

Effect of Berberine on MUC5AC Mucin Gene Expression and Mucin Production from Human Airway Epithelial Cells

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Abstract

We conducted this study to investigate whether berberine significantly affects MUC5AC mucin gene expression and mucin production induced by epidermal growth factor (EGF), phorbol 12-myristate 13-acetate (PMA) or tumor necrosis factor- α (TNF- α) from human airway epithelial cells. Confluent NCI-H292 cells were pretreated with varying concentrations of berberine for 30 min and then stimulated with EGF, PMA or TNF- α for 24 h. MUC5AC mucin gene expression and mucin production were measured by reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. Berberine was found to inhibit the expression of MUC5AC mucin gene induced by EGF, PMA or TNF- α . Berberine also inhibited the production of MUC5AC mucin protein stimulated by the same inducers. This result suggests that berberine can regulate the expression of mucin gene and production of mucin protein, by directly acting on human airway epithelial cells.

Key Words: Airway, Mucin, Berberine

INTRODUCTION

Mucus in the human respiratory system is crucial in defensive action against invading pathogenic microorganisms, chemicals and particles. This defense mechanism of airway mucus is known to be due to the viscoelasticity of mucins. Mucins are multimillion dalton glycoproteins which are present in the airway mucus and produced by goblet cells in the surface epithelium and mucous cells in the submucosal gland (Rogers and Barnes, 2006). Hypersecretion of airway mucus, however, is one of the major symptoms associated with severe pulmonary diseases including asthma, chronic bronchitis, cystic fibrosis and bronchiectasis (Voynow and Rubin, 2009). Therefore, we suggest it is valuable to determine the potential activities of compounds derived from various medicinal plants for inhibiting excess mucin production. We investigated the possible activities of some natural products on mucin secretion and/or production in cultured airway epithelial cells. As a result of our study, we previously reported that several natural compounds affected mucin secretion (release) and/or production from airway epithelial cells (Lee *et al.*, 2003; Lee *et al.*, 2004; Heo *et al.*, 2007; Heo *et al.*, 2009). According to a num-

ber of reports, *Coptis japonica* Makino and its components, berberine, have been used to control airway allergic or inflammatory diseases and reported to have anti-inflammatory and anti-cancer effects (Yoo *et al.*, 2008; Sun *et al.*, 2009; Tang *et al.*, 2009). On the other hand, it was previously reported that berberine stimulated basal mucin release from airway goblet cells, by our group (Lee *et al.*, 2003; 2004). However, to the best of our knowledge, there are no reports about the effect of berberine on MUC5AC mucin gene expression and mucin production induced by epidermal growth factor (EGF), phorbol 12-myristate 13-acetate (PMA) or tumor necrosis factor- α (TNF- α), from human airway epithelial cells. Therefore, in this study, we investigated whether berberine affects the expression of MUC5AC mucin gene and the production of mucin protein induced by EGF, PMA or TNF- α , from the NCI-H292 human pulmonary mucoepithelial cell line.

MATERIALS AND METHODS

Materials

All chemicals and reagents used in this study, including ber-

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berine (purity: 98.0%), were purchased from Sigma (St. Louis, MO, USA) unless otherwise specified.

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, USA) and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, in the presence of penicillin (100 units/ml), streptomycin (100 µg/ml) and HEPES (25 mM) at 37°C in a humidified, 5% CO₂/95% air, water-jacketed incubator. For serum deprivation, confluent cells were washed twice with phosphate-buffered saline (PBS) and recultured in RPMI 1640 with 0.2% fetal bovine serum for 24 h.

Treatment of cells with berberine

After 24 h of serum deprivation, cells were pretreated with varying concentrations of berberine (1-100 µM) for 30 min and then treated with EGF (25 ng/ml), PMA (10 ng/ml) or TNF-α (0.2 nM) for 24 h in serum-free RPMI 1640, respectively. 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 mucin protein in a 24-well culture plate. The total RNA was extracted for measuring the expression of MUC5AC mucin gene in a 6-well culture plate.

MUC5AC mucin analysis using ELISA

MUC5AC 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°C until dry in a 96-well

plate. 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 (NeoMarkers, CA, USA) (1:200), 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 reactions were developed using 3,3',5,5'-tetramethylbenzidine (TMB) peroxide solution and stopped with 1N H₂SO₄. Absorbance was measured at 450 nm.

Total RNA isolation and RT-PCR

Total RNA was isolated using an 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 volume by using Thermoprime 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 R18S1 rRNA, which encodes a small ribosomal subunit protein (a housekeeping gene that was constitutively expressed), were used. Primers for R18S1 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 sec, 60°C

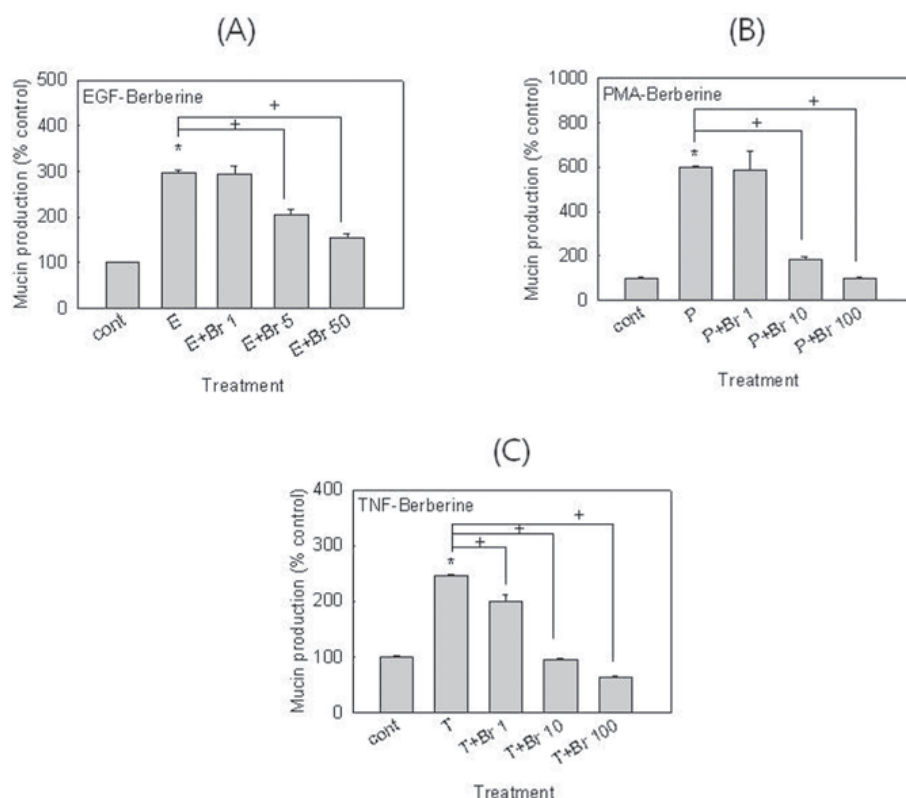


Fig. 1. Effect of berberine on EGF-, PMA- or TNF-α-induced MUC5AC production from NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of berberine for 30 min and then stimulated with EGF (25 ng/ml), PMA (10 ng/ml) or TNF-α (0.2 nM) for 24 h. Cell lysates were collected for measurement of MUC5AC mucin production by ELISA. Each bar represents a mean ± S.E.M. of 3-4 culture wells in comparison with that of a control set at 100%. *Significantly different from control ($p < 0.05$). *Significantly different from EGF, PMA or TNF-α alone ($p < 0.05$). cont: control, Br: berberine, concentration unit is µM, E: EGF, P: PMA, T: TNF-α.

for 30 sec and 72°C for 45 sec. Following 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 groups were converted to percent control and expressed as mean \pm S.E.M. The difference between groups was assessed using one-way ANOVA and Student's t-test for unpaired samples. $p < 0.05$ was considered as significantly different.

RESULTS

Effect of berberine on EGF-, PMA- or TNF- α -induced MUC5AC production

Fig. 1 shows that berberine significantly inhibited EGF-, PMA- or TNF- α -induced MUC5AC production from NCI-H292 cells. The amounts of mucin in the cells of berberine-treated and EGF-induced cultures were $100 \pm 2\%$, $298 \pm 6\%$, $295 \pm 18\%$, $206 \pm 10\%$ and $155 \pm 7\%$ for control, 25 ng/ml of EGF alone, EGF plus berberine 10^{-6} M, EGF plus berberine 5×10^{-6} M and EGF plus berberine 5×10^{-5} M, respectively (A). The amounts of mucin in the cells of berberine-treated and PMA- induced cultures were $100 \pm 2\%$, $600 \pm 8\%$, $586 \pm 89\%$, $186 \pm 9\%$ and $96 \pm 10\%$ for control, 10 ng/ml of PMA alone, PMA plus berberine 10^{-6} M, PMA plus berberine 10^{-5} M and PMA plus berberine 10^{-4} M, respectively (B). The amounts of MUC5AC mucin in the cells of berberine-treated and TNF- α -induced cultures were $100 \pm 2\%$, $245 \pm 4\%$, $200 \pm 12\%$, $95 \pm 2\%$ and $64 \pm 3\%$ for control, TNF- α 0.2 nM only, berberine 10^{-6} M+TNF- α , berberine 10^{-5} M+TNF- α and berberine 10^{-4} M+ TNF- α , respectively (C).

Effect of berberine on MUC5AC gene expression induced by EGF, PMA or TNF- α

Fig. 2 shows that berberine at the concentration of 5×10^{-5} M or 10^{-4} M inhibited MUC5AC gene expression induced by EGF, PMA or TNF- α (Fig. 2).

DISCUSSION

An important approach to the effective control of severe pulmonary diseases involving the hyperproduction and hy-

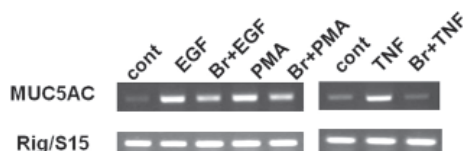


Fig. 2. Effect of berberine on EGF-, PMA- or TNF- α -induced MUC5AC gene expression from NCI-H292 cells. NCI-H292 cells were pretreated with berberine (50 µM for EGF-induced MUC5AC gene expression and 100 µM for PMA- or TNF- α -induced MUC5AC gene expression) for 30 min and then stimulated with EGF (25 ng/ml), PMA (10 ng/ml) or TNF- α (0.2 nM) for 24 h. MUC5AC gene expression was measured by RT-PCR. As quantitative control, R19/S15 rRNA, which encodes a small ribosomal subunit protein, a housekeeping gene that was constitutively expressed, was used. cont: control, Br: berberine.

persecretion of airway mucin is to develop a potential pharmacological tool for regulating production and/or secretion of mucus. Mucins are macromolecular glycoproteins present in the airway mucus and have peptide backbones and carbohydrate branches (Voynow and Rubin, 2009). Up to now, 20 MUC genes have been reported as coding the peptide backbone of human mucins and, among them, MUC5AC is strongly expressed in airway goblet cells (Rogers and Barnes, 2006; Yuan-Chen Wu *et al.*, 2007). Also, EGF and PMA were reported to regulate MUC5AC mucin gene expression in the lung (Takeyama *et al.*, 1999; Hewson *et al.*, 2004). MUC5AC mRNA expression was increased after ligand binding to the EGF receptor and activation of the mitogen-activated protein kinase (MAPK) cascade (Takeyama *et al.*, 1999; 2000). PMA is an inflammatory stimulant affecting gene transcription, cell growth and differentiation and induces MUC5AC gene expression in NCI-H292 cells (Park *et al.*, 2002; Hewson *et al.*, 2004). On the other hand, TNF- α levels in sputum were reported to be increased, with further increases during exacerbation of diseases (Chung, 2001; Cohn *et al.*, 2002). TNF- α converting enzyme (TACE) mediated MUC5AC mucin expression in cultured human airway epithelial cells (Shao *et al.*, 2003) and TNF- α induced MUC5AC gene expression in normal human airway epithelial cells (Song *et al.*, 2003). It also induced mucin secretion from guinea pig tracheal epithelial cells (Fischer *et al.*, 1999). Based on these reports, we investigated whether berberine affects EGF-, PMA- or TNF- α -induced MUC5AC mucin gene expression and mucin production from NCI-H292 cells, a human pulmonary mucoepidermoid cell line which is frequently used for studying intracellular signaling pathways involved in airway mucin production (Li *et al.*, 1997; Takeyama *et al.*, 1999; Shao *et al.*, 2003). As can be seen in Results, berberine inhibited the production of MUC5AC mucin protein induced by EGF, PMA and TNF- α , respectively. Also, berberine suppressed the expression of MUC5AC mucin gene induced by the same stimulators (Fig. 1, 2). These results suggest that berberine can regulate mucin gene expression and production of mucin protein, by directly acting on human airway epithelial cells. However, based on our previous report, berberine mildly stimulated the basal mucin release from cultured airway goblet cells (Lee *et al.*, 2003). Although the production and secretion of mucin can be controlled independently at the molecular levels, we do not know the reason why berberine inhibits the production and gene expression of MUC5AC mucin without affecting basal secretion (release) of mucin, solely based on the data we got from this study. Therefore, it is to be elucidated why berberine can stimulate the release (secretion) of airway mucin with the activity of suppression of airway mucin production and gene expression, through future research. Berberine, an isoquinoline alkaloid, can inhibit the enzyme activity of phospholipase A₂ (PLA₂), an enzyme which plays an important role in inflammation, through structural binding of berberine and PLA₂ (Chandra *et al.*, 2011). Therefore, we suggest that, in NCI-H292 cells, berberine might inhibit the activity of PLA₂ and then suppress inflammation-related mucin production e.g. TNF- α (and/or PMA- and EGF-) induced NF- κ B-mediated MUC5AC gene expression and production, as a potential mechanism of action of berberine. Of course, the underlying mechanism of action of these compounds is not clear at present and should be elucidated through future research, based on aforementioned hypothesis and, also, we are trying to examine whether berberine act as potential regulators of

the MAPK cascade after ligand binding to the EGF receptor and NF- κ B signaling pathway, in mucin-producing NCI-H292 cells. Taken together, the inhibitory action of berberine on airway mucin production and gene expression might explain, at least in part, the traditional use of *Coptis japonica* Makino as an anti-inflammatory agent for the control of airway inflammatory diseases, in oriental medicine. We suggest it is valuable to find the natural products that have specific inhibitory effect on mucin production and/or gene expression - in view of both basic and clinical sciences - and to search the optimal chemical moieties derived from the chemical structure of berberine which can be useful as an efficacious regulator for mucin production in hypersecretory status of diverse chronic airway diseases, through further studies.

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