Effect of *Helicobacter pylori* and its eradication on gastric juice ascorbic acid

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Abstract

The presence of ascorbic acid in gastric juice may protect against gastric carcinoma and peptic ulceration. This study examined the effect of *Helicobacter pylori* (*H. pylori*) on the secretion of ascorbic acid into gastric juice by measuring fasting plasma and gastric juice ascorbic acid concentrations in patients with and without the infection and also before and after its eradication. Gastric juice ascorbic acid concentrations in 19 *H. pylori* positive patients were significantly lower (median 2.8, range 0-28.8 µg/ml) than those in 10 *H. pylori* negative controls (median 17.8, range 5.6-155.4 µg/ml) ($p < 0.0005$) despite similar plasma ascorbic acid concentrations in both groups. The median gastric juice:plasma ascorbic acid ratio in the *H. pylori* positive patients was only 1.16 (range 0.02-6.67), compared with a median ratio of 4.87 (range 0.76-21.33) in *H. pylori* negative controls ($p < 0.01$). In the patients with *H. pylori* infection there was a significant negative correlation between the severity of the antral polymorphonuclear infiltrate and gastric juice ascorbic acid concentrations (correlation coefficient -0.52 , $p = 0.02$). After eradication of *H. pylori* in 11 patients, gastric juice ascorbic acid concentrations rose from 2.4 (0-12.8 µg/ml) to 11.2 (0-50 µg/ml) ($p = 0.01$). The median gastric juice:plasma ascorbic acid ratio also increased from 1.33 (0.05-6.67) to 2.89 (0.01-166) ($p = 0.01$). In conclusion, the high gastric juice:plasma ascorbic acid ratio in *H. pylori* negative subjects shows active secretion of ascorbic acid into gastric juice. Secondly, *H. pylori* infection causes a reversible lowering of gastric juice ascorbic acid concentrations, which may predispose to gastric carcinoma and peptic ulceration.

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Ascorbic acid, the reduced form of vitamin C, is present in the gastric juice of healthy subjects in concentrations considerably higher than those in plasma.^{1,4} This high gastric juice:plasma ascorbic acid ratio implies active secretion of ascorbic acid by the gastric mucosa. The importance of this secretory mechanism may lie in the ability of ascorbic acid to protect against the development of gastric cancer⁵⁻⁹ and possibly also against developing peptic ulceration.

In subjects with chronic gastritis, gastric juice ascorbic acid concentrations are considerably lower than those in healthy controls.^{1,4} The major cause of chronic gastritis is *Helicobacter pylori* (*H. pylori*) infection and the change in gastric juice ascorbic acid concentrations may play a part in the association of *H. pylori* infection

with both peptic ulceration¹⁰⁻¹² and gastric carcinoma.¹³⁻¹⁵

Previous studies have failed to establish whether the low gastric juice ascorbic acid concentrations associated with *H. pylori* related gastritis are a consequence of the infection, or a predisposing cause to the infection. A solitary case report has suggested that acquisition of *H. pylori* infection may lead to a small fall in gastric juice ascorbic acid concentrations.¹⁶ The study subject, however, was atypical in that his preinfection gastric juice ascorbic acid concentrations were already very low, similar to median values for patients with chronic gastritis.¹⁴ It is possible that there might exist two population groups - low secretors and high secretors of ascorbic acid into gastric juice, with the former being more susceptible to infection with *H. pylori*. This possibility is supported by the finding that ascorbic acid inhibits the growth and urease activity of *H. pylori* in vitro.¹⁷ The reduced antioxidant activity of the gastric juice of low secretors would also make them more susceptible to gastric carcinoma and peptic ulceration, and therefore the link between these diseases and *H. pylori* gastritis could be indirect.

To examine more fully the relation between *H. pylori* infection and gastric juice ascorbic acid, we have studied subjects with and without the infection and also patients before and after its eradication.

Patients and methods

We studied 19 *H. pylori* positive patients (nine male) attending a gastrointestinal clinic for investigation of dyspepsia. Ten *H. pylori* negative subjects (seven male) were also studied, seven of these being dyspeptic patients and the others asymptomatic volunteers. None of the patients had had any previous gastrointestinal surgery, or suffered from other medical conditions. The finding of upper gastrointestinal disease other than erythematous gastritis or duodenitis at the time of initial endoscopy led to exclusion from entry into the study. As antisecretory drugs may lower gastric juice ascorbic acid concentrations by raising intragastric pH,⁴ patients taking these drugs were asked to stop treatment a minimum of four weeks before entry into the study. None of the patients were receiving any other treatment.

The *H. pylori* state of the study subjects was determined by carrying out a ¹⁴C urea breath test, and in all subjects except the three asymptomatic volunteers, confirmed subsequently at endoscopy by a urease slide test (CLOtest - Delta West Pty Ltd, Australia) and microscopy of antral biopsy specimens. Patients attended for endoscopy after a 12 hour overnight fast. To

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correct for the circadian rhythm in the production of free radicals,^{18,19} which might affect gastric juice ascorbic acid concentrations, all patients were studied at the same time (9 00 am). A 5 ml sample of venous blood was withdrawn into a lithium heparin tube for measurement of plasma ascorbic acid. Patients then had an endoscopy under sedation with intravenous midazolam. On entering the stomach 5 ml of fasting gastric juice was aspirated through a sterile plastic cannula inserted through the suction channel of the endoscope. After endoscopic evaluation of the upper gastrointestinal tract, two endoscopic biopsy specimens for histopathological examination were obtained 10 cm distal to the cardia from the anterior and posterior walls of the gastric corpus, and two further specimens were taken from the anterior and posterior walls of the antrum, 2 cm proximal to the pylorus. A further antral biopsy specimen was obtained for a CLO test. The three *H pylori* negative asymptomatic volunteers did not have endoscopy, fasting gastric juice being collected by aspiration by a nasogastric tube.

Eradication of *H pylori* was attempted in 13 of the *H pylori* positive patients using triple therapy (amoxicillin 500 mg thrice daily, tripotassium dicitratobismuthate (De-Nolab, Brocades) 240 mg four times daily, and metronidazole 400 mg thrice daily, for two weeks). These patients had a repeat breath test, venesection, and endoscopy four weeks after completing their eradication therapy to check their *H pylori* state and ascorbic acid and vitamin C concentrations in plasma and gastric juice. Eight of the successfully eradicated patients were reassessed on a further occasion, four months after completion of treatment. The six *H pylori* positive patients who did not receive eradication therapy were also reassessed six weeks later to check the reproducibility of the measurements.

BIOCHEMICAL ANALYSIS

The venous blood samples were centrifuged and a 0.5 ml aliquot of plasma was added to 1 ml of 2% metaphosphoric acid. The gastric juice samples were centrifuged for five minutes immediately after collection, and a 0.8 ml aliquot of the supernatant was added to 0.8 ml of 2% metaphosphoric acid. Both plasma and gastric juice samples were then frozen in liquid nitrogen and were subsequently transferred to a -70°C freezer for storage. Analysis was carried out within seven days. The plasma and gastric juice samples were thawed, recentrifuged, and one portion of the supernatant was used to estimate ascorbic acid concentrations by high performance liquid chromatography using the methodology of Schorah *et al.*³ Further 0.5 ml aliquots of the plasma and gastric juice supernatants were used to determine total vitamin C concentrations. This was done by adding dithiothreitol to reduce any dehydroascorbic acid to ascorbic acid, and then using high performance liquid chromatography as above. Three mg of solid dithiothreitol was added to the 0.5 ml aliquots of plasma and gastric juice, the samples being mixed and allowed to stand at 20°C for 60 minutes before refreezing at -40°C for 16 hours.

The samples were then thawed once again and reanalysed. The amount of dehydroascorbic acid in the samples could be calculated by the difference between the second and first high performance liquid chromatography values for ascorbic acid for each sample. The limit of detection for the assay in our laboratory was 0.1 µg/ml. After correcting for dilution, the limits of detection were 0.2 µg/ml for gastric juice ascorbic acid, and 0.3 µg/ml for plasma ascorbic acid. The intra-assay coefficient of variation was <5% and the inter-assay coefficient of variation was <10%. In the course of the study, five gastric juice samples and two plasma samples were found to have ascorbic acid concentrations below the limits of detection. In calculating the gastric juice:plasma ascorbic acid ratio for these samples, the problem of division involving the value '0' was overcome by replacing '0' with values for the limits of detection – that is, 0.2 µg/ml for gastric juice and 0.3 µg/ml for plasma.

HISTOPATHOLOGICAL EXAMINATION

The two gastric corpus and two antral biopsy specimens were fixed in formalin and were subsequently stained with haematoxylin and eosin. All specimens were reviewed by a single histopathologist and the antral and corpus gastritis scores were assessed by a previously validated method,²⁰ final scores being expressed as the mean score of the two specimens. The polymorphonuclear infiltrate intraepithelially and the polymorphonuclear infiltrate in the lamina propria were each graded on a scale of 0 to 3, and the chronic inflammatory cell infiltrate in the lamina propria was graded on a scale of 0 to 2. The acute inflammatory score was determined by summing the polymorphonuclear infiltrate scores intraepithelially and in the lamina propria. The chronic inflammatory score was assessed from the severity of the chronic inflammatory cell infiltrate in the lamina propria.

STATISTICAL ANALYSIS

Statistical analysis was carried out using non-parametric methods. The Mann-Whitney U test and the Wilcoxon matched pairs test were used where appropriate. The Spearman rank correlation test was used to calculate correlation coefficients.

Ethical approval for this study was obtained from the ethical committee at the Southern General Hospital, Glasgow.

Results

Endoscopy at entry in the 19 *H pylori* positive patients showed erythematous antral gastritis in 13 patients and erythematous antral gastritis with erythematous duodenitis in six patients. Endoscopy was unremarkable in the seven *H pylori* negative subjects who had the procedure. Of the 13 patients who received triple therapy, *H pylori* was successfully eradicated in 11, as determined by a negative ¹⁴C urea breath test, a negative CLO test, and absence of the bacterium on microscopy of antral biopsy specimens at four weeks after treatment had finished.

TABLE II Comparison of values before, at four weeks, and four months after eradication of *H pylori*

	Plasma ascorbic acid (µg/ml)	Plasma vitamin C (µg/ml)	Gastric juice ascorbic acid (µg/ml)	Gastric juice vitamin C (µg/ml)	Ratio gastric juice/ plasma ascorbic acid	Ratio gastric juice/ plasma vitamin C	% Vitamin C as ascorbic acid in gastric juice
<i>H pylori</i> +ve before eradication (n=11)	2.1 (0.4-10.2)	2.7 (0.63-9.6)	2.4 (0-12.8)	4.4 (1-14)	1.33 (0.05-6.67)	1.87 (0.21-9.33)	63.6 (0-100)
4 Weeks after eradication (n=11)	2.1 (0-15)	2.7 (1.5-14.7)	11.2 (0-50)	11.2 (1-43)	2.89 (0.03-166.67)	2.4 (0.35-15.92)	82.1 (0-100)
Statistics*	p=0.2	p<0.05	p=0.01	p=0.01	p<0.05	p=0.1	p=0.1
4 Months after eradication (n=8)	5.7 (0-14.7)	6.1 (0.4-13.8)	5.4 (0-104.2)	6.9 (2.2-118)	4.44 (0.01-38)	5.3 (0.22-10.92)	78.4 (0-100)
Statistics†	p=0.6	p=0.6	p<0.05	p=0.01	p=0.01	p=0.01	p=0.1

Results expressed as medians (range). *p values represent comparison of values at entry with those at four weeks after eradication for the 11 patients; †p values represent comparison of values at entry with those at four months after eradication for the eight patients.

increases in median plasma and gastric juice ascorbic acid and vitamin C concentrations, in the gastric juice:plasma ascorbic acid and vitamin C ratios, and in the proportion of gastric juice vitamin C in the active ascorbic acid form, compared with median levels at four weeks after eradication. The increase in the gastric juice:plasma vitamin C ratio, which had failed to reach significance at four weeks, reached significance at four months after eradication (Table II).

The six *H pylori* positive controls who received no treatment showed no significant change in their plasma and gastric juice ascorbic acid and total vitamin C concentrations at their two assessments six weeks apart (Table III). Similarly, there was no significant change in their gastric juice:plasma ascorbic acid and vitamin C ratios, and in the proportion of gastric juice total vitamin C in the active ascorbic acid form.

There was a significant negative correlation between gastric juice ascorbic acid concentrations and the antral acute inflammatory score (correlation coefficient -0.52, p=0.02), whereas there was no correlation with the antral chronic inflammatory score. Gastric juice vitamin C concentrations showed a weaker though

significant correlation with the antral acute inflammatory score (correlation coefficient -0.46, p=0.04), but again there was no correlation with the antral chronic inflammatory score. There was no correlation between either the gastric juice ascorbic acid or vitamin C concentrations and the gastric corpus inflammatory scores. In addition, there was no correlation between the 20 minute reading of the ¹⁴C urea breath test and gastric juice ascorbic acid and vitamin C concentrations.

Discussion

Our findings confirm that *H pylori* gastritis is associated with low gastric juice ascorbic acid and total vitamin C concentrations, median concentrations of the first being six times lower, and of the second around five times lower than in *H pylori* negative subjects. The reason for the comparatively lower gastric juice ascorbic acid concentrations is that *H pylori* positive patients have a smaller proportion of the gastric juice total vitamin C in the ascorbic acid form, with larger amounts in the oxidised dehydroascorbic acid form, which does not confer protection against N-nitrosation. Our study has shown that these

Figure 3: Gastric juice ascorbic acid concentrations (µg/ml) before and after eradication of *H pylori*.

Figure 4: Gastric juice:plasma ascorbic acid ratios before and after eradication of *H pylori*.

TABLE III *H pylori* positive controls: comparison of values on two visits six weeks apart

	Plasma ascorbic acid (µg/ml)	Plasma vitamin C (µg/ml)	Gastric juice ascorbic acid (µg/ml)	Gastric juice vitamin C (µg/ml)	Ratio gastric juice/ plasma ascorbic acid	Ratio gastric juice/ plasma vitamin C	% Vitamin C as ascorbic acid in gastric juice
<i>H pylori</i> +ve controls (visit I) (n=6)	4.2 (2.1-6.6)	4.3 (2.4-6.6)	3.6 (0-28)	4.7 (1.6-50.8)	0.71 (0.02-5.33)	1.24 (0.2-12.09)	52.6 (0-100)
<i>H pylori</i> +ve controls (visit II) (n=6)	2.2 (0.3-8.4)	3.4 (0.3-8.4)	4.3 (0.2-18)	7.2 (2-25.2)	1.13 (0.45-12)	1.95 (0.8-16.8)	53.5 (10-71.4)
Statistics	p=NS	p=NS	p=NS	p=NS	p=NS	p=NS	p=NS

Results expressed as medians (range). NS=not significant.

abnormalities are secondary to *H pylori* infection as they resolve after its eradication.

The physiological mechanism by which ascorbic acid is normally concentrated in gastric juice is unclear. The pK_a of ascorbic acid is 4.1, and thus plasma ascorbic acid is largely in the ionised state. This rules out the mechanism of non-ionic diffusion followed by ionic trapping and concentration in the acidic gastric juice. It therefore seems probable that the gastric mucosa actively secretes ascorbic acid into gastric juice. Studies have shown that ascorbic acid concentrations are highest in the antral mucosa,¹ suggesting that this is the region of the stomach participating in the secretion of ascorbic acid.

The mechanism whereby *H pylori* infection lowers gastric juice ascorbic acid concentrations is also unclear. Cytopathic toxins released by the bacterium may impair the mucosal ascorbic acid secretory mechanisms. An alternative explanation for the low gastric juice ascorbic acid concentrations associated with *H pylori* infection is that it is a result of the antral gastritis induced by the infection. Consistent with this is our finding of a significant negative correlation between gastric juice ascorbic acid concentration and the acute inflammatory score in the antral mucosa. A product of the polymorphonuclear cells could impair the ascorbic acid secretory mechanism itself, or increase the consumption/degradation of ascorbic acid in the antral mucosa, resulting in lower amounts being available for secretion into the gastric juice. *H pylori* has been shown to potentiate the polymorphonuclear leucocyte oxidative burst,²¹ which is accompanied by a considerable production of reactive oxygen metabolites. Plasma ascorbic acid within the microcirculation of the gastric mucosa may be consumed in the course of scavenging these reactive oxygen metabolites. Ascorbic acid in gastric juice may be similarly degraded by the gastritic process, thus further lowering ascorbic acid concentrations, and resulting in the comparatively higher concentrations of dehydroascorbic acid seen in the gastric juice of *H pylori* positive patients.

Plasma total vitamin C concentrations showed a small but significant increase after eradication of *H pylori*. This may be explained by decreased tissue degradation of vitamin C after resolution of gastric inflammation. It is difficult, however, to absolutely rule out improved nutrition after eradication of *H pylori*, as patient's eating habits may change after resolution of dyspepsia. Although systemic inflammatory diseases such as rheumatoid arthritis are associated with low plasma ascorbic acid concentrations,²² localised inflammation in the stomach will probably not influence plasma ascorbic acid concentrations to

the same extent. Thus, in *H pylori* positive patients, dietary vitamin C intake would remain the main determinant of plasma ascorbic acid concentrations, whereas the intensity of the antral acute inflammatory infiltrate may be the main determinant of gastric juice ascorbic acid concentrations.

The depletion of gastric juice ascorbic acid caused by *H pylori* infection may be important in the association between infection and subsequent development of gastric carcinoma.¹³⁻¹⁵ Epidemiological studies have shown that diets rich in vitamin C reduce the risk of gastric carcinoma.⁵⁻⁹ The protective effect may be because of ascorbic acid in gastric juice inhibiting the formation of carcinogenic *N*-nitroso compounds. Intragastric bacteria in the hypochlorhydric stomach convert dietary nitrates to nitrites, which in turn combine with dietary amines and amides to form carcinogenic *N*-nitroso compounds. Ascorbic acid scavenges nitrites, thus preventing formation of *N*-nitroso compounds.²³⁻²⁷ The ability of ascorbic acid to scavenge reactive oxygen metabolites²⁸ may be a further mechanism whereby it protects against gastric carcinoma. Reactive oxygen metabolites may damage DNA, leading to strand scission, fragmentation, and destruction of bases and deoxyribose sugars, thus predisposing to chromosomal aberrations, mutations, and possibly to eventual neoplastic transformation.²⁹⁻³¹

The ability of ascorbic acid to scavenge reactive oxygen metabolites may also protect against mucosal ulceration. Reactive oxygen metabolites cause lipid peroxidation and adversely affect the basement membrane, epithelial function, and the mucus layer.^{32,33} Duodenal ulceration produced in rats by stimulating excess acid secretion by pentagastrin or carbachol can be prevented by administration of the free radical scavengers allopurinol and dimethyl sulphoxide.³⁴ Together with other synergistic factors, reactive oxygen metabolites may also participate in peptic ulcer disease in humans. Peptic ulceration is associated with high circulating concentrations of markers of free radical activity and reduced plasma antioxidant concentrations.³⁵ In addition, allopurinol has been reported to be more effective than cimetidine in preventing duodenal ulcer relapse,³⁶ and given concurrently with H_2 receptor blockers, may accelerate the healing of intractable duodenal ulcers.³⁷

In conclusion, these studies show that *H pylori* causes considerable but reversible lowering of gastric juice ascorbic acid concentrations. This may be an important factor in the link between *H pylori* infection and both gastric carcinoma and peptic ulceration.

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- Rathbone B, Johnson A, Wyatt J, *et al.* Ascorbic acid: a factor concentrated in human gastric juice. *Clin Sci* 1989; 76: 237-41.
- Sobala G, Schorah C, Sanderson M, *et al.* Ascorbic acid in the human stomach. *Gastroenterology* 1989; 97: 357-63.
- Sobala G, Pignatelli B, Schorah C, *et al.* Levels of nitrite, nitrate, N-nitroso compounds, ascorbic acid and total bile acids in gastric juice of patients with and without pre-cancerous conditions of the stomach. *Carcinogenesis* 1991; 12: 193-8.
- Schorah C, Sobala G, Sanderson M, *et al.* Gastric juice ascorbic acid: effects of disease and implications for gastric carcinogenesis. *Am J Clin Nutr* 1991; 53: 287-93S.
- Stahelin H, Rosel F, Buess E, Brubacher G. Dietary risk factors for cancer in the Basel Study. *Bibl Nutr Dieta* 1986; 37: 144-53.
- Jedrychowski W, Wahrendorf J, Popiela T, Rachtan J. A case-control study of dietary factors and stomach cancer risk in Poland. *Int J Cancer* 1986; 37: 837-42.
- Bjelke E. Epidemiological studies of cancer of the stomach, colon and rectum; with a special emphasis on the role of diet. *Scand J Gastroenterol* 1979; 9 (suppl): 231-5.
- Cuello C, Correa P, Haenszel W, *et al.* Gastric cancer in Columbia. Cancer risk and suspect environmental agents. *J Natl Cancer Inst* 1976; 57: 1015-20.
- Risch H, Jain M, Choi N, *et al.* Dietary factors and the incidence of cancer of the stomach. *Am J Epidemiol* 1985; 122: 947-59.
- Coghlan J, Gilligan D, Humphries H, *et al.* Campylobacter pylori and recurrence of duodenal ulcers - a 12 month follow up study. *Lancet* 1987; ii: 1109-11.
- Rauws E, Langenberg W, Houthoff H, *et al.* Campylobacter pyloridis associated chronic active antral gastritis: a prospective study of its prevalence and the effects of antibacterial and antiulcer treatment. *Gastroenterology* 1988; 94: 33-40.
- Marshall B, McGeachie D, Rogers P, *et al.* Pyloric Campylobacter infection and gastroduodenal disease. *Med J Aust* 1985; 142: 439-44.
- Parsonnet, Friedman G, Vandersteen D, *et al.* Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; 325: 1127-31.
- Nomura A, Stemmermann G, Chyou P, *et al.* Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991; 325: 1132-6.
- Forman D, Newell D, Fullerton F, *et al.* Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991; 302: 1302-5.
- Sobala GM, Crabtree JE, Dixon MF, Schorah CJ, Taylor JD, Rathbone BJ, *et al.* Acute Helicobacter pylori infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. *Gut* 1991; 32: 1415-8.
- Goldie J, Jalali S, Van Zanten S, Stowe C, Hunt RH. Ascorbic acid inhibits the growth and urease activity of Campylobacter pylori. *Gut* 1989; 30: A1484.
- Armstrong F, Sohler R, Slater T. *Free radicals in molecular biology, ageing and disease*. New York: Raven Press, 1984.
- Bridges A, Fisher T, Scott N, *et al.* Circadian variation of white blood cell function and endothelial cell proliferation in normal volunteers. *Br J Haematol* 1990; 76 (suppl 1): 37.
- Nujumi AE, Rowe P, Dahill S, Dorrian CA, Neithercut WD, McColl KEL. Role of ammonia in the pathogenesis of the gastritis, hypergastrinaemia, and hyperpepsinogaemia I caused by Helicobacter pylori infection. *Gut* 1992; 33: 1612-6.
- Mooney C, Keenan J, Munster D, Wilson I, Allardice R, Bagshaw P, *et al.* Neutrophil activation by Helicobacter pylori. *Gut* 1991; 32: 853-7.
- Lunec J, Blake D. The determination of dehydroascorbic acid and ascorbic acid in the serum and synovial fluid of patients with rheumatoid arthritis. *Free Radic Res Commun* 1985; 1: 31-9.
- Licht W, Tannenbaum S, Deen W. Use of ascorbic acid to inhibit nitrosation: kinetic and mass transfer conditions for an in vitro system. *Carcinogenesis* 1988; 9: 365-72.
- Mirvish S, Wallcave L, Eagen M, Shubik P. Ascorbate - nitrate reaction: possible means of blocking the formation of carcinogenic N-nitroso compounds. *Science* 1972; 177: 65-8.
- Wagner D, Shuker D, Bilmazes C, *et al.* Effect of vitamins C and E on endogenous synthesis of N-nitrosoamino acids in humans: precursor product studies with $[15\text{N}]$ nitrate. *Cancer Res* 1985; 45: 5619-22.
- Oshima H, Bartsh H. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. *Cancer Res* 1981; 41: 3658-62.
- Reed P, Johnstone B, Walters C, Hill M. Effect of ascorbic acid on the intragastric environment in patients at increased risk of developing gastric cancer. *IARC* 1991; 105: 139-42.
- Niki E. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *Am J Clin Nutr* 1991; 54: 1119-24S.
- Aruoma O, Halliwell B, Dizdaroglu M. Iron ion-dependent modification of bases in DNA by the superoxide radical generating system hypoxanthine/xanthine oxidase. *J Biol Chem* 1989; 264: 13024-8.
- Clemens M. Free radicals in chemical carcinogenesis. *Klin Wochenschr* 1991; 69: 1123-34.
- Santamaria L, Bianchi-Santamaria A. Free radicals as carcinogens and their quenchers as carcinogens. *Med Oncol Tumor Pharmacother* 1991; 8: 121-40.
- Maestro RD, Thaw H, Bjork J, *et al.* Free radicals as mediators of tissue injury. *Acta Physiol Scand* 1980; 492 (suppl): 43-57.
- Grisham M, Von Ritter C, BF S, *et al.* Interaction between oxygen radicals and gastric mucin. *Am J Physiol* 1987; 253: G93-6.
- Salim A. Role of oxygen derived free radicals in mechanism of acute and chronic duodenal ulceration in the rat. *Dig Dis Sci* 1990; 35: 73-9.
- Jankowski J, Bridges A, Scott N. Circulating free radical markers and peptic ulcer disease. *Eur J Gastroenterol Hepatol* 1991; 3: 823-8.
- Salim A. Oxygen derived free radicals and the prevention of duodenal ulcer relapse: a new approach. *Am J Med Sci* 1990; 300: 1-6.
- Salim A. A possible new approach to the problem of refractory peptic ulceration. A role for free radical scavengers. *Scott Med J* 1991; 36: 19-20.