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Effects of Kimchi on Stomach and Colon Health of *Helicobacter pylori*-Infected Volunteers

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Abstract

The effects of kimchis intake on *Helicobacter pylori* infection in the stomach, the counts of lactic acid bacteria in the large intestine, and bacterial enzymes (β-glucosidase, β-glucuronidase) and pH in feces were examined. A total of 20 participants (age range 34~57) were assessed for *H. pylori* infection status by ^{13}C urea breath test. Fourteen participants were eliminated because they were *H. pylori*-negative. This study consisted of 4 consecutive phase, each of which lasted 4 weeks. Three hundred grams of kimchi were administered to *H. pylori*-infected subjects during the kimchi phase, followed by 4 weeks of control phase. During the control phase, subjects consumed 60 g of kimchi, the minimum amount in their customary diets. All participants were found to be *H. pylori*-positive during all experimental periods. During the kimchi phase, delta over baseline (DOB) level was lower than during the control phase, although significant difference between the kimchi and control phases were not found (p=0.9439). However, the counts of *Lactobacillus* sp. and *Leuconostoc* sp. significantly ($p<0.0005$) increased during the kimchi phase. β-Glucosidase and β-glucuronidase activities and pH were significantly decreased by kimchi intake compared to control (p=0.0001). These results suggested that kimchi consumption did not show any therapeutic effect on *H. pylori* in the stomach. However, kimchi seemed to be a good food for colon health, since it increased the beneficial bacteria such as lactobacillus and decreased toxic enzyme (β-glucosidase and β-glucuronidase) activity and pH.

Key words: kimchi, *Helicobacter pylori*, fecal lactic acid bacteria, β-glucosidase, β-glucuronidase

INTRODUCTION

Kimchi is a major Korean traditional fermented food. Recent reports demonstrate that kimchi reduces the risk of many type of cancers, including those of lung, esophagus, stomach, colon, liver, etc (1-5). We previously reported the antimutagenic effects of both kimchi and its ingredients as revealed by the Ames test, SOS chromotest and *Drosophila* wing spot assay system (1,6). And the anticancer effects of kimchi have been confirmed in several cellular and animal experimental trials (1-5). Kimchi inhibited the growth of human cancer cells and reduced carcinogen-induced cytotoxicity and transformation in C3H/10T1/2 cells (4). In addition, kimchi extracts reduced tumor formation in Balb/c mice and increased the phagocytic activity of macrophages, which was confirmed in an *in vivo* experimental system (5). Because its major ingredients are vegetables, kimchi has many active compounds such as ascorbic acid, carotenoids, dietary fiber, chlorophylls and flavonoids which are known to suppress the formation of carcinogenic or mutagenic compounds, and to inhibit mutagenicities induced by several carcinogens (7-10).

H. pylori infection is recognized as a major risk factor for chronic gastritis, peptic ulcer disease, and gastric cancer (11,12). There have been several reports (13-17) that natural foods such as garlic, honey and capsaicin can inhibit *H. pylori in vitro*, and each report has suggested that the natural ingredient can be used for treatment of the infection. Korea is a country where garlic and red pepper powder are used extensively in the diet, and yet Korea has one of the highest rates of *H. pylori* infection as well as gastric cancer (18,19). Since baechu kimchi is prepared with red pepper powder and garlic, baechu kimchi has the potential for use as a functional food with efficacy for the treatment or prevention of *H. pylori* infection.

The concept that dietary changes can influence gastrointestinal microflora and microbial metabolism has been well established (20,21). Cancer incidence data from descriptive epidemiology imply a hypothetical relationship between the incidence of colon cancer and a high intake

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of fermented cabbage (22). In humans, consumption of a strict vegetarian diet increased the number of potentially beneficial bacteria, such as lactobacillus and bifidobacteria and decreased both the number of potentially harmful bacteria and microbial enzyme activity (20,21).

In this study, the effects of kimchis intake on *Helicobacter pylori* infection in the stomach, the counts of lactic acid bacteria in the large intestine, and bacterial enzyme activities (β-glucosidase, β-glucuronidase) and pH in feces were examined.

MATERIALS AND METHODS

Subjects

A total of 20 participants (age range 34~57) were assessed for *H. pylori* infection status by ^{13}C urea breath test (UBT) (Helikit^{TM}, Isotechnika Inc., Canada) at the Department of Preventive Medicine of Pusan National University. Fourteen participants were eliminated because they were *H. pylori*-negative. The study consisted of 4 consecutive phases, each of which was 4 weeks long. During the kimchi phase, subjects consumed 300 g of kimchi with their normal diet. During the control phase, subjects consumed 60 g of kimchi, the minimum amounts in their customary diets. Two variations of kimchi were used and three of volunteers were given kimchi I and the other 3 kimchi II.

Preparation of kimchi and experimental samples kimchi

The method and the proportion of ingredients for preparing kimchis (kimchi I and kimchi II) were described in a previous report (23).

Urea breath test

The urea breath test (UBT) assay was performed using the Helikit^{TM}(Isotechnika Inc., Canada), which contains 75 mg of ^{13}C urea as well as citric acid, flavor enhancers and stabilizers in a single plastic cup. The powder is dissolved in 75 mL of water for oral administration. No extra mixing or dilution steps are required. Fasting patients (at least 4 h) were instructed to fully exhale using a straw into the baseline tube, which was then quickly capped and labeled. The participants then consumed the drink and after 30 min they were instructed to provide another breath sample. All the analysis was performed on an Analytical Precision AP2003 isotope ratio mass spectrometer (Analytical Precision, Manchester, UK). The raw data were produced in units of delta per mil (δ‰), which refers to the ^{13}C content relative to the Pee Dee Belemnite international standard. The delta over baseline (DOB) refers to the difference in δ‰ between the baseline and 30 min samples (24).

Sampling and cultivation of human feces

Fresh fecal samples from the 6 volunteers were suspended in anaerobic phosphate buffer (pH 7.0) to give a final concentration of 10% (wet weight/volume). The slurry was homogenized and filtered to remove large particles and debris, and was used to count viable bacteria, assay fecal bacterial enzyme activities, and measure pH.

Enumeration of viable bacteria using plate culture

Ten-fold serial dilutions (from 10^1 to 10^{12}) were prepared from the fecal slurries. The samples were plated in triplicate onto different specific media at all dilutions using the procedure and conditions of cultivation given by Lee et al. (25). The modified LBS agar medium (m-LBS medium) for *Lactobacillus* sp. and phenylethyl alcohol sucrose agar medium (PES medium) for *Leuconostoc* sp. were used. The ratio of ingredients in m-LBS medium was 1.0 g peptone, 1.0 g beef extract, 0.5 g yeast extract, 2.0 g glucose, 0.1 g Tween 80, 0.2 g K_2HPO_4, 0.2 g sodium acetate, 0.2 g ammonium citrate, 0.02 g $MgSO_4 \cdot 7H_2O$, 2.5 mL acetic acid, 35 g sodium acetate in 1 L distilled water. And the ratio of PES medium was 5.0 g trypticase, 0.5 g yeast extract, 20.0 g sucrose, 2.0 g $(NH_4)_2SO_4$, 0.244 g $MgSO_4 \cdot 7H_2O$, 2.5 g phenylethyl alcohol in 1 L distilled water. Using appropriate dilutions, 100 μL of each sample were plated on m-LBS and PES. The plates were incubated at 20°C for 5 day before colonies were enumerated.

Fecal bacterial enzyme activities

Fecal homogenates were thawed to +4°C, homogenized in 0.1 mol/L potassium phosphate buffer (pH 7.0), sonicated in an ice bath, centrifuged at 500×g for 15 min and the supernatant used for analysis. Activities of β-glucuronidase (substrate phenolphtalein nomo-β-D-glucuronic acid; Sigma, St. Louis, MO; in 0.1 mol/L potassium phosphate buffer, pH 6.8), and β-glucosidase (substrate p-nitrophenyl-β-D-glucopyranoside; Sigma; in 0.1 mol/l potassium phosphate buffer, pH 7.4) were determined at 37°C as described by Freeman (26) with modification (27). Enzyme activities were also calculated as nmoles substrate metabolized · min^{-1} · g fecal wet $weight^{-1}$.

Fecal pH: The fecal pH was measured using a Corning model 440 pH meter (Fisher Scientific, Pittsburgh, PA) equipped with a flat electrode.

Statistical analysis

Data collection and statistical analysis were performed using the SAS system. Repeated ANOVA test was used. Statistical significance was reached with p values <0.05.

RESULTS AND DISCUSSION

Effect of kimchi intake on *H. pylori* (by UBT)

The urea breath test exploits the presence of the urease enzyme, a characteristic feature of *H. pylori*. Labeled urea is ingested by the patients who then exhales into an appropriate container for subsequent analysis of breath. In the ^{13}C-urea breath test, an increase in the $^{13}CO_2/^{12}CO_2$ ratio over a baseline (pre-drink) sample is consistent with *H. pylori* infection (28-30). The Helikit^{TM} offers an easy, safe and accurate approach to the diagnosis of *H. pylori* infection (24).

Table 1 shows the results of UBT test during experimental periods. All participants were found to be *H. pylori*-positive during all experimental periods. During the kimchi phase, the DOB level was lower than during the control phase, but not significantly (p=0.9439). And difference of DOB levels between kimchi I and kimchi II was not revealed. Therefore, kimchi intake did not eradicate *H. pylori* in this clinical trial.

In a previous *in vitro* study, three kimchi variations were studied : a standard recipe (kimchi I) and two functional variations for cancer prevention and treatment made with organically grown ingredients (kimch II and III). Methanol extracts and juices from kimchi I and III did not inhibit the growth of *H. pylori*. However, 10 mm and 12 mm inhibition zones were formed by methanol extract and juice from kimchi II, which had higher concentrations of red pepper powder (RPP) than those of kimchi I and III. Among the major kimchi ingredients, methanol extracts of RPP, garlic and ginger substantially inhibited the growth of *H. pylori*. The maximal inhibition zone (30 mm) was attained with garlic treatment. These results suggested that kimchi is a weak inhibitor of *H. pylori in vitro* and no effect on eradication of *H. pylori* was observed after administration of kimchi.

In various studies on eradication of *H. pylori*, anti-*H. pylori* action of *Lactobacillus* species have been observed *in vitro*, while clinical trials produced conflicting evidence. *L. acidophillus* was found to be the only agent that showed this activity in published trials (31). Thus, further studies of the dosage and duration of regimen required for efficacy are needed to determine if kimchi can inhibit the growth of *H. pylori* in clinical trials.

Effects of kimchi intake on fecal kimchi lactic acid bacteria and pH

The presence of a stable, diverse population of luminal bacteria is important to a healthy gastrointestinal system, and to overall health. The normal microbial population helps prevent overgrowth of potentially pathogenic bacteria that may promote diarrhea, colitis or bacterial translocation (32,33). To investigate the probiotic effect of kimchi intake on colon health, fecal lactic acid bacteria (*Lactobacillus* sp. and *Leuconostoc* sp., which are found in kimchi) was measured. The counts of *Lactobacillus* sp. significantly increased during the kimchi phase and declined during the control phase (Fig. 1, p=0.0003). The counts of *Leuconostoc* sp. exhibited a similar trend as *Lactobacillus* sp. (Fig. 1, p=0.0004). Both *Lactobacillus* sp. and *Leuconostoc* sp. contents of kimchi II phase were significantly higher than that of kimchi I. Lee et al. (34) reported that a portion of the lactic acid bacteria present in kimchi could pass through the human stomach, remain viable, and reside in the large intestinal tract when 200 g of kimchi was administered. These results suggested that kimchi has a beneficial effect on colonic health by increasing lactobacillus colonization and that the efficacy depends on the kinds or types of ingredients and preparation methods of kimchi.

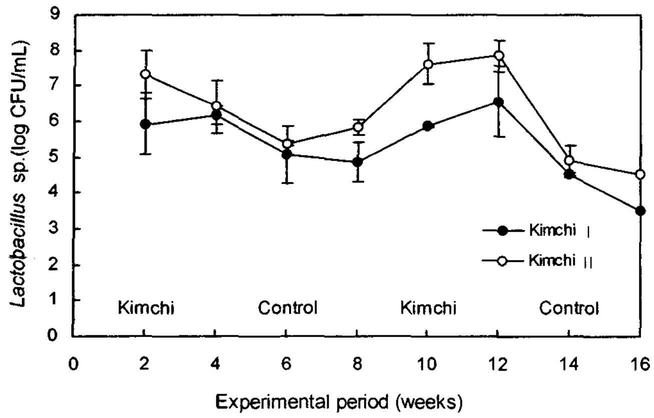
Effects of kimchi intake on fecal bacterial enzyme activities and pH

Intestinal bacteria are involved in the formation of putrefactive compounds and hydrolytic and reductive enzymes, which may have detrimental effects on intestinal health. Microbial hydrolytic and reductive enzymes are implicated in the activation and production of mutagenic compounds that may be involved in the development of colon cancer (35). β-Glucosidase is involved in the formation of toxic aglycone from plant glycosidase, and β-glucuronidase hydrolyzes glucuronic acid conjugates,

Table 1. The effects of kimchi intake on ^{13}C-urea breath test

	Periods of study (weeks)			
	4 (Kimchi)	8 (Control)	12 (Kimchi)	16 (Control)
Kimchi I[1)]	28±11[3)] (+)[4)]	34±30 (+)	24±8 (+)	21±9 (+)
Kimchi II[2)]	35±17 (+)	41±21 (+)	44±15 (+)	32±24 (+)

[1)]Standardized kimchi.
[2)]Functional kimchi for cancer prevention with organically cultivated ingredients.
[3)]Mean±SD of DOB (delta over baseline) 30 value.
[4)]+ was the positive of *H. pylori* (DOB30 > 4).
P-value between kimchi I and kimchi II was 0.3806.
P-value between kimchi phase and control phase was 0.9439.



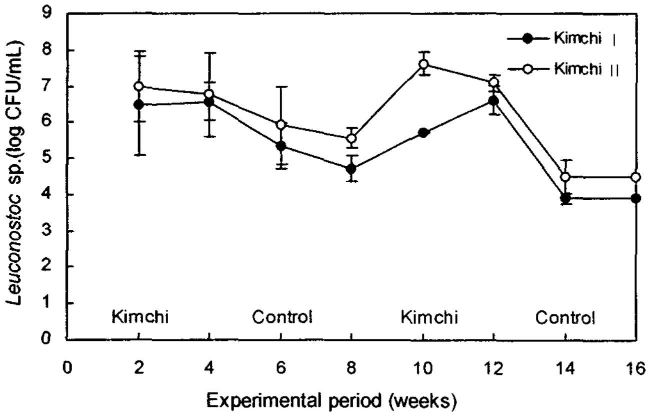
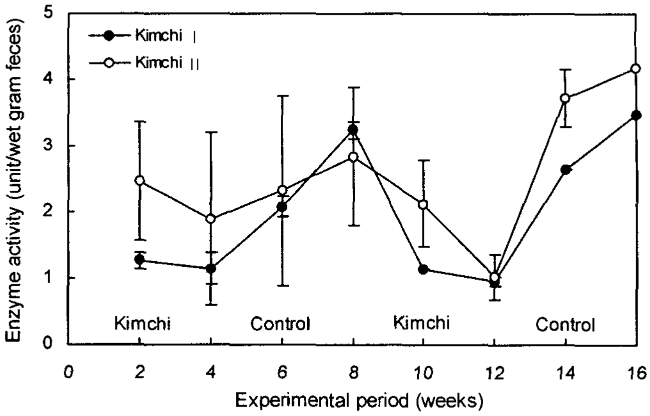


Fig. 1. Change in the *Lactobacillus* sp. and *Leuconostoc* sp. in fecal samples during the kimchi intake phase.
Kimchi I: Standardized kimchi (23).
Kimchi II: Functional kimchi for cancer prevention with organically cultivated ingredients (23).
Lactobacillus sp. (P-value between kimchi I and kimchi II was 0.0104*, P-value between kimchi phase and control phase was 0.0003*).
Leuconostoc sp. (P-value between kimchi I and kimchi II was 0.0400*, P-value between kimchi phase and control phase was 0.0004*).

A. β-Glucosidase activity



B. β-Glucuronidase activity

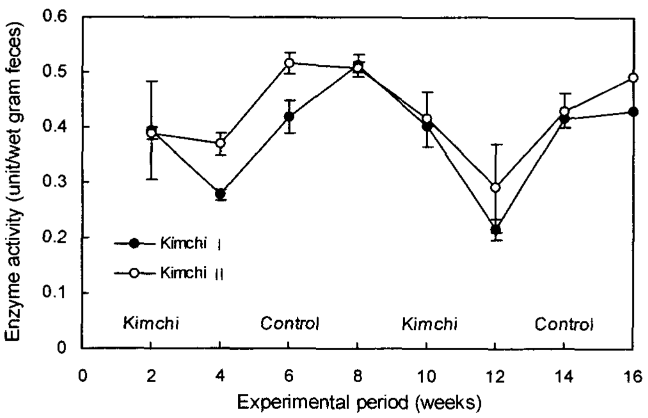


Fig. 2. Effect of kimchis intake on the fecal β-glucosidase and β-glucuronidase activities.
Kimchi I: Standardized kimchi.
Kimchi II: Functional kimchi for cancer prevention with organically cultivated ingredients.
P-value between kimchi I and kimchi II was 0.0065*.
P-value between kimchi phase and control phase was 0.0001*.

which increases the enterohepatic circulation of toxic parent compounds. Low pH has inhibitory effects on the growth of many pathogenic bacteria and reduces the intestinal absorption of potentially toxic compounds, such as ammonia (36).

To investigate the effect of kimchi intake on colon health, fecal bacterial enzyme activities (β-glucosidase, β-glucuronidase) and pH were measured. β-Glucosidase activities significantly decreased during the kimchi phase (p=0.0001) compared to the control phase (Fig. 2A). The intestinal β-glucosidase activity was significantly different between subjects eating kimchi I and kimchi II (p=0.0065). The activity of β-glucuronidase were significantly decreased during kimchi intake periods, but was significantly increased during the control phase (p=0.0001, Fig. 2B). β-Glucuronidase activity of the kimchi I phase was significantly lower than that of kimchi II phase (p=0.0065). Therefore, kimchi intake significantly

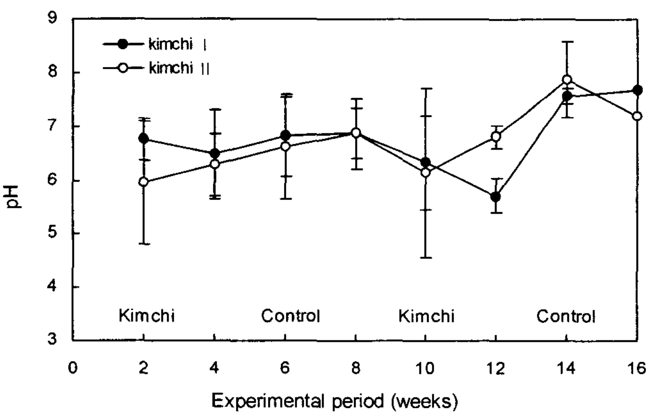


Fig. 3. *Effect of kimchi intake on the fecal pH during experimental period.*
Kimchi I: Standardized kimchi.
Kimchi II: Functional kimchi for cancer prevention with organically cultivated ingredients.
P-value between kimchi I and kimchi II was 0.617*.
P-value between kimchi phase and control phase was 0.0267*.

reduced β-glucosidase and β-glucuronidase, toxic enzymes known to transform precarcinogens into carcinogens in the human colon. Fecal pH was decreased during the kimchi phase, and increased during control phase (Fig. 3). Oh et al. (22) reported that the regular consumption (300 g) of kimchi causes a decrease in fecal bacterial enzyme activities (β-glucurunidase, nitroreductase), which are believed to be associated with colon cancer. The enzyme levels of β-glucosidase and β-glucuronidase decreased when 200 g of kimchi were administered to 10 healthy young volunteers every day for 2 weeks, followed by 2 weeks of non-intake period (34).

It can be concluded that kimchi consumption did not have a therapeutic effect in stomach since it had no effect on the eradication of *H. pylori*. However, kimchi did have a beneficial effect on colon health by increasing beneficial bacteria such as lactobacillus and by decreasing toxic enzyme activities (β-glucosidase and β-glucuronidase) and pH.

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