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Research Article

Compound K ameliorates airway inflammation and mucus secretion through the regulation of PKC signaling in vitro and in vivo

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ABSTRACT

Background: Cigarette smoke (CS) is considered a principal cause of chronic obstructive pulmonary disease (COPD) and is associated with mucus hypersecretion and airway inflammation. Ginsenoside compound K (CK), a product of ginsenoside metabolism, has various biological activities. Studies on the effects of CK for the treatment of COPD and mucus hypersecretion, including the underlying signaling mechanism, have not yet been conducted.

Methods: To study the protective effects and molecular mechanism of CK, phorbol 12-myristate 13-acetate (PMA)-induced human airway epithelial (NCI–H292) cells were used as a cellular model of airway inflammation. An experimental mouse COPD model was also established via CS inhalation and intranasal administration of lipopolysaccharide. Mucin 5AC (MUC5AC), monocyte chemoattractant protein-1, tumor necrosis factor-α (TNF-α), and interleukin-6 secretion, as well as elastase activity and reactive oxygen species production, were determined through enzyme-linked immunosorbent assay. Inflammatory cell influx and mucus secretion in mouse lung tissues were estimated using hematoxylin and eosin and periodic acid–schiff staining, respectively. PKCδ and its downstream signaling molecules were analyzed via western blotting.

Results: CK prevented the secretion of MUC5AC and TNF-α in PMA-stimulated NCI–H292 cells and exhibited a protective effect in COPD mice via the suppression of inflammatory mediators and mucus secretion. These effects were accompanied by an inactivation of PKCδ and related signaling in vitro and in vivo.

Conclusion: CK suppressed pulmonary inflammation and mucus secretion in COPD mouse model through PKC regulation, highlighting the compound's potential as a useful adjuvant in the prevention and treatment of COPD.

1. Introduction

As the mortality and prevalence of chronic obstructive pulmonary disease (COPD) increase annually, novel pharmacological strategies that prevent COPD exacerbation, thus reducing the associated clinical burden, are necessary [1]. The mucus hypersecretion and chronic inflammation are characteristics of patients with COPD, and cigarette smoke (CS) is a leading reason of both [2,3]. The levels of inflammatory mediators and the numbers of immune cells are upregulated in bronchoalveolar lavage fluid (BALF) from cigarette smokers [4]. Since there is sufficient evidence

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Abbreviations

BALF	bronchoalveolar lavage fluid
CK	compound K
COPD	chronic obstructive pulmonary disease
CS	cigarette smoke
IL-6	interleukin-6
LPS	ipopolysaccharide
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MUC5AC	Mucin 5AC
NF-κB	nuclear factor-κB
PMA	phorbol 12-myristate 13-acetate
ROF	roflumilast
ROS	reactive oxygen species
TNF-α	tumor necrosis factor-α.

that CS triggers the release of mucin 5AC (MUC5AC), cytokines (tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)), as well as reactive oxygen species (ROS) in the lung, CS is considered a principal threat factor for COPD [2,5–7]. In addition, bacterial respiratory infections were reported to accelerate progressive airway inflammation [3]. Thus, inhalation of CS and lipopolysaccharide (LPS) can synergistically promote inflammatory responses in mice, leading to lung injury similar to that observed in patients with COPD [4,8].

CS induces lung inflammation through a complex signal cascade involving transcription factors such as nuclear factor-κB (NF-κB), early growth response gene (EGR)-1, and cAMP response element-binding protein (CREB) regulated by mitogen-activated protein kinases (MAPKs) [6,7,9]. MAPKs include three major serine/threonine kinases, namely extracellular receptor kinase-1/2 (ERK1/2), p38 MAPK, and c-Jun N-terminal kinase (JNK), all of which are closely related to COPD pathogenesis [10]. Of them, ERK1/2 triggers the secretion of inflammatory mediators including ROS, IL-6, and TNF-α through downstream targets EGR-1 and RSK/CREB in airway epithelial cells [11–13]. JNK drives the inflammatory response to allergens through the activation of transcription factors including NF-κB [14–16]. Therefore, MAPKs and their downstream signal molecules are recognized as potential therapeutic targets in suppressing airway inflammation.

Protein kinase C (PKC) is a serine/threonine kinase for several enzymes with diverse biological functions. Out of these, PKCδ is considered a potential biomarker and therapeutic target in lung injury [17]. As an upstream regulator of MAPKs and inflammatory transcription factors, PKCδ is intimately associated with the immune response in pulmonary conditions, including chronic inflammation, COPD, and emphysema [18,19]. The activation of PKCδ by inflammatory stimuli such as CS, phorbol 12-myristate 13-acetate (PMA), and ovalbumin (OVA) enhances the inflammatory response, while pharmacological and genetic PKCδ inhibition suppress airway inflammation [20–23]. We previously demonstrated that, targeting PKCδ could inhibit the inflammatory response through downregulation of TNF-α-induced IKK/NF-κB in human lung epithelial cells [18]. Moreover, rottlerin, a selective PKCδ inhibitor, inhibits MUC5AC expression via suppression of MAPKs and NF-κB activity in human lung epithelial cells [24]. Therefore, PKCδ and MAPK signaling present major therapeutic targets for anti-inflammatory modalities in the cure of inflammatory respiratory diseases including COPD.

Panax ginseng Meyer has been applied as a tonic and herbal medicine in East Asia, including Korea, China, and Japan for a long period [25]. It reveals various pharmacological activities, including anti-inflammatory and neuroprotective activities [26,27]. *P. ginseng* and its constituents, ginsenosides, are attractive therapeutic agents for the protection of inflammatory lung diseases including COPD [28,29]. Of these, ginsenoside compound K (CK, [20-*O*-(β-$_D$-glucopyranosyl)-20(*S*)-protopanaxadiol], known as IH-901) is a major ginsenoside metabolite with anti-inflammatory and antioxidant properties [30–32].

However, the preventive activities of CK in COPD experimental models have not yet been confirmed. Thus, in the current work, we aimed to study the effects and molecular mechanism of CK against mucus production and airway inflammation in PMA-treated human lung epithelial cells and a CS/LPS-exposed COPD mouse model.

2. Materials and methods

2.1. Chemicals and reagents

Black ginseng extracts were prepared, and ginsenoside compound K (CK, purity >95%) was provided by Dr. Dae Young Lee (Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, Soi-myeon, Eumseong-gun, Chungbuk, Republic of Korea). Research cigarette 2R4F was obtained from the Tobacco and Health Research Institute (University of Kentucky, Lexington, KY). PMA, LPS, DMSO (Dimethyl Sulfoxide), roflumilast (ROF), and rottlerin were obtained from Sigma-Aldrich (St. Louis, MO). Anti-phospho(p)–NF–κB, –NF–κB, -p-p70S6K, -p70S6K, -p-mTOR, -mTOR, -EGR-1, -p-RSK, -RSK, -p-CREB, -CREB, and -p-PKCδ antibodies were obtained from Cell Signaling Technology (Beverly, MA). Anti-p-JNK, -JNK, -p-ERK, -ERK, -PKCδ, and -β-actin antibodies were acquired from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Cell preparation and viability assessment

Human airway epithelial NCI–H292 (CRL-1848) cells and human embryonic kidney HEK293T (CRL-3216) cells were purchased from the ATCC. NCI–H292 and HEK293T cells were grown in RPMI 1640 and DMEM (Hyclone, Logan, UT), respectively, supplemented with 10% fetal bovine serum (FBS) and 100 μg/mL streptomycin 100 units/mL plus penicillin (Hyclone) at 37 °C under a humidified 5% CO_2 atmosphere. In order to determine cell viability, NCI–H292 and HEK293T cells were grown in a 96-well plate at an average density of 8×10^3 cells/well and incubated with the respective medium for 16 h. The media were changed for medium without serum. After culture for another 16 h, cells were grown with respective concentrations of CK presence or absence PMA for 24 h. Cell viability was determined in triplicate via Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Rockville, ML) as per the manufacturer's instructions. Absorbance was estimated on an Epoch microplate reader (BioTek instruments, Inc., Winooski, VT) and switched to a percentage (%) of the control value.

2.3. ELISA assay

NCI–H292 cells were seeded in a 96-well plate at an average density of 8×10^3 cells/well for 16 h. The media were changed for medium without serum for 16 h. Cells were pre-treated with CK (1, 2, and 4 μM) for 2 h and were then cultured with PMA (100 nM) for measuring at individual response times before collecting the supernatant. The levels of TNF-α in the supernatant were counted by ELISA kit (BD Pharmingen, San Diego, CA), following recommended manufacturer's instructions. The quantity of MUC5AC protein in cell culture supernatant was measured as depicted previously, with slight modifications [20,33]. The concentration was determined by

calculating absorbance at 450 nm on an Epoch microplate reader (BioTek instruments, Inc., Winooski, VT).

2.4. Transient dual-luciferase reporter analysis

HEK293T cells were grown at seeding density of 5×10^4 cells per well in a 24-well plate. After 24 h, cells were transfected using reporter plasmids with transfection reagent (Invitrogen, Carlsbad, CA), following manufacturer's instructions. In brief, to express the luciferase reporter gene fused to the *MUC5AC* promoter (HEK293T/*MUC5AC* promoter-*Luc*), HEK293T cells were transfected for 16–18 h [34]. Cells were pre-treated with CK for 2 h, and PMA (100 nM) was then stimulated for 12 h. The lysed cells were measured luciferase activity by the dual-luciferase assay system (Promega, Madison, WI) on a spectraMax® M4 multi-mode microplate reader (Molecular Devices, Sunnyvale, CA). The values of transient luciferase activity are indicated as a percentage (%) of the control value measured in DMSO-treated cells.

2.5. Experimental animal model of COPD

C57BL/6 N mice (male, 6 week-old, 18–20 g) were obtained from Koatech Co., Ltd. (Pyeongtaek-si, Korea), transferred to specific pathogen-free (SPF) conditions, and allowed to acclimatize prior to experiments. The experimental mouse model of COPD was established as previously described [6,35]. Briefly, CS inhalation in mice was performed for 8 days using 3R4F research cigarettes (Lexington, KY) and a smoking machine (SciTech Korea, Inc., Seoul, Korea). In parallel, intranasal administration of LPS (5 μg LPS dissolved in 30 μL PBS per mouse) was conducted on day 7. The oral administration of CK and ROF was performed for 8 days, 1 h before CS exposure. On day 9, the mice were sacrificed, followed by the collection BALF and lung samples. Experimental mice were divided into four groups (n = 6 per subgroup) as follows: i) NC, normal control; ii) COPD; CS exposure/intranasal LPS administration; iii) ROF, COPD + 5 mg/kg ROF oral administration; iv) CK 2.5, COPD + 2.5 mg/kg CK oral administration. CK and ROF were dissolved with 1% Tween-20 and 1% DMSO in PBS, respectively. Animal experiments were approved by the ACUC of the Korea Research Institute of Bioscience and Biotechnology (KRIBB, Chungbuk, Korea; KRIBB-AEC-20216).

2.6. Inflammatory cell number and mediators in BALF

On day 9, the mice were anesthetized using zoletil as well as xylazine, and tracheal cannulation using PBS was conducted (two times; total volume 1.4 mL) as previously described [7]. The acquired BALF was separated into cells and supernatant via centrifugation at 1500 rpm and 4 °C for 10 min. Immune cells in BALF were fixed and stained by Diff-Quik staining kit (Cat. No. 38721, Sysmex, Kobe, Hyogo, Japan) to distinguish inflammatory cells. Further, the cells were used to determine ROS production and elastase activity as per previously described methods [36]. The levels of TNF-α, monocyte chemoattractant protein (MCP)-1, and IL-6 in BALF supernatant were detected by respective ELISA kits, following manufacturer's protocol (BD bioscience, NJ, and R&D Systems, MN, USA).

2.7. Western blot analysis

Total protein extraction from cultured cells and mouse lung tissues was performed using lysis buffer containing phosphatase and protease inhibitors (Roche, Switzerland). At least 30 μg from cell lysate was separated via SDS-PAGE and transferred onto PVDF membranes. Each membrane was blocked using 5% skim milk for 1 h, followed by incubation with specific antibodies against p–NF–κB, NF-κB, p-p70S6K, p70S6K, p-mTOR, mTOR, EGR-1, p-RSK, RSK, p-CREB, CREB, p-PKCδ, PKCδ, p-JNK, JNK, p-ERK, ERK, and β-actin at 4 °C overnight (1:1000 dilution). The membranes were incubated with HRP-conjugated secondary antibodies (1:2000, Jackson ImmunoResearch Laboratories, Inc., USA) for 2 h at room temperature after washing with TBST. Finally, the membranes were developed with ECL solution (Thermo Fisher Scientific, Inc., Rockford, IL, USA). Each band was visualized and measured by an image analyzer (Fujifilm, Tokyo, Japan) with Multi Gauge software (Version 3.0).

2.8. Histology

For histological analysis, each lung tissue sample was cleaned with PBS, fixed with 10% formalin, embedded in paraffin, sectioned with a rotary microtome (each section was 4-μm thick), and then stained with hematoxylin and eosin (H&E, Sigma-Aldrich St. Louis, MO) as well as periodic acid–schiff (PAS, IMEB, Inc., San Marcos, CA) stain solution, following the manufacturer's instruction. Lung tissue samples were visualized under a light microscope (H&E staining: × 100 magnification; scale bar, 100 μm; PAS staining: × 400 magnification; scale bar: 25 μm).

2.9. Statistical analysis

All data are indicated as the mean ± standard deviation. Statistical significance was assessed via two-tailed Student's t-tests to compare the between two groups. One-way ANOVA followed by Dunnett's multiple comparisons was analyzed the differences between multiple groups. Data analysis was performed using IBM SPSS 20.0 (IBM Corporation, Armonk, NY, USA). Data with values of $p < 0.05$ were counted statistically significant. Single (*), double (**), and triple (***) asterisk marks employed statistical significance as $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

3. Results

3.1. CK suppresses TNF-α and MUC5AC expression in PMA-stimulated human lung epithelial cells (NCI–H292)

Before assessing the effects of CK in NCI–H292 cells, we investigated whether cell viability is affected by CK with or without of PMA (100 nM) for 24 h (Fig. 1A). CCK-8 assay results indicated that CK exhibited no considerable cytotoxicity at concentrations below 4 μM. Thus, we applied CK at concentrations below 4 μM in the next experiments. Next, we assessed whether PMA-induced TNF-α secretion is suppressed by CK using ELISA. CK pre-treatment suppressed TNF-α secretion in NCI–H292 cells (Fig. 1B). Further, 2 μM CK also reduced the secretion of MUC5AC in NCI–H292 cells from 6 h after PMA treatment (Fig. 1C).

To investigate the suppressive activities of CK on *MUC5AC* transcription, we assessed *MUC5AC* promoter activity in response to CK. HEK293T cells were transiently transfected plasmid DNA with *MUC5AC* promoter (HEK293T/*MUC5AC* promoter-*Luc*), and luciferase reporter activity was evaluated at each CK concentration in the presence of PMA. As shown in Fig. 1D, PMA-stimulated *MUC5AC* promoter-*Luc* reporter activity was decreased by CK pretreatment. Similar to the data presented in Fig. 1A, CK exhibited no significant cytotoxicity in HEK293T cells at concentrations below 4 μM (Fig. 1E). These results indicated that CK effectively inhibits both the PMA-induced transcription and secretion of MUC5AC.

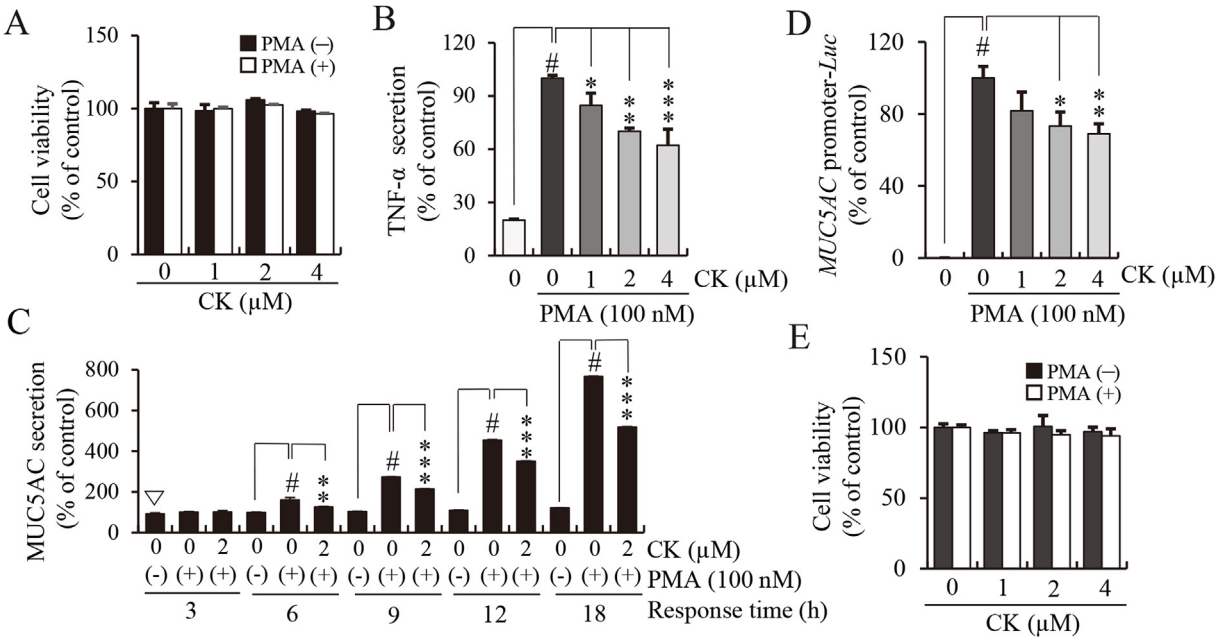

Fig. 1. Effects of CK on TNF-α and MUC5AC expression in PMA-stimulated NCI–H292 cells. (A) The viability of NCI–H292 cells was evaluated after treatment with CK (1, 2, and 4 μM) in the presence (white bars) or absence (black bars) of PMA using CCK-8 assay. (B–C) The effects of CK on TNF-α and MUC5AC secretion were analyzed by ELISA. NCI–H292 cells were pretreated with the respective concentration of CK for 2 h, followed by treatment with PMA (100 nM). TNF-α was measured at 8 h, and MUC5AC was assessed at various time points. The control in MUC5AC secretion is group without PMA in response time for 3 h (white triangle). (D) The suppressive effects of CK on *MUC5AC* transcription are shown. HEK293T cells were transiently transfected with a luciferase reporter gene fused to the *MUC5AC* promoter (HEK293T/*MUC5AC* promoter-*Luc*) for 16–18 h, pretreated with corresponding concentration of CK for 2 h, and were then treated with PMA (100 nM) for 12 h. (E) The viability of HEK293T cells was measured by CCK-8 assay after treatment with CK in the presence (white bars) or absence (black bars) of PMA. Data are represented as the mean ± standard deviation from three independent experiments. #$p < 0.05$ vs. negative control group (without PMA); *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. PMA only group.

3.2. CK regulates the activation of PKCδ and its downstream molecules in PMA-stimulated NCI–H292 cells

Since NF-κB and MAPK signaling activation are targeted for asthma and COPD treatment [10,16], we assessed whether CK affected these as well as associated upstream effector molecules. As shown in Fig. 2A, CK considerably inhibited PMA-induced NF-κB phosphorylation. The activation of mTOR in human brochial epithelial cells promotes the activation of NF-κB signaling, essential for the inflammatory response [37,38]. Similar to p–NF–κB results,

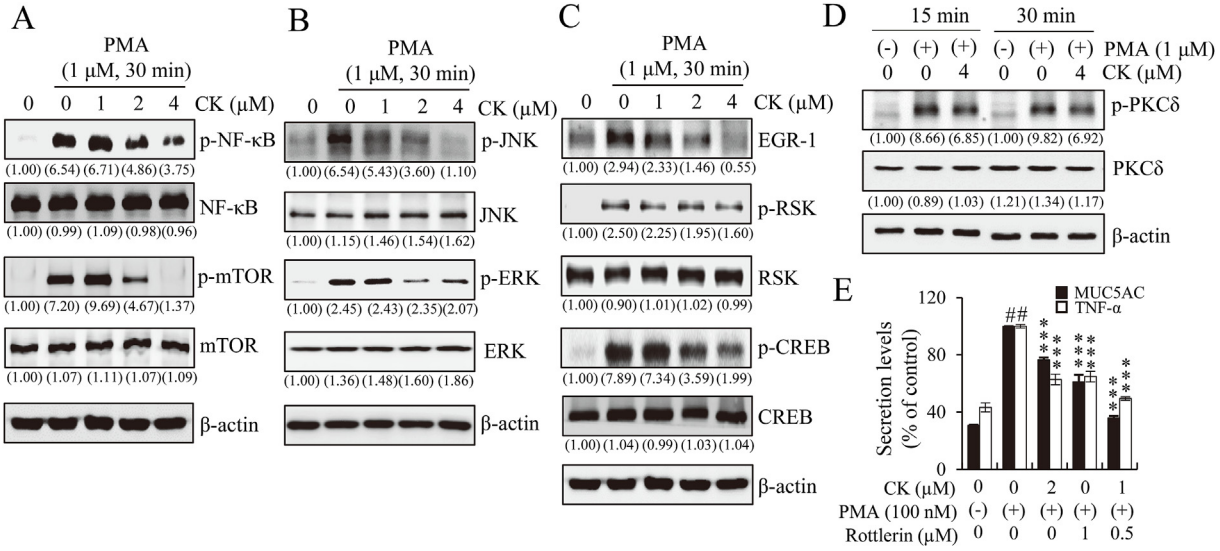

Fig. 2. Effects of CK on PMA-induced PKCδ and its downstream molecules in NCI–H292 cells. NCI–H292 cells were added with the respective concentration of CK for 2 h and subsequently treated with PMA (1 μM) for 15 or 30 min. Total cell lysates were analyzed via western blotting using (A) phospho(p)–NF–κB, NF-κB, p-mTOR, and mTOR; (B) p-JNK, JNK, p-ERK, and ERK; (C) EGR-1, p-RSK, RSK, p-CREB and CREB; (D) p-PKCδ and PKCδ antibodies. Anti-β-actin was chosen as an internal loading control. The numbers underneath the bands indicate the relative band intensity (fold change relative to the control). (E) The effect of CK combined with rottlerin (selective PKCδ inhibitor). TNF-α and MUC5AC secretion levels were measured using an ELISA assay. Individual bars indicate means ± S.D. for three separate experiments. #$p < 0.05$ vs. negative control group (without PMA); ***$p < 0.001$ vs. PMA only group.

CK pretreatment downregulated PMA-induced mTOR phosphorylation (Fig. 2A).

Next, we assessed the inhibitory effect of CK on MAPK activation. PMA-induced JNK and ERK phosphorylation was confirmed in NCI–H292 cells, with both attenuated via CK pretreatment (Fig. 2B). Based on the observed suppression of ERK activation, we assessed the molecular events downstream of ERK. The PMA-induced upregulation of both EGR-1 and RSK phosphorylation decreased with CK pretreatment. CK also suppressed CREB phosphorylation downstream of RSK (Fig. 2C).

Since the PKCδ/EGFR axis is known to induce the activation of MAPK and NF-κB signaling [39], we investigated whether CK suppresses PKCδ activation in PMA-stimulated NCI–H292 cells. The PMA-induced phosphorylation of PKCδ was downregulated following CK pretreatment for 15 and 30 min (Fig. 2D). Next, we evaluated the combined effect of CK treatment with a selective inhibitor for PKCδ (rottlerin) to confirm the inhibitory effects of CK on PMA-induced TNF-α or MUC5AC secretion by using ELISA. We observed that PMA-induced TNF-α or MUC5AC expression was more effectively suppressed by the combined treatment of CK with rottlerin, compared with CK alone (TNF-α, black bars and MUC5AC, white bars; Fig. 2E). Overall, our results indicate that CK could reduce the protein levels of the downstream target genes by negatively regulating MAPKs and downstream inflammation-promoting transcription factors, such as NF-κB, EGR-1, and CREB, through the suppression of PKCδ activation.

3.3. CK reduces inflammatory mediator production in an experimental animal model of COPD

Using CS- and LPS-exposed (CS/LPS) mice, we explored whether administration of CK could ameliorate CS/LPS exposure-stimulated lung inflammation. As shown in Fig. 3A and B, the significant increase in ROS production and elastase activity observed in BALF of COPD model mice was reduced by CK treatment. Moreover, CK also suppressed the upregulation of IL-6 and TNF-α in BALF (Fig. 3C and D). The inhibitory activity of 2.5 mg/kg CK was comparable to that of positive control ROF, which has been shown to decrease symptoms in patients with COPD [40]. Our results revealed that CK exerts anti-inflammatory activities in the COPD mouse model as well as in the NCI–H292 cellular model.

3.4. CK prevents inflammatory cell influx and mucus secretion in COPD mouse model

As shown in Fig. 4A and B, a remarkable increase of neutrophils and macrophages was confirmed in the BALF of COPD model mice via Diff-Quik staining. The increase was suppressed through CK pretreatment. Moreover, CK inhibited the secretion of MCP-1, a chemoattractant regulating the migration and infiltration of monocytes/macrophages (Fig. 4C). These points were consistent with the histological assessment of lung tissues. H&E staining revealed the presence of inflammatory cells in airway epithelium (Fig. 4D, blue arrow). This histopathological change was considerably suppressed by CK pretreatment (Fig. 4D). Consistent with Fig. 1C, PAS staining revealed the increased mucus production within the airways of COPD model mice (Fig. 4E, triangular arrow). CK also inhibited the increased mucus levels (Fig. 4E). These results demonstrate that CK exerts its anti-COPD activity through suppressing inflammation and mucus secretion in COPD mouse model.

3.5. CK regulates PKC signaling in the experimental animal model of COPD

In order to explore the molecular mechanism of CK in the lung tissue of COPD mouse model, we assessed PKC signaling as earlier on the NCI–H292 cellular model (Fig. 2A). In agreement with in vitro results, CK inhibited the phosphorylation of NF-κB, mTOR, and mTOR downstream target p70S6K1 in the lung tissue lysates of COPD model mice (Fig. 5A). The effect of CK on JNK and ERK activity was also confirmed in lung tissue from COPD model mice. In agreement with the results from Fig. 2B, CK markedly inhibited JNK activation (Fig. 5B). Moreover, the enhanced EGR-1 expression as well as the phosphorylation of RSK and CREB, all three being downstream of ERK, were considerably attenuated by CK treatment in mice (Fig. 5C). Further, CK as well as ROF inhibited PKCδ activation to suppress its downstream targets such as MAPK and NF-κB (Fig. 5D). Overall, CK preferentially inhibited PKCδ, downregulating

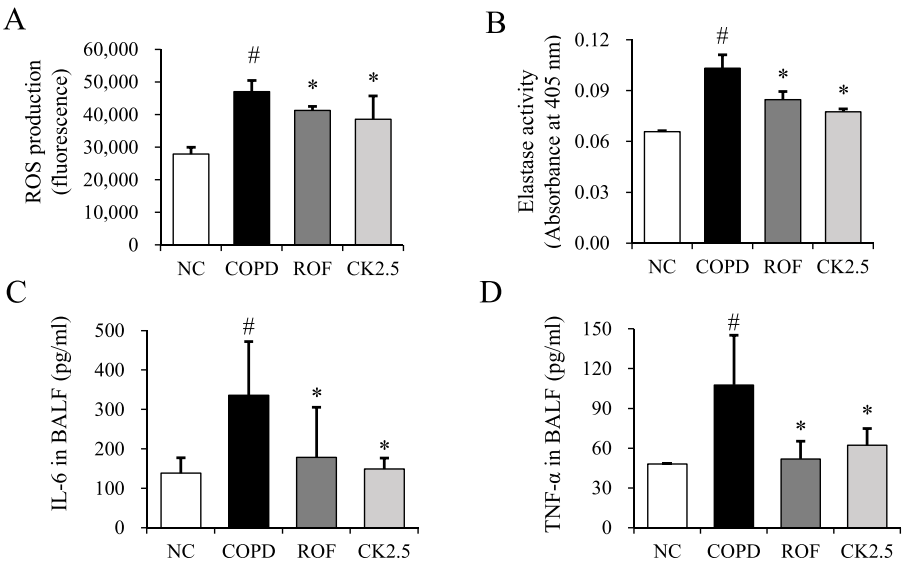

Fig. 3. Effects of CK on airway inflammation in COPD mouse models. The results of (A) ROS production, (B) elastase activity, (C) IL-6, and (D) TNF-α production in the BALF of COPD mice. NC: normal control mice; COPD: CS- and LPS (CS/LPS)-exposed COPD mice; ROF: CS/LPS-exposed mice treated with roflumilast (ROF, 5 mg/kg); CK2.5: CS/LPS-exposed mice treated with compound K (CK, 2.5 mg/kg). Data are expressed as the mean ± standard deviation ($n = 6$). #p < 0.05 vs. negative control group; *p < 0.05 vs. COPD only group.

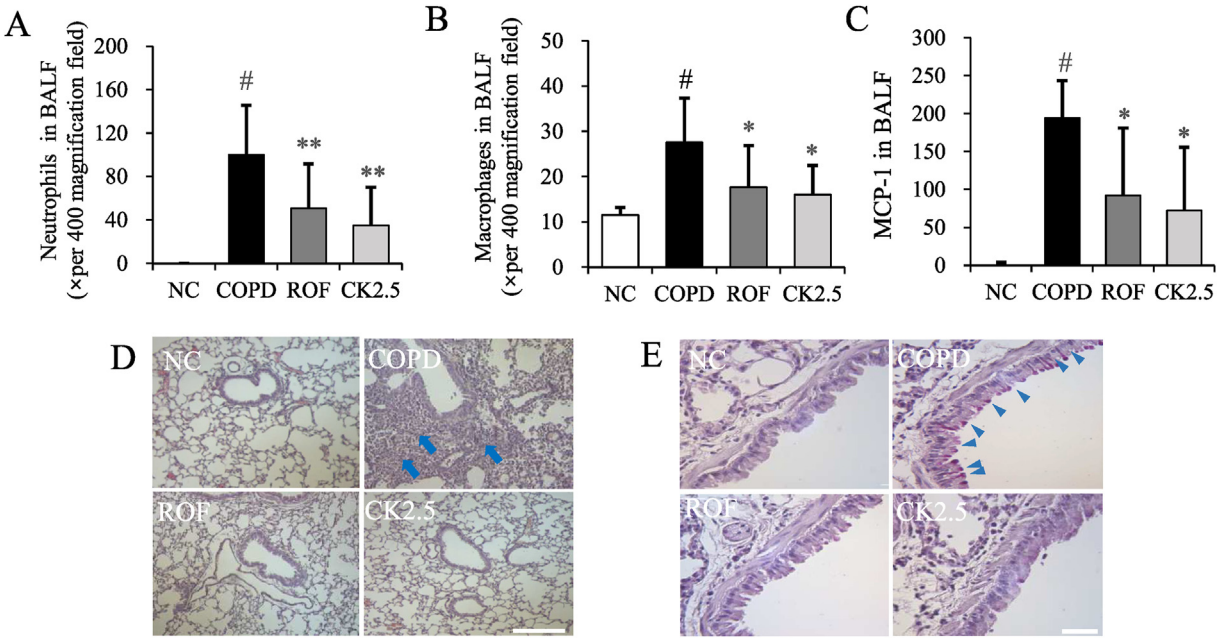

Fig. 4. Effects of CK on inflammatory cell influx and mucus secretion in the COPD mouse model. The numbers of (A) neutrophils and (B) macrophages in BALF from COPD mice were determined via Diff-Quik staining. (C) ELISA was employed for the detection of MCP-1 levels in BALF from COPD mice. (D) H&E stain was used to assess the presence of inflammatory cells around peribronchial lesions (× 100 magnification; scale bar, 100 μm). Blue arrows indicate inflammatory cells. (E) Periodic acid-Schiff staining was done to assess the production of mucus in airway epithelium (× 400 magnification; scale bar, 25 μm). The triangular arrows indicate mucus production. NC: normal control mice; COPD: CS and LPS (CS/LPS)-exposed COPD mice; ROF: CS/LPS expose-induced mice treated with roflumilast (ROF, 5 mg/kg); CK2.5: CS/LPS expose-induced mice treated with compound K (CK, 2.5 mg/kg). Data are expressed as the mean ± standard deviation ($n = 6$). #p < 0.05 vs. negative control group; *p < 0.05 and **p < 0.01 vs. COPD only group.

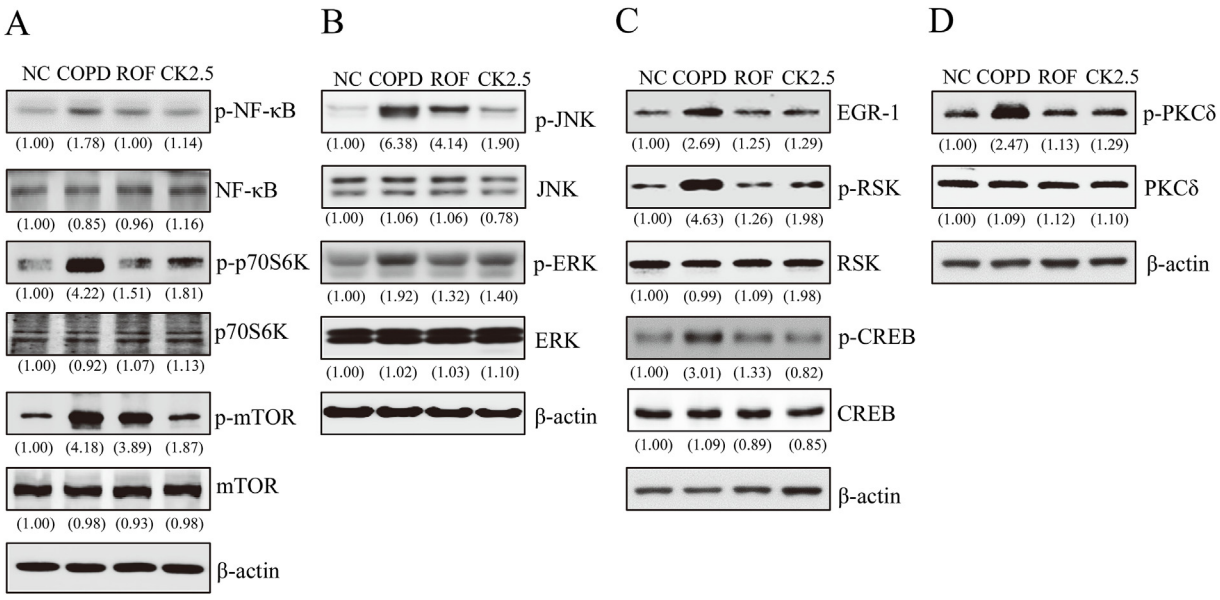

Fig. 5. Effect of CK on PKCδ and its downstream molecules in the lung tissue of COPD mouse. (A) The activation levels of p−NF−κB, NF-κB, p-p70S6K, p70S6K, p-mTOR, and mTOR; (B) p-JNK, JNK, p-ERK, and ERK; (C) EGR-1, p-RSK, RSK, p-CREB, and CREB; (D) p-PKCδ and PKCδ in lung tissue lysates from COPD mice were detected via Western blot analysis. Anti-β-actin was chosen as an internal loading control. NC: normal control mice; COPD: CS and LPS (CS/LPS)-exposed COPD mice; ROF: CS/LPS-exposed mice treated with roflumilast (ROF, 5 mg/kg); CK2.5: CS/LPS-exposed mice treated with compound K (CK, 2.5 mg/kg). The numbers underneath the bands indicate the relative band intensity (fold change relative to control).

MAPK (ERK and JNK) as well as mTOR signaling. As a result, inflammation-related transcription factors such as NF-κB, EGR-1, and CREB were suppressed. Our finding provide insight into the molecular mechanism of CK as a therapeutic agent for prevention of inflammatory airway diseases such as COPD by suppressing PKCδ activation and subsequent JNK/mTOR/NF-κB, MEK/ERK/EGR-1, and ERK/RSK/CREB axis (Fig. 6).

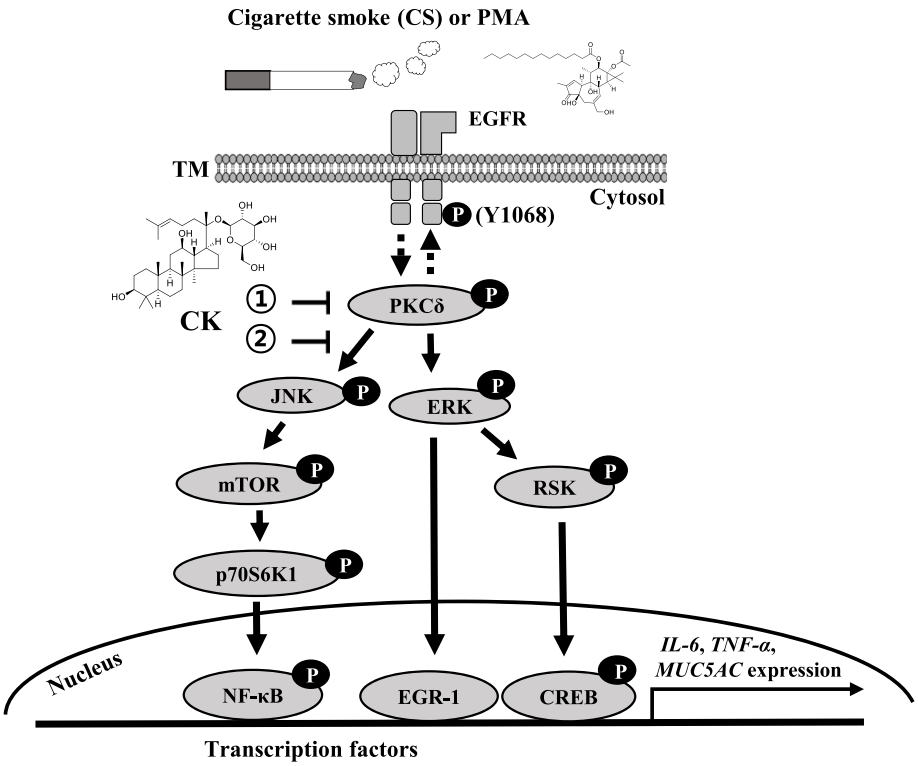

Fig. 6. Proposed molecular mechanism for the CK-induced inhibition of inflammatory mediators and mucus production in human lung airway epithelial cells and COPD mouse models. PMA and CS promote EGFR-mediated PKCδ phosphorylation and vice versa. In this experiment, the increased phosphorylation of PKCδ, JNK, and ERK as confirmed in both CS/LPS-exposed mice and PMA-treated cells. CK inhibited the expression level of inflammatory factors such as IL-6 and TNF-α as well as that of MUC5AC, both in vitro and in vivo. Our results suggest that the inhibitory effect of CK on cytokine expression may be mediated via inhibition of PKCδ, and its downstream effectors such as MAPKs and mTOR. PKCδ (number 1) and MAPKs (ERK and JNK, number 2) are presumably affected by CK, leading to the inhibition of inflammation-related transcription factors including NF-κB, EGR-1, and CREB. Taken together, CK inhibits JNK/mTOR/NF-κB, MEK/ERK/EGR-1, and ERK/RSK/CREB axis through the suppression of PKCδ activity. TM: transmembrane.

4. Discussion

COPD patients and cigarette smokers produce high levels of inflammatory moderators, including MUC5AC and TNF-α within respiratory tract epithelium [41]. TNF-α is a pivotal pro-inflammatory cytokine that may be utilized for COPD diagnosis and assessing disease progression [42]. Airway mucus hypersecretion leads to decreased lung function, a deprived quality of life, acute COPD exacerbation, as well as increased hospitalization and mortality [41]. Since MUC5AC is a major mucin component secreted within the lung, it is closely associated with COPD progression, similarly to TNF-α [20,43]. In the present study, CK inhibited the secretion of MUC5AC and TNF-α in PMA-stimulated human lung cells (Fig. 1).

Neutrophil-derived ROS and elastase cause alveolar destruction, resulting in lung damage, perpetuation of the inflammatory state, stimulation of mucus secretion, and breathing difficulties [44]. Further, the amplified number of macrophages and neutrophils in the airways reflect COPD severity [45]. Macrophage-derived TNF-α and IL-6 promote airway inflammation, while MCP-1 recruits ever greater numbers of immune cells to the inflamed sites [5,46]. Thus, the suppression of neutrophil/macrophage influx and associated inflammatory mediators in BALF may indicate the amelioration of airway inflammation.

Since CS/LPS exposure trigger lung inflammation and mucus secretion in a way comparable to that observed in COPD patients [6,35,45], we evaluated whether CK exerts protective effects in the COPD-like mouse model. Our results indicated that CK inhibited neutrophil/macrophage recruitment, ROS production, elastase activity, as well as TNF-α, IL-6, and MCP-1 secretion in BALF (Figs. 3 and 4). Histological analysis revealed that the extensive influx of inflammatory cell nearby the peribronchial region was notably attenuated by CK (Fig. 4D). Furthermore, mucus secretion was effectively reduced following CK pretreatment (Fig. 4E). Taken together, the current results strongly support the possibility of CK as a therapeutic agent for inflammatory airway diseases including COPD.

Growing evidence suggests that COPD inflammatory markers including MUC5AC, IL-6, TNF-α, and MCP-1, are associated with MAPK and NF-κB activation [6,7,33]. Of MAPKs, JNK activates mTOR signaling, thus representing a potential therapeutic target for COPD [47,48]. Moreover, mTOR and its downstream target p70S6K1 induce NF-κB activation in lung epithelial cells [37,49]. Thus, JNK/mTOR/NF-κB axis has been considered as a molecular target for the treatment of COPD.

Previous studies reported that CS-induced pulmonary inflammation was mediated by ERK and EGR-1, which were essential for MUC5AC production in lung epithelial cells [50,51]. Moreover, MUC5AC expression was modulated by ERK and its downstream target RSK, resulting in CREB activation in brochial epithelial cells [52]. Thus, ERK-mediated EGR-1 expression/activation and RSK/CREB pathway are considered as major targets for the prevention of inflammatory respiratory diseases, including COPD.

Our findings indicate that CK suppressed PMA-induced JNK phosphorylation. CK also exerted inhibitory effects on mTOR, p70S6K1, and NF-κB phosphorylation. In addition, PMA-induced ERK/RSK/CREB phosphorylation and EGR-1 expression were effectively attenuated following CK pretreatment. In agreement with these in vitro results, CK inhibited JNK and ERK phosphorylation as well as mTOR, p70S6K1, NF-κB, RSK, and CREB phosphorylation and

EGR-1 expression in mouse tissue. These data potently support that CK has anti-COPD effects through suppression of MAPK (ERK and JNK) as well as mTOR signaling, regulating inflammation-related transcription factors including NF-κB, EGR-1, and CREB.

Accumulating evidence has highlighted PKCδ as a crucial inflammatory regulator in various epithelial cells, acting through the activation of downstream molecules such as MAPKs and NF-κB [53,54]. PKCδ activation is closely related to lung inflammatory diseases including asthma and COPD [7,20]. Previous studies have shown that CS and PMA could activate EGFR-mediated PKCδ tyrosine phosphorylation [55,56]. PKCδ was also suggested to induce EGFR activation as well as that of downstream MAPKs and NF-κB [57]. Therefore, we suggest that targeting PKCδ and downstream signaling could be an effective strategy for the amelioration of airway inflammation and mucus secretion in COPD. Importantly, the negative regulation of CK on PKCδ phosphorylation was confirmed in both in vitro and in vivo.

CK is a major metabolite of *P. ginseng* saponin, known to be a more bioactive in its soluble form [58]. CK content in white ginseng is naturally low, increased via heating, fermentation, and enzymatic conversion [59]. Our results on the anti-inflammatory activity of CK in the airway suggest that processed *P. ginseng* products, such as red ginseng and black ginseng, may have greater physiological activity than white ginseng.

5. Conclusions

In summary, the present study confirmed the preventive effects of CK in airway inflammation and mucus secretion both in vitro and in vivo, comparable to those of ROF, an FDA-approved drug. These effects were closely related to the inactivation of PKCδ signaling. CK preferentially inhibited PKCδ and its downstream molecules, including MAPKs (ERK and JNK) and mTOR, thereby suppressing inflammation-related transcription factors such as NF-κB, EGR-1, and CREB. Targeting these pathways may be valuable for the treatment and prevention of airway inflammation and mucus secretion. Taken together, CK represents a pharmacological or nutraceutical candidate for airway inflammatory diseases, such as COPD.

Declaration of competing interest

All authors have no conflicts of interest to declare.

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References

[1] Lopez-Campos JL, Tan W, Soriano JB. Global burden of COPD. Respirology 2016;21(1):14–23.
[2] Yang T, Wang H, Li Y, Zeng Z, Shen Y, Wan C, Wu Y, Dong J, Chen L, Wen F. Serotonin receptors 5-HTR2A and 5-HTR2B are involved in cigarette smoke-induced airway inflammation, mucus hypersecretion and airway remodeling in mice. Int Immunopharm 2020;81:106036.
[3] Bagdonas E, Raudoniute J, Bruzauskaite I, Aldonyte R. Novel aspects of pathogenesis and regeneration mechanisms in COPD. Int J Chronic Obstr Pulm Dis 2015;10:995–1013.
[4] O'Donnell R, Breen D, Wilson S, Djukanovic R. Inflammatory cells in the airways in COPD. Thorax 2006;61(5):448–54.
[5] Barnes PJ. Mediators of chronic obstructive pulmonary disease. Pharmacol Rev 2004;56(4):515–48.
[6] Min JH, Kim MG, Kim SM, Park JW, Chun W, Lee HJ, Oh SR, Ahn KS, Lee JW. 3,4,5-Trihydroxycinnamic acid exerts a protective effect on pulmonary inflammation in an experimental animal model of COPD. Int Immunopharm 2020;85:106656.
[7] Lee JW, Ryu HW, Lee SU, Kim MG, Kwon OK, Kim MO, Oh TK, Lee JK, Kim TY, Lee SW, et al. Pistacia weinmannifolia ameliorates cigarette smoke and lipopolysaccharideinduced pulmonary inflammation by inhibiting interleukin8 production and NFkappaB activation. Int J Mol Med 2019;44(3):949–59.
[8] Moazed F, Burnham EL, Vandivier RW, O'Kane CM, Shyamsundar M, Hamid U, Abbott J, Thickett DR, Matthay MA, McAuley DF, et al. Cigarette smokers have exaggerated alveolar barrier disruption in response to lipopolysaccharide inhalation. Thorax 2016;71(12):1130–6.
[9] Li D, Xu D, Wang T, Shen Y, Guo S, Zhang X, Guo L, Li X, Liu L, Wen F. Silymarin attenuates airway inflammation induced by cigarette smoke in mice. Inflammation 2015;38(2):871–8.
[10] Kyriakis JM, Avruch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. Physiol Rev 2012;92(2):689–737.
[11] Boucherat O, Nadeau V, Berube-Simard FA, Charron J, Jeannotte L. Crucial requirement of ERK/MAPK signaling in respiratory tract development. Development 2015;142(21):3801.
[12] Sun S, Ning X, Zhai Y, Du R, Lu Y, He L, Li R, Wu W, Sun W, Wang H. Egr-1 mediates chronic hypoxia-induced renal interstitial fibrosis via the PKC/ERK pathway. Am J Nephrol 2014;39(5):436–48.
[13] Kim SW, Hong JS, Ryu SH, Chung WC, Yoon JH, Koo JS. Regulation of mucin gene expression by CREB via a nonclassical retinoic acid signaling pathway. Mol Cell Biol 2007;27(19):6933–47.
[14] Zhou JS, Zhao Y, Zhou HB, Wang Y, Wu YF, Li ZY, Xuan NX, Zhang C, Hua W, Ying SM, et al. Autophagy plays an essential role in cigarette smoke-induced expression of MUC5AC in airway epithelium. Am J Physiol Lung Cell Mol Physiol 2016;310(11):L1042–52.
[15] Basu S, Rajakaruna S, Reyes B, Van Bockstaele E, Menko AS. Suppression of MAPK/JNK-MTORC1 signaling leads to premature loss of organelles and nuclei by autophagy during terminal differentiation of lens fiber cells. Autophagy 2014;10(7):1193–211.
[16] Schuliga M. NF-kappaB signaling in chronic inflammatory airway disease. Biomolecules 2015;5(3):1266–83.
[17] Mondrinos MJ, Zhang T, Sun S, Kennedy PA, King DJ, Wolfson MR, Knight LC, Scalia R, Kilpatrick LE. Pulmonary endothelial protein kinase C-delta (PKCdelta) regulates neutrophil migration in acute lung inflammation. Am J Pathol 2014;184(1):200–13.
[18] Skaletz-Rorowski A, Eschert H, Leng J, Stallmeyer B, Sindermann JR, Pulawski E, Breithardt G. PKC delta-induced activation of MAPK pathway is required for bFGF-stimulated proliferation of coronary smooth muscle cells. Cardiovasc Res 2005;67(1):142–50.
[19] Lee S, Ro H, In HJ, Choi JH, Kim MO, Lee J, Hong ST, Lee SU. Fisetin inhibits TNF-alpha/NF-kappaB-induced IL-8 expression by targeting PKCdelta in human airway epithelial cells. Cytokine 2018;108:247–54.
[20] Lee SU, Lee S, Ro H, Choi JH, Ryu HW, Kim MO, Yuk HJ, Lee J, Hong ST, Oh SR. Piscroside C inhibits TNF-alpha/NF-kappaB pathway by the suppression of PKCdelta activity for TNF-RSC formation in human airway epithelial cells. Phytomedicine 2018;40:148–57.
[21] Shih RH, Cheng SE, Hsiao LD, Kou YR, Yang CM. Cigarette smoke extract upregulates heme oxygenase-1 via PKC/NADPH oxidase/ROS/PDGFR/PI3K/Akt pathway in mouse brain endothelial cells. J Neuroinflammation 2011;8:104.
[22] Lee TH, Chen JL, Liu PS, Tsai MM, Wang SJ, Hsieh HL. Rottlerin, a natural polyphenol compound, inhibits upregulation of matrix metalloproteinase-9 and brain astrocytic migration by reducing PKC-delta-dependent ROS signal. J Neuroinflammation 2020;17(1):177.
[23] Choi YH, Jin GY, Li LC, Yan GH. Inhibition of protein kinase C delta attenuates allergic airway inflammation through suppression of PI3K/Akt/mTOR/HIF-1 alpha/VEGF pathway. PLoS One 2013;8(11):e81773.
[24] Choi JH, Hwang YP, Han EH, Kim HG, Park BH, Lee HS, Park BK, Lee YC, Chung YC, Jeong HG. Inhibition of acrolein-stimulated MUC5AC expression by Platycodon grandiflorum root-derived saponin in A549 cells. Food Chem Toxicol 2011;49(9):2157–66.
[25] Park HJ, Kim DH, Park SJ, Kim JM, Ryu JH. Ginseng in traditional herbal prescriptions. J Ginseng Res 2012;36(3):225–41.
[26] Irfan M, Kwak YS, Han CK, Hyun SH, Rhee MH. Adaptogenic effects of Panax ginseng on modulation of cardiovascular functions. J Ginseng Res 2020;44(4):538–43.
[27] Mohanan P, Subramaniyam S, Mathiyalagan R, Yang DC. Molecular signaling of ginsenosides Rb1, Rg1, and Rg3 and their mode of actions. J Ginseng Res 2018;42(2):123–32.
[28] Shergis JL, Di YM, Zhang AL, Vlahos R, Helliwell R, Ye JM, Xue CC. Therapeutic potential of Panax ginseng and ginsenosides in the treatment of chronic obstructive pulmonary disease. Compl Ther Med 2014;22(5):944–53.
[29] Gao Y, Yan J, Li J, Li X, Yang S, Chen N, Li L, Zhang L. Ginsenoside Rg3 ameliorates acetaminophen-induced hepatotoxicity by suppressing inflammation and oxidative stress. J Pharm Pharmacol 2021;73(3):322–31.
[30] Kim HK. Pharmacokinetics of ginsenoside Rb1 and its metabolite compound K after oral administration of Korean Red Ginseng extract. J Ginseng Res 2013;37(4):451–6.
[31] Yi YS. Ameliorative effects of ginseng and ginsenosides on rheumatic diseases. J Ginseng Res 2019;43(3):335–41.

[32] Hossen MJ, Hong YD, Baek KS, Yoo S, Hong YH, Kim JH, Lee JO, Kim D, Park J, Cho JY. In vitro antioxidative and anti-inflammatory effects of the compound K-rich fraction BIOGF1K, prepared from Panax ginseng. J Ginseng Res 2017;41(1):43–51.
[33] Sikder MA, Lee HJ, Mia MZ, Park SH, Ryu J, Kim JH, Min SY, Hong JH, Seok JH, Lee CJ. Inhibition of TNF-alpha-induced MUC5AC mucin gene expression and production by wogonin through the inactivation of NF-kappaB signaling in airway epithelial cells. Phytother Res 2014;28(1):62–8.
[34] Lee SU, Sung MH, Ryu HW, Lee J, Kim HS, Hj In, Ahn KS, Lee HJ, Lee HK, Shin DH, et al. Verproside inhibits TNF-alpha-induced MUC5AC expression through suppression of the TNF-alpha/NF-kappaB pathway in human airway epithelial cells. Cytokine 2016;77:168–75.
[35] Lee SU, Ryu HW, Lee S, Shin IS, Choi JH, Lee JW, Lee J, Kim MO, Lee HJ, Ahn KS, et al. Lignans isolated from flower buds of magnolia fargesii attenuate airway inflammation induced by cigarette smoke in vitro and in vivo. Front Pharmacol 2018;9:970.
[36] Lee JW, Shin NR, Park JW, Park SY, Kwon OK, Lee HS, Hee Kim J, Lee HJ, Lee J, Zhang ZY, et al. Callicarpa japonica Thunb. attenuates cigarette smoke-induced neutrophil inflammation and mucus secretion. J Ethnopharmacol 2015;175:1–8.
[37] Hu Y, Lou J, Mao YY, Lai TW, Liu LY, Zhu C, Zhang C, Liu J, Li YY, Zhang F, et al. Activation of MTOR in pulmonary epithelium promotes LPS-induced acute lung injury. Autophagy 2016;12(12):2286–99.
[38] Temiz-Resitoglu M, Kucukkavruk SP, Guden DS, Cecen P, Sari AN, Tunctan B, Gorur A, Tamer-Gumus L, Buharalioglu CK, Malik KU, et al. Activation of mTOR/IkappaB-alpha/NF-kappaB pathway contributes to LPS-induced hypotension and inflammation in rats. Eur J Pharmacol 2017;802:7–19.
[39] Burgel PR, Nadel JA. Epidermal growth factor receptor-mediated innate immune responses and their roles in airway diseases. Eur Respir J 2008;32(4):1068–81.
[40] Gillissen A, Buhl R, Rabe KF, Vogelmeier C, Welte T. [Bronchodilators in chronic obstructive pulmonary disease (COPD)]. Med Klin 2005;100(5):246–54.
[41] Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016;138(1):16–27.
[42] Profita M, Chiappara G, Mirabella F, Di Giorgi R, Chimenti L, Costanzo G, Riccobono L, Bellia V, Bousquet J, Vignola AM. Effect of cilomilast (Ariflo) on TNF-alpha, IL-8, and GM-CSF release by airway cells of patients with COPD. Thorax 2003;58(7):573–9.
[43] Krishn SR, Ganguly K, Kaur S, Batra SK. Ramifications of secreted mucin MUC5AC in malignant journey: a holistic view. Carcinogenesis 2018;39(5):633–51.
[44] Thulborn SJ, Mistry V, Brightling CE, Moffitt KL, Ribeiro D, Bafadhel M. Neutrophil elastase as a biomarker for bacterial infection in COPD. Respir Res 2019;20(1):170.
[45] Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, Mapp CE, Fabbri LM, Donner CF, Saetta M. Severity of airflow limitation is associated with severity of airway inflammation in smokers. Am J Respir Crit Care Med 1998;158(4):1277–85.
[46] Lee JW, Park HA, Kwon OK, Jang YG, Kim JY, Choi BK, Lee HJ, Lee S, Paik JH, Oh SR, et al. Asiatic acid inhibits pulmonary inflammation induced by cigarette smoke. Int Immunopharm 2016;39:208–17.
[47] Mitani A, Ito K, Vuppusetty C, Barnes PJ, Mercado N. Restoration of corticosteroid sensitivity in chronic obstructive pulmonary disease by inhibition of mammalian target of rapamycin. Am J Respir Crit Care Med 2016;193(2):143–53.
[48] Lin Z, Liu T, Kamp DW, Wang Y, He H, Zhou X, Li D, Yang L, Zhao B, Liu G. AKT/mTOR and c-Jun N-terminal kinase signaling pathways are required for chrysotile asbestos-induced autophagy. Free Radic Biol Med 2014;72:296–307.
[49] Lin CH, Shih CH, Jiang CP, Wen HC, Cheng WH, Chen BC. Mammalian target of rapamycin and p70S6K mediate thrombin-induced nuclear factor-kappaB activation and IL-8/CXCL8 release in human lung epithelial cells. Eur J Pharmacol 2020;868:172879.
[50] Shen N, Gong T, Wang JD, Meng FL, Qiao L, Yang RL, Xue B, Pan FY, Zhou XJ, Chen HQ, et al. Cigarette smoke-induced pulmonary inflammatory responses are mediated by EGR-1/GGPPS/MAPK signaling. Am J Pathol 2011;178(1):110–8.
[51] Wang SB, Zhang C, Xu XC, Xu F, Zhou JS, Wu YP, Cao C, Li W, Shen HH, Cao JF, et al. Early growth response factor 1 is essential for cigarette smoke-induced MUC5AC expression in human bronchial epithelial cells. Biochem Biophys Res Commun 2017;490(2):147–54.
[52] Kim YO, Jung MJ, Choi JK, Ahn do W, Song KS. Peptidoglycan from Staphylococcus aureus increases MUC5AC gene expression via RSK1-CREB pathway in human airway epithelial cells. Mol Cell 2011;32(4):359–65.
[53] Masaki T, Kojima T, Okabayashi T, Ogasawara N, Ohkuni T, Obata K, Takasawa A, Murata M, Tanaka S, Hirakawa S, et al. A nuclear factor-kappaB signaling pathway via protein kinase C delta regulates replication of respiratory syncytial virus in polarized normal human nasal epithelial cells. Mol Biol Cell 2011;22(13):2144–56.
[54] Bae CH, Kim HS, Song SY, Kim YD. Phorbol 12-myristate 13-acetate induces MUC16 expression via PKCdelta and p38 in human airway epithelial cells. Clin Exp Otorhinolaryngol 2012;5(3):161–9.
[55] Goldkorn T, Filosto S. Lung injury and cancer: mechanistic insights into ceramide and EGFR signaling under cigarette smoke. Am J Respir Cell Mol Biol 2010;43(3):259–68.
[56] Amos S, Martin PM, Polar GA, Parsons SJ, Hussaini IM. Phorbol 12-myristate 13-acetate induces epidermal growth factor receptor transactivation via protein kinase Cdelta/c-Src pathways in glioblastoma cells. J Biol Chem 2005;280(9):7729–38.
[57] Denning MF, Dlugosz AA, Threadgill DW, Magnuson T, Yuspa SH. Activation of the epidermal growth factor receptor signal transduction pathway stimulates tyrosine phosphorylation of protein kinase C delta. J Biol Chem 1996;271(10):5325–31.
[58] Sharma A, Lee HJ. Ginsenoside compound K: insights into recent studies on pharmacokinetics and health-promoting activities. Biomolecules 2020;10(7).
[59] Shin KC, Choi HY, Seo MJ, Oh DK. Compound K production from red ginseng extract by beta-glycosidase from sulfolobus solfataricus supplemented with alpha-L-arabinofuranosidase from caldicellulosiruptor saccharolyticus. PLoS One 2015;10(12):e0145876.