Int J Clin Exp Med 2016;9(2):2479-2485
www.ijcem.com /ISSN:1940-5901/IJCEM0016631

Original Article

The green tea polyphenol epigallocatechin-3-gallate effectively inhibits *Helicobacter pylori*-induced gastritis in Mongolian gerbils

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Received September 22, 2015; Accepted January 21, 2016; Epub February 15, 2016; Published February 29, 2016

Abstract: Background: Epigallocatechin-3-gallate (EGCG) is a major catechin of green tea; it has protective effects against injury and exhibits anti-inflammatory activity. *Helicobacter pylori* (*H. pylori*) eradication rates with clarithromycin-based triple therapy are declining, and an alternative strategy is urgently needed. The activity of EGCG against *H. pylori*-infected gastritis was investigated in an in vivo Mongolian gerbil model. Methods: Mongolian gerbils were randomly divided into *H. pylori*-infected, *H. pylori*-infected + drinking water containing 0.05% EGCG, triple treatment (amoxicillin, clarithromycin and esomeprazole), and control groups. After 12 weeks, gastric pH tests and histopathological evaluations were performed, and mucosal interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) levels in the gastric mucosa were investigated. Results: Significant inflammatory mucosal changes were observed in the *H. pylori* infection groups. The EGCG and triple-drug treatments significantly decreased the severity of gastritis in the antrum and the corpus. The mRNA levels of IL-1β, TNF-α, COX-2 and iNOS were increased in the *H. pylori*-infected gastric mucosa and obviously lower in the EGCG group than in the *H. pylori*-infection groups. Conclusions: The activations of IL-1β, TNF-α, COX-2 and iNOS were essential for *H. pylori*-induced gastritis in Mongolian gerbils. EGCG exhibited anti-inflammatory effects that might be mediated through the inhibitions of IL-1β, TNF-α, COX-2 and iNOS in the gerbil model of *H. pylori*-induced inflammation.

Keywords: Epigallocatechin-3-gallate, *Helicobacter pylori*, gastritis, inflammation

Introduction

Helicobacter pylori (*H. pylori*) colonizes over 50% of the world's population and is definitely associated with various gastro-duodenal diseases [1]. Epidemiological studies have demonstrated that *H. pylori* infection in the gastric mucosa is related to chronic gastritis, peptic ulcers, and gastric cancer [2, 3]. *H. pylori* was designated as a class I gastric carcinogen by the WHO in 1994 [4]. *H. pylori* eradication is very important for the prevention of gastric carcinogenesis. Triple therapy consisting of a proton pump inhibitor and a combination of two antibiotics (i.e., amoxicillin and clarithromycin or amoxicillin and metronidazol) is commonly used for *H. pylori* eradication [5, 6]. However, recently, the occurrence of drug-resistant H. pylori and the adverse effects of the antibiotics have severely limited eradication therapy [7, 8]. Thus, alterative agents to antibiotics are needed, and the development of more effective and safer therapy using such agents has become urgent. There are many reports that some non-antibiotic substances from natural products potentially exhibit anti-*H. pylori* properties. Numerous phytochemicals have been reported to have cancer preventive activities [9-13].

EGCG is a major catechin in green tea that accounts for 50% to 80% of the catechin in green tea [14]. EGCG has been demonstrated to have beneficial effects in studies of diabetes and obesity and possesses antioxidant activity;

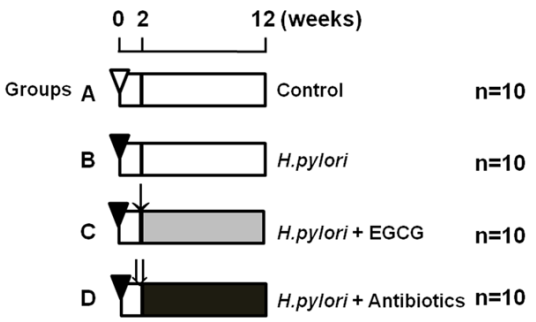

Figure 1. Experimental design. Animals: Six-week-old male Mongolian gerbils; ▼: *H. pylori*-infected (ATCC43504). ▽: broth; □: antibiotics: clarithromycin (CAM), amoxicillin (AMPC), and esomeprazole; ↓: EGCG: epigallocatechin gallate.

however, there are no reports of the anti-*H. pylori* infection effects of EGCG based on in vivo experiments [15, 16].

Currently, the Mongolian gerbil is considered a stable animal model of *H. pylori*-infection induced gastric disease. In this study, we established an *H. pylori*-infected Mongolian gerbil animal model to study the anti-*H. pylori* infection effects EGCG and the mechanisms of these effects.

Materials and methods

Animals and H. pylori inoculation

Six-week-old male specific pathogen-free Mongolian gerbils (*Meriones unguiculatus*; MGS/Sea) were purchased from the Zhejiang Medical Research Institute (Hangzhou, China) and maintained in an air-conditioned biohazard room with free access to a commercial rodent diet and water.

H. pylori ATCC 43504 [American Type Culture Collection (ATCC), Manassas, VA, USA] was grown in Brucella broth supplemented with 7% fetal calf serum at 37°C under microaerophilic conditions for 48 hours. The animals were fasted for 24 h before the first *H. pylori* inoculation, and the animals were then inoculated with 0.8 ml of broth culture containing 1×10^8 colony-forming units (cfus) of *H. pylori* via gastric intubation three times at 48 hours intervals. All experiments and procedures involving the animals were approved by the Animal Care Committee of the First Hospital of Jilin University.

Experimental protocol

The Mongolian gerbils were divided into the following four groups: a blank control group, a simple *H. pylori* infection pathological model group, an *H. pylori* infection with amoxicillin, clarithromycin and esomeprazole triple-drug control group, and an *H. pylori* infection with EGCG group. Groups A, B, and C were inoculated with *H. pylori* at 0 weeks. Group D received Brucella broth without *H. pylori*. At 12 weeks, the animals were sacrificed (**Figure 1**). All animals were subjected to deep ether anesthesia after 24 h of fasting, laparotomized, exsanguinated from the inferior vena cava, and their stomachs were then excised. H&E staining-based pathological examinations were performed, and gastric tissue specimens were immunohistochemically examined. For the intragastric pH measurements, the stomachs were excised, and the pHs of the gastric contents were immediately measured using an Orion 9863BN Micro pH electrode (Thermo Fisher Scientific Inc, Waltham, MA, USA).

Tissue collection, H&E staining and pathological assessment

The stomachs were resected and cut along the greater curvature. The samples were divided into two parts. One part was immediately snap frozen in liquid nitrogen and stored at -70°C for RNA extraction. The other part was fixed in 10% neutral buffered formalin and wax embedded for H&E and immunohistochemical analyses.

Tissue sections were stained with hematoxylin-eosin for histological analysis. The glandular mucosa was histologically examined for any inflammatory and epithelial changes. Active chronic gastritis was characterized by the infiltration of neutrophils and lymphocytes. The degree of inflammatory change was graded on a 4 point scale (0, normal; 1, mild; 2, moderate; and 3, marked gastritis) according to criteria modified from the updated Sydney System [17].

RNA isolation and qRT-PCR quantification

The cytokines were analyzed with real-time quantitative PCR. Total RNA was extracted from the glandular stomach mucosa at the antrum and corpus using an RNA extraction kit (Invitrogen Life Technologies, Carlsbad, CA, USA). After DNase treatment, first-strand cDNAs were synthesized using the Thermoscript

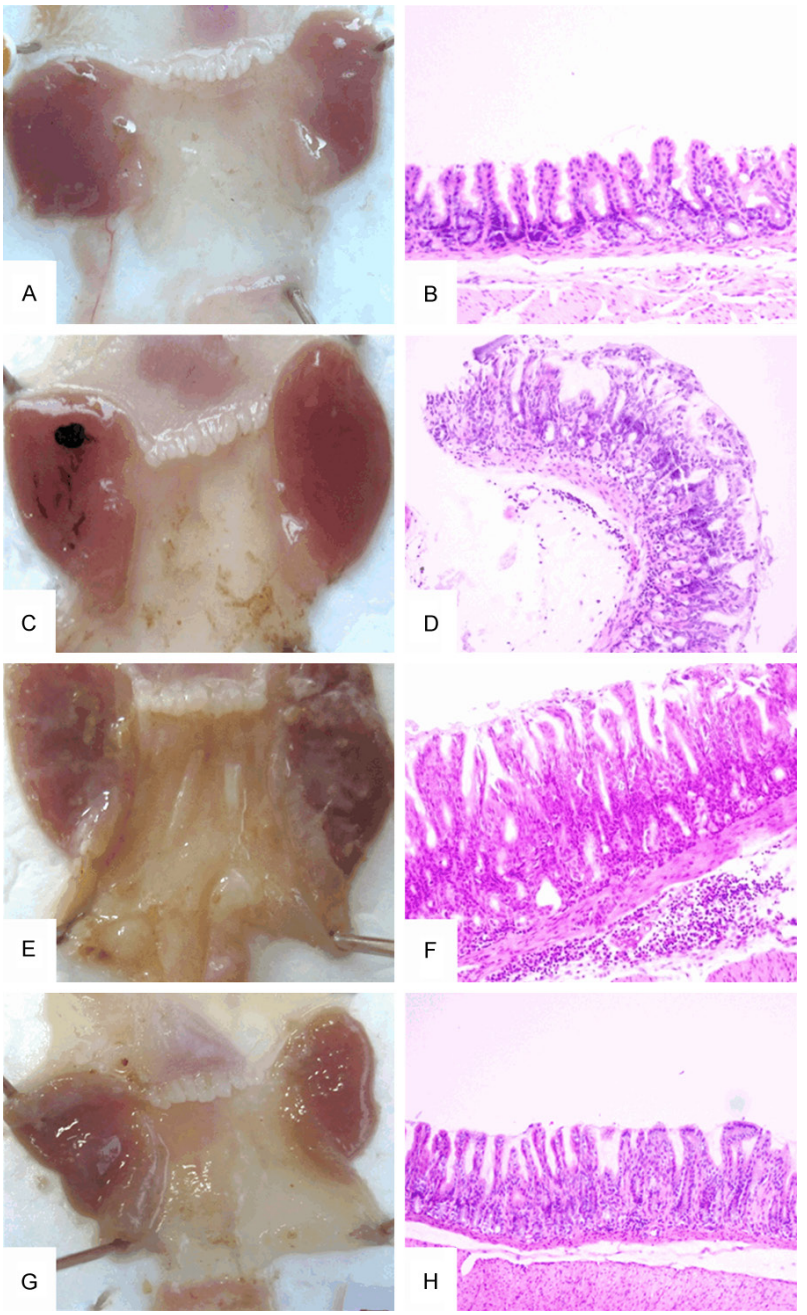

Figure 2. Morphological and histopathological findings from the gastric mucosae of the gerbils. *A*, *E*: Normal gastric mucosae from the control groups. *B, F*: Marked inflammation and erosion in the *H. pylori*-infected groups. *C, G*: Marked gastritis with lymphoid follicle formation in an *H. pylori* infected gerbil. *D, H*: Mild gastritis in an EGCG-treated gerbil (H&E; × 10).

RT-PCR System (Invitrogen, Carlsbad, CA, USA). Quantitative PCRs for interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) were performed with the ABI7500 Real-time PCR system. The gerbil-specific glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control. The primer sequences are listed in a previous study [10]. The expression levels of the cytokine mRNAs are expressed relative to 1.0 as defined by the control group.

Statistical analyses

The gastritis scores, mRNA expression levels, and PH values are given as the means ± the SDs. Fisher's exact tests and the Bonferroni multiple-comparison tests were performed to establish the significance of the differences at the cut-off of $P < 0.05$. All analyses were performed using SPSS software (version 11.0).

Results

The survival rates in all groups were 100% at the end point of the experiment. The bacterial culture and pathological examinations revealed that the *H. pylori* infection rate was 100% in this study.

Histopathology

All gastric mucosal specimens from the control gerbils exhibited normal histomorphologies. At 12 weeks, significant inflammatory mucosal changes were observed in the *H. pylori* infection group. The *H. pylori*-infected gerbils (group B) exhibited greater lymphoplasmocytic infiltration and submucosal lymphoid follicle formation than the control groups in the antral mucosa. The inflammatory changes were significantly attenuated in the *H. pylori* infection + EGCG group and the triple-drug control group (**Figure 2**). The histological findings from the

Table 1. Histopathological findings of gastric mucosa in *H.pyrori*-infected gerbils

	Infiltration of inflammatory cells	Hyperplasia	Erosion and ulcer	Infiltration of inflammatory cells	Hyperplasia	Erosion and ulcer
Group A	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Group B	1.5 ± 0.1	1.5 ± 0.1	1.8 ± 0.3	2.9 ± 0.3	2.2 ± 0.3	2.1 ± 0.3
Group C	0.9 ± 0.2*	0.7 ± 0.2*	0.9 ± 0.2*	2.1 ± 0.4*	1.5 ± 0.1*	1.2 ± 0.3*
Group D	0.8 ± 0.2*	0.6 ± 0.3*	0.8 ± 0.2*	1.8 ± 0.4*	1.6 ± 0.3*	1.0 ± 0.0*

*$P < 0.05$, compared with group B. Values for results are expressed as means ± SD.

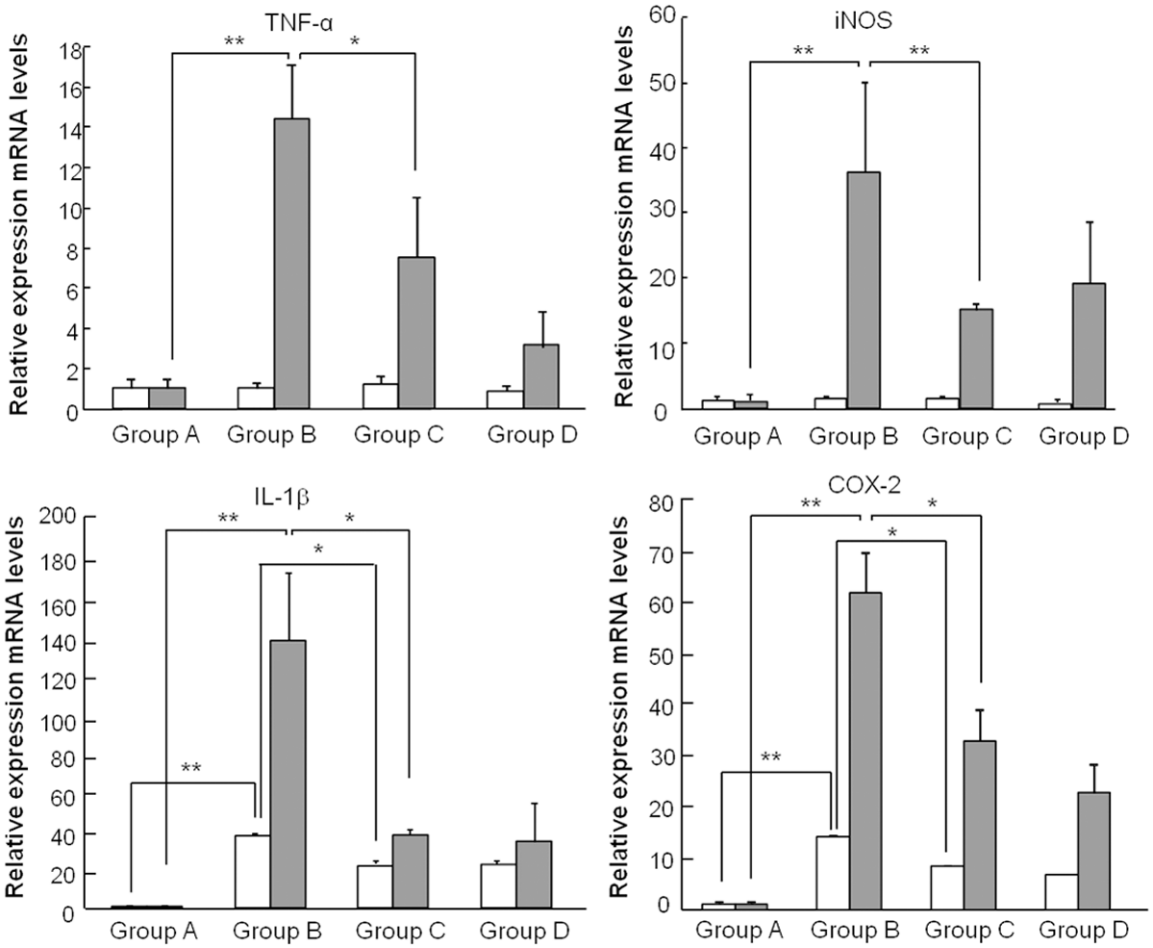

Figure 3. Relative expression levels of IL-1β, TNF-α, COX-2 and iNOS mRNAs in the glandular stomachs of the gerbils. Note the relative increases in IL-1β, TNFα, COX-2 and iNOS mRNA expression levels in Group B compared to Group A. The cytokines were significantly down-regulated following the administration of EGCG (Group C) and triple antibiotics (Group D). The values are relative to those of Group A (1.0) and are expressed as the means ± the SEs.*$P < 0.05$ and **$P < 0.01$. □: corpus; ■: antrum.

gastric mucosal specimens from the gerbils are summarized in **Table 1**.

Expressions of the cytokine mRNAs in the gastric mucosa

The IL-1β, TNF-α, COX-2 and iNOS mRNA levels were very low in the uninfected gerbils. The *H. pylori* infections increased the IL-1β and COX-2 expressions in both the antrum and corpus, whereas TNF-α and iNOS were only increased in antrum (**Figure 3**). The cytokines were significantly down-regulated by the EGCG and triple-drug administrations. In the *H. pylori*-infected gerbils, the IL-1β, TNF-α, COX-2, and iNOS

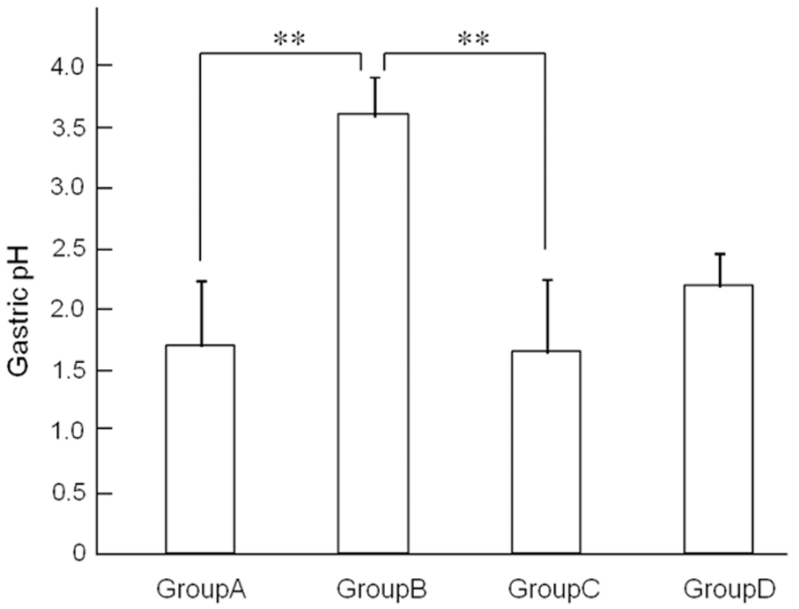

Figure 4. Gastric pH changes following *H. pylori* infection and treatment. Note that the pHs of the gastric contents increased after 12 weeks of *H. pylori* infection and significantly decreased after EGCG administration. The data are presented as the means ± the SEs.

mRNA levels were significantly correlated with the inflammation scores for the gastric mucosa.

Gastric juice pH values

The average pH value of the gastric juice of the uninfected groups was 1.7 ± 0.4 and increased to 3.6 ± 0.6 after 12 weeks of *H. pylori* infection ($P < 0.01$); however, the pH values decreased to 2.2 ± 0.3 following triple-antibiotic administration ($P < 0.01$) and to 1.8 ± 0.5 following EGCG administration ($P < 0.01$; **Figure 4**).

Discussion

In the present study, we successful established an *H. pylori* infection-induced gastritis model in Mongolian gerbils and proved that EGCG exhibited an *H. pylori* infection-inhibiting effect in an in vivo experiment. Our findings proved that EGCG inhibited inflammation and proliferation in the gastric mucosa and that EGCG treatment was not significantly different from the commonly clinical used amoxicillin, clarithromycin and esomeprazole triple treatment.

Green tea is an extremely popular beverage worldwide, and its habitual consumption has long been associated with health benefits including chemo-preventive efficacy [18]. The major catechins in green tea are EGCG, (-)-epicatechin-3-gallate, (-)-epigallocatechin, and (-)-epicatechin [19]. In 1987, Yoshizawa S et al. reported that the repeated application of EGCG prevents tumor promotion in murine skin [20]. Recently, many studies have indicated that EGCG has the potential to influence a variety of human diseases, and a new trend had demonstrated that green tea catechins act synergistically with anticancer compounds [21-23].

The current results indicated that the mechanisms of the anti-inflammation activity of EGCG are associated with the inhibitions of the activities of IL-1β and COX activity and the inhibition of the expressions of iNOS and TNFα. EGCG has been reported to inhibit the activity of COX-2. Inappropriate COX-2 activity has been observed in practically every premalignant and malignant condition involving the colon, liver, and stomach [24]. EGCG has been demonstrated to reduce the activity of COX-2 following the stimulation of human chondrocytes with interleukin-1β stimulation [25]. Pan H et al. reported that EGCG attenuates cisplatin-induced TNFα and IL1β mRNA expression in mice to 30% and 41%, respectively [26]. The actions of the EGCG have been evidenced by reductions in inflammatory factors, including the suppression of the expressions of TLR4, NFκB and iNOS and reductions in the release of TNF-α, IL-1β and IL-6 [27].

Basically, natural products that are consumed daily are safe and beneficial for humans. If components that are identified as effective in vitro actually exhibit weaker anti-*H. pylori* activities in vivo, the intake of these foodstuffs will cause no serious problems to human health. Further studies are warranted to identify the underlying mechanisms by which *H. pylori* infection affects the liver and the clinical impor-

tance of these effects. Therefore, the present experiments provide a reference drug for studies of the clinical prevention and treatment of *H. pylori* infection and the primary prevention of gastric cancer [28].

Acknowledgements

This study was partially supported by the National Natural Science Foundation of China (No. 81273065 and 81373084) and the Norman Bethune Program of Jilin University (No. 2013025).

Disclosure of conflict of interest

None.

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