Effects of Baicalin, Baicalein and Schizandrin on Airway Mucin Production Induced by Epidermal Growth Factor and Phorbol Ester

Hyun Jae Lee¹,a, Su Yel Lee¹,a, Young Sik Kim², Byeong Kyou Jeon³, Jae Woo Lee⁴, Heung Seog Bae¹, and Choong Jae Lee¹,*

¹Department of Pharmacology, School of Medicine, Chungnam National University, Daejeon 303-131, ²Department of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, ³Department of Radiologic Technology, Daegu Health College, Daegu 702-722, ⁴LG Life Science, Seoul 150-721, Republic of Korea

(Received July 28, 2010; Revised August 18, 2010; Accepted August 19, 2010)

Abstract - We conducted this study to investigate whether baicalin, baicalein or schizandrin significantly affect MUC5AC mucin production induced by epidermal growth factor (EGF) or phorbol ester (PMA) in human airway epithelial cells. Confluent NCI-H292 cells were pretreated with varying concentrations of baicalin, baicalein or schizandrin for 30 min and then stimulated with EGF or PMA for 24 h, respectively. MUC5AC mucin protein production was measured by ELISA. The results were as follows: (1) Baicalin was found to inhibit the production of MUC5AC mucin protein induced by both EGF and PMA. (2) Baicalein, the aglycone of baicalin, also inhibited MUC5AC mucin production. (3) Schizandrin, derived from Schizandrae Fructus, inhibited MUC5AC mucin production by the same inducers. These results suggest that baicalin, baicalein and schizandrin can regulate the production of mucin protein by directly acting on human airway epithelial cells.

Keywords: Airway mucin, Baicalin, Baicalein and schizandrin

INTRODUCTION

Mucus present in the human respiratory system is very important in defense against invading pathogenic microorganisms, chemicals and particles. This defensive action of airway mucus is 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. 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 in cultured airway epithelial cells. As a result of our study, we previously reported that several natural compounds affected mucin secretion by airway epithelial cells (Lee et al., 2003; Heo et al., 2006; Heo et al., 2007). According to a number of reports, Scutellariae Radix has been used to control airway allergic or inflammatory diseases, and their components, baicalin and baicalein, were reported to have diverse biological effects (Chou et al., 2003; Dong et al., 2005; Van Leyen et al., 2006; Hsieh et al., 2007). Baicalein was reported to be a potent antioxidant and free radical scavenger and has been regarded as a 12/15-lipoxygenase inhibitor and xanthine oxidase inhibitor (Van Leyen et al., 2006). Baicalein has been shown to antagonize the expression of adhesion molecules induced by interleukin-β1 (IL-β1) and tumor necrosis factor (TNF-α) (Hsieh et al., 2007). Baicalin demonstrated anti-inflammatory and analgesic effects through the inhibition of important inflammatory mediators and proinflammatory cytokines, as well as through neutrophil infiltration at sites of inflammation (Chou et al., 2003). Inhalation of baicalin showed an inhibition of airway hyperresponsiveness (Dong et al., 2005). Also, Schizandraceae Fructus and one of its components (schizandrin), were re-
ported to have various biological effects including free radical scavenging effect (Li et al., 1990), hepatoprotective effect (Liu, 1989; Chiu et al., 2003) and to offer protection of neuronal cells from excitotoxicity (Kim et al., 2004). On the other hand, it was previously reported that baicalin and schizandrin inhibited ATP-stimulated mucin release from airway goblet cells, although baicalein did not affect ATP-stimulated airway mucin release in studies conducted by our group (Lee et al., 2003; Heo et al., 2006; Heo et al., 2007). However, to the best of our knowledge, there are no reports about the effects of baicalin, baicalein or schizandrin on mucin production induced by epidermal growth factor or phorbol ester, in human airway epithelial cells. Therefore, in this study, we investigated whether baicalin, baicalein or schizandrin affect the production of mucin induced by epidermal growth factor or phorbol ester, in the NCI-H292 human pulmonary mucoepidermoid cell line.

MATERIALS AND METHODS

Materials
All chemicals and reagents used in this study, including baicalin (purity: 98.0%) and baicalein (purity: 98.0%), were purchased from Sigma (St. Louis, MO, U.S.A.) unless otherwise specified. Schizandrin (purity: 95.0%) was isolated, purified and identified by analytical chemists at the Research Institute of Natural Products of Seoul National University (Seoul, Korea).

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°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 agents
After 24 h of serum deprivation, cells were pretreated with baicalin, baicalein or schizandrin (1, 10 and 100 μM) for 30 min and then treated with EGF (25 ng/ml) or PMA (10 ng/ml) 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, U.S.A.) and collected to measure the production of MUC5AC protein in a 24-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, U.S.A.) (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-ant-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.

Statistics
Means of individual groups were converted to percent control and expressed as mean ± 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 baicalin on EGF-induced MUC5AC production
Fig. 1 shows that baicalin significantly inhibited EGF-induced MUC5AC production from NCI-H292 cells at the highest concentration. The amounts of mucin in the cells of baicalin-treated cultures were 100 ± 7%, 150 ± 9%, 193 ± 10%, 145 ± 5% and 104 ± 6% for control, 25 ng/ml of EGF alone, EGF plus baicalin 10−6 M, EGF plus baicalin 10−5 M and EGF plus baicalin 10−4 M, respectively (Fig. 1).

Effect of baicalin on PMA-induced MUC5AC production
Fig. 2 shows that baicalin significantly inhibited PMA-induced MUC5AC production from NCI-H292 cells at concentrations between 10−5 M and 10−4 M. The amounts of mucin in the cells of baicalin-treated cultures were 100 ± 8%, 303 ± 20%, 330 ± 15%, 54 ± 8% and 57 ± 7% for control, 10 ng/ml of PMA alone, PMA plus baicalin 10−6 M, PMA plus baicalin 10−5 M and PMA plus baicalin 10−4 M, respectively (Fig. 2).