

Vitamin C supplementation in relation to inflammation in individuals with atrophic gastritis: a randomised controlled trial in Japan

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Abstract

Evidence has shown that both C-reactive protein (CRP) and serum amyloid component A (SAA) are increased in individuals with gastritis and stomach cancer. Controlling the level of these biomarkers by inhibiting the gastric infection with high doses of ascorbic acid may reduce the risk of carcinogenesis. A population-based double-blind randomised controlled trial in a Japanese population with atrophic gastritis in an area of high stomach cancer incidence was conducted between 1995 and 2000. Daily doses of 50 or 500 mg vitamin C were given, and 120 and 124 participants completed the 5-year study, respectively. Although serum ascorbic acid was higher in the high-dosage group (1.73 (SD 0.46) µg/l) than in the low-dosage group (1.49 (SD 0.29) µg/l, $P < 0.001$), at the end of the study, no significant difference was observed for CRP between the low- and high-dosage groups (0.39 (95% CI 0.04, 4.19) mg/l and 0.38 (95% CI 0.03, 4.31) mg/l, respectively; $P = 0.63$) or for SAA between the low- and high-dosage groups (3.94 (95% CI 1.04, 14.84) µg/ml and 3.85 (95% CI 0.99, 14.92) µg/ml, respectively; $P = 0.61$). Vitamin C supplementation may not have a strong effect on reducing infections in individuals with atrophic gastritis.

Key words: Ascorbic acid: C-reactive protein: Serum amyloid component A: Atrophic gastritis

Chronic gastritis, caused by *Helicobacter pylori* infection, is an early-stage precursor for gastric adenocarcinoma^(1,2). However, gastric carcinogenesis may result from a combination of factors, particularly in individuals who react strongly to inflammation or demonstrate a strong immune response⁽³⁾. C-reactive protein (CRP) and serum amyloid component A (SAA) are acute-phase inflammatory reactants in the human body that increase in parallel^(3,4). Evidence has shown that both CRP and SAA are increased in individuals with gastritis and stomach cancer^(3,5,6). Vitamin C has been suggested to have roles in inhibiting the growth of *H. pylori*, inhibiting intragastric formation of nitrosamines and regulating the immune response^(7–9). Controlling the level of these biomarkers may reduce the risk of carcinogenesis in the stomach. Therefore, we hypothesise that a high serum level of ascorbic acid may reduce stomach cancer risk via control of the inflammatory markers CRP and SAA.

A population-based double-blind randomised controlled trial in a Japanese population with gastritis in an area of high stomach cancer incidence was conducted between 1995 and 2000, with the aim of examining the effect of vitamin C supplementation on the primary prevention of gastric cancer^(10,11). We report the impact of vitamin C supplementation on CRP and SAA status in trial subjects at the end of the 5-year period.

Materials and methods

Study participants

The trial was initially intended to examine the effects of supplementation with β-carotene (0 or 15 mg/d) and vitamin C (50 or 500 mg/d) on the incidence of gastric cancer, whereby participants were randomised in a double-blind manner to one of four groups by using a 2 × 2 factorial design. A total

Abbreviations: CRP, C-reactive protein; PG, pepsinogen; SAA, serum amyloid component A.

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of 1231 subjects who were aged 40–69 years and living in four municipalities of the Yokote Public Health Center District of Akita Prefecture were selected to participate in the randomised clinical trial. After the first year of participants' recruitment in 1995, β -carotene supplementation was reported to have potential harmful effects for individuals at high risk for lung cancer^(12,13), and the study protocol was modified by removing subjects who were using β -carotene and stopping recruitment of new subjects in three municipalities⁽¹⁰⁾. The primary endpoint of the trial was changed from a 10-year accumulated incidence of gastric cancer to 5-year changes of the serum levels of pepsinogen (PG) and other biomarkers⁽¹⁰⁾. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the ethics committee of the National Cancer Center and the Hiraka General Hospital. Written informed consents were obtained from all individuals willing to participate and those remaining in the study. Finally, 120 and 124 subjects in the low-dosage and high-dosage groups of vitamin C supplementation, respectively, completed the 5-year study (Fig. 1). The details of the study rationale, design, methodology and protocol amendment have been described previously^(10,11).

Eligible subjects were diagnosed with chronic atrophic gastritis by the cut-off value of PGI <70 ng/ml and a ratio of PGI:II of <3.0, of which the sensitivity was 80% and specificity was 70% as reported⁽¹⁴⁾. Miki⁽¹⁵⁾ reported that the values measured by the same kit showed a good correlation (correlation coefficient 0.983 for PGI, 0.991 for PGII and 0.935 for PGI:II) with those measured by RIA (PGI/PGII RIA-BEAD; Dinabot Company Limited), in which a sensitivity of 70.5% and a specificity of 97.0% for atrophic gastritis, compared with histology, have been reported⁽¹⁶⁾. Selection criteria were no history of gastric cancer or related surgery; no history of cirrhosis, liver cancer or other cancer within the last 5 years; no abnormal liver function; no use of diet supplements containing β -carotene or vitamin C; and no expectation of moving outside the study area within 1 year.

Participant follow-up and dietary intake assessment

Participants were asked to visit the community centres every 3 months where their clinical symptoms and side effects from vitamin C supplementation were assessed, compliance was checked based on the number of unconsumed capsules, and capsules for further use were dispensed^(10,11). Compliance averaged 92.6 and 92.2% in the low- and high-dosage groups,

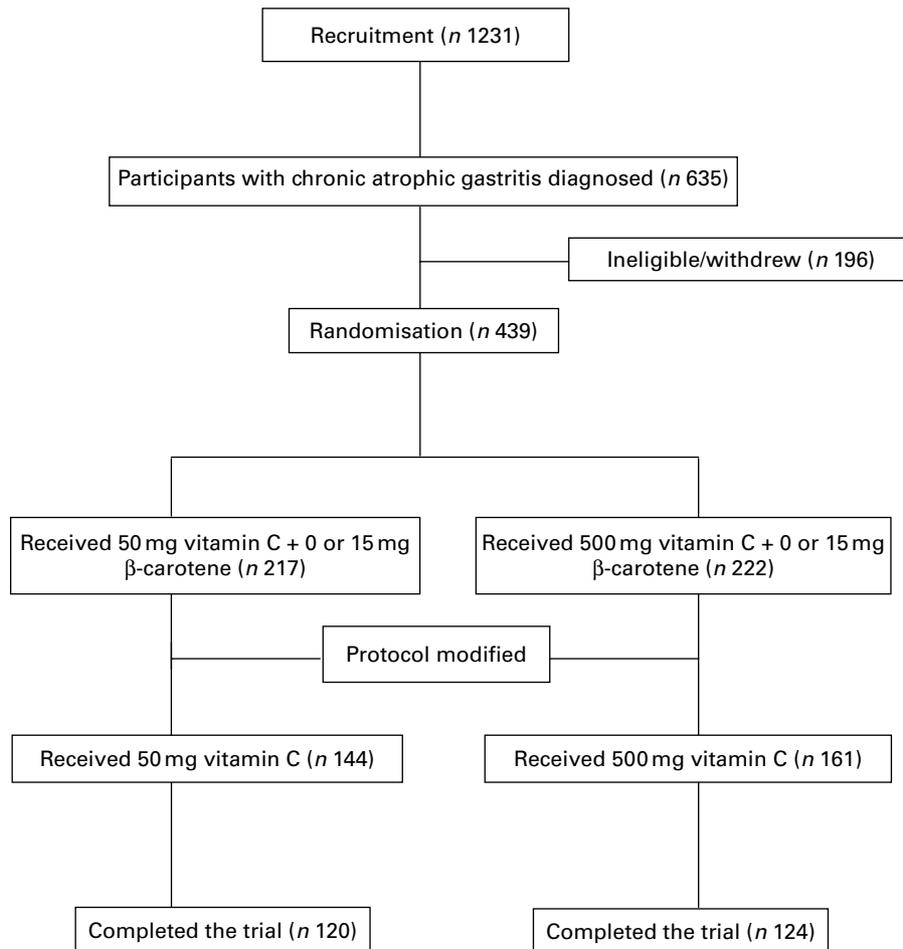


Fig. 1. Flow chart of participant recruitment before and after the protocol amendment and of participants at the 5-year follow-up.

respectively⁽¹⁷⁾. A validated 138-item FFQ was used to assess dietary intake, for which participants were asked how often they consumed individual food items and to estimate the representative size of their portions relative to the size of a standard portion. Daily intake of vitamin C and other nutrients were calculated by using the fifth revised and enlarged edition of the Standard Tables of Food Composition in Japan⁽¹⁸⁾. The details of the FFQ have been described in a previous report^(11,17).

Biochemical analysis

Fasting blood samples were collected at baseline and after 5 years and analysed for serum ascorbic acid levels, CRP and SAA. The subjects were asked not to eat or drink anything except water after 21.00 hours on the day before blood sampling. The serum was sampled between 07.00 and 10.00 hours. All samples were stored at -70 to -85°C and were analysed simultaneously after completion of the 5-year follow-up. All assays were conducted by persons who were blinded as to the intervention assignment and the questionnaire data.

Serum for ascorbic acid measurement was stabilised by the addition of metaphosphoric acid, and serum ascorbic acid concentration was measured fluorimetrically (iodine oxidation and condensation with 1,2-phenylenediamine). CRP and SAA concentrations were determined by the latex agglutination nephelometric immunoassay test (LZ test 'Eiken' CRP-HG and LZ test 'Eiken' SAA, respectively; Eiken Kagaku Company Limited). IgG antibodies to *H. pylori* were measured with a direct ELISA kit (E Plate 'Eiken' *H. pylori* antibody; Eiken Kagaku Company Limited). Levels of IgG were categorised as seropositive and seronegative for *H. pylori* according to the selected cut-off value (492 nm)⁽¹⁹⁾.

Statistical analysis

We followed the intent-to-treat analysis, which included all subjects remaining in the study after the protocol was modified. The per-protocol analysis included subjects who completed the study to the 5-year follow-up. Baseline comparisons between the low- and high-dosage groups and the dropout group as the control were examined by one-way ANOVA for continuous variables and by the χ^2 test for categorical variables. Differences of values within the low- and high-dosage groups were tested by the paired *t* test for continuous variables and by the one-sample *z* test for proportions.

CRP was categorised into positive and negative groups by using a cut-off point of 1.8 mg/l, while SAA was grouped as positive or negative based on a cut-off point of 8.0 $\mu\text{g/ml}$ ⁽³⁾. Subjects' status on combined biomarkers of CRP and SAA was determined by the defined positive and negative statuses of CRP and SAA. Log transformation was done for dietary intake of vitamin C, serum CRP and SAA, and *H. pylori* titre when conducting the comparisons between the two dosage groups; and data are presented as geometric means with their standard errors. The difference between the two

dosage groups for changes in CRP and SAA at the end of the 5-year follow-up compared with baseline was calculated by using the geometric means, respectively.

Adjusted analysis of the means of serum CRP and SAA for covariates was performed by one-way ANOVA. Results were adjusted for age (continuous), sex, dietary intake of vitamin C (quartile), alcohol consumption (never or occasional, regular), smoking status (never, ever), BMI (<25 , ≥ 25 kg/m²), *H. pylori* status (no, yes) and menopausal status (no, yes, for women). Stratified analysis was performed for age groups, alcohol consumption, smoking status, BMI and menopausal status. *P* values less than 0.05 in two-tailed tests were considered as significant, and all statistical analyses were performed using SAS version 9.1 (SAS Institute).

Results

The baseline characteristics of the trial participants are shown in Table 1. Subjects in the low-dosage group were older than those in the high-dosage group. There were more CRP-positive subjects in the high-dosage group than in the low-dosage group both in the intent-to-treat and per-protocol analyses (borderline significance). *H. pylori* titres were higher in the high-dosage group than in the low-dosage group, with a significant difference in the per-protocol analysis.

At the 5-year follow-up, serum ascorbic acid was higher in the high-dosage group (increased 0.37 $\mu\text{g/l}$) compared with the low-dosage group (increased 0.10 $\mu\text{g/l}$ from baseline, $P<0.001$) (Table 2). Correlation of the log-transformed CRP and SAA in all participants at the 5-year follow-up was 0.541 ($P<0.001$). A slight increase in the low-dose group and a decrease in the high-dose group both in CRP and SAA levels were observed at the 5-year follow-up; thus the absolute 0.07 mg/l reductions in CRP and the 0.31 $\mu\text{g/ml}$ reduction in SAA were in the high-dose group compared with those in the low-dose group, if taking consideration of the baseline values. However, there were no significant differences for CRP between the low- and high-dosage groups (0.39 (95% CI 0.04, 4.19) mg/l and 0.38 (95% CI 0.03, 4.31) mg/l, respectively; $P=0.63$) or for SAA between the low- and high-dosage groups (3.94 (95% CI 1.04, 14.84) $\mu\text{g/ml}$ and 3.85 (95% CI 0.99, 14.92) $\mu\text{g/ml}$, respectively; $P=0.61$) (Table 2). CRP status changed from positive to negative for 60% (six out of ten) of the low-dosage group and 68.4% (thirteen out of nineteen) of the high-dosage group between baseline and the 5-year follow-up ($P=0.33$), while SAA status for 57.1% (eight out of fourteen) in the low-dosage group and 70.0% (seven out of ten) in the high-dosage group of SAA-positive participants changed from positive to negative ($P=0.27$). The combined positive and negative statuses for CRP and SAA were also not significantly different between the two groups at the 5-year follow-up (47.4% (nine out of nineteen) *v.* 59.1% (thirteen out of twenty-two); $P=0.23$). When we deleted two outliers that were both CRP- and SAA-positive at baseline, similar null results for CRP and SAA were observed, respectively, between the two dosage groups at the 5-year follow-up.

Table 1. Baseline characteristics of the participants in the trial
(Mean values and standard deviations or standard errors; number of participants and percentages)

	Intent-to-treat				<i>P</i> *	Per-protocol				<i>P</i> *
	Low-dosage vitamin C, 50 mg (<i>n</i> 144)		High-dosage vitamin C, 500 mg (<i>n</i> 161)			Low-dosage vitamin C, 50 mg (<i>n</i> 120)		High-dosage vitamin C, 500 mg (<i>n</i> 124)		
	<i>n</i>	%	<i>n</i>	%		<i>n</i>	%	<i>n</i>	%	
Age (years)					0.01					0.02
Mean	58.56		56.55			58.67		56.29		
SD	6.64		8.74			6.53		8.66		
BMI (kg/m ²)					0.66					0.52
Mean	23.38		23.18			23.42		23.23		
SD	2.92		2.69			2.86		2.65		
Men	54	37.5	58	36.0	0.79	41	34.2	45	36.3	0.73
Current smoking	18	12.5	26	16.2	0.65	12	10.0	19	15.3	0.38
Alcohol consumption	62	43.1	69	42.9	0.99	53	42.2	57	46.0	0.69
Serum ascorbic acid (μg/l)	1.37	0.35	1.35	0.37	0.96	1.38	0.32	1.35	0.37	0.51
Dietary vitamin C (μg/l)					0.65					0.41
Mean	121.03		120.25			123.62		123.52		
SE	1.06		1.06			1.06		1.06		
CRP (mg/l)†					0.16					0.77
Mean	0.35		0.43			0.35		0.41		
SE	1.11		1.13			1.12		1.14		
CRP positive†	11	7.6	24	14.9	0.05	10	8.3	19	15.3	0.07
SAA (μg/ml)†					0.26					0.29
Mean	3.82		4.29			3.87		4.09		
SE	1.06		1.08			1.07		1.09		
SAA positive†	16	11.1	16	9.9	0.74	14	11.7	10	8.1	0.35
<i>Helicobacter pylori</i> titre (RU/ml)					0.15					0.01
Mean	59.19		68.73			57.13		73.73		
SE	1.07		1.07			1.08		1.07		
<i>H. pylori</i> positive	140	97.2	157	97.5	0.87	116	96.7	122	98.4	0.39
PGI (ng/ml)					0.83					0.65
Mean	38.38		39.03			38.35		39.8		
SD	17.06		16.43			17.2		16.35		
PGII (ng/ml)					0.15					0.11
Mean	19.62		20.60			19.46		20.79		
SD	7.14		7.34			7.22		7.34		
PGI:II					0.20					0.24
Mean	1.95		1.89			1.97		1.92		
SD	0.63		0.61			0.62		0.59		

CRP, C-reactive protein; SAA, serum amyloid component A; RU, relevant unit; PG, pepsinogen.

* By one-way ANOVA test or χ^2 test.

† 117 subjects in the per-protocol analysis were available in the low- and high-dosage groups, respectively.

Stratified analysis showed that there were no significant differences in the decrease in CRP or SAA levels between the two dosage groups by age categories (40s, 50s and 60s), sex, smoking or alcohol consumption. Similar results were observed after adjusting for sex, dietary intake of vitamin C (quartile), *H. pylori* titre, smoking status, alcohol consumption and BMI (data not shown).

Discussion

We did not observe any significant reduction of CRP or SAA levels in the low- or high-dosage groups after 5 years of ascorbic acid supplement use, although serum ascorbic acid concentration was higher in the high-dosage group than in the low-dosage group. We also did not observe any significant differences between the two groups in age, sex, smoking, alcohol consumption or body weight status.

The CRP and SAA levels in the present study were similar to those reported in other studies^(3,20). In the present study,

based on cut-off points of 1.8 mg/l for CRP and 8.0 μg/ml for SAA, there were small numbers of CRP- or SAA-positive participants and there was no significant difference for either between the two dosage groups at baseline, respectively. We also applied other cut-off points for CRP- and SAA-positive status such as a CRP of 10 mg/l⁽²¹⁾ or by areas under the received curve⁽²²⁾. By these criteria, the numbers of CRP- or SAA-positive participants remained similar and no significant differences existed between the two dosage groups. Nevertheless, the small number of CRP- and SAA-positive participants at baseline made it difficult to evaluate changes in CRP and/or SAA status at follow-up. It might be possible that CRP and SAA were not highly sensitive markers for measuring chronic infection status, which contributed to the null outcome in the present study. On the other hand, the 500 mg/d supplement in the present study might not be sufficient to control chronic gastric infection, although cancer chemoprevention trials with more than 500 mg/d of vitamin C

Table 2. Comparisons of serum ascorbic acid and inflammatory biomarkers between baseline and the 5-year follow-up (Mean values and standard deviations or standard errors)

	Low-dosage vitamin C, 50 mg (n 117)					High-dosage vitamin C, 500 mg (n 117)					
	Baseline		5 years		P*	Baseline		5 years		P*	P†
	Mean	SE	Mean	SE		Mean	SE	Mean	SE		
Serum ascorbic acid (µg/l)					<0.01					<0.001	<0.001
Mean	1.38		1.49			1.35		1.73			
SD	0.32		0.29			0.37		0.46			
Dietary vitamin C (µg/l)	123.62	1.06	121.14	1.06	0.79	123.52	1.06	123.11	1.06	0.88	0.78
CRP (mg/l)	0.35	1.12	0.39	1.12	0.35	0.41	1.14	0.38	1.12	0.64	0.63‡
SAA (µg/ml)	3.87	1.07	3.94	1.06	0.88	4.09	1.09	3.85	1.07	0.57	0.61‡

CRP, C-reactive protein; SAA, serum amyloid component A.

* By paired *t* test.

† By one-way ANOVA test for the difference between the two dose groups at the 5-year follow-up.

‡ Adjusted for age, sex, BMI, smoking status, alcohol consumption, dietary vitamin C, *Helicobacter pylori* status and baseline level of CRP or SAA.

supplementation have not shown consistent results on the beneficial effects^(23,24).

Human gastric carcinogenesis is a multistep and multifactorial process, with the initial stages of gastritis and atrophy linked to excessive salt intake and *H. pylori* infection^(17,25). *H. pylori* eradication can prevent the progression of precancerous gastric lesions and probably reduce the incidence of gastric cancer in those without advance lesions⁽²⁶⁾. In the present study, CRP and SAA were not significantly reduced and the positive proportions of *H. pylori* were consistently higher ($\geq 92\%$) after 5 years of follow-up in both the low- and high-dosage groups⁽¹⁷⁾. It was possible that in the achlorhydric stomach, *H. pylori* infection might disappear, although the antibodies in the serum might maintain a longer time. Nevertheless, *H. pylori* infection potentially modulates the effects of vitamin C or vice versa⁽⁹⁾. Without eradicating the infection, ascorbic acid supplementation for participants with atrophic gastritis might have fewer effects on CRP/SAA control. However, studies on changes in CRP after *H. pylori* eradication are contradictory. Some studies have reported a significant reduction of CRP levels in subjects after *H. pylori* eradication by antibiotics⁽²⁷⁾ or vitamin C supplementation⁽²⁸⁾, while others have shown no significant reduction of CRP by anti-inflammatory or antibiotic treatment^(20,29,30). A Colombian study in gastritis patients, applying a 2-week anti-*H. pylori* treatment and/or a 6-year antioxidant supplement, showed that acute inflammation disappeared soon after the *H. pylori* treatment, while chronic inflammation responded at a slower pace, and the antioxidant effect was transient and disappeared after the 6 years of follow-up, while the anti-*H. pylori* treatment effect persisted for as long as patients remained free of *H. pylori*⁽²³⁾. Also, subjects with non-metaplastic multifocal atrophic gastritis had the steepest declines if they cleared the bacteria, but had the sharpest increases if they did not⁽²³⁾. The present study results appear to support the finding that ascorbic acid supplementation does not have much beneficial effect on chronic gastric infections, particularly without assigning the anti-*H. pylori* treatment.

There are several limitations in the present study. The most critical one is that we did not have a placebo group for comparison with the 50 and 500 mg dosage groups⁽³¹⁾. However,

the mean dietary intakes of vitamin C were 151.95 (SD 111.98) µg/l and 147.93 (SD 99.81) µg/l for the high- and low-dose groups, respectively, and the low-dose supplementation group was similar to or within 1 SD of the estimated vitamin C intake level from foods. In the pilot study⁽³²⁾ for the present trial, there were no significant differences in serum vitamin C concentrations between the placebo (0 mg/d) and the low-dose groups at 1, 2 and 3 months of supplementation, respectively. Moreover, the purpose of the present study was to evaluate the effect of vitamin C supplementation (500 mg/d) compared with the normal level (the average consumption level of Japanese). Additionally, the similar mean dietary intake of vitamin C in the placebo group was seen in another trial⁽³³⁾. Therefore, the low-dose vitamin C supplementation group (50 mg/d) in the present study could be regarded as the placebo group for interpretation⁽³⁴⁾. Second, the initial sample size was considered with estimated differences in accumulated gastric cancer incidence between the two study groups in 10 years rather with the changes in these biomarkers of atrophic gastritis in 5 years⁽¹⁰⁾. For example, to detect the 0.15 µg/ml difference in SAA levels between the two dose groups at the 5-year follow-up, using the standard deviation in each group, 5% type I error and 20% type II error for estimation, 1030 subjects in each group are needed. The limited number of study subjects after changes in the initial study protocol had less statistical power for identifying the significance of CRP and SAA reductions between the two dosage groups. Third, IL-6 and other immunological factors are thought to be mediators that stimulate CRP production^(5,28,35); however, we could not evaluate the CRP reduction as modified by ascorbic acid by using these factors because the data were unavailable. Since we did not conduct endoscopy for gastritis participants, we therefore could not evaluate the progression or regression of gastric lesions after ascorbic acid supplementation at the 5-year follow-up^(23,36,37). Finally, since we only tested CRP and SAA two times, at baseline and the 5-year follow-up, any changes in their levels in the intervening time were not evaluated.

Some studies have reported that antioxidant supplementation, even at low doses, can have adverse effects on subjects at high risk for cancer or those with undiagnosed cancer^(38,39).

It should be noted that some of the well-known beneficial effects of ascorbic acid administration are still only understood at the phenomenological level⁽⁴⁰⁾. Currently, the Asia–Pacific guidelines on gastric cancer prevention do not recommend vitamin C supplementation for reducing the risk of gastric cancer⁽²⁶⁾.

In summary, we did not observe a significant reduction in CRP or SAA levels in atrophic gastritis participants with ascorbic acid supplementation of less than 500 mg/d at the 5-year follow-up. The present study suggests that ascorbic acid supplementation might not have much beneficial effect in individuals with chronic *H. pylori* infection. Further studies are needed in larger populations on the control of chronic infection and inflammation through ascorbic acid supplementation.

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