Epicatechin-gallate inhibits hydrogen peroxide-induced *MUC5AC* gene expression and MUC5AC secretion in human airway epithelial cells

JUN WAN YIM

Department of Medicine

The Graduate School, Yonsei University

Epicatechin-gallate inhibits hydrogen peroxide-induced *MUC5AC* gene expression and MUC5AC secretion in human airway epithelial cells

Directed by Professor Joo-Heon Yoon

The Master's Thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirement for the degree of Master of Medical Science

Jun Wan Yim

December 2006

This certifies that the Master's
Thesis of Jun Wan Yim is approved.

Thesis Supervisor : **Joo-Heon Yoon**

Thesis Committee Member : **Jeung-Gweon Lee**

Thesis Committee Member : **Joon Chang**

The Graduate School
Yonsei University

December 2006

Acknowledgements

I have started writing my master’s thesis 5 years after graduating medical college. I send my first thanks to my parents who have stood beside me all through my college years. I also thank professor J.H. Yoon, professor J.G. Lee, and professor J. Chang who has watched over me with a warm smile through the course of writing my master’s thesis. My great thanks also goes to H.J. Kim M.D who have given me a lot of help. I look forward to the day when I become able to write a better thesis and end my acknowledgement.

< Table of Contents >

< List of figures, list of tables >

LIST OF FIGURES

LIST OF TABLES

Abstract

Epicatechin-gallate inhibits hydrogen peroxide-induced *MUC5AC* gene expression and MUC5AC secretion in human airway epithelial cells

Jun Wan Yim

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Joo-Heon Yoon)

Oxidative stress induces overexpression of mucin genes and has been implicated in the pathogenesis of inflammatory airway diseases. Polyphenolic components of green tea, such as epicatechin gallate (ECG) have potent anti-oxidative properties. This study examines the effect of ECG on H_2O_2-induced mucin gene expression and mucin secretion in human airway epithelial cells. H_2O_2 induced overexpression of the mucin gene MUC5AC, and ECG markedly inhibited H_2O_2-induced *MUC5AC* gene expression and MUC5AC secretion. In addition, the MAP kinase signal pathway, in particular ERK MAP kinase, was associated with H_2O_2-induced *MUC5AC* gene expression and ERK MAP kinase activity was suppressed by ECG. This results show that the green tea polyphenol ECG is a potent inhibitor of H_2O_2-induced *MUC5AC* gene

expression and MUC5AC protein secretion in human airway epithelium through a mechanism involving inhibition of ERK MAP kinase-dependent signaling.

Key words : Mucin hypersecretion · Oxidative stress · Green tea · Polyphenol

<본문>

Epicatechin-gallate inhibits hydrogen peroxide-induced *MUC5AC* gene expression and MUC5AC secretion in human airway epithelial cells

Jun Wan Yim

Department of Medicine
The Graduate School, Yonsei University
(Directed by Professor Joo-Heon Yoon)

Ⅰ. Introduction

Mucin hypersecretion is commonly observed in many respiratory diseases, such as rhinitis, sinusitis, otitis media, nasal allergy, chronic bronchitis and cystic fibrosis [1-3]. To date, 20 different mucin genes have been identified and these are subdivided into two groups, membrane-bound mucins and secreted mucins. MUC5AC, MUC5B, MUC6, MUC7 and MUC19 are generally recognized to be the secreted mucins [4-8]. Of these, MUC5AC and 5B are known to be the major mucins, and are highly expressed in the goblet cells of the human airway epithelium and submucosal gland [9-11]. Mucin genes have been shown to be stimulated by a wide variety of stimuli, including proinflammatory cytokines such as IL-9, IL-1β and TNF-α, cellular proteins such as

neutrophil elastase and epidermal growth factor receptor (EGFR) ligands [9,10,12].

The oxidative injury triggered by both inhaled and locally generated reactive oxygen species (ROS) elicits an inflammatory response that can profoundly impair the structural integrity and biological properties of bronchial epithelium. A large number of studies have demonstrated that ROS such as hydrogen peroxide (H_2O_2), superoxide anion (O_2-), and hydroxyl radical, play a critical role in the initiation and progression of a great diversity of airway diseases [13]. Oxidative stress has also been shown to induce mucin synthesis in airway epithelial cells [14].

Mitogen-activated protein (MAP) kinase pathways play an important role in transmitting inflammatory signals from the cell surface to the nucleus and influence cell proliferation, differentiation, apoptosis, cytoskeletal remodeling, and cell cycle regulation [15]. It has been reported that exposure of cells to H_2O_2 activates several MAP kinase signal molecules including extracellular signal-regulated kinases (ERK), c-Jun amino-terminal kinases (JNK) and p38 Map kinases [14,16].

Polyphenols derived from green tea, the product of dried leaves of *Camellia sinensis*, especially catechins, are known to have anti-inflammatory, anti-oxidative, anti-mutagenic, anti-carcinogenic and apoptotic effects [17-20]. The major catechins are: (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epicatechin (EC), and (-)-epigallocatechin (EGC). EGCG is the most abundant bioactive polyphenolic constituent. Therefore, most of the previous studies on the beneficial effects of green tea have been performed with EGCG. The role of other catechins has not been well evaluated, especially with regard to the antioxidative effect.

In the present study we examined the effect of H_2O_2 on expression of the secreted mucin genes MUC5AC, MUC5B, MUC6, MUC7, MUC19 and the ability of ECG to suppress H_2O_2-induced secreted mucin gene expression in human airway epithelial cells. Our results show that H_2O_2 induced *MUC5AC* gene overexpression but not MUC5B, MUC6, MUC7 or MUC19, and induced MUC5AC protein secretion in a dose dependent manner. ECG suppressed H_2O_2-induced *MUC5AC* gene overexpression and MUC5AC secretion in a dose dependent manner by inhibiting ERK MAP kinase activity. This study provides new insight that ECG may be an effective therapeutic agent for secreted mucin hypersecretion due to oxidative stress.

Ⅱ. Materials and Methods

Materials

Epicatechin gallate (ECG) and Hydrogen peroxide (H_2O_2) were purchased from Sigma Aldrich. Anti-phospho-p44/42 MAP kinase(Thr202/Tyr204) antibody, anti-phospho-p38 MAP kinase (Thr180/Tyr182) antibody, and anti-phospho-SAPK/JNK MAP kinase (Thr183/Tyr185) were purchased from Cell Signaling (Beverly, MA, USA). α-tubulin antibody was purchased from Calbiochem and anti-MUC5AC antibody was purchased from Santa Cruz Biotechnology Inc.

Cell culture

The human lung mucoepidermoid carcinoma cell line (NCI-H292) was purchased from the American Type Culture Collection (CRL-1848, Manassas, VA, USA) and cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) 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.

Experimental conditions

ECG was diluted in DMSO to stock concentrations of 10, 50, 100 mM. The ECG stock was further diluted in RPMI to experimental concentrations of 10, 50, and 100 μM. For RT-PCR, NCI-H292 cells were treated with H_2O_2 (100, 250, 500, 1000 μM)

for 24 h prior to evaluation of *MUC5AC, MUC5B, MUC6, MUC7,* and *MUC19* gene expression. To test the effect of ECG on gene expression, cells were pretreated with ECG (10, 50, 100 μM) for 1 h and then they were incubated in fresh medium containing 250 μM H_2O_2 and ECG (10,50,100 μM) for 24 h.

For western blot analysis of the signal pathway proteins, ERK, p38 and JNK, cells were treated with 250 μM H_2O_2 for 10, 30, 60, and 120 min. To test the effect of ECG, cells were treated with 100μM ECG for 1 h prior to incubation in fresh medium containing 250 μM H_2O_2 and 100 μM ECG for 10 or 30 min.

RT-PCR

Total RNA was isolated from NCI-H292 cells treated with H_2O_2 (100, 250, 500 1000 μM) using TRIzol (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized with random hexamer primers (PerkinElmer Life Sciences and Roche Applied Science, Boston, MA, USA) using Molony murine leukemia virus-reverse transcriptase (PerkinElmer Life Sciences, Boston, MA, USA). Oligonecleotide primers for PCR were designed based on the GenbankTM sequence of MUC5AC, MUC5B, MUC6, MUC7, and MUC19 (Table 1).

Table 1. The experimental conditions of the polymerase chain reaction (PCR) and sequences of oligonucleotides used in PCR

Product	Cycle	Annealing Temp('C)	Primer	Sequence of oligonucleotide
MUC5AC	32	60	Forward	CGA CAA CTA CTT CTG CGG TGC
			Reverse	GCA CTC ATC CTT CCT GTC GTT
MUC5B	35	55	Forward	CTG CGA GAC CGA GGT CAA CAT C
			Reverse	TGG GCA GCA GGA GCA CGG AG
MUC6	35	55	Forward	TCA CCT ATC ACC ACA CAA C
			Reverse	GGA GAA GAA GGA AAA AGA G
MUC7	35	55	Forward	CCA CAC CTA ATT CTT CCC
			Reverse	CTA TTG CTC CAC CAT GTC
MUC19	30	55	Forward	TTT AGA GGC ACT GGG ACC AC
			Reverse	ACC ATT GCC CAA AGA AGT TG
*β2-M	23	55	Forward	CTCGCCCTACTCTCTCTTTCTGG
			Reverse	GCTTACATGTCTCGATCCCACTTAA

* β2-M : β2 microglobulin

PCR products were resolved in a 1% agarose gel (FMC, Rockland, ME, USA) and visualized with ethidium bromide under a transilluminator. To confirm that the amplified products were from mRNA and not genomic DNA contamination, negative controls were performed omitting the reverse transcriptase and no PCR products were observed. Specific amplification of target genes was confirmed by sequencing of PCR products (dsDNA Cycle Sequencing System; GibcoBRL, Rockville, MD, USA).

Immunodetection and quantitation of secretions

Methods for the detection of secretions from cultured cells have previously been

described [21]. Secreted MUC5AC mucins were detected by an immunoblot assay using a polyclonal anti-MUC5AC antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA).

Dilutions of apical secretions were applied to a nitrocellulose membrane, which was then incubated with the appropriate primary antibody followed by reaction with horseradish peroxidase-conjugated goat anti-mouse IgG. The signal was detected by chemiluminescence (ECL kit; Amersham, Little Chalfont, UK), and a standard curve was generated by linear regression analysis to determine the concentrations of the individual samples.

Western Blot analysis

NCI-H292 cells were grown to confluence in 6-well plates. After treatment with 250 μM H_2O_2 for 10, 30, 60, or 120 min, cells were lysed with 2X lysis buffer (250 mM Tris-Cl(pH6.5), 2% SDS, 4% β-mercaptoethanol, 0.02% bromphenol blue, 10% glycerol). Equal amounts of whole cell lysates were resolved by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane in Tris-buffered saline (50 mM Tris-Cl (pH7.5), 150 mM NaCl) for 1 h at room temperature. The blot was incubated overnight with primary antibody in TTBS (0.5% Tween 20 in Tris-buffered saline). After washing with TTBS, the blot was further incubated for 1 h at room temperature with anti-rabbit or anti-mouse antibody (Cell signaling) in TTBS and visualized using the ECL system (Amersham, Little Chalfont, UK).

Statistical analysis

Data are presented as the mean ± SD of triplicate cultures from the same experiment. Statistical comparisons were performed using Student's *t*-test. $p<0.05$ was considered statistically significant.

Ⅲ. Results

H_2O_2 induced gene expression of MUC5AC, but not MUC5B, MUC6, MUC7, or MUC19, in a dose-dependent manner

To determine whether H_2O_2 can induce the expression of secreted mucin genes, RT-PCR was performed after treatment of cells with H_2O_2 (100, 250, 500, 1000 μM) for 24 h. Levels of MUC5AC mRNA increased after treatment with H_2O_2 in a dose-dependent manner, while levels of MUC5B, MUC6, MUC7, and MUC19 mRNA did not increase (Fig. 1A). *MUC5AC* gene expression was increased significantly at 250 μM H_2O_2 (9.25$\pm$0.59 times greater than control; $p<0.01$), at 500 μM H_2O_2 (9.17$\pm$0.04 times greater than control; $p<0.01$), and at 1 mM H_2O_2 (9.81$\pm$0.49 times greater than control; $p<0.01$) (Fig. 1B). No corresponding change was found in the expression of the internal control, β2-microglobulin.

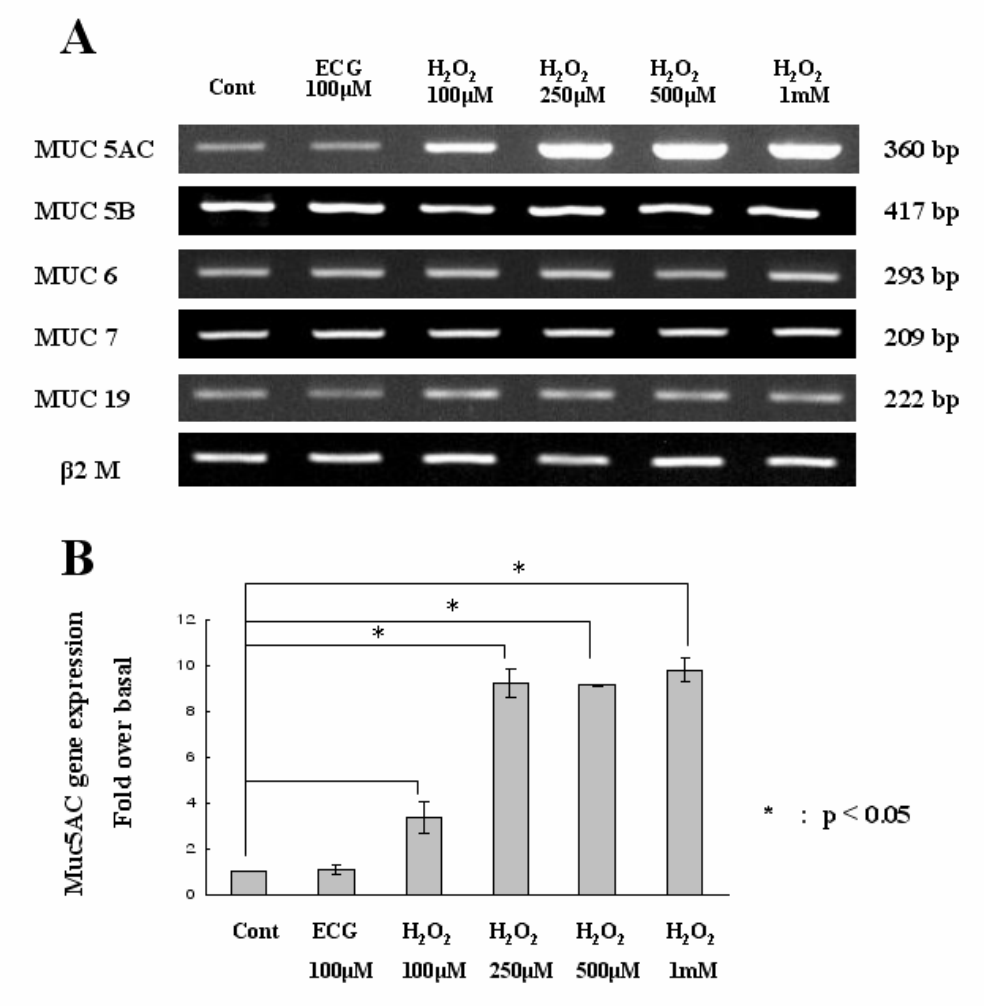

Figure 1. H_2O_2 induces *MUC5AC* gene expression in a dose-dependent manner. Confluent cells were treated with H_2O_2 (100 μM, 250 μM, 500 μM, 1 mM) for 24 h. C, control. β2-microglobulin (β2 M) was used as an internal control. H_2O_2 induced *MUC5AC* gene expression but did not affect expression of other secreted mucin genes (A). Densitometry demonstrating the dose-dependent effect of H_2O_2 on *MUC5AC* gene expression. All experiments were conducted at least four separate times (B). (* : $p < 0.05$)

ECG suppressed H_2O_2-induced MUC5AC gene expression

We next evaluated whether ECG could suppress the gene expression of MUC5AC. Cells (1 x 106/ml) were stimulated with H_2O_2 (250 μM) or H_2O_2 (250 μM) + ECG (10, 50, 100μM) for 24 h. RT-PCR analysis showed that treatment with H_2O_2 induced MUC5AC gene expression compared to control cells (6.41$\pm$0.27 times greater than control; $p<0.01$) (Fig 2A). Pre-treatment with ECG for 1h followed by 24-h treatment with H_2O_2 and ECG suppressed *MUC5AC* gene expression relative to H_2O_2 alone in a dose-dependent manner, with significant inhibition at 10 μM ECG (3.97$\pm$0.14 times greater than control; $p<0.01$), 50 μM ECG (3.47$\pm$0.31 times greater than control; $p<0.01$) and 100 μM ECG (1.39$\pm$0.31 times greater than control; $p<0.01$) (Fig. 2B).

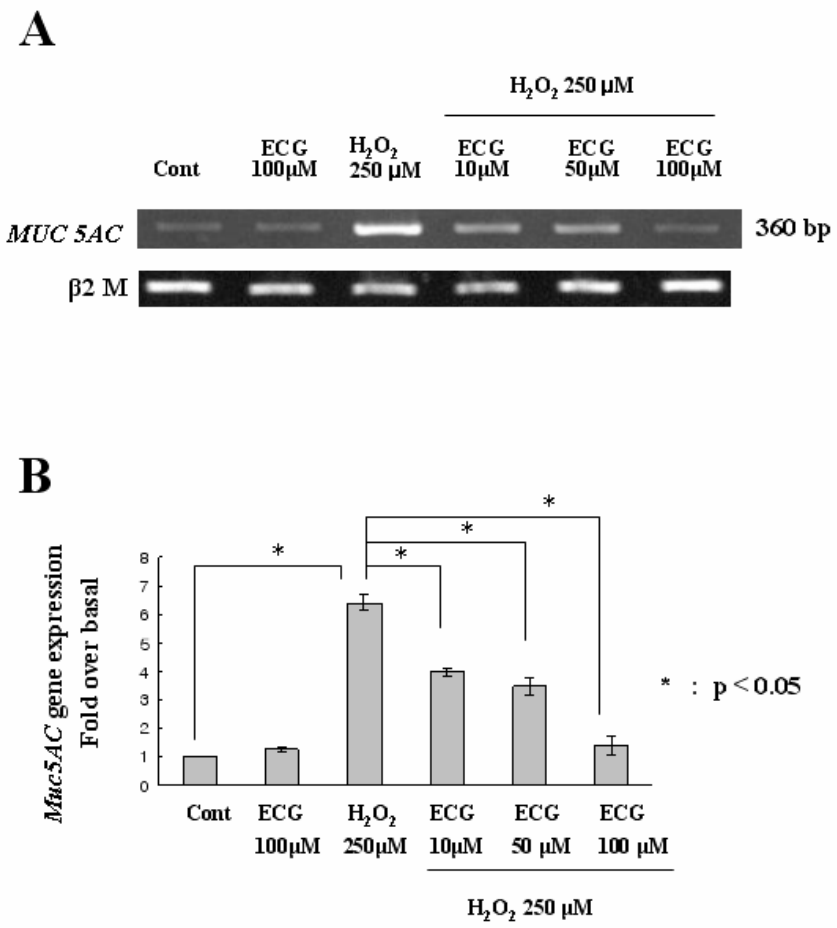

Figure 2. ECG suppresses H_2O_2-induced *MUC5AC* gene expression. Confluent cells were pretreated with ECG for 1 h and then treated with H_2O_2 (250μM) and ECG at increasing concentrations (10, 50, 100 μM) for 24 h. C, control. β2-microglobulin (β2 M) was used as an internal control. ECG suppressed H_2O_2-induced *MUC5AC* gene expression in a dose dependent manner (A). Densitometry demonstrating the effect of ECG on H_2O_2-mediated production of *MUC5AC* gene. All experiments were conducted at least four separate times (B). (* : $p < 0.05$)

These results show that ECG inhibits H_2O_2-induced *MUC5AC* gene overexpression.

ECG suppressed H_2O_2-induced MUC5AC secretion and intracellular MUC5AC protein

The secretion of MUC5AC protein in NCI-H292 cells was also measured. Treatment with 250 μM H_2O_2 increased MUC5AC mucin secretion (4.5±0.17 times greater than control; $p<0.05$, Fig. 3A). The increased MUC5AC secretion was suppressed by ECG treatment in a dose-dependent manner, with significant inhibition at 50 and 100 μM ECG (2.5±0.09 times greater than control, $p<0.05$; 1.5±0.2 times greater than control, $p<0.05$ respectively Fig. 3A). The intracellular MUC5AC protein was measured. . Treatment with 250 μM H_2O_2 increased intracellular MUC5AC protein (2.59±0.09 times greater than control; $p<0.05$, Fig. 3B). The increased MUC5AC secretion was suppressed by ECG treatment, with significant inhibition at 100 μM ECG (1.32±0.35 times greater than control, $p<0.05$, Fig. 3B).

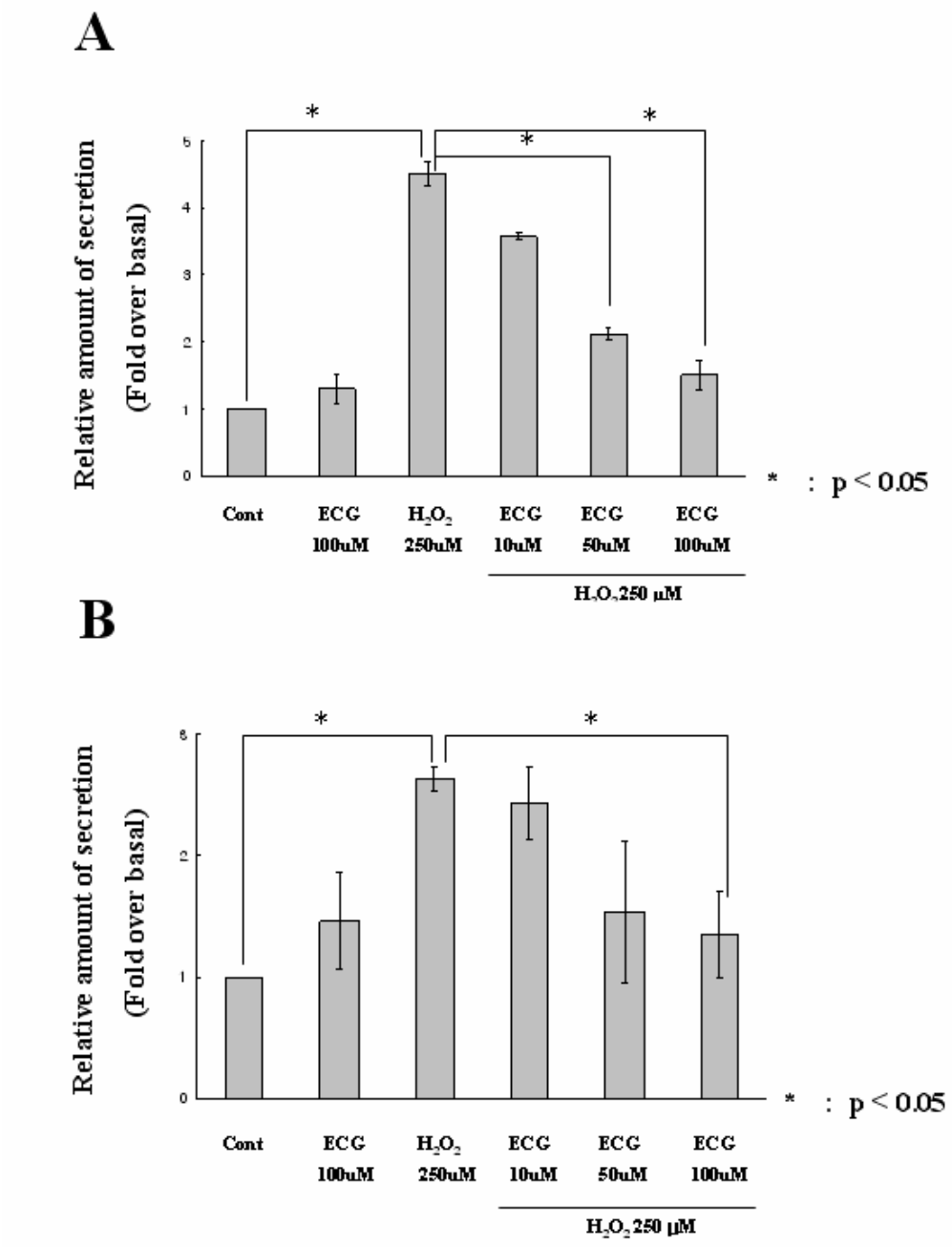

Figure 3. ECG suppresses H_2O_2-induced MUC5AC secretion and intracellular MUC5AC protein. Representative immunoblot assay demonstrating the effect of ECG

on H_2O_2-mediated MUC5AC secretion (A) and protein (B). ECG suppressed H_2O_2-induced MUC5AC secretion and intracellular MUC5AC protein (* : $p < 0.05$)

These results demonstrate that ECG suppresses H_2O_2- induced mucin hypersecretion.

H_2O_2 induced MUC5AC gene expression via ERK MAP kinases.

Treatment with 250 μM H_2O_2 activated ERK MAP kinase. This activation was maximal at 10 and 30 min after treatment and decreased after 60 min. Activity of p38 and JNK MAP kinases was not increased by treatment with H_2O_2 (Fig. 4A). It appears that stimulation by H_2O_2 induced both *MUC5AC* gene expression and ERK MAP kinase activity in human airway epithelial cells. We therefore investigated whether the induction of *MUC5AC* gene expression by H_2O_2 involves the ERK MAP kinase pathway. Cells were pretreated with 30 μM PD98059, a specific MEK1/2 inhibitor, for 1 hr before treatment with H_2O_2. RT-PCR and Western blot analysis clearly showed that pretreatment of NCI-H292 cells with PD98059 for 1 hr inhibited *MUC5AC* gene expression and phosphorylation of ERK MAP kinase (Fig. 4B). MEK1 DN mutant stable cells were preincubated for 48 hrs and stimulated for 24 hr or 10 min with H_2O_2 (250 μM) prior to real-time PCR or Western blot, respectively. The increased phosphorylation of ERK MAP kinase and *MUC5AC* expression were inhibited after transfecting MEK1 DN mutant cells (Fig. 4C).

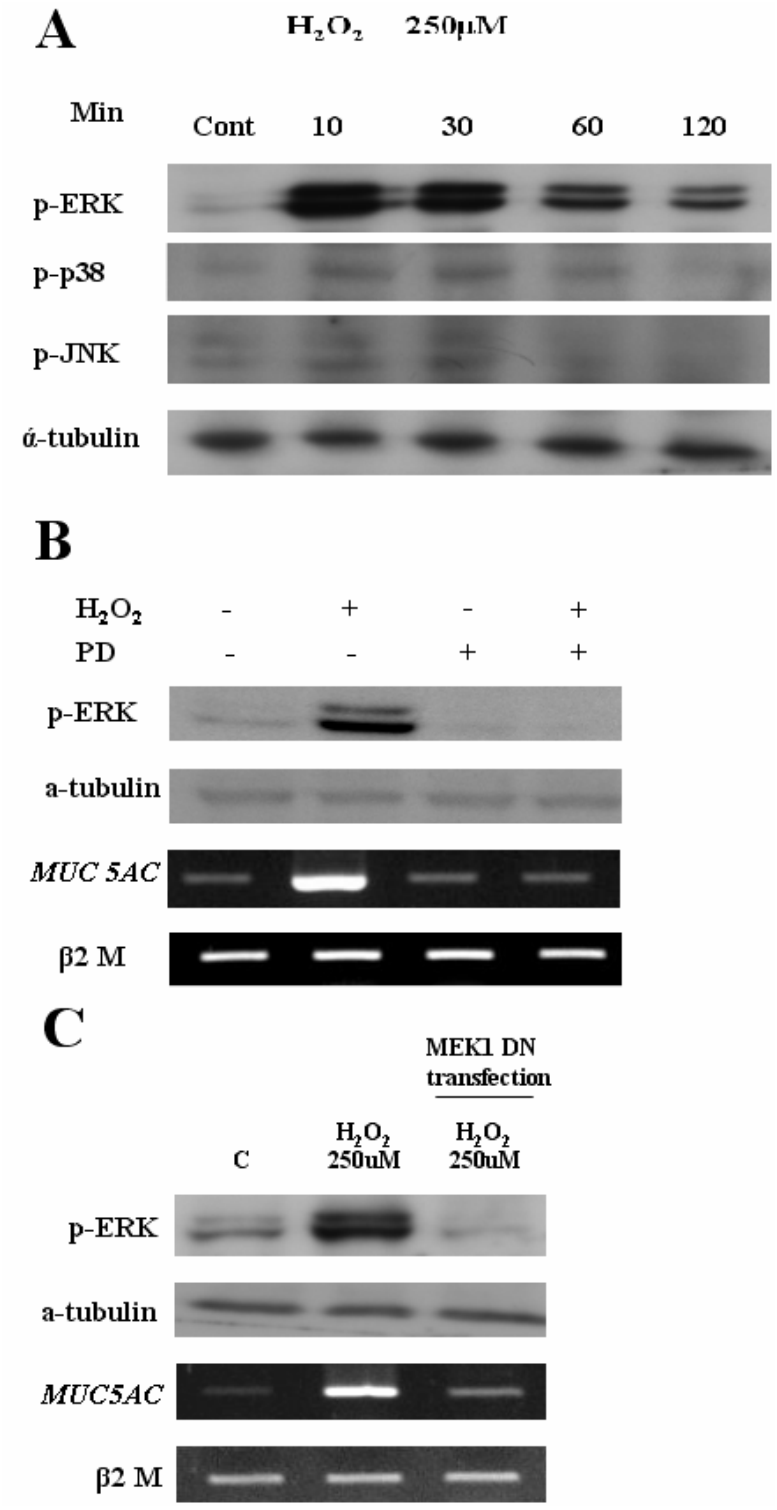
A
H2O2 250μM
Min
Cont
10
30
60
120
p-ERK
p-p38
p-JNK
ά-tubulin
B
H2O2
PD
p-ERK
a-tubulin
MUC 5AC
β2 M
C
MEK1 DN transfection
C
H2O2 250uM
H2O2 250uM
p-ERK
a-tubulin
MUC5AC
β2 M

Figure 4. H_2O_2 induces *MUC5AC* gene expression via ERK MAP kinase. A: Control cells were maintained in basal growth medium with DMSO. H_2O_2-treated cells were treated with H_2O_2 (250 μM) for 10, 30, 60, and 120 min. Western blot analysis demonstrated the effect of H_2O_2 on MAP kinase phosphorylation. H_2O_2 activated ERK, but not p38 or JNK MAP kinase, at 10 and 30 min. B: Cells were pretreated for 1 h with 30 μM PD98059 and then stimulated for 10 min with H_2O_2 (250 μM). Western blot analysis showed that and the phosphorylation of ERK MAP kinase was inhibited by treating cells with PD98059. Cells were pretreated for 1 h with 30 μM PD98059 and then stimulated for 24 hr with H_2O_2 (250 μM). Real time-PCR demonstrated that the increased *MUC5AC* gene expression was suppressed by pretreating cells with PD98059. C: MEK1 DN mutant stable cells were preincubated for 48 hrs and stimulated for 24 hr or 10 min with H_2O_2 (250 μM) prior to real-time PCR or Western blot, respectively. The increased phosphorylation of ERK MAP kinase and *MUC5AC* expression were inhibited after transfecting MEK1 DN mutant cells.

These results show that the activation of ERK MAP kinase is essential for H_2O_2-induced *MUC5AC* gene expression in NCI-H292 cells

ECG inhibited H_2O_2-induced MUC5AC gene expression via the inhibition of ERK MAP kinases

We next investigated whether the increased activity of ERK MAP kinase after stimulation with H_2O_2 could be inhibited by ECG treatment. After 1-h pre-treatment of 100 μM ECG, cells were treated with H_2O_2 (250 μM) with ECG (100 μM) for 10 and 30 min. Western blot analysis showed that ECG inhibited ERK MAP kinase activity (Fig. 5A). Interestingly, inhibition of the ERK MAP kinase pathway inhibited the induction of MUC5AC mRNA by H_2O_2 in NCI-H292 cells (Fig. 5B).

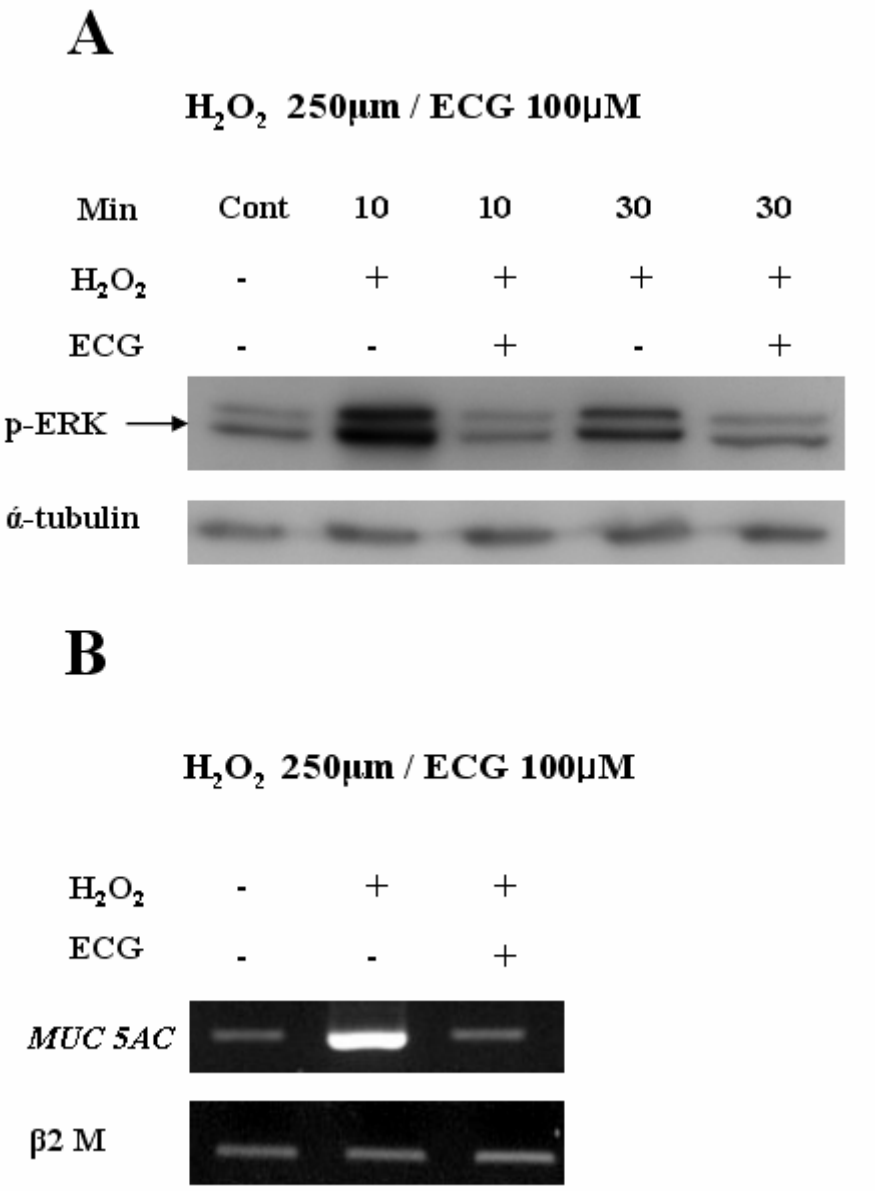

Figure 5. ECG inhibits H_2O_2-induced *MUC5AC* gene expression and phosphorylation of ERK MAP kinase. Cells were treated with ECG (100 μM) for 1 h before the addition of H_2O_2 for 10 or 30 min. ECG suppressed H_2O_2-induced phosphorylation of ERK MAP kinase in western blot analysis (A). RT-PCR showing

that *MUC5AC* gene expression was suppressed after treatment with ECG (B).

ECG clearly inhibited both H_2O_2-induced activation of ERK MAP kinase and *MUC5AC* gene expression. Thus, H_2O_2-induced activation of ERK MAP kinase appears to be closely related to *MUC5AC* gene expression and MUC5AC secretion.

Ⅳ. Discussion

Mucin hypersecretion causes many clinical problems such as rhinorrhea, nasal stuffiness, and sputum in the respiratory tract, and is commonly observed in many inflammatory airway diseases. Mucin genes are usually subdivided into two groups based on membrane-bound and secreted mucins, and it is known that secreted mucins may play an important role in mucin hypersecretion. It has been reported that MUC5AC is the predominant secreted mucin in human airways [9-11] and both the protein back bone and carbohydrates of MUC5AC contribute to the viscoelastic properties of airway mucus [22]. An understanding of the various stimuli of MUC5AC secretion, including inflammatory cytokines, cellular proteins, and oxidative stress is very important and may offer new therapeutic strategies for the inhibition of airway mucin hypersecretion [10].

The airway epithelium is continuously exposed to both exogenous oxidants, including air pollutants and cigarette smoke, and endogenous oxidants generated by activated inflammatory cells through mitochondrial electron transport [16]. Reactive oxygen species (ROS) are classically thought of as cytotoxic and mutagenic or as inducers of oxidative stress and recent evidence suggests that ROS play a role in stimulation or inhibition of cell proliferation, apoptosis, and inflammation [22,23]. In the present study, we investigate whether oxidative stress induces overexpression of secreted mucin genes in human airway epithelial cells. Our results showed that H_2O_2 increased both *MUC5AC* mRNA expression in a dose-dependent manner and

MUC5AC protein secretion. Interestingly, H_2O_2 did not have any effect on *MUC5B, MUC6, MUC7* and *MUC19* mRNA expression. These results suggest that H_2O_2 induced mucin gene overexpression and mucin secretion through only MUC5AC.

Previous studies have shown that polyphenols present in green tea (mainly catechins) are potent anti-oxidants [18]. The majority of the biological effects of green tea are mimicked by its principal constituent catechin EGCG [23-25] and there have been few reports about the anti-oxidant effects of other catechins. Our results show that treatment with the catechin ECG inhibits H_2O_2-induced *MUC5AC* gene overexpression in a dose-dependent manner and inhibits H_2O_2-induced MUC5AC protein secretion. It is likely that ECG inhibits H_2O_2-induced *MUC5AC* gene expression and MUC5AC secretion via an antioxidant effect.

The mechanism by which oxidative stress induces *MUC5AC* gene overexpression and MUC5AC secretion is important with respect to therapeutic approaches for respiratory inflammatory disease. Several studies have shown that activation by H_2O_2 is dependent on the MAP Kinase signal pathway [26,27]. The EGFR-MEK-MAPK transduction pathway is known to be involved in *MUC5AC* gene overexpression and hypersecretion [14]. In primary cultures of human bronchial epithelial cells H_2O_2 elicits a cytotoxic effect mediated by phosphorylation-dependent activation of MAPKs, and ERK, p38 and JNK MAP kinases are also involved in the inflammatory changes induced by H_2O_2 [16].

To examine which MAP kinases are involved in H_2O_2-induced *MUC5AC* gene expression in human airway epithelium, we investigated the effects of H_2O_2 treatment

on NCI-H292 cells. While ERK MAP kinase was phosphorylated after treatment with H_2O_2 the p38 and JNK MAP kinases were not activated. Pretreatment with PD98059 clearly suppressed *MUC5AC* gene expression and phosphorylation of ERK MAP kinase. This result indicates that H_2O_2 induces *MUC5AC* gene expression via ERK MAP kinase.

ERK MAP kinase activity was inhibited by ECG in a dose-dependent manner, indicating that ECG inhibits H_2O_2-induced *MUC5AC* gene overexpression and decreases MUC5AC secretion via suppression of ERK MAP kinase.

The fact that ECG inhibited H_2O_2-induced *MUC5AC* gene expression and decreased MUC5AC secretion suggests that H_2O_2 increases *MUC5AC* gene expression through transcriptional regulation and that ECG may have an effect on this regulatory mechanism.

It should be noted that some of the mechanistic studies of tea catechin, including our study, were performed in the concentration ranges of 10-1000 μM, which is unlikely to be achieved under physiologic conditions, except in the tissues of the gastrointestinal tract that come into direct contact with the tea solution [28]. The maximum achievable peak plasma level of catechin concentration *in vivo* is significantly lower than the oral consumptive concentrations of green tea solution [28-30]. We propose that the nasal topical application may be more useful than oral green tea solution in order to achieve the effective experimental dosage of ECG and to make use of ECG as a therapeutic agent against the nasal mucus hypersecretion.

V. Conclusion

In summary, oxidative stress, including H_2O_2, is a central feature in airway inflammatory diseases and antioxidants may play a critical role in the prevention of airway inflammation. The present study shows that H_2O_2 up-regulates *MUC5AC* gene expression and secretion and that ERK MAP kinase plays an important role in H_2O_2-induced *MUC5AC* overexpression. ECG, a green tea derived polyphenol, has a potent inhibitory effect on the induction by H_2O_2 of *MUC5AC* gene expression and MUC5AC secretion through suppression of ERK MAP kinase.

VI. References

1. Yuta A, Ali M, Sabol M, Gaumond E, Baraniuk JN. Mucoglycoprotein hypersecretion in allergic rhinitis and cystic fibrosis. Am J Physiol 1997;273: 1203-1207.
2. Kim SS, Kim KS, Lee JG, Park IY, Koo JS, Yoon JH. Levels of intracellular protein and messenger RNA of mucin and lysozyme in normal human nasal and polyp epithelium. Laryngoscope 2000;112:276-280.
3. Nadel JA. Role of epidermal growth factor receptor activation in regulating mucin synthesis. Respir Res 2001;2:85-89.
4. Van de Bovenkamp JH, Hau CM, Strous GJ, Buller HA, Dekker J, Einerhand AW. Molecular cloning of human gastric mucin MUC5AC reveals conserved cysteine-rich D- domain and a putative leucin zipper motif. Biochem Biophys Res Commun 1998;245:853-859.
5. Keates AC, Nunes DP, Afdhal NH, Troxler RF, Offner GD. Molecualr cloning of major human gall bladder mucin: complete C-terminal sequence and genomic organization of MUC5B. Biochem J 1997;324:295-303.
6. Ho SB, Roberton AM, Shekels LL, Lyftogt CT, Niehans GA, Toribara NW Expression cloning of gastric mucin complementary DNA and localization of mucin gene expression. Gastroenterology 1995;109:735-747.
7. Bobek LA, Tsai H, Biesbrock AR, Levine MJ. Molecular cloning, sequence, and specificity of expression of the gene encoding the low molecular weight human salivary mucin(MUC7). J Biol Chem 1993;268:20563-20569.

8. Chen Y, Zhao YH, Kalaslavadi TB, Hamati E, Nehrke K, Le AD, et al. Genome-wide search and identification of a novel-gel forming mucin MUC19/muc19 in glandular tissues. Am J Respir Cell Mol Biol 2004;30:155-165.
9. Song KS, Lee WJ, Chung KC, Koo JS, Yang EJ, Choi JY, et al. Interleukin-1β and Tumor necrosis factor-α induce MUC5AC overexpression through a mechanism involving ERK/p38 mitogen-activated protein kinases-MSK1-CREB activation in human airway epithelial cells. J Biol Chem 2003;276:23243-23250.
10. Hewson CA, Edbrooke MR, Johnston SL. PMA induces the MUC5AC respiratory mucin in human bronchial epithelial cells, via PKC, EGF/TGF-α, Ras/Raf, MEK, ERK and Sp1-dependent mechanisms. J Mol Biol 2004;344:683-695.
11. Hovenberg HW, Davies JR, Carlstedt I. Different mucins are produced by the surface epithelium and the submucosa in human trachea: identification of MUC5AC as a major mucin from the goblet cells. Biochem J 1996;318:319-324.
12. Wang B, Lim DJ, Han J, Kim YS, Basbaum CB, Li JD. Novel cytoplasmic proteins of nontypeable Haemophilus influenzae up-regulate human MUC5AC mucin transcription via a positive p38 mitogen-activated protein kinase pathway and a negative phosphoinositide 3-kinase-Akt pathway. J Biol Chem 2002;277:949-957.
13. Barnes PJ. Reactive oxygen species and airway inflammation. Free Radical Biol Med 1990;9:235-245.
14. Takeyama K, Dabbagh K, Shim JJ, Pick TD, Ueki I, Nadel JA. Oxidative stress

causes mucin synthesis via transactivation of epidermal growth factor receptor: Role of neurtophils. J Immunol 2000;164:1546-1552.

15. Garrington TP, Johnson GL. Organization and regulation of mitogen-activated protein kinase signaling pathways. Curr Opin Cell Biol 1999;11:211-218.
16. Pelaia G, Cuda G, Vatrella A, Gallelli L, Fratto D, Gioffre V, et al. Effects of hydrogen peroxide on MAPK activation, IL-8 production and cell viability in primary cultures of human bronchial epithelial cells. J Cell Biochem 2004;93:142-152.
17. Mutoh M, Takayashi M, Fukuda K, Komatsu H, Enya T, Masushima HY, et al. Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure-activity relationship. Jpn J Cancer Res 2000;91:686-791.
18. Ho CT, Chen Q, Shi H, Zhang KQ, Rosen RT. Antioxidative effect of polyphenol extract prepared from various Chinese teas. Prev Med 1992;21:520-525.
19. Kuroda Y, Hara Y. Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat Res 1999;436:69-97.
20. Ahmad N, Feyes DK, Nieminen AL, Agarwal R, Mukhtar H. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J Natl Cancer Inst 1997;89:1881-1886.
21. Yoon JH, Gray T, Guzman K, Koo JS, Nettesheim P. Regulation of the secretory phenotype of human airway epithelium by retinoic acid, triiodothyronine, and extracellular matrix. Am J Respir Cell Mol Biol 1997;16:724-731.

22. Shao MXG, Nadel JA. Dual oxidase 1-dependent MUC5AC mucin expression in cultured human airway epithelial cells. Proc Natl Acad Sci USA 2005;102:767-772.
23. Arnold RS, Shi J, Murad E, Whalen AM, Sun CQ, Polavarapu R, et al. Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1. Proc Natl Acad Sci USA 2001;98:5550-5555.
24. Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, Goldsmith E, et al. Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. Cell 1998;93:605-615.
25. Li WQ, Dehnade F, Zafarullah M. Thiol antioxidant, N-acetylcysteine, activates extracellular signal-regulated kinase signaling pathway in articular chondrocytes. Biochem Biophys Res Commun 2000;275:789-794.
26. Pelaia G, Cuda G, Vatrella A, Grembiale RD, Fratto D, Tagliaferri P, et al. Effects of transforming growth factor-β and budesonide of mitogen-activated protein kinase activation and apoptosis in airway epithelial cells. Am J Respir Cell Mol Biol 2003;29:12-18.
27. Nabeyrat E, Jones GE, Fenwick PS, Barnes PJ, Donnelly LE. Mitogen-activated protein kinases mediate peroxynitrite-induced cell death in human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2003;284: 1112-1120.
28. Koo MWL, Cho CH. Pharmacological effects of green tea on the gastrointestinal system. Eur J Pharmacol 2004;500:177-185.
29. Lee MJ, Maliakal P, Chen L, Meng XF, Bondoc FY, Prabhu S, et al. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-

epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomark Prev 2002;11:1025-1032.

30. Yang CS, Chen LS, Lee MJ. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol Biomark Prev 1998;7:351-354.

<Abstract (in Korean)>

사람 기도 상피세포에서 Hydrogen peroxidase 에 의해 유도된 점액 과분비에 대한 ECG 의 억제 효과

<지도 교수 윤 주 헌>

연세대학교 대학원 의학과
임 준 완

체내에서 발생하는 활성 산소는 기도에 염증을 일으키고, 점액 분비를 증가시키며, 만성 기도 염증 질환을 일으킨다. 점액유전자인 MUC5AC 의 과발현이 유발되며 이는 MAP kinase pathway 신호전달체계를 이용한다고 알려져 있다. 녹차 성분의 일부인 polyphenol 은 항산화 물질로 알려져 있다. 본 연구자는 사람 기도 상피세포에서 H2O2 가 점액 유전자의 발현에 미치는 영향을 알아보고 이에 의해 유도된 점액의 과분비 및 점액 유전자의 과발현에 대한 polyphenol 의 성분인 epicatechin-gallate(ECG)의 효과를 알아보고자 하였다. NCI-H292 cell 에 H2O2 를 농도 별(100,250,500,1000 μM) 로 처치하여 H2O2 에 의한 *MUC5AC* 의 mRNA 의 발현을 보았으며 ECG(10,50,100μM)를 전처치한 후 H2O2(250μM)를 처치하여 *MUC5AC* 의 mRNA 의 발현의 변화를 역전사 중합효소반응을 통해 측정하였고 MUC5AC 분비의 변화를 Dot blotting 을 통해 관찰하였다. H2O2 를 처치하여 MAP kinase pathway 의 신호전달 단백들의 발현량과 ECG(100μM)를 처치한 후 신호전달 단백들의 변화를 Western blotting 을 이용하여 분석하였다. H2O2 를 처치 한 경우, 농도 별로 *MUC5AC* mRNA 의 발현이 증가 하였으며, H2O2 에 의해 과발현된 *MUC5AC* mRNA 는 10,50,100μM 용량의 ECG 를 전처치하였을 때 현저히 감소하였고 이는 MUC5AC 의 secretion 에도 같은 양상을 보였다. H2O2 를 처치한 경우 *MUC5AC* 의 발현에 미치는 영향은 MAP kinase pathway 중 ERK 에 의한 것이었으며 증가된 ERK 의 활성화가 ECG 에 의해 억제되는 것을 알 수 있었다. 결론적으로 H2O2 에 의해 *MUC5AC* 의 발현이 증가하며 녹차의 성분인 ECG 가 H2O2 에 의해 유도된 *MUC5AC* 유전자의 과발현 및 단백의 분비를 억제하는 것을 알 수 있었고, 이는 MAP kinase pathway 중 ERK 의 억제에 의한 것임을 알 수 있었다.

핵심되는 말 : 점액 과분비 · 활성 산소 · 녹차 · Polyphenol