Naringenin Regulates Lipopolysaccharide-Induced Abnormal Airway Surface Liquid Secretion

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Abstract
Airway surface liquid (ASL) is one of the key factors affecting the respiratory system’s physiological function. Abnormal ASL secretion can increase the incidence of various respiratory diseases. Lipopolysaccharide (LPS) stimulation can damage the airway epithelial barrier, affect the concentration of ASL contents, and down-regulate ion channel expression, which in turn causes abnormal ASL secretion. Naringenin, which exists in many Citrus foods, has the ability to promote airway surface liquid secretion. This work is designed to investigate the regulatory mechanism of naringenin on LPS-induced abnormal ASL secretion. The effects of naringenin and LPS on the viability of Calu-3 cells were measured by CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS). ASL secretion volume was measured by a micropipette on air–liquid interface cultured cells. The concentration of Cl⁻, Na⁺, lysozyme, and total protein in ASL were respectively measured by assay kits. The mRNA expressions were determined by quantitative real-time polymerase chain reaction, and proteins were measured by enzyme-linked immunosorbent assay. The results indicated that LPS could affect ASL secretion and regulate cystic fibrosis transmembrane conductance regulator (CFTR), aquaporin 1 (AQP1) and aquaporin 5 (AQP5) expression. Naringenin had the ability to regulate the ASL secretion by increasing secretion volume, and Cl⁻ and Na⁺ concentrations, reducing lysozyme and total protein content, and regulating CFTR, AQP1, and AQP5 expression. This study indicated that naringenin had regulating effects to attenuate LPS-induced abnormal ASL secretion.

Keywords
naringenin, lipopolysaccharide, airway surface liquid, secretion

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Introduction
Airway surface liquid (ASL) lies apically on top of airway epithelia, which can adhere foreign bodies and provide a microenvironment for mucociliary oscillates to remove the adhering foreign bodies.¹ ASL is one of the key factors affecting the respiratory system’s physiological function. The ion content, protein abundance, and pH all have important effects on the physical and chemical properties of ASL, and, therefore, the ability to regulate ASL is crucial for normal respiratory system physiology.² Airway epithelial cells can affect the diversion of water by regulating the secretion of ions, mucins, and proteases, thereby regulating the composition of ASL solutes and solvents. Abnormal ASL secretion can increase the incidence of various respiratory diseases.³,⁴

Lipopolysaccharide (LPS) is a toxic substance present in the cell wall of Gram-negative bacteria. In the respiratory system, LPS stimulation can down-regulate the expression levels of cell tight junction proteins, which, in turn, damage the airway epithelial barrier and affect the concentration of ASL contents. In addition, LPS can activate the mitogen-activated protein kinase (MAPK) signaling pathway and induce increased mucin secretion, causing ASL to become viscous, affecting sputum discharge.⁵ Furthermore, LPS can also down-regulate ion channel expression by activating phosphoinositol-3-kinase (PI3K)-Akt and tumor necrosis factor-alpha (TNF-α) signaling pathways,⁶ resulting in variable secretion of ASL, blocking of
mucociliary movement, and aggravation of tissue inflammation. It has been observed that LPS can affect the ASL secretory function in many ways, resulting in increased sputum and inflammation.

Naringenin (Figure 1) is a flavonoid found in many Citrus foods such as grapefruit, tangerine, and orange. Previous studies have shown that naringenin has significant biological activities such as antitussive, expectorant, and anti-inflammatory.8-15 Our previous research results indicate that naringenin can promote airway surface liquid secretion.8,9,11 This work is designed to investigate the regulatory mechanism of naringenin on LPS-induced abnormal ASL secretion.

Materials and Methods

Cell Culture

Calu-3 cells were cultured in Dulbecco’s modified Eagle’s medium/F12 (DMEM/F12, Gibco) to which was added 10% fetal bovine serum (HyClone), 1% MEM non-essential amino acid solution (MEM NEAA) (Gibco, USA), and 100 U/mL penicillin–streptomycin solution (HyClone), in a humidified atmosphere of 5% CO₂ at 37 °C.

Chemicals

Lipopolysaccharides (LPS), naringenin, dexamethasone, and forskolin were purchased from Sigma-Aldrich. All four were dissolved in dimethyl sulfoxide (DMSO) for incubating Calu-3 cells. The final concentration of DMSO was 0.1%, which had no significant effects in the follow-up experiments.

Cell Viability Assay

Viability of cells exposed to different concentrations of LPS, naringenin, dexamethasone, and forskolin for 24 h was evaluated by MTS (CellTiter 96® AQueous One Solution Cell Proliferation Assay) using the method referred to by Shi et al.11

Measurement of Cl⁻, Na⁺, Protein, and Lysozyme Concentrations

Tightly connected Calu-3 cells were air–liquid interface cultured in Transwell® with different concentrations of LPS, naringenin, dexamethasone, and forskolin for 24 h. The ASL collected by a micropipette was used to assay the volume and the Na⁺, Cl⁻, total protein, and lysozyme concentrations.11,16,17

The concentrations of Cl⁻, Na⁺, and lysozyme in ASL were respectively measured using chlorine, sodium, and Lysozyme Assay Kits. Protein content was determined by the bicinchoninic acid (BCA) Protein Assay Kit (Beyotime).

Measurement of aquaporin 1 and aquaporin 5 mRNA Expression

Calu-3 cells grown in 6-well plates were induced with different concentrations of LPS, naringenin, dexamethasone, and forskolin for 8 h. GoScript™ Reverse Transcription System (Promega) was used to reverse transcript mRNA, and GoTaq® quantitative polymerase chain reaction (qPCR) Master Mix (Promega) was used to quantitatively determine the mRNA level of AQP1 and AQP5 by quantitative real-time polymerase chain reaction (qRT-PCR). The specific method used was that referred to by Shi et al.9

Gene-specific primers: AQP1 forward 5′-GGT GGG GAA CAA CCA GACG-3′ and reverse 5′-TAC ATG AGG GCA CGG AAG ATG-3′, AQP5 forward 5′- GGTT GTG CTC CGT GCC CT TCT CCT-3′ and reverse 5′- CTT CCG CTC TTC CCG CTG CTC-3′, β-actin forward 5′-CCT GTA CGC CAA CAC AGT GC-3′ and reverse 5′-ATA CTC CTG CTT GCT GAT CC-3′. β-Actin was used as internal control.

Measurement of aquaporin 1, aquaporin 5 and cystic fibrosis transmembrane conductance regulator Protein Expression

Calu-3 cells were respectively treated with LPS, DMSO, naringenin, dexamethasone, and forskolin for 24 h. The aquaporin 1 (AQP1) content was assayed using the Aquaporin 1 Assay Kit (SEA579Hu, USCN KIT Inc.). The aquaporin 5 (AQP5) content was assayed using the Aquaporin 5 Assay Kit (SEA583Hu, USCN KIT Inc.). The cystic fibrosis transmembrane conductance regulator (CFTR) expression content was assayed using the Cystic Fibrosis Transmembrane Conductance Regulator Assay Kit (SEC425Hu, USCN KIT Inc.). Protein content was determined by the BCA Protein Assay Kit (Beyotime). The data were expressed in nanograms per mg of total protein.

Data Analysis and Statistics

The data were represented as the mean±SEM (standard error of the mean)(n is the number of experiment replications). Student’s t-test was used to evaluate statistical significance.
Results and Discussion

Effect of Naringenin and lipopolysaccharide on the Viability of Calu-3 Cells

To determine the appropriate concentrations of naringenin and LPS for subsequent studies, MTS technology was applied to investigate their effects on cell viability. As it was considered that 24 h were required to detect protein changes, a 24-h cell viability study was executed with selected safe doses. There was no significant effect on cell viability after incubation with naringenin (25 μM, 50 μM, and 100 μM) for 24 h (Figure 2a). In the co-incubation study with LPS (10 μg/mL) and naringenin, there were no significant effects on cell viability from low to high doses after 24 h (Figure 2b). The above experimental results indicated that the drug dose was safe for the Calu-3 cells and would not cause apoptosis. Therefore, safe drug dosages were chosen to use in subsequent experiments.

Effect of Naringenin on lipopolysaccharide-induced airway surface liquid Secretion Volume of Calu-3 Cells

Calu-3 cells were cultured in air–liquid-interface conditions to examine the effect of naringenin on the volume of ASL and the regulation of naringenin on LPS-induced ASL secretion. The results showed that, after 24 h of administration, naringenin (100 μM) could significantly increase the ASL secretion volume of Calu-3 cells, and this secretion-promoting effect showed a dose-dependent relationship in the concentration range of 25–100 μM (Figure 3a). A total of 10 μg/mL LPS could significantly increase the volume of ASL in the airway epithelium after 24-h treatment. Based on treatment with LPS, the addition of naringenin, dexamethasone, and forskolin could respectively increase the ASL volume (Figure 3b). The secretory effect of naringenin presented in a dose-dependent manner with no significant difference compared with the LPS group, while 25 nM dexamethasone and 10 μM forskolin could significantly increase the secretory volume. Among these, the secretion-promoting effect of forskolin was most obvious.

Effect of Naringenin on lipopolysaccharide-Induced Cl− and Na+ Secretion in Calu-3 Cells

Calu-3 cells with air–liquid-interface culture were utilized to investigate the effect of naringenin-induced Cl− and Na+ secretion and the regulation of naringenin on LPS-induced cells. Naringenin (25-100 μM) dose-dependently increased the Cl− concentration of ASL; naringenin 50 μM and 100 μM were significantly different from the control group (Figure 4a). Naringenin also could increase Na+ concentration, which was significantly different at a high dose (100 μM) (Figure 4c). There was no significant effect on the concentration of Cl− and Na+ after treatment with 10 μg/mL LPS for 24 h. Based on treatment with LPS, the addition of naringenin could increase the concentration of Cl− with no statistical difference with the LPS group, while forskolin could significantly increase the concentration of Cl− (Figure 4b). From the aspect of Na+ concentration, the addition of naringenin, dexamethasone, and forskolin on the LPS-treated cells could increase the concentration of Na+, and both dexamethasone and forskolin groups were statistically different from the LPS group (Figure 4d).

Figure 2. Effect of naringenin and LPS on calu-3 cell viability. (a) After 24 h of administration, naringenin did not significantly influence cell viability. (b) The co-incubation of LPS and naringenin did not significantly influence cell viability. Data are presented as the mean ± SEM (n = 3; *P < 0.05; **LPS: *P < 0.05).

Abbreviations: LPS, lipopolysaccharide; SEM, standard error of the mean.
Effect of Naringenin on lipopolysaccharide-Induced Lysozyme and Total Protein Secretion in Calu-3 Cells

After the administration of air–liquid-cultured Calu-3 cells for 24 h, naringenin could reduce the concentrations of lysozyme (Figure 5a) and total protein (Figure 5c) secreted in the ASL in a dose-dependent manner, but there was no significant difference compared with the control group. A total of 10 μg/mL LPS could significantly increase the concentration of lysozyme in ASL. The addition of naringenin dose-dependently reduced the lysozyme concentration in the concentration range of 25 to 100 μM, as well as dexamethasone and forskolin (Figure 5b). Both high-dose naringenin (100 μM) and forskolin significantly reduced total protein concentrations of LPS-treated cells (Figure 5d). The results suggested that naringenin could increase the secretion volume of ASL while reducing the concentration of lysozyme and total protein.

Effect of Naringenin on lipopolysaccharide-Induced Expression of aquaporin 1 and aquaporin 5 mRNA

Our previous experiments had investigated the effect of naringenin on LPS-induced low CFTR expression in Calu-3 cells.9 The results showed that naringenin could increase CFTR expression while organismal CFTR expression was seriously disrupted; but it did not affect normal cells. Therefore, in this experiment, we focused on the effect of naringenin on the expression of AQP1 and AQP5 mRNA. Compared with the control group, the three doses of naringenin (25 μM, 50 μM, 100 μM) could not significantly change the expression levels of AQP1 (Figure 6a) and AQP5 (Figure 6c) mRNA in Calu-3 cells. After LPS was incubated with Calu-3 cells for 8 h, AQP1 (Figure 6b) and AQP5 (Figure 6d) mRNA expression were significantly decreased, indicating that LPS could inhibit the expression of AQP1 and AQP5 mRNA, while different doses of naringenin (25–100 μM) could dose-dependently enhance the AQP1 and AQP5 mRNA expression. The high-dose naringenin (100 μM) group had significant differences compared to the LPS group. Dexamethasone and forskolin could restore LPS-induced low expression of AQP1 mRNA, but did not affect the low expression of AQP5 mRNA. It showed that naringenin could restore the AQP1 and AQP5 mRNA expression levels of cells reduced by LPS stimulation.

Effect of Naringenin on lipopolysaccharide-Induced Expression of cystic fibrosis transmembrane conductance regulator, aquaporin 1, and aquaporin 5 Proteins

Consistent with mRNA detection, after 24 h of administration, naringenin (25 μM, 50 μM, and 100 μM) did not significantly change the expression levels of CFTR (Figure 7a), AQP1 (Figure 7c), and AQP5 (Figure 7e) proteins compared with the control group. Incubation of 10 μg/mL LPS for 24 h reduced CFTR, AQP1, and AQP5 protein expression, which indicated that LPS could inhibit these three proteins’ expression.
in Calu-3 cells. Different doses of naringenin were added to test the expression change in these three proteins. Naringenin dose-dependently increased the expression of CFTR protein in the concentration range of 25–100 μM; all different dose groups had significant differences from the LPS model group. A total of 10 μM forskolin also restored LPS-induced low CFTR protein expression, whereas 25 nM dexamethasone did not (Figure 7b). Similarly, adding different doses of naringenin could up-regulate the expression of AQP5 (Figure 7f) proteins, but only the high-dose (100 μM) group could significantly up-regulate AQP5 protein compared to the LPS model group. The up-regulation of the AQP1 (Figure 7d) protein was not significant. Forskolin could regulate the expression of both AQP1 and AQP5 proteins, while dexamethasone only regulated the expression of AQP1 protein. The results showed that naringenin had no effect on the protein expression of CFTR, AQP1, and AQP5 in normal Calu-3 cells, but could up-regulate the CFTR and AQP5 protein expression level of cells reduced by LPS stimulation.

Discussion

Abnormal secretion of ASL is one of the main causes of clinical respiratory system disease. It can damage the respiratory system

Figure 4. Effect of naringenin on LPS-induced Cl\(^{-}\) and Na\(^{+}\) secretion in Calu-3 cells. Naringenin could dose-dependently increase the (a) Cl\(^{-}\) and (c) Na\(^{+}\) concentration of ASL. Based on treatment with LPS, the addition of naringenin, dexamethasone, and forskolin could increase (b) Cl\(^{-}\) and (d) Na\(^{+}\) secretion to varying degrees. Data are presented as the mean ± SEM (n = 3; vs Control: * P < 0.05, ** P < 0.01, *** P < 0.001; vs LPS: ## P < 0.01, ### P < 0.001).

Abbreviations: LPS, lipopolysaccharide; ASL, airway surface liquid; SEM, standard error of the mean.
from multiple angles, which leads to functional damage. Therefore, it is necessary to carry out relevant treatment research. Previous studies have shown that LPS can cause abnormal ASL secretion, the main reason being that LPS can inhibit the expression of AQPs and CFTR protein, promote mucin secretion, and increase inflammatory factors and apoptotic cells.\textsuperscript{18-22} Our previous research indicated that naringenin has the effect of promoting ASL secretion by up-regulating the intracellular cyclic adenosine monophosphate (cAMP) content to activate CFTR for promoting $\text{Cl}^-$ secretion, and then change the osmotic pressure to induced water transport.\textsuperscript{9} In addition, naringenin can also increase LPS-induced low expression of CFTR mRNA.\textsuperscript{9} However, the regulation effect of naringenin on the key AQPs’ expression during the

![Figure 5](image_url)

**Figure 5.** Effect of naringenin on LPS-induced lysozyme and total protein secretion in Calu-3 cells. Naringenin dose-dependently reduced the concentrations of (a) lysozyme and (c) total protein secreted in the ASL. LPS significantly increased the concentration of lysozyme in ASL. The addition of naringenin dose-dependently reduced the (b) lysozyme and (d) total protein concentration. Data are presented as the mean ± SEM ($n = 3$; $\nu\nu$ Control: * $P < 0.05$; $\nu\nu$ LPS: $^\# P < 0.05$, $^\## P < 0.01$).

Abbreviations: LPS, lipopolysaccharide; ASL, airway surface liquid; SEM, standard error of the mean.

secretion, and the influence of naringenin on the physicochemical properties of the secreted ASL in the LPS-induced abnormal ASL secretion cell model had not been investigated.

Using MTS to screen the safe dose, it was found that neither LPS (10 μg/mL) nor naringenin (25 μM, 50 μM, and 100 μM) had significant effect on Calu-3 cell activity within 24 h (Figure 2), indicating that the selected dose was safe and could be used in subsequent studies. The addition of naringenin to air–liquid interface cultured Calu-3 cells could promote the secretory function of normal respiratory epithelial cells. By increasing the concentration of $\text{Cl}^-$ (Figure 4a) and $\text{Na}^+$ (Figure 4c) in ASL, naringenin was able to increase the volume of secreted fluid (Figure 3a), thereby reducing the content of lysozyme (Figure 5a) and total protein (Figure 5c).
Our previous research results demonstrated that naringenin had the ability to stimulate Cl\(^{-}\) secretion, which was mediated by CFTR through a signaling pathway by increasing cAMP content, and this secretion process was supported by Na\(^{+}\)–K\(^{+}\)–2Cl\(^{-}\)cotransporters and K\(^{+}\) channels on the basolateral membrane.\(^9\) Combined with this result, we speculated that naringenin activated CFTR and promoted Cl\(^{-}\) transepithelial transport. In addition, CFTR could regulate the function of endogenous Epithelial sodium channel (ENaC) in airway cells.\(^{23}\) Therefore, naringenin-activated CFTR could simultaneously promote Cl\(^{-}\) and Na\(^{+}\) secretion, causing an increase in the concentration of both ions in ASL. By changing the osmotic pressure of the solution, naringenin affected water transport and promoted the increase in the volume of ASL. The increased water not only diluted the lysozyme in the solution but also reduced the total protein concentration. Therefore, naringenin could increase ASL secretion and regulate the microenvironmental homeostasis of the respiratory tract.

LPS stimulation can cause inflammation of airway epithelial cells and down-regulate the expression levels of cell tight junction proteins, which in turn cause increased permeability of the airway epithelium.\(^{24,25}\) Therefore, LPS stimulation could increase ASL secretion volume (Figure 3b). Since LPS affected the paracellular permeability by decreasing the cell tight junction proteins,\(^{25}\) stimulation with LPS did not change the ion concentration in the solution (Figure 4b and d). However, because LPS could cause an inflammatory response, it could increase the lysozyme secretion (Figure 5b) at the same time. Based on treatment with LPS, the addition of naringenin, dexamethasone, and forskolin could regulate the secretion volume (Figure 3b) and content of ASL (Figures 4 and 5) to varying degrees. Among them, dexamethasone and forskolin were used as positive

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**Figure 6.** Effect of naringenin on LPS-induced expression of AQP1 and AQP5 mRNA. After 8 h of administration, naringenin significantly changed the expression levels of (a) AQP1 and (c) AQP5 mRNA in Calu-3 cells. After LPS was incubated with Calu-3 cells for 8 h, (b) AQP1 and (d) AQP5 mRNA expression were significantly decreased, while naringenin dose-dependently enhanced the mRMA expression. The 100 \(\mu\)M naringenin group had significant differences compared to the LPS group. Data are presented as the mean ± SEM (\(n = 3\); vs Control: *\(P < 0.05\), **\(P < 0.01\); vs LPS: #\(P < 0.05\)).

Abbreviations: LPS, lipopolysaccharide; SEM, standard error of the mean; AQP1: aquaporin1; AQP5: aquaporin5
controls to evaluate the regulation efficiency of naringenin in LPS-induced abnormal ASL secretion. The regulation of dexamethasone was related to its anti-inflammatory mechanism and effect of enhancing airway epithelial barrier integrity through the nuclear factor-2 erythroid related factor-2/aldehyde oxidase 1 pathway. The regulation of forskolin was due to its activation of CFTR as a cAMP agonist to promote the secretion of ASL. In addition, studies had shown that cAMP/PKA signaling could attenuate the disruption of the structure and functions of the airway epithelial barrier by mechanisms involving the stabilization of epithelial junctions and inhibition of viral biogenesis. Therefore, as a cAMP agonist, forskolin could reduce the damage of LPS to the epithelial cell barrier through enhancement cell junction stabilization, and ultimately improve ASL secretion. Previous studies had shown that naringenin could activate the cAMP/PKA and Nrf2 signal pathway, regulate the function of CFTR to promote the secretion of ions, and reduce the inflammatory response of cells with significant anti-inflammatory activity by inhibiting the nuclear factor kappaB (NF-κB) activity via the EGFR-PI3K-Akt/ERK MAPKinase signaling pathway. Therefore, naringenin could regulate LPS-induced abnormal ASL secretion by activating CFTR function, enhancing the cell junction stabilization, and reducing cellular inflammation.

CFTR and AQPs in cells play an important role in the secretion of ASL, so changes in their expression can affect ASL. The regulation of dexamethasone was related to its anti-inflammatory mechanism and effect of enhancing airway epithelial barrier integrity through the nuclear factor-2 erythroid related factor-2/aldehyde oxidase 1 pathway. The regulation of forskolin was due to its activation of CFTR as a cAMP agonist to promote the secretion of ASL. In addition, studies had shown that cAMP/PKA signaling could attenuate the disruption of the structure and functions of the airway epithelial barrier by mechanisms involving the stabilization of epithelial junctions and inhibition of viral biogenesis. Therefore, as a cAMP agonist, forskolin could reduce the damage of LPS to the epithelial cell barrier through enhancement cell junction stabilization, and ultimately improve ASL secretion. Previous studies had shown that naringenin could activate the cAMP/PKA and Nrf2 signal pathway, regulate the function of CFTR to promote the secretion of ions, and reduce the inflammatory response of cells with significant anti-inflammatory activity by inhibiting the nuclear factor kappaB (NF-κB) activity via the EGFR-PI3K-Akt/ERK MAPKinase signaling pathway. Therefore, naringenin could regulate LPS-induced abnormal ASL secretion by activating CFTR function, enhancing the cell junction stabilization, and reducing cellular inflammation.

Figure 7. Effect of naringenin on LPS-induced expression of CFTR, AQP1, and AQP5 proteins. Incubation of naringenin for 24 h did not significantly change the expression levels of (a) CFTR, (c) AQP1 and (e) AQP5 proteins compared with the control group. LPS reduced CFTR, AQP1, and AQP5 protein expression. Adding naringenin, dexamethasone, and forskolin to the LPS-induced Calu-3 cell model resulted in up-regulation of the expression of (b) CFTR, (d) AQP1 and (f) AQP5 proteins to varying degrees. Data are presented as the mean ± SEM (n = 3; ns Control: * P < 0.05, *** P < 0.001; vs LPS: # P < 0.05, ## P < 0.01). Abbreviations: LPS, lipopolysaccharide; SEM, standard error of the mean; AQP1: aquaporin 1; AQP5: aquaporin 5; CFTR, cystic fibrosis transmembrane conductance regulator.
secretion. LPS can down-regulate CFTR expression by activating the PI3K-Akt pathway, decrease AQP1 and AQP5 expression through the TNF-α signaling pathway, which involves tumor necrosis factor-alpha receptor 1 and activated nuclear factor kappa B (NF-κB).

Our previous results confirmed that naringenin could up-regulate LPS-induced low CFTR mRNA expression in the respiratory epithelial cell without affecting normal cell CFTR expression, and this recovery effect had nothing to do with the mechanism of increasing CAMP concentration. In this experiment, we found that LPS could reduce CFTR, AQP1, AQP5 mRNA expression via NF-κB and MAPK pathways and two classes of transcription factors, NF-κB and p-c-Jun/c-Fos, thereby reducing the stability of the epithelial barrier.

Conclusions

The above studies show that naringenin has the ability to regulate the abnormal secretion of ALS induced by LPS through increasing the secretion volume, adjusting Na⁺, Cl⁻, total protein, and lysozyme concentrations, and up-regulating the expression of CFTR, AQP1, and AQP5. We proved that naringenin has a positive therapeutic effect on abnormal ASL secretion, thus expanding its potential application in the dietary supplement industry.

Ethical Approval

Ethical approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

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Trial Registration

Not applicable, because this article does not contain any clinical trials.

References

1. Derichs N, Jin BJ, Song Y, Finkheiner WE, Verkman AS. Hyperviscous airway periciliary and mucous liquid layers in cystic fibrosis measured by confocal fluorescence photobleaching. FASEB J. 2011;25(7):2325-2332.
2. Webster MJ, Tarran R. Slippery when wet: airway surface liquid homeostasis and mucus hydration. Curr Top Membr. 2018;81:293-355.
3. Rogers DF. Physiology of airway mucus secretion and pathophysiology of hypersecretion. Respir Care. 2007;52(9):1134-1146, 1146-1149.
4. Zhang T, Zhou X. Clinical application of expectorant therapy in chronic inflammatory airway diseases (review). Exp Ther Med. 2014;7(4):763-767.
5. Shen H, Yoshida H, Yan F, et al. Synergistic induction of MUC5AC mucin by nontypeable Haemophilus influenzae and Streptococcus pneumoniae. Biochem Biophys Res Commun. 2008;365(4):795-800.
6. He Z, Gao Y, Deng Y, et al. Lipopolysaccharide induces lung fibroblast proliferation through toll-like receptor 4 signaling and the phosphoinositide3-kinase-Akt pathway. PLoS One. 2012;7(4):e35926.
7. Yang Y, Cheng Y, Lian Q, et al. Contribution of CFTR to alveolar fluid clearance by lipoxin A4 via PI3 K/Akt pathway in LPS-induced acute lung injury. Mediators Inflamm. 2013;2013:862628.
8. Lin BQ, Li PB, Wang YG, et al. The expectorant activity of naringenin. Pulm Pharmacol Ther. 2008;21(2):259-263.
9. Shi R, Xiao ZT, Zheng YJ, et al. Naringenin regulates CFTR activation and expression in airway epithelial cells. *Cell Physiol Biochem.* 2017;44(3):1146-1160.

10. Shi R, Xu JW, Xiao ZT, et al. Naringenin and naringenin relax rat tracheal smooth by regulating BKCa activation. *J Med Food.* 2019;22(9):963-970.

11. Shi R, Su WW, Zhu ZT, et al. Regulation effects of naringin on diesel particulate matter-induced abnormal airway surface liquid secretion. *Phytomedicine.* 2019;63:153004.

12. Tsai SJ, Huang CS, Meng MC, Kam WY, Huang HY, Yin MC. Anti-inflammatory and antibacterial effects of naringenin in diabetic mice. *J Agric Food Chem.* 2012;60(1):514-521.

13. Yang J, Li Q, Zhou XD, Kolosov VP, Perelman JM. Naringenin attenuates mucous hypersecretion by modulating reactive oxygen species production and inhibiting NF-kB activity via EGFR-PI3K-Akt/ERK MAPK signaling in human airway epithelial cells. *Mol Cell Biochem.* 2011;351(1-2):29-40.

14. Yu DH, Ma CH, Yue ZQ, Yao X, Mao CM. Protective effect of naringenin against lipopolysaccharide-induced injury in normal human bronchial epithelium via suppression of MAPK signaling. *Inflammation.* 2015;38(1):195-204.

15. Shi Y, Dai J, Liu H, et al. Naringenin inhibits allergen-induced airway inflammation and airway responsiveness and inhibits NF-kappaB activity in a murine model of asthma. *Can J Physiol Pharmacol.* 2009;87(9):729-735.

16. Babu PB, Chidekel A, Shaffer TH. Protein composition of apical surface fluid from the human airway cell line Calu-3: effect of ion transport mediators. *Clin Chim Acta.* 2004;347(1-2):81-88.

17. Zhang Y, Reenstra WW, Chidekel A. Antibacterial activity of apical surface fluid from the human airway cell line Calu-3: pharmacologic alteration by corticosteroids and beta(2)-agonists. *Am J Respir Cell Mol Biol.* 2001;25(2):196-202.

18. Hasan B, Li FS, Sijt A, et al. Expression of aquaporins in the lungs of mice with acute injury caused by LPS treatment. *Respir Physiol Neurobiol.* 2014;200:40-45.

19. Jiang YX, Dai ZL, Zhang XP, Zhao W, Huang Q, Gao LK. Desmocollin Imediates pulmonary edema by upregulating AQP1 and AQP5 expression in rats with acute lung injury induced by lipopolysaccharide. *J Huazhong Univ Sci Technol Med Sci.* 2015;35(5):684-688.

20. Shen Y, Wang X, Wang Y, et al. Lipopolysaccharide decreases aquaporin 5, but not aquaporin 3 or aquaporin 4, expression in human primary bronchial epithelial cells. *Respirology.* 2012;17(7):1144-1149.

21. Su X, Song Y, Jiang J, Bai C. The role of aquaporin-1 (AQP1) expression in a murine model of lipopolysaccharide-induced acute lung injury. *Respir Physiol Neurobiol.* 2004;142(1):1-11.

22. Yao C, Purwanti N, Karabasli MR, et al. Potential down-regulation of salivary gland AQP5 by LPS via cross-coupling of NF-kappaB and p-c-Jun/c-Fos. *Am J Pathol.* 2010;177(2):724-734.