Natural Product Sciences
21(1) : 59-65 (2015)

Effects of Cynaroside, Cynarin and Linarin on Secretion, Production and Gene Expression of Airway MUC5AC Mucin in NCI-H292 Cells

Yong Pill Yoon[1,†], Hyun Jae Lee[1,†], Young Ho Kim[2], Bui Thi Thuy Luyen[2], Jang-Hee Hong[1], and Choong Jae Lee[1,*]

[1]Department of Pharmacology, School of Medicine, Chungnam National University, Daejeon, Korea
[2]Department of Pharmacy, College of Pharmacy, Chungnam National University, Daejeon, Korea

Abstract – In this study, we investigated whether cynaroside, cynarin and linarin derived from *Chrysanthemum indicum* L. affect the secretion, production and gene expression of MUC5AC mucin in airway epithelial cells. Confluent NCI-H292 cells were pretreated with cynaroside, cynarin or linarin for 30 min and then stimulated with PMA (phorbol 12-myristate 13-acetate) for 24 h. The MUC5AC mucin gene expression, mucin protein production and secretion were measured by RT-PCR and ELISA, respectively. Effect of linarin on EGF (epidermal growth factor) - or TNF-α (tumor necrosis factor-α)-induced MUC5AC mucin gene expression and mucin protein production was also examined. The results were as follows: (1) Cynaroside and cynarin did not significantly affect PMA-induced MUC5AC mucin secretion from NCI-H292 cells. However, linarin decreased MUC5AC mucin secretion; (2) Cynaroside did not affect PMA-induced MUC5AC mucin production and gene expresion from NCI-H292 cells. However, cynarin and linarin inhibited the production and gene expression of MUC5AC mucin; (3) Linarin also inhibited the production and gene expression of MUC5AC mucin induced by EGF- or TNF-α from NCI-H292 cells. These results suggest that linarin can regulate the gene expression, production and secretion of mucin, by directly acting on airway epithelial cells.
Keywords – Airway, MUC5AC, Linarin, Cynarin, Cynaroside

Introduction

Mucus in the pulmonary system is very important in defensive action against invading pathogenic microorganisms, chemicals and particles. This defensive action of pulmonary mucus is attributed to the physicochemical property of mucins i.e. viscoelasticity. Mucins are high molecular weight glycoproteins present in the airway mucus and produced by goblet cells in the surface epithelium as well as mucous cells in the submucosal gland. However, hypersecretion of airway mucus is one of the major symptoms associated with severe pulmonary diseases including asthma, chronic bronchitis, cystic fibrosis and bronchiectasis.[1,2] Therefore, we suggest it is valuable to find the potential activity of regulation of the excessive mucin secretion and/or production by the compounds derived from various medicinal plants. We have tried to investigate the possible activities of some natural products on mucin secretion and/or production in cultured airway epithelial cells. As a result of our trial, we previously reported that several natural compounds affected the secretion and/or production of mucin in airway epithelial cells.[3-5] According to traditional oriental medicine, *Chrysanthemum indicum* L. has been utilised for regulating the allergic and inflammatory diseases. Also, linarin, cynarin and cynaroside - its components - were reported to have antioxidative, hepatoprotective, antimicrobial and some other biological effects.[6-11] However, to the best of our knowledge, no other studies on the effect of linarin, cynarin and cynaroside on the secretion, production and gene expression of mucin in airway epithelial cells have been carried out. Among the twenty one or more MUC genes coding human mucins, MUC5AC was reported to be mainly expressed in goblet cells in the airway surface epithelium.[2,12] Therefore, in this study, we checked whether linarin, cynarin and cynaroside affect the secretion, production and gene expression of MUC5AC mucin in NCI-H292 cells, a human pulmonary mucoepidermoid cell line, which are frequently used for the purpose of studying the airway mucin production and gene expression.[13-15]

*Author for correspondence
Choong Jae Lee, Department of Pharmacology, School of Medicine, Chungnam National University, 6 Munhwa-Dong, Joong-Gu, Daejeon, Korea.
Tel: +82-42-580-8255; E-mail: LCJ123@cnu.ac.kr
†These two authors equally contributed to this work.

Experimental

General experimental procedures – All the chemicals and reagents used in this experiment were purchased from Sigma (St. Louis, MO, U.S.A.) unless otherwise specified.

Preparation of cynaroside, cynarin and linarin – Cynaroside (purity: 98.0%), cynarin (purity: 98.0%) and linarin (purity: 98.0%) were isolated, purified and identified by analytical chemist, Professor Dr. Young Ho Kim, in the Laboratory of Pharmacognosy, Department of Pharmacy, Chungnam National University (Daejeon, Korea). Briefly, dried flowers of *Chrysanthemum indicum* L. (3.5 kg) were collected in Jeju, Korea in 2011 and taxonomically identified by Professor Jae Hyun Lee in Dongguk University. A voucher specimen (CNU 11102) was deposited at the Herbarium of College of Pharmacy, Chungnam National University. The dried flowers of *Chrysanthemum indicum* were extracted with methanol under reflux. Evaporation of the solvent under reduced pressure gave MeOH extract (500 g). The MeOH extract was suspended in H_2O and successively extracted with CH_2Cl_2 and EtOAc. Removal of the solvent under reduced pressure yielded CH_2Cl_2 extract (200 g) and EtOAc extract (40 g), respectively. The EtOAC-soluble extract was subjected to silica gel column chromatography eluting with MeOH in CH_2Cl_2 (0 - 100%, step-wise) to afford eight fractions [fr. 1(1.0 g), fr.2 (1.2 g), fr.3 (4.7 g), fr.4 (5.2 g), fr.5 (10.0 g), fr.6 (12.3 g), fr. 7(5.0 g) fr.8 (3.4 g)]. Fraction 5 was separated by CC over Sephadex LH-20, eluting with MeOH-H_2O (1 : 1), and further puried by an YMC reverse-phase (RP) CC, using MeOH-H_2O (1 : 2) as eluents, to obtain cynarin (500.0 mg). Fraction 6 was chromatographed over silica gel, eluting with CH_2Cl_2-MeOH-H_2O (4 : 1 : 0.1) to afford cynaroside (1.0 g). Fraction 7 was subjected over silica gel, eluting with CH_2Cl_2-MeOH-H_2O (4 : 1 : 0.1) to provide three subfractions (f7.1-f7.3). Subfraction f7.3 was separated by CC over silica gel, using EtOAc-MeOH (5 : 1) as eluents and then puried by RP CC, using MeOH-H_2O (1 : 2) to provide linarin (5.0 mg).

NCI-H292 cell culture – NCI-H292 cells, a human pulmonary mucoepidermoid carcinoma cell line, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, U.S.A.) and cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) in the presence of penicillin (100 units/mL), streptomycin (100 μg/mL) and HEPES (25 mM) at 37 ℃ in a humidified, 5% CO_2/95% air, water-jacketed incubator. For serum deprivation, confluent cells were washed twice with PBS and recultured in RPMI 1640 with 0.2% FBS for 24 h.

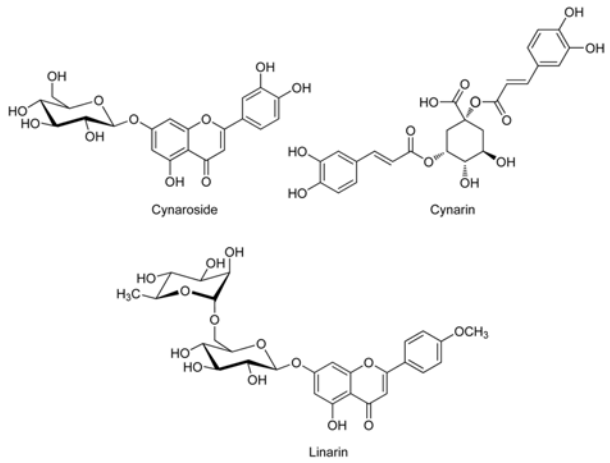

Fig. 1. Chemical structure of cynaroside, cynarin and linarin.

Treatment of cells with cynaroside, cynarin and linarin – After 24 h of serum deprivation, cells were pretreated with cynaroside, cynarin or linarin (1, 10 and 100 μM, the chemical structure of each compound can be seen in Fig. 1), for 30 min and then treated with phorbol 12-myristate 13-acetate (PMA) (10 ng/mL), epidermal growth factor (EGF) (25 ng/mL) or tumor necrosis factor-α (TNF-α) (10 ng/mL) for 24 h in serum-free RPMI 1640. Cynaroside, cynarin and linarin were dissolved in dimethylsulfoxide, diluted in PBS and treated in culture medium (final concentrations of dimethylsulfoxide were 0.5%). The final pH values of these solutions were between 7.0 and 7.4. Culture medium and 0.5% dimethylsulfoxide in medium did not affect mucin secretion, production and gene expression from NCI-H292 cells. After 24 h, the spent media were collected to measure the secretion of MUC5AC protein and 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, U.S.A.) and collected to measure the production of MUC5AC protein (in 24-well culture plate). The total RNA was extracted for measuring the expression of MUC5AC gene (in 6-well culture plate) by using RT-PCR.

MUC5AC mucin analysis – MUC5AC mucin protein was measured by using ELISA. Cell lysates were prepared with PBS at 1 : 10 dilution, and 100 μL of each sample was incubated at 42℃ in a 96-well plate, until dry. Plates were washed three times with PBS and blocked with 2% BSA (fraction V) for 1 h at room temperature. Plates were again washed three times with PBS and then incubated with 100 μL of 45M1, a mouse monoclonal MUC5AC antibody (1 : 200) (NeoMarkers, CA, U.S.A.), 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.

Total RNA isolation and RT-PCR – Total RNA was isolated by using Easy-BLUE Extraction Kit (INTRON Biotechnology, Inc. Kyung-gi-do, Korea) and reverse transcribed by using AccuPower RT Premix (BIONEER Corporation, Daejeon, Korea) according to the manufacturer's instructions. 2 μg of total RNA was primed with 1 μg of oligo (dT) in a final volume of 50 μL (RT reaction). 2 μL of RT reaction product was PCR amplified in a 25 μL by using Thermorprime Plus DNA Polymerase (ABgene, Rochester, NY, U.S.A.). 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'. As quantitative controls, primers for Rig/S15 rRNA, which encodes a small ribosomal subunit protein, a housekeeping gene that was constitutively expressed, were used. 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 ºC for 2 min followed by 40 cycles at 94 ºC for 30 s, 60ºC for 30 s and 72ºC 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.

Statistics – Means of individual group were converted to percent control and expressed as mean ± S.E.M. The difference between groups was assessed using one-way ANOVA and Holm-Sidak test as a post-hoc test. $P < 0.05$ was considered as significantly different.

Results and Discussion

Cynaroside, cynarin and linarin were reported to be derived from *Chrysanthemum indicum* L. and there are many reports with regard to the various biological effects of cynaroside, cynarin and linarin.[6-11] However, as aforementioned in introduction, there are no reports about the potential effects of cynaroside, cynarin and linarin on the secretion, production and gene expression of mucin in airway epithelial cells. Among the twenty one or more MUC genes coding human mucins reported, MUC5AC was mainly expressed in goblet cells in the airway surface epithelium.[2,12] Phorbol 12-myristate 13-acetate (PMA) was reported to stimulate the endogenous activator of protein kinase C (PKC), diacylglycerol (DAG)[16] and to be an inflammatory stimulant that can control a gene transcription[17], cell growth and differentiation.[18] PMA also can induce MUC5AC gene expression in NCI-H292 cells. PMA activates a type of PKC isoforms. This activates matrix metalloproteinases (MMPs), which cleave pro-EGFR ligands from the cell surface to become mature EGFR ligands. These ligands bind to the EGF receptor, provoking the phosphorylation of its intracellular tyrosine kinase. This leads to activation of MEK leading to ERK activation. Following is the activation of the transcription factor, Sp1, and binding of the factor to specific sites with the MUC5AC gene promoter. Finally, the promoter is activated and produced the gene transcription and translation to MUC5AC mucin protein.[17] Based on these reports, we investigated the effects of cynaroside, cynarin and linarin on PMA-induced MUC5AC mucin secretion, production and gene expression from NCI-H292 cells, a human pulmonary mucoepidermoid cell line.

As shown in results, linarin decreased mucin secretion stimulated by PMA. The amounts of mucin in the spent medium of linarin-treated cultures were 100 ± 7%, 215 ± 3%, 218 ± 1%, 198 ± 3% and 185 ± 5% for control, 10 ng/ml of PMA alone, PMA plus linarin 10^{-6} M, PMA plus linarin 10^{-5} M and PMA plus linarin 10^{-4} M, respectively (Fig. 2(A)). However, cynaroside and cynarin did not affect PMA-induced MUC5AC secretion. The amounts of mucin in the spent medium of cynaroside-treated cultures were 100 ± 4%, 297 ± 3%, 275 ± 5%, 255 ± 6% and 293 ± 10% for control, 10 ng/ml of PMA alone, PMA plus cynaroside 10^{-6} M, PMA plus cynaroside 10^{-5} M and PMA plus cynaroside 10^{-4} M, respectively (Fig. 2(B)). The amounts of mucin in the spent medium of cynarin-treated cultures were 100 ± 7%, 215 ± 3%, 198 ± 6%, 200 ± 2% and 200 ± 6% for control, 10 ng/ml of PMA alone, PMA plus cynarin 10^{-6} M, PMA plus cynarin 10^{-5} M and PMA plus cynarin 10^{-4} M, respectively (Fig. 2(C)).

Also, linarin and cynarin dose-dependently suppressed the production and gene expression of MUC5AC mucin induced by PMA. The amounts of mucin in the linarin-treated cells were 100 ± 1%, 208 ± 2%, 152 ± 1%, 61 ± 3% and 49 ± 1% for control, 10 ng/ml of PMA alone, PMA plus linarin 10^{-6} M, PMA plus linarin 10^{-5} M and PMA plus linarin 10^{-4} M, respectively (Fig. 3(A)). The amounts of mucin in the cynarin-treated cells were 100 ± 4%, 226 ± 2%, 198 ± 8%, 85 ± 11% and 47 ± 1% for control, 10 ng/ml of PMA alone, PMA plus cynarin 10^{-6} M, PMA plus cynarin 10^{-5} M and PMA plus cynarin 10^{-4} M, respectively (Fig. 3(B)). However, cynaroside did not affect PMA-induced MUC5AC production. The amounts

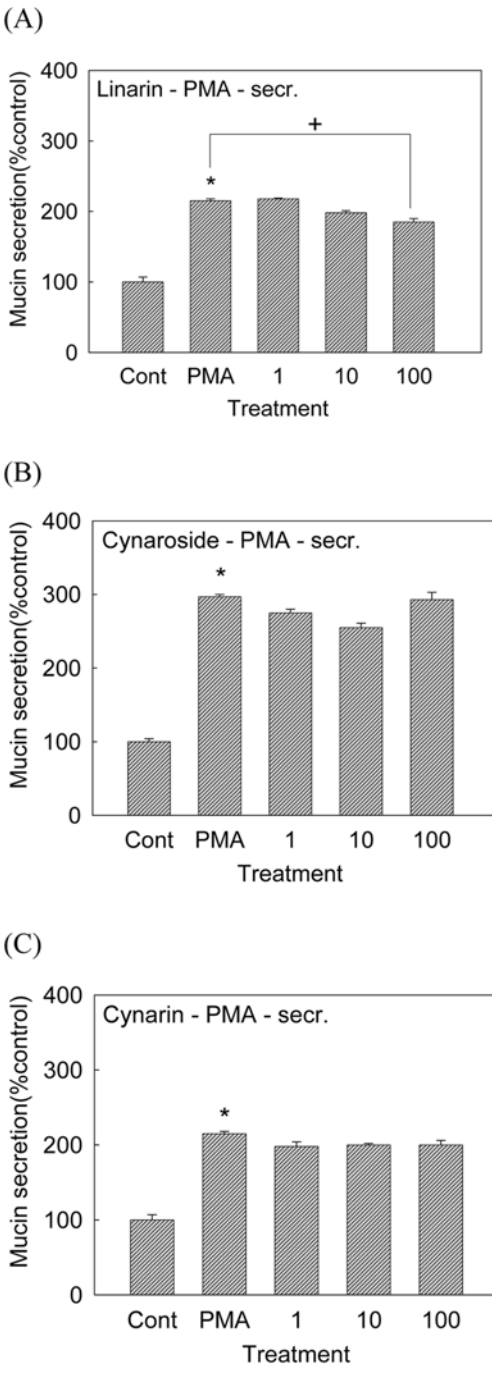

Fig. 2. Effect of cynaroside, cynarin or linarin on PMA-induced MUC5AC mucin secretion in NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of cynaroside, cynarin or linarin for 30 min and then stimulated with PMA (10 ng/mL) for 24 h. Spent media were collected for measurement of MUC5AC mucin secretion by ELISA. Three independent experiments were performed and the representative data were shown. Each bar represents a mean ± S.E.M. of three culture wells in comparison with that of control set at 100%.

*significantly different from control ($p < 0.05$).

+significantly different from PMA alone ($p < 0.05$).

(cont: control, concentration unit is μM)

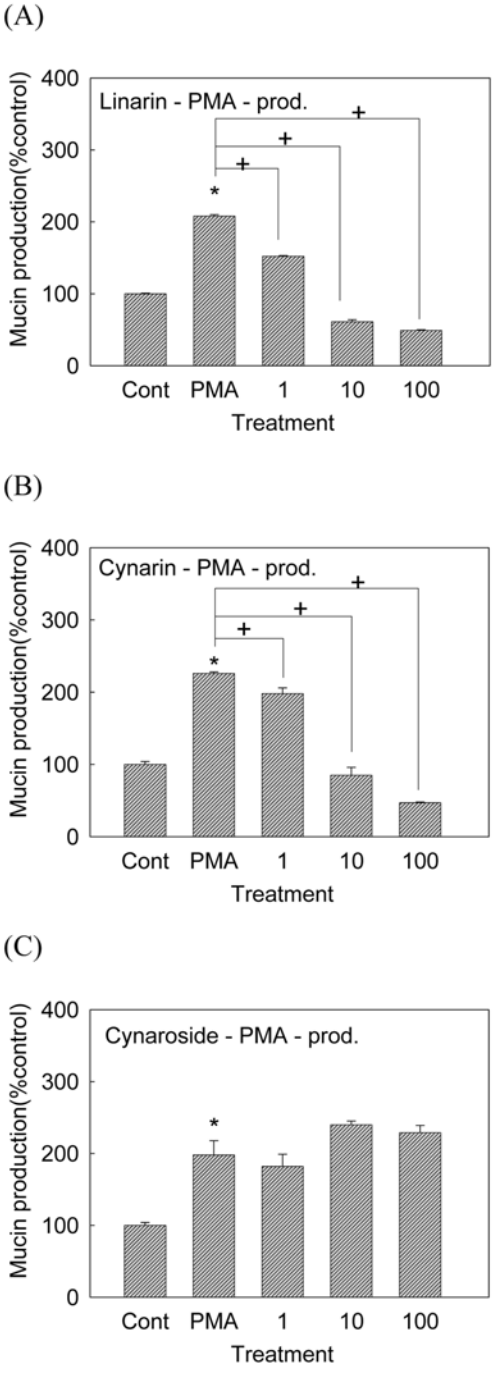

Fig. 3. Effect of cynaroside, cynarin or linarin on PMA-induced MUC5AC mucin production in NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of cynaroside, cynarin or linarin for 30 min and then stimulated with PMA (10 ng/mL) for 24 h. Cell lysates were collected for measurement of MUC5AC mucin production by ELISA. Three independent experiments were performed and the representative data were shown. Each bar represents a mean ± S.E.M. of three culture wells in comparison with that of control set at 100%.

*significantly different from control ($p < 0.05$).

+significantly different from PMA alone ($p < 0.05$).

(cont: control, concentration unit is μM)

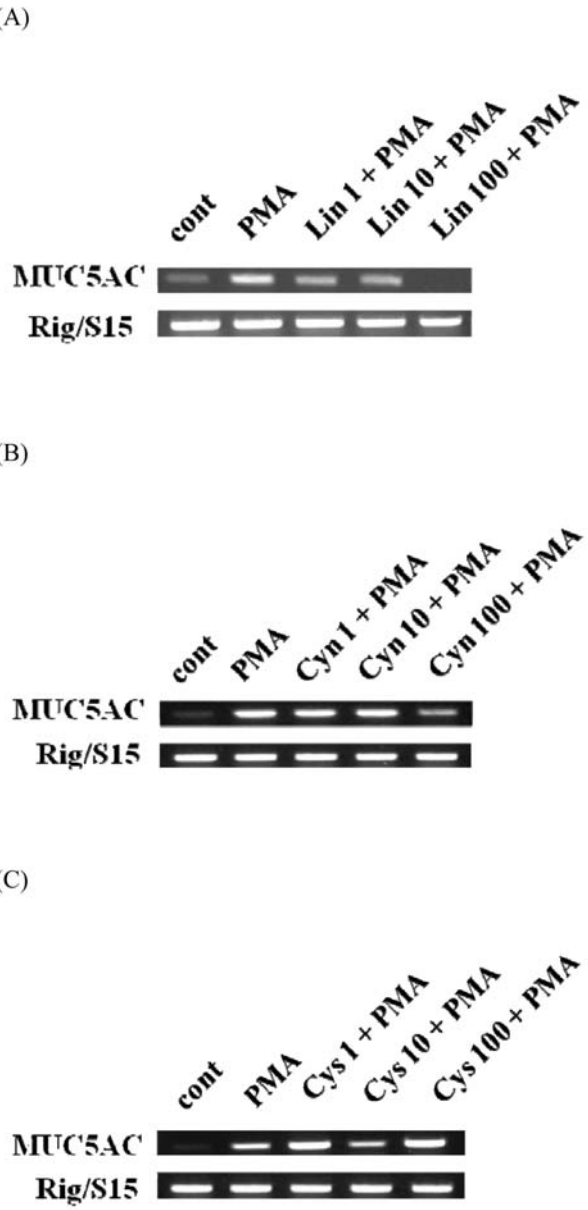

Fig. 4. Effect of cynaroside, cynarin or linarin on PMA-induced MUC5AC mRNA expression in NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of cynaroside, cynarin or linarin for 30 min and then stimulated with PMA (10 ng/mL) for 24 h. MUC5AC mRNA expression was measured by RT-PCR. Three independent experiments were performed and the representative data were shown.
(cont: control, Lin: linarin, Cyn: cynarin, Cys: cynaroside)

of mucin in the cynaroside-treated cells were $100 \pm 4\%$, $198 \pm 20\%$, $182 \pm 17\%$, $240 \pm 5\%$ and $229 \pm 10\%$ for control, 10ng/ml of PMA alone, PMA plus cynaroside 10^{-6} M, PMA plus cynaroside 10^{-5} M and PMA plus cynaroside 10^{-4} M, respectively (Fig. 3(C)). As can be seen in Fig. 4, MUC5AC mRNA expression induced by PMA in NCI-H292 cells was inhibited by pretreatment with linarin or cynarin (Fig. 4 (A), (B)). However, cynaroside did not show consistent inhibitory effect on MUC5AC gene expression (Fig. 4(C)).

These results suggest that, among the three natural products, only linarin showed the consistent inhibitory activities on the secretion, production and gene expression of airway MUC5AC mucin. Since linarin showed the inhibitory activities on mucin secretion, production and gene expression induced by PMA, we examined the potential effect of linarin on production and gene expression of airway MUC5AC mucin induced by EGF- or TNF-α, the other two prominent stimulators of production and gene expression of airway MUC5AC mucin. Takeyama and his colleagues reported that epidermal growth factor (EGF) regulated MUC5AC mucin gene expression in the pulmonary system. According to their reports, MUC5AC mRNA expression was increased after ligand binding to the EGF receptor and activation of the MAPK (mitogen-activated protein kinase) cascade.[15,19] Also, TNF-α is a well-known stimulant for secretion and gene expression of airway mucin.[14,20,21] TNF-α levels in sputum were reported to be increased, with further increases during exacerbation of diseases.[15,22] TNF-α converting enzyme (TACE) mediated MUC5AC mucin expression in cultured human airway epithelial cells[14] and TNF-α induced MUC5AC gene expression in normal human airway epithelial cells.[21] It also induced mucin secretion from guinea pig tracheal epithelial cells.[20] As shown in results, linarin also suppressed the production and gene expression of MUC5AC mucin induced by EGF or TNF-α.

As can be seen in Fig. 5, MUC5AC mRNA expression induced by EGF or TNF-α from NCI-H292 cells was inhibited by pretreatment with linarin (Fig. 5(A), (B)). Linarin inhibited EGF-induced MUC5AC production from NCI-H292 cells. The amounts of mucin in the linarin-treated cells were $100 \pm 10\%$, $176 \pm 13\%$, $143 \pm 17\%$, $141 \pm 8\%$ and $74 \pm 20\%$ for control, 25 ng/mL of EGF alone, EGF plus linarin 10^{-6} M, EGF plus linarin 10^{-5} M and EGF plus linarin 10^{-4} M, respectively (Fig. 5 (C)). Linarin also inhibited TNF-α-induced MUC5AC mucin production. The amounts of MUC5AC mucin in the linarin-treated cells were $100 \pm 7\%$, $190 \pm 14\%$, $109 \pm 4\%$, $100 \pm 3\%$ and $94 \pm 4\%$ for control, TNF-α 0.2 nM only, TNF-α plus linarin 10^{-6} M , TNF-α plus linarin 10^{-5} M and TNF-α plus linarin 10^{-4} M, respectively (Fig. 5(D)).

These results suggest that linarin can regulate the secretion, production and gene expression of mucin, by directly acting on airway epithelial cells. The underlying mechanisms of action of linarin on the secretion, production and gene expression of MUC5AC mucin are not clear at

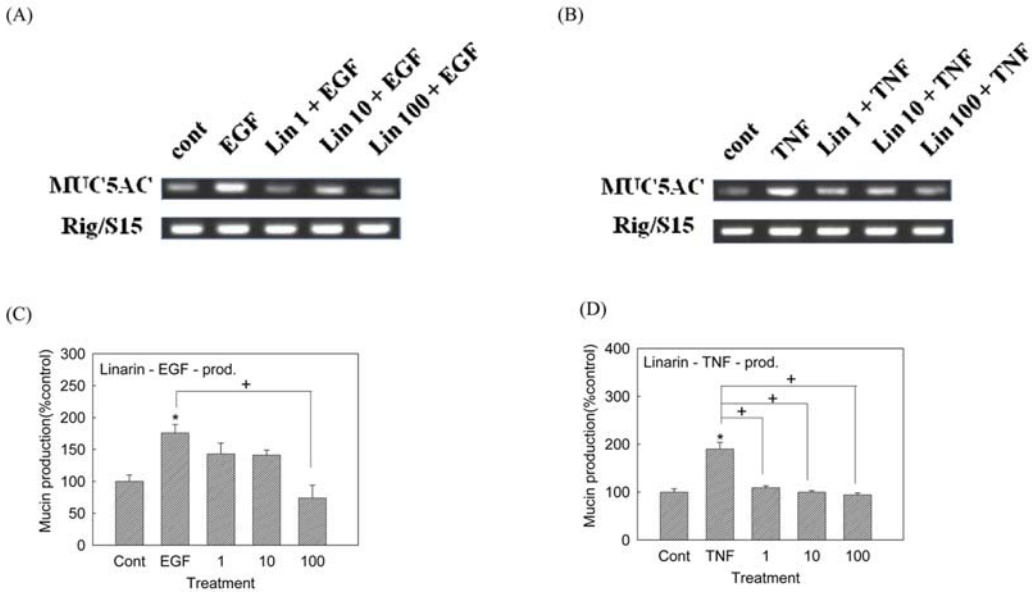

Fig. 5. Effect of linarin on EGF- or TNF-α-induced MUC5AC mRNA expression and production in NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of linarin for 30 min and then stimulated with EGF (25 ng/mL) (A, C) or TNF-α (0.2 nM) (B, D) for 24 h. MUC5AC mRNA expression was measured by RT-PCR (A, B). Cell lysates were collected for measurement of MUC5AC mucin production by ELISA (C, D). Three independent experiments were performed and the representative data were shown. Each bar represents a mean ± S.E.M. of three culture wells in comparison with that of control set at 100%.

*significantly different from control ($p < 0.05$).

+significantly different from inducer (EGF or TNF-α) alone ($p < 0.05$).

(cont: control, Lin: linarin, concentration unit is μM)

present, although we are investigating whether linarin act as potential regulators of the MAPK (mitogen-activated protein kinase) cascade after ligand binding to the EGF receptor and/or potential regulators of NF-kB signaling pathway, in mucin-producing NCI-H292 cells.[21]

Taken together, the inhibitory action of linarin on the secretion, production and gene expression of airway mucin might explain, at least in part, the traditional use of *Chrysanthemum indicum* L., as an anti-inflammatory and anti-allergic agent for pulmonary inflammatory diseases, in traditional oriental medicine. We suggest it is valuable to find the natural products that have specific inhibitory effects on the secretion, production and gene expression of mucin - in view of both basic and clinical sciences - and the result from this study suggests a possibility of developing linarin as a candidate for the new efficacious mucoregulators for pulmonary diseases, although further studies are required.

Acknowledgements

This work was supported by research fund of Chungnam National University.

References

(1) Lee, C. J.; Park, S. H.; Ko, K. H.; Kim, K. C. *Inflamm. Res.* **2002**, *51*, 490-494.

(2) Voynow, J. A.; Rubin, B. K. *Chest* **2009**, *135*, 505-512.

(3) Heo, H. J.; Kim, C.; Lee, H. J.; Kim, Y. S.; Kang, S. S.; Seo, U. K.; Kim, Y. H.; Park, Y. C.; Seok, J. H.; Lee, C. J. *Phytother. Res.* **2007**, *21*, 462-465.

(4) Heo, H. J.; Lee, S. Y.; Lee, M. N.; Lee, H. J.; Seok, J. H.; Lee, C. J. *Phytother. Res*. **2009**, *23*, 1458-1461.

(5) Kim, K. D.; Lee, H. J.; Lim, S. P.; Sikder, A.; Lee, S. Y.; Lee, C. J. *Phytother. Res*. **2012**, *26*, 1301-1307.

(6) Gebhardt, R.; Fausel, M. *Toxicol. In Vitro* **1997**, *11*, 669-672.

(7) Kim, Y. H.; Lee, Y. S.; Choi, E. M. *Cell. Immunol*. **2011**, *268*, 112-116.

(8) Lou, H.; Fan, P.; Perez, R. G.; Lou, H. *Bioorg. Med. Chem*. **2011**, *19*, 4021-4027.

(9) Mamadalieva, N. Z.; Herrmann, F.; El-Readi, M. Z.; Tahrani, A.; Hamoud, R.; Egamberdieva, D. R.; Azimova, S. S.; Wink, M. *J. Pharm. Pharmacol.* **2011**, *63*, 1346-1357.

(10) Adzet, T.; Camarasa, J.; Laguna, J. C. *J. Nat. Prod*. **1987**, *50*, 612-617.

(11) Zhu, X.; Zhang, H.; Lo, R. *J. Agric. Food Chem*. **2004**, *52*, 7272-7278.

(12) Rogers, D. F.; Barnes, P. J. *Ann. Med*. **2006**, *38*, 116-125.

(13) Li, J. D.; Dohrman, A. F.; Gallup, M.; Miyata, S.; Gum, J. R.; Kim, Y. S.; Nadel, J. A.; Prince, A.; Basbaum, C. B. *Proc. Natl. Acad. Sci. U S A* **1997**, *94*, 967-972.

(14) Shao, M. X.; Ueki, I. F.; Nadel, J. A. *Proc. Natl. Acad. Sci .U S A* **2003**, *100*, 11618-11623.

(15) Takeyama, K.; Dabbagh, K.; Lee, H. M.; Agustí, C.; Lausier, J. A.; Ueki, I. F.; Grattan, K. M.; Nadel, J. A. *Proc. Natl. Acad. Sci. U S A* **1999**, *96*, 3081-3086.
(16) Hong, D. H.; Petrovics, G.; Anderson, W. B.; Forstner, J.; Forstner, G. *Am. J. Physiol*. **1999**, *277*, G1041-G1047.
(17) Hewson, C. A.; Edbrooke, M. R.; Johnston, S. L. *J. Mol. Biol.* **2004**, *344*, 683-695.
(18) Park, S. J.; Kang, S. Y.; Kim, N. S.; Kim, H. M. *Immunopharmacol. Immunotoxicol*. **2002**, *24*, 211-226.
(19) Takeyama, K.; Dabbagh, K.; Jeong Shim, J.; Dao-Pick, T.; Ueki, I. F.; Nadel, J. A. *J. Immunol*. **2000**, *164*, 1546-1552.
(20) Fischer, B. M.; Rochelle, L. G.; Voynow, J. A., Akley, N. J.; Adler, K. B. *Am. J. Respir. Cell Mol. Biol*. **1999**, *20*, 413-422.
(21) Song, K. S.; Lee, W. J.; Chung, K. C.; Koo, J. S.; Yang, E. J.; Choi, J. Y.; Yoon, J. H. *J. Biol. Chem.* **2003**, *278*, 23243-23250.
(22) Cohn, L.; Whittaker, L.; Niu, N.; Homer, R. J. *Novartis Found Symp*. **2002**, *248*, 201-213.

Received September 24, 2014
Revised January 22, 2015
Accepted January 26, 2015