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Effects of Three Compounds from Schizandrae Fructus and Uridine on Airway Mucin Secretion

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Abstract – In this study, we investigated whether schizandrin, schizandrin-A, gomisin-A and uridine affect mucin secretion from cultured airway epithelial cells and compared the potential activities of these agents with the inhibitory action on mucin secretion by poly-l-lysine (PLL) and the stimulatory action by adenosine triphosphate (ATP). Confluent primary hamster tracheal surface epithelial (HTSE) cells were metabolically radiolabeled using ^{3}H-glucosamine for 24 h and chased for 30 min in the presence of varying concentrations of each agent to assess the effects on ^{3}H-mucin secretion. The results were as follows: schizandrin-A and uridine increased mucin secretion at the highest concentrations (2×10^{-4}M - 10^{-3}M). We conclude that schizandrin-A and uridine can stimulate mucin secretion via direct effect on airway mucin-secreting cells and suggest that these agents be further investigated for the potential use as mucoregulators during the treatment of chronic airway diseases.

Key words □ Airway mucin, Schizandrin, Schizandrin-A, Gomisin-A, Uridine

INTRODUCTION

Airway mucus plays an important role in host defenses against airborne chemicals, paticles and invading microorganisms through a mechanism called the mucociliary clearance. Its protective function is due mainly to the viscoelastic property of mucous glycoproteins or mucins. Mucins are high molecular weight glycoproteins produced by goblet cells in the surface epithelium as well as mucous cells in the submucosal gland. 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 (Newhouse and Biennenstock, 1983). Therefore, we suggest it is valuable to find the possible activity of controlling (inhibiting) the excess mucin secretion by the components from oriental herbs that have been used for the management of airway diseases. We have tried to investigate the possible activities of some components from medicinal plants on mucin secretion from airway goblet cells using a primary hamster tracheal surface epithelial (HTSE) cell culture - an established in vitro model for secretory cell metaplasia (Wasano et al., 1988). As a result of our trial, we previously reported that a few natural compounds affected mucin secretion from HTSE cells (Lee *et al.*, 2003, Lee *et al.*, 2004a, Lee *et al.*, 2004b). According to oriental medicine, *Schizandrae Fructus* has been used for controlling respiratory allergic or inflammatory diseases (National Association of Professor of Herbology in Oriental Medical School, 2005a) and their components, schizandrin, schizandrin-A and gomisin-A, respectively, were reported to have diverse biological effects (Jang, 2003). Additionally, we investigated the possible effect of uridine, a nucleoside containing uracil, on airway mucin secretion. We compared the possible activities of these agents with the inhibitory action on mucin secretion by PLL, a non-steroidal polycationic inhibitor of mucin secretion (Ko *et al.*, 1999) and the stimulatory action by ATP, a stimulator of mucin secretion (Kim *et al.*, 1997).

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MATERIALS AND METHODS

Materials

All the chemicals and reagents used in this experiment were purchased from Sigma (St. Louis, MO, U.S.A.) unless otherwise specified. Schizandrin (purity ; above 95%), schizandrin-A (purity ; above 95%) and gomisin-A (purity ; above 95%) were isolated, purified and identified by analytical chemists in Chungnam National University (Daejeon, Korea) and Research Institute of Natural Products of Seoul National University (Seoul, Korea).

Primary hamster tracheal surface epithelial (HTSE) cell culture

Tracheas were obtained from male Golden Syrian hamsters, 8 weeks of age (Harlan Sprague Dawley, Indiana, U.S.A.). HTSE cells were harvested and cultured on a thick collagen gel substratum as previously reported (Wasano *et al.*, 1988). Briefly, animals were euthanized in a CO_2 chamber and the tracheas were exposed under aseptic conditions. The tracheas were canulated using a polyethylene tube through which the tracheal lumen was filled with 0.1% pronase (Type XIV) prepared in Ca^{++}, Mg^{++} free Minimum Essential Medium (MEM, GIBCO) and incubated at 4°C for 16 h. The luminal contents were flushed, and cells were washed twice with MEM containing 10 % fetal bovine serum by centrifuging at 200 × g. The washed cell pellets were dissociated in a growth medium containing Medium 199 and Dulbecco's Modified Eagle's medium (DME) (1:1) supplemented with insulin (5 μg/mL), epidermal growth factor (12.5 ng/mL), hydrocortisone (0.1 μM), fetal bovine serum (5% v/v, Hyclone, Logan, UT, U.S.A.), sodium selenite (0.01 μM), retinoic acid (0.1 μM), Penicillin G (100 U/mL, GIBCO), Streptomycin (100 μg/mL, GIBCO), and Gentamicin (50 μg/mL) ("complete" medium). At this stage, most of the cells were in small aggregates and plated at a density of 10^4 cells/cm^2 into tissue culture dishes containing a thick collagen gel (0.15 mL/ cm^2) using collagen type I (Regenmed, Seoul, Korea). Cultures were incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO_2 and culture medium were changed on day 1, 3, 5 and 7.

Metabolic labeling of mucins and treatment of cultures

Mucins were metabolically radiolabeled for 24 h by incubating confluent cultures (24 well plate, 5×10^5 cells/well) with 0.2 mL/well of the "complete" medium containing 10 μCi/mL of [6-^{3}H] glucosamine (39.2 Ci/mmol, New England Nuclear, Boston, MA, U.S.A.) for 24 h, as previously reported (Kim *et al.*, 1987). At the end of the 24 h incubation, the spent media (the pretreatment sample) were collected, and the labeled cultures were washed twice with Dulbecco's phosphate-buffered saline (PBS) without Ca^{++} and Mg^{++} before chasing for 30 min in PBS containing varying concentrations of each agent (the treatment sample). Uridine, PLL (average molecular weight 7,500) and ATP were prepared and administered to cultures in PBS. Schizandrin, schizandrin-A, and gomisin-A were dissolved in dimethylsulfoxide and administered in PBS (final concentrations of dimethylsulfoxide were 0.5%). The final pH values of these solutions were between 7.0 and 7.4. PBS solution between this range and 0.5% dimethylsulfoxide did not affect mucin secretion from HTSE cells. Floating cells and cell debris were removed by centrifugation of samples at 12,000×g for 5 min. The samples were stored at -80°C until assayed for their ^{3}H-mucin contents.

Quantitation of ^{3}H-mucins

High molecular weight glycoconjugates excluded after Sepharose CL-4B gel-filtration column chromatography and resistant to hyaluronidase were defined as mucins and measured by the column chromatography as previously reported (Kim *et al.*, 1985). Media samples were adjusted to pH 5.0 with 0.1 M citric acid and treated with 100 U/mL of testicular hyaluronidase (Type VI-S) at 37°C for 16 h. At the end of the incubation, the digestion mixture were neutralized to pH 7.4 using 0.2 M NaOH, boiled for 2 min and centrifuged. The supernatants were applied to Sepharose CL-4B columns (1×50 cm) equilibrated with PBS containing 0.1% (w/v) Sodium Dodecyl Sulfate (SDS). Columns were eluted with the same buffer at a constant flow rate of 0.336 mL/min and fractions of 0.42 mL were collected. Void volume fractions (4 peak fractions) were mixed with 4 mL of scintillation cocktail and the radioactivity of fractions were counted using a liquid scintillation counter (LSC). The sum of radioactivity in four peak fractions will be defined as the amount of mucin in the sample. The effect of agents on mucin secretion will be measured as follows : the amount of mucin secreted during the treatment period were divided by the amount of mucin secreted during the pretreatment period and the ratio were expressed as a secretory index. Means of secretory indices of each group were compared and the differences were assessed using statistics.

Statistics

Means of individual group were converted to percent control

and expressed as mean ± S.E.M. The difference between groups was assessed using Student's t-Test for unpaired samples. $p<0.05$ was considered as significantly different.

RESULTS AND DISCUSSION

Schizandrin, schizandrin-A and gomisin-A were reported to occur in *Schizandrae Fructus* and have various biological effects including free radical scavenging effect (Jang, 2003). On the basis of above report, we tried to test the possible effects of schizandrin, schizandrin-A and gomisin-A on airway mucin secretion. As shown in Fig. 1, schizandrin did not affect mucin secretion, significantly. The amounts of mucin in the spent media of schizandrin-treated cultures were 100 ± 2%, 107 ± 3 %, 110 ± 14%, 132 ± 15% and 133 ± 20% for control, 10^{-7}M, 10^{-6}M, 10^{-5}M and 10^{-4}M, respectively. Also, Gomisin-A did not affect mucin secretion, significantly. The amounts of mucin in the spent media of gomisin-A-treated cultures were 100 ± 7%, 101 ± 3%, 93± 2% and 105 ± 6% for control, 10^{-5}M, 10^{-4} M and 10^{-3}M, respectively (Fig. 3). However, schizandrin-A significantly increased mucin secretion from airway. The amounts of mucin in the spent media of schizandrin-A-treated cultures were 100 ± 2%, 89 ± 5%, 98± 2% and 167 ± 5% for control, 10^{-5}M, 10^{-4}M and 10^{-3}M, respectively (Fig. 2). This result suggests schizandrin-A shows a direct effect on mucin-secreting cells. Actually, we expected that components derived from *Schizandrae Fructus* would show the possible inhibitory activity on mucin secretion based on the use of this medicinal plant in traditional oriental medicine. On the contrary to this expectation, schizandrin-A stimulated mucin secretion. The underlying mechanisms of action of these agents on mucin secretion are not clear at present and should be investigated through ongoing research. In traditional Chinese (oriental) medicine, *Angelica Radix* was administered to nourish and make wet the respiratory system (including cardiovascular, liver and gastrointestinal system), in order to enhance the removal of respiratory mucus from dry respiratory tract observed in 'yin(or blood)-deficient' patient (National Association of Professor of Herbology in Oriental Medical School, 2005b). We supposed, among many components derived from *Angelica Radix*, a nutrient-like compound might show the above activities described in traditional Chinese medicine. On the other hand, Lin and colleagues isolated uracil, from aqueous extract of *Angelica Radix* (Lin *et al.*, 1979). Therefore, we

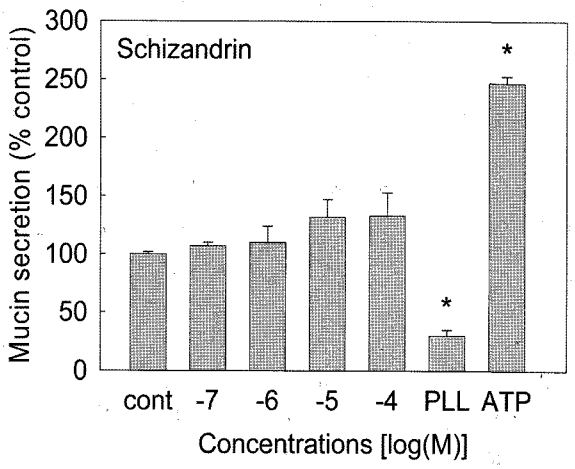

Fig. 1. Effect of schizandrin on mucin secretion. Confluent HTSE cells were metabolically radiolabeled with ^{3}H-glucosamine for 24 h and chased for 30 min in the presence of varying concentrations of schizandrin. For comparison, both 200 μM of ATP, a well-known mucin secretagogue and 10 μM of PLL (MW 7,500) which is reported to be an inhibitor of mucin secretion were used as positive controls. The amounts of ^{3}H-mucins in the spent media were measured as described in Materials and Methods. Each bar represents a mean ± S.E.M. of four culture wells in comparison with that of control set at 100 %. *significantly different from control ($p<0.05$) (ATP : adenosine triphosphate, PLL : poly-L-lysine)

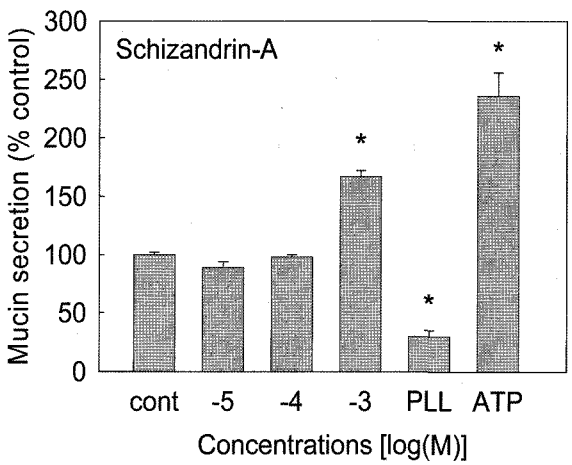

Fig. 2. Effect of schizandrin-A on mucin secretion. Confluent HTSE cells were metabolically radiolabeled with ^{3}H-glucosamine for 24 h and chased for 30 min in the presence of varying concentrations of schizandrin-A. For comparison, both 200 μM of ATP, a well-known mucin secretagogue and 10 μM of PLL (MW 7,500) which is reported to be an inhibitor of mucin secretion were used as positive controls. The amounts of ^{3}H-mucins in the spent media were measured as described in Materials and Methods. Each bar represents a mean ± S.E.M. of four culture wells in comparison with that of control set at 100 %. *significantly different from control ($p<0.05$) (ATP : adenosine triphosphate, PLL : poly-L-lysine)

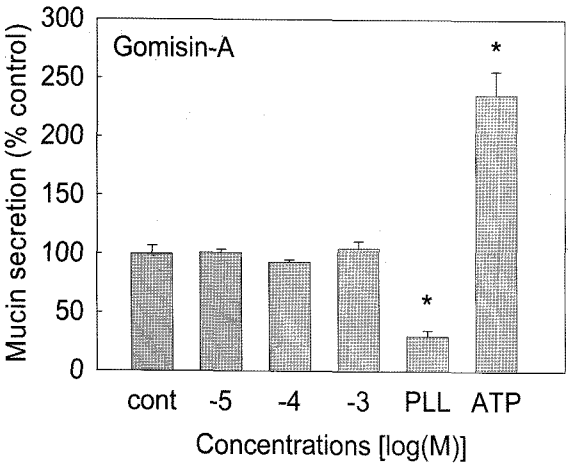

Fig. 3. Effect of gomisin-A on mucin secretion. Confluent HTSE cells were metabolically radiolabeled with ^{3}H-glucosamine for 24 h and chased for 30 min in the presence of varying concentrations of gomisin-A. For comparison, both 200 μM of ATP, a well-known mucin secretagogue and 10 μM of PLL (MW 7,500) which is reported to be an inhibitor of mucin secretion were used as positive controls. The amounts of ^{3}H-mucins in the spent media were measured as described in Materials and Methods. Each bar represents a mean ± S.E.M. of four culture wells in comparison with that of control set at 100%. *significantly different from control ($p<0.05$) (ATP : adenosine triphosphate, PLL : poly-L-lysine)

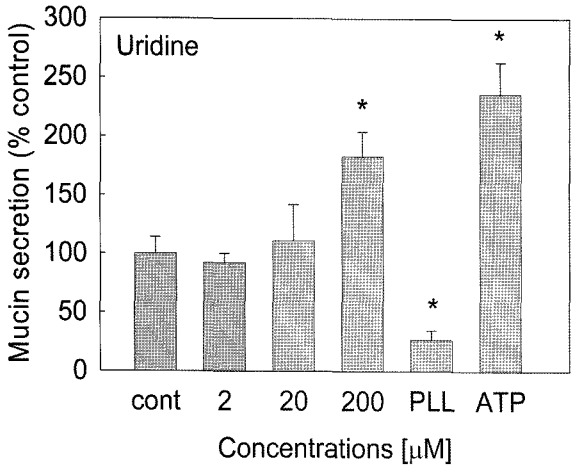

Fig. 4. Effect of uridine on mucin secretion. Confluent HTSE cells were metabolically radiolabeled with ^{3}H-glucosamine for 24 h and chased for 30 min in the presence of varying concentrations of uridine. For comparison, both 200 μM of ATP, a well-known mucin secretagogue and 10 μM of PLL (MW 7,500) which is reported to be an inhibitor of mucin secretion were used as positive controls. The amounts of ^{3}H-mucins in the spent media were measured as described in Materials and Methods. Each bar represents a mean ± S.E.M. of four culture wells in comparison with that of control set at 100%. * significantly different from control ($p<0.05$) (ATP : adenosine triphosphate, PLL : poly-L-lysine)

suggest uracil is a major component that shows effect of enhancing the removal of respiratory mucus by *Angelica Radix*, and selected uridine, a nucleoside containing uracil, as an agent that simulate the possible action of uracil in *Angelica Radix*. On the basis of this idea, we tried to test the possible effect of uridine on airway mucin secretion. As shown in Fig. 4, uridine significantly increased mucin secretion at 2×10^{-4}M. The amounts of mucin in the spent media of uridine-treated cultures were 100 ± 14%, 92 ± 8%, 111 ± 31% and 183 ± 21% for control, 2×10^{-6}M, 2×10^{-5}M and 2×10^{-4}M, respectively. This result suggests uridine shows a direct effect on mucin-secreting cells. This stimulatory action of uridine on mucin secretion might explain, at least in part, the use of *Angelica Radix* as a therapeutic for airway diseases in traditional oriental medicine. Also, the underlying mechanism of action on mucin release should be investigated through ongoing research. Taken together, schizandrin-A and uridine increased mucin secretion from airway epithelial cells. We suggest that it is valuable to find the natural products with specific inhibitory effects on mucin secretion. These results suggest a potential use of schizandrin-A and uridine as mild mucoregulators or expectorants for respiratory diseases, which warrants further studies.

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