The Effects of Broccoli Sprout Extract Containing Sulforaphane on Lipid Peroxidation and Helicobacter pylori Infection in the Gastric Mucosa

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Background/Aims: The aims of this study were to investigate whether a broccoli sprout extract containing sulforaphane (BSES) inhibited the Helicobacter pylori infection density and exerted an antioxidative effect on gastric mucosal damage. Methods: The enrolled subjects were randomized in a double-blinded manner into three groups. Finally, 33 H. pylori (+) BSES treatment subjects (group A), 28 H. pylori (+) placebo subjects (group B), and 28 H. pylori (-) BSES treatment subjects (group C) were studied. H. pylori infection density was indirectly quantified by a $^{13}$C-urea breath test (UBT), and the ammonia concentration in gastric juice aspirates was measured through gastroscopic examination. Malondialdehyde (MDA), an oxidative damage biomarker, and reduced glutathione (GSH), an antioxidant biomarker, were measured in the gastric mucosa by an enzyme-linked immunosorbent assay. Results: BSES treatment did not significantly affect the UBT values or ammonia concentration in group A (p=0.634 and p=0.505, respectively). BSES treatment did significantly reduce mucosal MDA concentrations in group A (p<0.05) and group C (p<0.001), whereas the gastric mucosal GSH concentrations did not differ before and after treatment in any of the groups. Conclusions: BSES did not inhibit the H. pylori infection density. However, BSES prevented lipid peroxidation in the gastric mucosa and may play a cytoprotective role in H. pylori-induced gastritis. (Gut Liver 2015;9:486-493)

Key Words: Helicobacter pylori; Sulforaphane; Malondialdehyde; Glutathione

INTRODUCTION

Helicobacter pylori infects approximately 50% of the adult population worldwide and is associated with gastritis, peptic ulcer disease, and gastric cancer. In Korea, the H. pylori infection rate in adults 16 to 79 years is approximately 60%, and gastric cancer is the second most frequently diagnosed malignancy. Successful eradication of H. pylori could be beneficial in alleviating H. pylori-related gastroduodenal diseases and reduce the risk of gastric cancer in countries such as Korea, which have a high prevalence of H. pylori infection and high incidence of gastric cancer.

Proton pump inhibitor (PPI)-based triple therapy is the most effective treatment for H. pylori eradication, but has become less successful, with eradication rates as low as 50% to 70%, due to low compliance and high rates of antibiotic resistance. The standard eradication therapy for H. pylori infection involves a 1-week triple therapy, combining a PPI with two antibiotics (i.e., clarithromycin plus amoxicillin or metronidazole). However, primary and acquired metronidazole or clarithromycin resistance has been discovered worldwide, resulting in treatment failure when using regimens including these antibiotics. In Korea, resistance to metronidazole and clarithromycin has been reported in 66.2% and 38.5% of patients, respectively. In addition, high rates of antibiotic-associated side effects may result in poor patient compliance. Therefore, it is necessary to develop new treatment strategies that increase the eradication rate and reduce adverse effects.

Gastric mucosal damage in H. pylori-associated gastritis could be caused directly by H. pylori or as a consequence of the inflammatory reaction. Oxygen free radicals are released by polymorphs and other inflammatory cells in H. pylori infection, initiating lipid peroxidation by oxygen free radicals. Both oxygen free radical formation and lipid peroxidation are strongly linked to tissue damage and may play a role in the multistep pathogenesis of chronic lesions and possibly of cancer.

Sulforaphane is a molecule within the isothiocyanate group of organosulfur compounds. It is obtained from cruciferous
vegetables such as broccoli, Brussels sprouts, or cabbages. Sulforaphane has strong bactericidal activity against *H. pylori* in vitro. Moreover, sulforaphane is highly active against a large number of clinical isolates of *H. pylori*, many of which are resistant to conventional antibiotics. Recently, broccoli sprout extract containing sulforaphane (BSES) was reported to reduce colonization and attenuate gastritis in *H. pylori*-infected mice and humans. Sulforaphane is also a potent inducer of phase 2 detoxification enzymes, such as glutathione S-transferase, and exhibits antioxidative, anti-inflammatory, and anticancer effects. Glutathione S-transferases catalyze the conjugation of glutathione (GSH) to xenobiotic substrates for detoxification. Therefore, intracellular GSH is one of the preventive factors against oxidative damage.

This prospective study investigated whether BSES inhibited *H. pylori* infection and stimulated an antioxidative effect on inflamed gastric mucosa in patients with *H. pylori*-infected functional dyspepsia.

**MATERIALS AND METHODS**

1. Subjects and study design

One hundred volunteer subjects with functional dyspepsia were randomized double blindly from March 2009 to October 2011. Medical history interviews were conducted in all participants, followed by a brief physical examination. Exclusion criteria were previous gastric surgery, peptic ulcer disease, gastric malignancy, use of nonsteroidal anti-inflammatory drugs or anticoagulant drugs, and systemic diseases such as diabetes, hypertension, and heart disease. We included the subjects with nonatrophic erythematous gastritis (mild to moderate) in accordance with the updated Sydney system to avoid the bias of divergent gastritis patterns.

We performed upper gastrointestinal endoscopy with measurements of urea breath test (UBT), malondialdehyde (MDA), and GSH concentration twice before and after intervention in all subjects. Follow-up examinations were performed within 1 week after taking all the BSES capsules. After an overnight fast, upper gastrointestinal endoscopy and a UBT were performed. Sixty-seven of the 100 subjects were *H. pylori* positive. Subjects with *H. pylori* infection were randomized double blindly to receive either one BSES capsule containing a 250-mg standardized broccoli sprout yielding 1,000 μg sulforaphane (Oregon Health, Phoenix, AZ, USA) or placebo twice daily for 4 weeks. The placebo capsules were identical to the BSES capsules in appearance. Thirty-four of the 67 *H. pylori* (+) subjects received BSES and 33 subjects received the placebo. The 33 subjects who were *H. pylori* (-) received BSES. One of the 34 *H. pylori* (+) BSES subjects, five of the 33 *H. pylori* (+) placebo subjects, and five of the 33 *H. pylori* (+) BSES subjects were lost during follow-up. Therefore, the study comprised 89 subjects: 33 in the *H. pylori* (+) BSES group (group A), 28 *H. pylori* (+) in the placebo group (group B), and 28 *H. pylori* (-) in the BSES group (group C). Informed consent was obtained from all individuals before entering the study. This study was approved by the Institutional Review Board of Kyung Hee University Hospital (KMC IRB 0401-0410).

2. 13C-UBT

In all instances, the 13C-UBT was performed on the first day after an overnight fast or at least after an 8-hour fast. A baseline breath sample was collected into a collection tube. An aliquot of 75 mg 13C-urea dissolved in 75 mL of citric acid solution was given orally (Helikit; Isodiagnostika, Edmonton, Canada). Another breath sample was collected after 30 minutes. Breath samples were subsequently analyzed by mass spectrometry to determine the 13C/12C ratio (HeliView; Medichems, Seoul, Korea). The 13C/12C ratio of each breath sample was expressed as a milli-percentage (‰). Changes in the 12C value compared to baseline were expressed as Δ13C. A positive result was defined as an increase >4‰.

3. Gastroscopic examination

Eighty-nine subjects underwent gastroscopy before and after BSES or placebo treatment. Upon entering the stomach, 10 to 20 mL of gastric juice were aspirated from the gastric fundus and collected in a trap. During the gastroscopic examination, one biopsy specimen was taken from the antrum, and four pieces were taken from the lower gastric corpus. The antral and one corpus biopsy specimen were submitted for a rapid urease test (CLO test; Green Cross Co., Seoul, Korea). The three remaining corpus mucosal tissues were used for measurement of MDA and GSH concentrations.

4. Ammonia concentration in gastric Juice

The ammonia concentration in gastric juice was used as an additional indicator of *H. pylori* colony density in the gastric mucosa. We used a timed endpoint method to determine ammonia concentration based on a reaction between NH3, NADPH and 2-oxoglutarate in the presence of the enzyme glutamate dehydrogenase. The stoichiometry of the reaction can be determined by NADPH disappearance. Aspirated gastric juice was frozen at -70°C until analysis. After thawing, the gastric juice specimen was centrifuged at 3,000g for 15 minutes to separate gastric mucus and debris. The centrifuged samples were diluted 1:10 in Tris buffer (pH 7.2) and the concentration of ammonia in the samples determined from spectrophotometric readings at 340 nm (Synchron LX20; Beckman Coulter, Fullerton, CA, USA).

5. Indirect quantification of *H. pylori* infection density

*H. pylori* infection was diagnosed upon a positive result in the rapid urease test and 13C-UBT. The successful eradication of *H. pylori* was defined as a decrease to <4‰ in the 13C-UBT. Also, *H.