Original Research

Extract of *Bambusae Caulis in Taeniam* inhibits cigarette smoke-induced pulmonary and intestinal inflammation

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Abstract

The pulmonary and intestinal systems have several characteristics in common. It is believed that these similarities somehow function to cause pulmonary–intestinal crosstalk during inflammation. Many studies have shown that pulmonary disease occurs in association with inflammatory bowel disease more often than is commonly recognized. *Bambusae Caulis in Taeniam*, a medicinal herb originated from the inner bark of *Phyllostachys nigra var. henosis* (Milford) Rendle (Poaceae), has been used to cure fever, diarrhea, and chest inflammation in Korea as well as in China. Cigarette smoke is a well-known risk factor for several inflammatory disorders. In this study, we induced pulmonary and bowel inflammation in mice using cigarette smoke and investigated whether *Bambusae Caulis in Taeniam* extract modulates the inflammatory response in both the lung and the bowel. C57BL/6 mice were exposed to cigarette smoke for 90 min per day for three weeks, and *Bambusae Caulis in Taeniam* extract was administered via oral injection 2 h before cigarette smoke exposure. The bronchoalveolar lavage cells were counted and hematoxylin and eosin staining were performed. Levels of inflammatory mediators in lung and large intestine were determined by enzyme-linked immunosorbent assay, real-time polymerase chain reaction, and Western blotting. Our results showed that *Bambusae Caulis in Taeniam* attenuated cigarette smoke-induced inflammatory response in both the lung and the bowel of mice by inhibiting the production of pro-inflammatory cytokines, chemokines, and protease as well as NF-κB signaling factor. Therefore, we suggest that *Bambusae Caulis in Taeniam* extract might be a candidate therapeutic agent for inhibiting pulmonary and intestinal inflammation.

Keywords: *Bambusae Caulis in Taeniam*, cigarette smoke, intestinal inflammation, NF-κB, pulmonary inflammation

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Introduction

Cigarette smoke (CS) contains more than 4000 deadly chemicals, of which hundreds are toxic and nearly 70 are carcinogenic. Accordingly, CS can induce major health problems and diseases, which may lead to death in severe cases. Although it is well recognized that CS has adverse effects on the respiratory system, little is known about the harmful nature of CS in the intestinal bowel. Accumulating evidence suggests a detrimental effect of CS on the gut by showing a strong correlation between CS and bowel inflammation.[1–4] Therefore, CS is thought to be a major risk factor for inflammation in both the lung and the intestine. CS affects the generation of cytokines and chemokines and induces uncontrolled protease productions, which are possible mechanisms of respiratory–intestinal crosstalk.[5–7]

Inflammation in the lung is prevalent worldwide and is therefore an important ongoing multinational research topic.[8] Pulmonary inflammation is characterized by activated pro- and anti-inflammatory cytokines such as TNF-α, IL-6, IL-1β, and MCP-1 that cause cell damage in the lung.[9,10] Intestinal inflammation is characterized by an excessive gut inflammatory response that affects the gastrointestinal tract and has shown an increasing incidence over the last 50 years.[11,12] Recent studies showing a remarkable upsurge of tissue levels of TNF-α, IL-6, IL-1β, and MCP-1 during inflammatory bowel disease (IBD) have supported the significance of these factors during intestinal inflammation. The marked increase in cytokine and chemokine levels led to the hypothesis that intestinal epithelial cells might provide early signals to immune cells in the mucosa during inflammation.[13–15]

Although several factors such as cytokines are known to be associated with the pathogenesis of pulmonary and intestinal inflammation, no cure for this inflammation has

yet been developed and continuing treatment is required for patients.[5] Despite clinical acceptance that pulmonary inflammation and intestinal inflammation are linked, there are few research studies that explain their association.[16–19]

The pulmonary and intestinal systems have several characteristics in common. It is believed that these similarities somehow function to cause pulmonary–intestinal crosstalk during inflammation. Many clinical studies of Western populations have shown that pulmonary disease occurs in association with IBD more often than is commonly recognized. Interestingly, the Dongui-Bogam, which was published about 500 years ago in Korea, proposes a specific linkage theory between lung and large intestinal diseases and suggests several medicinal herbs for their treatment.

Bambusae Caulis in Taeniam (BC), a medicinal herb originated from the inner bark of *Phyllostachys nigra var. henosis* (Milford) Rendle (Poaceae), has shown many pharmacologic activities. The material media of past dynasties in Chinese history indicated that BC was used to treat fever, diarrhea, and chest inflammation.[20–22] Accordingly, Ministry of Health in China approved BC as an ethnopharmacological functional food material. Other study demonstrated BC's anti-inflammatory effects with anti-oxidative properties.[22] Our previous study showed the efficacy of BC in the OVA-induced asthma model.[23]

In our present study, we induced and identified pulmonary and intestinal inflammatory response using a cigarette smoking murine model and investigated whether BC modulates smoke-induced inflammation in both the lung and the bowel. We also investigated both the inhibitory activities of BC on the production of pro-inflammatory cytokines and chemokines, as well as its activation of the NF-κB signal transduction pathway, which is known to be involved in the regulation of the inflammatory response.

Materials and methods

Preparation of BC extracts

The inner bark middle layer of BC was purchased from Omniherb Co. (Daegu, Korea) and its identity was confirmed by one of the authors (Y.P. Jang). The voucher specimen (KHOP00315) was deposited at Kyung Hee Korean Traditional Herbal Medicine Museum of College of Pharmacy, Kyung Hee University. Extraction was performed according to the method described previously.[23]

High-performance liquid chromatography analysis of BC extracts

High-performance liquid chromatography (HPLC) grade acetonitrile was purchased from J. T. Baker (NJ, USA), and analytical-reagent grade acetic acid was obtained from Wako (Osaka, Japan). Caffeic acid, ferulic acid, and *p*-coumaric acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The total extract was dissolved at a concentration of 10 mg/mL in 50% (v/v) methanol aqueous solution. As a standard, *p*-coumaric acid was dissolved at 1 mg/mL in methanol and then diluted 5-, 10-, 20-, and 40-fold. All samples and standard solutions were filtered through a 0.45-μm syringe filter (Millipore, MA, USA). HPLC analysis was performed on a Waters system (Milford, MA, USA) equipped with a Waters 996 photodiode array detector running Empower software. A Capcellpak (Shiseido, Tokyo, Japan) C18 column (250 mm × 4.6 mm, i.d. 5 μm) was selected for the analysis of BC extract. The UV chromatogram was monitored at 280 nm. The flow rate was 0.8 mL/min. The mobile phase consisted of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in methanol (solvent B). The gradient program started with 10% B for 5 min, followed by a linear gradient to 30% B in 15 min, isocratic elution to 45 min, and linear gradient to 50% B at 55 min. The injection volume of total extract and standard solutions was 10 μL.

Animals

Specific pathogen-free female C57BL/6 mice (seven weeks old) were purchased from Orient Bio Inc. (Seoul, South Korea). The animals were housed in an air-conditioned room maintained at 24°C with 55% humidity and were provided a standard sterile rodent diet (Purina Mills, St. Louis, MO, USA) with water given ad libitum. All experimental procedures were carried out in accordance with the requirements of the Animal Care and Ethics Committee of Kyung Hee University.

CS exposure and drug treatment

Control group mice ($n = 5$) were exposed to fresh air with distilled water (DW). Experimental groups ($n = 5$) were exposed to CS (Reference Cigarette 3R4F without a filter, University of Kentucky, Lexington, KY, USA) with either DW (CS group), dexamethasone (DEX group, treated with 1 mg/kg of dexamethasone), or BC (BC group, treated with 100 mg/kg of BC extract). Mice were subjected to whole-body exposure to CS in a smoke chamber for three 30-min periods with recovery in a fresh air environment for 1 h between each exposure for five days per week for three weeks. In our experiment, the mice were exposed to the side stream smoke, and each cigarette was completely burned out during the first 1 min due to the pressure generated from an oil-less air pump (M-technology, Incheon, South Korea). In addition, the oil-less pump was set to the inhalation rate of 30 per 1 min. Mice were orally treated with DEX or BC 2 h before exposure to CS. Individual body weights were measured twice a week, and the mice were sacrificed on day 21. These experiments were performed in duplicate. The experimental procedure of CS exposure is described in Figure 1.

Analysis of bronchoalveolar lavage fluid

After the mice were sacrificed, bronchoalveolar lavage fluid (BALF) was collected by infusion and extraction of PBS. Total cell numbers were counted using a hemocytometer, and differential cell counts were performed on slides prepared by cytocentrifugation and Diff-Quick staining (Life Technologies, Auckland, New Zealand) using light microscopy. BALF samples were then centrifuged and the

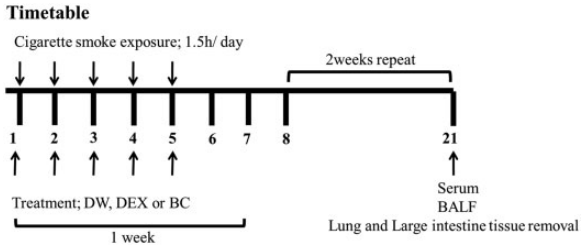

Figure 1 Time course of cigarette smoke exposure. Mice were divided into four groups ($n = 5$/group). CON: fresh air with DW; CS: cigarette smoke (reference cigarettes 3R4F, University of Kentucky, Lexington, Kentucky, USA) with DW; DEX: CS with dexamethasone (DEX, 1 mg/kg, p.o.); and BC: CS with BC extract (BC, 100 mg/kg, p.o.). Mice were subjected to whole-body exposure to CS for 30-min periods in the smoking chamber, after which they rested in a fresh air environment for 1 h. This process was repeated three times for 3 weeks

supernatants were stored at −80°C for measurement of cytokines. Levels of TNF-α, IL-6, and MCP-1 in the BALF were measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (OptELATM Kits; BD Biosciences, San Diego, CA, USA) according to the manufacturer's protocols.

Histological study

Lung and colon tissue was fixed in 10% formaldehyde for 24 h and embedded in paraffin. Tissue sections (4 μm) were stained with hematoxylin and eosin solution (H&E, Sigma-Aldrich, MO, USA) for inflammation score assessment. H&E-stained sections were evaluated using light microscopy at a magnification of 100×. The air space of lung was determined by digital image analysis. Micrographs were obtained using Image Pro-Plus 5.1 software (Media Cybernetics, Inc. Silver Spring, MD, USA).

Collection of colonic tissue samples and analysis of large intestine

The whole colon was pulled out, the surrounding mesentery was detached, and the weight/length ratio of the colon was measured. The large intestine tissue was washed with PBS using a gavage needle attached to a 5 mL syringe and the tissue was stored at −80°C for analysis. Proteins were extracted using T-PER tissue protein extraction reagent (Pierce, Rockford, IL, USA) containing a protease inhibitor cocktail (Roche, Indianapolis, IN, USA). Protein concentrations were determined using the Bradford method. The amount of TNF-α, IL-6, IL-1β, and MCP-1 in the intestinal tissue was measured by ELISA using a commercial kit.

Real-time polymerase chain reaction

Lung and intestinal tissues were lysed using TRIzol reagent (Invitrogen) and total RNA was isolated in accordance with the manufacturer's guidance. The total RNA was reverse transcribed for cDNA synthesis using M-MuLV Reverse Transcriptase (Invitrogen), and the synthesized cDNA was used as a template for polymerase chain reaction (PCR) amplification. mRNA levels of TNF-α, IL-6, IL-1β, MCP-1, and MMP-12 were determined by performing real-time PCR using a Thermal Cycler Dice™ real-time (RT) PCR system (Takara, Katsushika, Japan). The primers used for SYBR Green real-time PCR were as follows: TNF-α, forward, 5′-CAAGGGACAAGGCTGCCCCG-3′ and reverse, 5′-TAGACCTGCCCGGACTCCGC-3′; IL-6, forward, 5′-TGCTGGTGACAACCACGGCCT-3′ and reverse, 5′-ACAGGTCTGTTGGGAGTGGTATCCT-3′; IL-1β, forward, 5′-ACCTGCTGGTGTGTGACGTT-3′ and reverse, 5′-TCGTTGCTTGGTTCTCCTTG-3′; MCP-1, forward, 5′-TCACAGTTGCCGGCTGGAGC-3′ and reverse, 5′-CAGCAGGTGAGTGGGGCGTT-3′; MMP-12, forward, 5′-GGCCATTCCTTGGGGCTGCA-3′ and reverse, 5′-GGGGGTTTCACTGGGGCTCC-3′; and GAPDH, forward, 5′-TCTCAGGTGCCGCCTGGAGA-3′ and reverse, 5′-TGGGCCCTCAGATGCCTGCT-3′ (Cosmogentech Ltd, Seoul, Korea). The specificity of amplification was confirmed by a melting curve with a single peak. Real-time PCR was performed with the following conditions: denaturation at 95°C for 10 s, annealing at 60°C for 10 s, and elongation at 72°C for 12 s. GAPDH was used as the endogenous control for normalization.

Western blotting

NF-κB protein expression in the lung and large intestinal tissue was detected by Western blotting. Proteins (20 μg/lane) were separated by SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. The transblotted membranes were blocked with 0.5% defatted milk in TBS for 1h and were incubated overnight with primary antibodies against β-actin and NF-κB (Santa Cruz Biotechnology, Dallas, TX, USA; diluted 1:1,000 in TBS-T). The blots were washed, and then incubated with secondary antibodies. Blots were washed again, and then developed using an enhanced chemiluminescence Western blot analysis system (AbClon Inc., Seoul, South Korea).

Statistical analysis

Statistical analysis of the data was carried out using Prism 4 software (GraphPad Software Inc., CA, USA). Data are presented as mean ± standard error of the mean (SEM), and multiple comparisons were performed using one-way ANOVA. Results with $P < 0.05$ were considered statistically significant.

Results

HPLC analysis of BC extracts

The HPLC chromatogram of total BC extract is shown in Figure 2. The main peak in the chromatogram (Rt = 38 min) showed an UV maximum absorption at 310 nm. This peak was assumed to be a phenylpropanoid because a simple phenylpropanoid was reported to be a major component of bamboo extract.[24] To identify the peak, standard compounds containing ferulic acid, caffeic acid, and *p*-coumaric acid were injected into the HPLC under exactly the same conditions and their retention time and UV spectra were compared with those of the major peak. In this way, the main peak was identified as *p*-coumaric acid, which was previously reported to be a component of the leaves of this plant.[25]

To quantify the *p*-coumaric acid in the BC extract, a calibration curve was established using a stock solution of *p*-coumaric acid serially diluted to specified concentrations. The coefficients value (R^2) was 0.9997, demonstrating that the linearity in this range was sufficient to provide a highly accurate value of the content in each sample. Precision was determined using a triplicate measurement of each standard, and the relative standard deviations (RSD) were less than 2.4% (Table 1). Using the established calibration curves, the content of *p*-coumaric acid in the extract was calculated to be 4.19 ± 0.46 μg/mg.

BC alleviated weight loss in mice exposed to CS

The body weights of the control (CON) group increased from 17.3 ± 0.5 g to 19.2 ± 0.3 g (10.6% increase) over three weeks. In comparison, the weight of the CS (CS)-exposed group decreased to 16 ± 0.4 g (7% decrease). Compared with the CS group, the body weights of the DEX and BC group were higher at 18.1 ± 0.3 g (6.1% increase) and 18.2 ± 0.72 g (5.2% increase), respectively (Figure 3). The DEX group was used as a positive control.

BC inhibited the infiltration of inflammatory cells into the lung

The number of inflammatory cells in the BALF was examined. The CS group showed a significant increase in the number of total cells, neutrophils, and macrophages compared to the CON group. Compared with the CS group, the DEX group (as a positive control) showed a significant reduction in total cells (84%), macrophages (85%), and neutrophils (84%). Treatment with BC reduced the amount of total cells (85%), macrophages (85%), and neutrophils (86%), respectively (Figure 4). These results demonstrated that CS inhalation induced lung inflammation and treatment with BC inhibited lung inflammation in the CS group.

BC reduced the production of inflammatory cytokines and chemokines in BALF

The amount of TNF-α, IL-6, and MCP-1 in the BALF was evaluated using ELISA. Compared to the CON group, the CS group showed significant increase in the productions of TNF-α, IL-6, and MCP-1. However, in the DEX group, the levels of TNF-α, IL-6, and MCP-1 were significantly

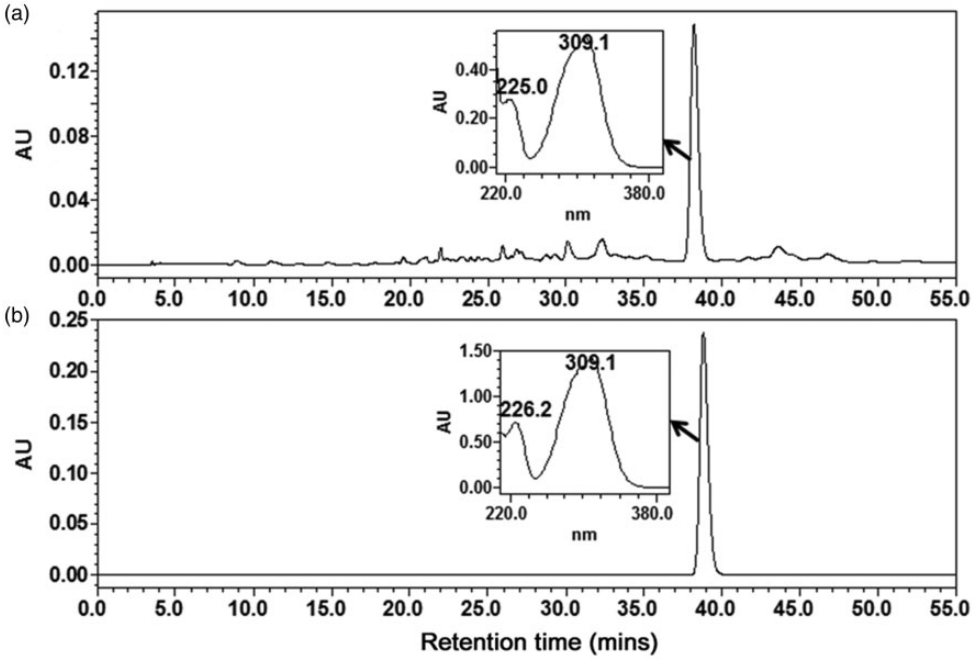

Figure 2 High-performance liquid chromatography (HPLC) chromatogram of the water extract of *Bambusae Caulis* (BC) (a) and p-coumaric acid (b). UV-visible absorption spectra of major peak in the water extract of BC (a) and p-coumaric acid (b) are also shown on the chromatogram

Table 1 The regression data, precision and quantification of *p*-coumaric acid from the total extract of *Bambusae Caulis.*

Compound	Regression equation	R^2	Linear range (μg/mL)	RSD (%) ($n = 3$)	Contents of *p*-coumaric acid in the water extract (μg/mg)
p-coumaric acid	$y = 7 \times 10^7 \times + 401{,}809$	0.9997	35–290	0.07–2.40	4.19 ± 0.46

reduced by 40%, 50%, and 77%, respectively, compared with the CS group. Similarly, the BC group showed significantly reduced levels of TNF-α (38%), IL-6 (46%), and MCP-1 (49%) (Figure 5). These results further demonstrated that treatment with BC inhibited lung inflammation in the CS group.

BC alleviated the CS-induced emphysematous changes

To determine the effects of BC on pathologic changes in lung tissues, we performed histologic examination by H&E staining. The CS group showed mild emphysematous changes with cells infiltration into surrounding the bronchi regions compared to the CON group. In contrast, treatment with DEX or BC significantly alleviated the CS-induced emphysematous changes and cell infiltration in the lungs of CS-exposed mice (Figure 6). The DEX group was used as a positive control. These results provided further evidence that CS inhalation induces lung inflammation that is ameliorated by treatment with BC.

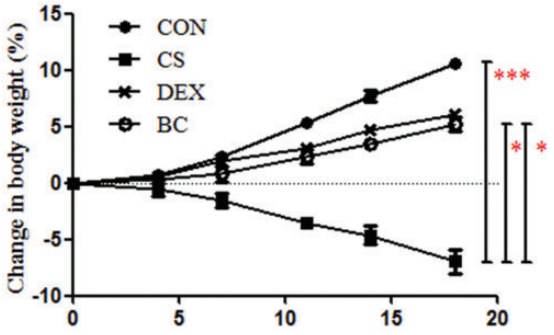

Figure 3 Effect of BC on variation in body weight. Body weights were determined for each group of mice twice a week. Statistical analysis was performed on day 19 after CS exposure. Values are mean ± SEM. Statistical analysis was by one-way ANOVA; $^{*}P < 0.05$ and $^{***}P < 0.001$. (A color version of this figure is available in the online journal.)

BC attenuated CS-induced macroscopic and histopathological changes in the intestine

The colon weight/length ratio was used as a marker of disease-related intestinal wall thickening and intensity of inflammation. Thus, we evaluated colonic inflammation index by measuring the weight/length ratio of the colon.[26] As shown in Figure 7, compared with CON group, CS group colon weight/length ratio was increased, and BC group showed reduction. These results demonstrated that CS exposure induces intestinal wall thickening and BC inhibited CS-induced intestinal wall thickening. Furthermore, the histologic study data supported these results. We performed histologic examination by H&E staining to determine the effects of BC on pathologic changes in colon tissues. The CS group showed a marked thickening of the colonic wall with cells infiltration compared to the CON group. In contrast, treatment with DEX or BC significantly alleviated the CS-induced edematous swelling of the colonic wall and cell infiltration in the colon of CS-exposed mice. The DEX group was used as a positive control. These results provided further evidence

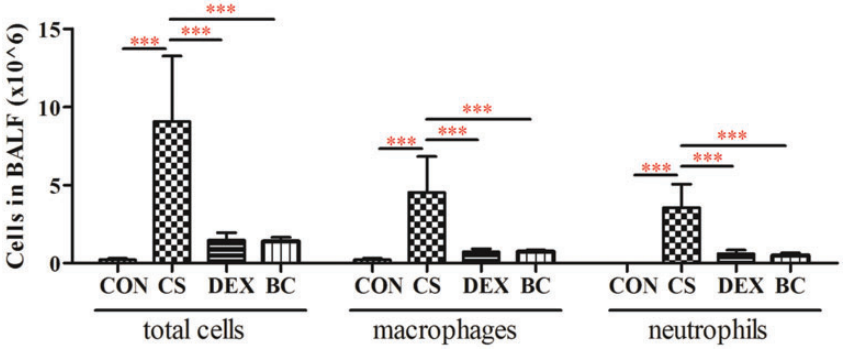

Figure 4 Effects of BC on inflammatory cell infiltration in bronchoalveolar lavage fluid. The amount of inflammatory cells was determined three weeks after CS exposure. Total cells, macrophages, and neutrophils in BALF were counted with light microscopy after Diff-Quick staining. Values are mean ± SEM. Statistical analysis was by one-way ANOVA; $^{***}P < 0.001$. (A color version of this figure is available in the online journal.)

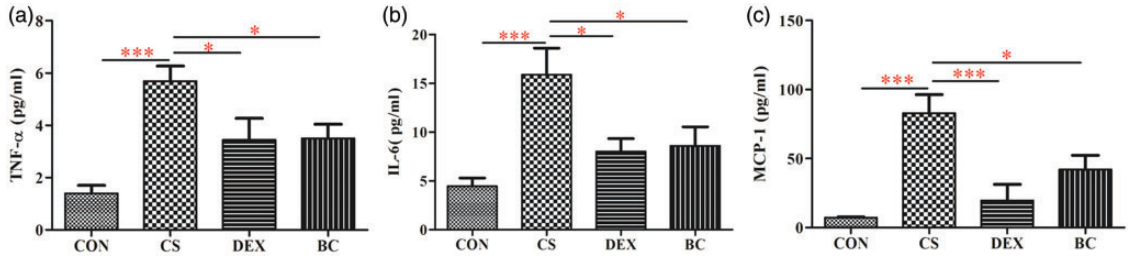

Figure 5 Effects of BC on proteins production of inflammatory cytokines and chemokines in bronchoalveolar lavage fluid. The amount of TNF-α (a), IL-6 (b), and MCP-1 (c) was measured by enzyme-linked immunosorbent assay. Values are mean ± SEM. Statistical analysis was by one-way ANOVA; $^{*}P < 0.05$ and $^{***}P < 0.001$. (A color version of this figure is available in the online journal.)

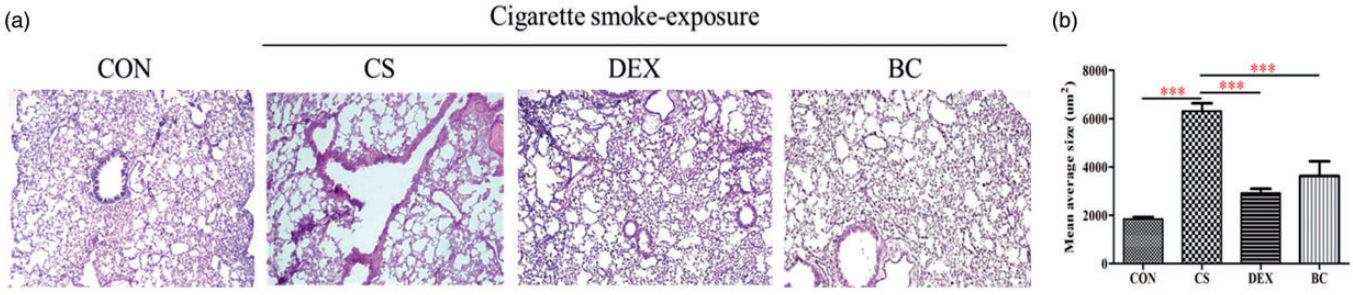

Figure 6 Effects of BC on emphysematous changes. Lung tissue sections (4 μm thickness) were stained with H&E solution (magnification × 100) (a). The air space size was evaluated by digital image analysis (b). Values are mean ± SEM. Statistical analysis was by one-way ANOVA; ***$P < 0.001$. (A color version of this figure is available in the online journal.)

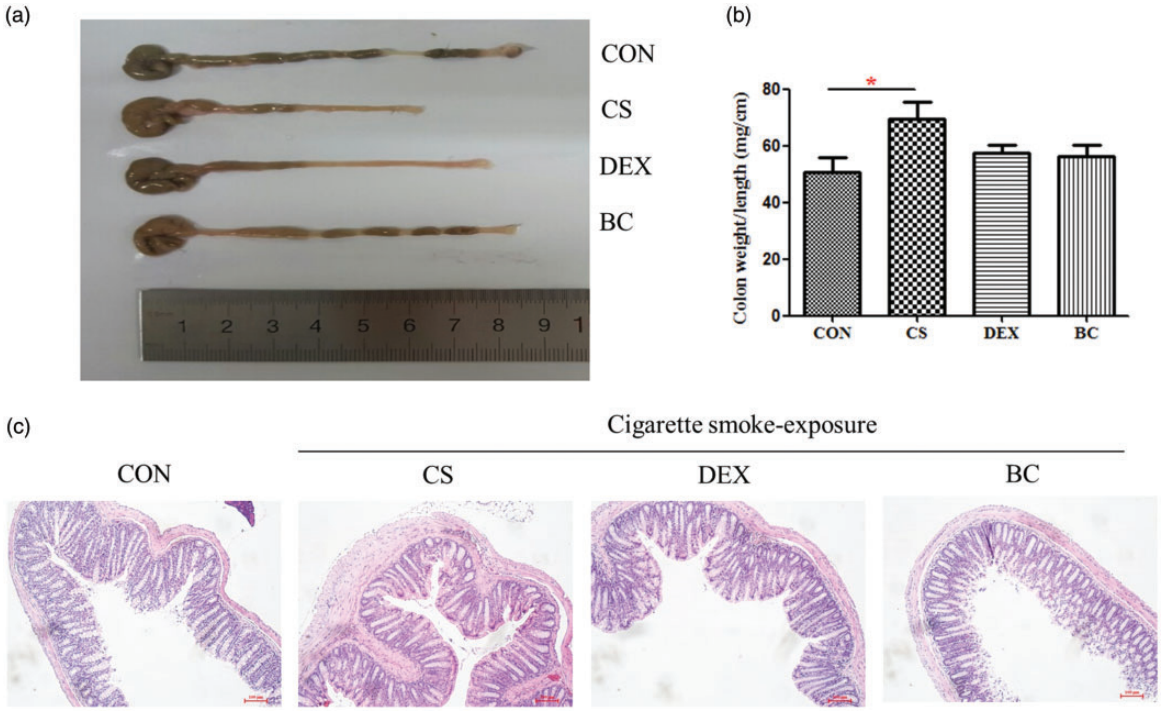

Figure 7 Effect of BC on CS-induced macroscopic and histopathological changes in the colon. Mice were exposed to CS to induce inflammation. The colon length was measured with a ruler under macroscopic examination (a), and the weight/length ratio was depicted in a graph (b). Colon tissue sections (4 μm thickness) were stained with H&E solution (magnification × 100) (c). Values are mean ± SEM. Statistical analysis was by one-way ANOVA; *$P < 0.05$. (A color version of this figure is available in the online journal.)

that CS inhalation induces colonic inflammatory response that is ameliorated by treatment with BC.

BC decreased the secretion of inflammatory cytokines and chemokines in the large intestine tissue

The protein productions of TNF-α, IL-6, IL-1β, and MCP-1 in the intestine were determined by ELISA. As shown in Figure 8, the CS group showed significantly increased levels of these cytokines and chemokine compared to the CON group. In contrast, treatment with DEX as a positive control reduced the levels of TNF-α (74%), IL-6 (91%), IL-1 β (45%), and MCP-1 (85%) compared with the CS group. The BC group similarly showed a reduction in TNF-α (69%), IL-6 (91%), IL-1 β (80%), and MCP-1 (95%) compared to the CS group. These results further demonstrated that CS induces large intestine inflammation and treatment with BC inhibits this inflammatory response.

BC reduced TNF-α, IL-6, IL-1β, MCP-1, and MMP-12 mRNA expression in lung tissue and large intestinal tissue

ELISA results showed that BC reduced inflammatory proteins in the BALF and colon. After that, we confirmed the inhibitory effect of BC on the mRNA expression of inflammatory mediators in the lung and colon tissue together. We evaluated TNF-α, IL-6, IL-1β, MCP-1, and MMP-12 mRNA expression in lung and large intestine by real-time PCR. In the lung tissue, the CS group showed significantly increased mRNA expressions of TNF-α, IL-6, IL-1β, MCP-1, and MMP-12 compared to the control group. Compared

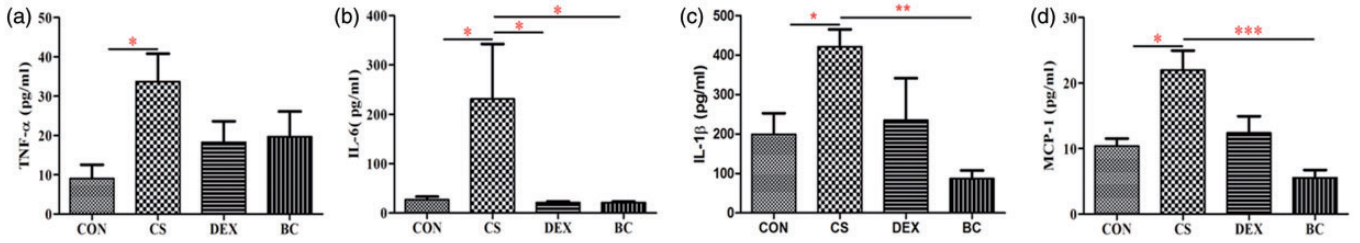

Figure 8 Effects of BC on the protein production of inflammatory cytokines and chemokines in the large intestine. Protein levels of TNF-α (a), IL-6 (b), IL-1β (c), and MCP-1 (d) were evaluated by ELISA. Values are mean ± SEM. Statistical analysis was by one-way ANOVA; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. (A color version of this figure is available in the online journal.)

with the CS group, the DEX group showed a significant reduction in these mRNAs (56%, 50%, 84%, 69%, and 66%, respectively.) The BC group showed significant decrease in mRNA expression (56%, 47%, 58%, 52%, and 58%, respectively). Similarly, the CS group showed significant increase in the levels of TNF-α, IL-6, IL-1β, MCP-1, and MMP-12 mRNA in the large intestinal tissue compared with the CON group. Compared to the CS group, the levels of TNF-α, IL-6, IL-1β, MCP-1, and MMP-12 mRNA were decreased by treatment with DEX (75%, 87%, 95%, 85%, and 77%, respectively) or BC (69%, 63%, 96%, 95%, and 51%, respectively) (Figure 9). Thus, BC ameliorated the expression of inflammatory cytokines and chemokines in the lung and large intestine following exposure to CS.

BC inhibited expression of NF-kB in lung and the large intestinal tissues

The effects of BC on NF-κB protein expression in the lung and large intestine of CS-exposed mice model were examined by Western blotting. Compared to the normal group, the CS group showed a significant increase in NF-κB expression in the lung and large intestine, and BC treatment significantly reduced CS-induced NF-κB production in both tissues (Figure 10).

Discussion

Current body of scholarship strongly demonstrates the link between the lung and the intestine. It is supported by numerous studies that organs sharing common embryological origins may communicate with each other even if there is no anatomical association.[27–36] The primitive foregut has been shown from a growing number of literatures as the embryological origin of respiratory and gastrointestinal epithelia.[37,38] Wang *et al*.[39] indicated the existence of the lung–intestine communication by presenting the lung's duplication of inflammatory reactions in the bowel. Recently, Western medical theory recognized that the pulmonary and intestinal systems have much in common.[40] The respiratory and intestinal tracts both have a sophisticated luminal surface area covered by protective epithelia barrier and a mucus gel layer,[5] and connective and lymphoid tissue under the surface that plays a crucial role in immune defenses.[41,42] It is believed that these similarities somehow function to cause pulmonary–intestinal crosstalk during inflammation.[5]

Although the mechanism is unknown, the association between pulmonary and intestinal inflammation is supported by many clinical studies. Comparison of two representative inflammatory diseases, chronic obstructive pulmonary disease (COPD) and IBD, reveals the stark correlation between pulmonary and intestinal inflammation. Indeed, exacerbation of pulmonary and intestinal inflammation leads to COPD and IBD, respectively. COPD is characterized by gradual air blockage in the lung and accounts for the third most common cause of death and the fifth most common cause of disability.[43] IBD has two phenotypes, ulcerative colitis (UC) and Crohn's disease (CD), characterized by inflammatory damage to the intestinal area.[44] A population-based cohort study reported a higher chance of having CD in COPD patients than in healthy controls, and indicated an increased susceptibility to CD in first-degree relatives of COPD patients.[16] Another study showed that the small intestine of COPD patients shows aberrations in absorbing essential nutrients such as fats, protein, and carbohydrates.[17] Conversely, the study by Jess *et al*.[18] shows an increased risk of dying from COPD in CD patients. Black *et al*.[19] reported the similar finding that thoracic abnormalities are common in IBD patients.

CS is a major possible risk factor for IBD and COPD and is considered to have a significant influence on the possible mechanisms of respiratory–intestinal crosstalk inflammation. Crosstalk between these two organs is induced by uncontrolled inflammatory cytokine secretion when immune-inflammatory responses occur.[5–7] Reactive oxygen species (ROS) from CS degrade epithelial cells through peroxidation of cell membrane components and other constituents activate signaling pathways to activate inflammatory factors such as TNF-α and NF-κB.[45]

C57BL/6 mice are good responders to CS and present a well-formed injurious airway or intestinal states.[46–48] In our experiment, we exposed C57BL/6 mice to CS using the 3R4F reference cigarette. After three weeks of CS inhalation, we confirmed a significant weight loss in the CS-exposed mice. These data are meaningful since CS is reported to decrease body weight by affecting the amount of caloric intake,[49] and chronic disorders such as IBD and COPD are characterized by body weight loss. Next we identified infiltration of inflammatory cells, including macrophages and neutrophils, in the BAL fluid. Furthermore, we observed abnormal airspace enlargement, a pathologic change in the lung parenchyma

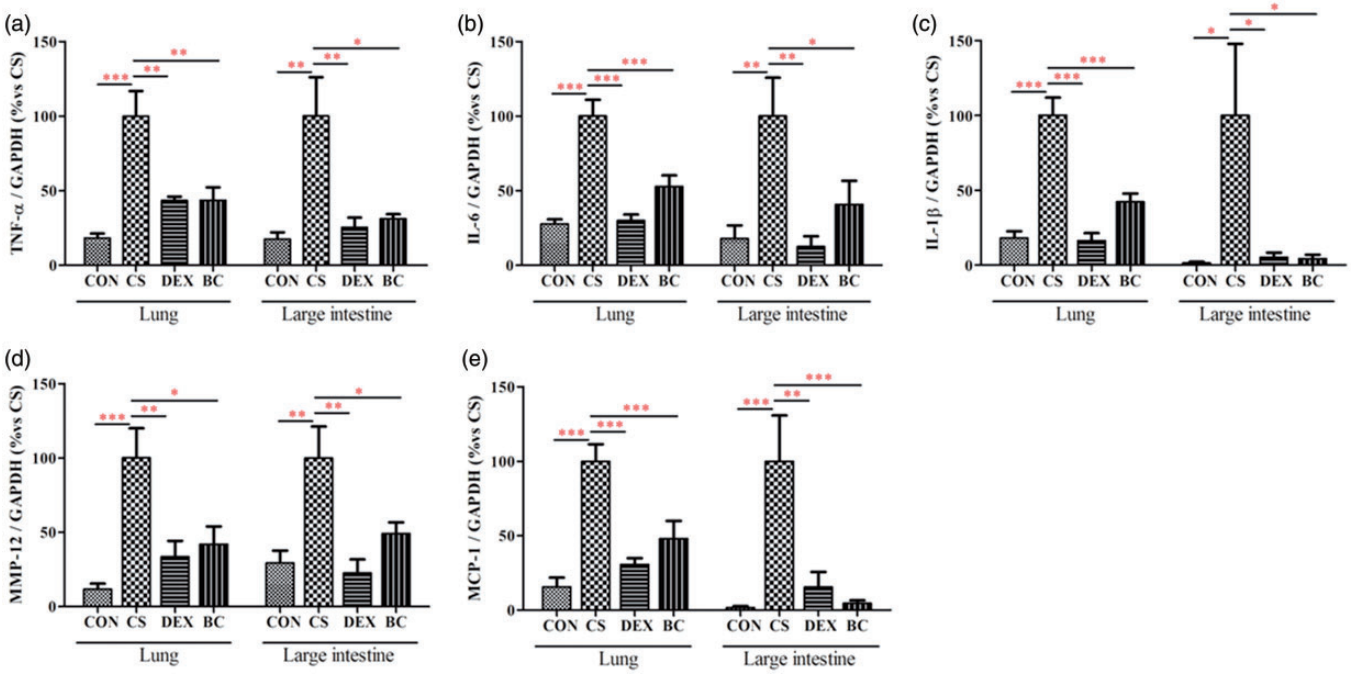

Figure 9 Effects of BC on cytokine and chemokine mRNA production in the lung and the large intestine. mRNA levels of TNF-α (a), IL-6 (b), IL-1β (c), MCP-1 (d), and MMP-12 (e) were determined by real-time PCR. GAPDH was used as the endogenous control for normalization. Values are mean ± SEM. Statistical analysis was by one-way ANOVA; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. (A color version of this figure is available in the online journal.)

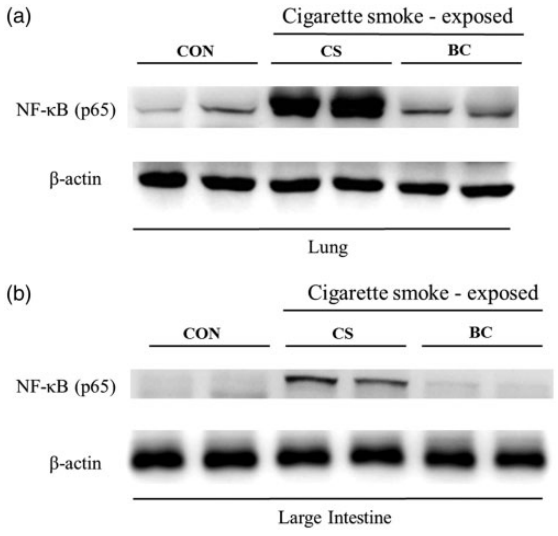

Figure 10 Effects of BC on NF-κB activation in the lung and the large intestine tissue. Tissue proteins were extracted by using T-PER tissue protein extraction reagent containing protease inhibitor cocktail and total protein concentration was measured using Bradford assay. NF-κB expression in lung (a) and large intestine (b) tissue was determined by Western blotting

caused by severe inflammatory response, and macroscopic changes in the large intestine. In particular, colonic shortening in CS-exposed mice provides evidence that a CS-induced inflammatory response occurred in the large intestine.[26] We also confirmed an increase in the concentration of several cytokines, including TNF-α and IL-6, the chemokine MCP-1, the protease MMP-12, and the transcription factor NF-κB in both the lung and the large intestine tissues of the CS group compared to the control group (Figure 9).

TNF-α is an important cytokine that plays a crucial role in inflammatory and immune reactions[50] and has been implicated to show a strong correlation with COPD[51] and the progression of CD.[52] TNF-α is believed to activate not only pro-inflammatory cytokines such as IL-6 and IL-8[53] but also the transcription factor NF-κB.[54] IL-6 is necessary for regulation of local or systemic acute inflammatory responses[55] and is thought to have a direct role in pulmonary–intestinal crosstalk by driving cross-organ inflammation. To our knowledge, we are the first to present experimental evidence for an elevated level of IL-6 during pulmonary–intestinal crosstalk inflammation in an animal model. MCP-1 is a CC chemokine that fights infection by attracting macrophages to areas of inflammation.[56,57] Indeed, the expression of MCP-1 in lung and intestinal inflammation has been reported in many studies.[58–61] MMP-12 is a member of the metalloproteinase (MMP) family and plays a role in tissue remodeling.[62] MMP-12 is reported to be involved in recruiting neutrophils, macrophages, and pro-inflammatory cytokines.[63] Also, the lack of MMP-12 neutralization resulting from A1AT deficiency exacerbates tissue damage during mucosal inflammation.[64,65] MMP-12 has been associated with the pathogenesis of both COPD,[66–68] and IBD.[69–72] On the basis of these results, we confirmed that CS-exposure induces inflammatory response in the lung and large intestine together. However, there is still a possibility where intestinal inflammation occurred from the licking behavior of CS-exposed mice rather than from the lung and intestinal crosstalk.

Thus, further study about the mechanism of pulmonary–intestinal crosstalk inflammation is needed.

In this study, BC treatment attenuated the CS-induced weight loss, airspace enlargement, and colonic shortening. Also, the BC-treated group had significantly lower numbers of total cells, macrophages, and neutrophils in BALF compared to the CS group. Furthermore, the increased levels of the cytokines TNF-α and IL-6 and the chemokine MCP-1 induced by CS were significantly ameliorated by BC treatment. Real-time PCR and ELISA analyses indicated a decrease in MMP-12 levels in both the lung and the large intestine in the BC-treated group. Lastly, we observed a reduction in the production of NF-κB after pro-inflammatory cytokine stimulation in lung and large intestine of the BC-treated mice.

In conclusion, we showed that BC is involved in the regulation of CS-induced lung and intestinal inflammation through inhibition of pro-inflammatory cytokines, chemokines, and protease as well as downregulation of the NF-κB signaling factor in both the lungs and large intestine tissues of mice exposed to CS. Therefore, we suggest that BC extract may be a candidate resource for the development of a therapeutic that inhibits pulmonary and intestinal inflammation.

Authors' contributions: DL and YC were involved in drafting the manuscript and made substantial contributions to the animal experiments, acquisition of data, analysis and interpretation of data. WK made substantial contributions to the animal experiments. JS and YJ made substantial contributions to the HPLC analysis and acquisition of data, analysis and interpretation of data. JK made substantial contributions to the conception and design, revising it critically for important intellectual content and giving final approval of the version to be published. All authors read and approved the final manuscript.

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DECLARATION OF CONFLICTING INTERESTS

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