Dietary Sulforaphane-Rich Broccoli Sprouts Reduce Colonization and Attenuate Gastritis in *Helicobacter pylori*–Infected Mice and Humans

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Abstract The isothiocyanate sulforaphane [SF; 1-isothiocyanato-4(*R*)-methylsulfinylbutane] is abundant in broccoli sprouts in the form of its glucosinolate precursor (glucoraphanin). SF is powerfully bactericidal against *Helicobacter pylori* infections, which are strongly associated with the worldwide pandemic of gastric cancer. Oral treatment with SF-rich broccoli sprouts of C57BL/6 female mice infected with *H. pylori* Sydney strain 1 and maintained on a high-salt (7.5% NaCl) diet reduced gastric bacterial colonization, attenuated mucosal expression of tumor necrosis factor-α and interleukin-1β, mitigated corpus inflammation, and prevented expression of high salt-induced gastric corpus atrophy. This therapeutic effect was not observed in mice in which the *nrf2* gene was deleted, strongly implicating the important role of Nrf2-dependent antioxidant and anti-inflammatory proteins in SF-dependent protection. Forty-eight *H. pylori*–infected patients were randomly assigned to feeding of broccoli sprouts (70 g/d; containing 420 μmol of SF precursor) for 8 weeks or to consumption of an equal weight of alfalfa sprouts (not containing SF) as placebo. Intervention with broccoli sprouts, but not with placebo, decreased the levels of urease measured by the urea breath test and *H. pylori* stool antigen (both biomarkers of *H. pylori* colonization) and serum pepsinogens I and II (biomarkers of gastric inflammation). Values recovered to their original levels 2 months after treatment was discontinued. Daily intake of sulforaphane-rich broccoli sprouts for 2 months reduces *H. pylori* colonization in mice and improves the sequelae of infection in infected mice and in humans. This treatment seems to enhance chemoprotection of the gastric mucosa against *H. pylori*–induced oxidative stress.

Evidence that *Helicobacter pylori* infection is strongly associated with the development of stomach cancer is widely accepted (1, 2). Environmental factors, specifically diet, are also known to play an important role in the development of this and other cancers. Past research has shown that chemical compounds, such as salt (sodium chloride; ref. 3), secondary amine compounds found in burned fish (4), and nitroso compounds generated within the stomach (5), accelerate the development of stomach cancer. Many vegetables and fruits, on the other hand, are known to possess cancer-inhibitory effects (6), although a consensus as to their mechanisms of action has been slower to emerge. Cruciferous vegetables, sulforaphane (the primary isothiocyanate from broccoli), and glucosinolate/isothiocyanate-rich foods have been of special interest to those involved in dietary strategies for cancer prevention.

Glucoraphanin, the inert glucosinolate precursor of sulforaphane (the biologically active isothiocyanate) in cruciferous plants, is hydrolyzed by the enzyme myrosinase, which is present in fresh (uncooked) broccoli and broccoli sprouts (7, 8). This process begins as soon as the fresh vegetable is chewed or otherwise damaged, releasing membrane-associated myrosinase and vacuole-contained glucoraphanin. Thus, there is partial conversion even before the compound reaches the stomach. Myrosinase is also present in the microbial flora of the lower intestine of animals and humans; thus, a significant fraction of glucoraphanin is expected to be hydrolyzed and become bioavailable as sulforaphane by the time it fully transits the gastrointestinal system. Broccoli sprouts are therefore an efficient delivery vehicle for sulforaphane.

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Note: The clinical study was approved by an ethics committee at the University of Tsukuba Hospital (Approval 282) and has been assigned UMIN-Clinical Trials Registry UMIN000001415. All participants provided written informed consent, and the study was carried out in accordance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki.

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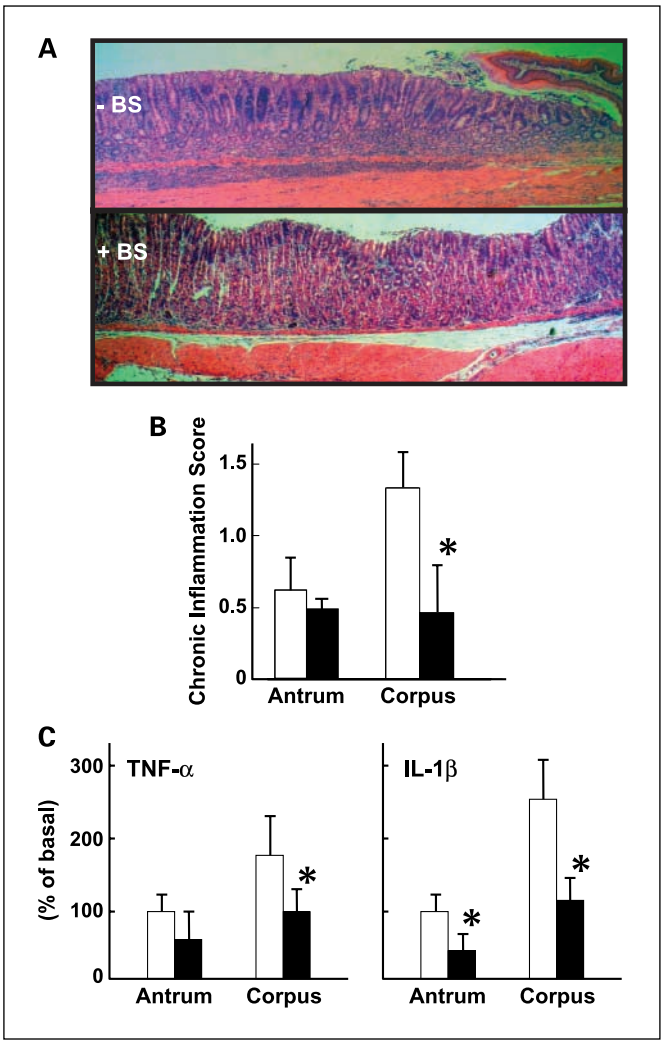

Fig. 1. Effects of feeding broccoli sprouts (+*BS*; ■) compared with untreated controls (–BS;□) on *H. pylori*–induced gastric mucosal inflammation in *nrf2*$^{+/+}$ mice on a high-salt diet (*, $P < 0.05$, for broccoli sprout versus alfalfa sprout; $n = 5$ per treatment). *A*, effect on gastric mucosal histology. *B*, effect on chronic inflammation score. *C*, effect on TNF-α and IL-1β expression in gastric mucosa.

In mammalian systems, sulforaphane is an extremely potent inducer of protective (phase 2) enzymes, and it manifests its cellular antioxidative, anti-inflammatory, and antiangiogenic effects largely via the transcription factor Nrf2 (NF-E2 p45-related factor-2; refs. 8–10). It is also a selective and highly potent antimicrobial agent against *H. pylori in vitro* and it prevents development of chemically induced stomach tumors in a rodent model (11). Several studies have confirmed the anti–*H. pylori* activity of sulforaphane (12, 13), and an early attempt to evaluate its efficacy *in vivo* with only one biomarker of efficacy has identified some of the issues that would be likely to occur in a trial in which complete eradication of *H. pylori* is not required as an end point (14). A subsequent pilot study in Detroit reported eradication of infection in three of nine *H. pylori*–infected patients (15).

We have here evaluated the efficacy of fresh broccoli sprouts in reducing *H. pylori* infection and its sequelae both in a high-salt, *H. pylori*–infected mouse model developed by Fox and colleagues over 10 years ago (16) as well as in infected human volunteers. The expectation was that ingestion of fresh broccoli sprouts would result in both glucoraphanin and its bioactive metabolite, sulforaphane, reaching the stomach for potential direct antibiotic activity against *H. pylori*. We also expect uptake of sulforaphane in the digestive tract and the subsequent induction of systemic cytoprotective enzymes. Biomarkers that were evaluated included both the direct (antibiotic) effects and systemic and local anti-inflammatory consequences.

Materials and Methods

Animal model

Infections with *H. pylori* Sydney strain 1 were established in 6-wk-old female wild-type C57BL/6 mice and 6-wk-old female Nrf2 knockout (Nrf2$^{-/-}$) mice by administration via a feeding needle of 0.5 mL of a broth culture containing 5×10^7 colony-forming units of *H. pylori* (17). This was repeated thrice at 48-h intervals and the mice were fasted for 24 h before each inoculation but had free access to food thereafter. The *H. pylori*–positive mice were followed for 2 mo with a high-salt diet (7.5% NaCl) because high-salt treatment has been shown to exaggerate *H. pylori*–induced gastritis in mice (16). These mice were divided into two groups: one of which was treated by administration of homogenized broccoli sprouts in the drinking water and the other was untreated. Wild-type mice ($n = 10$) were randomized equally into a group to receive broccoli sprout homogenate (+BS) and a control group receiving plain drinking water (–BS). Nrf2$^{-/-}$ mice ($n = 10$) were similarly randomized into a +BS group and a –BS group. Glucoraphanin-rich 3-d-old broccoli sprouts (containing 6 μmol/g glucoraphanin; Broccoli Super Sprout, Murakami Farm) were homogenized and diluted in distilled water such that average consumption was calculated to be ~3 μmol/mouse/day of glucoraphanin equivalents. When these raw sprouts were homogenized, we verified that autolysis by the endogenous enzyme myrosinase had proceeded to at least 60% conversion of glucoraphanin to sulforaphane, monitoring the appearance of sulforaphane in these homogenates by high-performance liquid chromatography (8). This sulforaphane-rich drinking water was provided *ad libitum* and was replaced with freshly prepared homogenate every other day to compensate for degradative loss of sulforaphane.

Tests described below were done using gastric mucosa that was excised from stomachs removed following euthanasia with phenobarbital, done after 8 wk of the study diet. All animal experiments were done according to the guidelines of the National Research Council Guide for the Care and Use of Laboratory Animals and approved by the Review Board of Tsukuba University.

Assessment of inflammation and atrophy of gastric mucosa. The degree of inflammation and atrophy of the gastric mucosa was measured as defined in the updated Sydney system (18).

Assessment of apoptosis in the gastric mucosa. Apoptosis was assessed immunohistochemically by counting the numbers of cells in which the nucleus was stained with antibody against ssDNA. Briefly, after deparaffinization and inactivation of endogenous peroxidases, gastric mucosal sections were incubated in 5% normal goat serum for 10 min to block nonspecific binding of the antibody. The specimens were then incubated with either anti–peroxiredoxin I polyclonal antibody (Alexis Corp.) diluted 1:500 or anti-ssDNA polyclonal antibody (DakoCytomation Co. Ltd.) diluted 1:400 at room temperature for 1 h. The samples were further incubated with biotinylated goat anti-rabbit IgG antibody for 1 h followed by treatment with peroxidase conjugated with streptavidin (DakoCytomation) for 1 h. The sections were washed thrice with cold TBS between each procedure. Apoptosis of both the glands and the surface epithelial cells in the gastric corpus mucosa was quantified by a single observer, who was unaware of the experimental treatment of each specimen. The apoptotic index (%) was determined by counting the number of ssDNA-positive cells per total number of epithelial cells under high-power magnification.

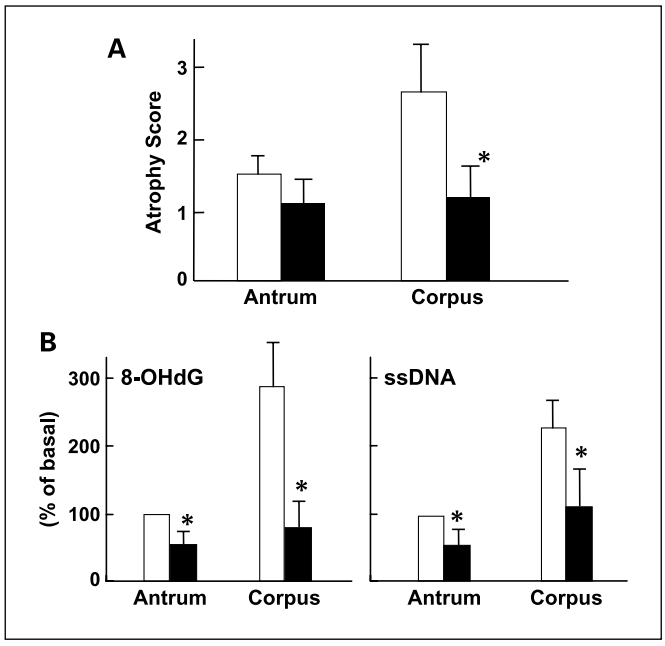

Fig. 2. Effects of feeding broccoli sprouts (■) compared with untreated controls (□) on progression of *H. pylori*–induced gastric mucosal atrophy and mucosal cell apoptosis in *nrf2*$^{+/+}$ mice (*, $P < 0.05$, for broccoli sprout versus control; $n = 5$ per treatment). *A*, atrophy score. *B*, mucosal 8-OHdG and ssDNA.

Oxidative DNA injury. This was quantified by measurement of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the gastric mucosa (19). DNA was extracted from gastric antral mucosa by use of a DNA extractor WB kit (Wako Pure Chemical Industries Ltd.). The DNA sample was dissolved in sodium acetate and digested with Nuclease P1 (Seikagakukogyo) at 37°C for 60 min. The digested DNA was then treated with alkaline phosphatase (Wako Pure Chemical Industries) in Tris-HCl buffer at 37°C for 60 min to hydrolyze the polynucleotides to free nucleosides. The mucosal levels of 8-OHdG were determined using an ELISA kit: "Highly Sensitive 8-OHdG Check" from JaICA.

Phase 2 detoxification enzyme activity in gastric mucosal membranes. NAD(P)H:quinone oxidoreductase 1 (NQO1) activity was measured as described by Prochaska and Santamaria (20). Glutathione *S*-transferase (GST) levels were measured by "Express ELISA kit (Mouse)" (L00252) from GenScript Corp.

H. pylori *colonization of mouse gastric mucosae.* Colonization was evaluated by homogenizing and culturing excised mucosal tissue following euthanasia as described by Lee et al. (17). The level of colonization was also determined numerically in the animals by doing viable counts of the bacteria. In brief, half of the stomach was placed in 1 mL of "Brain Heart Infusion broth" and homogenized with an UltraTurrax homogenizer. Ten-fold dilutions were done and 100 μL of the dilutions were plated onto "Glaxo Selective Supplement A" plates. Bacterial counts were expressed as colony-forming units per gram of tissue.

Human feeding study

This randomized controlled study recruited 50 *H. pylori*–positive volunteers whose endoscopy showed no other abnormality except gastritis. Persons who took medication such as antibiotics, proton pump inhibitors, or antiulcer drugs were excluded from participation in the study. Informed consent was obtained. Study subjects were randomized to two groups of 25 as described in the following paragraph. There were two dropouts, both in the alfalfa group: one due to proton pump inhibitor use and one due to antibiotic use after enrollment. Therefore, final results are from 25 subjects in the broccoli sprout group and 23 subjects in the alfalfa group.

Treatment. Subjects were instructed to consume 70 g/d of glucoraphanin-rich 3-d-old germinated broccoli sprouts (Broccoli Super Sprout) for 8 wk; these sprouts are validated to have a content of glucoraphanin, the precursor of sulforaphane, of ~6 μmol/g, or ~420 μmol/70 g dose. Only a fraction of glucoraphanin was expected to be converted to sulforaphane (21, 22).

Control. Subjects in the placebo (alfalfa sprouts) group were instructed to consume an equivalent amount of alfalfa sprouts (also supplied by Murakami Farms), which do not contain any glucoraphanin or other glucosinolates or isothiocyanates, for 8 wk (Fig. 4).

Sprouts were maintained at 5°C to 7°C at all times and delivered in consumer-sized packages to all participants' homes by Yamato Transport Company on a weekly basis. The same lot of broccoli sprouts was sent to the study center every week. Urine samples collected for 8 h after consumption were stored at −20°C for measurement of dithiocarbamates (DTC), a group of metabolites of sulforaphane.

Compliance was evaluated from the diary provided by the participants, which showed the time and amount of the sprouts they had consumed or if they had missed their dose. According to the diaries, all of the participants had eaten >95% of their sprout doses. All participants were required to visit the hospital for collection of blood, stool, and urine samples at days 0, 28, 56, and 112 and were required to be interviewed by a physician if they had any symptoms during or after the intervention period. Stool samples for *H. pylori* stool antigen (HpSA) analysis were obtained in the morning of their visit to the hospital. The overnight urine collected was used to measure urinary levels of DTC by using the cyclocondensation reaction (23, 24). Total overnight urinary excretion of DTC was estimated as the product of DTC concentration times urine volume.

HpSA values were measured with a HpSA-ELISA kit from Meridian Bioscience, Inc. as described previously (25) using stool collections made at 0, 4, 8, and 16 wk (8 wk after the completion of the intervention; dates correspond to study days 0, 28, 56, and 112, respectively). The manufacturer's recommended cutoff value was 0.100 (unitless ratio of A_{450}/A_{630}), and *H. pylori* was scored as negative when the HpSA value was below this threshold. Serum pepsinogens I and II (PGI and PGII) were measured in blood samples collected from volunteers at these same time points, and PGI/PGII ratios were computed (26, 27). Severity of current *H. pylori* colonization was assessed by the urea breath test (UBT; UBiT-IR300, Otsuka Electronics, Inc.; ref. 14). We adopted the manufacturer's recommended cutoff value of 2.5‰ (parts per thousand), below which subjects were scored as *H. pylori* negative.

Heme oxygenase-1 (HO-1) expression was assessed by real-time PCR in blood samples taken from subjects before and 24 h after eating 50 g of broccoli sprouts. HO-1 mRNA was measured in polymorphonuclear granulocytes, which had been purified from whole blood samples using Polymorphprep (28).

All tests results were compared using a Student's *t* test. Error bars on all figures represent ±1 SD from the mean.

Results

Mouse studies

Effects on wild-type mice. There was inflammation of the gastric corpus mucosa in *H. pylori*–infected mice, which was substantially attenuated by treatment with broccoli sprouts (Fig. 1A). Broccoli sprouts protected against inflammation in the antrum and the corpus of the high-salt mouse model, although the degree of protection was greater in the corpus mucosa than the antral mucosa (Fig. 1B and C). Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are major proinflammatory cytokines that are elevated in gastric mucosa by *H. pylori* infection. Broccoli sprouts suppressed up-regulation

of these cytokines (Fig. 1C). This protective effect against inflammation of the gastric mucosa was paralleled by significant inhibition of gastric atrophy and decreased levels of both 8-OHdG and ssDNA, which are markers of apoptosis (Fig. 2A and B).

Effects on* nrf2*-deficient mice. Activation of the cytoprotective enzymes NQO1 and GST after the administration of broccoli sprouts also increased significantly in wild-type mice, but not in *nrf2*$^{-/-}$ mice (Fig. 3A), as would be expected based on the central position of Nrf2 as a signal molecule for protection against oxidative and inflammatory stress (29). The protective effect of broccoli sprouts in both the antrum and the corpus of wild-type mice was not observed in *nrf2*$^{-/-}$ mice (Fig. 3B). Broccoli sprouts also suppressed up-regulation of TNF-α and IL-1β in gastric mucosa by *H. pylori* infection in wild-type but not in *nrf2*$^{-/-}$ mice (Fig. 3C), suggesting a systemic protective effect against gastritis. Additionally, there was an almost 2-log reduction in *H. pylori* density in both the antrum and corpus only in wild-type mice and not in *nrf2*$^{-/-}$ mice (Fig. 3D), thus providing strong support for the integral role of the Nrf2 mechanism in protection against *H. pylori*–induced inflammation and gastritis.

Human feeding study

We conducted a preliminary experiment to confirm that the doses of broccoli sprouts that we had planned to use in the double-blind portion of the trial would be expected to upregulate cytoprotective (phase 2) enzymes. Thus, we measured HO-1 expression in polymorphonuclear granulocytes by reverse transcription-PCR before and 24 hours after eating 50 g of broccoli sprouts by a few volunteers. HO-1 expression increased dramatically (2- to 3-fold) 24 hours after eating 50 g of broccoli sprouts (data not shown). Because the average body weight in this preliminary experiment was ~40% less than the body weights of subjects recruited to the double-blind study, the dose regimen in the latter study was increased from 50 to 70 g/d.

The study protocol (outlined in Fig. 4) randomized 50 subjects to daily consumption of either 70 g of broccoli sprouts or a placebo (alfalfa sprouts) containing no sulforaphane. Mean age of subjects at randomization was 54.5 years. There were more female (n = 28) than male (n = 19) subjects, and there was no difference in preintervention *H. pylori* infection and inflammation status for the two experimental groups (Table 1). Subjects tolerated the intervention well and reported no notable objection to the daily sprout consumption, and there were no notable gastrointestinal or other abnormalities that could be attributed to the intervention. Urinary excretion of DTC at the two time points measured during the intervention period was elevated markedly in the subjects consuming broccoli sprouts. These levels were significantly higher than DTC levels in the placebo control group, where levels remained in the baseline range as expected (Fig. 5). Thus, compliance with the intervention was validated and correlated well with dietary records kept by subjects (self-report data not shown).

Serum PG values are indicators of inflammation in the gastric lumen. During the intervention period, there were significant reductions in both PGI and PGII ($P < 0.05$), only in the broccoli sprout group, compared with baseline levels, and there was a return to baseline values 2 months after the intervention (Fig. 6A and B). The ratio of PGI to PGII, used by many clinicians as a more robust indicator of change in gastric inflammation (27), rose significantly ($P < 0.05$) only during the intervention and only in the broccoli sprout group (Fig. 6C). In addition, individual urinary DTC levels measured late in the intervention (week 8) were highly correlated with the magnitude of changes in PGI/PGII ratio after the 8-week feeding with broccoli sprouts ($P < 0.01$; Fig. 6D).

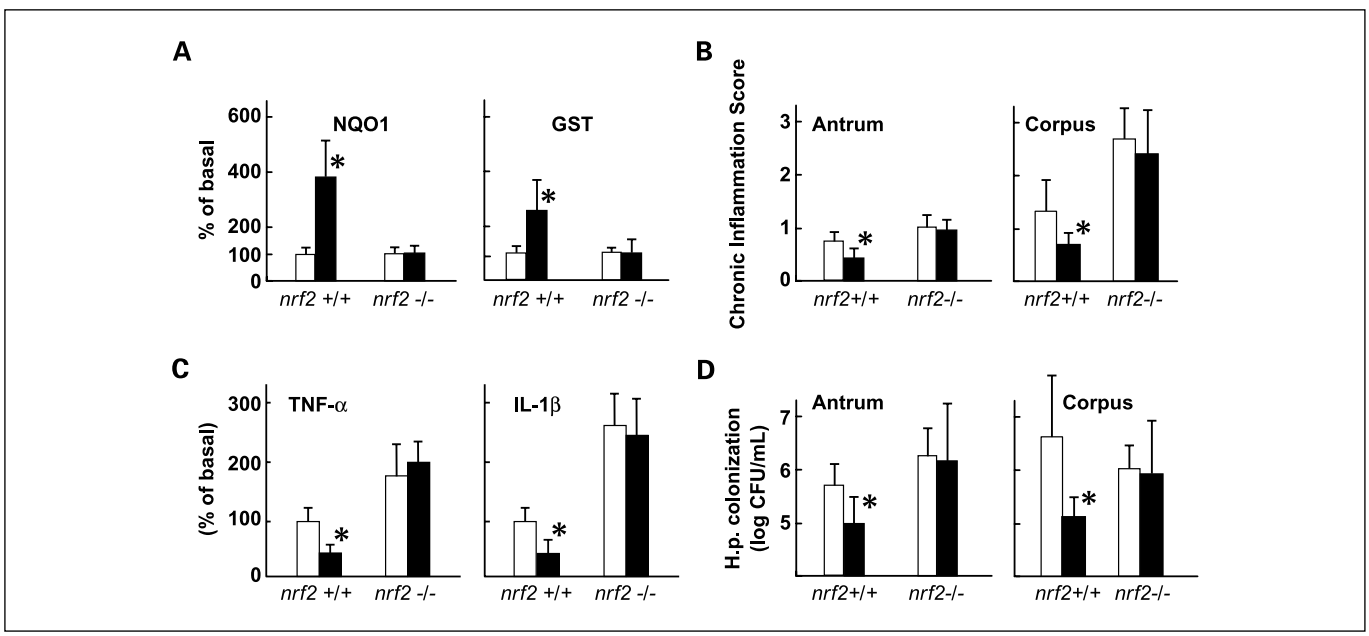

Fig. 3. Effects of feeding broccoli sprouts (■) compared with untreated controls (□) on gastric mucosa of *H. pylori*–infected, high-salt diet–treated *nrf2*$^{-/-}$ mice compared with *nrf2*$^{+/+}$ mice (*, $P < 0.05$, for broccoli sprout versus control; n = 5 per treatment). *A,* phase 2 enzyme activity: NQO1 and GST. *B,* chronic inflammation. *C,* TNF-α and IL-1β expression. *D, H. pylori* colonization.

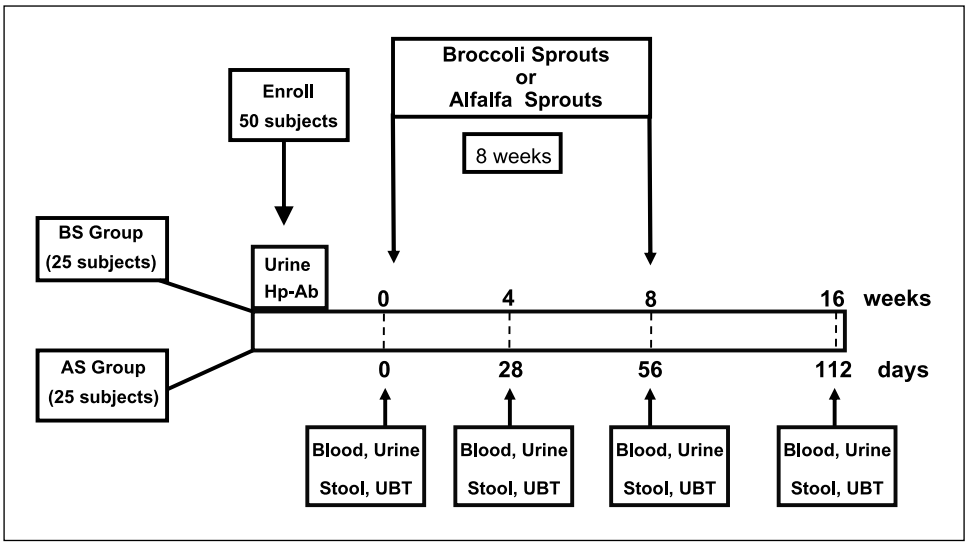

Fig. 4. Protocol for the human clinical intervention.

The HpSA levels and UBT values measured in the broccoli sprout intervention arm were significantly lower ($P < 0.05$) during the intervention than at baseline and returned to baseline levels ($P < 0.05$) at 16 weeks (2 months after intervention). The placebo group receiving alfalfa sprouts had no significant change in HpSA (Fig. 7A and B). Although HpSA values respond less rapidly to changes in *H. pylori* infection status than do UBT or direct urease tests, these measures serve as a robust measure of colonization on a population basis (30, 31). In this study, no subjects had UBT values below the cutoff value (2.5‰) at any of the measurement periods. However, 8 of 25 subjects in the broccoli sprout treatment group had HpSA values below the cutoff (0.100) at the end of the 8-week broccoli sprout treatment period. Six of these subjects' HpSA values again became positive 8 weeks after cessation of broccoli sprout consumption (Fig. 7B), and the HpSA values of the other two subjects turned positive a further 6 months after intervention (data not shown), thus indicating that broccoli sprout treatment does reduce *H. pylori* colonization but does not provide complete eradication of *H. pylori*.

Discussion

This study was designed to determine whether regular dietary consumption of broccoli sprouts rich in the sulforaphane precursor glucoraphanin inhibits *H. pylori* colonization and the attendant gastric atrophy in mice fed a high-salt diet and whether this intervention ameliorates gastritis in *H. pylori*–infected human subjects. The major findings were as follows.

First, we determined that oral delivery of broccoli sprouts decreased corpus gastritis and protected against gastric mucosal inflammation *in vivo* in a well-established *H. pylori*–infected animal model. Oral dosing of animals in this model (~3 μmol/mouse/day) was consistent with dosing of sulforaphane in a variety of other mouse experiments in which carcinogenesis or biomarkers were end points [e.g., (*a*) a reduction in chemically induced lung tumors by using either 6.7 or 13.43 μmol/mouse/day gavaged 1 day per week for 8 weeks (32) or (*b*) with 5 and 10 μmol/mouse/day supplied in food for 21 weeks (33); (*c*) a reduction in intestinal tumors and biomarkers with 10.2 μmol/mouse/day for 10 weeks (34); (*d*) suppression of intestinal polyps and histone deacetylase inhibition with a dose of 6 μmol/mouse/day for 10 weeks

Table 1. Baseline data for *H. pylori*–infected subjects ($n = 50$) after randomization to broccoli sprout and alfalfa sprout supplemented diets

	Broccoli sprout ($n = 25$)		Alfalfa sprout ($n = 23$)	
	Mean ± SD	Range	Mean ± SD	Range
Age (y)	53.4 ± 11.6	25-70	55.7 ± 12.9	23-73
UBT (‰)	32.7 ± 14.4	10.0-56.8	35.7 ± 25.3	6.7-106.1
PGI (ng/mL)	70.3 ± 21.4	33.3-118	69.4 ± 26.7	31.5-135
PGII (ng/mL)	30.5 ± 14.8	14.7-43.7	29.4 ± 8.63	18.3-52.5
PGI/PGII (ratio)	2.41 ± 1.00	0.68-4.10	2.38 ± 0.70	1.03-3.93

NOTE: Note that there were two dropouts, both in the alfalfa group, one due to proton pump inhibitor use and one due to antibiotic use after enrollment, and these subjects are not included in the table or the data presented in Figs. 5 to 7.

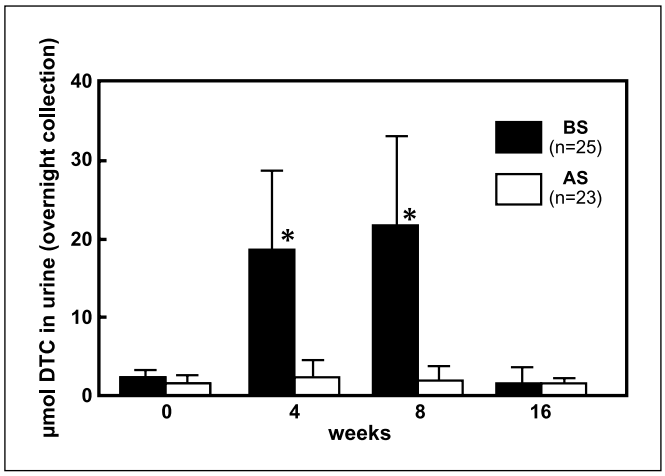

Fig. 5. Changes in urinary excretion of sulforaphane metabolites before, during weeks 4 and 8, and after (week 16) the dietary intervention with broccoli sprouts (BS; *, significant, $P < 0.05$) or alfalfa sprouts (*AS*; no significant differences).

(35); (*e*) a reduction in chemically induced forestomach tumors following 7.5 μmol/mouse/day feeding for 21 weeks (11); and (*f*) eradication (presumably a direct, antibiotic effect) of *H. pylori* with direct instillation of 7.5 μmol/mouse/day into human gastric xenografts (13)].

Second, the findings strongly suggest that the gastritis-moderating effect of sulforaphane was due at least in part to induction of cytoprotective enzymes in the gastric mucosa cells via the host animal Nrf2 signaling pathway. Significant leukocyte infiltration occurs in *H. pylori*–colonized gastric mucosa, exposing the membrane mucosa to enhanced oxidative stress. Sulforaphane is well known as an activator of cytoprotective enzymes and we have shown herein that they are up-regulated in broccoli sprout–treated animals.

Third, we determined that *H. pylori* colonization was reduced in sulforaphane-treated wild-type mice but not in *nrf2*$^{-/-}$ mice. This *in vivo* finding suggests that sulforaphane may not only have a direct antibiotic effect on the level of *H. pylori* colonization. Rather, its primary effect may be via the up-regulation of the host's systemic protection against oxidative stress and inflammation, which results in reduced *H. pylori* colonization. The mechanisms and effector pathways for the detoxification effects of sulforaphane and related compounds have been studied extensively (9, 36-39). Sulforaphane induces cytoprotective, antioxidant, and anti-inflammatory enzymes via the transcription factor Nrf2, which activates the genes that control these endogenous protective responses (29). In the development of gastric cancer, it is further suggested that *H. pylori* and sodium chloride cooperate as cancer promoters by enhancing chronic gastric mucosal membrane inflammation (16). Sulforaphane

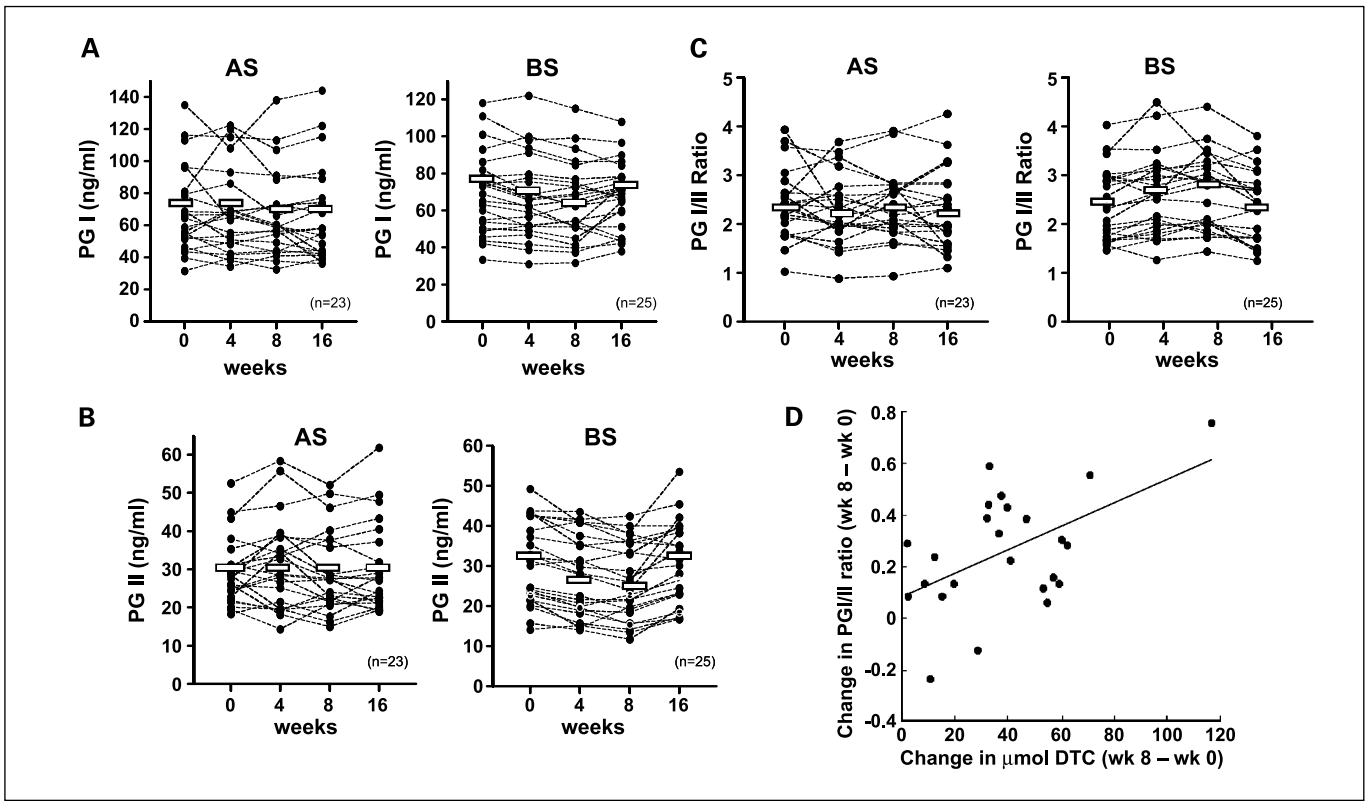

Fig. 6. Effects of broccoli sprouts (BS) or alfalfa sprouts (AS) on levels of serum PGs [alfalfa sprout: no significant differences between treatments; broccoli sprout: weeks 4 and 8 are significantly different from week 0, and week 16 is significantly different from week 8 ($P < 0.05$)]. *A*, PGI. *B*, PGII. *C*, PGI/PGII ratio. Rectangular overlays represent means for each time period. *D*, correlation of changes in PGI/PGII ratio and the increase in DTC after intervention with broccoli sprouts; values represent the differences between measurements made during the intervention (week 8) and those made before intervention (week 0) on individual subjects.

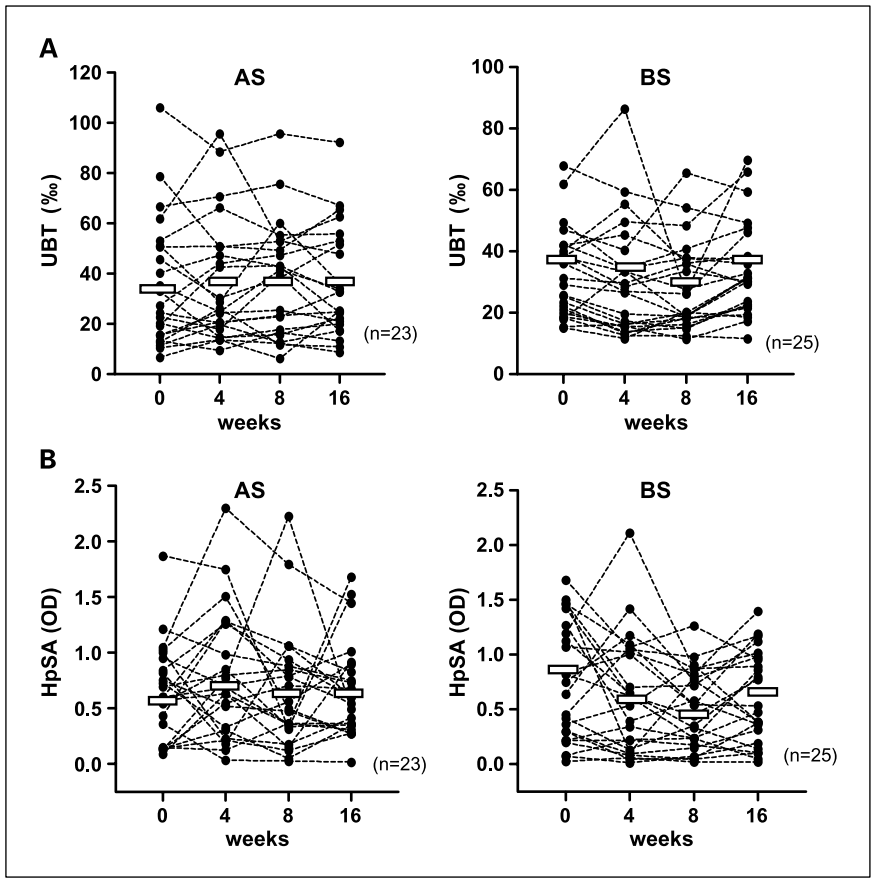

Fig. 7. Effects of broccoli sprouts (BS) or alfalfa sprouts (AS) on *H. pylori* colonization of human subjects. *A,* UBT (cutoff value is 2.5‰). *B,* HpSA [cutoff value is 0.100 (ratio of A_{450}/A_{630})]. Rectangular overlays represent means for each time period [alfalfa sprout: no significant differences between treatments; broccoli sprout: weeks 4 and 8 are significantly different from week 0, and week 16 is significantly different from week 8 ($P < 0.05$)].

may be inhibiting this inflammation either via the Nrf2 pathway or by yet unknown mechanisms. Our data show that gastritis is mitigated following broccoli sprout treatment. The data do not permit us to differentiate whether this effect is induced either by the inhibition of *H. pylori* colonization (*in vitro*, sulforaphane is a potent antibiotic against *H. pylori*) or by up-regulation of Nrf2-dependent cytoprotective and antioxidant enzyme activity (a plethora of *in vitro*, animal, and clinical studies have shown this to occur) or by some combination of these two modes of protection. *H. pylori* infection generates a variety of reactive oxygen species within the mucosa, which enhances gastric mucosal injury and inflammation. Thus, by enhancing Nrf2-dependent antioxidant enzyme activity in gastric mucosal cells, sulforaphane could promote the reduction of reactive oxygen species from gastric mucosae, resulting in the mitigation of *H. pylori* infection-induced gastritis.

Fourth, a 2-month course of daily intake of broccoli sprouts (70 g), which delivers a quantity of sulforaphane equivalent to between two and three servings of broccoli per day (40), decreased serum values of PGI and PGII and increased the PGI/PGII ratio during the 2-month intervention period, consistent with extensive clinical observations correlating increased PGI/PGII ratio with reduced inflammation of gastric mucosa but not gastric mucosal atrophy (26, 27). We also measured reductions in HpSA (an indicator of recent infection) and UBT (an indicator of instant infection) after the intake of broccoli sprouts. All three biomarkers returned to baseline levels once the intervention was discontinued.

Complementary mouse and human evidence thus suggests that sulforaphane may have both a direct antibacterial effect on *H. pylori*, leading to reduced gastritis, as well as having an indirect (systemic) effect by increasing the mammalian cytoprotective (phase 2) response. It is not possible to determine the relative contributions of these two mechanisms from this study; however, in light of other evidence, which suggests a strong anti–*H. pylori* effect of sulforaphane *in vitro* (11), the findings in this study strongly suggest that sulforaphane has promise both as an antibacterial agent directed against *H. pylori* and as a dietary preventive agent against the development of human gastric cancer.

Disclosure of Potential Conflicts of Interest

J.W. Fahey is a cofounder of Brassica Protection Products LLC (BPP), a company that is licensed by Johns Hopkins University to produce broccoli sprouts. A portion of the proceeds of BPP is used to support cancer research, but no funds were provided to support this study. J.W. Fahey may be entitled to royalty payments from the sale of broccoli sprouts, and terms of this arrangement are being managed by Johns Hopkins University in accordance with its conflict of interest policies.

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