Dietary polyphenols affect *MUC5AC* expression and ciliary movement in respiratory cells and nasal mucosa

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ABSTRACT

Background: *Dietary polyphenols have been widely consumed in food, and their anti-inflammatory and anticancer activities have been recently studied. Although the effects of dietary polyphenols on mucin hypersecretion have been studied to some extent, the effects of mucociliary movement have not been elucidated thus far. Therefore, we investigated whether dietary polyphenols inhibit* MUC5AC *gene expression in NCI-H292 cells, and, if so, whether they would have an effect on ciliary beat frequency (CBF) of human nasal mucosa.*

Methods: *NCI-H292 cells were pretreated with four different dietary polyphenols ([6]-gingerol, epigallocatechin gallate (EGCG), curcumin, and quercetin) and were treated with IL-1beta (10 ng/mL). Proliferation of NCI-H292 cells was analyzed. The mRNA expression of* MUC5AC *was determined by real-time polymerase chain reaction. CBF of normal nasal mucosa, which was obtained from the ethmoid sinus and treated with the polyphenols, was assessed* via *inverted microscope and computerized program.*

Results: *Minimal inhibitory concentration (MIC) of* MUC5AC *expression of each polyphenol was found as follows: [6]-gingerol, 1 μM; EGCG, 20 μM; quercetin, 40 μM; and curcumin, 10 μM. No polyphenol influenced cell proliferation at this MIC. CBF was not affected by [6]-gingerol, quercetin, or EGCG, but ciliary movement decreased in curcumin.*

Conclusion: *[6]-Gingerol, quercetin, and EGCG may be considered as antihypersecretory agents because they effectively inhibit mucus secretion of respiratory epithelial cells while maintaining normal nasal ciliary movement.*

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Key words: Anti-hypersecretion, ciliary beat frequency, human nasal mucosa, *MUC5AC*, mucin, polyphenol, respiratory cells

Dietary polyphenols are contained in our daily intake and there are many varieties of polyphenols, including[6]-gingerol; the chief active component of ginger, EGCG; green tea extract, quercetin; red wine extract, curcumin; curry extract, genistein, and indole-3-carbinol; and bean extracts. Because there has been increased interest in food, several recent studies have focused on the influence of polyphenols such as anti-inflammatory and anticancer effects.[1–5] The effects of dietary polyphenols on nasal mucin secretion have been studied to some extent,[6–8] but the effects on mucociliary movement have not been elucidated thus far.

Chronic rhinosinusitis (CRS) is presented when mucin genes of *MUC5AC* and *MUC5B* are overexpressed.[9] *MUC5AC* is well known as the most vital secretory mucin gene among the identified mucin genes and is clearly expressed in nasal epithelial cells, nasal polyps, and nasal turbinates.[10–13] Thus, the research on *MUC5AC* is essential in antihypersecretory treatment. Although this treatment has been studied extensively, only dexamethasone and macrolide have been proved to reduce the overexpression of mucin genes.[14–16] Another hallmark of CRS is a reduced ciliary beat frequency (CBF).[17] Even though the mucin-reducing treatment is discovered, it can cause the reduction or acceleration of CBF, which in turn maintains CRS status.[18,19]

Therefore, we first evaluated whether polyphenols inhibit the expression of *MUC5AC* by treating NCI-H292 cells (human lung mucoepidermoid cell line) with various dietary polyphenols ([6]-gingerol, epigallocatechin gallate (EGCG), curcumin, and quercetin). Second, we assessed the effects of polyphenols on mucociliary movement by applying polyphenols with minimal inhibitory concentration (MIC) of *MUC5AC* expression to human nasal mucosa. Through this study, we discovered that dietary polyphenols inhibit *MUC5AC* expression in respiratory cells and do not influence ciliary movement of human nasal mucosa.

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MATERIALS AND METHODS

Reagents

EGCG, curcumin, and quercetin were purchased from Sigma Co. (St. Louis, MO), [6]-gingerol was purchased from Calbiochem Biochemicals (San Diego, CA), and IL-1β was purchased from R&D Systems (Minneapolis, MN). IL-1β concentration for *MUC5AC* induction was adjusted to 10 ng/mL, which was established from previous studies.[6–8] [6]-Gingerol was adjusted to 0.1, 1, and 10 μM with DMSO as solvent, and curcumin and quercetin were adjusted to 10, 20, and 40 μM with DMSO as solvent. EGCG concentration was adjusted to 10, 20, and 40 μM with H_2O as solvent.

Cell Culture

NCI-H292 cell line was purchased from the American Type Culture Collection (Rockville, MD) and cultured in RPMI-1640 (Gibco BRL, Grand Island, NY) with 10% fetal bovine serum (Cellgro, Hemdon, VA), 2 mM of L-glutamine, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37°C in a humidified chamber with 95% air and 5% CO_2. When cultures were confluent, cells were incubated in RPMI-1640 medium containing 0.5% fetal bovine serum for 24 hours.

Real-Time Polymerase Chain Reaction (PCR) of *MUC5AC* mRNA

For real-time PCR of *MUC5AC* mRNA, the previously published methods were used using TaqMan probes on *MUC5AC* and β_2-

microglobulin.[7] Real-time PCR was performed on an Applied Biosystems ABI PRISM 7700 Sequence Detection System (PE Biosystem, Foster City, CA). All reactions were performed in triplicate. Relative quantities of *MUC5AC* mRNA were obtained using the comparative cycle threshold method and were normalized using β_2-microglobulin as an endogenous control.

Cell Proliferation Analysis

For cell proliferation analysis, the previously published methods were used.[7] Optical density (O.D.) was measured by a spectrophotometer (490 nm). The mean percentage of living cells was calculated as follows: (1-[mean O.D. of experimental group/mean O.D. of control]) $\times$ 100.

CBF Analysis

Normal nasal mucosa was collected from ethmoid sinuses of patients who underwent transsphenoidal pituitary tumor surgery, intranasal biopsy, or endoscopic maxillary sinus surgery without any inflammation in the ethmoid sinus. The tissue was collected and cultured in culture media used in normal human nasal epithelial cells.[6] The tissue was harvested in a shape of a square the sides of which were 1–1.5 cm. The harvested tissue was divided into two parts and cultured at 37°C in a 5% CO_2 incubation chamber for 6 hours; one was used for the control group and the other was used for the experiment group. Then, the tissue was treated with the polyphenols with MIC of *MUC5AC* expression and was observed at specific time points (0, 3, 6, 12, and 24 hours) under an inverted microscope (Axiovert 40CLF; Carl Zeiss, Göttingen, Germany) at 1000× magnification. Five spots presenting the most active CBF were selected and were recorded by a microscope equipped with high-speed digital video camera (Moticam 2000; Mikron Instruments, Inc., Oakland, NJ), and saved on the computer. The files were reloaded, and CBF was determined with the optical flow technique with peak detection analysis.[20]

Statistical Analysis

Data were expressed as mean $\pm$ SD. A minimum of at least three separate experiments were performed for each measurement. Differences between treatment groups were assessed by analysis of variance with *post hoc* tests, and differences were considered significant at $p < 0.05$.

RESULTS

Decrease in IL-1β–Induced *MUC5AC* Expression by Polyphenols

Cultured NCI-H292 cells were pretreated with various concentrations of the polyphenols for 1 hour, and then they were treated with 10 ng/mL of IL-1β for 24 hours. Dose-dependent decreases of IL-1β–induced *MUC5AC* expression were observed in quercetin, EGCG, and[6]-gingerol. Curcumin, on the other hand, showed decreased *MUC5AC* expression at 10 μM, but the expression increased at 40 μM ($p < 0.05$). MIC of *MUC5AC* expression in each polyphenol was found as follows: [6]-gingerol, 1 μM; EGCG, 20 μM; quercetin, 40 μM; and curcumin, 10 μM (Table 1).

Suppression in Proliferation of NCI-H292 Cells by Polyphenols

At respective MICs, [6]-gingerol, quercetin, EGCG, and curcumin did not significantly lower cell proliferation, showing that cell proliferation was uninfluenced at the studied MIC (Table 2).

Change in CBF by Treatment with Polyphenols

After treating normal nasal mucosa with polyphenols at the respective MIC, changes in CBF were evaluated. Quercetin, [6]-gingerol, and EGCG did not show any difference between the control and experiment groups at all time points, from 30 minutes to 24 hours. Curcumin, however, showed a significant decrease in CBF compared with the control group from the 1- to 24-hour period ($p < 0.05$; Table 3).

DISCUSSION

Our results showed that *MUC5AC* expression in NCI-H292 cells was significantly suppressed when pretreated with 1 and 10 μM of [6]-gingerol, but cell proliferation was not influenced at either concentration. Pretreatment with 40 μM of quercetin and 20 μM of EGCG decreased mucin gene expression, and cell proliferation was not influenced at any of the concentrations. These results agree with the suppression of *MUC2* and *MUC5AC* expression by dexamethasone in NCI-H292 cells[14] and that of IL-1β–induced *MUC2/5AC* expression and mucin production by budesonide.[21,22] Our results also agree with other studies on the suppression of the mucin gene by [6]-gingerol and EGCG.[6–8] In a previous study, EGCG on normal human nasal epithelial cells suppressed *MUC5AC* expression at 50 μM in normal human nasal epithelial cells.[6] However, our study used a cell line instead and showed the suppression at 10 μM. This can be explained by homeostasis activity of normal cells, which tend to require higher reagent concentrations than in cell lines.

Even though *MUC5AC* expression was suppressed with the treatment of [6]-gingerol, quercetin, and EGCG at respective MICs, the concentrations were different. Different MIC seems to be attributable to chemical structure and properties of the polyphenols.[1,2,4] However, the differences in MIC will need to be studied further. Extracellular signal–regulated kinase and p38 mitogen-activated protein kinase are involved in the mechanism of the suppression of *MUC5AC* expression by polyphenols, according to our previous studies.[6–8] This needs to be further investigated as well.

Curcumin significantly suppressed expression of *MUC5AC* mRNA at 10 μM, but the expression increased at 40 μM. Cell proliferation was not influenced at 10 μM but was suppressed at 40 μM. Thus, we used 10 μM of curcumin for CBF assessment. Curcumin, a key ingredient for curry's hot flavor, seemed to suppress mucin secretion at low concentrations, but it stimulated mucin secretion at higher concentrations. Therefore, curcumin at high concentrations may act as a stimulus, increasing mucin secretion and inhibiting cell proliferation in NCI-H292 cells. Other studies have also reported mucin secretion

Table 1 **Change of IL-1β−induced *MUC5AC* gene expression by polyphenols**

	0	0.1 μM	1 μM	10 μM	20 μM	40 μM
[6]-Gingerol	4.8 ± 0.7	2.7 ± 0.3	1.1 ± 0.4	0.8 ± 0.2		
Quercetin	5.1 ± 0.6			3.3 ± 0.5	2.4 ± 0.2	1.1 ± 0.3
EGCG	5.0 ± 0.7			2.4 ± 0.3	1.2 ± 0.2	0.9 ± 0.2
Curcumin	5.3 ± 0.5			1.2 ± 0.1	2.3 ± 0.4	9.5 ± 1.2

Ratio: MUC5AC expression of polyphenol-treated cells in treatment with IL-1β/MUC5AC expression of the control cells.
Value: mean ± SD.

Table 2 **Change of cell proliferation in treatment with polyphenols**

	0.1 μM	1 μM	10 μM	20 μM	40 μM
[6]-Gingerol	97.5 ± 1.5	94.1 ± 2.5	86.8 ± 2.9		
Quercetin			92.8 ± 4.7	86.3 ± 7.5	81.0 ± 4.1
EGCG			96.1 ± 2.2	89.8 ± 3.8	82.3 ± 4.8
Curcumin			93.2 ± 1.9	90.2 ± 2.5	68.3 ± 3.6

Cell proliferation: proliferation of experimental group/proliferation of the control × 100 (%).
Value: mean ± SD.

Table 3 **Change of ciliary beat frequency (CBF) in treatment with polyphenols**

Hour Materials	0	0.5	1	3	6	24
[6]-Gingerol (1 μM)	100.0	103.7 ± 1.6	98.1 ± 1.6	97.2 ± 4.9	92.6 ± 1.4	88.9 ± 4.4
Quercetin (40 μM)	100.0	93.6 ± 1.1	91.8 ± 2.2	87.1 ± 2.2	89.2 ± 10.3	86.5 ± 10.2
EGCG (20 μM)	100.0	96.5 ± 1.4	96.5 ± 3.0	95.7 ± 5.3	94.7 ± 2.5	91.3 ± 6.4
Curcumin (10 μM)	100.0	87.4 ± 3.2	76.3 ± 3.7	81.2 ± 0.5	79.8 ± 5.9	77.6 ± 3.9

CBF: CBF of experimental group/CBF of control (0 hr) × 100 (%).
Value: mean ± SD.

promoted by curcumin at 100 μM,[23,24] supporting our findings. This may be a characteristic nature of curcumin because the same phenomenon can be observed in hesperidine, a major polyphenol in citrus fruits.[24]

After treating human nasal mucosa with polyphenols at respective MICs except for curcumin, changes in CBF were evaluated. No significant difference in ciliary movement was observed between the control and polyphenols during the entire observation period, from 30 minutes to 24 hours. Therefore, our study may be used as the basis for further *in vivo* study on secure application of these polyphenols. When pretreated with curcumin, CBF started to drop at 30 minutes and was down by 75% at 1 hour. Then, a significant decrease was maintained until 24 hours. Because curcumin might cause nasal dysfunction by hindering ciliary movement, its clinical application seems inappropriate.[25] The mechanism of 10 μM of curcumin reducing CBF is not clear and needs to be clarified by future studies.

Our results indicated that [6]-gingerol, quercetin, and EGCG can be clinically applied to decrease mucin secretion without influencing normal ciliary movement. However, curcumin can not be used for the same purpose because it hinders ciliary movement. The main drawback of this study is that the decrease of *MUC5AC* expression was verified in NCI-H292 cells, not in normal human nasal epithelial cells. Even though NCI-H292 cells are a good candidate to study mucin expression, the study using normal human nasal epithelial cells should be conducted to confirm these results.

CONCLUSION

[6]-Gingerol, quercetin, and EGCG may be considered as antihypersecretory agents because they effectively inhibit mucus secretion of respiratory epithelial cells, while maintaining normal nasal ciliary movement.

REFERENCES

1. Cragg GM, Newman DJ, and Weiss RB. Coral reefs, forests, and thermal vents: The worldwide exploration of nature for novel antitumor agents. Semin Oncol 24:156–163, 1997.
2. Kuhnau J. The flavonoids. A class of semi-essential food components: Their role in human nutrition. World Rev Nutr Diet 24:117–191, 1976.
3. Dewick PM. The Biosynthesis of shikimate metabolites. Nat Prod Rep 12:579–607, 1995.
4. Matuschek E, and Svanberg U. Oxidation of polyphenols and the effect on in vitro iron accessibility in a model food system. J Food Sci 67:420–424, 2002.
5. Enomoto T, Nagasako-Akazome Y, Kanda T, et al. Clinical effects of apple polyphenols on persistent allergic rhinitis: A randomized double-blind placebo-controlled parallel arm study. J Investig Allergol Clin Immunol 16:283–289, 2006.
6. Kim HJ, Park SH, Park SY, et al. Epigallocatechin-3-gallate inhibits interleukin-1β-induced MUC5AC gene expression and MUC5AC secretion in normal human nasal epithelial cells. J Nutr Biochem 19: 536–544, 2008.
7. Kim JH, Chang JH, Yoon JH, et al. [6]-Gingerol suppresses interleukin-1β-induced MUC5AC gene expression in human airway epithelial cells. Am J Rhinol Allergy 23:385–391, 2009.
8. Kwon SH, Nam JI, Kim SH, et al. Kaempferol and quercetin, essential ingredients in ginkgo biloba extract, inhibit interleukin-1β-induced MUC5AC gene expression in human airway epithelial cells. Phytother Res 23:1708-1712, 2009. (Epub ahead of print April 15, 2009.)
9. Ding GQ, and Zheng CQ. The expression of MUC5AC and MUC5B mucin genes in the mucosa of chronic rhinosinusitis and nasal polyposis. Am J Rhinol 21:359–366, 2007.
10. Yoon JH, and Park IY. Mucin gene expression and mucin secretion in human airway epithelium. Rhinology 36:146–152, 1998.
11. Aust MR, Madsen CS, Jennings A, et al. Mucin mRNA expression in normal and vasomotor inferior turbinates. Am J Rhinol 11:293–302, 1997.
12. Voynow JA, Selby DM, and Rose MC. Mucin gene expression (MUC1, MUC2, and MUC5/5AC) in nasal epithelial cells of cystic fibrosis, allergic rhinitis, and normal individuals. Lung 176:345–354, 1998.
13. Voynow JA, Young LR, Wang Y, et al. Neutrophil elastase increases MUC5AC mRNA and protein expression in respiratory epithelial cells. Am J Physiol 276:L835–L843, 1999.
14. Kai H, Yoshitake K, Hisatsune A, et al. Dexamethasone suppresses mucus production and MUC-2 and MUC-5AC gene expression by NCI-H292 cells. Am J Physiol 271:484–488, 1996.
15. Cervin A, and Wallwork B. Macrolide therapy of rhinosinusitis. Rhinology 45:259–267, 2007.
16. Hatipoglu U, and Rubinstein I. Anti-inflammatory treatment of chronic rhinosinusitis: A shifting paradigm. Curr Allergy Asthma Rep 8:154–161, 2008.
17. Abdel-Hak B, Gunkel A, Kanonier G, et al. Ciliary beat frequency, olfaction and endoscopic sinus surgery. ORL 60:202–205, 1998.
18. Wyatt TA, Forget MA, and Sisson JH. Ethanol stimulates ciliary beating by dual cyclic nucleotide kinase activation in bovine bronchial epithelial cells. Am J Pathol 163:1157–1166, 2003.

19. Braverman I, Wright ED, Wang CG, et al. Human nasal ciliary beat frequency in normal and chronic sinusitis subjects. J Otolaryngol 27:145–152, 1998.
20. Kim WJ, Han TH, Kim HJ, and Park RW. Optical flow techniques with peak detection method for ciliary beat frequency measurement. AMIA Annu Symp Proc 11:1069, 2007.
21. Kim YD, Cho JS, Chang KY, et al. Budesonide down-regulates IL-1β-mediated MUC2/MUC5AC genes expression and mucin secretion in human airway epithelial cells. Korean J Otolaryngol 45:873–877, 2002.
22. Kim YD, Kwon EJ, Kwon TK, et al. Regulation of IL-1β-mediated MUC2 gene in NCI-H292 human airway epithelial cells. Biochem Biophys Res Commun 274:112–116, 2000.
23. Lee CJ, Lee JH, Seok JH, et al. Effects of betaine, coumarin, and flavonoids on mucin release from cultured hamster tracheal surface epithelial cells. Phytother Res 18:301–305, 2004.
24. Lee CJ, Lee JH, Seok JH, et al. Effects of baicalein, berberine, curcumin and hesperidin on mucin release from airway goblet cells. Planta Med 69:523–526, 2003.
25. Joki S, Toskala E, and SannoV. Correlation between ciliary beat frequency and the structure of ciliated epithelia in pathologic human nasal mucosa. Laryngoscope 108:426–430, 1998. □