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Original Article

Effects of Curcumin and Genistein on Phorbol Ester or Tumor Necrosis Factor-α-Induced Mucin Production from Human Airway Epithelial Cells

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Background: We investigated whether curcumin and genistein affect the MUC5AC mucin production from human airway epithelial cells that is induced by phorbol 12-myristate 13-acetate (PMA) or tumor necrosis factor-α (TNF-α).
Methods: Confluent NCI-H292 cells were pretreated with each agent for 30 min and then stimulated with PMA or TNF-α for 24 hours. MUC5AC mucin production was measured by an ELISA.
Results: (1) Curcumin dose-dependently inhibited the production of MUC5AC mucin that was induced by PMA or TNF-α; (2) Genistein inhibited PMA-induced MUC5AC mucin production. However, it did not decrease TNF-α-induced MUC5AC mucin production.
Conclusion: These results suggest that curcumin and genistein inhibit the production of airway mucin induced by PMA.

Key Words: Mucin 5AC; Curcumin; Genistein

Introduction

Airway mucus is crucial in defense against airborne chemicals, particles and pathogenic microorganisms. The protective function of airway mucus is due mainly to the viscoelastic property of mucous glycoproteins or mucins. Mucins are macromolecular glycoproteins present in the airway mucus and have peptide backbones and carbohydrate branches[1]. Currently, 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[2,3]. However, any abnormality in the quality or quantity of mucins not only cause altered airway physiology but may also impair host defenses often leading to serious airway pathology as exemplified in chronic bronchitis, cystic fibrosis, asthma, and bronchiectasis[1]. Therefore, we suggest it is valuable to find the possible activity of controlling the excess mucin production by the components from medicinal plants that have been used for the management of airway diseases. We have tried to investigate the possible activities of some natural products on mucin secretion from cultured airway epithelial cells. As a result of our trial, we previously reported that several natural compounds affected mucin secretion from airway epithelial cells[4-6]. According to oriental medicine, Curcumae Longae Rhizoma and Puerariae radix have been used for controlling diverse inflammatory diseases including respiratory disease[7] and their components, curcumin and genistein were reported to have various biological effects including effects on asthma and cystic fibrosis[8-17]. However, to the best of our knowledge, there

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are no reports about the effect of two compounds on airway mucin production from airway epithelial cells induced by phorbol 12-myristate 13-acetate (PMA) or tumor necrosis factor (TNF)-α, except for the effect of curcumin on basal airway mucin release from cultured hamster tracheal surface epithelial cells[5] and the effects of genistein and curcumin on EGF-induced mucin production from NCI-H292 cells[18], reported by our group. Therefore, we checked whether curcumin and genistein affect mucin production induced by PMA or TNF-α from NCI-H292 cells, a human pulmonary mucoepidermoid cell line.

Materials and Methods

1. Materials

All the chemicals and reagents used in this experiment including curcumin (purity, 80.0%) and genistein (purity, 98.0%) were purchased from Sigma (St. Louis, MO, USA) unless otherwise specified.

2. 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 (FBS) in the presence of penicillin (100 units/mL), 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 recultured in RPMI 1640 with 0.2% fetal bovine serum for 24 hours.

3. Treatment of cells with curcumin and genistein

After 24 hours of serum deprivation, cells were pretreated with genistein (1, 10, 100 μM) or curcumin (1, 10, 100 μM) for 30 min and treated with PMA (10 ng/mL) or TNF-α (0.2 nM) for 24 hours in serum-free RPMI 1640, respectively. Genistein and curcumin were dissolved in dimethylsulfoxide, diluted in PBS and treated in culture medium (final concentrations of dimethylsulfoxide were 0.5%). Culture medium, PBS solution and 0.5% dimethylsulfoxide in medium did not affect mucin production from NCI-H292 cells. After 24 hours, 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 Diagnostic Systems, Indianapolis, IN, USA) and collected to measure the production of MUC5AC protein (in 24-well culture plate).

4. MUC5AC mucin analysis using ELISA

MUC5AC mucin 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 in a 96-well plate, until dry. Plates were washed three times with PBS and blocked with 2% BSA (fraction V) for 1 hour 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, Freemont, CA, USA) (1 : 200), which was diluted with PBS containing 0.05% Tween 20 and dispensed into each well. After 1 hour, 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 hour, plates were washed three times with PBS. Color reaction was developed with 3,3',5,5'-tetramethylbenzidine (TMB) peroxide solution and stopped with 1N H_2SO_4. Absorbance was read at 450 nM.

5. Statistics

Means of individual group were converted to percent control and expressed as mean±standard error of the mean (SEM). 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.

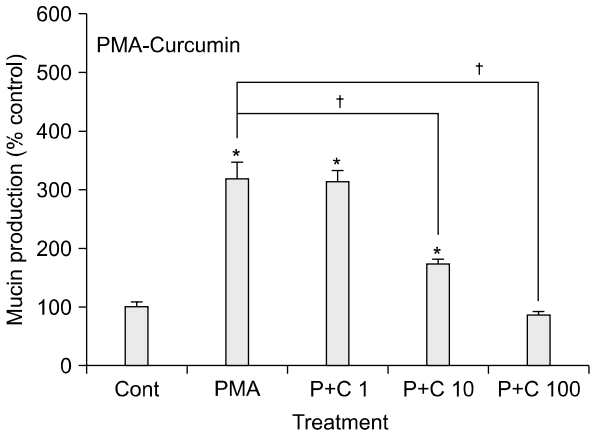

Figure 1. Effect of curcumin on PMA-induced MUC5AC mucin production from NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of curcumin for 30 min and then stimulated with PMA (10 ng/mL) for 24 hours. Cell lysates were collected for measurement of MUC5AC mucin production by ELISA. Means of individual group were converted to percent control and expressed as mean±SEM. 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. Each bar represents a mean±SEM. 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 PMA alone ($p<0.05$). SEM: standard error of the mean; cont: control; C: curcumin, concentration unit is μM.; PMA: phorbol 12-myristate 13-acetate.

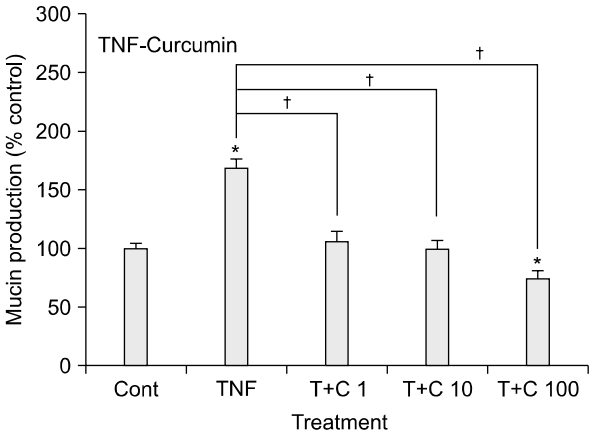

Figure 2. Effect of curcumin on TNF-α-induced MUC5AC mucin production from NCI-H292 cells. NCI-H292 cells were pretreated with various concentrations of curcumin for 30min and then stimulated with TNF-α (0.2 nM) for 24 hours. Cell lysates were collected for measurement of MUC5AC mucin production by ELISA. Means of individual group were converted to percent control and expressed as mean±SEM. 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. Each bar represents a mean±SEM. of 3~4 culture wells in comparison with that of control set at 100%. *Significantly different from control ($p<0.05$), †Significantly different from TNF-α alone ($p<0.05$). SEM: standard error of the mean; cont: control; C: curcumin, concentration unit is μM.; PMA: phorbol 12-myristate 13-acetate; TNF: tumor necrosis factor.

Results

1. Effect of curcumin on PMA-induced MUC5AC production

As can be seen in Figure 1, curcumin significantly inhibited PMA-induced MUC5AC production from NCI-H292 cells at the concentrations between 10^{-5} M and 10^{-4} M. The amounts of mucin in the cells of curcumin-treated cultures were 100±8%, 320±26%, 313±19%, 173±8% and 87±5% for control, 10 ng/mL of PMA alone, PMA plus curcumin 10^{-6} M, PMA plus curcumin 10^{-5} M and PMA plus curcumin 10^{-4} M, respectively (Figure 1).

2. Effect of curcumin on TNF-α- induced MUC5AC production

As can be seen in Figure 2, curcumin significantly inhibited TNF-α-induced MUC5AC production from NCI-H292 cells at the concentrations between 10^{-6} M and 10^{-4} M. The amounts of mucin in the cells of curcumin-treated cultures were 100±5%, 169±8%, 106±9%, 100±7% and 75±6% for control, 0.2 nM of TNF-α alone, TNF-α plus curcumin 10^{-6} M, TNF-α plus curcumin 10^{-5} M and TNF-α plus curcumin 10^{-4} M, respectively (Figure 2).

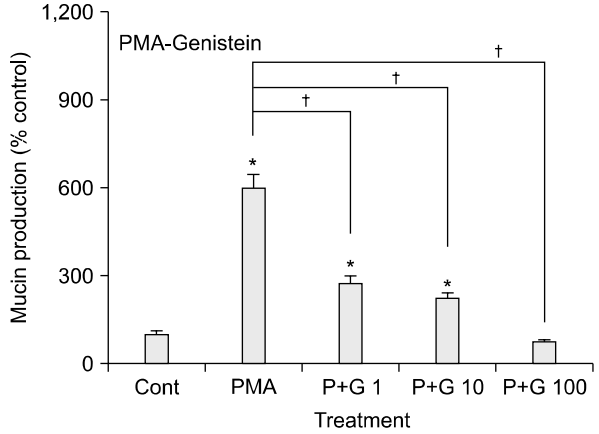

Figure 3. Effect of genistein on PMA-induced MUC5AC mucin production from NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of genistein for 30 min and then stimulated with PMA (10 ng/mL) for 24 hours. Cell lysates were collected for measurement of MUC5AC mucin production by ELISA. Means of individual group were converted to percent control and expressed as mean±SEM. 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. Each bar represents a mean±SEM 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 PMA alone ($p<0.05$). SEM: standard error of the mean; cont: control; PMA: phorbol 12-myristate 13-acetate; G: genistein, concentration unit is μM.

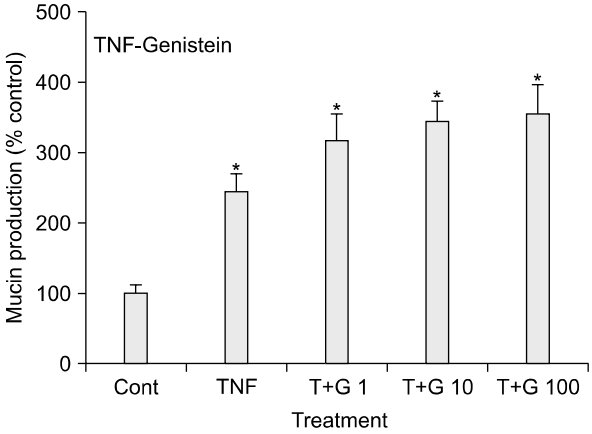

Figure 4. Effect of genistein on TNF-α-induced MUC5AC mucin production from NCI-H292 cells. NCI-H292 cells were pretreated with various concentrations of genistein for 30 min and then stimulated with TNF-α (0.2 nM) for 24 hours. Cell lysates were collected for measurement of MUC5AC mucin production by ELISA. Means of individual group were converted to percent control and expressed as mean±SEM. 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. Each bar represents a mean±SEM. of 3~4 culture wells in comparison with that of control set at 100%. *Significantly different from control ($p<0.05$). ELISA: enzyme-linked immunosorbent assay; cont: control; G: genistein, concentration unit is μM.

3. Effect of genistein on PMA-induced MUC5AC production

As can be seen in Figure 3, genistein significantly inhibited PMA-induced MUC5AC production from NCI-H292 cells at the concentrations between 10^{-6} M and 10^{-4} M. The amounts of mucin in the cells of genistein-treated cultures were 100±10%, 600±46%, 275±24%, 225±16% and 75±8% for control, 10 ng/mL of PMA alone, PMA plus genistein 10^{-6} M, PMA plus genistein 10^{-5} M and PMA plus genistein 10^{-4} M, respectively (Figure 3).

4. Effect of genistein on TNF-α- induced MUC5AC production

As can be seen in Figure 4, genistein did not decrease TNF-α-induced MUC5AC production from NCI-H292 cells. The amounts of mucin in the cells of genistein-treated cultures were 100±12%, 245±25%, 318±37%, 345±28% and 355±42% for control, 0.2 nM of TNF-α alone, TNF-α plus genistein 10^{-6} M, TNF-α plus genistein 10^{-5} M and TNF-α plus genistein 10^{-4} M, respectively (Figure 4).

Discussion

Assem and his colleagues reported that genistein, a tyrosine kinase inhibitor, relaxed the contraction of airway smooth muscle induced by histamine or carba-

chol[16]. It also showed anti-inflammatory effects on a guinea pig model of asthma[17]. Genistein was reported to activate cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel in airway epithelial cell lines, at the similar concentrations obtained in plasma by a soy-rich diet[14]. Genistein was able to affect asthma by inhibition of eosinophil p38-dependent leukotriene synthesis[15]. Curcumin was reported to stimulate CFTR chloride channel activity[13] and attenuated allergen-induced airway hyperresponsiveness in sensitized guinea pigs[11]. Moon and his colleagues reported that curcumin attenuated ovalbumin-induced airway inflammation by regulating nitric oxide[12]. On the other hand, TNF-α is a stimulator of secretion and gene expression of mucin in the airway epithelium[19-21]. TNF-α level in sputum was reported to be increased, with further increases during exacerbation of diseases[22,23]. TNF-α converting enzyme (TACE) mediated MUC5AC mucin expression in cultured human airway epithelial cells[20] 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[19]. Phorbol 12-myristate 13-acetate (PMA) acts as an alternative stimulus to the endogenous activator of protein kinase C (PKC), diacylglycerol (DAG) and a model inflammatory stimulant that can modulate a variety of cellular events, including gene transcription[24], cell growth and differentiation[25]. Especially, PMA was reported to induce MUC5AC gene expression in NCI-H292 cells[24]. Based on these reports, we examined the effects of curcumin and genistein on airway MUC5AC mucin production induced by PMA or TNF-α from NCI-H292 cells, a human pulmonary mucoepidermoid cell line, which are frequently used for the purpose of elucidating intracellular signaling pathways involved in airway mucin production[10,20,26]. As shown in results, curcumin and genistein inhibited the production of MUC5AC mucin protein induced by PMA, dose-dependently. Curcumin also inhibited the production of MUC5AC mucin induced by TNF-α, although genistein did not decrease TNF-α-induced MUC5AC mucin production. Instead, genistein showed tendency of increasing mucin production induced by TNF-α (Figure 4). In this group of treatment, there is a possibility that, genistein can not affect TNF-α-induced mucin production and TNF-α itself might show more potent action than in TNF-α alone group, although we do not suggest the exact cause based on data from the current study. This result suggests that curcumin and genistein might inhibit the production of mucin induced by PMA, by directly acting on airway epithelial cells. The underlying mechanisms of action of these two compounds on MUC5AC production are not clear at present, although we are trying to investigate whether curcumin and genistein act as possible regulators of NF-kB signaling pathway and/or selective inhibitors of MAPK pathway in mucin-producing NCI-H292 cells. In summary, the inhibitory actions of curcumin and genistein on airway mucin production might explain, at least in part, the traditional use of Curcumae Longae Rhizoma and Puerariae radix as anti-inflammatory agents and mucoregulators for airway inflammatory diseases, in oriental medicine. We suggest it is valuable to find the natural products that have specific inhibitory effects on mucin production - in view of both basic and clinical sciences - and the result from this study suggest a possibility of using curcumin and genistein as potential mucoregulators for respiratory diseases, although further studies are essential.

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