N-(p-Coumaroyl) serotonin mitigates inflammatory responses in lipopolysaccharide-stimulated RAW264.7 cells and phorbol 12-myristate 13-acetate-stimulated A549 cells through NF-κB and MAPK inactivation

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Abstract. Inflammation is a protective response of the body, but excessive inflammation can exacerbate conditions such as acute lung injury and asthma. *N*-(p-Coumaroyl) serotonin (CS) is known to have anti-inflammatory effects. The present study aimed to explore the anti-inflammatory effects of CS on lipopolysaccharide (LPS)-induced inflammatory responses in macrophages and lung epithelial cells. Cellular inflammatory responses and associated signaling pathways were analyzed using ELISA, Western blotting and immunocytochemistry. Initial increase in cytokine (including IL-6 and TNF-α) and chemokine [including monocyte chemoattractant protein-1 (MCP-1)] levels, nitric oxide formation and inducible NO synthase expression in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages, was reduced by CS pretreatment. In addition, CS blocked LPS-induced MAPK/NF-κB activation in RAW264.7 cells. CS led to heme oxygenase-1 (HO-1) upregulation in RAW264.7 cells. In PMA-stimulated A549 lung epithelial cells, the increase in IL-6, TNF-α and MCP-1 expression was also attenuated by CS. This was accompanied by decreased MAPK/NF-κB activation. Furthermore, CS elevated the expression of HO-1 in A549 cells. Collectively, the present study confirmed that CS exhibited anti-inflammatory effects in both macrophages and lung epithelial cell lines, suggesting that CS may alleviate systemic or lung inflammation.

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Abbreviations: MCP-1, monocyte chemoattractant protein-1; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; CS, N-(p-Coumaroyl) serotonin

Key words: N-(p-Coumaroyl) serotonin, RAW264.7 cell, A549 cell, cytokine, NF-κB

Introduction

Acute inflammatory diseases such as sepsis, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are serious conditions induced by viruses and bacterial endotoxins (1,2). The coronavirus disease of 2019 increased the incidence of these diseases and was reported to be associated with hospital mortality rates of sepsis that may exceed 40% and increased ARDS mortality rates by 23 to 56% (3,4). Additionally, chronic inflammatory diseases such as asthma affects >350 million people globally and causes 0.4 million deaths annually, posing a significant social burden (5).

Cytokine upregulation is a key feature of sepsis, ALI and asthma. Immune cells contribute to the pathogenesis of these diseases by secreting excessive amounts of cytokines (6,7). Macrophage-released cytokines (such as IL-6 and TNF-α), chemokines [such as monocyte chemoattractant protein-1 (MCP-1)] and mediators [such as inducible nitric oxide synthase (iNOS)] exacerbate systemic inflammation in septic conditions (8,9). These molecules also promote pulmonary inflammation in ALI (10). Airway epithelial cells promote airway inflammation and lung damage in ALI by secreting TNF-α (11) and induce pulmonary inflammation in asthma by expressing IL-6 (12). In addition, cell-derived MCP-1 promotes bronchitis by inducing immune cell recruitment (13-15).

MAPK activation is implicated in the formation of inflammatory molecules. p38 activation is associated with IL-6 and TNF-α expression and p38 inactivation ameliorates

endotoxin-induced sepsis and ALI (16). Additionally, p38 activation contributes to airway inflammation and is present in the epithelium of patients with asthma (17). ERK is activated in sepsis and ARDS (18,19). IL-13 leads to ERK activation and mucin gene expression in airway epithelial cells, influencing asthma progression (20). Furthermore, JNK activation occurs in airway epithelial cells and macrophages (21,22) and is associated with lung inflammation and oxidative stress in the lipopolysaccharide (LPS)-induced ALI model (22). JNK inhibition ameliorates endotoxin-induced sepsis and ovalbumin-induced asthma in mice (21,23).

The NF-κB signaling pathway is activated in response to a variety of stimuli, including bacterial endotoxins. This activation has been shown to promote hyperinflammation by increasing IL-6, TNF-α, MCP-1 and iNOS generation in both *in vitro* and *in vivo* studies of sepsis and ALI (24,25). Phorbol 12-myristate 13-acetate (PMA) has also been shown to upregulate IL-6, TNF-α and MCP-1 generation as well as NF-κB activation in A549 lung epithelial cells in an *in vitro* study of asthma (21,26-28).

Heme oxygenase-1 (HO-1) decreases inflammatory response in cell models such as RAW264.7 and A549 by decreasing the formation of cytokines/chemokines and inhibiting the activation of MAPK and NF-κB (29,30).

Bacterial endotoxin LPS increases cytokine and MAPK/NF-κB activation in the RAW264.7 macrophage cell line; therefore, LPS is used for *in vitro* studies on sepsis and ALI (31,32). PMA is an established MAPK/NF-κB activator that elevates cytokines and chemokines in the A549 airway epithelial cell line and is used as an inducer for *in vitro* studies of asthma (21,28). Based on the abilities of LPS and PMA, both molecules were selected to induce an inflammatory response in RAW264.7 and A549 cells in the present study.

The phenolic compound *N*-(p-Coumaroyl) serotonin (CS) is an active component of safflower seeds and exhibits various biological effects. For example, CS demonstrates antioxidant effects and decreases atherosclerosis *in vivo* (33). CS also ameliorates vascular smooth muscle cell activation by suppressing calcium release (34). Additionally, Lazari *et al* (35) reported that CS exhibits anticancer effects by inducing S-phase cell cycle arrest in both U251 and T98 glioblastoma cell lines. To the best of our knowledge, however, the anti-inflammatory effects of CS have not been reported in RAW264.7 macrophages and A549 airway epithelial cells. Therefore, the present study examined whether CS exerts anti-inflammatory effects in these cell lines.

Materials and methods

Cell culture and viability assay. RAW264.7 macrophages were obtained from American Type Culture Collection and cultured in DMEM (cat. no. LM001-05; Welgene, Inc.) with 10% FBS (cat. No. 6000044; Thermo Fisher Scientific, Inc.) at 37˚C in a CO_2 incubator for 24 h. To analyze cell viability, the cells were seeded into a 12-well plate ($1x10^5$/ml) and incubated with 6.3, 12.5, 25.0 and 50.0 μM CS (Wuhan ChemFaces Biochemical Co., Ltd.) for 24 h at 37˚C. Cell viability was then determined using MTT assay, following a previously described protocol (10). To detect cell viability, A549 cells (American Type Culture Collection) were seeded into a 12-well plate ($1x10^5$/ml) and incubated with CS (6.3, 12.5 and 25.0 μM) for 24 h at 37˚C. After adding the MTT solution (Amresco, LLC) to each well, the plate was incubated at 37˚C for 3 h. Subsequently, DMSO (Sigma-Aldrich; Merck KGaA) was used to dissolve the purple formazan, and the absorbance at 570 nm was measured using a SPARK® 10M microplate reader (Tecan Group, Ltd.).

NO assay. To analyze the NO content, cells were seeded into a 12-well plate ($1x10^5$/ml) and incubated with CS (6.3, 12.5 and 25.0 μM) for 1 h at 37˚C. Cells were incubated for 18 h with LPS (200 ng/ml) at 37˚C. NO levels were detected using a NO assay as previously described (36).

Cytokines and chemokine ELISA. To measure the levels of cytokines and chemokines secreted from RAW264.7 and A549 cells, $1x10^5$/ml cells were seeded into a 12-well plate, incubated with CS (6.3, 12.5 and 25.0 μM) for 1 h at 37˚C and maintained for 18 h at 37˚C with 200 ng/ml LPS or 50 nM PMA, respectively. The levels of IL-6, TNF-α and MCP-1 in the cell culture media (CCM) were analyzed using ELISA kits according to the manufacturers protocols of these kits [BD OptEIA™ Mouse IL-6 ELISA Set (cat. no. 555240), BD OptEIA™ Mouse TNF ELISA Set (cat. no. 558534), BD OptEIA™ Human IL-6 ELISA Set (cat. no. 555220), BD OptEIA™ Human TNF ELISA Set (cat. no. 555212) (BD Biosciences); Mouse CCL2/JE/MCP-1 DuoSet ELISA (cat. no. DY479) and Human CCL2/MCP-1 DuoSet ELISA (cat. no. DY279) (R&D Systems, Inc.)].

Western blotting. To detect the expression of iNOS in RAW264.7 cells and the levels of phosphorylated (p)-p38, p-ERK, p-JNK, p-p65 and p-IκBα in RAW264.7 and A549 cells, the cell lysate was isolated using RIPA lysis butter (0.15 M NaCl, 0.05 M Tris, 5 mM EDTA, pH 8.0) including protease (cat. no 11836153001; Roche Diagnostics) and phosphatase inhibitors (cat. no.4906837001; Roche Diagnostics). Protein was quantified using a BCA assay and protein samples (20-60 μg) were separated using 8-12% SDS-PAGE as previously described (31). Proteins were transferred onto PVDF membranes and each membrane was blocked with 5% skimmed milk in 1X TBST for 1 h at room temperature. Subsequently, membranes were incubated with the primary antibodies (Table I) diluted to 1:1,000 in 1% BSA for 24 h at 4˚C and the corresponding secondary antibodies [(goat anti rabbit-HRP; cat. no. 111-035-003; Jackson Laboratory) and (goat anti mouse-HRP; cat. no. 115-035-003; Jackson Laboratory)] diluted to 1:2,000 in 1% BSA for 2 h at room temperature. Finally, each membrane was exposed to ECL solution (cat. no. 32106; Thermo Fisher Scientific, Inc.) to visualize the protein bands. Protein semi-quantitative analysis of iNOS/β-actin, p-p38/p38, p-ERK/ERK, p-JNK/JNK, p-p65/p65, p-IκB/IκBα and HO-1/β-actin was performed using ImageJ version 1.52a software (National Institutes of Health).

Immunocytochemistry. To determine the nuclear translocation of NF-κB p65 in RAW264.7 cells, cells were fixed with 10% formalin for overnight at 4˚C, washed with ice-cold PBS, blocked with 5% BSA (Gibco; Thermo Fisher Scientific, Inc.) blocking buffer for 30 min at room temperature, incubated with

Table I. Primary antibodies used for western blotting.

Primary antibody	Dilution	Manufacturer	Cat. no.	Molecular weight, kDa[a]	Host
iNOS	1:1,000	Enzo Life Sciences, Inc.	ADI-KAS-NO001	130	Rabbit
HO-1	1:1,000	Invitrogen (Thermo Fisher Scientific, Inc.)	PA5-27338	32	Rabbit
p-p38	1:1,000	Cell Signaling Technology, Inc.	9211S	43	Rabbit
p38	1:1,000	Santa Cruz Biotechnology, Inc.	sc-7972	38	Mouse
p-ERK	1:1,000	Cell Signaling Technology, Inc.	9101S	42,44	Rabbit
ERK	1:1,000	Cell Signaling Technology, Inc.	9102S	42, 44	Rabbit
p-JNK	1:1,000	Cell Signaling Technology, Inc.	4668S	46, 54	Rabbit
JNK	1:1,000	Cell Signaling Technology, Inc.	9252S	46, 54	Rabbit
p-NF-κB p65	1:1,000	Cell Signaling Technology, Inc.	3033S	65	Rabbit
NF-κB p65	1:1,000	Santa Cruz Biotechnology, Inc.	sc-8008	65	Mouse
p-IκBα	1:1,000	Santa Cruz Biotechnology, Inc.	sc-8404	41	Mouse
IκBα	1:1,000	Invitrogen (Thermo Fisher Scientific, Inc.)	MA5-15132	36	Mouse
β-actin	1:5,000	Santa Cruz Biotechnology, Inc.	sc-47778	43	Mouse

[a]Isoform dependent. p-, phosphorylated; iNOS, inducible nitric oxide synthase.

the primary antibody (anti-NF-κB p65 subunit; Table I) for 24 h at 4˚C and the corresponding secondary antibody (Alexa Fluor 488-conjugated goat anti-rabbit IgG; cat no. A-11034; Invitrogen; Thermo Fisher Scientific, Inc.) diluted to 1:250 in 1% BSA for 1 h at room temperature and stained with Antifade Mounting Medium with DAPI (cat. no. H-1500; Vector Laboratories, Inc.) at room temperature as previously described (31). Finally, cells were visualized using a confocal microscope.

Statistical analysis. All data are expressed as the mean ± standard deviation (n=3). Data were analyzed using an unpaired two-tailed Student's t-test to assess the difference between two groups. One-way ANOVA followed by Tukey's multiple comparison test was performed to assess differences between >2 groups. Data were analyzed using SPSS software (version 20.0; IBM Corp.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

CS ameliorates LPS-stimulated inflammatory responses in RAW264.7 macrophages. Viability of RAW264.7 cells was assessed following treatment with CS (6.3, 12.5, 25.0 and 50.0 μM) by an MTT assay. No notable changes in cell viability were observed following treatment with ≤25.0 μM CS (Fig. 1A). Based on this result, the anti-inflammatory effects of CS at 6.3, 12.5 and 25.0 μM on LPS-stimulated RAW264.7 cells were examined. ELISA results confirmed the significant increase in IL-6, TNF-α and MCP-1 levels in the CCM of LPS-stimulated RAW264.7 cells (Fig. 1B-D) compared to the control group. This increase was significantly inhibited in the CS pretreated-LPS group. Overall, 25 μM CS had a notable inhibitory effect, resulting in a 56.93% decrease in IL-6, 52.62% decrease in TNF-α and a 45.73% decrease in MCP-1 levels.

Table II. Inhibitory effect of CS and DEX on MCP-1 and NO in lipopolysaccharide-stimulated RAW264.7 cells.

Treatment	MCP-1 inhibition rate (%)	NO inhibition rate (%)
25 μM CS	33.9±5.95	33.4±0.57
20 μM DEX	35.5±4.02	31.9±2.17

CS, *N*-(p-Coumaroyl) serotonin; DEX, dexamethasone; MCP-1, monocyte chemoattractant protein-1; NO, nitric oxide.

The notable elevation in NO formation in LPS-stimulated RAW264.7 cells was decreased by the addition of CS (Fig. 2A). Western blotting revealed increased expression of iNOS in the lysate of LPS-stimulated RAW264.7 cells. This expression was attenuated in the lysate of the CS-pretreated LPS group (Fig. 2B).

The inhibitory effects of dexamethasone (DEX), which is a synthetic pregnane corticosteroid on inflammatory molecules, were demonstrated in an experimental study of ALI and asthma (10,13), Notably, the present study confirmed that the inhibitory effect of 25 μM CS on LPS-induced MCP-1 and NO was similar to that of 20 μM DEX (Table II).

CS ameliorates LPS-stimulated MAPK activation in RAW264.7 cells. To elucidate the underlying mechanisms of CS, the levels of p38, ERK and JNK activation were investigated via western blotting. LPS significantly upregulated not only p-p38 and p-ERK expression but also p-JNK expression in RAW264.7 cells (Fig. 3A and B). However, this upregulation in LPS-stimulated RAW264.7 cells was inhibited by CS pretreatment.

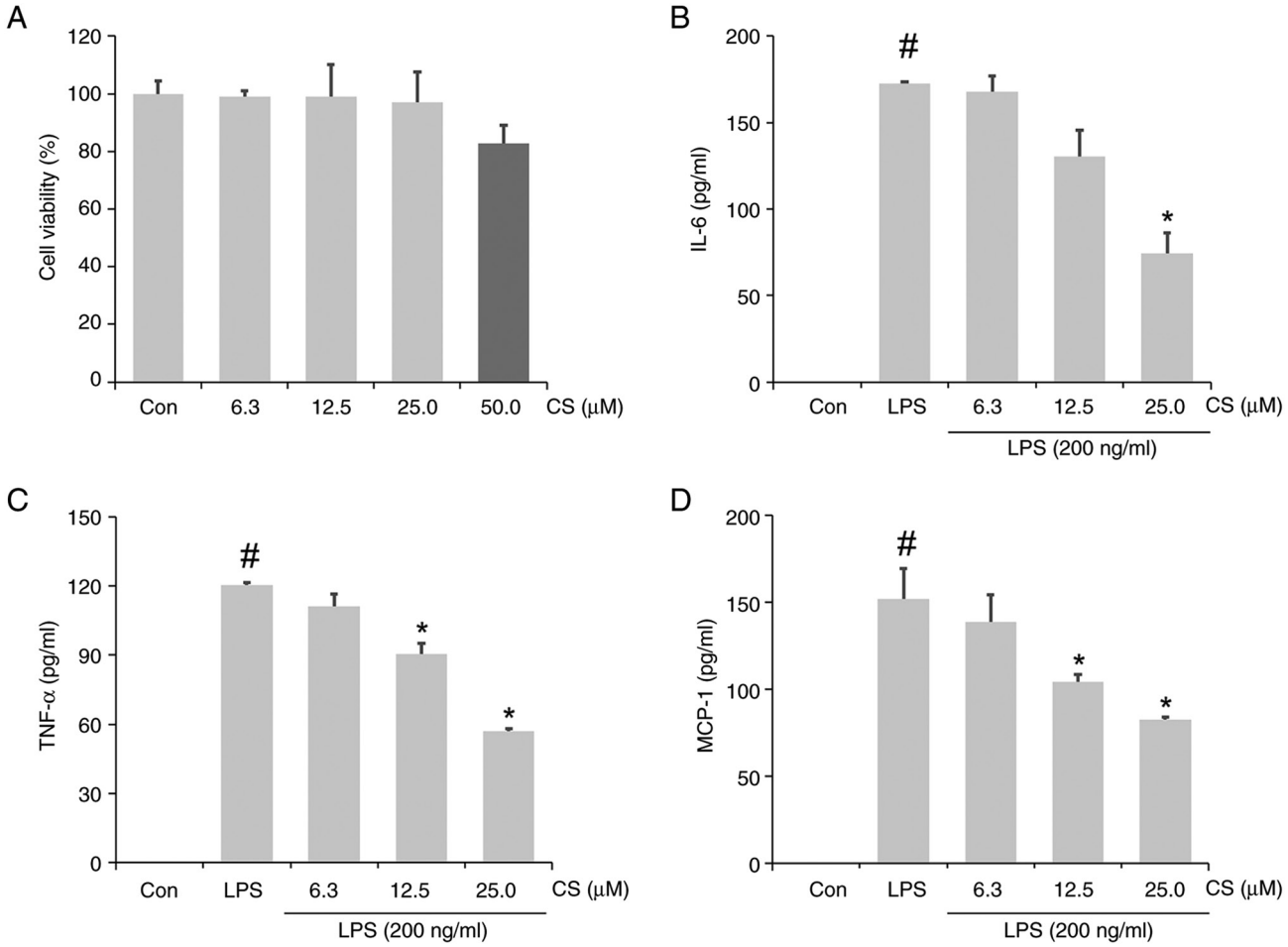

Figure 1. CS pretreatment decreases LPS-stimulated cytokine and chemokine generation in RAW264.7 cells. (A) Cell viability was analyzed using an MTT assay. RAW264.7 cells were incubated with CS for 1 h and maintained with 200 ng/ml LPS for 18 h. The levels of (B) IL-6, (C) TNF-α and (D) MCP-1 in the cell culture supernatant were detected using ELISA kits. #P<0.05 vs. con. *P<0.05 vs. 200 ng/ml LPS-only condition. CS, *N*-(p-Coumaroyl) serotonin; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; Con, control.

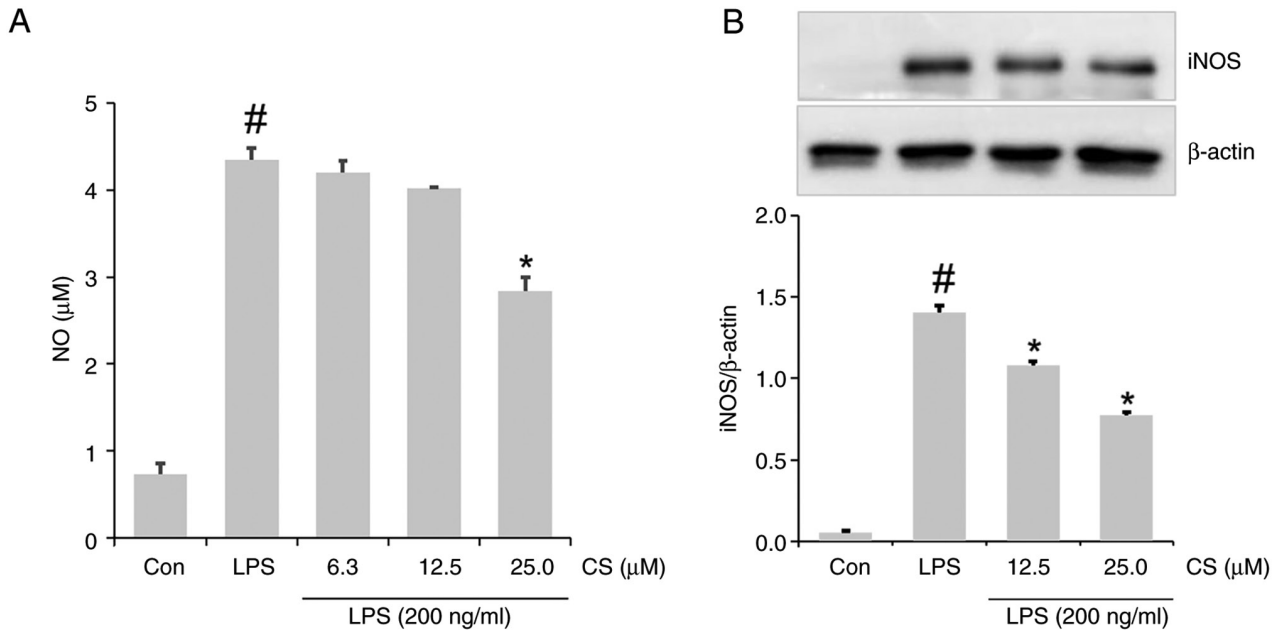

Figure 2. CS pretreatment decreases LPS-stimulated NO formation and iNOS expression in RAW264.7 cells. (A) NO levels in cell culture supernatant were detected using NO assays. RAW264.7 cells were incubated with CS for 1 h and maintained with 200 ng/ml LPS for 18 h. (B) Levels of iNOS in the cell lysates were detected using western blotting. #P<0.05 vs. con. *P<0.05 vs. 200 ng/ml LPS-only condition. CS, *N*-(p-Coumaroyl) serotonin; LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthase; Con, control.

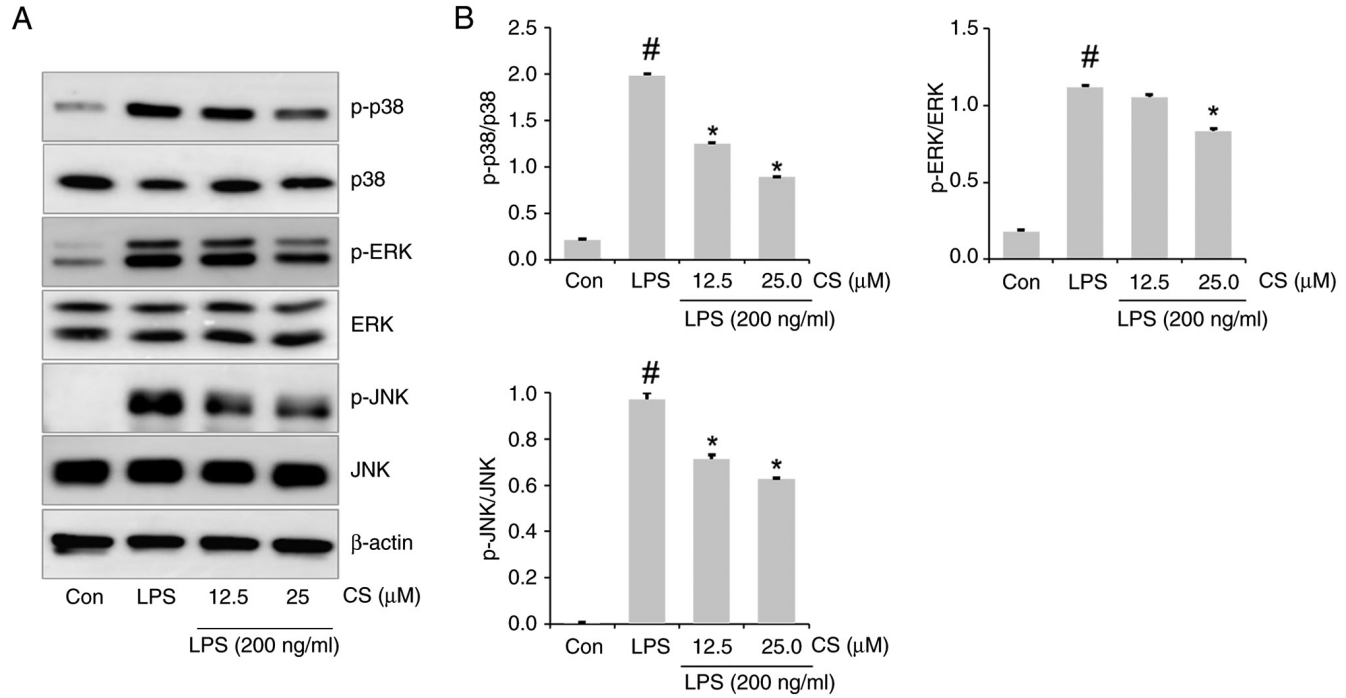

Figure 3. CS pretreatment decreases LPS-stimulated mitogen-activated protein kinase activation in RAW264.7 cells. (A) Levels of p-p38, p-ERK and p-JNK in the cell lysates were detected using western blotting. (B) Protein semi-quantitative analysis of p-p38/p38, p-ERK/ERK and p-JNK/JNK. #P<0.05 vs. con. *P<0.05 vs. 200 ng/ml LPS-only condition. CS, *N*-(p-Coumaroyl) serotonin; LPS, lipopolysaccharide; p-, phosphorylated; Con, control.

CS ameliorates LPS-stimulated NF-κB activation in RAW264.7 cells. As NF-κB signaling pathways are associated with the expression of inflammatory molecules (24,25), the abrogative effects of CS on LPS-induced NF-κB activation were explored. Increased expression of p-NF-κB and p-IκB was confirmed in the lysate of the LPS-treated group. However, this was effectively inhibited by CS (Fig. 4A and B). Furthermore, immunocytochemistry demonstrated that CS resulted decreased nuclear translocation of NF-κB p65 in LPS-stimulated RAW264.7 cells (Fig. 4C).

CS results in HO-1 upregulation in RAW264.7 cells. As HO-1 induction exhibits anti-inflammatory effects (29,30), the effect of CS on HO-1 upregulation was investigated. A significant increase in HO-1 expression was observed in the lysate from the CS-treated RAW264.7 cells compared to the control group (Fig. 5A and B).

CS ameliorates the PMA-stimulated inflammatory response in A549 cells. Based on the aforementioned anti-inflammatory effects of CS against endotoxin stimulation in RAW264.7 cells, the ameliorative effects of CS against PMA-induced inflammation in A549 airway epithelial cells were also investigated. Notable upregulation of IL-6, TNF-α and MCP-1 was observed in the CCM of PMA-stimulated A549 cells, which was mitigated by CS pretreatment (Fig. 6A-C). No notable changes in viability were observed in A549 cells within the treated concentrations (Fig. 6D). In general, 25 μM CS significantly suppressed cytokine and chemokine secretion. The inhibition rates of IL-6, TNF-α and MCP-1 secretion were 70.94, 60.01 and 46.05%, respectively.

CS ameliorates PMA-stimulated MAPK activation in A549 cells. p38, ERK and JNK phosphorylation were demonstrated in the lysate of PMA-stimulated A549 cells (Fig. 7A and B). p38, ERK and JNK phosphorylation was inhibited in the lysate of the 25 μM CS-pretreated PMA group (Fig. 7A and B).

CS ameliorates PMA-stimulated NF-κB activation in A549 cells. To determine whether the anti-inflammatory effects of CS in A549 cells were associated with the NF-κB signaling pathway, NF-κB and IκB activation levels in lysate were evaluated. NF-κB and IκB phosphorylation was increased in the lysate of PMA-stimulated A549 cells compared to the control group, whereas this was inhibited in the lysate of the CS-pretreated PMA group (Fig. 8A and B).

CS results in HO-1 upregulation in A549 cells. Increase in HO-1 expression was confirmed in the lysate of the 25 μM CS-treated A549 cells compared to the control group (Fig. 9A and B).

Discussion

Hyperinflammation leads to harmful effects, such as organ damage (1). Therefore, substances that modulate this are valuable as adjuvants or therapeutics to prevent or improve the development of systemic or pulmonary inflammatory disease. Similar to the suppression ability of CS on IL-6 and TNA-α in a previous study (37), in the present study, CS inhibited the secretion of IL-6, TNF-α and MCP-1 in both LPS-stimulated macrophages and PMA-stimulated lung epithelial cells. In addition, CS exerted a marked inhibitory effect on LPS-stimulated iNOS expression in macrophages.

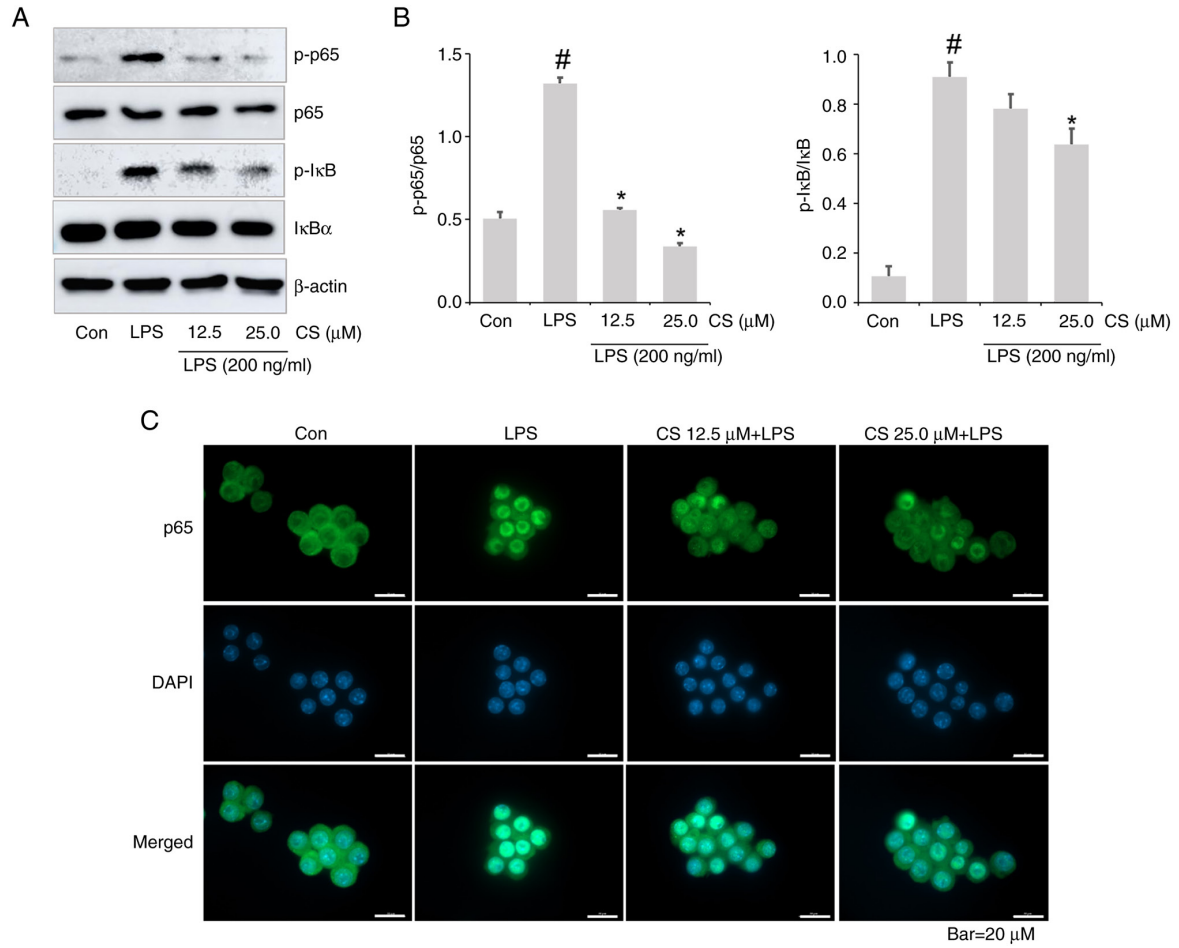

Figure 4. CS pretreatment decreases LPS-stimulated NF-κB activation in RAW264.7 cells. (A) Levels of p-NF-κB p65 and p-IκBα in the cell lysates were detected using western blotting. (B) Semi-quantitative analysis of p-p65/p65 and p-IκB/IκB. (C) Nuclear translocation of NF-κB p65 was detected using immunocytochemistry. Scale bar, 20 μm. #P<0.05 vs. con. *P<0.05 vs. 200 ng/ml LPS-only condition. CS, *N*-(p-Coumaroyl) serotonin; LPS, lipopolysaccharide; p-, phosphorylated; Con, control.

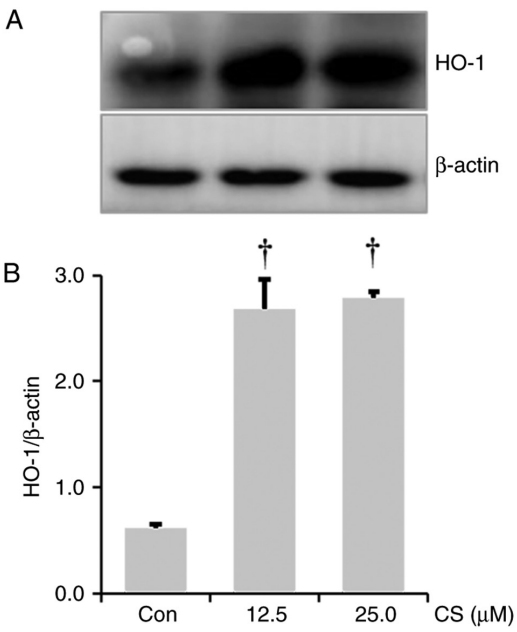

Figure 5. CS upregulates HO-1 expression in RAW264.7 cells. (A) Levels of HO-1 in the cell lysate were detected using western blotting. (B) Protein semi-quantitative analysis of HO-1/β-actin. †P<0.05 vs. con. CS, *N*-(p-Coumaroyl) serotonin; HO-1, heme oxygenase-1; Con, control.

Notably, 25 μM CS had a marked inhibitory effect on cytokine formation in both cell lines. In addition, the suppressive effects of 25 μM CS on chemokine and mediators, such as MCP-1 and NO were comparable to those of 20 μM DEX. These results suggested CS may prevent and improve the development of systemic or bronchial inflammatory disease.

MAPK and NF-κB inactivation mitigates hyperinflammation in various cell types, such as macrophages and lung epithelial cells (30,38-40). Therefore, MAPK and NF-κB pathways have been targeted as a therapeutic strategy to improve inflammatory disease. Notably, the present study demonstrated the inhibitory effect of CS on LPS- or PMA-induced MAPK and NF-κB activation *in vitro*. These observations suggested that CS may serve as an MAPK/NF-κB inactivator in systemic or bronchial inflammatory disease.

There is a marked focus on HO-1 induction to decrease the inflammatory response (30,31). Notably, in the present study, 25 μM CS significantly elevated HO-1 expression in both RAW264.7 and A549 cell lines, indicating that CS exerted not only anti-inflammatory, but also antioxidant effects.

Serotonin and its derivative (N-feruloylserotonin) decrease inflammatory responses both *in vivo* and *in vitro* (41,42). Similar to previous results (37,41,42), the present study

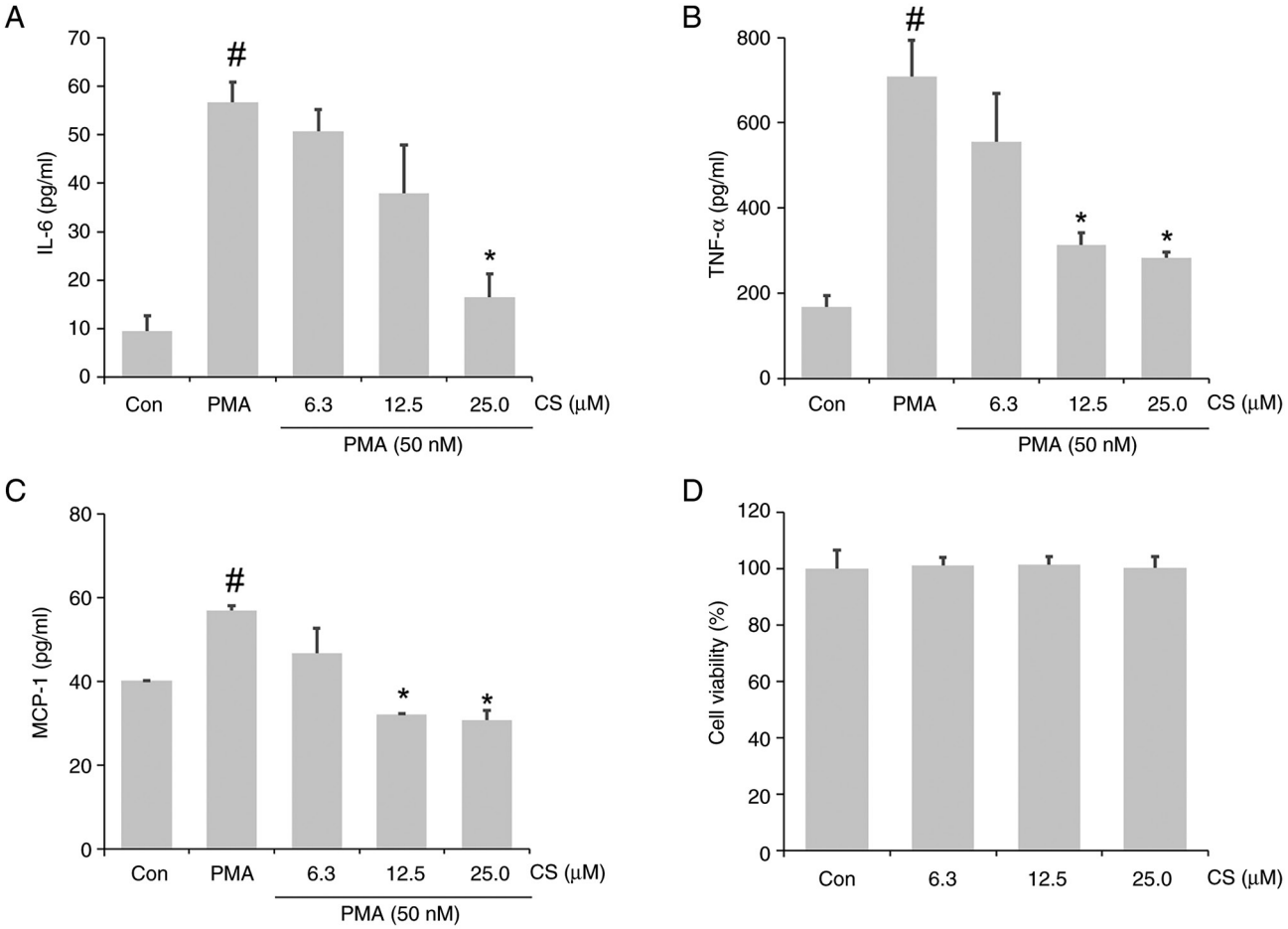

Figure 6. CS pretreatment decreases PMA-stimulated cytokine and chemokine secretion in A549 cells. The levels of (A) IL-6, (B) TNF-α and (C) MCP-1 in cell culture supernatant were detected using ELISA kits. The A549 cells were incubated with various concentrations of CS for 24 h. (D) Cell viability was analyzed using an MTT assay. #P<0.05 vs. con. *P<0.05 vs. 50 nM PMA only condition. CS, *N*-(p-Coumaroyl) serotonin; PMA, phorbol 12-myristate 13-acetate; MCP-1, monocyte chemoattractant protein-1; Con, control.

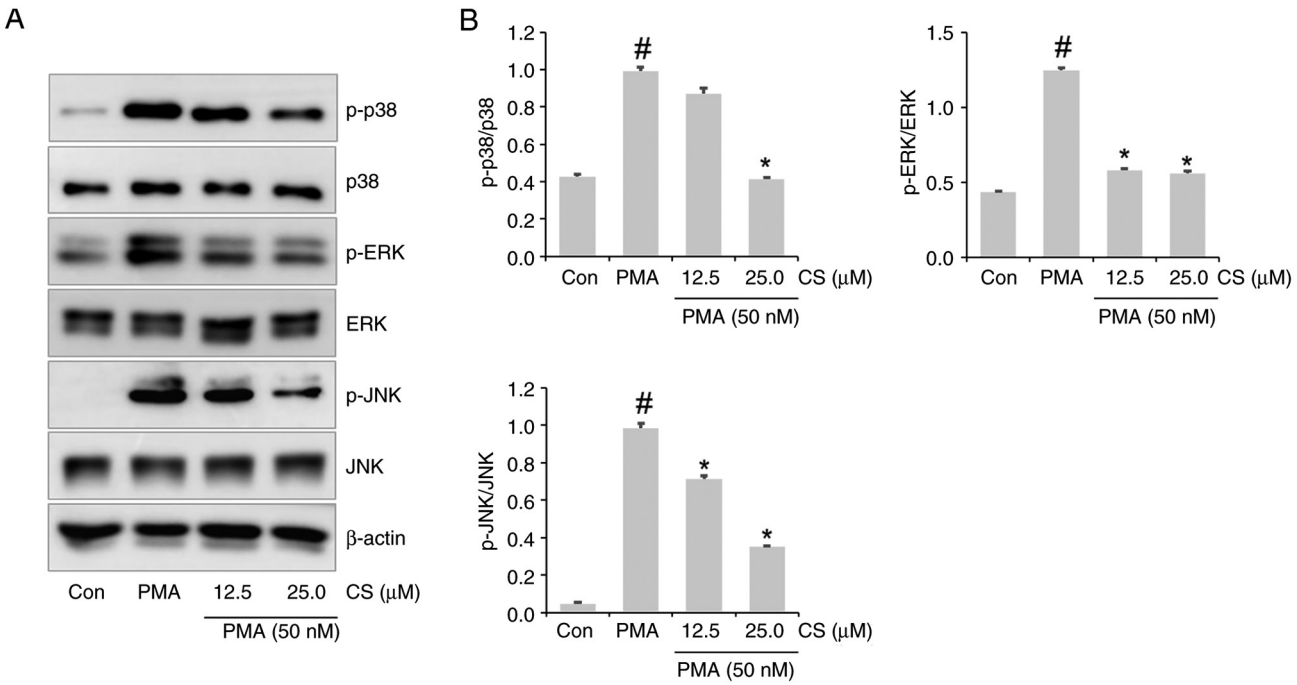

Figure 7. CS pretreatment decreases PMA-induced mitogen-activated protein kinase activation in A549 cells. (A) Levels of p-p38, p-ERK and p-JNK in cell lysate were detected using western blotting. (B) Protein semi-quantitative analysis of p-p38/p38, p-ERK/ERK and p-JNK/JNK. #P<0.05 vs. con. *P<0.05 vs. 50 nM PMA only condition. CS, *N*-(p-Coumaroyl) serotonin; PMA, phorbol 12-myristate 13-acetate; p-, phosphorylated; Con, control; p-, phosphorylated.

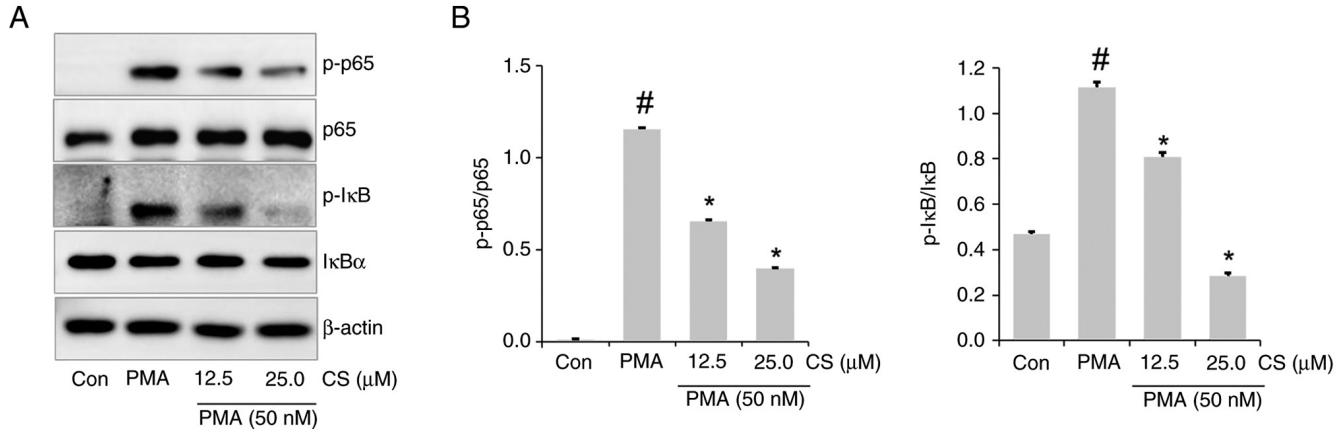

Figure 8. CS pretreatment decreases PMA-induced NF-κB activation in A549 cells. (A) Levels of p-NF-κB p65 and p-IκBα in the cell lysates were detected using western blotting. (B) Protein semi-quantitative analysis of p-p65/p65 and p-IκB/IκB was performed using ImageJ software. #P<0.05 vs. con. *P<0.05 vs. 50 nM PMA only condition. CS, *N*-(p-Coumaroyl) serotonin; PMA, phorbol 12-myristate 13-acetate; p-, phosphorylated.

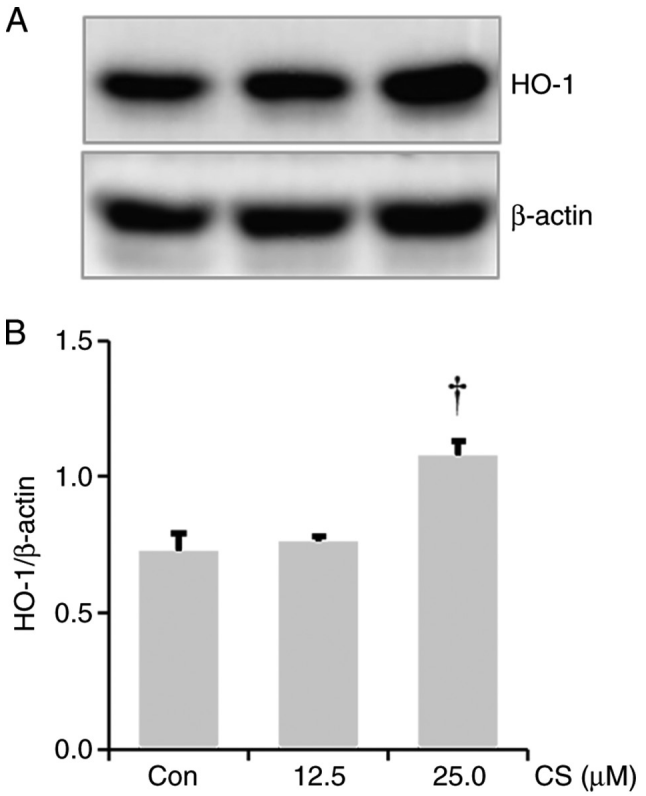

Figure 9. CS upregulates HO-1 expression in A549 cells. (A) Levels of HO-1 in the cell lysate were detected using western blotting. (B) Protein semi-quantitative analysis of HO-1/β-actin. †P<0.05 vs. con. CS, *N*-(p-Coumaroyl) serotonin; HO-1, heme oxygenase-1; Con, control.

demonstrated the anti-inflammatory properties of CS *in vitro* by evaluating its inhibitory ability on cytokine and chemokine secretion.

In summary, the present study demonstrated that CS ameliorated inflammation in both activated macrophages and lung epithelial cells via MAPK and NF-κB inactivation. Furthermore, 25 μM CS and 20 μM DEX showed similar effects on reducing MCP-1 and NO in activated-RAW264.7 cells. These results suggested CS may serve as an adjuvant or therapeutic for sepsis, ALI and asthma. However, further studies are required to determine whether CS affects the activation of other pathways, such as STAT3. In addition, animal studies are required to confirm the efficacy and mechanism of CS.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SHY, CHJ and SJP performed experiments. HJL, OKK and JWL conceived and designed the study. SHY, CHJ, SJP, HJL, OKK and JWL wrote the manuscript. SHY, CHJ, SJP, HJL, OKK and JWL confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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