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Research article

Protective effect of Korean Red Ginseng extract against *Helicobacter pylori*-induced gastric inflammation in Mongolian gerbils

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ABSTRACT

Helicobacter pylori-induced gastric inflammation includes induction of inflammatory mediators interleukin (IL)-8 and inducible nitric oxide synthase (iNOS), which are mediated by oxidant-sensitive transcription factor NF-κB. High levels of lipid peroxide (LPO) and increased activity of myeloperoxidase (MPO), a biomarker of neutrophil infiltration, are observed in *H. pylori*-infected gastric mucosa. *Panax ginseng* Meyer, a Korean herb medicine, is widely used in Asian countries for its biological activities including anti-inflammatory efficacy. The present study aims to investigate whether Korean Red Ginseng extract (RGE) inhibits *H. pylori*-induced gastric inflammation in Mongolian gerbils. One wk after intragastric inoculation with *H. pylori*, Mongolian gerbils were fed with either the control diet or the diet containing RGE (200 mg RGE/gerbil) for 6 wk. The following were determined in gastric mucosa: the number of viable *H. pylori* in stomach; MPO activity; LPO level; mRNA and protein levels of keratinocyte chemoattractant factor (KC, a rodent IL-8 homolog), IL-1β, and iNOS; protein level of phospho-IκBα (which reflects the activation of NF-κB); and histology. As a result, RGE suppressed *H. pylori*-induced mRNA and protein levels of KC, IL-1β, and iNOS in gastric mucosa. RGE also inhibited *H. pylori*-induced phosphorylation of IκBα and increases in LPO level and MPO activity of gastric mucosa. RGE did not affect viable *H. pylori* colonization in the stomach, but improved the histological grade of infiltration of polymorphonuclear neutrophils, intestinal metaplasia, and hyperplasia. In conclusion, RGE inhibits *H. pylori*-induced gastric inflammation by suppressing induction of inflammatory mediators (KC, IL-1β, iNOS), MPO activity, and LPO level in *H. pylori*-infected gastric mucosa.

1. Introduction

Helicobacter pylori infection leads to gastroduodenal inflammation, peptic ulceration, and gastric carcinoma [1,2]. *H. pylori* infection is reported to include pathologic changes of the stomach, including edema and congestive surface epithelium [3]. A characteristic event in gastritis is the infiltration of the subepithelial gastric lamina propria by phagocytes, mainly neutrophils and macrophages, that produce large amounts of reactive oxygen species (ROS). ROS activate the oxidant-sensitive transcription factor NF-κB, which induces expression of the inflammatory genes, oncogenes, and cell-cycle regulators [4,5]. *H. pylori*-induced gastric mucosal injury and inflammation are mediated by proinflammatory cytokines such as interleukin (IL)-8 and IL-1β as well as inflammatory enzymes, including inducible nitric oxide synthase (iNOS). Transcription of these inflammatory mediators is regulated by the oxidant-sensitive transcription factor NF-κB [6–10]. NF-κB is an inducible transcription factor composed of p50/p65 (heterodimer) or p50 (homodimer) [11]. NF-κB is retained in the cytoplasm by binding to the inhibitory protein IκBα. Extracellular stimuli

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trigger rapid degradation of IκBα by proteasomes, allowing NF-κB to translocate into the nucleus and bind to the DNA sites of target genes, including IL-8, IL-1β, and iNOS [12]. Therefore, degradation of IκBα represents activation of NF-κB.

H. pylori-elicited neutrophils produce ROS, which subsequently injure gastric mucosal cells [13]. ROS cause peroxidation of membrane lipids, thus increasing the level of lipid peroxide (LPO) in the damaged tissues. We previously demonstrated that LPO production increases in parallel with IL-8 production in *H. pylori*-infected cells [7]. Myeloperoxidase (MPO) is more abundantly expressed in neutrophils than other cells and thus, is used as a biomarker for neutrophil infiltration [14]. In neutrophils, MPO produces hypochlorous acid from hydrogen peroxide and chloride anion during respiratory bursts. Furthermore, it oxidizes tyrosine to form tyrosyl radicals using hydrogen peroxide. Both hypochlorous acid and tyrosyl radicals cause lipid peroxidation sequences [15]. Therefore, high levels of LPO and increased MPO activity could reflect oxidative damage and inflammatory responses of cells.

Korean Red Ginseng, which is the steamed root of a 6-year-old Korean ginseng (*Panax ginseng* Meyer), is used in Asian countries as a traditional medicine for the treatment of various diseases, including inflammatory disorders [16–18]. The most effective components of Korean Red Ginseng are triterpeneglysides known as ginsenosides [19]. Ginsenosides have anti-inflammatory [20,21] and anticancer effects [22]. An *in vitro* study showed that Korean Red Ginseng inhibited adhesion of *H. pylori* to gastric epithelial cells [23]. Korean Red Ginseng extract (RGE) inhibits *H. pylori*-induced oxidative damage in gastric epithelial cells [24,25]. Previously we showed hepatoprotective effects of Korean Red Ginseng in rats and mouse liver, which may be contributed by its antioxidant activity [26,27]. Therefore, the antioxidant or anti-inflammatory effects of RGE, containing ginsenosides, may protect gastric mucosa from inflammation caused by *H. pylori* infection.

In the present study, we investigated whether RGE protects against *H. pylori*-induced gastric inflammation in Mongolian gerbils. Animal models for *H. pylori* infection have been developed to replicate many features of human gastric inflammation and carcinogenesis in order to test potential therapeutic agents for the prevention and treatment of *H. pylori*-associated gastric disease. The Mongolian gerbil model is the best animal model for this purpose because *H. pylori* infection induces chronic gastritis, gastric ulcers, and intestinal metaplasia in these animals. Mongolian gerbils develop gastric neoplasia and gastric cancer after chronic infection by *H. pylori* strain 7.13 [28,29], as used in the present study. After the infection of gerbils with *H. pylori*, we determined: the changes in LPO level, which is an index of oxidative membrane damage; the activity of MPO, a biomarker of neutrophil infiltration; the induction of inflammatory mediator keratinocyte chemoattractant factor (KC), an IL-8 homolog in rodents [30]; IL-1β; iNOS; and the phosphorylation of IκBα, which reflects the activation of NF-κB. In addition, viable *H. pylori* colonization in the stomach, changes in food intake and body weight, stomach weight/total body weight, and histological analysis of gastric mucosa were compared between animals that received RGE and those that did not.

2. Materials and methods

2.1. Animals

Five-wk-old male specific-pathogen-free Mongolian gerbils (MGS/Sea) with an average weight of approximately 40 g were purchased from Charles River Laboratories (Wilmington, MA, USA). Gerbils were housed in polypropylene cages on hard wood chip bedding in groups of five/cage. Food and water were provided *ad libitum*. The animals were maintained in a temperature-controlled room (22 ± 2°C) with a 12-h light–dark cycle. The animal experiments were performed in accordance with institutional guidelines. Protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Yonsei University Medical Center (Seoul, Korea; Permit No.: 10-107). Ten gerbils were included in each group. Histological observations are reported for 10 gerbils/group. All animals were maintained in the specific pathogen-free facility at Yonsei University Medical Center.

2.2. Bacterial inoculation

H. pylori strain 7.13 was maintained as frozen stock at −80°C in brain–heart infusion medium supplemented with 20% glycerol and 10% fetal bovine serum. Bacteria were grown on horse blood agar plates containing 4% Columbia agar base (Oxoid, Basingstoke, Hampshire, UK), 5% defibrinated horse blood (HemoStat Labs, Dixon, CA, USA), 0.2% β-cyclodextrin, 10 μg/mL vancomycin, 5 μg/mL cefsulodin, 2.5 U/mL polymyxin B, 5 μg/mL trimethoprim, and 8 μg/mL amphotericin B at 37°C under microaerophilic conditions. A microaerobic atmosphere was generated using a CampyGen sachet (Oxoid) in a gas pack jar. For liquid culture, *H. pylori* was grown in brucella broth (Difco & BBL Diagnostics, Franklin Lakes, NJ, USA) containing 10% FBS (Gibco-BRL, Grand Island, NY, USA). Cultures were shaken in a microaerobic environment. According to the growth curve, 10^8 bacteria were collected and resuspended in 500 μL of brucella broth for the infection of each animal.

2.3. Preparation of RGE

A standardized water extract of Korean Red Ginseng was prepared and supplied by the Korea Ginseng Corporation (Daejeon, Korea) as described previously [31]. The content of crude saponin in RGE is approximately 7%, and it is composed of the following ginsenosides: 8.27 mg/g of Rb1, 3.22 mg/g of Rb2, 3.90 mg/g of Rc, 1.09 mg/g of Rd, 2.58 mg/g of Re, 1.61 mg/g of Rf, 2.01 mg/g of Rg1, 1.35 mg/g for (20*S*)-Rg2, 1.04 mg/g for (20*S*)-Rg3, and 0.95 of Rh1, respectively [31].

2.4. Experimental design

One wk after inoculation with *H. pylori*, Mongolian gerbils were fed control AIN76A diet (Research Diets, Inc, New Brunswick, NJ, USA) or a diet containing RGE (200 mg RGE/each gerbil) for 6 wk. As a negative control, Mongolian gerbils that were not inoculated with *H. pylori* were fed the control diet AIN76A. Gerbils that were inoculated with *H. pylori* were fed the control diet AIN76A and considered as a positive *H. pylori* control.

This level of RGE supplementation (200 mg RGE/gerbil) was adapted from previous studies showing the protective effect of RGE against oxidative stress-mediated epithelial damage [32,33]. Body weight and food intake were measured every wk during the experimental period. At the end of experimental period, gastric mucosal tissues were examined histologically and *H. pylori* colonization was confirmed. For biochemical analyses, gastric mucosal samples were homogenized in 10 mM Tris buffer (pH 7.4). The homogenates were used for determining LPO level, MPO activity, and protein levels of KC, iNOS, phospho-specific IκBα and IκBα. For mRNA level of KC, IL-1β, and iNOS, total RNA was isolated from a gastric mucosal sample by the guanidine thiocyanate extraction method. RGE supplementation had no effect on any of these parameters in animals not infected with *H. pylori*, determined in our preliminary study.