Naringin inhibits chemokine production in an LPS-induced RAW 264.7 macrophage cell line

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Abstract. Naringin has been reported to act as an effective anti-inflammatory compound. In a previous study, we demonstrated that the anti-inflammatory effect of naringin on lipopolysaccharide (LPS)-induced acute lung injury in mice correlated with the inhibition of the nuclear factor-κB (NF-κB) pathway. However, the effects and mechanism of action of naringin on LPS-induced chemokine expression are not yet fully understood. This study aimed to evaluate the effect of naringin on chemokine expression in LPS-induced RAW 264.7 macrophages and to provide insights into the possible underlying mechanisms. We found that the *in vitro* pre-treatment with naringin led to a significant attenuation in the LPS-induced secretion of interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α). RT-qPCR demonstrated that naringin significantly reduced the LPS-induced upregulation of IL-8, MCP-1 and MIP-1α mRNA expression in a dose-dependent manner. Additionally, western blot analysis revealed that naringin effectively suppressed NF-κB activation by inhibiting the degradation of IκB-α and the translocation of p65. Naringin also attenuated MAPK activation by inhibiting the phosphorylation of ERK1/2, JNK and p38 MAPK. Taken together, these demonstrate that naringin reduces IL-8, MCP-1 and MIP-1α secretion and mRNA expression, possibly by blocking the activation of the NF-κB and MAPK signaling pathways in LPS-induced RAW 264.7 macrophages.

Introduction

Inflammation is considered to be closely associated with a number of diseases, such as infection, and environmental, autoimmune and chronic diseases. Following injury, inflammation is one of the main mechanisms involved in the repair of tissue and consists of a cascade of cellular and microvascular reactions that help remove damaged tissue and generate new tissue. The cascade includes elevated permeability in microvessels, the attachment of circulating cells to the vessels in the vicinity of the injury site, the migration of several cell types, macrophage/monocyte chemotaxis, the release of inflammatory mediators, cell apoptosis, as well as the growth of new tissue and blood vessels (1,2). Inflammation is an important aspect of the pathogenesis of several types of acute and chronic airway and lung diseases, such as acute lung injury (ALI), acute respiratory distress syndrome (ARDS), asthma and chronic obstructive pulmonary disease (COPD) (3-5). Severe persistent asthma is associated with an increased number of neutrophils in the airways and in the peripheral blood (6). Airway obstruction and chronic expectoration in smokers are associated with increased levels of sputum neutrophils (7). The number of airway neutrophils is also increased in COPD (8).

The recognition of pathogen-associated molecular patterns, such as lipopolysaccharide (LPS), induces the expression of chemokines, such as interleukin-8 (IL-8), to promote the recruitment of various leukocytes, primarily neutrophils, to the site of infection. Upon recruitment, neutrophils undergo phagocytosis and then mediate inflammatory responses (9). Neutrophil recruitment into the lung occurs in a cascade-like sequence of activation/adhesion, sequestration in pulmonary vessels, transendothelial and transepithelial migration (10). Both CXC and CC chemokines have been found to participate in inflammation related to leukocyte trafficking, recruiting and recirculation, and to interact with specific receptors on the surface of the target cells (11). The increased numbers of macrophages in inflammatory sites are partly due to the recruitment of monocytes from the circulation in response to monocyte-selective chemokines, such as monocyte chemoattractant protein-1 (MCP-1) (12). Macrophages are considered to play an essential role in inflammation. When activated by endotoxin, macrophages produce inflammatory cytokines, which in turn activate other macrophages and other nearby cells to promote inflammatory gene expression (13). Macrophages also contribute to immune responses through the production of chemokines, such as regulated on activation, normal T cell expressed and secreted (RANTES), MCP-1 and macrophage inflammatory protein-1α (MIP-1α) (14). A number of previous

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studies have demonstrated that the production of various chemokines may be regulated by both mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) signaling pathways in LPS-induced RAW 264.7 macrophages (15-17).

Naringin is a bioflavonoid derived from grapefruit and related citrus species (18). Naringin or its metabolite, naringenin, has been reported to possess diverse biological and pharmacological properties. Recently, growing evidence has indicated that naringin or naringenin displays anti-inflammatory effects (19-20). In a study conducted in our laboratory, we recently reported that naringin significantly alleviates LPS-induced ALI, by mainly reducing neutrophil migration into the lungs, attenuating pro-inflammatory cytokine production and subsequent lung disease (21). An *in vitro* study using RAW 264.7 cells demonstrated that naringin inhibited the LPS-induced production of nitric oxide (NO), by suppressing the activation of NF-κB (22). Naringin and naringenin have previously reported to inhibit chemokine production. They have also been reported to inhibit aortic MCP-1 gene expression in high cholesterol diet-fed rabbits (23). Furthermore, naringin has been proven to inhibit chemokine RANTES secretion in tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ)-induced human keratinocytes through the NF-κB pathway (24). When cultured with 250 and 500 μM naringin, the production of RANTES in TNF-α/IFN-γ-stimulated HaCaT cell supernatants was reduced and the expression of NF-κB p65 protein in nuclei was also decreased (25). The abovementioned results contributed to our research regarding the properties of naringin for attenuating chemokine production. In this study, we aimed to evaluate the chemokine production and gene expression in LPS-induced RAW 264.7 macrophages with or without pre-treatment with various doses of naringin, and to clarify the possible mechanisms involved.

Materials and methods

Culture and treatment of macrophages. The mouse RAW 264.7 macrophage cell line was purchased from Boster Biological Technology Co., Ltd. (Wuhan, China). Cells were cultured in DMEM supplemented with 10% fetal bovine serum (HyClone, Fisher Scientific International, Logan, UT, USA) and 100 U/ml of penicillin/streptomycin in a humidified 5% CO_2 37˚C incubator. Macrophages were plated in 96-well plates for cell viability analysis or in 6-well plates for other experiments. Macrophages were pre-incubated with naringin (50, 100 or 200 μM), or dexamethasone (DEX, 200 μM) for 1 h prior to the addition of 1 μg/ml LPS.

Reagents. Naringin was extracted from *Citrus grandis* '*Tomentosa*' by water and deposited in ethanol (with concentrated filtrate obtained after 1:10 recrystallization, purity >98.3%, determined by peak area normalization). LPS (from *Escherichia coli* 055:B5) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dexamethasone sodium phosphate injection was purchased from Baiyunshan Medicine Co. (Guangzhou, China, batch no. 100304). The ELISA kits for IL-8, MCP-1 and MIP-1α were purchased from RB Systems (Laredo, TX, USA). All primers for RT-qPCR were synthesized by Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China). Rabbit anti-p65, anti-IκB-α monoclonal antibodies and mouse anti-pERK (Thr202/Tyr204), anti-pJNK (Thr183/Tyr185), anti-p-p38 MAPK (Thr180/Tyr182) and anti-β-actin monoclonal antibodies were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). The purity of all chemical reagents was at least of analytical grade.

Cell viability assay. Cell viability was evaluated by MTT assay as previously described (26). To evaluate the effect of LPS on cell viability, RAW 264.7 macrophages were seeded in 96-well plates at $5x10^4$ cells/well for 24 h. The cells were treated with the various concentrations of LPS (1, 2, 4, 8 and 16 μg/ml) for 24 h at 37˚C. MTT solution was added (20 μl, 1 mg/ml; Sigma-Aldrich) to each well and the cells were further incubated for 4 h at 37˚C. The medium was discarded and 100 μl of DMSO were added to each well for the solubilization of formazan. Optical density was measured at 492 nm on a Tecan Infinite M200 microplate reader.

Chemokine secretion measured by ELISA kits. RAW 264.7 macrophages were plated at a density of $5x10^4$ cells/ml in a 12-well plate and cultured for 24 h in the aforementioned culture medium. The cells were pre-treated with various doses of naringin (50, 100 and 200 μM), or DEX (200 μM) for 1 h and challenged by LPS (1 μg/ml) for 24 h. The culture supernatants were then harvested and IL-8, MCP-1 and MIP-1α levels were analyzed using commercially available ELISA kits (RB Systems), according to the manufacturer's instructions.

Chemokine transcription measured by RT-qPCR. RAW 264.7 macrophages were pre-treated with various doses of naringin (50, 100 and 200 μM), DEX (200 μM) for 1 h and then challenged by LPS (1 μg/ml) for 6 h. IL-8, MCP-1 and MIP-1α mRNA expression was measured by RT-qPCR, as previously described (27). Total RNA was isolated from the cultured RAW 264.7 macrophages using TRIzol (Beyotime), according to the manufacturer's instructions. Prior to cDNA synthesis, RNA was treated with DNase I (Fermentas #EN0521, Thermo Scientific Fermentas, Glen Burnie, MD, USA) to remove traces of DNA, according to the manufacturer's instructions. Synthesis of the first-strand cDNA was primed with oligo$(dT)_{18}$ primer. The synthesis conditions were: 42˚C for 60 min and 70˚C for 5 min. The resulting cDNA was subjected to RT-qPCR. The RT-qPCR amplification was performed with Maxima™ SYBR-Green/ROX qPCR Master Mix (2X) (Thermo Scientific Fermentas) for the IL-8, MCP-1 and MIP-1α genes and GADPH. Cycle-to-cycle fluorescence emission readings were monitored and analyzed using the Bio-Rad iCycler iQ5 detection system (Bio-Rad, Hercules, CA, USA). The quantification of IL-8, MCP-1 and MIP-1α mRNA expression was calculated using the comparative CT method. The primer sequences were as follows: IL-8 sense, 5'-GACGAGACCAGGAGAAACAGGG-3' and antisense, 5'-AACGGAGAAAGAAGACAGACTGCT (530 bp); MCP-1 sense, 5'-GGAAAAATGGATCCACACCTTGC-3' and antisense, 5'-TCTCTTCCTCCACCACCATGCAG-3' (582 bp); MIP-1α sense, 5'-GAAGAGTCCCTCGATGTGGCTA-3' and antisense, 5'-CCCTTTTCTGTTCTGCTGACAAG-3' (561 bp); and GAPDH sense, 5'-TGAAGGTCGGTGTGAACGGATTT GGC-3' and antisense, 5'-CATGTAGGCCATGAGGTCCA CCAC-3' (983 bp). The RT-qPCR conditions were: 95˚C for

5 min and 40 PCR cycles, each consisting of denaturation (95˚C for 15 sec), annealing (58˚C for 15 sec) and extension (72˚C for 30 sec). An additional 81 cycles were performed to measure the melt curve by lowering the temperature by 0.5˚C/cycle/10 sec from 55˚C. For the data analysis, the comparative threshold cycle (CT) value for GADPH was used to normalize loading variations in the RT-qPCRs. The ΔΔCt of each sample was obtained by subtracting the ΔCt value of the sample from the ΔCt value of the reference. The ΔΔCT values were compared with the control by raising 2 to the ΔΔCT power. The RT-qPCR was performed in triplicates for each treatment group.

Western blot analysis for nuclear and cytoplasmic NF-κB and MAPK family proteins. RAW 264.7 macrophages were pre-treated with various doses of naringin (50, 100 and 200 μM), or DEX (200 μM) for 1 h and then challenged by LPS (1 μg/ml) for 6 h. Western blot analyses of NF-κB and MAPK family proteins were performed as previously described, with slight modifications (21). Briefly, proteins were extracted from the cells using the Nuclear and Cytoplasmic Protein Extraction kit (Beyotime) and boiled for 5 min with loading buffer. The extracts were then subjected to SDS-polyacrylamide gel electrophoresis on an 8% gel and transferred onto nitrocellulose membranes (Beyotime). The membranes were washed with PBST and 5% skim milk for 1 h and incubated with diluted primary antibody for 4 h at room temperature. After 3 additional washes, diluted HRP-conjugated secondary antibody was added followed by incubation for 1 h at room temperature. The blots were developed using BeyoECL Plus reagent (Beyotime) and then exposed to film (Fuji, Japan) for 10 sec and placed into developing and fixing solutions for 1 min, respectively. The bands were visualized and quantified using Quantity One imaging software (Bio-Rad). The p65 and IκB-α band intensity was adjusted by the β-actin band intensity. The p-p38, pJNK and pERK1/2 band intensity was adjusted by the p38, JNK and ERK1/2 band intensity, respectively.

Statistical analysis. The results in this study are expressed as the means ± SD of 3 independent measurements performed in triplicate. Statistical analyses were carried out using SPSS 16.0 software. Statistically significant differences in the mean values were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test. P-values <0.05 were considered to indicate statistically significant differences.

Results

Effects of LPS on cell viability. In order to determine the appropriate dose of LPS used in the study of chemokine secretion and mRNA expression, we first investigated the effect of LPS (1, 2, 4 and 8 μg/ml) on the cell proliferation of RAW 264.7 macrophages using MTT assay. As shown in Fig. 1, LPS suppressed the proliferation of RAW 264.7 macrophages at 4-8 μg/ml in a dose-dependent manner. The results indicated that at an increased dose (2 μg/ml for 24 h) of treatment no cytotoxicity was observed in the RAW 264.7 macrophages. Therefore, 1 μg/ml dose of LPS was used in the subsequent experiments.

Naringin inhibits LPS-induced IL-8, MCP-1 and MIP-1α production. IL-8 is an important chemokine for the initiation of innate immune responses that function by recruiting immune cells to inflamed sites (28). MCP-1 is a potent chemoattractant for monocytes and macrophages to areas of inflammation (29). MIP-1α plays a potentially important role in the development of inflammatory responses during infection by recruiting mononuclear cells, modulating cytokine production and mediating fever (30). In order to evaluate the effect of naringin on chemokine production, IL-8, MCP-1 and MIP-1α levels in the culture supernatants of RAW 264.7 macrophages were analyzed. As shown in Fig. 2, the production of IL-8, MCP-1 and MIP-1α in the LPS group was significantly increased, compared to the control group ($p<0.01$). The groups treated with various doses of naringin (50, 100 and 200 μM) showed a significant decrease in the IL-8, MCP-1 and MIP-1α levels in a dose-dependent manner, compared to the LPS group ($p<0.01$).

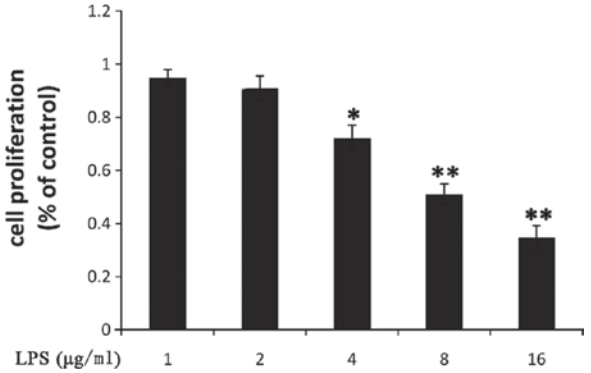

Figure 1. Effects of LPS on cell viability. RAW 264.7 macrophages were treated with various doses of LPS (1, 2, 4, 8 and 16 μg/ml) for 24 h. MTT assay was then performed, as described in Materials and methods. Results are presented as the percentage absorbance of the control group. Data are expressed as the means ± SD. $^{*}p<0.05$, $^{**}p<0.01$ vs. the control group.

Naringin attenuates LPS-induced IL-8, MCP-1 and MIP-1α mRNA expression. The cells treated with LPS at 1 μg/ml showed a noticeable increase in IL-8, MCP-1 and MIP-1α mRNA expression compared to the control group (Fig. 3). When the cells were exposed to various concentrations (50, 100 and 200 μM) of naringin for 1 h prior to LPS stimulation, the IL-8, MCP-1 and MIP-1α mRNA expression was significantly inhibited in a dose-dependent manner, compared to the LPS group ($p<0.01$).

Naringin inhibits the phosphorylation of MAPK and nuclear translocation of NF-κB. The MAPK and NF-κB pathways play significant roles in the expression and production of IL-8, MCP-1 and MIP-1α (15-17). To assess the inhibitory activity of naringin on MAPK and NF-κB pathways, cytoplasm IκB-α, p65, pERK1/2, pJNK, p-p38 and nuclear p65 expression were examined by western blot analysis. As shown in Fig. 4A, in the LPS group, the band densities of pERK1/2, pJNK and p-p38 MAPK were significantly increased compared to the control group, demonstrating that LPS induced MAPK signaling pathway activation. The naringin-treated groups (50 and 200 μM) revealed lower densities of pERK1/2, pJNK and p-p38 MAPK when compared to the LPS group, demonstrating that naringin attenuated the phosphorylation of MAPK induced by 1 μg/ml LPS for 6 h.

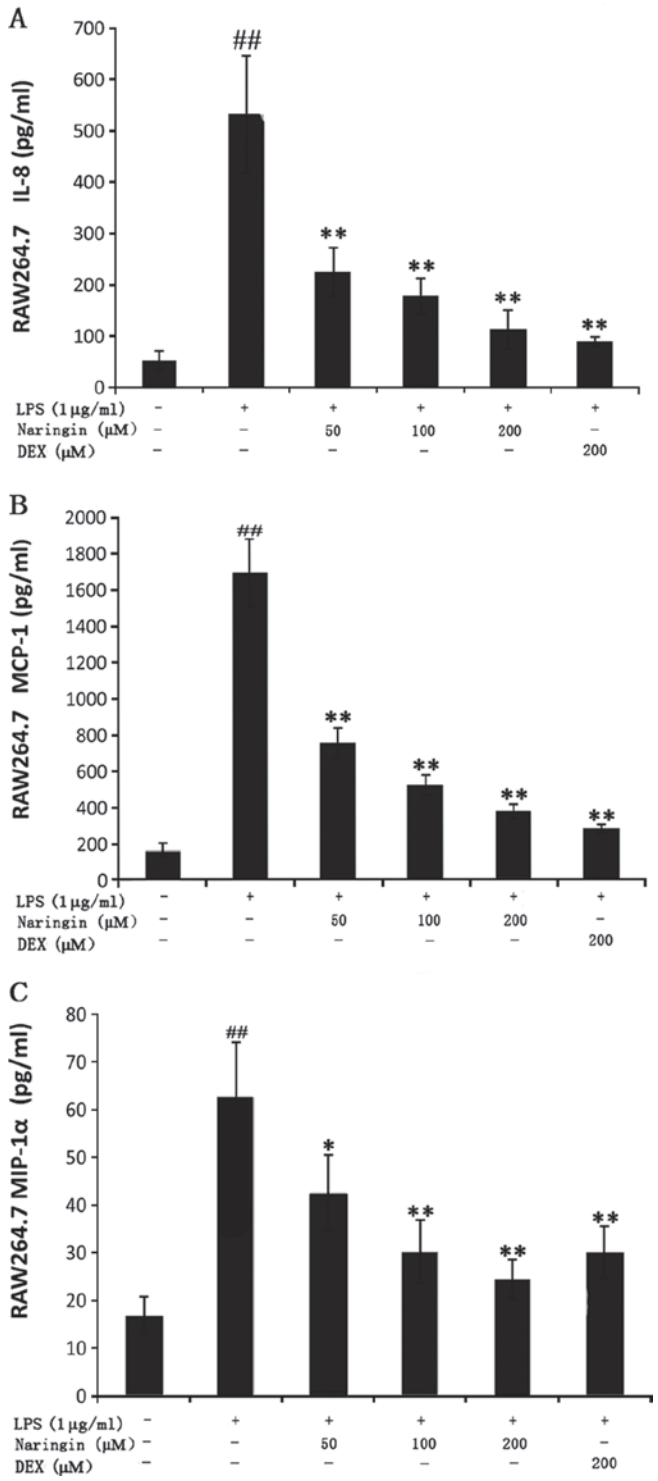

Figure 2. Effects of naringin on LPS-induced chemokine production. RAW 264.7 macrophages were pre-treated with various doses of naringin (50, 100 and 200 μM), or DEX (200 μM) for 1 h and challenged by LPS (1 μg/ml) for 24 h. (A) IL-8 production in supernatants. (B) MCP-1 production in supernatants. (C) MIP-1α production in RAW 264.7 supernatants. Data are expressed as the means ± SD. $^{\#\#}$p<0.01 vs. the control group. *p<0.05, **p<0.01 vs. the LPS group.

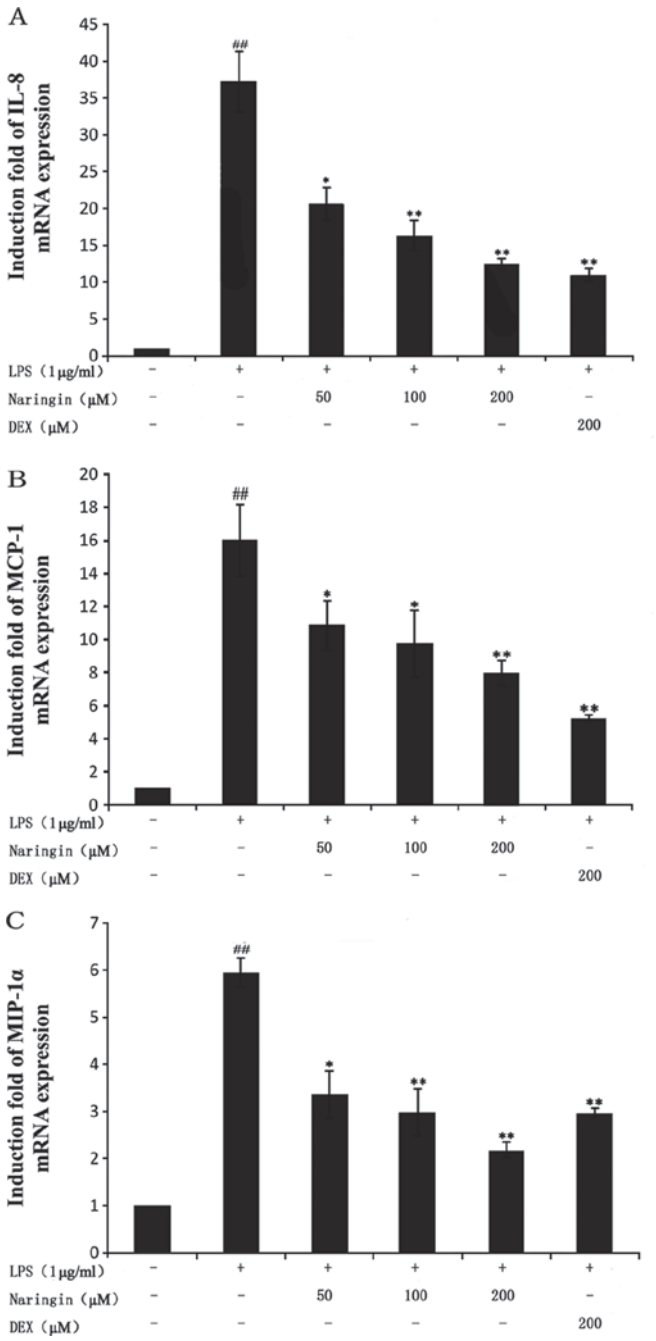

Figure 3. Effects of naringin on LPS-induced chemokine mRNA production. RAW 264.7 macrophages were pre-treated with various doses of naringin (50, 100 and 200 μM), or DEX (200 μM) for 1 h and challenged by LPS (1 μg/ml) for 6 h. IL-8, MCP-1, MIP-1α and GADPH mRNA was detected by RT-qPCR. Quantification of IL-8, MCP-1 and MIP-1α mRNA expression was calculated using the comparative CT method. RT-qPCR was performed in triplicate for each treatment group. Data are expressed as the means ± SD of 3 replicate experiments. $^{\#\#}$p<0.01 vs. the control group. *p<0.05; **p<0.01 vs. the LPS group.

As shown in Fig. 4B, the blotting band of IκB-α, the inhibitory protein of NF-κB by binding to NF-κB dimers and making them inactive, was markedly decreased in the LPS group compared to the control group. By contrast, IκB-α degradation in the naringin-treated groups (50 and 200 μM) was significantly reduced compared with the LPS group, indicating that naringin inhibited IκB-α degradation. The LPS group also showed a decrease in NF-κB density in the cytoplasm and an increase in the nuclear extracts of the p65 subunit of NF-κB. In contrast to this, naringin at a dose of 200 μM inhibited the translocation of p65 from the cytoplasm to the nucleus, since the naringin-treated group remained almost at the same level as the control group for cytoplasm and nuclear p65.

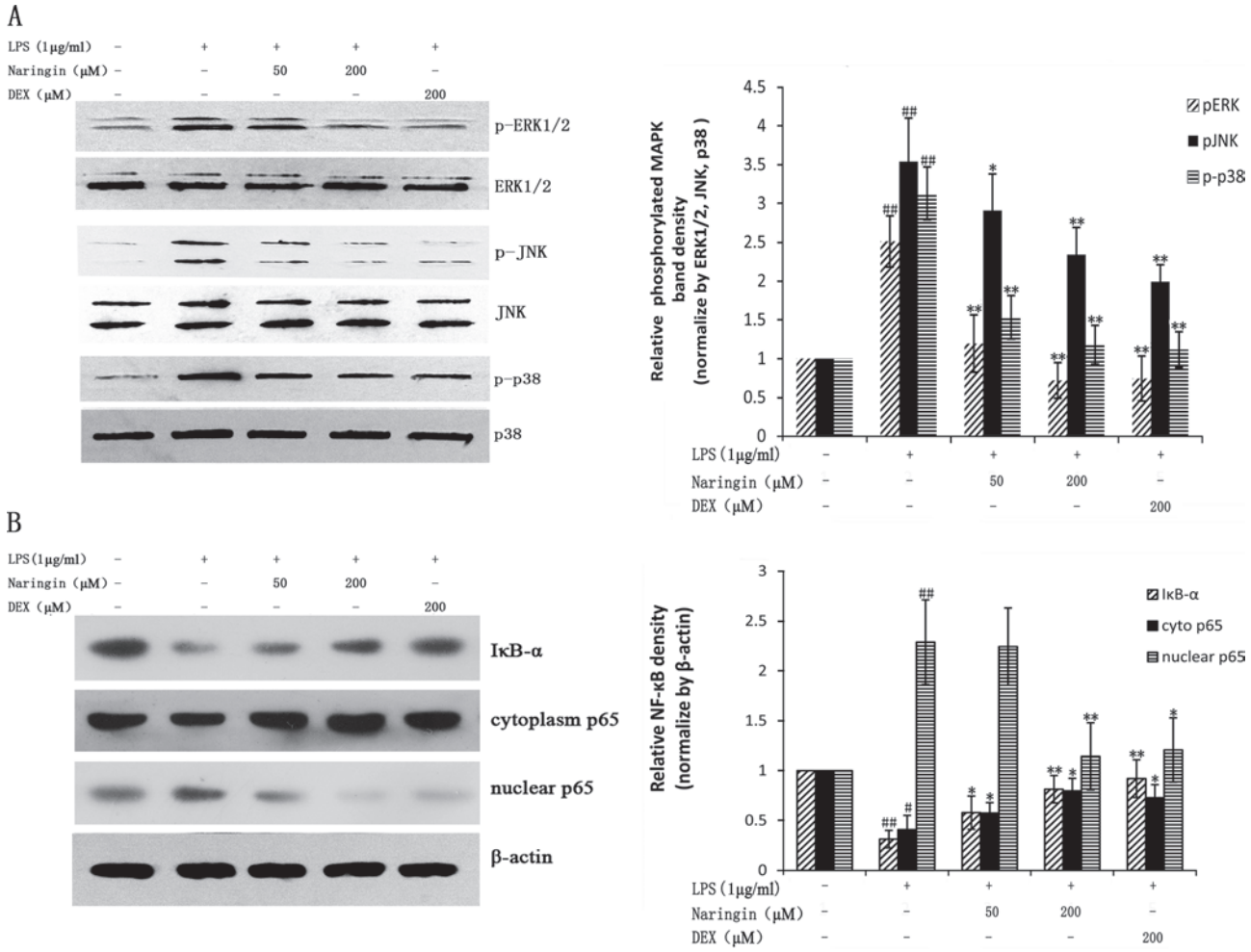

Figure 4. Naringin inhibited NF-κB and MAPK signal transduction. RAW 264.7 macrophages were pre-treated with various doses of naringin (50, 100 and 200 μM), or DEX (200 μM) for 1 h and challenged by LPS (1 μg/ml) for 6 h. Nuclear and cytoplasmic extracts were prepared from the cells and subjected to western blot analysis. (A) The results are expressed as relative ratios of band density of phosphorylated forms of p38, JNK and ERK1/2 to the respective p38, JNK and ERK1/2. (B) Results are expressed as relative ratios of band density of p65 and IκB-α to the respective β-actin. Data are expressed as the means ± SD of 3 replicate experiments. $^{\#\#}$p<0.01 vs. the control group. *p<0.05, **p<0.01 vs. the LPS group.

Discussion

Inflammation is a significant aspect of the pathogenesis of several different types of acute or chronic airway and lung diseases, such as ALI, ARDS, asthma and COPD (3-5). Tissue injury associated with bacterial infections results, in part, from infiltrating leukocytes recruited by local mediators to the site of infection (31). LPS is one of the major constituents of the outer membrane of Gram-negative bacteria and LPS recognition and signal transmission are among the key events in the host defense reaction towards Gram-negative bacteria (32). LPS induces the accumulation of neutrophils into the alveolar space related to the expression of pro-inflammatory cytokines and chemokines (31,32). Chemokines regulated the recruitment of neutrophils, monocytes and macrophages to sites of inflammation and infection (33).

Pulmonary macrophages, with a key role in the defence against respiratory infections, are a heterogeneous family of cells with phagocytic antigen processing and immunomodulatory functions (34). Activated pulmonary macrophages have the capacity to release multiple chemokines leading to the recruitment of several cell types, including monocytes, neutrophils and T-lymphocytes. Neutrophil recruitment has been recognized as one of the key molecular mechanisms inducing chronic airway inflammation. The chemokines released by activated alveolar macrophages recruit neutrophils through a process known as chemotaxis, a biological phenomenon whereby a cell migrates through physiological barriers (e.g., vessel walls or epithelial layers) and tissues towards sites of inflammation or infection, usually in response to concentration gradients of chemoattractant substances (35).

Two major chemokine families, the CC and the CXC chemokines, are distinguished based on their function, sequence and chromosomal location. CC chemokines preferentially attract monocytes and lymphocytes, whereas CXC chemokines mainly have a chemotactic effect on neutrophils (36). Neutrophils are the first cells to reach the infectious loci and it is crucial that they remain there until macrophages and other inflammatory cells arrive to control the infection (37). Prolongation of neutrophil functional longevity by inflammatory mediators causes severe tissue damage in a number of diseases characterized by neutrophilic inflammation, including SIRS and adult ARDS (38,39). IL-8 is a member of the CXC chemokine family that plays a pivotal role in controlling neutrophil and

monocyte chemotaxis towards sites of infection and the induction of airway inflammation (40). Narigenin has been reported to inhibit IL-8 secretion in PMA-induced HL-60 cells (41). In the present study, we demonstrated that naringin (50, 100 and 200 μM) significantly attenuated LPS-induced IL-8 production and mRNA expression in a dose-dependent manner, which indicates that naringin has an inhibitory effect on IL-8 production.

Along with IL-8, MCP-1 has been proven to trigger the firm adhesion of monocytes to the vascular endothelium (42). MCP-1 is a member of the CC chemokines. As a potent chemokine for monocytes and macrophages, MCP-1 has been implicated in the pathogenesis of several diseases characterized by monocyte infiltration (43). The influx of macrophages into these sites of inflammation has been suggested to exacerbate inflammation-related diseases. Thus, the expression of MCP-1, which is likely to be critical for fighting infectious diseases, has to be tightly regulated (44). Elevated MCP-1 levels are found in the bronchial epithelium of idiopathic pulmonary fibrosis and asthma patients, in tuberculosis effusions, as well as during pleural infections (45). In this study, we demonstrated that naringin (50, 100 and 200 μM) significantly attenuated LPS-induced MCP-1 production and mRNA expression in a dose-dependent manner, which indicates that it may inhibit monocyte chemotaxis and control the progress of inflammation.

MIP-1α is one of the major factors produced by macrophages stimulated by bacterial endotoxins. MIP-1α activates human neutrophils, eosinophils and basophils, leading to acute neutrophilic inflammation (46). MIP-1α is produced by several cells, particularly macrophages, dendritic cells and lymphocytes (47). Increased levels of MIP-1α have been described in a variety of human inflammatory conditions, suggesting its importance in the induction and development of chronic inflammatory processes (48). MIP-1α has also been reported to participate in the selective recruitment of mononuclear cells in interstitial lung disease (49). The present study revealed that naringin (50, 100 and 200 μM) significantly attenuated LPS-induced MIP-1α production and mRNA expression in a dose-dependent manner, which indicates that naringin may inhibit macrophage chemotaxis and suppress chronic inflammation.

During acute and chronic diseases, LPS-induced inflammation activates multiple intracellular signalling cascades, including the MAPK signal transduction pathway, which plays a significant role in the recruitment of leukocytes to the sites of inflammation. Activated MAPKs can phosphorylate and activate other kinases or nuclear proteins, such as transcription factors in either the cytoplasm or the nucleus (50). The LPS-mediated activation of the TLR4 complex has been shown to induce specific signaling pathways, involving the phosphorylation of 3 major MAPK pathways, ERK1/2, JNK and p38 MAPKs, which may lead to the liberation of the NF-κB family (51). These signaling pathways induce the production of pro-inflammatory cytokines and chemokines in macrophages, including TNF-α, IL-8 and MCP-1 (52). Naringin and naringenin have been reported to regulate the phosphorylation of ERK1/2, JNK and p38 MAPK in a variety of cells, treated with different stimuli (53-56). In high fat diet-fed mice, naringin exerts inhibitory effects on metabolic syndrome by activating AMP-activated protein kinase (AMPK) to inhibit MAPK-dependent signaling, including the ERK1/2, JNK1/2 and p38 pathways (56). Pre-treatment with 50 μg/ml naringenin, however, has been reported to increase the phosphorylation of ERK5 and p38a MAPK in U937-derived macrophages stimulated with 1 μg/ml LPS for 1 h (55). In the present study, to our knowledge, we demonstrate for the first time that pre-treatment with 50 and 200 μM of naringin attenuates the LPS-induced phosphoration of the ERK1/2, JNK and p38 MAPK pathways in RAW 264.7 macrophages.

NF-κB has been reported to regulate more than 150 genes, including those involved in immunity and inflammation, anti-apoptosis, cell proliferation and the negative feedback of the NF-κB signal (57). NF-κB positively regulates genes encoding cytokines, cell adhesion molecules, chemokines (e.g., IL-8, MIP-1α, MCP-1 and RANTES) (58). NF-κB plays a fundamental role in the inflammatory and acute response (52). NF-κB subunits are normally inactive, being bound to a protein known as IκB-α. Signaling through cytokines such as LPS, TNF-α and IL-1 causes the dissociation of NF-κB from IκB-α. The transcription factor, p65, translocates to the nucleus and binds to DNA, regulating the transcription of various inflammatory mediators (59). Similarly to other members of the NF-κB family, NF-κB p65 also resides in the cytoplasm in an inactive form, bound to inhibitory IκB proteins. Cellular activation leads to the nuclear translocation of NF-κB p65 to initiate gene transcription. The translocation of NF-κB p65 from the cytoplasm to the nucleus is often taken as an indication of NF-κB-activation and correlates with the cellular response to oxidants or with the inflammatory and acute immune response (60). Naringin has been reported to reduce nuclear p65 activation in LPS-treated J774 macrophages (61). However, another study demonstrated that 30 μM naringin had no significant effect on attenuating NF-κB activation, which was induced by 50 ng/ml LPS for 1 h in a RAW 264.7 cell line (62). In the present study, we demonstrate that pre-treatment with 50 and 200 μM of naringin significantly inhibits IκB-α degradation and attenuates NF-κB p65 subunit translocation in RAW 264.7 macrophages, induced by 1 μg/ml LPS for 6 h.

On the basis of the results presented in this study, we demonstrate that naringin attenuates the production of chemokines, such as IL-8, MCP-1 and MIP-1α at least in part, by interfering with the NF-κB and MAPK signaling pathways, which may partially account for its anti-inflammatory effects.

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