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Original Article

Broccoli Consumption and Chronic Atrophic Gastritis among Japanese Males: An Epidemiological Investigation

Kyoko Sato[a]*, Norito Kawakami[a], Tadahiro Ohtsu[a], Akizumi Tsutsumi[a], Shogo Miyazaki[b], Takeshi Masumoto[c], Seichi Horie[d], Takashi Haratani[e], Fumio Kobayashi[f], and Shunichi Araki[e]

[a]Department of Hygiene and Preventive Medicine, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8558, Japan, [b]Koukankai Keihin Occupational Health Center, Kawasaki 210-0866, Japan, [c]Mitsubishi Chemical Corporation, Kashima 314-0102, Japan, [d]Department of Health Policy and Management, Institute of Industrial Ecological Science, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan, [e]Institute of Industrial Health, Kawasaki 214-8585, Japan, and [f]Department of Psychosocial Health, Aichi Medical University, Aichi 480-1195, Japan

Previous *in vitro* and animal experiments have shown that sulforaphane, which is abundant in broccoli, inhibits *Helicobacter pylori (H. pylori)* infection and blocks gastric tumor formation. This suggests that broccoli consumption prevents chronic atrophic gastritis (CAG) introduced by *H. pylori* infection and, therefore, gastric cancer. For an epidemiological investigation of the relationship between the broccoli consumption and CAG, a cross-sectional study of 438 male employees, aged 39 to 60 years, of a Japanese steel company was conducted. CAG was serologically determined with serum cut-off values set at pepsinogen I $\leq$ 70 ng/ml and a ratio of serum pepsinogen I/pepsinogen II $\leq$ 3.0. Broccoli consumption (weekly frequency) and diet were monitored by using a 31-item food frequency questionnaire. The prevalence of CAG among men who ate broccoli once or more weekly was twice as high as that among men who consumed a negligible amount ($P < 0.05$). Multiple logistic regression analysis indicated that broccoli consumption once or more weekly significantly increased the risk for CAG (odds ratio, 3.06; 95% confidence interval, 1.12-8.38; $P < 0.05$), after controlling for age, education, cigarette smoking, and alcohol consumption. The present study failed to show an expected association between frequent broccoli consumption and a low prevalence of CAG.

Key words: broccoli, sulforaphane, chronic atrophic gastritis, pepsinogen, *Helicobacter pylori (H. pylori)*

The relationship of *H. pylori* and gastric pathology was first described about 20 years ago [1]. *H. pylori* infection has been recognized as a strong predictor of chronic atrophic gastritis (CAG) and gastric cancer [2-6]. In Japan, the prevalence of *H. pylori* infection is relatively high at 70-80% among people born before 1950 [7]. Therefore, *H. pylori* infection has been considered as one of major problems of preventive against gastric cancer.

Recently, Fahey *et al*. [8] reported that sulforaphane [(−)-1-isothiocyanate-(4R)-(methylsulfinyl) butane], which is abundant in certain varieties of broccoli

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*Corresponding author. Phone: +81-86-235-7174; Fax: +81-86-235-7178
E-mail: kyoko-st@nifty.com (K. Sato)

and broccoli sprouts, inhibits *H. pylori* infection and blocks gastric tumor formation. They showed the bactericidal potency of sulphoraphane on *H. pylori* after exposure to 1× and 5× minimal inhibitory concentration (MIC) of sulforaphane at pH 5.8. The effect was nearly always concentration-dependent. These authors also tested the eradication of intracellular bacterial infection after treatment with sulforaphane at 0.5×, 1×, and 5× the MIC for *H. pylori*. They found that sulforaphane delivered at a concentration equivalent to its MIC completely killed intracellular bacteria within 24 h. Higher concentrations (*e.g.*, 5× the MIC) completely killed the intracellular bacteria within 4 to 8 h. Furthermore, they showed the effect of sulforaphane on benzo[a]pyrene-induced neoplasia of the forestomach in mice. The forestomachs of control mice developed 17.6 tumors per mouse 20 weeks after the first administration of benzo[a]pyrene. The addition of sulforaphane (estimated consumption 7.5 μmol per day) in the diet for a period of 7 days before the first dose to 2 days after the last dose of carcinogen reduced the number of tumors to 10.8 per mouse (a 39% reduction; $P < 0.001$). Fahey *et al*. concluded that sulforaphane might inhibit *H. pylori* infection and block gastric tumor formation in mice.

Based on these *in vitro* and animal experiments, Fahey *et al*. [8] further implied that broccoli consumption may decrease *H. pylori* infection and showed that it prevents CAG in a human stomach. However, the structure of the epithelium in the mouse stomach and human stomach is different: *e.g.*, the forestomach of the mouse has squamous epithelium, while human stomach is covered by mucosa. Sulforaphane, and broccoli consumption, may not be effective to prevent *H. pylori* infection and CAG in a human stomach. To date, no epidemiological investigation has been made on the relationship between broccoli consumption and CAG among people under the conditions of daily life.

It is possible that other diets and health-related habits affect the development of CAG. An increased consumption of alcoholic beverages increases the risk for CAG [9]; however, other studies have reported no association between alcohol consumption and CAG [6, 10–12]. Cigarette smoking has been reported to have no relationship with an increased risk of CAG [10–14]. Previous reports have shown that a high salt intake is associated with an increased risk of CAG [10, 15, 16]. Other factors, such as the ones mentioned above, should be considered in an investigation of the association of broccoli consumption and CAG.

If broccoli consumption decreases a risk of CAG through the suppression of *H. pylori* infection or tumor formation in human, we could observe a negative association between broccoli consumption and the prevalence of CAG. To test this hypothesis, we conducted a cross-sectional study on the relationship between frequency of broccoli consumption and a serological diagnosis of CAG in a sample of Japanese middle-aged male workers. We examined the relationship between broccoli consumption and CAG, controlling for age, education, the intake of other dietary components, cigarette smoking, and alcohol consumption.

Materials and Methods

In August 1997, Japanese male steel-company employees (n = 496) aged 39 to 60 years old were invited to participate in an annual health checkup and in the study. Blood samples were taken, and serum pepsinogen I and II levels were measured. They were also asked to complete a dietary and life-style questionnaire. This study design was deliberated and approved by the humans subject committee at the Gifu University School of Medicine, which was affiliation of one of the authors (NK) was affiliated with when the survey was conducted. The aims and study procedures were fully explained to the subjects, and written consents were obtained. The data obtained (including questionnaires and serum data) were collected and kept by occupational physicians in the company and only anonymous data were provided to researchers outside the company after deleting the subjects' names and IDs. A total of 456 (response rate, 92 %) participated and returned the questionnaire. Some were excluded because of the following conditions: one with a past history of gastric cancer, 16 currently being treated for gastric ulcers, and one that failed to answer every item in the questionnaire. The data from the remaining 438 respondents (average age, 47.7 with standard deviation, 4.8) were analyzed. The subjects included three managers, 11 professionals, 33 technicians, 13 clerks, 2 salesmen, 119 mechanics/repairmen, 174 machine operators, 11 laborers, 60 non-classified workers, and 12 with undetermined occupations.

Serological Diagnosis of CAG. Serum levels of pepsinogen I and II were measured using Riabead Kits (Dinabot Co., Ltd., Matsudo, Japan). The ratio of the serum pepsinogen I to serum pepsiongen II has been know as an indicator of degree of atrophy of the gastric mucosa. Miki and coworkers have applied the pepsiogen I and II levels to screen [17, 18]: they proposed a combination of less than 70 ng/dl of a pepsinogen I level and less than 3.0 of the pepsinogen I/II ratio as a cut-off point for CAG [17, 18]. In this study we applied their criteria to determine CAG.

Consumption of Broccoli and Other Foods. Diets were assessed by using a revised version of a 31-item dietary history questionnaire (DHQ) [19], which was developed from a longer DHQ [20]. The DHQ included a question regarding the monthly frequency of broccoli consumption with a seven-point response option. First, we divided the subjects into those who consumed broccoli almost not at all (n = 49) and those who did at least once per month. Those who consumed broccoli at least one per month were further divided into 2 equally-sized groups based on the distribution of broccoli consumption: less than once per week (n = 203) and once or more per week (n = 186).

The DHQ was designed to measure the daily consumption of 17 macro and micro nutrients: total calories (kcal/day), proteins (g/day), fat (g/day), carbohydrates (g/day), fiber (g/day), calcium (mg/day), retinoid (mg/day), carotinoid (mg/day), vitamins A (IU/day), C (mg/day), D (mg/day), and E (mg /day), salt (g/day as NaCl), cholesterol (mg/day), saturated fat (g/day), polyunsaturated fat (g/day), and monounsaturated fat (g/day). The original questionnaire sought information about portion size and frequency regarding each food item. The portion size questions were deleted in order to reduce the burden on the respondents. In their place, a sex-specific average portion size was used to estimate the volume of each item. This modification slightly decreased the validity of the questionnaire. However, the revised 31-item DHQ still showed moderate-to-high correlation coefficients with 12 three-day dietary records over a one-year period for 37 volunteers: Pearson's correlation coefficients were 0.53 for total calories, 0.41 for protein, 0.24 for fat, 0.43 for carbohydrates, 0.55 for fiber, 0.57 for calcium, 0.28 for retinoid, 0.39 for carotenoid, 0.26 for vitamin A, 0.39 for vitamin C, 0.55 for vitamin D, 0.43 for vitamin E, 0.47 for salt, 0.33 for cholesterol, 0.33 for saturated fat, 0.36 for polyunsaturated fat, and 0.23 for monounsaturated fat (Takatsuka, Shimizu, Kawakami *et al*. unpublished data). Comparisons of estimated average values with those from the three-day records indicated an apparent overestimation of vitamin C (+112%), retinol (+58%), carotenoid (+52%), and fiber (+16%); otherwise, the differences in the estimated averages between the DHQ and the three-day records were within 10%.

Other covariates. Other covariates included age, education, cigarette smoking, and alcohol consumption, which were also assessed by the questionnaire. Age (years) was used as a continuous variable. Education was dichotomized into "high school or less" and "college or higher". Subjects were classified into current smokers and non-smokers (never smokers + past smokers). Alcohol consumption was classified as none, 0–14 drinks weekly, and 15 or more drinks weekly (1 drink contains approximately 9 g of ethanol), calculated based on responses to questions regarding weekly frequency of consumption and volume of drinking per session.

Statistical Analysis. First, we compared variables relevant to CAG (age, education, consumption of total calories and salt, cigarette smoking, and alcohol consumption) [6, 9–16] among the 3 groups classified based on frequency of broccoli consumption in order to determine whether broccoli intake was associated with known risk factors of CAG (one-way analysis of variance). The prevalence of CAG was then compared among the groups; crude and age-adjusted odds ratios (prevalence ratios) were calculated by using multiple logistic regression. Finally, multiple logistic regression was conducted in order to examine a unique association between frequency of broccoli consumption and CAG, adjusting for age, education, consumption of total calories and salt, cigarette smoking, and alcohol consumption. Adjusted odds ratios of CAG and their 95% confidence interval were estimated. In addition to these analyses, we also compared the dietary intakes of 17 nutrients between those who had CAG and those who did not, in order to examine whether any dietary factor other than broccoli and salt was associated with CAG (t-test and ANOVA controlling for age). Probability values of less than 0.05 were considered statistically significant.

Results

Total energy was significantly different among the groups classified on the basis of frequency of broccoli consumption ($P < 0.05$, Table 1): total energy intake was greater among the groups with higher frequency of broccoli consumption more. The same tendency was observed for salt consumption ($P < 0.05$). There was no

Table 1 Comparison of age, education, total energy, salt consumption, alcohol consumption, and smoking among the groups classified on the basis of frequency of broccoli consumption

	Frequency of broccoli consumption											
	None (n = 49)				Less than once per week (n = 203)				Once or more per week (n = 186)			
	n	%	Mean	SD	n	%	Mean	SD	n	%	Mean	SD
Age (years)			47.4	5.3			47.8	4.7			47.7	4.7
Education												
High school or less	48	98			198	98			177	95		
Some college and higher	1	2			5	2			9	5		
Total energy (kcal)*			2610	650			2710	660			3110	740
Salt consumption (g)*			14.5	5.3			14.8	4.5			17.8	5.3
Alcohol consumption												
None	5	10			24	12			28	15		
14 drinks or less weekly	19	39			98	48			83	45		
15 drinks or more weekly	25	51			81	40			75	40		
Smoking												
Non-smoker	13	27			78	38			56	30		
Current smoker	36	73			125	62			130	70		

*$P < 0.05$, difference among the 3 groups classified on the basis of frequency of broccoli consumption (one-way ANOVA or chi-square test).

Table 2 Prevalence of serological diagnosis of CAG (chronic atrophic gastritis) by frequency of broccoli consumption among 438 Japanese employed men aged 39 to 60 years old

Frequency of broccoli consumption	CAG positive			POR	95%CI	Age adjusted	
	N	n	%			POR	95%CI
None	49	5	10.2	1.00		1.00	
Less than once weekly	203	35	17.2	1.83	0.68–4.95	1.81	0.67–4.93
Once or more weekly	186	46	24.7	2.89*	1.08–7.73	2.90*	1.08–7.80

POR, prevalence odds ratio. A logistic regression model was used to estimate age-adjusted OR. 95%CI, 95% confidence interval. *$P < 0.05$.

significant difference in age among the 3 groups classified on the basis of frequency of broccoli consumption ($P > 0.05$). Education, cigarette smoking, or alcohol consumption was not significantly different among the 3 groups ($P > 0.05$, Table 1). These patterns were unchanged after controlling for age.

According to the results of a serological test a total of 86 of the subjects (n = 438) were to have CAG. The prevalence of CAG was 19.6%. The prevalence of CAG among those who consumed broccoli once or more weekly was statistically significantly higher than that among those who consumed a negligible amount (Table 2). The tendency was unchanged after controlling for age. When the group who ate broccoli once or more weekly was further divided into subgroups based on the frequency of intake, the prevalence of CAG was 17% for those who ate broccoli once weekly (n = 114), 33% for those who ate it 2–3 times weekly (n = 57), and 53% for those who ate it 4 times or more per week (n = 15); a statistically significant difference was observed between those who ate broccoli once weekly and those who ate it 4 times or more ($P = 0.002$ in the crude analysis and after controlling for age).

Table 3 shows a result of multiple logistic regressions on the association between CAG and age, education, broccoli consumption, total energy, salt consumption, alcohol consumption, and smoking. The odds ratio of CAG was still significantly higher among those who ate broccoli once or more weekly than it was among those who rarely consumed broccoli. This trend was also

Table 3 Association of broccoli consumption with serological diagnosis of CAG among 438 Japanese employed men aged 39 to 60 years old: Multiple logistic regression controlling for other covariates

	POR	95%CI
Age (for 10 year increase)	1.93*	1.16–6.92
Education		
High school or less	1.00	
College or higher	0.68	0.14–3.21
Frequency of broccoli consumption		
None	1.00	
Less than once weekly	1.90	0.70–5.18
Once or more weekly	3.06*	1.12–8.38
Total energy (for 1000 kcal)	1.14	0.47–2.75
Salt consumption (for 1 g increase)	0.98	0.86–1.11
Alcohol consumption		
None	1.00	
14 drinks or less weekly	1.06	0.48–2.34
15 drinks or more weekly	1.36	0.68–2.98
Smoking		
Non-smoker	1.00	
Current smoker	1.27	0.74–2.18

POR, prevalence odds ratios. 95%CI, 95% confidence interval. $^{*}P < 0.05$.

statistically significant ($P = 0.008$). Age was also significantly associated with increased risk of CAG. There was no significant relationship between salt consumption and CAG. A slightly increased odds ratio of CAG was observed among those who consumed 15 drinks of alcohol weekly compared with those who were non-drinkers, and among smokers compared with non-smokers. However, theses associations were not statistically significant ($P > 0.05$).

There were no statistically significant differences in the consumption of the 17 nutrients between the groups with and without CAG (data not shown). Prevalence of CAG was not statistically significantly different among groups classified on the basis of frequency of cabbage intake ($P > 0.05$): 16% for those who ate cabbage once or less weekly (n = 132), 22% for those who had 2–3 times weekly (n = 170), and 21% for those who ate it 4 times or more weekly (n = 136). The results were unchanged after controlling for age.

Discussion

This study failed to show that the prevalence of CAG was lower among those who frequently consumed broccoli. Rather, we observed a higher prevalence of CAG among those who consumed broccoli once or more weekly than among those who rarely ate it. This finding was contrary to a previous finding based on *in vitro* and animal experiments [8], because it did not support the preventive effect of broccoli consumption against CAG.

There are several possible explanations for this apparent discrepancy between our findings and those by Fahey *et al*. [8]. First, the discrepancy is attributable to a difference between the mouse stomach and the human stomach. Broccoli intake may not be enough effective to prevent CAG in the human stomach. Second, in the *in vitro* study by Fahey *et al*. [8], a high dose of sulforaphane was given to suppress *H. pylori* activity. On the other hand, 100 g of broccoli contains only 0.025 mg of sulforaphane. For this reason, the average person's consumption of broccoli would be insufficient to suppress *H. pylori* and prevent CAG. However, broccoli sprouts contains a much greater dose, *i.e.*, 2.19 mg of sulforaphane per 100 g. A future study might focus on broccoli sprouts rather than broccoli.

We unexpectedly observed an increased risk of CAG among those who consumed broccoli once or more weekly. Those who felt discomfort in the stomach may generally consume more vegetables, including broccoli. However, we found no statistical difference in nutritional or vitamin consumption between those who had or did not have CAG. In this study, the subjects who consumed broccoli frequently tended to have more salt, which is a possible risk factor of CAG [10, 15, 16]. However, the possible association between broccoli consumption and CAG remained after controlling for salt consumption. In fact, in our study, there was no significant relationship was seen between salt consumption and CAG. This may be attributable to a limited variety of salt consumption in this sample from workers of the same company, the difficulty of measuring salt intake by means of the DHQ, or the influence of seasonality. Another possibility includes that a greater vegetable intake including broccoli may increase the chance of transmission of *H. pylori* to a person, which results in an increased risk of CAG. However, a recent study has shown no association between vegetable intake and of *H. pylori* infection [21].

A major limitation of this study was that *H. pylori* infection was not measured. It is not clear whether broccoli consumption reduces *H. pylori* activity or whether it reduces the risk of CAG only when is *H. pylori* present. Further study is needed to address these issues. Another limitation of the current study is that serologic criteria were used to diagnose CAG. While the sensitivity and specificity of the serological test were high, 59% and 79%, respectively, when pathologically diagnosed CAG was used as a gold standard [18], there was still a chance that random measurement error overlook a true association between broccoli consumption and CAG when pathologically diagnosed CAG was used as the test of certainty [17, 18]. Furthermore, only the frequency of broccoli consumption was measured with no consideration given to volume. This may have resulted in an underestimation of the relationship between broccoli consumption and CAG. A future study should be conducted to replicate our findings, using a more accurately measure of food consumption and to precisely determine the existence CAG, as well as examinations of *H. pylori*. Previous studies have reported that there is no association between the consumption of alcohol and CAG [6, 10–12]. The one exception is a study by Dixon *et al.* [9]. Reports also show that cigarette smoking is not associated with an increased risk of CAG [10–14]. In our study, cigarette smoking and alcohol consumption were not significantly associated with an increased risk of CAG, which agrees with earlier findings. Psychological stress may also affect the development of CAG, as it correlates with ulcer disease [22]. If those who felt stress tended to consume broccoli more frequently, an apparent association between broccoli consumption and CAG may arise as a result of confounding by stress. Because we did not measure stress in our study, this should be investigated in future research.

In conclusion, the present study failed to demonstrate any association between frequent broccoli consumption and a low prevalence of CAG. On the contrary, CAG was more frequent among those who consumed broccoli frequently. To make a clear conclusion regarding the association between broccoli consumption and CAG, a further follow-up study with a better assessment method of broccoli consumption in a community-based sample, or an intervention or experimental study is needed.

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