Ascorbic acid and total vitamin C concentrations in plasma, gastric juice, and gastrointestinal mucosa: effects of gastritis and oral supplementation

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Abstract

Epidemiological evidence suggests that high dietary ascorbic acid reduces gastric cancer risk. It may do this by either reducing *N*-nitroso compound formation in gastric juice, or by scavenging reactive oxygen species in gastric mucosa. The aim of this study was to discover if potential ascorbic acid protection might be increased by supplementation. Thirty two patients were supplemented with ascorbic acid, 500 mg twice daily for two weeks. Gastric juice, plasma, and upper gastrointestinal biopsy ascorbate concentrations were measured and compared with values in 48 unsupplemented patients. It was found that ascorbic acid and total vitamin C concentrations were considerably higher in biopsy specimens from oesophagus, body, antrum, duodenum, and rectum, compared with values in plasma or gastric juice. Plasma and mucosal concentrations were unaffected by the presence of chronic gastritis but gastric juice concentrations were substantially lower in patients with chronic gastritis than in patients with normal histological assessment ($p < 0.01$). Patients receiving ascorbic acid supplements had higher ascorbic acid concentrations in plasma ($p < 0.001$), gastric juice ($p < 0.001$), and at all biopsy sites in the upper gastrointestinal tract ($p < 0.05$). Gastric juice ascorbic acid and total vitamin C concentrations in gastritic patients, however, were still less after supplementation than in normal subjects ($p < 0.01$). These data suggest that high ascorbic acid intake could reduce gastric cancer risk, but its protective effect might be greater if gastritis is treated (for example, by *Helicobacter pylori* eradication).

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Keywords: gastritis, *Helicobacter pylori*, gastric cancer, ascorbic acid, supplementation.

Epidemiological data suggest that ascorbic acid, the reduced form of vitamin C, protects against gastric cancer.¹ *N*-nitroso compounds are implicated in gastric carcinogenesis² and experimental evidence suggests that ascorbic acid is protective through its ability to reduce nitrous acid and prevent the formation of

carcinogenic *N*-nitroso compounds in the human stomach.³⁻⁶ As well as inhibiting chemical nitrosation in the acid stomach, ascorbic acid prevents nitrosation by bacteria at neutral pH.⁷ The concentration of ascorbic acid in gastric juice may therefore, be a critical factor in the prevention of intragastric *N*-nitrosation and cancer.

Ascorbic acid is secreted into the histologically normal stomach leading to gastric juice concentrations greater than those in plasma, but this secretion is impaired in the presence of chronic gastritis.⁸⁻¹⁰ When gastritis or drug treatment causes hypochlorhydria ($\text{pH} \geq 4$) ascorbic acid concentrations are reduced almost to zero, eventually leading to a loss of total vitamin C (ascorbic acid and dehydroascorbic acid) as well.^{11,12} This dramatic decrease in juice ascorbate could be caused by a combination of decreased excretion, instability of the vitamin at neutral pH, and its oxidation by nitrite.¹² Whatever the cause of low gastric juice ascorbate in patients with atrophic gastritis and a tendency towards hypochlorhydria, it is these patients who are at increased risk of gastric cancer.^{13,14} This would suggest that ascorbic acid is indeed important in reducing the risk of gastric cancer and that the effectiveness of its protection might be considerably impaired in the diseased stomach.

There is, therefore, a need to examine ways of increasing the concentration of ascorbic acid in the stomach. This may not be easy in the presence of disease, especially where hypochlorhydria has led to accumulation of potential oxidants, such as nitrite. Here any transient acidification leads rapidly to the oxidation of large quantities of ascorbate.¹² There has only been one study examining the effect of oral ascorbic acid supplementation on gastric ascorbate concentrations in vivo and this showed that in Venezuelan patients with premalignant gastric histology (atrophic gastritis, intestinal metaplasia) oral supplementation did not raise ascorbic acid concentrations in gastric juice.¹⁵ O'Connor *et al.*¹⁶ however, found that supplementation with ascorbic acid reduced genotoxicity of gastric juice showing that such treatment could have some impact on carcinogenesis. The aim of this study was therefore to discover if oral supplements of ascorbic acid can indeed raise juice and mucosal concentrations in vivo in healthy patients and those with chronic gastritis. We also measured ascorbic acid and total vitamin

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C concentrations in oesophageal, duodenal, and rectal tissue to find out whether or not the stomach is unique in the gastrointestinal tract in concentrating high values of the vitamin.

Methods

Patient selection

Patients were recruited from those undergoing endoscopy for dyspepsia. Any patients with a history of gastric surgery were excluded. Approval was granted by the local ethics committee, and written informed consent was obtained. The supplemented subjects were asked to take ascorbic acid tablets 500 mg twice daily for the two weeks prior to endoscopy. Supplementation ceased the day before the investigation to ensure that gastric juice values reflected secreted ascorbic acid rather than residual ingested ascorbic acid. The unsupplemented patients were recruited in 1991, and the supplemented group during 1992. Also in 1992 we recruited additional unsupplemented patients to ensure that our high performance liquid chromatography (HPLC) and homogenisation techniques still produced comparable tissue results with those obtained the year previously. As the values in these were all within the range of our earlier unsupplemented results, all unsupplemented patients have been considered together.

Endoscopic procedures

At endoscopy a sterile Teflon catheter was passed through the biopsy channel immediately after intubation and about 5 ml of gastric juice aspirated. Ten ml of blood was also drawn from each patient into a heparinised tube and centrifuged immediately. Both gastric juice and plasma were each divided into two aliquots. Gastric juice was stored at -70°C ; (a) in an equal volume of 2% metaphosphoric acid containing 0.5% sulphamic acid until analysed for ascorbic acid, (b) in 2% metaphosphoric acid 0.5% sulphamic acid supplemented with 6 mg/ml dithiothreitol for analysis of total vitamin C (ascorbic acid and dehydroascorbic acid). Plasma was stored at -70°C ; (a) in two volumes of 2% metaphosphoric acid for ascorbic acid analysis, (b) in 2% metaphosphoric acid supplemented with 6 mg/ml dithiothreitol for total vitamin C analysis.

In addition, at least two biopsy specimens were taken from each of the following sites; oesophagus, gastric body, gastric antrum, first part of the duodenum, and second part of the duodenum. One or more of the specimens were sent for histological examination, which included examination using modified Giemsa staining. One specimen from each site was immediately frozen and stored indefinitely in liquid nitrogen for vitamin C analysis.

Samples were later thawed and blotted dry on filter paper prior to weighing. They were then homogenised in 0.5–1.0 ml 2% metaphosphoric acid in a glass hand-held

homogeniser. Each sample was halved and dithiothreitol added to a final concentration of 6 mg/ml to one of these aliquots. Both samples were stored at -70°C . Preliminary work (unpublished data) showed close correlation between ascorbic acid content of the stomach expressed per g wet weight and per mg protein estimated on the acid precipitates. Values here are all reported as μmol per wet weight tissue.

Biochemical analysis

On thawing, all the samples were centrifuged and the supernatant solution analysed by HPLC using reversed phase ion pair chromatography on a C18 column.¹⁰ An electrochemical detector set at a low voltage selectively measured ascorbic acid content. To determine total vitamin C content the supernatant containing dithiothreitol was incubated at 45°C for 120 minutes prior to analysis by HPLC.

The recovery of ascorbic acid and total vitamin C has been investigated by adding ascorbic acid to tissue homogenates prepared from resected stomach tissue, and using ascorbate oxidase spatulas (Boehringer-Mannheim), which oxidise ascorbic acid, to assess recovery of dehydroascorbic acid. In all cases ascorbate oxidase reduces measured ascorbic acid values to zero showing specificity of the measurements. Recovery of ascorbic acid in tissue was measured as mean (SD) 90 (3)% ($n=4$), and recovery of dehydroascorbic acid (produced by ascorbic acid oxidation) was 75 (5)% ($n=4$).

Rectal tissue collection

To determine the mucosal concentrations of vitamin C elsewhere in the gastrointestinal tract, rectal biopsy specimens and blood samples were obtained from 15 patients undergoing colonoscopy. Plasma and tissue samples were analysed for ascorbic acid and total vitamin C concentrations using the same HPLC techniques applied to those samples obtained from the upper gastrointestinal tract.

Statistical analysis

The data were skewed so non-parametric tests were used. As the unsupplemented and supplemented groups were different patients, the data were unpaired and comparisons were made with the Mann-Whitney test. Comparisons between those patients with gastritis and those without were also made with the Mann-Whitney test. Data comparing vitamin C concentrations in different parts of the gastrointestinal tract within the same patient were paired and the Wilcoxon signed ranks test used.

All analyses were performed using Oxstat V (1.00), (c) Holman, Jones, Walter and Wiggins.

Results

There was a total of 51 unsupplemented

Figure 1: Gastric juice ascorbic acid concentrations (median values and ranges): effect of gastritis (N, normal histology; G, chronic gastritis) in unsupplemented and supplemented patients. Significantly different from; *normal histology, $p < 0.01$; †unsupplemented, $p < 0.001$.

patients of whom 37 had chronic gastritis. Antral and body biopsy specimens were obtained from all of them, but only the five patients recruited in 1992 with the supplemented patients also had oesophageal and duodenal samples taken. The supplemented group contained 33 patients of whom 20 had chronic gastritis. One patient in both the unsupplemented and supplemented group had reactive gastritis and these were both excluded from subsequent analysis. Also excluded were a patient with gastric lymphoma, and another with lymphocytic gastritis, both in the unsupplemented group. This left final totals of 48 unsupplemented and 32 supplemented patients.

The unsupplemented and supplemented patient groups had a similar sex distribution with 28 males and 20 females in the unsupplemented group and 20 males and 12 females in the supplemented group. The unsupplemented group was slightly older, having a

Figure 2: Plasma ascorbic acid concentrations (median values and ranges): effect of gastritis (N, normal histology; G, chronic gastritis) in unsupplemented and supplemented patients. Difference from; *normal histology, NS; †unsupplemented, $p < 0.001$.

TABLE 1 Gastric juice ascorbic acid and total vitamin C concentrations: effect of gastritis in supplemented and unsupplemented patients

	Unsupplemented		Supplemented	
	Ascorbic acid	Total vitamin C	Ascorbic acid	Total vitamin C
No gastritis	87 (3–483) n=11	100 (12–474) n=11	140 (25–298)† n=12	216 (47–351)† n=12
Gastritis	17 (0–246)* n=32	39 (4–268)* n=32	75 (0–235)*† n=20	80 (17–352)*† n=20

Values represent medians with ranges in parentheses (μmol/l). Significantly different from: *no gastritis, $p < 0.01$; †unsupplemented, $p < 0.001$.

mean age of 52.7 years (range 19–86) compared with 43.1 years (range 18–69) in the supplemented group.

Gastric juice and plasma

Figures 1 and 2 show the distribution of gastric juice and plasma ascorbic acid concentrations respectively.

Effect of gastritis – reduced concentrations of both ascorbic acid and total vitamin C were found in the gastric juice of patients with gastritis when compared with patients with normal histological tests (Table I). The difference persisted even after supplementation (unsupplemented group: $p < 0.01$ ascorbic acid, $p < 0.01$ total vitamin C, supplemented group: $p < 0.01$ ascorbic acid, $p < 0.01$ total vitamin C). There were no differences, however, in plasma ascorbic acid or total vitamin C concentrations between patients with and without gastritis in either supplemented or unsupplemented groups ($p > 0.05$ in all cases) (Table II).

Effect of supplementation – there were significantly higher concentrations of ascorbic acid and total vitamin C in gastric juice (Table I) and plasma (Table II) in supplemented compared with unsupplemented patients ($p < 0.001$ in all cases).

Effect of pH – a pH of 4 was taken as the cut off point to divide juice into acid (pH < 4) or hypochlorhydric juice (pH > 4) as this is the value above which bacterial overgrowth is a feature,¹⁷ which might lead to increased nitrite and N-nitroso compound formation.

The concentration of ascorbic acid in the unsupplemented group with hypochlorhydric juice was significantly lower ($p < 0.05$) than in juice with pH < 4 (Table III). Total vitamin C also tended to be lower in the hypochlorhydric juice, but this did not reach conventional levels of significance ($p > 0.05$).

All the patients in the supplemented group had gastric juice with pH < 4.

Tissue

Figure 3 shows the distribution of gastric mucosal ascorbic acid concentrations. Ascorbic acid and total vitamin C concentrations throughout the gastrointestinal tract, in supplemented and unsupplemented patients, are summarised in Tables IV and V respectively. Ascorbic acid and total vitamin C concentrations in biopsy samples from all sites in the gastrointestinal tract were approximately 10-times higher than those found in gastric juice and 20-times those found in plasma from subjects without gastritis.

Comparison by biopsy site – in the unsupplemented patients antral concentrations of ascorbic acid and total vitamin C are significantly higher than those in the gastric body ($p < 0.001$). Duodenal and oesophageal specimens were too few to make meaningful statistical comparison, but values were similar to the antrum and body suggesting no important differences in different parts of the upper gastrointestinal tract. The ascorbic acid and total vitamin C concentrations in rectal specimens

TABLE II Plasma ascorbic acid and total vitamin C concentrations: effect of gastritis in supplemented and unsupplemented patients

	Unsupplemented		Supplemented	
	Ascorbic acid	Total vitamin C	Ascorbic acid	Total vitamin C
No gastritis	31 (12-81) n=11	29 (12-88) n=11	81 (47-122)* n=12	83 (55-122)* n=12
Gastritis	21 (3-91) n=36	21 (5-89) n=36	74 (40-97)* n=19	74 (49-91)* n=19

Values represent medians with ranges in parentheses ($\mu\text{mol/l}$). There were no significant differences between gastritis and no gastritis groups. Significant difference from: *unsupplemented, $p<0.001$.

TABLE III Gastric juice ascorbic acid and total vitamin C concentrations: effect of pH in supplemented and unsupplemented patients

	Unsupplemented		Supplemented	
	Ascorbic acid	Total vitamin C	Ascorbic acid	Total vitamin C
pH<4	39 (0-483) n=33	51 (6-474) n=33	85 (0-298) n=32	140 (17-352) n=32
pH>4	9 (0-82)* n=10	34 (4-111) n=10	n=0	n=0

Values represent medians with ranges in parentheses ($\mu\text{mol/l}$). Significant difference from: *pH <4, $p<0.05$.

were also comparable with those found in the upper gastrointestinal tract specimens and much higher than plasma concentrations in unsupplemented subjects. In the supplemented group of patients, concentrations of ascorbic acid and total vitamin C were significantly higher in the second part of the duodenum than in the oesophagus ($p<0.05$ for ascorbic acid, $p<0.01$ for total vitamin C) or gastric body ($p<0.001$ for ascorbic acid, $p<0.001$ for total vitamin C). Concentrations of total vitamin C and ascorbic acid were also significantly higher in the first part of the duodenum ($p<0.05$ for ascorbic acid, $p<0.01$ for total vitamin C) and antrum ($p<0.01$ for ascorbic acid, $p<0.05$ for total vitamin C) than in the gastric body.

Effect of gastritis – in the unsupplemented group, the numbers of duodenal and oesophageal specimens were too small for statistical

analysis associated with the presence of gastritis. There was no significant difference between antral or body concentrations of ascorbic acid and total vitamin C in patients with gastritis compared with those with normal histological appearance. There was no significant difference between the supplemented tissue ascorbic acid and total vitamin C concentrations of patients with gastritis compared with those without gastritis. This was true of duodenum, antrum, gastric body, and oesophagus.

Effect of supplementation – despite the small number of unsupplemented oesophageal and duodenal specimens, we found that ascorbic acid and total vitamin C concentrations were significantly higher ($p<0.05$) in the supplemented group than in the unsupplemented group at all biopsy sites (oesophagus, gastric body, gastric antrum, first and second parts of the duodenum).

Discussion

Subjects with and without gastritis who were supplemented with ascorbic acid had higher gastric juice concentrations of total vitamin C and ascorbic acid but, as shown previously,⁸⁻¹² values were lower in patients with gastritis and this difference remained despite supplementation. Plasma and tissue concentrations were also increased by supplementation, but were unaffected by the presence of gastritis. These results suggest that antioxidant defences can be bolstered in subjects with gastritis by increasing dietary ascorbic acid but, in the gastric juice, not to the values seen in supplemented patients without gastritis. In unsupplemented subjects with hypochlorhydria gastric juice concentrations of vitamin C are very low and ascorbic acid concentrations are reduced almost to zero. This means that if intragastric nitrite concentrations are high secondary to bacterial overgrowth, ascorbic acid concentrations are too low to afford protection.¹² All of the patients given ascorbic acid were found to have gastric juice with pH below 4, so there were no data to show whether oral vitamin C would have increased gastric juice concentrations in patients with hypochlorhydria. In vitro work suggests that oral vitamin C taken by such subjects could be oxidised and cease to be effective.¹² Further investigation is needed to find out if oral ascorbic acid does raise gastric ascorbic acid in vivo in hypochlorhydric conditions.

Although this study would have been more powerful if the same subjects had been compared before and after supplementation, we are confident that the data here do show that oral ascorbate supplements will significantly increase gastric juice, plasma, and mucosal ascorbic acid concentrations in most patients. Our methodology produced consistent ascorbic acid concentrations in unsupplemented patients with and without gastric disease over the two year period of the study and is also in close agreement with the results from earlier studies.⁹⁻¹² It is unlikely therefore, that individual variation can be responsible for the

Figure 3: Mucosal ascorbic acid concentrations in gastric antrum and gastric body (median values and ranges): effect of gastritis (N, normal histology; G, chronic gastritis) in unsupplemented and supplemented patients. Differences from: *normal histology, NS; †unsupplemented, $p<0.05$; ‡antrum, $p<0.001$; §antrum, $p<0.01$.

TABLE IV Ascorbic acid concentrations in tissue from the upper gastrointestinal tract and rectum: effect of gastritis in supplemented and unsupplemented patients

Biopsy site	Unsupplemented		Supplemented	
	No gastritis	Gastritis	No gastritis	Gastritis
Oesophagus	357* n=1	385 (202–807) n=4	980 (182–1925) n=10	988 (278–1880) n=17
Body	473 (217–1016) n=12	628 (87–1228) n=36	730 (278–1488) n=10	795 (375–1630) n=16
Antrum	805 (158–1473) n=12	797 (122–1795) n=36	1045 (460–1562) n=9	1454 (312–1863) n=17
Duodenum 1st part	765* n=1	491 (434–735) n=4	1119 (562–1505) n=9	1369 (114–2397) n=17
Duodenum 2nd part	935* n=1	492 (257–750) n=4	1201 (284–1784) n=8	1670 (68–2778) n=17
Rectum	949 (454–1392)† n=15			

Values represent medians with ranges in parentheses ($\mu\text{mol/kg}$). In neither the unsupplemented nor the supplemented patients were there any significant differences between the no gastritis and gastritis groups but values were significantly higher at all biopsy sites in supplemented subjects compared with those who had received no vitamin C (for significance of differences between biopsy sites, see text). *Insufficient numbers for statistical analysis of no gastritis and gastritis groups. †No assessment of gastric pathology was made.

higher concentrations measured in the supplemented patients, particularly as the differences were so great. In addition, we also felt that it would be unethical to ask patients with normal endoscopic findings to undergo a second endoscopy purely for the purpose of determining the effect of ascorbic acid supplementation.

This study shows that both ascorbic acid and total vitamin C are concentrated in mucosa throughout the gastrointestinal tract at values much higher than those found in plasma. There were insufficient unsupplemented subjects to determine which areas of the gastrointestinal mucosa contained the greatest amount of vitamin C, except that in the stomach concentrations in antral mucosa are greater than the body, confirming earlier work.⁸ It is unclear in which part of the mucosa vitamin C is concentrated. Further work is needed to delineate whether it is intracellular, bound to cell membrane or in the interstitium. Values of ascorbic acid and total vitamin C measured in rectal biopsy specimens suggest that the large intestine also concentrates the vitamin, so ascorbic acid seems to be concentrated by all epithelial cells along the gastrointestinal tract. Clearly, if vitamin C is secreted into the stomach,⁹ it is possible that ascorbic acid is a universal protective agent in and on all gastrointestinal epithelial tissues subject to attack from external genotoxic agents. This, however, is conjecture and further studies are required to find out if ascorbic acid is present in duodenal or colonic secretions.

TABLE V Total vitamin C concentrations in tissue from the upper gastrointestinal tract and rectum: effect of gastritis in supplemented and unsupplemented patients

Biopsy site	Unsupplemented		Supplemented	
	No gastritis	Gastritis	No gastritis	Gastritis
Oesophagus	404* n=1	439 (273–653) n=4	900 (256–1806) n=10	1056 (136–1937) n=17
Body	684 (432–1139) n=12	639 (193–1226) n=36	903 (392–1369) n=10	897 (596–1766) n=16
Antrum	842 (369–1497) n=12	821 (182–1979) n=36	1153 (557–1467) n=9	1261 (386–1698) n=17
Duodenum 1st part	902* n=1	570 (522–866) n=4	1369 (682–2255) n=9	1414 (233–2681) n=17
Duodenum 2nd part	1076* n=1	493 (377–921) n=4	1329 (403–1806) n=9	1744 (261–3005) n=17
Rectum	932 (500–1494)† n=15			

See Table IV for footnotes.

Ascorbic acid might act to reduce gastric cancer risk in two ways. Firstly, it is a good nitrite scavenger and could thereby reduce the endogenous formation of carcinogenic N-nitroso compounds in the stomach lumen by its presence in gastric juice.^{3–7} Secondly, high mucosal concentrations of ascorbic acid may be important in limiting free radical mediated damage within the epithelium. This may be relevant in conditions such as *Helicobacter pylori* associated gastritis, where increased free oxygen radical activity has been shown by chemiluminescence.¹⁸ Free radicals have been implicated in carcinogenesis¹⁹ and ascorbic acid may partly exert its protective effect against gastric cancer by scavenging them and protecting DNA. As gastritis both reduces ascorbic acid concentrations in gastric juice and leads to mucosal reactive oxygen species formation, it may be that both of these mechanisms participate in the progression from gastritis to dysplasia and cancer. This study has confirmed, however, that gastritis has no effect on ascorbic acid or total vitamin C concentrations in supplemented or unsupplemented tissue⁸ and so, although gastric juice ascorbic acid concentrations are low in the diseased stomach, tissue ascorbic acid may still protect against carcinogenesis in gastritis by scavenging reactive oxygen species formed in the mucosa. Vitamin supplementation could increase this protection.

We conclude that vitamin C is concentrated in mucosa throughout the gastrointestinal tract, and is present in the normal stomach lumen at concentrations greater than in plasma. Although oral supplementation raises concentrations in plasma, mucosa, and gastric juice, the presence of gastritis impairs juice concentrations of vitamin C in supplemented patients just as it does in unsupplemented ones. Eradication of *H. pylori* leads to an increase in the gastric juice concentrations of ascorbic acid in patients with *H. pylori* associated gastritis.²⁰ In our study 75% of the patients with chronic gastritis also had *H. pylori* demonstrated, although this is probably an underestimate as we did not use culture or urease testing, and because of this, and the fact that both chronic gastritis and *H. pylori* have a similar effect on gastric juice ascorbate^{9–12 20 21} we have not analysed the data by the presence of *H. pylori*. However, all this implies that *H. pylori* eradication may be an important adjunct to supplementation if juice concentrations of vitamin C are to be raised maximally for the greatest protective effect against N-nitroso compound formation to be realised.

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