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Steamed Ginger Extract Exerts Anti-inflammatory Effects in *Helicobacter pylori*-infected Gastric Epithelial Cells through Inhibition of NF-κB

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Ginger (*Zingiber officinale*) has traditionally been used as a treatment for inflammatory diseases in the Asian region. Recently, anti-inflammatory effects of steamed ginger extract (GGE03) have been reported, but its association with *Helicobacter pylori* (*H. pylori*)-induced gastritis has not been investigated. The purpose of this study was to assess the anti-inflammatory activity of GGE03 in *H. pylori*-infected gastric epithelial cells. Our studies revealed that the GGE03 suppressed the growth of *H. pylori*. GGE03 markedly reduced the expression of the *H. pylori*-induced pro-inflammatory cytokines including interleukin (IL)-8, TNF-α, IL-6, inducible NOS (iNOS) and IFN-γ. We also demonstrated that GGE03 treatment inhibited the *H. pylori*-activated NF-κB signaling pathway. In addition, the treatment with GGE03 significantly attenuated nitric oxide production and myeloperoxidase activity in *H. pylori*-infected gastric epithelial cells. These anti-inflammatory effects of GGE03 were more effective than ginger extract. Finally, we investigated the minimum effective concentration of GGE03 to inhibit *H. pylori*-induced inflammation. Our findings suggest that GGE03 not only inhibits the growth of *H. pylori*, but also attenuates *H. pylori*-induced inflammation.

Key Words Steamed ginger extract, *Helicobacter pylori*, Gastritis, Anti-inflammation, NF-kappa B

INTRODUCTION

Ginger (*Zingiber officianale*) is one of the herbaceous perennial plants that belongs to the Zingiberaceae family. Ginger has been used to treat stomachache, fever, vomiting and primary dysmenorrhea [1,2], and has an anti-inflammatory, anti-microbial and anti-oxidative properties [3]. Interestingly, recent studies have revealed that steamed ginger has a lower toxicity and higher efficacy than the dried ginger [4,5]. During the process of steaming, the bioactive components were affected, and this apparently enhanced the biological activities of ginger [6]. Actually, steamed ginger has been shown to have a greater anti-hyperglycemic, anti-diabetic and anti-obesity activity [6].

Helicobacter pylori (*H. pylori*) infection is a major risk factor for gastric diseases, including chronic gastritis and gastric adenocarcinoma [7]. It is estimated that more than half of the world's population is infected with *H. pylori*, and the WHO classified this bacterium as a class 1 carcinogen [8]. Infection by *H. pylori* activates several pro-inflammatory signals. NF-κB is one of the major pro-inflammatory pathways activated during infection by *H. pylori* [9]. The inflammatory response induced by *H. pylori* infection is implicated in the pathogenesis of some gastric disorders. Therefore, inhibition of inflammatory response is important in the management of gastric disease associated with *H. pylori* infection [10]. It is well known that NF-κB activation by *H. pylori* infection is mediated through cytotoxin-associated gene A (CagA), lipopolysaccharide, and peptidoglycan [11]. In the present study, we investigated the inhibitory effect of steamed ginger extract (GGE03) against the expression of inflammatory cytokines, and production of cellular nitric oxide (NO), and myeloperoxidase (MPO) activity in AGS human gastric adenocarcinoma cells infected with *H. pylori*.

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MATERIALS AND METHODS

Preparation of steamed ginger extract (GGE03)

GGE03 was prepared as previously published [5]. Briefly, ginger was washed three times with distilled water, dried at 50°C for 30 hours, and then steamed at: 2 to 2.5 kgf/cm^2, 97°C, for 2 hours. GGE03 was obtained by extracting steamed ginger with fifteen-fold 70% ethanol (v/v) for 15 hours at 85°C, 1.5 kg/cm^2, and then passed through a 60-mesh filter, concentrated at –650 mmHg, 55°C. GE was prepared in the same way without steam process. The extract (GE and GGE03) was spray-dried to obtain a powder and stored at –20°C until use. GE and GGE03 were dissolved in distilled water and diluted with medium into appropriate concentrations.

Cell culture

AGS gastric adenocarcinoma cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained according to the ATCC's instructions. Cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium (GE Healthcare, Chicago, IL, USA) supplemented with 10% (v/v) fetal bovine serum (ATCC), 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Bacterial culture and infection of gastric cells

The *H. pylori* strain ATCC 43504 (American Type Culture Collection, a cagA+ and vacA s1-m1 type's strain) was obtained from ATCC. *H. pylori* were cultured at 37°C in BBL Trypticase soy (TS) agar plate with 5% sheep blood (TSAII; BD Biosciences, Franklin Lakes, NJ, USA) under microaerophilic condition (BD GasPaK EZ Gas Generating Systems; BD Biosciences) for 3 days. The bacteria were harvested in clean TS broth, centrifuged at 3,000 ×*g* for 5 minutes, and resuspended in broth at a final density of 10^9 CFUs/mL. For infection, an absorbance of 0.4 units at 600 nm was considered equivalent to 1.4×10^6 bacteria. Cells were infected with a multiplicity of infection (MOI) of 1:50.

Cytotoxicity assay

For measuring the viability of AGS cells, the cells were treated with GE and GGE03 at the concentrations of 1, 10, 100, or 200 μg/mL. After incubation for 24 hours, cells were rinsed with PBS and then MTT (Sigma-Aldrich, St. Louis, MO, USA) solution was added to the cells followed by incubation for additional 3 hours. After the conversion of the substrate to a chromogenic product by metabolically active cells, the medium was removed and the purple MTT formazan crystals were solubilized with dimethyl sulfoxide (DMSO). The absorbance of each samples measured at 570 nm as a reference wavelength.

Western blotting

This assay was performed as previously described [12]. Briefly, proteins were separated by SDS PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with optimal concentrations of a primary antibody (pp65, p65, pp50, p50, p-IκBα, IκBα or β-actin) at 4°C overnight and then incubated with an appropriate secondary antibody for 1 hour at room temperature. The immune-labeled proteins were visualized using an enhanced chemiluminescence system (Thermo Fisher Scientific, Waltham, MA, USA). The primary antibodies to detect pp65, p65, pp50, p50, p-IκBα, and IκBα used in this study were purchased from Cell Signaling Technology (Danvers, MA, USA) and β-actin from Santa Cruz Biotechnology (Dallas, TX, USA).

RNA preparation and gene expression analysis

AGS cells were washed twice with PBS, and total mRNA was isolated from the cells using TRIzol™ Reagent (Invitrogen, Waltham, MA, USA), and cDNA was prepared using a Labopass cDNA synthesis kit (Cosmogenetech, Seoul, Korea) according to the manufacturer's instructions. The mRNA levels were assessed by reverse transcription PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR). qRT-PCR was performed as previously and assessed on the ViiA™ 7 real-time PCR system (Applied Biosystems, Waltham, MA, USA) using Luna universal qPCR master mix (New England Biolabs, Beverly, MA, USA). The relative quantities of target genes were calculated from triplicate samples after normalization by an internal control, 18S rRNA. The PCR primer sequences used in this study are listed in Table 1.

Myeloperoxidase activity assay

Samples obtained from cellular or bacterial pellets were washed twice with PBS and then lysed with a buffer containing 20 mM HEPES. Then, measurement of MPO activity was performed with kits (Abcam, Cambridge, UK) according to the manufacturer's instructions. The activity was measured as the absorbance at 450 nm read by a 96-well microplate reader.

Measurement of NO production

Culture medium supernatants were collected from the culture of AGS cells. The NO production in the culture medium was quantified colorimetrically using a nitric oxide detection kit (iNtRON Biotechnology, Seongnam, Korea). The absorbance at 550 nm was read with a 96-well microplate reader.

Disk diffusion assay

H. pylori (1.4×10^6 CFU/mL) suspended in 100 μL of PBS were applied onto the 5% Sheep Blood Agar (BD Bioscience) plate and spread on it. Then, 6-mm paper disks impregnated with different concentrations of GE and GGE03 (10 and 100 mg/mL) were positioned on an agar plate to evaluate their anti-bacterial effect. After incubation at 37°C in a microanaerobic chamber for 72 hours, the diameters of the growth inhibition zone were measured.

Table 1. Primer sequences used in RT-PCR and qRT-PCR primers

Species	Gene		Primer sequence
Human (RT-PCR)	*18S rRNA*	Foward	CCCAACTTCTTAGAGGGACAAGT
		Reverse	TAGTCAAGTTCGACCGTCTTCTC
	iNOS	Foward	GGCCTCTCAGCTCACCCCGA
		Reverse	CCAGGCGCACTGTCTGGTGG
Human (qRT-PCR)	*18S rRNA*	Foward	GCAATTATTCCCCATGAACG
		Reverse	GGCCTCACTAAACCATCCAA
	IL-8	Foward	TCC TTG TTC CAC TGT GCC TTG
		Reverse	TGC TTC CAC ATG TCC TCA CAA
	TNF-α	Foward	TCA GAG GGC CTG TAC CTC AT
		Reverse	GGA AGA CCC CTC CCA GAT AG
	IL-6	Foward	AGGGCTCTTCGGCAAATGTA
		Reverse	GAAGGAATGCCCATTAACAACAA
	IL-1β	Foward	TTA AAG CCC GCC TGA CAG A
		Reverse	GCG AAT GAC AGA GGG TTT CTT
	iNOS	Foward	AGGTCCAAATCTTGCCTGGG
		Reverse	ATCTGGAGGGGTAGGCTTGT
	IFN-γ	Foward	ACTGTCGCCAGCAGCTAAAA
		Reverse	TATTGCAGGCAGGACAACCA

RT-PCR, reverse transcription PCR; qRT-PCR, quantitative real-time PCR; iNOS, inducible NOS; IL, interleukin.

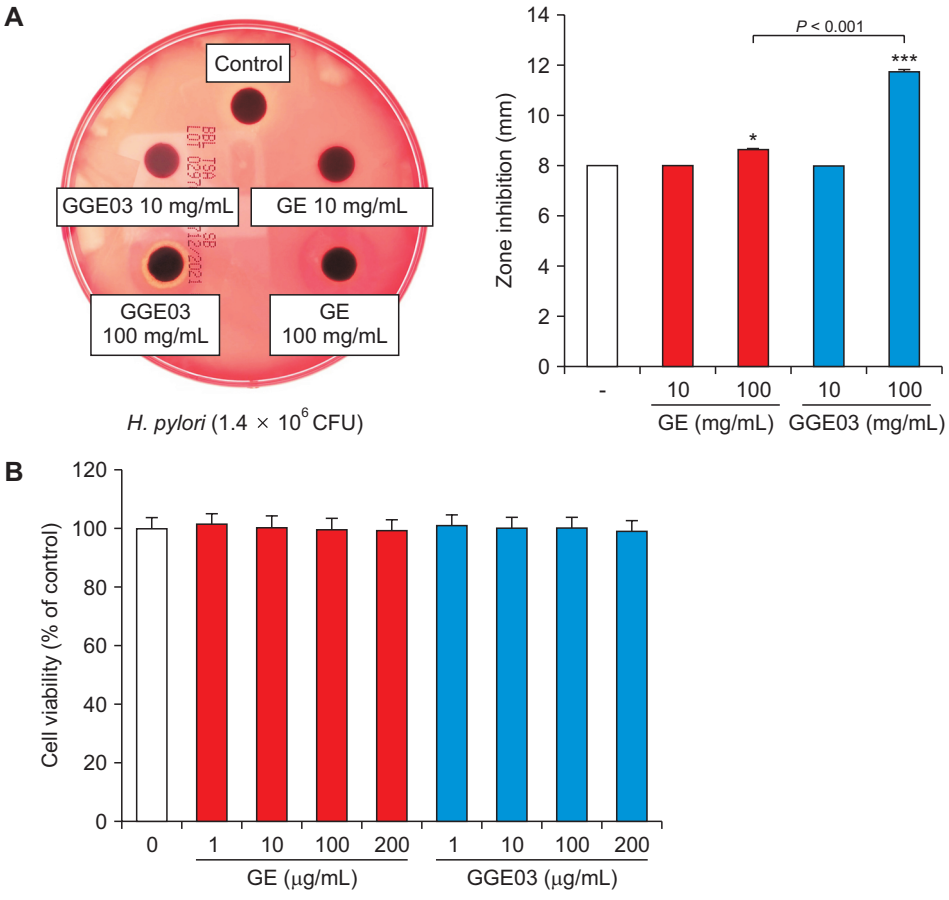

Figure 1. The steamed ginger extract (GGE03) inhibits the growth of *Helicobacter pylori* (*H. pylori*) and has no cytotoxic effect in AGS cells. (A) *H. pylori* strains (ATCC 43504; ATCC, Manassas, VA, USA) were grown on tryptic soy agar plate with 5% sheep blood containing indicated concentrations of GE or GGE03 (10 and 100 mg/mL). *H. pylori* with GE and GGE03 were determined after 72 hours of incubation. The diameters were calculated against inhibition zone. The results were evaluated by ANOVA. Data are the mean ± SD (n = 3). Statistical significance was analyzed by ANOVA. *$P < 0.05$ and ***$P < 0.001$ compared to control. (B) AGS cells were treated with GE and GGE03 at indicated concentrations for 24 hours and cell viability was measured by the MTT assay.

Statistical analysis

Results are expressed as the mean ± SD. The statistical significance was analyzed by one-way ANOVA. Statistical significance was accepted at $P < 0.05$.

RESULTS

Inhibitory effect of GGE03 on the growth of *H. pylori* in AGS cells

We conducted the investigation of relative inhibitory potency of GGE03 and GE against *H. pylori* growth, using the disk agar diffusion assay. To quantify the inhibitory effect of *H. pylori*, the diameter of growth inhibition area was measured and expressed in millimeters. GGE03 and GE were tested at concentrations of 10 and 100 mg/mL. As shown in Figure 1A, GGE03 at 100 mg/mL showed an inhibitory effect on *H. pylori* growth with inhibition zone ranging up to 12 mm. However, a lower concentration of GGE03 and GE (10 mg/mL) was not inhibitory. Next, we investigated the cytotoxic effect of GGE03 and GE on AGS cells. The AGS cells were treated with GGE03 and GE at various concentrations (1, 10, 100, or 200 μg/mL) for 24 hours. The results showed that GGE03 and GE did not exhibit cytotoxic effects when treated to AGS cells for 24 hours (Fig. 1B).

Anti-inflammatory effect of GGE03 in *H. pylori*-infected AGS cells

It has been reported that *H. pylori* infection stimulates the production of pro-inflammatory cytokines such as interleukin (IL)-8, TNF-α, IL-6 and IL-1β [13]. The qRT-PCR results showed that the levels of aforementioned cytokines were significantly increased in *H. pylori*-infected AGS cells, and this was signifi-

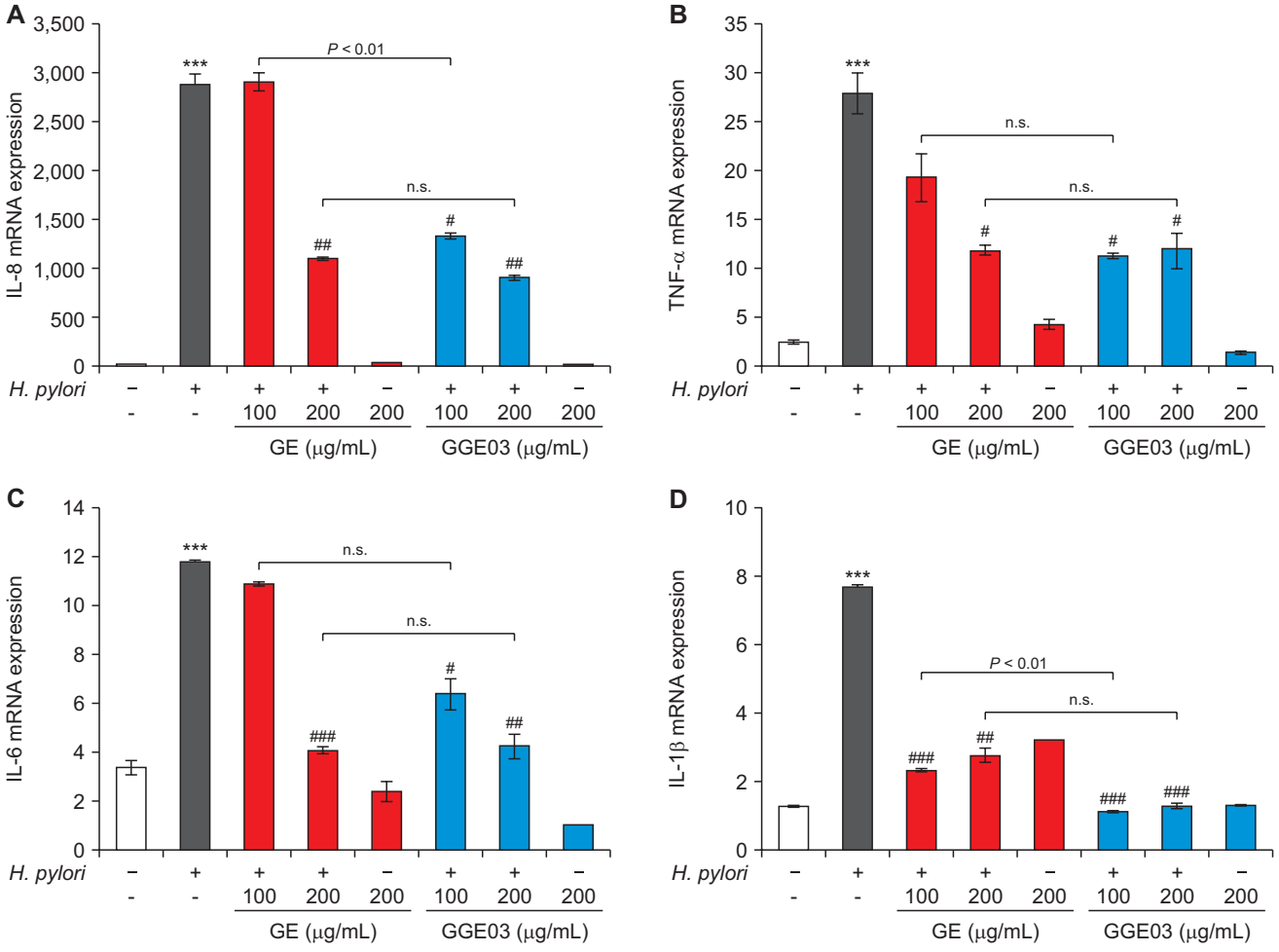

Figure 2. GGE03 inhibits pro-inflammatory cytokine gene expression in *H. pylori*-infected AGS cells. AGS cells were pretreated with GE and GGE03 at indicated concentrations for 1 hour, followed by co-culture with *H. pylori* (50 multiplicity of infection) for additional 3.5 hours. The effects of GE and GGE03 on the mRNA expression of IL-8 (A), TNF-α (B), IL-6 (C), and IL-1β (D) in AGS cells were analyzed by quantitative real-time PCR (qRT-PCR). 18S rRNA was used as an internal control for the expression of pro-inflammatory cytokines. Data are the mean ± SD. Statistical significance was analyzed by ANOVA. IL, interleukin; *H. pylori*, *Helicobacter pylori*; n.s., not significant. ***$P < 0.001$ compared to control; #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ compared to the *H. pylori* group.

cantly reduced by GGE03 and GE treatment (Fig. 2). GGE03 was shown to more effectively inhibit *H. pylori*-induced inflammation than GE in AGS cells.

GGE03 exerts anti-inflammatory effects through blockade of *H. pylori*-induced NF-κB signaling pathway in AGS cells

The NF-κB signaling pathway is one of the most important mediators of pro-inflammatory cytokine production upon *H. pylori* infection [14,15]. As shown in Figure 3A, the phosphorylation of IκBα was enhanced by *H. pylori* infection, and GGE03 and GE treatment significantly reduced the levels of phosphorylated IκBα. GGE03 was more effective in inhibiting the phosphorylation of IκBα induced by *H. pylori* than GE. Moreover, phosphorylation of p65, a major functionally active subunit of NF-κB, was increased in *H. pylori*-infected AGS cells, which was significantly inhibited by the treatment with GGE03. In contrast, there was no change in the phosphorylation of p50 by infection with *H. pylori* or treatment with GGE03 (Fig. 3B). Collectively, these results suggest that GGE03 suppresses *H. pylori*-induced inflammatory response by inhibiting the NF-κB signaling pathway.

GGE03 inhibits the production of NO in *H. pylori*-infected AGS cells

Several lines of evidence indicate that NO is involved in the pathogenesis of *H. pylori*-mediated gastritis and gastric cancer. NO has the ability to diffuse freely across the cells and has strong reactivity as a free radical molecule [16]. Therefore, we measured the expression of inducible NOS (iNOS), a prototype pro-inflammatory enzyme responsible for NO production. The results showed that GGE03 treatment significantly decreased the mRNA expression of iNOS as determined by RT-PCR and qRT-PCR in *H. pylori*-infected AGS cells in a dose-dependent manner (Fig. 4A). GGE03 also inhibited the iNOS-induced production of NO in AGS cells to a greater extent than GE (Fig. 4B).

Effect of GGE03 on *H. pylori*-induced MPO activity and production of IFN-γ in AGS cells

MPO activity is used as an indicator of neutrophil infiltration in inflamed site [17]. Because of the abundance of this enzyme, it is used as an index for *H. pylori*-induced infiltration of neutrophils [18]. As shown in Figure 5, MPO activity was significantly increased in *H. pylori*-infected AGS cells compared to that in non-treated cells. GGE03 treatment attenuated the

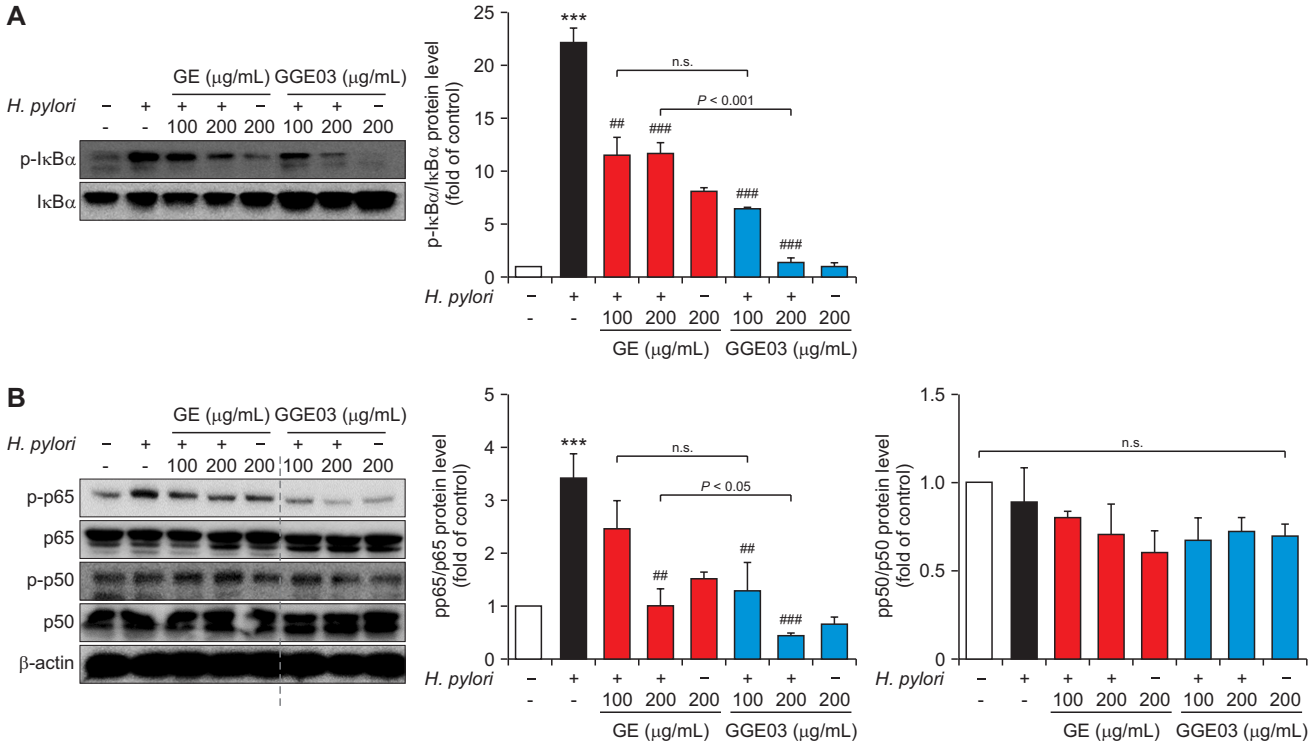

Figure 3. GGE03 exerts anti-inflammatory effects by inhibiting the NF-κB signaling pathway in *Helicobacter pylori* (*H. pylori*)-infected AGS cells. (A) AGS cells were pretreated with indicated concentrations of GE and GGE03 for 1 hour and then infection was induced by *H. pylori* (50 multiplicity of infection [MOI]) for 1 hour. The expression of p-IκBα and IκBα in AGS cells were determined by Western blotting. (B) AGS cells were pretreated with indicated concentrations of GE and GGE03 for 1 hour and then infection was induced by *H. pylori* (50 MOI) for 1 hour. The expression of p-p65, p65, p-p50, p50 and β-actin in AGS cells was determined by Western blotting. Results are presented as the mean ± SD. Statistical significance was analyzed by ANOVA. n.s., not significant. $^{***}P < 0.001$ compared to control; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to the *H. pylori* group.

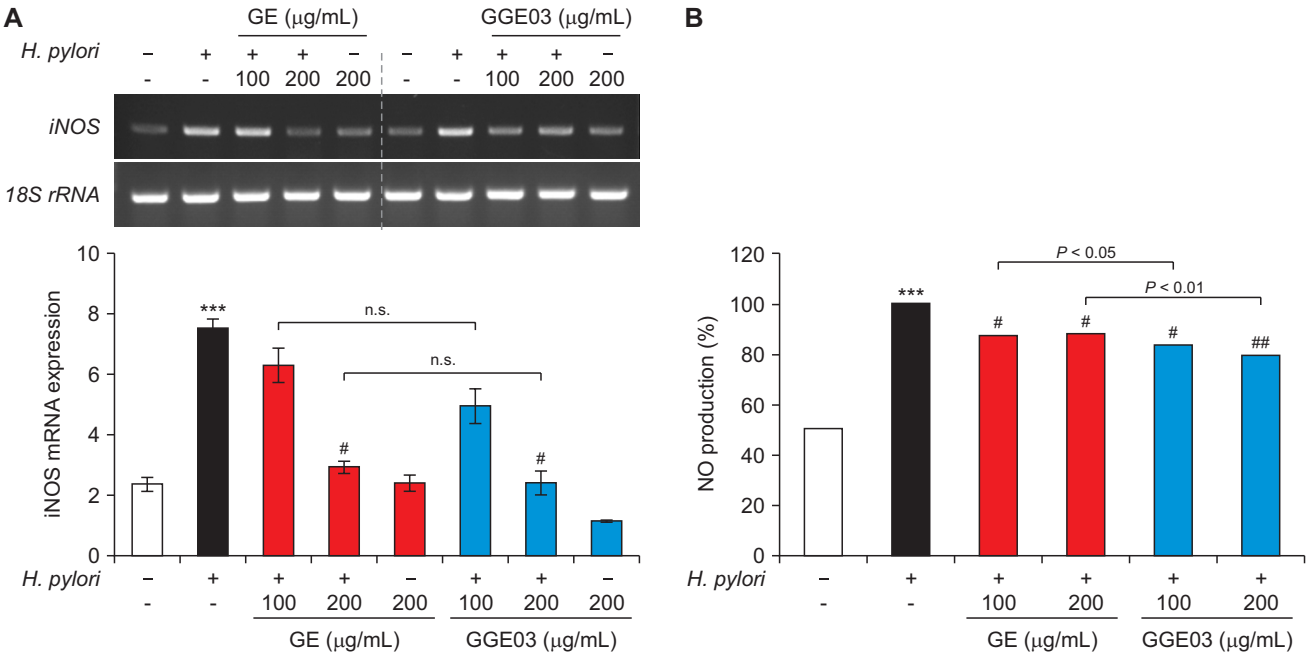

Figure 4. GGE03 inhibits the production of nitric oxide (NO) through inducible NOS (iNOS) in *Helicobacter pylori* (*H. pylori*)-infected AGS cells. (A) AGS cells were pretreated with GE and GGE03 indicated concentrations for 1 hour, followed by 1.5 hours co-culture with *H. pylori* (50 multiplicity of infection [MOI]). After incubation, the culture supernatants were collected for measurement of NO. (B) AGS cells were pretreated with GE and GGE03 at indicated concentrations for 1 hour, followed by 3.5 hours co-culture with *H. pylori* (50 MOI). The effect of GE and GGE03 on the expression of iNOS in AGS cells was analyzed by reverse transcription PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR). 18S rRNA was used as an internal control for the expression of cytokines. Data are the mean ± SD. Statistical significance was analyzed by ANOVA. n.s., not significant. ***$P < 0.001$ compared to control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ compared to the *H. pylori* group.

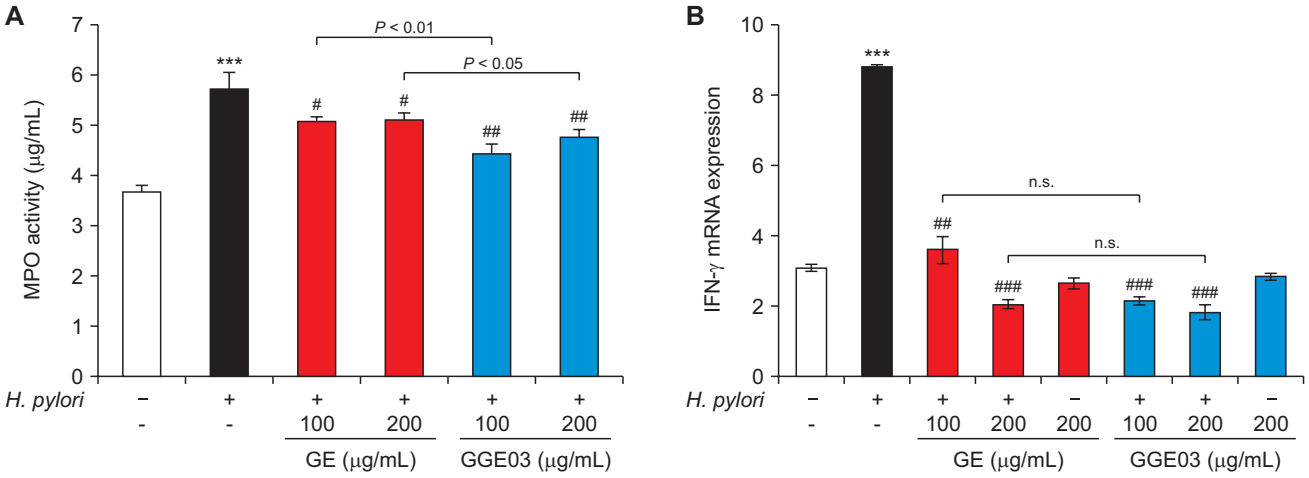

Figure 5. GGE03 inhibits *Helicobacter pylori* (*H. pylori*)-induced myeloperoxidase (MPO) activity and expression of IFN-γ in AGS cells. (A) AGS cells were pretreated with GE and GGE03 at indicated concentrations for 1 hour, followed by 1.5 hours co-culture with *H. pylori* (50 multiplicity of infection [MOI]). After incubation, the cell lysates were collected for determination of MPO activity as described in Materials and Methods. (B) AGS cells were pretreated with GE and GGE03 indicated concentrations for 1 hour, followed by 12 hours co-culture with *H. pylori* (50 MOI). The effect of GE and GGE03 on the expression of IFN-γ in AGS cells was analyzed by qRT-PCR. 18S rRNA was used as an internal control. Data are the mean ± SD. Statistical significance was analyzed by ANOVA. n.s., not significant. ***$P < 0.001$ compared to control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ compared to the *H. pylori* group.

H. pylori-induced elevation of MPO activity in AGS cells (Fig. 5A). Increased expression of IFN-γ after *H. pylori* infection has been reported in the gastric tissues of humans [19]. *H. pylori* is known to stimulate IFN-γ production from peripheral blood mononuclear cells and lymphocytes [20]. *H. pylori* infection significantly increased the mRNA levels of IFN-γ, and the treatment with GGE03 lowered IFN-γ levels in a dose dependent manner (Fig. 5B). These results demonstrate that

GGE03 may inhibit *H. pylori*-induced neutrophil influx and the expression of inflammatory mediators in AGS cells.

The minimum inhibitory concentration for the anti-inflammatory effect of GGE03 in *H. pylori*-infected AGS cells

To assess the minimum concentration of GGE03 exerting anti-inflammatory responses in *H. pylori*-infected AGS cells, we monitored the expression of the pro-inflammatory cytokines upon treatment with GGE03 at the concentrations of 50, 100, and 200 μg/mL. As qRT-PCR data shown in Figure 6, GGE03 differentially inhibits the mRNA levels of IL-8, TNF-α, IL-6 and iNOS at the concentration of 50 μg/mL or higher in *H. pylori*-infected AGS cells. Thus, 50 μg/mL of GGE03 significantly inhibited the expression of IL-8 mRNA, a major pro-inflammatory cytokine expressed upon *H. pylori* infection in AGS cells whereas TNF-α, IL-6 and iNOS were not altered (Fig. 6).

DISCUSSION

H. pylori infection occurs more than 50% of the world's population and is widely known as the cause of gastric diseases [21]. *H. pylori* infection promotes gastritis, gastric ulcer, chronic gastritis, and gastric cancer by changing the stomach pH [8]. When *H. pylori* is attached to gastric epithelial cells, it releases various virulence factors such as CagA and vacuolating cytotoxin (VacA) into the epithelial cells using a type IV secretion system [22]. In particular, CagA acts as a signaling molecule in the cell and induces upregulation of NF-κB, extracellular signal-regulated kinase and IL-8 [23]. Chronic inflammation caused by *H. pylori* can lead to the development of gastric adenocarcinoma [24]. However, there are limitations for treatment for *H. pylori* infection due to antibiotic resistance [25,26]. Therefore, development of new agents to prevent or treat gastric damage caused by *H. pylori* is still needed.

It has been reported that *H. pylori* has different virulence factors released by each strain [27]. There are several differ-

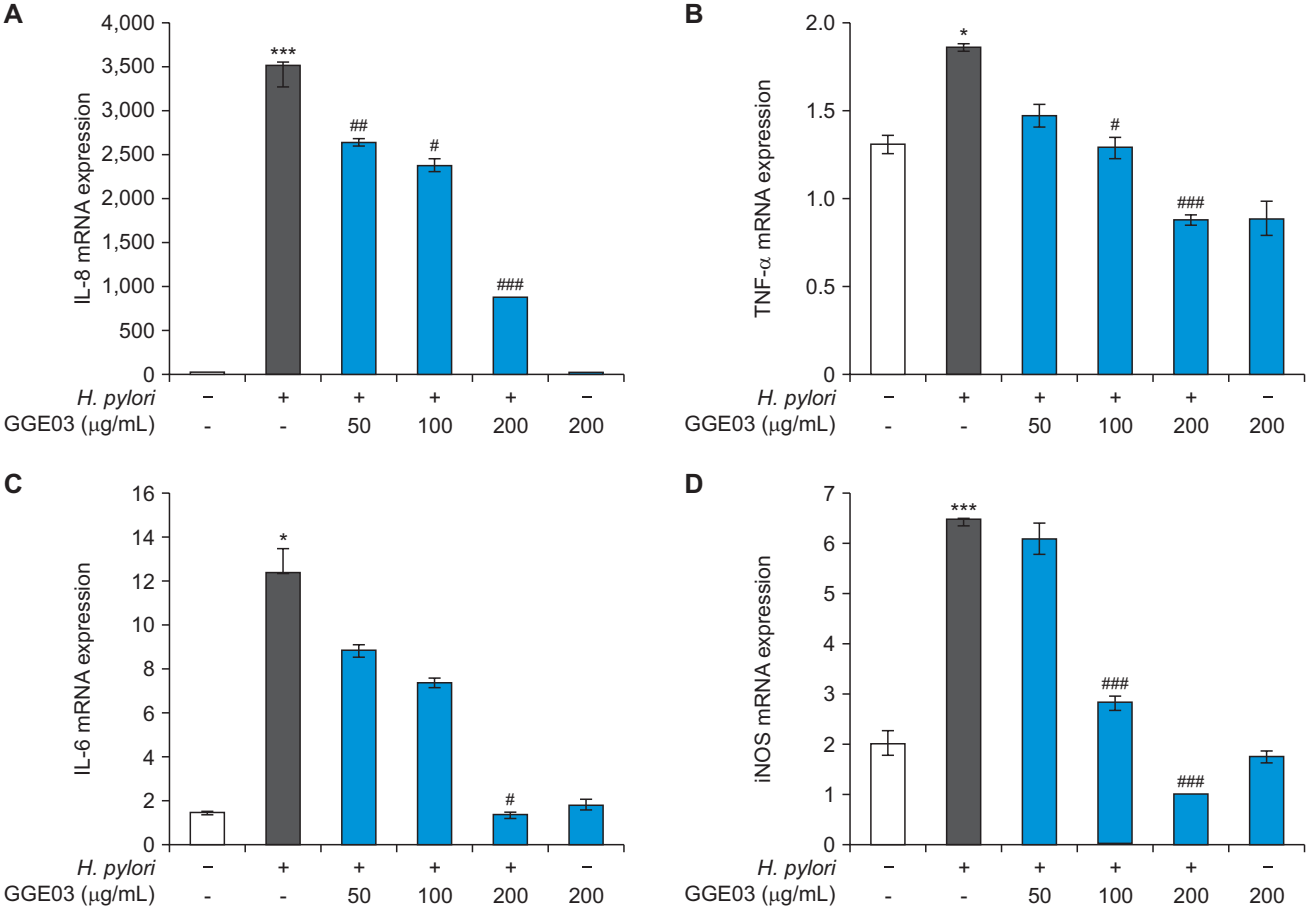

Figure 6. The minimum dose of GGE03 required for exerting anti-inflammatory effects in *Helicobacter pylori* (*H. pylori*)-infected AGS cells. AGS cells were pretreated with GGE03 at indicated concentrations for 1 hour, followed by 3.5 hours co-culture with *H. pylori* (50 multiplicity of infection [MOI]). The effects of GGE03 on the mRNA expression of IL-8 (A), TNF-α (B), IL-6 (C), and iNOS (D) in AGS cells were determined by quantitative real-time PCR (qRT-PCR). 18S rRNA was used as an internal control for the expression of pro-inflammatory cytokines. Data are the mean ± SD. Statistical significance was analyzed by ANOVA. IL, interleukin; iNOS, inducible NOS. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared to control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ compared to the *H. pylori* group.

ent types of *H. pylori* strains. In this study, we used the 43504 strain. Because the *H. pylori* 43504 strain is positive for CagA and VacA, it is widely used in gastric inflammation studies [28]. In this study, we compared the inhibitory effects of GE and GGE03 on *H. pylori* growth and *H. pylori*-induced inflammation. The growth inhibitory effect of GGE03 was demonstrated with the *H. pylori* 43504 strain whereas GE had an marginal effect. These results suggest that GGE03 has the potential to protect gastric epithelial cells from virulence factors such as CagA and VacA by inhibiting the growth of *H. pylori*.

Next, we investigated that the anti-inflammatory responses using cultured AGS cells infected with *H. pylori*. The transcription factor NF-κB regulates the initiation of inflammatory responses induced upon bacterial infection in gastrointestinal cells [11,15,29]. GGE03 was shown to reduce the *H. pylori*-induced up-regulation of pro-inflammatory cytokines such as IL-8, TNF-α, IL-6 and IL-1β by inhibiting NF-κB activation. This is associated with phosphorylation and subsequent ubiquitin-mediated degradation of IκBα, leading to increased nuclear translocation of the NF-κB subunits, such as p65 and p50 [30]. This induces the expression of NF-κB-related target genes including IL-8, TNF-α, IL-6 and IL-1β [14]. The treatment with GGE03 inhibited the *H. pylori*-induced phosphorylation of IκBα protein and also significantly reduced the phosphorylation of p65. The results showed that the effect of GGE03 was significantly greater than GE in inflammation responses in *H. pylori*-infected AGS cells. Moreover, the suppression of pro-inflammatory cytokines such as IL-8 and IL-1β was significantly different between the GGE03 and GE at an 100 μg/mL concentration.

Production of NO is a feature of gastric epithelial cells as well as genuine immune cells including macrophages upon inflammatory insult [31-33]. In gastric epithelial cells, *H. pylori* produces NO, a ubiquitous free radical synthesized by the enzyme NO synthetase (NOS), via the oxidation of L-arginine [16]. iNOS, one of the NOS isoforms, is regulated at the transcriptional level when cells are challenged by cytokines or pathogens [31]. The previous studies revealed that generation of NO activates NF-κB, thereby triggering the inflammation in tumors and other pathological conditions [34,35]. As a consequence of *H. pylori* infection, iNOS expression and NO production were increased in gastric epithelial cells, which accelerates the inflammatory responses, implicated in gastric diseases including gastritis [16]. In this study, we confirmed that GGE03 treatment significantly reduced NO production and expression of iNOS mRNA in *H. pylori*-infected AGS cells. In addition, we revealed that NO production was inhibited by GGE03 to a greater extent than GE. According to the previous studies, *H. pylori* infection provokes the persistent neutrophil infiltration and up-regulation of IFN-γ in the gastric mucosa [19,36]. In line with this notion, our data showed that the IFN-γ expression and MPO activity were increased in *H. pylori* infection that were reduced by GGE03 treatment.

Finally, we investigated the minimum inhibitory concentration of GGE03 for anti-inflammatory effect on *H. pylori* infection. Our study revealed that the IL-8, a representative inflammatory cytokine released upon *H. pylori* infection, was significantly reduced at the 50 μg/mL concentration of GGE03. In contrast, TNF-α, IL-6 and iNOS were not significantly inhibited at the same concentration. Furthermore, we calculated the human equivalent dose for the effect of GGE03. GGE03 was found to protect against EtOH/HCL-induced rat gastric mucosal injury at concentrations of 100 to 300 mg/kg [5]. Based on our findings and others, we estimate that an amount of 400 to 1,000 mg/day GGE03 is required for a 60 kg subject as calculated by the recommended body surface area normalization method [37]. However, in order to determine the exact concentration of the anti-inflammation effect of GGE03 against *H. pylori* infection, an in vivo studies need to be conducted.

To sum up, GGE03 suppresses the expression of pro-inflammatory cytokines, NO production and MPO activity in *H. pylori*-infected gastric epithelial cells. GGE03 appears to have more potent anti-inflammation effects compared to GE. Collectively, it is possible that GGE03 might serve as a preventive formula for *H. pylori*-associated gastritis.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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