survival of inflammatory cells. TNF-α and IL-1β have long been known to be classical proinflammatory cytokines that contribute to the development of COPD [29–31]. IL-6 is stimulated by TNF-α and IL-1β and also plays a critical role in the pathogenesis of emphysematous change [32]. These proinflammatory cytokines influence one another and amplify the inflammatory response in COPD [16, 33]. TGF-β, a profibrotic cytokine, is one of the main mediators involved in tissue remodeling in the lungs and contributes to architectural changes in the lungs in COPD [34–37]. The blocking of TGF-β improves emphysematous changes [38, 39], although a low concentration of activated TGF-β is required to maintain alveolar homeostasis and prevent the development of emphysema [40, 41]. Therefore, inhibition of proinflammatory cytokines is one of the most promising treatments for COPD [42]. In this study, CP reduced the mRNA levels of these cytokines in the lung, suggesting the suppression of chronic inflammation and pathological changes as well as the associated neutrophil infiltration in the lung.

5. Conclusion

Previously, we have demonstrated the therapeutic effect of i.t. CP administration on ALI [7]. The current study provides experimental evidence that long-term administration of CP has a therapeutic effect on chronic lung injury in a COPD mouse model induced by PPE and LPS. The anti-inflammatory effect exerted by i.t. CP administration suggests that it could be a new therapeutic formula and that inhalation
of herbal medicine can be a promising strategy for treatment of COPD.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**Authors’ Contribution**

Myungsoo Joo and Sung-Ki Jung designed research and contributed to the editing of the paper. Joon-Ho Hwang and Beom-Joon Lee contributed equally as first authors. All authors read and approved the final paper.

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