BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 31(5), 544-549 (2023)

Regulation of the Gene Expression of Airway MUC5AC Mucin through NF-κB Signaling Pathway by Artesunate, an Antimalarial Agent

Kyung-il Kim[1,2,†], Rajib Hossain[1,2,†], Jiho Ryu[3], Hyun Jae Lee[4,*] and Choong Jae Lee[1,2,*]

[1]Department of Pharmacology, School of Medicine, Chungnam National University, Daejeon 35015,
[2]Brain Korea 21 FOUR Project for Medical Science, Chungnam National University, Daejeon 35015,
[3]Department of Pharmacy, College of Pharmacy, Chungbuk National University, Cheongju 28644,
[4]Smith Liberal Arts College and Department of Addiction Science, Graduate School, Sahmyook University, Seoul 01795, Republic of Korea

Abstract

In this study, artesunate, an antimalarial agent, was investigated for its potential effect on the gene expression of airway MUC5AC mucin. The human pulmonary epithelial NCI-H292 cells were pretreated with artesunate for 30 min and then stimulated with phorbol 12-myristate 13-acetate (PMA), for the following 24 h. The effect of artesunate on PMA-induced nuclear factor kappa B (NF-kB) signaling pathway was also examined. Artesunate inhibited the glycoprotein production and mRNA expression of MUC5AC mucins, induced by PMA through the inhibition of degradation of inhibitory kappa Bα (IkBα) and NF-kB p65 nuclear translocation. These results suggest artesunate suppresses the gene expression of mucin through regulation of NF-kB signaling pathway, in human pulmonary epithelial cells.

Key Words: MUC5AC, Pulmonary mucin, Artesunate

INTRODUCTION

Mucus, a thin layer of gels present in the luminal surface of respiratory tract, contains ions, water, and various molecules, exerting anti-microbial and anti-oxidative effects. The majority of macromolecules assigning the viscoelasticity to mucus is mucous glycoproteins, mucins. Mucins maintains the normal function of pulmonary system and protects it from inhaled noxious factors including various particles, many bacteria and viruses, and irritating gases. However, the qualitative and/or quantitative changes of mucins like the over-secretion and/or overproduction of respiratory mucus interfere with the normal defensive action of pulmonary system, thereby contributing to the pathogenesis of bronchiectasis, chronic bronchitis, cystic fibrosis, and asthma. Also, it was reported that, among the various subtypes of human mucins, MUC5AC mucin is the major type of gel-forming pulmonary mucin (Adler and Li, 2001; Lillehoj and Kim, 2002; Rose and Voynow, 2006; Voynow and Rubin, 2009).

For the efficient controlling of mucus in the pulmonary system, hypertonic saline solution, S-carboxymethyl cysteine, bromhexine, thymosin β-4, ambroxol, mannitol dry powder, 2-mercaptoethane sulfonate sodium, erdosteine, azithromycin, glyceryl guaiacolate, dornase alfa, letocysteine, myrtol, N-acetyl L-cysteine (NAC), and sobrerol have been used, although these agents might provoke diverse side effects including the rebound over-secretion of mucus and the irritation of luminal wall of respiratory tract (Rogers, 2007; Li *et al*., 2020).

Therefore, it is ideal to develop a novel agent regulating the biosynthesis and/or degradation of mucin, for controlling its production and/or secretion. This might be a pivotal approach to control the over-secretion of pulmonary mucus. Although corticosteroids have been utilized to decrease the secretion and/or production of respiratory mucus, they showed various adverse effects, pharmacologically (Voynow and Rubin, 2009; Sprenger *et al*., 2011).

In this context, we tried to examine the potential activity of controlling the abnormal production and/or secretion of

Open Access **https://doi.org/10.4062/biomolther.2023.048**

Received Mar 5, 2023 **Revised** Apr 24, 2023 **Accepted** May 10, 2023
Published Online May 31, 2023

***Corresponding Authors**
E-mail: hjy1213@syu.ac.kr (Lee HJ), LCJ123@cnu.ac.kr (Lee CJ)
Tel: +82-2-3399-1909 (Lee HJ), +82-42-580-8255 (Lee CJ)
Fax: +82-2-3399-1909 (Lee HJ), +82-42-585-6627 (Lee CJ)
[†]The first two authors contributed equally to this work.

mucins, using natural products isolated from a multitude of medicinal plants used empirically for ameliorating the inflammatory respiratory diseases. We reported that various natural compounds affected the production of pulmonary mucin (mucous glycoprotein), resulting from its gene expression (Kim *et al*., 2012; Seo *et al*, 2014; Choi *et al*., 2019; Li *et al*., 2020).

Artesunate (Fig. 1) has been reported to be a drug that is used in the treatment of malaria. It is a chemical derivative of artemisinin, a natural product isolated from Artemisia annua. It is one of the most rapidly acting and effective drugs for treating severe malaria evoked by a type of malaria parasite responsible for most malaria-related deaths over the world, Plasmodium falciparum. The main pharmacological action of artesunate was reported to be killing the malaria parasite infecting the erythrocytes, thereby helping to mitigate the severity of the infection and prevent complications. Artesunate has been reported to provoke the oxidative stress and the damage of malarial protein through alkylation reaction by producing reactive oxygen species (Cui and Su, 2009) and to suppress a kind of membrane glutathione S-transferase, Plasmodium falciparum exported protein 1 (Lisewski *et al*., 2014). Artesunate showed a regulative effect on ferroptosis, thereby ameliorating hepatic fibrosis (Kong *et al*., 2019). Artesunate alleviated osteoclastogenesis (Zeng *et al*., 2020), experimental allergic asthma (Cheng *et al*., 2011), and oxidative lung damage (Ho *et al*., 2012). Artesunate suppressed the proliferation of primary cultured airway smooth muscle cells (Tan *et al*., 2014) and showed a potential for controlling COVID-19 (Krishna *et al*., 2021).

However, there is no report about the activity of artesunate on the gene expression of mucin in airway epithelial cells. Thus, we suggest it is promising to investigate the potential activity of artesunate on mRNA expression and glycoprotein production of pulmonary MUC5AC mucin stimulated by phorbol ester, in NCI-H292 cells. A human pulmonary mucoepidermoid cell line, NCI-H292 cells, has been utilized for specifying the intracellular signaling pathways involved in the gene expression of pulmonary mucin (Li *et al*., 1997; Takeyama *et al*., 1999; Shao *et al*., 2003). Phorbol ester provokes MUC5AC mucin gene expression, and intracellular nuclear factor kappa B (NF-κB) signaling is involved in the activity of phorbol ester in airway epithelial cells (Ishinaga *et al*., 2005; Laos *et al*., 2006; Wu *et al*., 2007; Kim *et al*., 2012; Choi *et al*., 2019; Li *et al*., 2020). Therefore, to elucidate an action mechanism of artesunate, we examined whether artesunate regulates the activation of NF-kB signaling pathway stimulated by phorbol ester, in NCI-H292 cells.

MATERIALS AND METHODS

Materials

Artesunate (purity: 98.0%) was donated by Shinpoong pharmaceutical company Ltd. (Seoul, Korea). Phospho-specific anti-IκBα (serine 32/36, #9246), and phospho-specific anti-p65 (serine 536, #3036S) antibodies were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). Anti-β-actin (sc-8432), anti-NF-κB p65 (sc-8008), and anti-inhibitory kappa Bα (IkBα) (sc-371) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-nuclear matrix protein p84 (ab-487) antibody was purchased from Abcam (Cambridge, MA, USA). Either Goat Anti-mouse IgG (#401215) or Goat Anti-rabbit IgG (#401315) was purchased from Calbiochem (Carlsbad, CA, USA) and used as the secondary antibody. The other chemicals used in the current experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA).

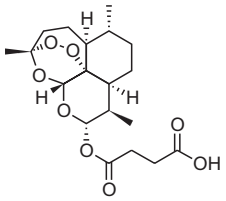

Fig. 1. Chemical structure of artesunate.

Culture of NCI-H292 cells

NCI-H292 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) in the presence of pen-strep mixture (penicillin (100 units/mL) plus streptomycin (100 μg/mL)) and HEPES (25 mM) at 37°C in a humidified, 5% CO_2/95% air, water-jacketed incubator. For serum deprivation, confluent cells were washed twice with phosphate-buffered saline (PBS) and then cultured in RPMI 1640 with 0.2% FBS, for 24 h.

Treatment of cells with artesunate

After serum deprivation, cells were pretreated with varying concentrations of artesunate for 30 min and then treated with phorbol 12-myristate 13-acetate (PMA) (10 ng/mL) for 24 h in serum-free RPMI 1640. Artesunate was dissolved in dimethyl sulfoxide and treated in culture medium (final concentrations of dimethyl sulfoxide were 0.5%). The final pH values of these solutions were between 7.0 and 7.4. Culture medium and 0.5% dimethyl sulfoxide did not affect mucin gene expression, and activity and expression of molecules involved in NF-κB signaling pathway, in NCI-H292 cells. After 24 h, cells were lysed with buffer solution containing 20 mM Tris, 0.5% NP-40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA and protease inhibitor cocktail (Roche Diagnostics, IN, USA) and collected to measure the production of MUC5AC glycoproteins (in a 24-well culture plate). The total RNA was extracted to check the expression of MUC5AC gene (in a 6-well culture plate) using RT-PCR. For the western blot analysis, cells were treated with artesunate for 24 h and then with PMA for 30 min.

Quantitative analysis of MUC5AC mucin

Airway MUC5AC mucin production was measured using enzyme-linked immunosorbent assay (ELISA). Cell lysates were prepared with PBS at 1:10 dilution, and 100 μL of each sample was incubated at 42°C in a 96-well plate, until it would be dry. Plates were washed three times with PBS and blocked with 2% bovine serum albumin (BSA) (fraction V) for 1 h at room temperature. Plates were washed another three times with PBS and then incubated with 100 μL of 45M1, a mouse monoclonal MUC5AC antibody (1:200) (NeoMarkers, CA, USA), which was diluted with PBS containing 0.05% Tween 20, and dispensed into each well. After 1 h, the wells were washed three times with PBS, and 100 μL of horseradish peroxidase-goat anti-mouse IgG conjugate (1:3,000) was dispensed into

each well. After 1 h, plates were washed three times with PBS. Color reaction was developed with 3,3',5,5'-tetramethylbenzidine (TMB) peroxide solution and stopped with 1 N H_2SO_4. Absorbance was read at 450 nm.

Isolation of total RNA and RT-PCR

Total RNA was isolated by using Easy-BLUE Extraction Kit (INTRON Biotechnology, Inc., Sungnam, Korea) and reverse transcribed by using AccuPower RT Premix (BIONEER Corporation, Daejeon, Korea) according to the manufacturer's instructions. Two μg of total RNA was primed with 1 μg of oligo (dT) in a final volume of 50 μL (RT reaction). Two μL of RT reaction product was PCR-amplified in a 25 μL by using Thermorprime Plus DNA Polymerase (ABgene, Rochester, NY, USA). Primers for MUC5AC were (forward) 5′-TGA TCA TCC AGC AGG GCT-3′ and (reverse) 5′-CCG AGC TCA GAG GAC ATA TGG G-3′. Primers for Rig/S15 rRNA, which encodes a small ribosomal subunit protein, a housekeeping gene that was constitutively expressed, were used as quantitative controls. Primers for Rig/S15 were (forward) 5′-TTC CGC AAG TTC ACC TAC C-3′ and (reverse) 5′-CGG GCC GGC CAT GCT TTA CG-3′. The PCR mixture was denatured at 94°C for 2 min followed by 40 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 45 s. After PCR, 5 μL of PCR products were subjected to 1% agarose gel electrophoresis and visualized with ethidium bromide under a transilluminator.

Whole cell extract preparation

NCI-H292 cells (confluent in 100 mm culture dish) were pretreated for 24 h at 37°C with 1, 5, 10 or 20 μM of artesunate, and then stimulated with PMA (50 ng/mL) for 30 min, in serum-free RPMI 1640. After the treatment of the cells with artesunate, media were aspirated, and the cells washed with cold PBS. For the cell collection, the cells were scraped and centrifuged at 3,000 rpm for 5 min. After the supernatant was discarded, the cell pellet was mixed with RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) for 30 min with continuous agitation. The lysate was centrifuged in a microcentrifuge at 14,000 rpm for 15 min at 4°C. The supernatant was either used, or was immediately stored at –80°C. The amount of protein in extract was quantified by Bradford method.

Nuclear and cytosolic extracts preparation

After the treatment with artesunate as stated, the cells (confluent in 150 mm culture dish) were harvested using Trypsin-EDTA solution and then centrifuged in a microcentrifuge (1,200 rpm, 3 min, 4°C). After the supernatant was discarded, the cell pellet was washed by suspending in PBS. The cytoplasmic and nuclear protein fractions were extracted using NE-PER® nuclear and cytoplasmic extraction reagent (Thermo-Pierce Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Both extracts were stored at –20°C. The amount of protein in extracts was quantified by Bradford method.

Western blotting for the detection of proteins

Whole cell, cytosolic, and nuclear extracts containing proteins (each 50 μg as proteins) were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto the polyvinylidene difluoride (PVDF) membrane. The blots were blocked using 5% skim milk and probed with appropriate primary antibody in blocking buffer overnight at 4°C. The membrane was washed with PBS and then probed with the secondary antibody conjugated with horseradish peroxidase. Immunoreactive bands were detected by an enhanced chemiluminescence kit (Pierce ECL western blotting substrate, Thermo Scientific, Waltham, MA, USA).

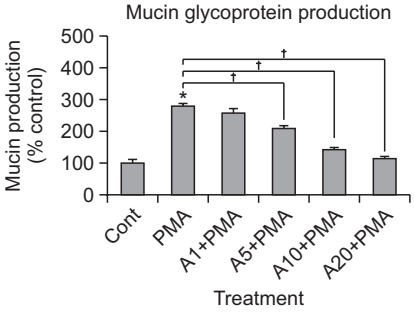

Fig. 2. Effect of artesunate on PMA-induced MUC5AC mucin glycoprotein production from NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of artesunate for 30 min and then stimulated with PMA (10 ng/mL), for 24 h. Cell lysates were collected for measurement of MUC5AC mucin production by ELISA. Each bar represents a mean ± SEM. of 3 culture wells compared to the control set at 100%. Three independent experiments were performed, and the representative data were shown. *Significantly different from control (p<0.05). †Significantly different from PMA alone (p<0.05). cont: control, A: artesunate, concentration unit is μM.

Statistical analysis

The means of individual groups were converted to percent control and expressed as mean ± SEM. The difference between groups was assessed using a one-way ANOVA and the Holm-Sidak test as a post-hoc test. A p-value of <0.05 was considered significantly different.

RESULTS

Effect of artesunate on PMA-induced MUC5AC mRNA expression and glycoprotein production of MUC5AC mucin

Artesunate inhibited PMA-induced MUC5AC mucin glycoprotein production, dose-dependently. The amounts of MUC5AC mucin in the cells of artesunate-treated cultures were 100 ± 11% (control), 279 ± 8% (10 ng/mL of PMA alone), 258 ± 13% (PMA plus artesunate 1 μM), 210 ± 7% (PMA plus artesunate 5 μM), 143 ± 5% (PMA plus artesunate 10 μM) and 114 ± 6% (PMA plus artesunate 20 μM), respectively (Fig. 2). MUC5AC mRNA expression induced by PMA was also inhibited by pretreatment with artesunate (Fig. 3). Cytotoxicity was checked by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and there was no cytotoxic effect of artesunate, at 1, 5, 10, or 20 μM concentration (data were not shown).

Effect of artesunate on PMA-induced phosphorylation and degradation of IκBα

In order for NF-κB to be activated, PMA provokes the phosphorylation of IKK and this phosphorylated IKK, in turn, phosphorylates the IκBα. The phosphorylated IκBα dissociates

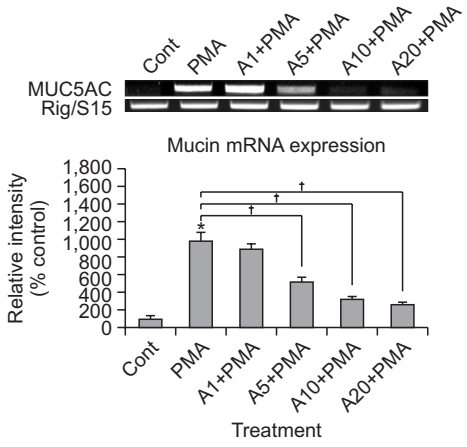

Fig. 3. Effect of artesunate on PMA-induced MUC5AC mucin mRNA expression from NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of artesunate for 30 min and then stimulated with PMA (10 ng/mL), for 24 h. Cell lysates were collected for measurement of MUC5AC gene expression using RT-PCR. Three independent experiments were performed, and the representative data were shown. *Significantly different from control (p<0.05). †Significantly different from PMA alone (p<0.05). cont: control, A: artesunate, concentration unit is μM.

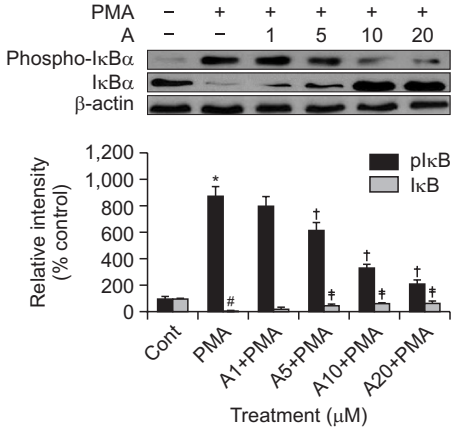

Fig. 4. Effect of artesunate on PMA-induced IkBα phosphorylation and IkBα degradation in NCI-H292 cells. NCI-H292 cells were incubated with varying concentrations of artesunate for 24 h and treated with 50 ng/mL PMA for 30 min. Cytoplasmic extracts were fractionated and then subjected to western blot analysis using phospho-specific IκBα (Ser 32/36) antibody or antibody against anti-IκBα. Equal protein loading was evaluated by β-actin levels. *,#Significantly different from control (p<0.05). †,‡Significantly different from PMA alone (p<0.05). cont: control, A: artesunate, IkBα: inhibitory kappa Bα, concentration unit is μM.

from NF-κB and degraded. Thus, we checked whether artesunate affects the phosphorylation of IκBα and degradation of IκBα, provoked by PMA. As can be seen in Fig. 4, PMA stimulated the phosphorylation of IκBα, whereas artesunate inhibited its phosphorylation. Also, PMA increased the degradation of IκBα, whereas artesunate inhibited its degradation (Fig. 4).

Effect of artesunate on PMA-induced phosphorylation and nuclear translocation of NF-κB p65

After the activation of NF-κB, it translocates from the cytosol to the nucleus, followed by being combined to the specific site of DNA. This assembly of DNA/NF-κB recruits the RNA polymerase and then the resulting mRNA is translated into the specific proteins, including MUC5AC mucins. The transcriptional activity of NF-κB p65 is dependent upon its phosphorylation. As can be seen in Fig. 5, PMA stimulated the phosphorylation of p65, whereas artesunate suppressed its phosphorylation. Finally, artesunate blocked the nuclear translocation of NF-κB p65, stimulated by PMA.

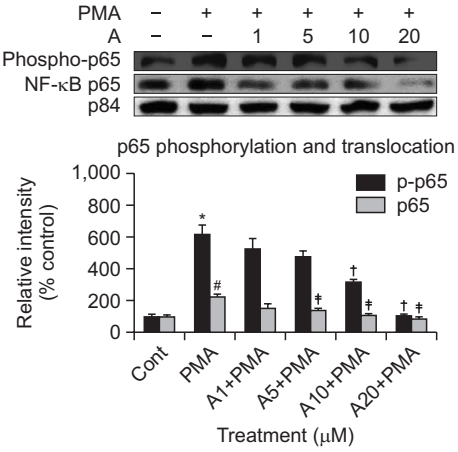

Fig. 5. Effect of artesunate on PMA-induced phosphorylation and translocation of NF-κB p65 in NCI-H292 cells. Nuclear protein extracts were prepared and subjected to western blot analysis using phospho-specific p65 (Ser 536) antibody and antibody against p65. As a loading control, p84 levels were analyzed. The results shown are the representative of three independent experiments. *,#Significantly different from control (p<0.05). †,‡Significantly different from PMA alone (p<0.05). cont: control, A: artesunate, concentration unit is μM.

DISCUSSION

As mentioned in introduction, there is no specific agent efficiently regulating the secretion and/or production of pulmonary mucus, despite that a multitude of clinical medicine have been used for exerting such a pharmacological activity. In order to regulate a multitude of inflammatory airway diseases efficiently, controlling inflammatory response might be the ultimate goal. We have investigated the potential effect of various natural compounds and reported that diverse anti-inflammatory natural compounds showed a regulative effect on the gene expression of MUC5AC mucin, thereby trying to develop the novel candidate affecting the production and/or secretion of airway MUC5AC mucin (Kim *et al*., 2012; Ryu *et al*., 2013, 2014; Seo *et al*, 2014; Sikder *et al*., 2014; Lee *et al*., 2015; Kim *et al*., 2016; Choi *et al*., 2018, 2019).

As can be seen in results, artesunate significantly suppressed the production of MUC5AC mucin proteins and expression of MUC5AC mucin mRNA (Fig. 2, 3). This is the first report, as far as we recognize, on the effect of artesunate on MUC5AC mucin gene expression in airway epithelial cells. Also, artesunate suppressed the phosphorylation and nuclear translocation of NF-κB p65 via affecting the steps of the phosphorylation of IκBα and degradation of IκBα, in NCI-H292

cells (Fig. 4, 5). Thus, the pharmacological effect of artesunate on the gene expression of MUC5AC might be manifested through the degradation of IkBα and nuclear translocation of NF-κB p65.

Also, there is a possibility that artesunate might inhibit MUC5AC gene expression through a certain intracellular signaling pathway other than NF-kB signaling. Thus, we investigated whether artesunate inhibits the gene expression of MUC5AC mucin through an intracellular signaling pathway mediated by epidermal growth factor receptor (EGFR). The result shows that artesunate did not affect EGF-induced mitogen-activated protein kinase (MAPK) signaling pathway. Artesunate did not affect EGF-induced EGFR phosphorylation, p38 MAPK phosphorylation and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation, and the nuclear translocation of specificity protein-1 (Sp1) (unpublished data).

Various original research articles have reported that the gene expression of MUC5AC mucin might be stimulated by the inflammatory mediators activating the transcription factors including NF-κB (Fujisawa *et al*., 2009; Kurakula *et al*., 2015; Garvin *et al*., 2016). It was reported that PMA stimulates MUC5AC mucin gene expression, and nuclear factor kappa B (NF-kB) signaling is involved in the activity in airway epithelial cells (Ishinaga *et al*., 2005; Laos *et al*., 2006; Wu *et al*., 2007; Kim *et al*., 2012; Choi *et al*., 2018). On the basis of these publications and the present experimental results, we suggest it is reasonable to conclude that the pharmacological effect of artesunate on PMA-induced MUC5AC gene expression might be mediated, at least partly, through acting on the degradation of IkBα and nuclear translocation of NF-kB p65.

It has been reported that the side effects of artesunate are dizziness, abnormality in kidney function, decrease in leukocyte number, allergic reaction, and bradycardia, although artesunate has been known to be well-tolerated and might be safe in the treatment of malaria in pregnant and/or breast-feeding women. (Rosenthal, 2008; Kovacs *et al*., 2015). Potential adverse effects of artesunate should and could be resolved, by each developing step and stage of the novel drug, since it is very crucial to search a pharmacological agent specifically controlling the production and/or secretion of airway mucus by preclinical/clinical study, thereby regulating the abnormal secretion and/or production of mucus in the respiratory tract of pulmonary diseases. Also, it is promising to modify and optimize the chemical structure of artesunate through the medicinal chemistry, so that it exerts the adequate controlling effect on the secretion and/or production of pulmonary mucus.

In conclusion, we suggest it is encouraging to search a new candidate compound showing an inhibitory activity on the gene expression of MUC5AC. The results in this study suggest a possibility of utilizing artesunate as an efficacious mucoregulative agent for diverse respiratory diseases.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

ACKNOWLEDGMENTS

This research was supported by NRF-2014R1A6A1029617 Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education.

REFERENCES

Adler, K. B. and Li, Y. (2001) Airway epithelium and mucus: intracellular signaling pathways for gene expression and secretion. *Am. J. Respir. Cell Mol. Biol.* **25**, 397-400.

Cheng, C., Ho, W. E., Goh, F. Y., Guan, S. P., Kong, L. R., Lai, W. Q., Leung, B. P. and Wong, W. S. (2011) Anti-malarial drug artesunate attenuates experimental allergic asthma via inhibition of the phosphoinositide 3-kinase/Akt pathway. *PLoS One* **6**, e20932.

Choi, B. S., Kim, Y. J., Choi, J. S., Lee, H. J. and Lee, C. J. (2019) Obtusifolin isolated from the seeds of Cassia obtusifolia regulates the gene expression and production of MUC5AC mucin in airway epithelial cells via affecting NF-κB pathway. *Phytother. Res.* **33**, 919-928.

Choi, B. S., Kim, Y. J., Yoon, Y. P., Lee, H. J. and Lee, C. J. (2018) Tussilagone suppressed the production and gene expression of MUC5AC mucin via regulating nuclear factor-kappa B signaling pathway in airway epithelial cells. *Korean J. Physiol. Pharmacol.* **22**, 671-677.

Cui, L. and Su, X. Z. (2009) Discovery, mechanisms of action and combination therapy of artemisinin. *Expert Rev. Anti Infect. Ther.* **7**, 999-1013.

Fujisawa, T., Velichko, S., Thai, P., Hung, L. Y., Huang, F. and Wu, R. (2009) Regulation of airway MUC5AC expression by IL-1beta and IL-17A; the NF-kappa B paradigm. *J. Immunol.* **183**, 6236-6243.

Garvin, L. M., Chen, Y., Damsker, J. M. and Rose, M. C. (2016) A novel dissociative steroid VBP15 reduces MUC5AC gene expression in airway epithelial cells but lacks the GRE mediated transcriptional properties of dexamethasone. *Pulm. Pharmacol. Ther.* **38**, 17-26.

Ho, W. E., Cheng, C., Peh, H. Y., Xu, F., Tannenbaum, S. R., Ong, C. N. and Wong, W. S. (2012) Anti-malarial drug artesunate ameliorates oxidative lung damage in experimental allergic asthma. *Free Radic. Biol. Med.* **53**, 498-507.

Ishinaga, H., Takeuchi, K., Kishioka, C., Suzuki, S., Basbaum, C. and Majima, Y. (2005) Pranlukast inhibits NF-kappaB activation and MUC2 gene expression in cultured human epithelial cells. *Pharmacology* **73**, 89-96.

Kim, E. J., Yoon, Y. P., Woo, K. W., Kim, J. H., Min, S. Y., Lee, H. J., Lee, S. K., Hong, J. H., Lee, K. R. and Lee, C. J. (2016) Verticine, ebeiedine and suchengbeisine isolated from the bulbs of Fritillaria thunbergii Miq. inhibited the gene expression and production of MUC5AC mucin from human airway epithelial cells. *Phytomedicine* **23**, 95-104.

Kim, J. O., Sikder, M. A., Lee, H. J., Rahman, M., Kim, J. H., Chang, G. T. and Lee, C. J. (2012) Phorbol ester or epidermal growth-factor-induced MUC5AC mucin gene expression and production from airway epithelial cells are inhibited by apigenin and wogonin. *Phytother. Res.* **26**, 1784-1788.

Kong, Z., Liu, R. and Cheng, Y. (2019) Artesunate alleviates liver fibrosis by regulating ferroptosis signaling pathway. *Biomed. Pharmacother.* **109**, 2043-2053.

Kovacs, S. D., Rijken, M. J. and Stergachis, A. (2015) Treating severe malaria in pregnancy: a review of the evidence. *Drug Safety* **38**, 165-181.

Krishna, S., Augustin, Y., Wang, J., Xu, C., Staines, H. M., Platteeuw, H., Kamarulzaman, A., Sall, A. and Kremsner, P. (2021) Repurposing antimalarials to tackle the COVID-19 pandemic. *Trends Parasitol.* **37**, 8-11.

Kurakula, K., Hamers, A. A., van Loenen, P. and de Vries, C. J. (2015) 6-Mercaptopurine reduces cytokine and Muc5ac expression involving inhibition of NF-kB activation in airway epithelial cells. *Respir. Res.* **16**, 73.

Laos, S., Baeckstrom, D. and Hansson, G. C. (2006) Inhibition of NF-kappaB activation and chemokine expression by the leukocyte glycoprotein, CD43, in colon cancer cells. *Int. J. Oncol.* **28**, 695-704.

Lee, H. J., Park, J. S., Yoon, Y. P., Shin, Y. J., Lee, S. K., Kim, Y. S.,

Hong, J. H., Son, K. H. and Lee, C. J. (2015) Dioscin and methylprotodioscin isolated from the root of Asparagus cochinchinensis suppressed the gene expression and production of airway MUC5AC mucin induced by phorbol ester and growth factor. *Phytomedicine* **22**, 568-572.

Li, J. D., Dohrman, A. F., Gallup, M., Miyata, S., Gum, J. R., Kim, Y. S., Nadel, J. A., Prince, A. and Basbaum, C. B. (1997) Transcriptional activation of mucin by Pseudomonas aeruginosa lipopolysaccharide in the pathogenesis of cystic fibrosis lung disease. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 967-972.

Li, X., Jin, F., Lee, H. J. and Lee, C. J. (2020) Recent advances in the development of novel drug candidates for regulating the secretion of pulmonary mucus. *Biomol. Ther. (Seoul)* **28**, 293-301.

Lillehoj, E. R. and Kim, K. C. (2002) Airway mucus: its components and function. *Arch. Pharm. Res.* **25**, 770-780.

Lisewski, A. M., Quiros, J. P., Ng, C. L., Adikesavan, A. K., Miura, K., Putluri, N, Eastman, R. T., Scanfeld, D., Regenbogen, S. J., Altenhofen, L., Llinás, M., Sreekumar, A., Long, C., Fidock, D. A. and Lichtarge, O. (2014) Supergenomic network compression and the discovery of EXP1 as a glutathione transferase inhibited by artesunate. *Cell* **158**, 916-928.

Rose, M. C. and Voynow, J. A. (2006) Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiol. Rev.* **6**, 245-278.

Rogers, D. F. (2007) Mucoactive agents for airway mucus hypersecretory diseases. *Respir. Care* **52**, 1176-1193.

Rosenthal, P. J. (2008) Artesunate for the treatment of severe falciparum malaria. *N. Engl. J. Med.* **358**, 1829-1836.

Ryu, J., Lee, H. J., Park, S. H., Kim, J., Lee, D., Lee, S. K., Kim, Y. S., Hong, J. H., Seok, J. H. and Lee, C. J. (2014) Effects of the root of Platycodon grandiflorum on airway mucin hypersecretion *in vivo* and platycodin D(3) and deapi-platycodin on production and secretion of airway mucin *in vitro*. *Phytomedicine* **21**, 529-533.

Ryu, J., Lee, H. J., Park, S. H., Sikder, M. A., Kim, J. O., Hong, J. H., Seok, J. H. and Lee, C. J. (2013) Effect of prunetin on TNF-α-induced MUC5AC mucin gene expression, production, degradation of IκB and translocation of NF-κB p65 in human airway epithelial cells. *Tuberc. Respir. Dis. (Seoul)* **75**, 205-209.

Seo, H. S., Sikder, M. A., Lee, H. J., Ryu, J. and Lee, C. J. (2014) Apigenin inhibits tumor necrosis factor-α-induced production and gene expression of mucin through regulating nuclear factor-kappa b signaling pathway in airway epithelial cells. *Biomol. Ther. (Seoul)* **22**, 525-531.

Shao, M. X., Ueki, I. F. and Nadel, J. A. (2003) TNF-alpha converting enzyme mediated MUC5AC mucin expression in cultured human airway epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 11618-11623.

Sikder, M. A., Lee, H. J., Mia, M. Z., Park, S. H., Ryu, J., Kim, J. H., Min, S. Y., Hong, J. H., Seok, J. H. and Lee, C. J. (2014) Inhibition of TNF-α-induced MUC5AC mucin gene expression and production by wogonin through the inactivation of NF-κB signaling in airway epithelial cells. *Phytother. Res.* **28**, 62-68.

Sprenger, L., Goldmann, T., Vollmer, E., Steffen, A., Wollenberg, B., Zabel, P. and Hauber, H. P. (2011) Dexamethasone and N-acetylcysteine attenuate Pseudomonas aeruginosa-induced mucus expression in human airways. *Pulm. Pharmacol. Ther.* **24**, 232-239.

Takeyama, K., Dabbagh, K., Lee, H., Agusti, C., Lausier, J. A., Ueki, I. F., Grattan, K. M. and Nadel, J. A. (1999) Epidermal growth factor system regulates mucin production in airways. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 3081-3086.

Tan, S. S., Ong, B., Cheng, C., Ho, W. E., Tam, J. K., Stewart, A. G., Harris, T., Wong, W. S. and Tran, T. (2014) The antimalarial drug artesunate inhibits primary human cultured airway smooth muscle cell proliferation. *Am. J. Respir. Cell Mol. Biol.* **50**, 451-458.

Voynow, J. A. and Rubin, B. K. (2009) Mucins, mucus, and sputum. *Chest* **135**, 505-512.

Wu, D. Y., Wu, R., Reddy, S. P., Lee, Y. C. and Chang, M. M. (2007) Distinctive epidermal growth factor receptor/extracellular regulated kinase-independent and -dependent signaling pathways in the induction of airway mucin 5B and mucin 5AC expression by phorbol 12-myristate 13-acetate. *Am. J. Pathol.* **170**, 20-32.

Zeng, X. Z., Zhang, Y. Y., Yang, Q., Wang, S., Zou, B. H., Tan, Y. H., Zou, M., Liu, S. W. and Li, X. J. (2020) Artesunate attenuates LPS-induced osteoclastogenesis by suppressing TLR4/TRAF6 and PLCγ1-Ca2+-NFATc1 signaling pathway. *Acta Pharmacol. Sin.* **41**, 229-236.