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Epigallocatechin-3-gallate inhibits interleukin-1β-induced *MUC5AC* gene expression and MUC5AC secretion in normal human nasal epithelial cells

Hyun Jik Kim[a], Sang Ho Park[b], Sung-Yoon Park[a], Uk Yeol Moon[c], Byung Don Lee[b], Sung Hyun Yoon[d], Jeung-Gweon Lee[c], Seung Joon Baek[e], Joo-Heon Yoon[c,d,f,*]

[a]*Department of Otolaryngology—Head and Neck Surgery, Chung-Ang University College of Medicine, Seoul, Korea*
[b]*Department of Otorhinolaryngology—Head and Neck Surgery, Soonchunhyang University, Seoul, Korea*
[c]*Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
[d]*The Airway Mucus Institute, Yonsei University College of Medicine, Seoul, Korea*
[e]*The Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA*
[f]*The Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea*

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Abstract

It has been reported that the proinflammatory cytokine interleukin-1β (IL-1β) induces mucus hypersecretion in normal human nasal epithelial (NHNE) cells and that the MAP kinase pathway may be an important signal pathway in IL-1β-induced *MUC5AC* gene expression. Green tea (*Camellia sinensis*) polyphenols are potent anti-inflammatory agents and have been shown to inhibit inflammation in tumor cell lines and cultured respiratory epithelial cells. In this study, we examined the effect of (−)-epigallocatechin-3-gallate (EGCG), a green tea polyphenol, on IL-1β-induced *MUC5AC* gene expression and secretion in NHNE cells. After cells had been treated with IL-1β (10 ng/ml) and pretreated with EGCG (10, 50 and 100 μM), mRNA expression of MUC5AC was determined by real-time polymerase chain reaction. The suppression of each signal pathway protein was determined by Western blot analysis after treatment with IL-1β and EGCG, respectively. IL-1β increased *MUC5AC* gene expression and MUC5AC secretion. EGCG markedly suppressed IL-1β-induced *MUC5AC* gene expression and MUC5AC secretion via suppression of the phosphorylation of ERK MAP kinase, MSK1, and transcription factor, cAMP response element-binding protein. IL-1β increased the number of cells staining positive with MUC5AC antibodies, and EGCG treatment decreased this number. Our data suggest that EGCG may be an effective inhibitor of IL-1β-induced mucus hypersecretion.

Keywords: EGCG; IL-1β; MUC5AC

1. Introduction

Mucins, which are produced by airway epithelial cells, are essential components of airway mucus. Mucin hypersecretion is commonly observed in many respiratory diseases such as rhinitis, sinusitis, otitis media, nasal allergy and chronic bronchitis [1,2]. To date, 20 different mucin genes have been identified [3–5]. Of these, *MUC5AC* and *MUC5B* are generally recognized to be the major airway mucins, and *MUC5AC* is highly expressed in goblet cells of the human airway epithelium [6,7]. Moreover, during the course of airway inflammatory disease, a variety of cytokines, growth factors and free radicals are released, and the number of goblet cells, the expression of MUC5AC mRNA and the production of mucin increase [4,5,8]. Investigation of the signal transduction pathway for inflammatory-cytokine-induced *MUC5AC* gene expression would provide an important clue to the understanding of airway mucus hypersecretion and would offer new therapeutic strategies for the inhibition of airway mucus hypersecretion [9,10].

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* Corresponding author. Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul 120-752, Korea. Tel.: +82 2 2228 3610; fax: +82 2 393 0580.
E-mail address: jhyoon@yumc.yonsei.ac.kr (J.-H. Yoon).

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The proinflammatory interleukin-1β (IL-1β), a multifunctional cytokine produced by a variety of cells, is thought to be the principal inducer of inflammation [11,12]. The signaling cascade initiated by IL-1β has been characterized to some extent and has been shown to result in the activation of proinflammatory transcription factors, including nuclear factor κB (NF-κB), active protein-1 and cAMP response element-binding protein (CREB). We have previously shown that two different MAP kinases, ERK and p38 MAP kinases, are essential for IL-1β-induced *MUC5AC* gene expression in normal human nasal epithelial (NHNE) cells and that MSK1 mediates IL-1β-induced phosphorylation of CREB and transcription of MUC5AC [3].

Polyphenols, derived from green tea made from dried leaves of *Camellia sinensis*, and catechin, the major component of polyphenol, have demonstrated anti-inflammatory, antioxidative, antimutagenic, anticarcinogenic and apoptotic effects [13–15]. The major catechins are (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin, (−)-epigallocatechin and (−)-epicatechin gallate with (−)-epigallocatechin gallate. Of these, EGCG is the most abundant bioactive polyphenolic constituent [12].

In the present study, we investigated whether EGCG can suppress IL-1β-induced *MUC5AC* gene expression and the level of the signal pathway at which EGCG inhibits *MUC5AC* gene expression in NHNE cells. We found that EGCG suppressed IL-1β-induced *MUC5AC* gene expression, MUC5AC secretion and secretory granules in a dose-dependent manner, and that EGCG remarkably inhibited IL-1β-induced ERK MAP kinase (but not p38 MAP kinase) phosphorylation. In addition, EGCG also inhibited the phosphorylation of MSK1, CREB and the transcription of the MUC5AC promoter. This study provides new insight that EGCG may be an effective suppressor for IL-1β-induced *MUC5AC* overexpression.

2. Materials and methods

2.1. Materials

EGCG (10 mg) and α-tubulin antibody were purchased from Calbiochem. Anti-phospho-p44/42 MAP kinase (Thr^{202}/Tyr^{204}) antibody, anti-phospho-p38 MAP kinase (Thr^{180}/Tyr^{182}) antibody, anti-phospho-MSK1 (Thr^{581}) antibody and anti-phopho-CREB (Ser^{133}) antibody were purchased from Cell Signaling (Beverly, MA). Anti-MUC5AC antibody was purchased from Santa Cruz Biotechnology, Inc.

2.2. Cell cultures

The cell culture system used for NHNE cells has been described previously [16]. The human lung mucoepidermoid carcinoma cell line (NCI-H292) was purchased from the American Type Culture Collection (CRL-1848; Manassas, VA) and cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum in the presence of penicillin/streptomycin at 39°C in a humidified chamber with 5% CO_2. For serum deprivation, confluent cells were washed twice with phosphate-buffered saline and recultured in RPMI 1640 with 0.2% fetal bovine serum.

2.3. Experimental conditions

EGCG was diluted in DMSO to stock concentrations of 10, 50 and 100 mM, then further diluted to experimental concentrations of 10, 50 and 100 μM in Dulbecco's modified essential medium or RPMI. For polymerase chain reaction (PCR), immunoblot analysis, luciferase assay and immunocytochemistry, cells were treated with EGCG for 1 h before incubation with IL-1β (10 ng/ml). After this, the medium was replaced with a medium containing IL-1β (10 ng/ml) and EGCG (10, 50 or 100 μM) and incubated for 24 h. For Western blot analysis, cells were also treated with EGCG for 1 h, followed by the medium with IL-1β (10 ng/ml) and EGCG (100 μM) for 15, 30, 45 or 60 min.

2.4. Reverse transcriptase PCR

Total RNA was isolated, using TRIzol (Invitrogen), from NHNE cells treated with IL-1β (10 ng/ml). cDNA was synthesized with random hexamers (PerkinElmer Life Sciences and Roche Applied Science) using Moloney murine leukemia virus reverse transcriptase (PerkinElmer Life Sciences). Oligonucleotide primer sequences of MUC5AC and β_2-microglobulin for PCR were designed as follows: MUC5AC (forward: 5′-CGACAACTACTTCTGCGGTGC-3′; reverse: 5′-GCACTCATCCTTCCTGTCGTT-3′) and β_2-microglobulin (forward: 5′-CTCGCCCTACTCTCTCTTTCTGG-3′; reverse: 5′-GCTTACATGTCTCGATCCCACTTAA-3′). PCR products were run on a 2% agarose gel and visualized with ethidium bromide under a transilluminator. In order to verify whether amplified products were from mRNA and not from genomic DNA contamination, negative controls were obtained by omitting reverse transcriptase. No PCR products were observed in negative controls. Specific amplification of all target genes was confirmed by the sequencing (dsDNA Cycle Sequencing System; GibcoBRL, Rockville, MD) of PCR fragments.

2.5. Real-time PCR

Primers and probes were designed with PerkinElmer Life Sciences Prime Express software and purchased from PE Biosystems. Commercial reagents (*TaqMan* PCR Universal PCR Master Mix; PerkinElmer Life Sciences) and conditions were applied in accordance with the manufacturer's protocol. One microgram of cDNA (reverse transcription mixture) and oligonucleotides with final concentrations of 800 nM for primers and 200 nM for *TaqMan* hybridization probes were analyzed in a 25-μl volume. The real-time PCR probe was labeled with carboxyfluoroscein (FAM) at the 5′ end and with the quencher carboxytetramethylrhodamine (TARMA) at the 3′ end. The MUC5AC, β_2-microglobulin primers and *TaqMan* probe were designed as follows: MUC5AC (forward: 5′-CAGCCACGTCCCCTTCAATA-3′; reverse:

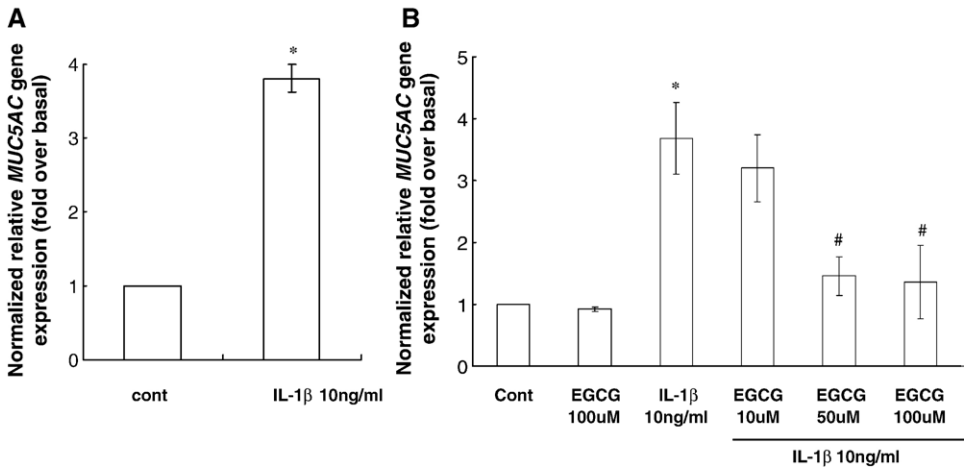


Fig. 1. Effect of IL-1β and EGCG on *MUC5AC* gene expression. (A) Confluent NHNE cells were treated with IL-1β (10 ng/ml) for 24 h, and cell lysate was harvested for real-time PCR. *MUC5AC* gene expression increased after 24 h of treatment with IL-1β. (B) Confluent NHNE cells were treated with IL-1β (10 ng/ml) and EGCG at increasing concentrations (10, 50 and 100 μM) for 24 h. Cont, control. IL-1β-induced MUC5AC mRNA expression was analyzed by real-time PCR. EGCG suppressed IL-1β-induced *MUC5AC* gene expression in a dose-dependent manner. The data are derived from three separate experiments. Values are presented as mean±S.D. **P*< 05 when compared with control; $^{\#}P$<.05 when compared with the treatment group with IL-1β. Results are representative of three independent experiments.

5′-ACCGCATTTGGGCATCC-3′; *TaqMan* probe: 6FAM-CCACCTCCGAGCCCGTCACTGAG-TAMRA) and β_2-microglobulin (forward: 5′-CGCTCCGTGGCCTTAGC-3′; reverse: 5′-GAGTACGCTGGATAGCCTCCA-3′; *TaqMan* probe: 6FAM-TGCTCGCGCTACTCTCTCTTTCTGGC-TAMRA). Real-time PCR was performed on a PerkinElmer Life Sciences ABI PRISM 7700 Sequence Detection System. The Thermocycler (ABI PRISM 7700 Sequence Detection System) parameters were 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All reactions were performed in triplicate. The relative quantity of MUC5AC mRNA was obtained using a comparative cycle threshold method and was normalized using β_2-microglobulin as an endogenous control.

2.6. Immunodetection and quantitation of secretions

The methods used to detect secretions from cultured cells have previously been described in detail [17]. Secreted MUC5AC mucins were detected using immunoblot analysis. MUC5AC was detected using a monoclonal anti-MUC5AC antibody (Santa Cruz Biotechnology, Inc.). Dilutions of apical secretions were applied to a nitrocellulose membrane, which was then incubated with the appropriate primary antibody, followed by a reaction with horseradish-peroxidase-conjugated goat anti-mouse IgG. The signal was detected by means of chemiluminescence (ECL kit; Amersham, Little Chalfont, UK), and a standard curve was generated by linear regression analysis to determine the concentration of individual samples.

2.7. Western blot analysis

NHNE cells were grown to confluence in six-well plates, and the cells were lysed with 2× lysis buffer [250 mM Tris–Cl (pH 6.5), 2% sodium dodecyl sulfate, 4% β-mercaptoethanol, 0.02% bromophenol blue and 10% glycerol]. Equal amounts of whole-cell lysates were resolved by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane in Tris-buffered saline [50 mM Tris–Cl (pH 7.5), 150 mM NaCl] for 2 h at room temperature. This blot was then incubated overnight with primary antibody in TTBS (0.5%

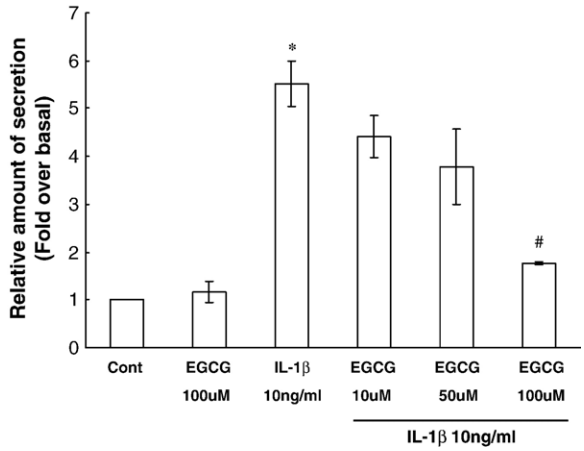


Fig. 2. EGCG suppressed IL-1β-induced MUC5AC secretion. After a 1-h pretreatment with EGCG, NHNE cells were stimulated with IL-1β (10 ng/ml) and then cotreated with EGCG (100 μM). This representative immunoblot analysis demonstrates that EGCG suppressed the IL-1β-induced secretion of MUC5AC. EGCG suppressed IL-1β-induced MUC5AC secretion significantly at 100 μM. The data are derived from three separate experiments. Values are presented as mean±S.D. **P*<.05 when compared with control; $^{\#}P$<.05 when compared with the treatment group with IL-1β. Results are representative of three independent experiments.

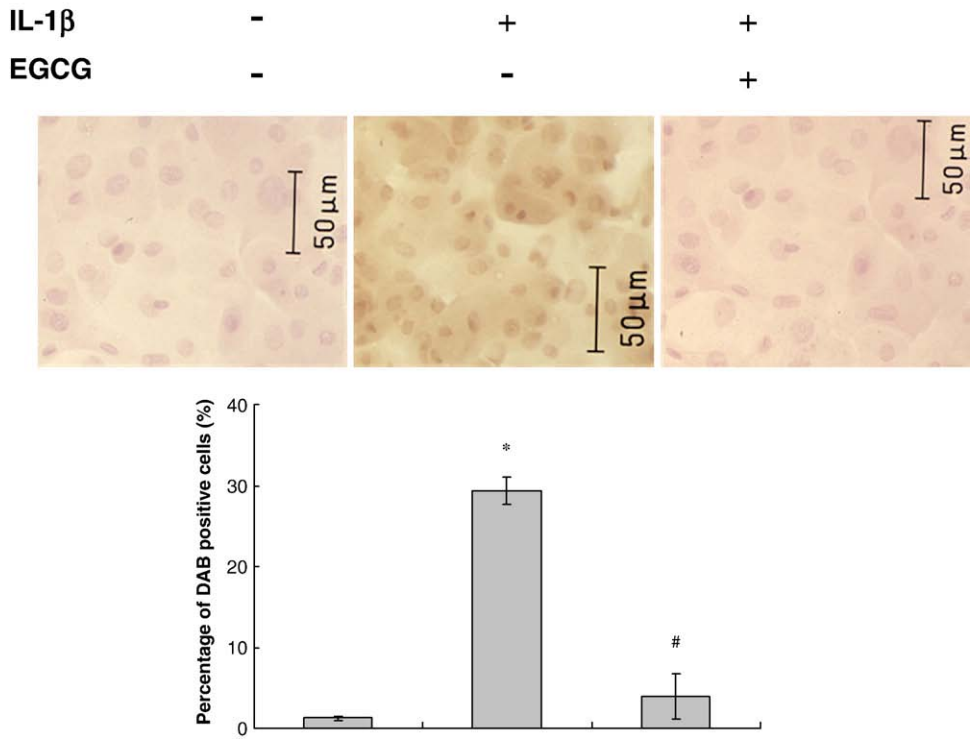


Fig. 3. EGCG suppressed IL-1β-induced MUC5AC secretion. After a 1-h pretreatment with EGCG, NHNE cells were stimulated with IL-1β (10 ng/ml) and then cotreated with EGCG (100 μM). Immunocytochemistry shows that IL-1β increased the number of MUC5AC-positive cells and that treatment with EGCG decreased the number of positive cells. Histogram data are derived from three separate experiments and display the percentage of MUC5AC-positive cells. Values are presented as mean±S.D. *P<.05 when compared with control; #P<.05 when compared with the treatment group with IL-1β. The results are derived from three independent experiments.

Tween-20 in Tris-buffered saline). After the blot had been washed with TTBS, it was further incubated for 1 h at room temperature with anti-rabbit or anti-mouse antibody (Cell Signaling) in TTBS and then visualized by chemiluminescence (ECL kit).

2.8. Luciferase assay

Cells were transiently transfected with plasmid containing either the promoterless pGL3 basic vector or the MUC5AC 5′ flanking region (−976/+1), including the CRE site, and fused to a luciferase reporter gene using FuGENE6 transfection reagent (Roche Applied Science) in accordance with the manufacturer's instruction. NCI-H292 cells were incubated for 24 h, harvested and assayed for luciferase activity using a luciferase assay system (Promega) in accordance with the manufacturer's instructions. β-Galactosidase activity was also assayed to standardize the transfection efficiency of each sample.

2.9. Immunocytochemistry assay

Cytospin slides to be used for immunostaining were made on the second day after confluency in NHNE cells. Positive cells were detected using monoclonal anti-MUC5AC antibodies (Santa Cruz Biotechnology, Inc.). DAB and hematoxylin–eosin staining was performed after the application of secondary antibodies. The mean number of positive cells for MUC5AC antibodies was determined by scoring 1000 cells on each slide.

2.10. Statistical analysis

Data are expressed as mean±S.D. 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 test, and differences were considered statistically significant at P<.05.

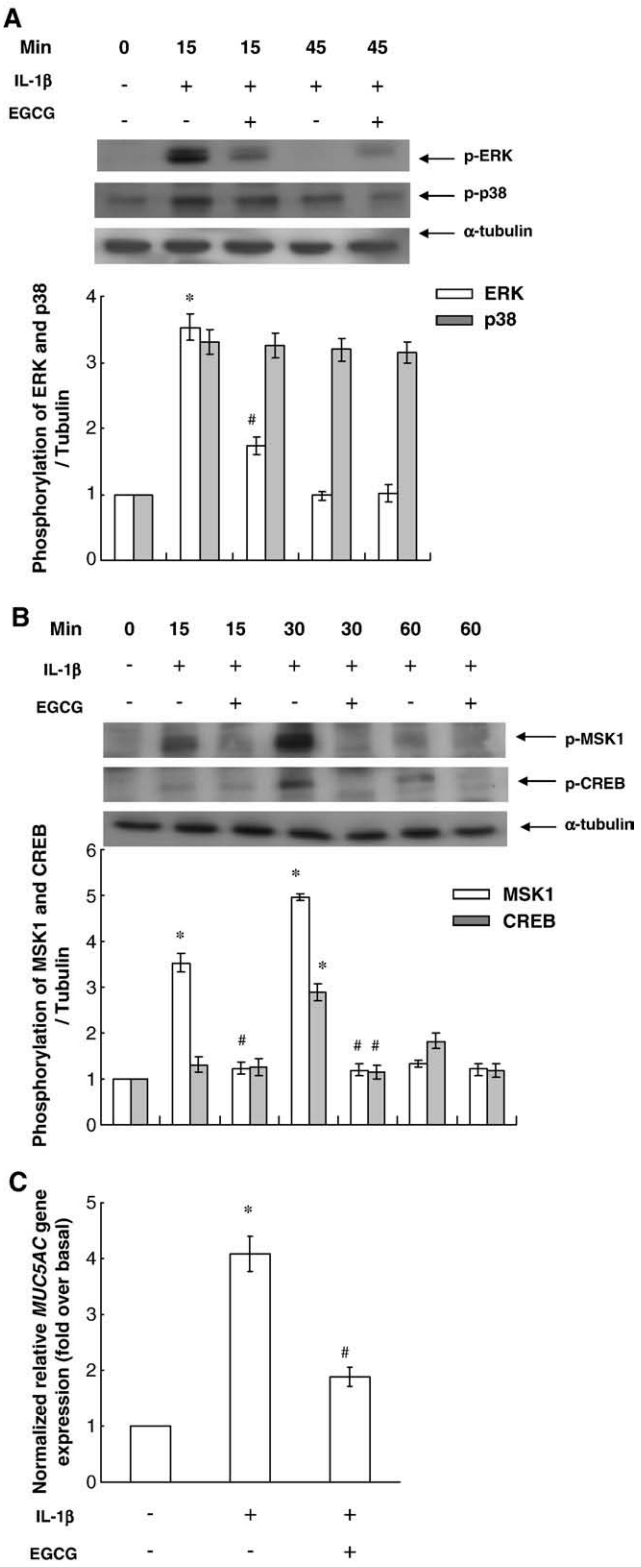
3. Results

3.1. EGCG suppressed IL-1β-induced MUC5AC gene expression in a dose-dependent manner

To confirm whether IL-1β can induce *MUC5AC* gene expression in NHNE cells, we carried out real-time PCR after treatment with IL-1β (10 ng/ml) for 24 h. MUC5AC mRNA expression was significantly greater after treatment with IL-1β (Fig. 1A). As a next step, we examined whether EGCG itself could influence MUC5AC mRNA expression in NHNE cells. We stimulated the NHNE cells with EGCG in a dose-dependent manner (10–100 μM) and found that

MUC5AC gene expression was not altered at any concentration (data not shown).

We next evaluated whether EGCG suppressed IL-1β-induced *MUC5AC* gene expression using real-time PCR. NHNE cells (1×10^6/ml) were stimulated with IL-1β (10 ng/ml) for 24 h or treated with EGCG 1 h before incubation with IL-1β and EGCG (10, 50 and 100 μM) for 24 h. Treatment with IL-1β induced *MUC5AC* gene expression (3.45±0.43 times greater than control, $P<.05$). Treatment with IL-1β and EGCG, including pretreatment with EGCG for 1 h, suppressed *MUC5AC* gene overexpression in a dose-dependent manner, with significant inhibition at 50 and 100 μM EGCG (3.45±0.43 vs. 1.49±0.25 and 1.07±0.43 times greater than control, respectively, $P<.05$; Fig. 1B). Our results demonstrate that EGCG impeded IL-1β-induced *MUC5AC* gene overexpression.



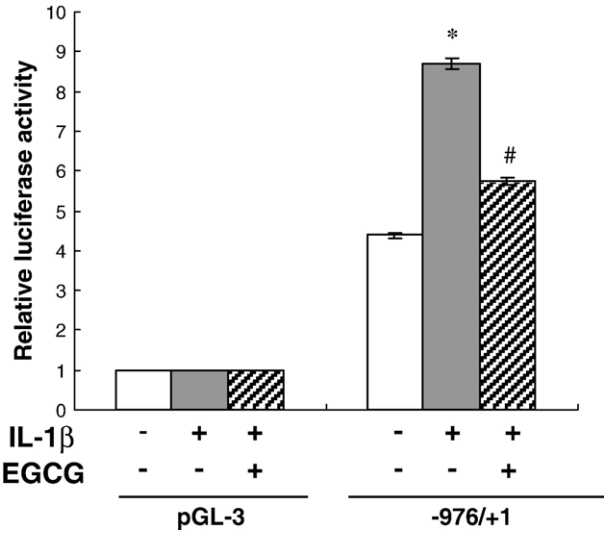
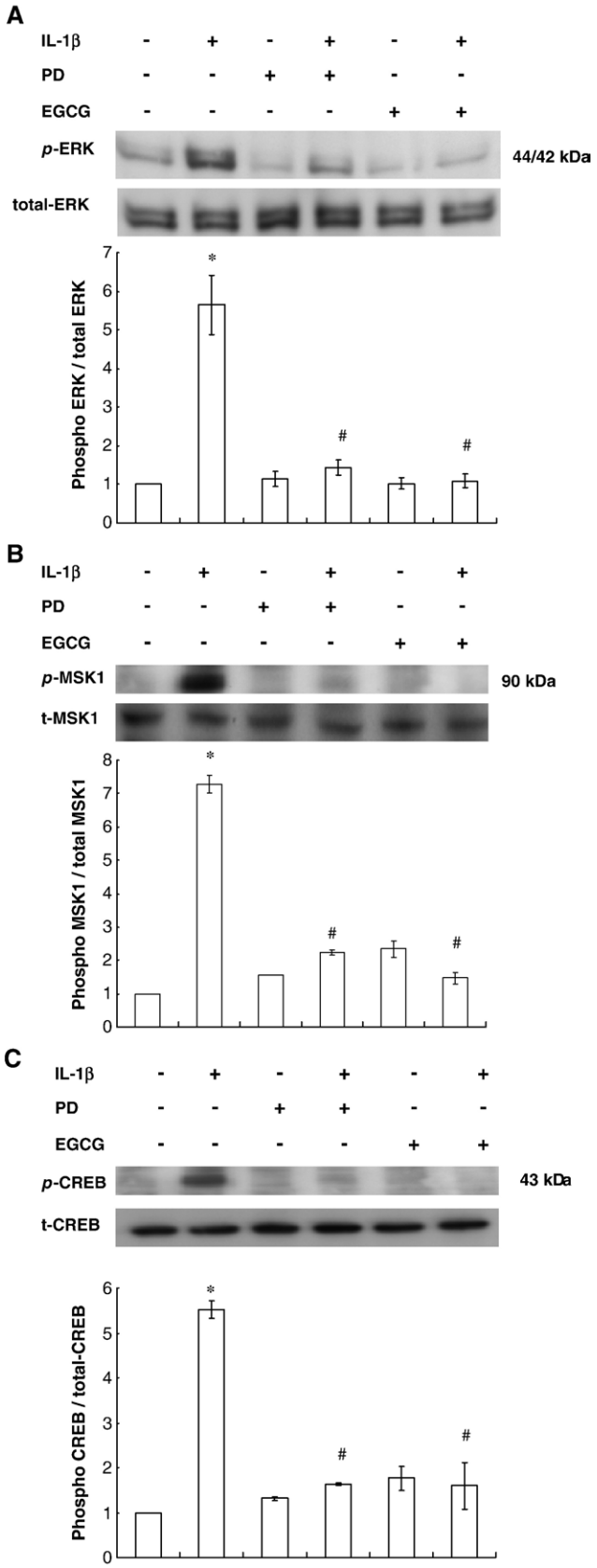


Fig. 5. EGCG inhibited the IL-1β-induced activation of the MUC5AC promoter. NCI-H292 cells were transiently transfected with a MUC5AC promoter (−976/+1) luciferase reporter plasmid containing the CRE region (−878 region). After 24 h of treatment with IL-1β (10 ng/ml), luciferase activity increased by 1.98±0.14-fold over the control and decreased after a 1-h pretreatment with EGCG, followed by cotreatment with 100 μM EGCG and IL-1β (10 ng/ml). The graph summarizes the relative luciferase activity derived from five separate experiments performed in duplicate. Values are presented as mean±S.D. *$P<.05$ when compared with control; #$P<.05$ when compared with the treatment group with IL-1β.

3.2. EGCG suppressed IL-1β-induced MUC5AC secretion

We also measured the secretion of MUC5AC protein by immunoblot analysis. MUC5AC mucin secretion increased

Fig. 4. EGCG inhibited the activation of ERK1/2 in the MAP kinase signal pathway and in downstream signal proteins. NHNE cells were stimulated with IL-1β (10 ng/ml), which increased the phosphorylation of ERK and p38 MAP kinases at 15 min posttreatment. After a 1-h pretreatment with EGCG, NHNE cells were cotreated with IL-1β (10 ng/ml) and EGCG (100 μM). EGCG inhibited the phosphorylation of ERK MAP kinase, but not of p38 MAP kinase. (B) Phosphorylation of MSK1 and CREB increased significantly 30 min after treatment with IL-1β (10 ng/ml), but this phosphorylation was reduced after cotreatment with EGCG (100 μM) and IL-1β (10 ng/ml). (C) Real-time PCR shows that *MUC5AC* expression decreased after the suppression of the phosphorylation of ERK MAP kinase. The graph summarizes the densitometry data derived from three separate experiments. Values are presented as mean±S.D. *$P<.05$ when compared with control; #$P<.05$ when compared with the treatment group with IL-1β. Results are representative of three independent experiments.



after 24 h of treatment with IL-1β (10 ng/ml) (5.51±0.47 times greater than control, $P<.05$; Fig. 3). EGCG treatment suppressed the increased MUC5AC secretion in a dose-dependent manner and significantly inhibited secretion at 100 μM EGCG (5.51±0.47 vs. 1.77±0.04 times greater than control, $P<05$; Fig. 2).

To further identify the intracellular effect of EGCG on secretory granules, we performed immunocytochemistry with monoclonal anti-MUC5AC antibody and DAB staining. The number of cells positive for MUC5AC increased after the addition of IL-1β (12.1±1.03 times greater than control; $P<.05$) and decreased after treatment with EGCG (12.1±1.03 vs. 5.8±1.6 times greater than control, $P<05$; Fig. 3). These results showed that EGCG can suppress IL-1β-induced MUC5AC hypersecretion.

3.3. EGCG inhibits the activation of ERK1/2 in the MAP kinase signal pathway and in downstream signal proteins

The MAP kinase signal pathway has been shown to activate in NHNE cells stimulated with IL-1β. In particular, ERK and p38 MAP kinases were maximally activated 15 min after treatment with IL-1β, and this effect decreased after 45 min [3]. As a next step, we investigated which MAP kinase signal pathway is inhibited by EGCG after stimulation with IL-1β. NHNE cells were pretreated with EGCG (100 μM) for 1 h, then stimulated with IL-1β (10 ng/ml) and EGCG (100 μM) for 15 and 45 min, respectively. Western blot analysis revealed that EGCG clearly inhibited ERK MAP kinase phosphorylation (3.52±0.20 vs. 1.72±0.13 times greater than control, $P<.05$), but not p38 MAP kinase phosphorylation (Fig. 4A). In addition, the inhibition of the ERK MAP kinase pathway inhibited MUC5AC mRNA expression in human airway epithelial cells (Fig. 4C). Thus, stimulation with IL-1β increased *MUC5AC* gene expression and MUC5AC secretion via ERK MAP kinase, and the inhibition of ERK MAP kinase with EGCG appeared to be closely related to the suppression of IL-1β-induced *MUC5AC* gene expression and MUC5AC secretion.

Fig. 6. EGCG inhibited the IL-1β-induced activation of the signal pathway to compare the inhibitory effect of MEK1 inhibitor. NHNE cells were pretreated for 1 h with 30 μM PD98059 and 100 μM EGCG. Pretreated cells were stimulated for 10 min with IL-1β (10 ng/ml) prior to the collection of cell lysate for Western blot analysis. (A) Pretreatment of PD98059 inhibited the phosphorylation of ERK MAP kinase (5.52±0.19- vs. 1.63±0.02-fold over the control). EGCG also inhibited the phosphorylation of ERK MAP kinase (5.52±0.19- vs. 1.60±0.51-fold over the control). (B) Pretreatment of PD98059 inhibited the phosphorylation of MSK1 (7.28±0.27- vs. 2.23±0.07-fold over the control). EGCG also inhibited the phosphorylation of MSK1 (7.28±0.27- vs. 1.47±0.16-fold over the control). (C) Pretreatment of PD98059 inhibited the phosphorylation of CREB (5.51±0.19- vs. 1.62±0.02-fold over the control). EGCG also inhibited the phosphorylation of CREB (5.51±0.19- vs. 1.59±0.51-fold over the control). The graph summarizes the densitometry data derived from three separate experiments. Values are presented as mean±S.D. *$P<.05$ when compared with control; #$P<.05$ when compared with the treatment group with IL-1β. Results are representative of three independent experiments.

IL-1β has been shown to induce the activation of MSK1 and CREB, mediated by ERK MAP kinase, with the phosphorylation of MSK1 and CREB by IL-1β, reaching a maximum at 30 min before declining at 60 min after IL-1β stimulation [3]. As seen in Fig. 4B, treating cells with EGCG (100 μM) inhibited MSK1 and CREB phosphorylation. These findings indicate that the inhibition of ERK MAP kinase by EGCG influenced downstream signal molecules of ERK MAP kinase and suggest that EGCG may suppress IL-1β-induced transcription factors in *MUC5AC* gene expression via suppression of ERK MAP kinase.

3.4. EGCG inhibits IL-1β-induced activation of the MUC5AC promoter

To confirm the activity of EGCG at the promoter level of *MUC5AC* gene expression, cells were transiently transfected with plasmid containing the CRE region (−878 region) of the MUC5AC promoter (−976/+1). Treatment with IL-1β (10 ng/ml) for 24 h increased luciferase activity by nearly 2-fold (1.98±0.14 times greater than control) in cells transfected with a MUC5AC promoter luciferase reporter plasmid containing the CRE site (−976/+1 region), compared to control cells that were transfected with the basic vector (pGL3) (Fig. 5). Pretreatment of the cells with EGCG (100 μM) for 1 h before incubation with IL-1β (10 ng/ml) and EGCG (100 μM) inhibited luciferase activity (1.98±0.14 vs. 1.31±0.08 times greater than control; Fig. 6). These data demonstrate that the inhibitory effects of EGCG on IL-1β-induced *MUC5AC* gene expression are associated with the inhibition of the MUC5AC promoter.

EGCG has a similar effect with MEK1 inhibitor (PD98059) on IL-1β-induced activation of ERK MAP kinase and downstream signal proteins.

To investigate the real effect of EGCG on IL-1β-induced phosphorylation of signal proteins, we next compared the effects of EGCG and MEK1 inhibitor (PD98059; 30 μM) on NHNE cells after treatment with IL-1β. NHNE cells were pretreated with PD98059 (30 μM) and EGCG (100 μM) for 1 h, then stimulated with IL-1β (10 ng/ml) for 15 min. Western blot analysis revealed that phosphorylation of ERK MAP kinase decreased after pretreatment with PD98059 (5.65±0.76 vs. 1.45±0.19 times greater than control, $P<.05$). In addition, EGCG clearly inhibited ERK MAP kinase phosphorylation (5.65±0.76 vs. 1.08±0.17 times greater than control, $P<.05$; Fig. 6A). NHNE cells were pretreated with PD98059 (30 μM) and EGCG (100 μM) for 1 h then stimulated with IL-1β (10 ng/ml) for 30 min, and Western blot analysis was performed. Phosphorylation of MSK1 decreased after pretreatment with PD98059 (7.28±0.27 vs. 2.23±0.07 times greater than control, $P<.05$), and EGCG also clearly inhibited MSK1 phosphorylation (7.28±0.27 vs. 1.47±0.16 times greater than control, $P<.05$) (Fig. 6B). Phosphorylation of CREB decreased after pretreatment with PD98059 (5.51±0.19 vs. 1.62±0.02 times greater than control, $P<.05$), and EGCG clearly inhibited CREB phosphorylation (5.51±0.19 vs. 1.59±0.51 times greater than control, $P<.05$) (Fig. 6C). These results showed that EGCG has a more potent inhibitory effect on the activation of signal proteins related to IL-1β-induced MUC5AC overexpression, in comparison to the effect of MEK1 inhibitor.

4. Discussion

The proinflammatory cytokine IL-1β is one of the principal mediators of inflammation and participates in airway diseases characterized by increased mucus production [3,6,18]. The mechanisms that lead to IL-1β-dependent signal transduction are also important in various inflammatory reactions and generally correlate with increased NF-κB activity [19–22]. However, it has been reported that IL-1β plays a role in airway diseases characterized by increased mucus production, and the MAP kinase signal pathway is thought to play a significant role in the signal transduction of mucin production [4,7,23]. Previously, we have shown that IL-1β increased *MUC5AC* gene expression in NHNE cells and that both ERK and p38 MAP kinases are essential for IL-1β-induced *MUC5AC* gene expression [3]. Moreover, the number MUC5AC-positive cells measured by immunocytochemistry also increased after stimulation with IL-1β.

A variety of studies have shown that polyphenols (mainly catechin) present in green tea possess diverse pharmacological properties, including anti-inflammatory effects, and the majority of the biological effects of green tea are mimicked by its principal constituent, catechin (EGCG) [15,24,25]. In our study, EGCG alone did not change *MUC5AC* gene expression (data were not shown), but EGCG did inhibit IL-1β-induced *MUC5AC* gene expression, MUC5AC secretion and the number of MUC5AC-positive cells. These results suggest that EGCG may have an inhibitory effect on inflammatory-cytokine-induced *MUC5AC* gene expression and that EGCG may actually suppress mucus secretion from MUC5AC in NHNE cells.

EGCG's inhibition of the signal pathway of IL-1β-induced *MUC5AC* gene expression and MUC5AC secretion may be important in several therapeutic approaches to respiratory inflammatory diseases. Several studies have shown that interleukin-receptor-associated kinase (IRAK) plays an important role in the IL-1β-mediated signal pathway [26–28] and that EGCG has a suppressive activity through the degradation of IRAK at the receptor level of the cell membrane [29,30]. However, little is known about the inhibitory mechanism of EGCG or the required inhibitory level for EGCG to impact on mucin gene overexpression through the IL-1β-induced MAP kinase signal pathway. We examined the effect of EGCG on the signal pathway of MAP kinases, which are known to be the major pathway in IL-1β-induced *MUC5AC* gene expression [3]. In our data, EGCG inhibited the phosphorylation of only the ERK MAP kinase signal pathway, suggesting that ERK MAP kinase

may be the main signal molecule involved in IL-1β-increased MUC5AC overexpression, and EGCG inhibited the upstream signal proteins of ERK MAP kinase from the cell surface receptor to MEK1 in the signal transduction of IL-1β-induced *MUC5AC* gene expression and MUC5AC secretion.

MSK1 and CREB are the primary downstream MAP kinase and transcription factors in IL-1β-induced *MUC5AC* gene expression [3,31,32]. We also found that IL-1β increased the phophorylation of MSK1 and CREB and that EGCG inhibited the IL-1β-induced phosphorylation of MSK1 and CREB. These results suggest that EGCG may suppress the downstream and transcription factors of ERK MAP kinase in IL-1β-induced *MUC5AC* gene expression and MUC5AC secretion. The fact that EGCG inhibited IL-1β-induced *MUC5AC* gene expression and decreased MUC5AC secretion suggests that IL-1β increases *MUC5AC* gene transcriptional regulation and that EGCG has an effect on this regulatory mechanism.

It has to be noted that some of the mechanistic studies of tea catechin, including our own, were performed in the concentration range of 10–1000 μM, which is unlikely to be achieved under physiologic conditions, except with tissues in the gastrointestinal tract that come into direct contact with tea solution [33]. The maximum achievable peak plasma level of catechin concentration in vivo is significantly less than the oral consumptive concentrations of green tea solution [33–35]. We propose that nasal topical application might achieve the effective experimental dosage of EGCG, allowing the use of EGCG as a therapeutic agent against nasal mucus hypersecretion.

In conclusion, IL-1β induces *MUC5AC* gene expression and MUC5AC secretion via ERK and p38 MAP kinases. EGCG, green tea polyphenol, is a potent inhibitor of IL-1β-induced *MUC5AC* gene expression and MUC5AC secretion. The mechanism of this effect involves, in part, inhibition of the phosphorylation of ERK MAP kinase and its downstream and transcription factors.

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