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

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Epicatechin-gallate inhibits hydrogen peroxide-induced \textit{MUC5AC} gene expression and \textit{MUC5AC} secretion in human airway epithelial cells

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December 2006
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.
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Abstract

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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 antioxidative properties. This study examines the effect of ECG on H₂O₂-induced mucin gene expression and mucin secretion in human airway epithelial cells. H₂O₂ induced overexpression of the mucin gene MUC5AC, and ECG markedly inhibited H₂O₂-induced *MUC5AC* gene expression and MUC5AC secretion. In addition, the MAP kinase signal pathway, in particular ERK MAP kinase, was associated with H₂O₂-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 H₂O₂-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
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I. Introduction

Mucin hypersecretion is commonly observed in many respiratory diseases, such as rhinitis, sinusitis, otitis media, nasal allergy, chronic bronchitis and cystic fibrosis. 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. 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. 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 ($\text{H}_2\text{O}_2$), superoxide anion ($\text{O}_2^-$), 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 $\text{H}_2\text{O}_2$ 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$_2$O$_2$ on expression of the secreted mucin genes MUC5AC, MUC5B, MUC6, MUC7, MUC19 and the ability of ECG to suppress H$_2$O$_2$-induced secreted mucin gene expression in human airway epithelial cells. Our results show that H$_2$O$_2$ induced *MUC5AC* gene overexpression but not MUC5B, MUC6, MUC7 or MUC19, and induced MUC5AC protein secretion in a dose dependent manner. ECG suppressed H$_2$O$_2$-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 (H2O2) 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% CO2. 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 H2O2 (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 H₂O₂ 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 H₂O₂ for 10, 30, 60, and 120 min. To test the effect of ECG, cells were treated with 100 µM ECG for 1 h prior to incubation in fresh medium containing 250 µM H₂O₂ and 100 µM ECG for 10 or 30 min.

**RT-PCR**

Total RNA was isolated from NCI-H292 cells treated with H₂O₂ (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). Oligonucleotide primers for PCR were designed based on the Genbank™ 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

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

* 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
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$_2$O$_2$ for 10, 30, 60, or 120 min, cells were lysed with 2X lysis buffer (250 mM Tris-Cl(pH 6.5), 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 (pH 7.5), 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.
### Results

**H$_2$O$_2$ induced gene expression of MUC5AC, but not MUC5B, MUC6, MUC7, or MUC19, in a dose-dependent manner**

To determine whether H$_2$O$_2$ can induce the expression of secreted mucin genes, RT-PCR was performed after treatment of cells with H$_2$O$_2$ (100, 250, 500, 1000 µM) for 24 h. Levels of MUC5AC mRNA increased after treatment with H$_2$O$_2$ 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$_2$O$_2$ (9.25±0.59 times greater than control; p<0.01), at 500 µM H$_2$O$_2$ (9.17±0.04 times greater than control; p<0.01), and at 1 mM H$_2$O$_2$ (9.81±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\textsubscript{2}O\textsubscript{2} induces \textit{MUC5AC} gene expression in a dose-dependent manner.

Confluent cells were treated with H\textsubscript{2}O\textsubscript{2} (100 \textmu M, 250 \textmu M, 500 \textmu M, 1 mM) for 24 h. C, control. \(\beta\)2-microglobulin (\(\beta\)2 M) was used as an internal control. H\textsubscript{2}O\textsubscript{2} induced \textit{MUC5AC} gene expression but did not affect expression of other secreted mucin genes (A). Densitometry demonstrating the dose-dependent effect of H\textsubscript{2}O\textsubscript{2} on \textit{MUC5AC} gene expression. All experiments were conducted at least four separate times (B). (*) : \(p < 0.05\)
ECG suppressed \( \text{H}_2\text{O}_2 \)-induced MUC5AC gene expression

We next evaluated whether ECG could suppress the gene expression of MUC5AC. Cells (1 x 10^6/ml) were stimulated with \( \text{H}_2\text{O}_2 \) (250 \( \mu \)M) or \( \text{H}_2\text{O}_2 \) (250 \( \mu \)M) + ECG (10, 50, 100\( \mu \)M) for 24 h. RT-PCR analysis showed that treatment with \( \text{H}_2\text{O}_2 \) induced MUC5AC gene expression compared to control cells (6.41±0.27 times greater than control; \( p<0.01 \)) (Fig 2A). Pre-treatment with ECG for 1h followed by 24-h treatment with \( \text{H}_2\text{O}_2 \) and ECG suppressed \textit{MUC5AC} gene expression relative to \( \text{H}_2\text{O}_2 \) alone in a dose-dependent manner, with significant inhibition at 10 \( \mu \)M ECG (3.97±0.14 times greater than control; \( p<0.01 \)), 50 \( \mu \)M ECG (3.47±0.31 times greater than control; \( p<0.01 \)) and 100 \( \mu \)M ECG (1.39±0.31 times greater than control; \( p<0.01 \)) (Fig. 2B).
Figure 2. ECG suppresses H$_2$O$_2$-induced *MUC5AC* gene expression. Confluent cells were pretreated with ECG for 1 h and then treated with H$_2$O$_2$ (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$_2$O$_2$-induced *MUC5AC* gene expression in a dose dependent manner (A). Densitometry demonstrating the effect of ECG on H$_2$O$_2$-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$_2$O$_2$-induced *MUC5AC* gene overexpression.
ECG suppressed H$_2$O$_2$-induced MUC5AC secretion and intracellular MUC5AC protein

The secretion of MUC5AC protein in NCI-H292 cells was also measured. Treatment with 250 µM H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$-induced MUC5AC secretion and intracellular MUC5AC protein. Representative immunoblot assay demonstrating the effect of ECG.
on H$_2$O$_2$-mediated MUC5AC secretion (A) and protein (B). ECG suppressed H$_2$O$_2$-induced MUC5AC secretion and intracellular MUC5AC protein (*: $p < 0.05$).

These results demonstrate that ECG suppresses H$_2$O$_2$-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  

\[ \text{H}_2\text{O}_2 \ 250\mu\text{M} \]

\[
\begin{array}{c|ccccc}
\text{Min} & \text{Cont} & 10 & 30 & 60 & 120 \\
\hline
\text{p-ERK} & \text{image} & \text{image} & \text{image} & \text{image} & \text{image} \\
\text{p-p38} & \text{image} & \text{image} & \text{image} & \text{image} & \text{image} \\
\text{p-JNK} & \text{image} & \text{image} & \text{image} & \text{image} & \text{image} \\
\text{\alpha-} \text{tubulin} & \text{image} & \text{image} & \text{image} & \text{image} & \text{image} \\
\end{array}
\]

B  

\[
\begin{array}{ccccccc}
\text{H}_2\text{O}_2 & - & + & - & + \\
\text{PD} & - & - & + & + \\
\text{p-ERK} & \text{image} & \text{image} & \text{image} & \text{image} \\
\text{\alpha-} \text{tubulin} & \text{image} & \text{image} & \text{image} & \text{image} \\
\text{MUC5AC} & \text{image} & \text{image} & \text{image} & \text{image} \\
\beta2 \text{ M} & \text{image} & \text{image} & \text{image} & \text{image} \\
\end{array}
\]

C  

\[
\begin{array}{cccc}
\text{C} & \text{H}_2\text{O}_2 \ 250\mu\text{M} & \text{H}_2\text{O}_2 \ 250\mu\text{M} \\
\text{p-ERK} & \text{image} & \text{image} & \text{image} \\
\text{\alpha-} \text{tubulin} & \text{image} & \text{image} & \text{image} \\
\text{MUC5AC} & \text{image} & \text{image} & \text{image} \\
\beta2 \text{ M} & \text{image} & \text{image} & \text{image} \\
\end{array}
\]

MEKI DN transfection
Figure 4. H$_2$O$_2$ induces $MUC5AC$ gene expression via ERK MAP kinase. A: Control cells were maintained in basal growth medium with DMSO. H$_2$O$_2$-treated cells were treated with H$_2$O$_2$ (250 µM) for 10, 30, 60, and 120 min. Western blot analysis demonstrated the effect of H$_2$O$_2$ on MAP kinase phosphorylation. H$_2$O$_2$ 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$_2$O$_2$ (250 µM). Western blot analysis showed that 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$_2$O$_2$ (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$_2$O$_2$ (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$_2$O$_2$-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 H₂O₂-induced MUC5AC gene expression and phosphorylation of ERK MAP kinase. Cells were treated with ECG (100 µM) for 1 h before the addition of H₂O₂ for 10 or 30 min. ECG suppressed H₂O₂-induced phosphorylation of ERK MAP kinase in western blot analysis (A). RT-PCR showing
that \textit{MUC5AC} gene expression was suppressed after treatment with ECG (B).

ECG clearly inhibited both H\textsubscript{2}O\textsubscript{2}-induced activation of ERK MAP kinase and \textit{MUC5AC} gene expression. Thus, H\textsubscript{2}O\textsubscript{2}-induced activation of ERK MAP kinase appears to be closely related to \textit{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 and both the protein backbone and carbohydrates of MUC5AC contribute to the viscoelastic properties of airway mucus. 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.

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. 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. 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$_2$O$_2$ increased both MUC5AC mRNA expression in a dose-dependent manner and
MUC5AC protein secretion. Interestingly, \( \text{H}_2\text{O}_2 \) did not have any effect on \textit{MUC5B}, \textit{MUC6}, \textit{MUC7} and \textit{MUC19} mRNA expression. These results suggest that \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \)-induced \textit{MUC5AC} gene overexpression in a dose-dependent manner and inhibits \( \text{H}_2\text{O}_2 \)-induced MUC5AC protein secretion. It is likely that ECG inhibits \( \text{H}_2\text{O}_2 \)-induced \textit{MUC5AC} gene expression and MUC5AC secretion via an antioxidant effect.

The mechanism by which oxidative stress induces \textit{MUC5AC} gene overexpression and MUC5AC secretion is important with respect to therapeutic approaches for respiratory inflammatory disease. Several studies have shown that activation by \( \text{H}_2\text{O}_2 \) is dependent on the MAP Kinase signal pathway \(^{26,27}\). The EGFR-MEK-MAPK transduction pathway is known to be involved in \textit{MUC5AC} gene overexpression and hypersecretion \(^{14}\). In primary cultures of human bronchial epithelial cells \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) \(^{16}\).

To examine which MAP kinases are involved in \( \text{H}_2\text{O}_2 \)-induced \textit{MUC5AC} gene expression in human airway epithelium, we investigated the effects of \( \text{H}_2\text{O}_2 \) treatment
on NCI-H292 cells. While ERK MAP kinase was phosphorylated after treatment with H$_2$O$_2$ the p38 and JNK MAP kinases were not activated. Pretreatment with PD98059 clearly suppressed $\textit{MUC5AC}$ gene expression and phosphorylation of ERK MAP kinase. This result indicates that H$_2$O$_2$ induces $\textit{MUC5AC}$ gene expression via ERK MAP kinase.

ERK MAP kinase activity was inhibited by ECG in a dose-dependent manner, indicating that ECG inhibits H$_2$O$_2$-induced $\textit{MUC5AC}$ gene overexpression and decreases MUC5AC secretion via suppression of ERK MAP kinase.

The fact that ECG inhibited H$_2$O$_2$-induced $\textit{MUC5AC}$ gene expression and decreased MUC5AC secretion suggests that H$_2$O$_2$ increases $\textit{MUC5AC}$ 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. The maximum achievable peak plasma level of catechin concentration \textit{in vivo} is significantly lower than the oral consumptive concentrations of green tea solution. 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


<Abstract (in Korean)>

사람 기도 상피세포에서 Hydrogen peroxidase 에 의해 유도된 점액 과분비에 대한 ECG의 역제 효과

Abstract

체내에서 발생하는 활성 산소는 기도에 염증을 일으키고, 점액 분비를 증가시키며, 만성 기도 염증 천사를 일으킨다. 점액유전자인 MUC5AC의 과발현이 유발되며 이는 MAP kinase pathway 신호전달체계를 이용한다고 알려져 있다. 녹차 성분의 일부인 polyphenol은 항산화 물질로 알려져 있다. 본 연구자는 사람 기도 상피세포에서 H2O2가 점액 유전자 발현에 미치는 영향을 알아보고 이를 통해 유도된 점액의 과분비 및 점액 유전자의 과발현에 대한 polyphenol의 성분인 epicatechin-gallate(ECG)의 효과를 알아보고자 하였다. NCI-H292 cell에 H2O2를 농도 별로(100,250,500,1000 μM)로 처리하여 H2O2에 의한 MUC5AC의 mRNA의 발현을 보았으며 ECG(10,50,100μM)를 전처치한 후 H2O2(250μM)를 처리하여 MUC5AC의 mRNA의 발현의 변화를 역전사 중합효소반응을 통해 측정하였고 MUC5AC 분비의 변화를 Dot blotting을 통해 관찰하였다. H2O2를 처리하여 MAP kinase pathway의 신호전달 단백들의 발현량과 ECG(100μM)를 처리한 후 신호전달 단백들의 변화를 Western blotting을 이용하여 분석하였다. H2O2를 처리한 경우, 농도 별로 MUC5AC mRNA의 발현이 증가하였으며, H2O2에 의해 과발현된 MUC5AC mRNA는 10,50,100μM 용량의 ECG를 전처치하였을 때 현저히 감소하였고 이는 MUC5AC의 secretion에도 같은 양상을 보았다. H2O2를 처리한 경우 MUC5AC의 발현에 미치는 영향은 MAP kinase pathway 중 ERK에 의한 것이었으며 증가된 ERK의 활성화가 ECG에 의해 억제되는 것을 알 수 있었다. 결과적으로 H2O2에 의해 MUC5AC의 발현이 증가하며 녹차의 성분인 ECG가 H2O2에 의해 유도된 MUC5AC 유전자의 과발현 및 단백의 분비를 억제하는 것을 알 수 있었고, 이는 MAP kinase pathway 중 ERK의 역제에 의한 것임을 알 수 있었다.

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