**β-Carotene and Lutein Inhibit Hydrogen Peroxide-Induced Activation of NF-κB and IL-8 Expression in Gastric Epithelial AGS Cells**

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**Summary** Reactive oxygen species (ROS) including hydrogen peroxide (H$_2$O$_2$) are involved in the pathogenesis of gastric inflammation. Interleukin-8 (IL-8) is a potent mediator of the inflammatory response by activating and recruiting neutrophils to the site of infection. Oxidant-sensitive transcription factor NF-κB regulates the expression of IL-8 in the immune and inflammatory events. Carotenoids (carotenes and oxygenated carotenoids) show antioxidant and anti-inflammatory activities. Low intake of β-carotene leads to high risk of gastric cancer. Oxygenated carotenoid lutein inhibited NF-κB activation in experimental uveitis. The present study aims to investigate whether β-carotene and lutein inhibit H$_2$O$_2$-induced activation of NF-κB and expression of IL-8 in gastric epithelial AGS cells. The cells were treated with carotenoids 2 h prior to the treatment of H$_2$O$_2$. mRNA expression was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and real time RT-PCR analyses. IL-8 level in the medium was determined by enzyme-linked immunosorbent assay. NF-κB activation was assessed by electrophoretic mobility shift assay. ROS levels of the cells were detected by confocal microscopic analysis for fluorescent dichlorofluorescein. As a result, H$_2$O$_2$ induced the activation of NF-κB and expression of IL-8 in AGS cells time-dependently. β-Carotene and lutein showed inhibitory effects on H$_2$O$_2$-induced increase in intracellular ROS levels, activation of NF-κB, and IL-8 expression in AGS cells. In conclusion, supplementation of carotenoids such as β-carotene and lutein may be beneficial for the treatment of oxidative stress-mediated gastric inflammation.

**Key Words** hydrogen peroxide, β-carotene, lutein, IL-8, gastric epithelial cells

Reactive oxygen species (ROS), including hydrogen peroxide (H$_2$O$_2$), have an important role in protecting the infected tissues against invading pathogens during the innate immune response (1). In addition, ROS activate signal transduction for the expression of inflammatory cytokines and chemokines (2). Interleukin-8 (IL-8), a member of the C-X-C chemokine family, is a potent chemoattractant and activator of neutrophils, monocytes, and T lymphocytes (3–5). ROS mediate the expression of IL-8 by activating oxidant-sensitive transcription factors in gastric epithelial cells (6–8).

One of the oxidant-sensitive transcriptional modulators is the NF-κB family of proteins (9–11). NF-κB is present in the cell as a hetero- or homo-dimer and remains inactive by binding to an inhibitory protein, IκB, within the cytoplasm (10). NF-κB plays an important role in the inflammatory response in the intestine (12) and accordingly, *Helicobacter pylori* activates this transcription factor (13–15). This activation may in turn cause gastritis via the induction of proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF)-α and chemokines like IL-8 (15, 16). The inducers of NF-κB activation, such as TNF-α, IL-1, lipopolysaccharide (LPS), phorbol myristate acetate (PMA), UV and ionizing radiation, produce the elevated levels of ROS in various cells which suggests that ROS may function as common mediators of NF-κB activation. NF-κB activation could be blocked by a variety of antioxidants, including pyrrolidine dithiocarbamate (PDTC), N-acetylcysteine (NAC), reduced glutathione (GSH) or by overexpression of antioxidant enzymes, including superoxide dismutase, glutathione peroxidase or thioredoxin peroxidase (17, 18).

Fruits and vegetables have been suggested to confer protection against diseases such as cancer through the effects of antioxidants, often represented by carotenoids (19). Patients with gastric ulcer have very low concentrations of gastric antioxidants (ascorbic acid, α-tocopherol, α-carotene, β-carotene, total carotenoids, lutein, cryptoxanthin, and lycopene levels) compared to patients with gastritis and normal mucosa (20). Epidemiological study demonstrated that very low plasma levels of carotenoids (α-carotene and β-carotene) are at a higher risk of gastric cancer while there is no statistically significant association between plasma levels of...
oxygenated carotenoids such as lutein and zeaxanthin and gastric cancer risk (21). Lutein showed anti-\textit{H. pylori} activity while \textit{\beta}-carotene has relatively lower anti-\textit{H. pylori} activity. It is because oxygenated carotenoids contain a monofuranoid ring or an allene bond in addition to an epoxy group and an additional two or three hydroxyl substituents on the side group which shows anti-bacterial effect (22). \textit{\beta}-Carotene is responsible for the orange color of the many fruits and vegetables. \textit{\beta}-Carotene induces cell growth inhibition and apoptosis in human breast cancer and colon cancer (23, 24). Moreover, \textit{\beta}-carotene inhibits inflammatory gene expression by suppressing the activation of NF-\kappaB (25–27). Lutein is a member of the oxygenated carotenoids found particularly in dark-green leafy vegetables. Lutein is considered to be beneficial in the prevention of cancers and eye diseases (28, 29). Even though like other Carotenoids lutein intake is reported to prevent certain cancers (19), lutein is uniquely concentrated in the retina and lens, indicating that it has a possible specific function in vital ocular tissues. Lutein has a potential role in the prevention and treatment of certain eye diseases such as age-related macular degeneration, cataract and retinitis pigmentosa (30).

In the present study, we investigated whether \textit{\beta}-carotene and lutein inhibit \textit{H. pylori}-induced activation of NF-\kappaB and the expression of IL-8 in gastric epithelial AGS cells. To determine the antioxidant activities of \textit{\beta}-carotene and lutein, intracellular levels of ROS were determined in AGS cells treated with carotenoids and cultured in the presence of \textit{H. pylori}. N-Acetylcysteine (NAC), a known antioxidant, was used as a positive control.

**MATERIALS AND METHODS**

**Cellculture.** The human gastric carcinoma AGS cell line (American Type Culture Collection, Manassas, VA, USA) was cultured in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (GIBCO), 2 mM glutamine, 100 U/mL penicillin, and 100 \mu g/mL streptomycin (Sigma, St. Louis, MO, USA) at 37°C in a humidified atmosphere of 5% CO\textsubscript{2} and 95% air. The cells were seeded in 6-well cell culture plates at 3×10\textsuperscript{5} cells per well and cultured to reach 80% confluency for the determination of ROS, mRNA expression of IL-8, IL-8 levels in the medium, and cell viability. For NF-\kappaB activation, the cells were seeded in 10 cm dish at 1×10\textsuperscript{5} cells per dish and cultured to reach 80% confluency.

**Preparation of carotenoids and NAC.** \textit{\beta}-Carotene (Catalog No. C4582, purity ≥95%, Sigma) or lutein (Catalog No. X95507, purity ≥90%, Fluka, Buchs, Switzerland) was freshly dissolved in tetrahydrofuran (THF, Catalog No. 401757, purity ≥99.9%, inhibitor-free, Sigma-Aldrich, St. Louis, MO, USA) before each experiment. The concentration of THF did not exceed 0.1%. The control group received THF instead of carotenoids. NAC (Catalog No. A7250, purity ≥99%, Sigma) was dissolved in phosphate buffered saline (PBS), pH 7.5, and neutralized with NaOH immediately before use.

**Experimental protocol.** For the time-course experiments, the cells were cultured in the presence of \textit{H. pylori} (100 \mu mol/L) for 30 min (ROS levels), 1 h (NF-\kappaB activation), 4 h (mRNA expression of IL-8) or 12 h (IL-8 levels in the medium). For the effect of carotenoids or NAC, the cells were treated with carotenoids (\textit{\beta}-carotene or lutein at a final concentration of 20 \mu mol/L) or NAC (at a final concentration of 10 mmol/L) 2 h prior to the treatment with \textit{H. pylori} (100 \mu mol/L). \textit{H. pylori} was added to the cells which were pretreated with carotenoids or NAC without washing the medium. Pretreatment time and concentration of carotenoids (20 \mu mol/L) were adapted from our previous study showing the inhibitory effect of \textit{\beta}-carotene on the expression of cyclooxygenase-2 and inducible nitric oxide synthase in AGS cells (31). Previously, we compared the concentrations of \textit{\beta}-carotene at 2–20 \mu mol/L and found 20 \mu mol/L as the most effective concentration to inhibit ROS-mediated expression of inflammatory enzymes in AGS cells. Cell viability was determined by the trypan blue exclusion test after treatment with \textit{H. pylori} (100 \mu mol/L) for 8 h.

In other sets of experiments to determine the effect of intracellular carotenoids on \textit{H. pylori}-induced IL-8 expression, carotenoids (20 \mu mol/L) or NAC (10 mmol/L) were applied to the cells for 2 h and washed out. Then the cells were cultured in the presence of \textit{H. pylori} for 4 h (IL-8 mRNA expression) or 12 h (IL-8 levels in the medium).

**Cell counting.** The cells were seeded in 6-well cell culture plates at 3×10\textsuperscript{5} cells per well and cultured to reach 80% confluency. The cells were cultured in the presence of \textit{H. pylori} (100 \mu mol/L) for 2, 4, and 8 h. Cell viability was determined by the trypan blue exclusion test.

Reverse transcription-polymerase chain reaction (RT-PCR) and real-time RT-PCR analyses for IL-8 mRNA expression. The cells were treated with or without carotenoids (\textit{\beta}-carotene, lutein) or NAC and cultured in the absence or presence of \textit{H. pylori}. mRNA expression of IL-8 was assessed using RT-PCR analysis standardized by coamplifying with the housekeeping gene \textit{\beta}-actin, which served as an internal control. Total RNAs isolated from the cells were reverse transcribed into cDNAs and used for PCR with human-specific primers for IL-8 and \textit{\beta}-actin. Sequences of IL-8 primers were 5’-ATGAC-TTCAAGACTGGCGGTGCT-3’ (forward primer) and 5’-TTCAGCCTTCTTCAAAACTTCT-3’ (reverse primer), giving a 297 bp PCR product. For \textit{\beta}-actin, the forward primer was 5’-ACCACTGGAGCACTGGAG-3’ and the reverse primer was 5’-GTGAGATCTCATGAGTACTGTC-3’, giving a 349 bp PCR product. PCR was amplified by 25–30 repeat denaturation cycles at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. During the first cycle, the 95°C step extended to 2 min, and on the final cycle the 72°C step extended to 5 min. PCR products were separated on 1.5% agarose gels containing 0.5 g/mL ethidium bromide and visualized by UV transillumination.

Real-time RT-PCR for quantifying IL-8 gene expression was conducted by the Chromo 4 detection system.
cDNA was added in a SYBR Green Realtime PCR Master Mix (TOYOBO Co, Japan) containing 10 pg/mL of forward and reverse primers for IL-8. For PCR amplification, the cDNA was amplified by 40 cycles, denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s. During the first cycle, the 95°C step extended to 30 s, and on the final cycle the 37°C step extended to 1 min. β-Actin gene was amplified in the same reaction to serve as the reference gene.

Enzyme-linked immunosorbent assay (ELISA). Levels of IL-8 in the medium were determined by enzyme-linked immunosorbent assay kits (Invitrogen Corporation, CA, USA) according to the manufacturer’s instructions.

Nuclear extracts and NF-κB activation by electrophoretic mobility shift assay. The cells, treated with or without carotenoids (β-carotene, lutein) or NAC and cultured in the absence or presence of H2O2, were washed with ice-cold PBS, harvested by scraping into PBS, and pelleted by centrifugation at 1,500 × g for 5 min. The cells were lysed in buffer containing 10 mM HEPES, 10 mM KCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1.5 mM MgCl2, 0.2% Nonidet P-40, 1 mM diithiothreitol (DTT), and 0.5 mM phenylmethylsulfonylfluoride (PMSF). The nuclear pellet was resuspended on ice in nuclear extraction buffer containing 20 mM HEPES, 420 mM NaCl, 0.1 mM EDTA, 1.5 mM MgCl2, 25% glycerol, 1 mM DTT, and 0.5 mM PMSF, and the nuclear protein concentration was determined by the method of Bradford (32).

Nuclear extracts (5 μg) were preincubated in buffer containing 12% glycerol; 12 mM HEPES, pH 7.9; 4 mM Tris-HCl, pH 7.9; 1 mM EDTA; 1 mM DTT; 25 mM KCl; 5 mM MgCl2; 0.04 μg/mL poly[d(I-C)] (Boehringer Mannheim, Indianapolis, IN, USA); 0.4 mM PMSF; and tris-EDTA buffer (TE). The labelled probe was added and samples were incubated on ice for 10 min. NF-κB gel shift oligonucleotide (5′-AGTTGAGGGGACTTTCCCA-GGC-3′) from Promega Corp (Madison, WI, USA) was labelled with [γ-32P]dATP (Amersham) using T4 polynucleotide kinase (GIBCO-BRL). The end-labelled probe was purified from unincorporated [γ-32P]dATP using a Bio-Rad purification column (Bio-Rad Laboratories, Hercules, CA, USA) and recovered in TE. Samples were subjected to electrophoretic separation at room temperature on a nondenaturing 5% acrylamide gel at 30 mA using 0.5×Tris borate EDTA buffer. The gels were dried at 80°C for 1 h and exposed to the radiography film for 6–18 h at −70°C with intensifying screens.

Measurement of ROS levels in the cells. The cells in a chamber slide (Nalge Nunc, Naperville, IL, USA) at 80% confluency were treated with or without carotenoids (β-carotene, lutein) or NAC for 2 h and cultured in the absence or presence of H2O2 for 30 min. The cells were washed with HEPES-buffered saline (HBS), and then loaded with 5 μg/mL of 2′,7′-dichlorofluorescein diacetate (DCF; Molecular Probes, Eugene, OR, USA) for 5 min. The fluorescent dichlorofluorescein was detected using a laser scanning confocal microscope (Leica TCS-NT, Heidelberg, Germany) with excitation and emission wavelengths of 488 and 520 nm, respectively as described in our previous study (33). ROS trapped in the cells were expressed as the relative increase. ROS of the cells cultured in the absence of H2O2 was considered as 1.

Statistical analysis. The statistical differences were determined using one-way ANOVA and Newman-Keuls’s test. All values are expressed as mean±SE of four different experiments. *p<0.05 vs 0 h. (B) IL-8 level in the medium was determined by ELISA. Each bar represents the mean±SE of four different experiments. *p<0.05 vs None (the cells cultured in the absence of H2O2).

RESULTS

Cell viability and IL-8 expression of AGS cells cultured in the presence of H2O2

Cell viability of the cells cultured in the presence of H2O2, determined by the trypan blue exclusion test, was expressed as % of those of the cells cultured in the absence of H2O2. Viable cell numbers of the cells cultured in the presence of H2O2 were 95, 70 and 61% of those cultured in the absence of H2O2 at 2, 4, and 8 h-culture.

IL-8 mRNA expression of the cells cultured in the presence of H2O2, determined by the trypan blue exclusion test, was expressed as % of those of the cells cultured in the absence of H2O2. Viable cell numbers of the cells cultured in the presence of H2O2 were 95, 70 and 61% of those cultured in the absence of H2O2 at 2, 4, and 8 h-culture.
presence of \( \text{H}_2\text{O}_2 \) was determined by RT-PCR and real-time RT-PCR analyses. mRNA expression of IL-8 was evident from 1 h-culture and increased up to 4 h-culture according to RT-PCR analysis (Fig. 1A, upper panel) and real-time RT-PCR analysis (Fig. 1A, lower panel). \( \beta \)-Actin was constitutively expressed in AGS cells and was not changed by \( \text{H}_2\text{O}_2 \) treatment. As shown in Fig. 1B, IL-8 protein levels in the medium were determined by ELISA. Each bar represents the mean±SE of four different experiments. *\( p<0.05 \) vs None (the cells cultured in the absence of \( \text{H}_2\text{O}_2 \)). †\( p<0.05 \) vs Control (the cells cultured in the presence of \( \text{H}_2\text{O}_2 \)). ‡\( p<0.05 \) vs \( \beta \)-Carotene (the cells treated with \( \beta \)-carotene and cultured in the presence of \( \text{H}_2\text{O}_2 \)).

**Effect of carotenoids on \( \text{H}_2\text{O}_2 \)-induced mRNA expression and protein levels of IL-8 in AGS cells**

To investigate whether carotenoids inhibit \( \text{H}_2\text{O}_2 \)-induced expression of IL-8, mRNA expression levels were determined by RT-PCR analysis (Fig. 2A, upper panel) and real-time RT-PCR analysis (Fig. 2A, lower panel). IL-8 protein levels in the medium were determined by ELISA (Fig. 2B). Carotenoids and NAC suppressed mRNA expression and protein levels of IL-8 in the medium released from AGS cells treated with \( \text{H}_2\text{O}_2 \). The inhibitory effect of lutein on \( \text{H}_2\text{O}_2 \)-induced expres-
IL-8 was higher than that of β-carotene at mRNA and protein levels.

**Effect of carotenoids on ROS levels of AGS cells treated with H₂O₂**

Time-course of ROS levels in the cells treated with H₂O₂ was determined by confocal microscopic analysis for fluorescent dichlorofluorescein (Fig. 3A). H₂O₂ increased ROS levels with time until 30 min. Therefore, the effect of carotenoids (β-carotene, lutein) or NAC on ROS levels of AGS cells treated with H₂O₂ were determined at 30 min (Fig. 3B). A very low level of ROS was detected in the cells cultured in the absence of H₂O₂ (None). Treatment with H₂O₂ for 30 min resulted in substantial increase in ROS which was reduced by treatment of carotenoids (β-carotene, lutein) or NAC in the cells. Lutein and NAC showed higher inhibitory effects on H₂O₂-induced increment of intracellular ROS levels than β-carotene did.

**Effect of carotenoids on H₂O₂-induced activation of NF-κB in AGS cells**

Nuclear extracts were subjected to EMSA for the activation of NF-κB. H₂O₂ induced activation of NF-κB at 1 h, which continued until 2 h (Fig. 4A). Therefore, a 1 h-culture period was used for the following experiments on the effect of carotenoids (β-carotene, lutein) or NAC on NF-κB activation. AGS cells were treated with carotenoids (β-carotene, lutein) or NAC and cultured in the presence of H₂O₂ for 1 h. Then the cells were cultured in the presence of H₂O₂ for 4 h (mRNA expression) or 12 h (IL-8 levels in the medium). NAC also inhibited NF-κB activation induced by H₂O₂ in AGS cells. The inhibitory effect of lutein on H₂O₂-induced activation of NF-κB was higher than that of β-carotene in AGS cells.

**Effect of carotenoids on H₂O₂-induced mRNA expression and protein levels of IL-8 in AGS cells**

Carotenoids or NAC was applied to the cells for 2 h and washed out prior to addition of H₂O₂ to determine the effect of intracellular carotenoids or NAC on H₂O₂-induced expression of IL-8 in AGS cells. Then the cells were cultured in the presence of H₂O₂ for 4 h (IL-8 mRNA expression) or 12 h (IL-8 levels in the medium). As shown in Fig. 5A, H₂O₂-induced mRNA expression of IL-8 was inhibited by intracellular carotenoids and NAC. The inhibitory effects of carotenoids and NAC on H₂O₂-induced mRNA expression of IL-8 were relatively
lower than those shown in Fig. 2A. In Fig. 2A, carotenoids and NAC were applied to the cells and then H$_2$O$_2$ was applied to the cells in the presence of carotenoids and NAC. In Fig. 5A, carotenoids and NAC were applied to the cells and washed out prior to H$_2$O$_2$ treatment. The inhibitory effects of intracellular carotenoids and NAC on IL-8 levels in the medium were similar to their effects on the mRNA expression of IL-8 shown in Fig. 5A. There are no significant differences among the inhibitory effect of intracellular β-carotene, lutein, and NAC on H$_2$O$_2$-induced expression of IL-8 in AGS cells.

**DISCUSSION**

IL-8 plays a role in the pathogenesis of gastric inflammation caused by ROS and inflammatory stimuli including *H. pylori* infection (6–8). The expression of IL-8 is mainly regulated by redox-sensitive transcription factor NF-κB (8). H$_2$O$_2$-induced expression of IL-8 was inhibited in the cells treated with carotenoids (β-carotene, lutein) and NAC antioxidant in the present study. These results confirm that the activation of NF-κB is directly induced by H$_2$O$_2$ and essential for IL-8 expression in gastric epithelial AGS cells. Since NF-κB performs transcription regulation of various inflammatory cytokines, carotenoids may inhibit the expression of various cytokines regulated by NF-κB.

Epidemiological study demonstrated that there is no statistically significant association between plasma levels of lutein/zeaxanthin, lycopene, retinol, α- or γ-tocopherol and gastric cancer risk. But those who have very low plasma levels of α-carotene and β-carotene are at a higher risk of gastric cancer (21). Plasma lutein, zeaxanthin, α-carotene, β-carotene, lycopene, and total carotenoids were significantly lower in head and neck squamous cell carcinoma (HNSCC) patients than controls (34). This study indicates that increasing plasma carotenoid concentration may reduce risk of premature death or recurrence of tumor in HNSCC patients. In the relation of NF-κB and carotenoids, lutein suppresses the production of nitric oxide (NO) and the expression of tumor necrosis factor (TNF)-α, interleukin-6, prostaglandin-E$_2$, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein (MIP)-2 on endotoxin-induced uveitis in rats by inhibiting NF-κB activation (35). β-Carotene inhibited *H. pylori*-induced increase in ROS level, the activation of mitogen-activated protein kinases (p38, the c-Jun NH$_2$-terminal protein kinases, the extracellular signal-regulated kinases), NF-κB, and AP-1 and the expression of iNOS and COX-2 in AGS cells (31). β-Carotene inhibited inflammatory gene expression in lipopolysaccharide-stimulated macrophages by suppressing NF-κB activation (27).

Antioxidant activity of carotenoids in multilamellar liposomes assayed by inhibition of formation of thiobarbituric acid-reactive substances was in the ranking: lycopene > α-tocopherol > α-carotene > β-cryptoxanthin > zeaxanthin = β-carotene > lutein (36). The mechanisms and rate of scavenging of free radicals by carotenoids in solution is strongly dependent on the nature of ROS itself (37). β-Carotene is very reactive to peroxyl radicals but much less so to hydroxyl radical and superoxide (38). Lycopene prevented oxidative damage by directly scavenging singlet oxygen and trapping peroxyl radicals (39, 40). Carotenoids interact with free radicals in three main ways: electron transfer, hydrogen abstraction, and addition of a radical species (41, 42). The quenching of singlet oxygen by carotenoids involves direct energy transfer between these molecules (43). In addition, the nature and position of substituent groups on the carotenoid molecule (e.g. cyclic or acyclic termini, polar or apolar end groups, redox properties) may affect its antioxidant activity (44, 45). However, under high oxygen pressures and at high β-carotene concentrations it can exhibit pro-oxidant behavior, possibly due to autooxidative processes. Therefore, carotenoids may serve as antioxidants or prooxidants depending on their intrinsic properties as well as on the redox potential of the biological environment in which they act. In the present study, even though the cells were exposed to H$_2$O$_2$, carotenoids inside the cells or in the medium inhibited H$_2$O$_2$-induced expression of IL-8 in the cells. In addition, the results clearly indicate that carotenoids and NAC showed high inhibitory effects if these antioxidants existed outside the cells, and that they showed just slight inhibitory effects if they were inside the cells. We used a relatively higher concentration of carotenoids (20 μM) than the concentration present in human plasma (less than 1 μM) in the present study. Our previous study showed that 2 μM of β-carotene had no antioxidant activity, but 20 μM of β-carotene did reduce intracellular levels of ROS in *H. pylori*-infected cells (31). The study demonstrates that physiological concentrations of carotenoids may not be enough to scavenge ROS if the cells produce relatively large amounts of ROS upon various inflammatory stimuli.

In the present study, the inhibitory effect of lutein on ROS level, NF-κB activation and IL-8 expression induced by H$_2$O$_2$ was higher than that of β-carotene. Even though the present results were different from the previous in vitro antioxidant study (36), the structural difference of β-carotene and lutein may result in different inhibitory effects on IL-8 expression in AGS cells.

NAC enhances the endogenous antioxidant defense mechanism by increasing the intracellular stores of glutathione in the cells (46). In addition, NAC directly scavenges ROS (hydroxyl radicals, hypochlorous acid) produced by inflamed cells (47). Therefore, the effect of NAC may be the combined effect of GSH and NAC itself. Our previous study showed that the effect of GSH on scavenging of H$_2$O$_2$ and inhibition of NF-κB activation and IL-8 expression was similar to that of NAC in *H. pylori*-infected AGS cells (48). These studies propose the ROS-scavenging effect of NAC may be mainly caused by its precursor role for GSH formation even though we could not exclude a direct scavenging effect of ROS by NAC.

In conclusion, the NF-κB pathway regulates the expression of inflammatory cytokine IL-8 in human gastric epithelial cells cultured in the presence of H$_2$O$_2$. H$_2$O$_2$-induced activation of NF-κB was inhibited by ca-
rotenoids (β-carotene, lutein) as well as an antioxidant (NAC). Supplementation of carotenoids may be beneficial for the treatment of oxidative gastric inflammation.

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