Ascorbic acid: a factor concentrated in human gastric juice

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SUMMARY

1. Concentrations of ascorbic acid (ascorbic and dehydro-ascorbic; A+D; measured by the 2,4-dinitrophenylhydrazine method) of nearly three times those of plasma are present in gastric juice samples from patients with normal gastric histology.

2. A significant reduction in gastric juice ascorbic acid (A+D) was observed in patients with chronic gastritis. This reduction in concentration was independent of the grade of gastritis.

3. Concentrations of ascorbic acid (A+D) in gastric biopsy specimens were consistently higher in the antrum than in the body of the stomach.

4. These data demonstrate that considerable quantities of ascorbic acid (A+D) are normally secreted into the stomach.

5. Ascorbic acid (ascorbic only; A; measured by h.p.l.c.) was present predominantly in its biologically active form in the patients with normal gastric histology. However, in patients with gastritis, independent of grade, ascorbic acid was present predominantly in its oxidized, biologically inactive form.

Key words: ascorbic acid, gastric carcinoma, gastritis, nitrates, nitrites, N-nitrosoamines, stomach.

Abbreviations: A, ascorbic acid (measured by h.p.l.c.); A+D, ascorbic and dehydro-ascorbic acid (measured by the 2,4-dinitrophenylhydrazine method).

INTRODUCTION

Considerable interest has recently been directed to the relationship between dietary nitrates, nitrites and N-nitroso compounds and the pathogenesis of gastric carcinoma [1–5]. Nitrites are the immediate precursors of carcinogenic N-nitroso compounds in a reaction which is favoured by the acid environment of the stomach [6] and is known to be prevented by the antioxidant properties of ascorbic acid [7, 8]. During eating and in the postprandial state, nitrate, nitrite and ascorbic acid will be present together in the stomach lumen. However, for a large proportion of any 24 h period the stomach will be devoid of dietary ascorbic acid, yet there is a continuous supply of nitrate through salivary secretions [2].

The presence of ascorbic acid in fasting gastric juices has been documented in the past [9–11]; however, the relationship with disease states has not been reported. Furthermore, no previous study has investigated whether the measured ascorbic acid activity is in the biologically active ascorbic acid form or the inactive dehydro form.

This study investigates the relative amounts and forms of ascorbic acid present in fasting gastric juice and gastric tissue of patients with normal gastric histology compared with a group of patients with chronic gastritis, a condition strongly associated with gastric colonization with the recently isolated organism Campylobacter pylori [12].

METHODS

Patients

Fifty-seven consecutive dyspeptic patients attending for endoscopy were studied. Ascorbic acid concentrations were measured in plasma and leucocytes taken before endoscopy and gastric juice sampled at endoscopy. Gastric biopsies were taken for ascorbic acid estimation and histological examination. The studies were approved by the Leeds Eastern Health Authority ethical committee and informed consent was obtained from all patients and controls before each test.

Endoscopy

Endoscopic examinations were carried out after a 12 h fast, by one endoscopist using an Olympus G1F-T gastroduodenoscope. The patients were sedated before the procedure with intravenous midazolam (2.5–10 mg). Gastric juice was collected immediately the stomach was
entered using a trap inserted into the suction line. Endoscopic biopsies (weighing approximately 20 mg) were taken from the gastric body and the antral floor. Those taken for histology were immediately fixed in 10% (v/v) buffered formalin, while those for ascorbic acid assay were rinsed in normal saline (150 mmol/l NaCl), blotted, weighed and placed in 10% (v/v) trichloroacetic acid.

**Histology**

Sections from routinely processed gastric antral and body biopsies were stained with haematoxylin and cosin for assessment of gastric inflammation. This was carried out by one pathologist using the criteria of Whitehead et al. [13], without knowledge of the clinical details. Gastric antral and body sections were also stained by a modified Giemsa technique [14] to assess colonization of the epithelium with C. pylori.

**Ascorbic acid assay**

Plasma and separated leucocytes were added to copper-free trichloroacetic acid to precipitate protein and cells, before storage at 20°C. Gastric juice and saliva samples were centrifuged at 2000 rev./min for 10 min before pH measurement and the supernatant was treated as for plasma. Gastric biopsies were sonicated in trichloroacetic acid and stored at -20°C. Before the ascorbic acid assay, thawed plasma and leucocyte samples were centrifuged at 2500 rev./min for 20 min and gastric juice, saliva and biopsies for 40 min. Ascorbic acid concentrations were estimated using the 2,4-dinitrophenylhydrazine method [15]. This method estimates both ascorbic acid and dehydro-ascorbic acid concentrations and all reference to this estimation are identified by the suffix (A+D).

The actual biochemical form of ascorbic acid (ascorbic acid as opposed to dehydro-ascorbic acid) was determined within 1 h of gastric sampling in 21 patients (11 normal, 17 gastritis). The method of analysis was by h.p.l.c., using a diode array detector (Hewlett Packard 1040), a NH2-bonded column (Phase-sep, U.K.) and previously described chromatography conditions [16]. Ascorbic acid measured by this method will be identified by the suffix (A). The use of the diode array detector enabled the ultra-violet spectrum of ascorbic acid to be identified and absolute comparison with pure standards to be made.

Studies were carried out into the stability of ascorbic acid (A+D) in gastric juice and the variation of ascorbic acid (A+D) content in duplicate biopsies from the same patients. There was a significant correlation (<P<0.05, Spearman’s rho) between duplicate gastric biopsies from the same area of the stomach in seven patients. In 12 gastric juice samples (five normal, seven gastritis) at 37°C there was no significant loss in ascorbic acid (A+D) concentration over 24 h (Table 1). However, when these specimens were analysed by h.p.l.c. the amount of ascorbic acid (A) was reduced. Hence immediate analysis for h.p.l.c. analysis was paramount.

**Statistical analysis**

Mean and SEM were used as descriptive statistics; non-parametric statistics were used for comparative purposes [17].

**RESULTS**

The mean (SEM) age of the patient group was 45.4 (2.2) years. Of the 57 patients studied, six patients had duodenal ulcers and two oesophageal ulcers. Overall, 34 patients had type B chronic gastritis histologically. Details of the antral histology are shown in Table 2.

Plasma, leucocyte and gastric juice ascorbic acid (A+D) concentrations for the normal and gastritis groups are shown in Table 3. The mean gastric juice ascorbic acid (A+D) concentration for the histologically normal patients was almost three times that of plasma. Gastric juice ascorbic acid (A+D) concentrations were significantly decreased in patients with chronic gastritis compared with the normal histology group. However, there was no significant difference between the measured ascorbic acid (A+D) concentrations of the various gastritic groups (Table 3). There was no significant difference in the leucocyte ascorbic acid (A+D) concentrations between the patients with gastritis and those with normal histology. Salivary ascorbic acid (A+D) was measured in nine patients and found to be less than 0.5 mg/l.

<table>
<thead>
<tr>
<th>Table 1. Ascorbic acid concentrations in fresh gastric juice and the same juice held at 37°C for 24 h assessed by the 2,4-dinitrophenylhydrazine dye method and by h.p.l.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascorbic acid (mg/l)</strong></td>
</tr>
<tr>
<td><strong>2,4-Dinitrophenylhydrazine method (A+D)</strong></td>
</tr>
<tr>
<td><strong>H.p.l.c. method (A)</strong></td>
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<tr>
<td><strong>Histology</strong></td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Gastritis</td>
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<table>
<thead>
<tr>
<th>Table 2. Age and sex distribution in the different histological groups</th>
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</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Mild gastritis</td>
</tr>
<tr>
<td>Moderate gastritis</td>
</tr>
<tr>
<td>Severe gastritis</td>
</tr>
<tr>
<td>All gastritis</td>
</tr>
</tbody>
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Ascorbic acid in human gastric juice

Table 3. Gastric juice pH and ascorbic acid (A+ D) concentrations in fasting gastric juice, plasma and leucocytes taking into account gastric histology

Statistical analysis was by using Kruskal-Wallis one-way analysis of variance. Results are presented as means (SEM).

<table>
<thead>
<tr>
<th>Histology</th>
<th>pH</th>
<th>Plasma (mg/l)</th>
<th>Gastric juice (mg/l)</th>
<th>Leucocytes (µg/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 23)</td>
<td>2.0</td>
<td>8.5</td>
<td>24.0</td>
<td>19.8</td>
</tr>
<tr>
<td>Mild gastritis (n = 8)</td>
<td>2.2</td>
<td>(0.9)</td>
<td>13.3</td>
<td>(1.8)</td>
</tr>
<tr>
<td>Moderate gastritis (n = 13)</td>
<td>2.9</td>
<td>(1.8)</td>
<td>(3.3)</td>
<td>(5.1)</td>
</tr>
<tr>
<td>Severe gastritis (n = 13)</td>
<td>4.6</td>
<td>(1.0)</td>
<td>(1.8)</td>
<td>(1.8)</td>
</tr>
</tbody>
</table>

P: all groups <0.02 = 0.14
P: gastritis patients = 0.39 = 0.29

The ascorbic acid (A+D) content of gastric biopsies was studied in eight histologically normal patients and eight with chronic gastritis. When these patients' groups were compared, there was no significant difference in the ascorbic acid (A+D) content of biopsies taken from the same site. However, a significant difference was found between the antral and body biopsies within patients irrespective of patient group, the mean (SEM) content being 368 (23) and 238 (15) µg/g, respectively.

When gastric ascorbic acid (A) from gastritic patients was assayed by h.p.l.c., minimal amounts of ascorbic acid (A) were found, although a peak identifying with dehydro-ascorbic acid (by both retention time and u.v. spectrum) was detected. The latter peak was difficult to quantify because of the low extinction coefficient of dehydro-ascorbic acid. On subsequent treatment with a reducing agent (dithiothreitol [18]), the ascorbic acid (A) peak was enhanced, while the dehydro-ascorbic acid peak disappeared. Gastric juice from patients with normal histology demonstrated ascorbic acid (A) to be present as the predominant form [97% ascorbic acid (A+D), range 83-100%]. These results are shown in Fig. 1.

DISCUSSION

The presence of high concentrations of ascorbic acid (A+D) in gastric juice has been noted previously [9-11]; however, these studies performed were before the advent of fibreoptic endoscopy and hence took no account of gastric pathology. In this study, ascorbic acid (A+D) in gastric juice was concentrated compared with that in plasma in subjects with normal gastric mucosa. From these data, assuming a gastric juice secretion of 2.5 litres per day [19], approximately 60 mg of ascorbic acid is 'secreted' into the stomach per day in the normal group. This large quantity of ascorbic acid, equivalent to almost twice the recommended daily allowance [20], is presumably reabsorbed by the small bowel, thus implying an entero-gastric circulation of ascorbic acid, the significance of which clearly requires further investigation. The situation with regard to the gastritis group is complicated by their not only having a reduced gastric juice ascorbic acid (A+D) concentration but also a reduced gastric juice secretion [21]. This implies that any entero-gastric circulation is reduced.

The conversion of ascorbic acid to dehydro-ascorbic acid does occur in the gastric juice of patients with normal gastric histology, but this is a slow process (Table 1). Furthermore, dehydro-ascorbic acid appears to be stable in gastric juice, contrary to its behaviour in aqueous solution [22]. We can only surmise that the ionic environment and the presence of protein somehow stabilizes this compound in a manner similar to ascorbic acid [22].
Patients with chronic gastritis demonstrated a significant reduction in gastric juice ascorbic acid (A+D) concentration. No significant difference in leucocyte ascorbic acid (A+D) concentration (a reflection of body ascorbic acid stores) was detected between the normal and gastritis groups. Hence the ascorbic acid status of both groups appears satisfactory, although no dietary record of input was taken. The reason for the higher antral than body tissue ascorbic acid (A+D) concentrations found in all biopsies studied is unclear, but does not appear to be a contributory factor in determining gastric juice ascorbic acid (A+D), because no differences were found between the biopsy specimens from the two patient groups.

The h.p.l.c. separation [16, 18] of gastric juice ascorbic acid (A) from gastritic patients showed it to be present in the biologically inactive dehydro form and thus unable to act as an antioxidant. Furthermore, this variable was independent of the severity of the mucosal lesion (Table 3), suggesting that there may be some common process influencing local metabolism.

One factor which is affected by the presence of chronic gastritis is gastric acid secretion. With increasing severity of chronic gastritis, acid secretion is increasingly impaired. The most extreme example of this is in patients with pernicious anaemia who have achlorhydria. The neutral gastric luminal environment in these latter patients would be expected to increase ascorbic and dehydro-ascorbic acid breakdown [23]. This does not, however, explain the diminished concentration or the predominance of dehydro-ascorbic acid in patients with mild chronic gastritis, in whom acid secretion is not significantly impaired. Another factor seen in the majority of patients with non-autoimmune chronic gastritis of any severity is the organism C. pylori [12, 24]. In this study all patients with chronic gastritis were colonized with this organism. C. pylori is a slow growing microaerophilic organism which colonizes gastric epithelium beneath the mucus layer. These organisms have high enzyme activities and have been shown to markedly influence the metabolism of certain endogenous organic molecules, most notably urea [25, 26]. The effects of these organisms in vivo, or the local biochemical alterations they cause, on ascorbic acid or dehydro-ascorbic acid metabolism are unknown. Alternatively, the damage caused to the gastric epithelium by the gastritic process may cause release of compounds which either destroy and/or oxidize ascorbic acid.

Clearly the finding of high concentrations of ascorbic acid (A+D) in the gastric juice of normals and the absence of ascorbic acid (A) in patients with chronic gastritis raises many interesting questions. Ascorbic acid (A) is ionized at normal physiological pH and thus passive transport is unlikely. Hence an active transport system would appear to be involved. The mechanism of secretion is unclear and an enterogastric circulation appears likely. The absence of a relationship between grade of chronic gastritis and the diminished ascorbic acid (A+D) activity raises the possibility that a factor such as C. pylori, which is also independent of the grade of gastritis, may be involved. The form of ascorbic acid in the gastric mucosa, on the epithelial surface and in the gastric mucus is unknown and it might be in these sites that ascorbic acid may have a protective antioxidant role.

Recent work has confirmed that citrus fruit and ascorbic acid appear to have a protective role in preventing gastric carcinoma [27]. The postulated mechanism is that by acting as an antioxidant ascorbic acid protects against the formation of potentially carcinogenic nitrosamines [28]. As the ascorbic acid in the gastric juice of gastritic patients is predominantly in the dehydro form, it is biologically inactive. Whether this influences nitrosamine formation or contributes to the aetiology of gastric carcinoma is, at present, highly speculative. Further work is clearly required to investigate the physiology of ascorbic acid in the upper gut and its possible role in disease.

ACKNOWLEDGMENT
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REFERENCES
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