Aliment Pharmacol Ther 2000; 14: 1303–1309.

Ascorbic acid and intestinal metaplasia in the stomach: a prospective, randomized study

A. ZULLO, V. RINALDI, C. HASSAN, F. DIANA, S. WINN, G. CASTAGNA* & A. F. ATTILI
*Department of Clinical Medicine – Gastroenterology, *Department of Experimental Medicine and Pathology, 'La Sapienza' University – Rome, Italy*

Accepted for publication 9 June 2000

SUMMARY

Background: Intestinal type metaplasia plays a role in intestinal type gastric carcinoma development. Ascorbic acid demonstrates a protective effect against gastric carcinogenesis, due to its ability to inactivate oxygen free-radicals as well as its nitrite-scavenging effects.
Aim: To assess whether long-term ascorbic acid administration following *Helicobacter pylori* eradication could affect intestinal metaplasia regression in the stomach.
Methods: Sixty-five patients were included in the study. The inclusion criterion was the presence of intestinal metaplasia on the gastric mucosa after *H. pylori* eradication. An upper gastrointestinal endoscopy was performed and 3 biopsy specimens were taken in the antrum, 3 in the gastric body, and 2 in the *incisura angularis*. Patients were randomized to receive 500 mg of ascorbic acid o.d., after lunch (32 patients) for 6 months or no treatment (33 patients). All patients underwent to endoscopic control at the end of the 6 months.
Results: *H. pylori* infection recurrence was detected in 6 (9.4%) patients (three from each group), and these patients were excluded from further analysis. We were unable to find evidence of intestinal metaplasia in any biopsied site of the gastric mucosa in 9/29 (31%) patients from the ascorbic acid group and in 1/29 (3.4%) of the patients from the control group ($P = 0.006$). Moreover, a further six (20.7%) patients from the ascorbic acid group presenting chronic inactive pangastritis with widespread intestinal metaplasia at entry, showed less extensive antritis with intestinal metaplasia at control, whilst a similar finding was only seen in one patient from the control group ($P = 0.051$).
Conclusion: The administration of ascorbic acid significantly helps to resolve intestinal metaplasia of the gastric mucosa following *H. pylori* eradication, and its use as a chemoprevention treatment should be considered.

INTRODUCTION

Evidence suggests that *Helicobacter pylori* causes chronic active gastritis.[1] Furthermore, a correlation between *H. pylori* infection and intestinal metaplasia in the stomach has been found,[2–4] with recent studies showing an estimated 4.5–9-fold increased risk of intestinal metaplasia in patients with this bacterial infection.[5–7] Moreover, the presence of chronic atrophic gastritis together with intestinal metaplasia is widely recognized as being the most prevalent precursor of intestinal type gastric carcinoma.[8]

Three variants of intestinal metaplasia have been identified, namely 'small intestinal' metaplasia (types I and II) and 'colonic' metaplasia (type III).[9] In general, it has been suggested that intestinal type metaplasia is the result of a reaction to the inflammatory process which tends to regress following healing of gastritis.[10] Despite this, other studies have reported no significant change in intestinal metaplasia presence at long-term follow-

Correspondence to: Dr A. Zullo, Ospedale Nuovo Regina Margherita, Servizio di Gastroenterologia ed Endoscopia Digestiva, Via E. Morisini 30, 00153, Roma, Italy.
E-mail: zullo@tiscalinet.it

up,[11–13] not even after *H. pylori* eradication,[14–16] and an increasing loss of differentiation over time from the intestinal type towards colonic type metaplasia has also been reported.[7, 17]

Ascorbic acid is the main antioxidant agent present in gastric juice, where its concentration is normally found to be fourfold higher than in the plasma.[18, 19] Ascorbic acid demonstrates a protective effect against gastric carcinogenesis, due to its ability to inactivate oxygen free-radicals as well as its nitrite-scavenging effects.[20] Interestingly, both blood and gastric juice ascorbic acid levels were found to be reduced in patients with intestinal metaplasia compared to controls.[21, 22] Moreover, a higher dietary consumption of ascorbic acid was seen to reduce the risk of intestinal metaplasia appearance, even in patients with an ongoing *H. pylori* infection.[5] However, other studies have reported that *H. pylori* causes a reduction in ascorbic acid secretion from the blood to the gastric juice, which is only recovered following bacterial eradication.[23, 24]

The present prospective, randomized study aimed to assess whether long-term ascorbic acid administration following *H. pylori* eradication could affect the presence of intestinal metaplasia in the stomach.

METHODS

All patients who had participated to our previous studies, aiming to evaluate the efficacy of different *H. pylori* eradication regimens,[25–27] were invited to participate in the present prospective study. The inclusion criterion was the persistence of small intestinal type metaplasia on the gastric mucosa (antrum, corpus or both), after *H. pylori* eradication. Bacterial eradication was assessed at endoscopy 4 weeks after eradication therapy cessation. During endoscopy, three biopsies were performed in the antrum, three in the gastric body, and a further two biopsies were taken from the *incisura angularis*, following guidelines recommended in the updated Sydney System classification of gastritis.[28] Two biopsy specimens (one each from the antrum and corpus) were utilized to perform a rapid urease test (CP-test, Yamanouchi, Milan, Italy). All remaining biopsy specimens were used to look for *H. pylori* (Giemsa staining), and for histological assessment (haematoxylin and eosin staining).

A total of 65 patients agreed to participate in the study, and they were randomized to receive 500 mg of ascorbic acid, o.d. after lunch (32 patients) for a total period of 6 months or otherwise, to receive no treatment (33 patients). The dose of ascorbic acid chosen for the study was almost 10-fold greater than the recommended daily amount and this pharmacological dose has been proven to not have any toxic effects.[29] All patients were asked to return for an endoscopic control at the end of the 6 months, after being thoroughly instructed to avoid proton pump inhibitor use. No changes to dietary regimen were suggested. Patient compliance and side-effects occurring during the therapy were assessed by personal interview at the end of treatment. At repeat endoscopy, a further eight biopsies were performed and utilized for *H. pylori* examination by histology (Giemsa staining), rapid urease test, and histological assessment (haematoxylin and eosin staining), as at entry. All slides were reviewed by a single experienced pathologist who was unaware of the clinical data and rapid urease test results. The study was approved by the local ethics committee, and all patients gave their informed consent to participate.

For statistical analysis, histological data regarding biopsies from the *incisura angularis* were grouped with those from the antrum. Comparison between groups was performed using *t*-test and two-tailed Fisher's exact test, as appropriate, whilst modifications within the each group were evaluated using the Wilcoxon rank signed test. Differences were considered significant at a 5% probability level.

RESULTS

Sixty-four of the 65 patients completed the study, whilst one patient was excluded from the control group since proton pump inhibitor therapy was not discontinued after *H. pylori* eradication due to persistent heartburn. At entry, the two groups did not significantly differ for age, sex, frequency of peptic ulcer, or gastric intestinal metaplasia distribution, as shown in Table 1. No patient complained of side-effects during therapy. Indeed, the overall compliance was good since consumption of prescribed drugs was > 95% for all but two (6.2%) out of 32 patients. In detail, one patient followed the ascorbic acid therapy for a total period of 4 months and a second patient for only 2 months, instead of the full 6 months.

At endoscopic control, a total of six (9.4%) patients (three in the ascorbic acid group and three in control

Table 1. Demographic and clinical characteristics of patients before *H. pylori* eradication

	Ascorbic acid group (n = 32)	Control group (n = 32)	P-value
Age, mean (range), years	58 (40–77)	59 (41–72)	N.S.
Male/female	15/17	11/21	N.S.
Non-ulcer dyspepsia	23	19	N.S.
Duodenal ulcer	5	10	N.S.
Gastric ulcer	4	3	N.S.
Pangastritis with intestinal type metaplasia	15	9	N.S.
Antritis with intestinal type metaplasia	17	23	N.S.

group) resulted *H. pylori* positive with histological signs of active gastritis. These patients were consequently excluded from further analysis. Modifications of the histological features in treated patients and in nontreated controls are given in Table 2 and shown in Figures 1 and 2. As demonstrated, we were unable to find evidence of intestinal metaplasia in any biopsied site of the gastric mucosa in 9/29 (31%) patients from the ascorbic acid group and in 1/29 (3.4%) of the patients from the control group ($P = 0.006$). Moreover, a further 6 (20.7%) patients from the ascorbic acid group presenting chronic inactive pangastritis with widespread intestinal metaplasia (both in antrum and corpus) at entry, showed less extensive antritis with intestinal metaplasia at control, whilst a similar finding was only seen in one patient from the control group ($P = 0.051$). In the antral mucosa, the persistence of intestinal metaplasia was always found on the *incisura angularis*.

Table 2. Histological features after 6 months in treated patients and in controls

	Ascorbic acid group (n = 29)		Control group (n = 29)	
Histological feature	Before	After*	Before	After
Normal mucosa	—	9	—	1
Antritis with intestinal type metaplasia	15	17	21	21
Pangastrititis with intestinal type metaplasia	14	3	8	7

*P = 0.0007 vs. before.

A repeat histological assessment in the six patients with *H. pylori* recurrence found no intestinal metaplasia regression, in neither the treated nor control groups.

DISCUSSION

It has been found that *H. pylori* infection may cause intestinal metaplasia in the stomach[2–7] and in addition, it is known that intestinal metaplasia plays a role in intestinal type gastric carcinoma development.[8] Therefore, any treatment that leads to intestinal metaplasia regression in the gastric mucosa could result in chemoprevention.[19] There is evidence that *H. pylori* eradication is associated with a significant improvement in gastritis, with a rapid disappearance of granulocytes and a more gradual reduction of infiltrated lymphocytes at follow-up.[30] It has also been suggested that intestinal type metaplasia is an early event in the response to a persistent irritant and that it may regress in some cases after improvement of gastritis.[10] However, no significant regression of intestinal metaplasia was observed after *H. pylori* eradication in some studies.[11–16] On the other hand, it has recently been found that higher dietary consumption of ascorbic acid tends to reduce the risk of intestinal metaplasia development in patients with *H. pylori* infection.[5, 18]

In the present study, we have assessed the long-term effect of ascorbic acid administration on intestinal metaplasia in the gastric mucosa following *H. pylori* eradication. Our data show that ascorbic acid significantly improves gastric histological features with complete intestinal metaplasia regression from all sites tested in one third of cases and partial regression (from corpus only) in a further one-fifth of patients treated. Thus, our findings show how intestinal metaplasia regression in the gastric body occurs more readily than in the antrum. This finding could depend on the likely briefer duration of intestinal metaplasia in the gastric body compared to that of the antral mucosa. In fact, it has widely been reported that intestinal metaplasia first appears on the antral mucosa (with its origin on the *incisura angularis*) and successively spreads to the gastric body mucosa.[8, 28] Intriguingly, this is analogous to the development of *H. pylori*-associated gastritis.[1]

Our results are based on findings obtained from biopsied material. Consequently, a risk due to sampling error could be associated with them. Indeed, it is true that intestinal metaplasia may be patchily distributed in the stomach, suggesting that certain biopsy samples

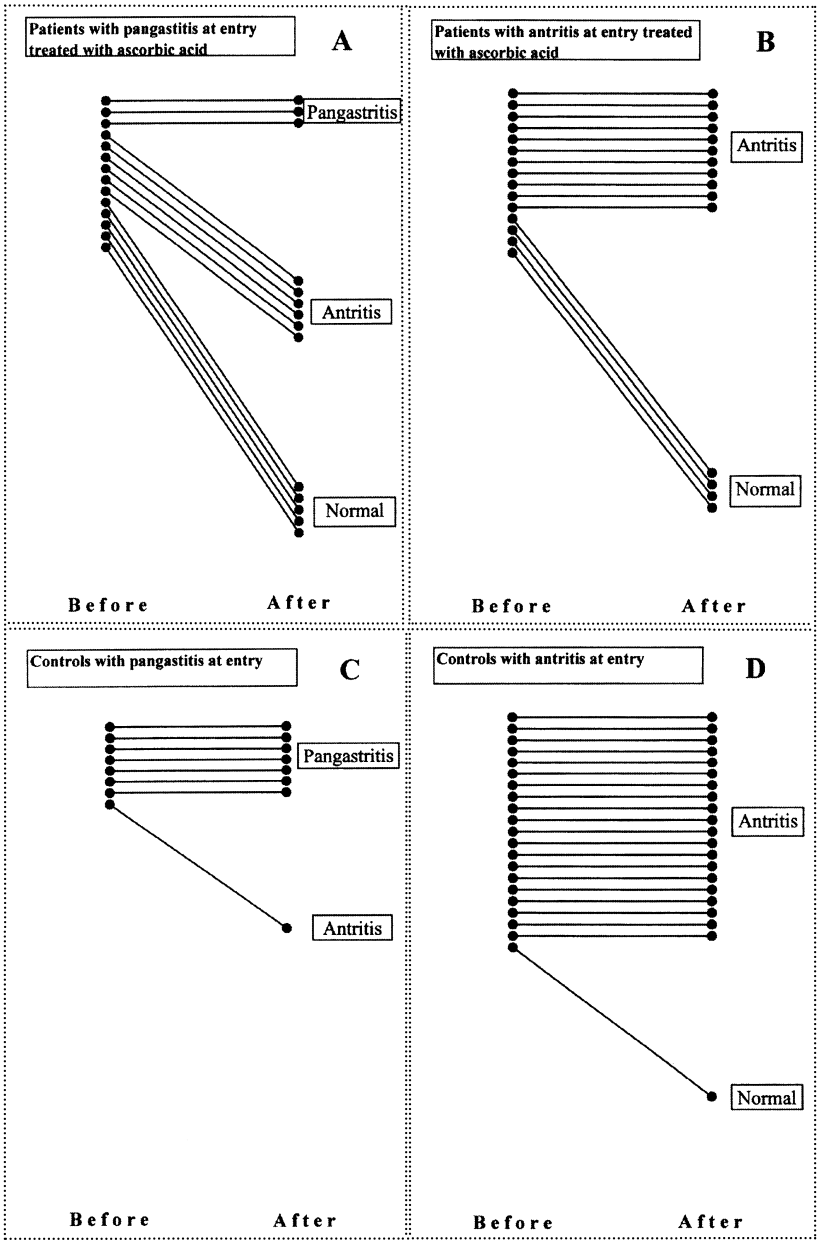

Figure 1. Histological modifications in patients (A and B), and in controls (C and D).

could have given false negative results.[31] Nevertheless, we maintain that this possibility may be marginal for at least two reasons. Firstly, in addition to other biopsies from the antrum and corpus, we systematically performed two separate biopsies along the *incisura angularis* (i.e. one more than that suggested for intestinal metaplasia assessment in the updated Sydney System classification of gastritis).[28] It is widely reported that the *angulus* is the principal gastric site of intestinal metaplasia presence, and often it is the only location.[28] Therefore, it would appear that biopsy specimens taken from the *incisura angularis* give the most representative information regarding intestinal metaplasia presence or absence in the entire stomach. Moreover, we recently found that this sampling procedure is also adequate for the histomorphometric analysis of gastritis.[32] Secondly, we also applied the same biopsy sampling procedures in the control group, and were able to find a persistence of intestinal metaplasia in all but one case. This observation suggests that if intestinal metaplasia was present in the stomach it would have been found with the sampling procedure used in this study. Thus, despite

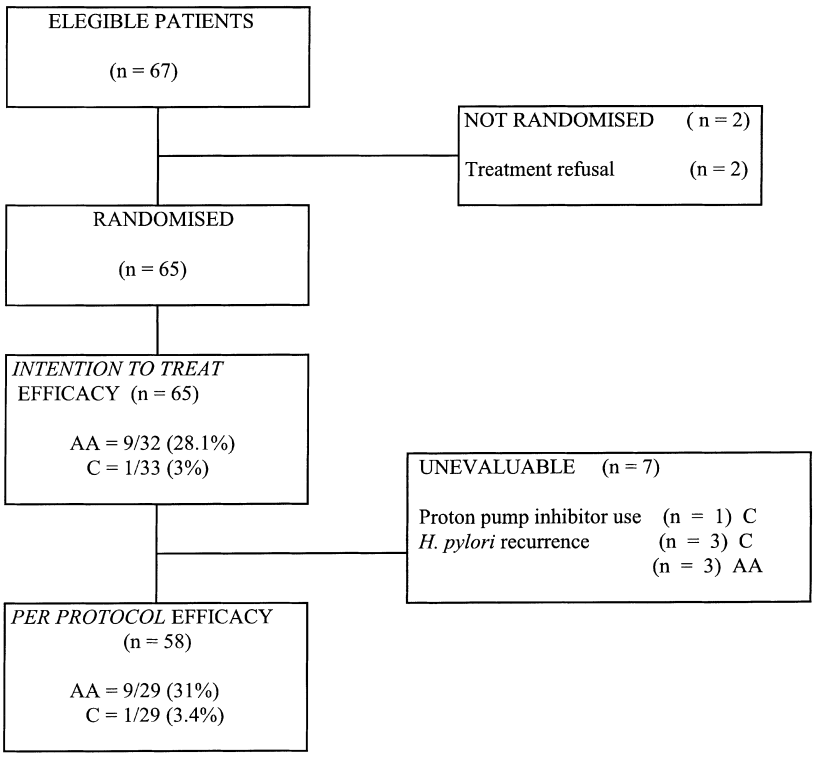

Figure 2. Flow chart presenting results for the ascorbic acid (AA) and control (C) groups.

the inevitable sampling error associated with this type of study, due to the impossibility of examining the entire gastric surface, the approach we adopted would seem to have greatly reduced such risks.

Factors involved in pathogenesis of intestinal metaplasia in the gastric mucosa are still being defined,[4, 5, 8] and it is not known why ascorbic acid administration following *H. pylori* eradication should help to resolve intestinal metaplasia in the stomach. Nevertheless, some hypotheses could be put forward. There is evidence that both blood and gastric ascorbic acid levels are reduced in patients with intestinal metaplasia.[21, 22] Moreover, it has been reported that gastric cell proliferation is significantly higher in the presence of intestinal metaplasia than in chronic gastritis without intestinal metaplasia,[33] and that this is not affected by *H. pylori* presence.[34] Therefore, intestinal metaplasia in itself is characterized by gastric cell hyperproliferation. It is reported that ascorbic acid administration significantly reduces cell proliferation throughout the gastrointestinal tract,[35] and therefore may well affect intestinal metaplasia persistence. Moreover, ascorbic acid is the major powerful antioxidant agent present in gastric juice,[19] it is able to inactivate oxygen free-radicals and it also has a nitrite-scavenging effect.[18] Furthermore, the metabolism of ascorbic acid to dehydroascorbic acid produces local nitric oxide[36]—a compound that is implicated in gastric mucosal repair phenomena.[37] All these characteristics contribute a protective action against gastric carcinogenesis.[20] Therefore, the effect of ascorbic acid on intestinal metaplasia could depend on several factors and other studies are warranted to clarify this aspect.

The recurrence rate of *H. pylori* seen in the present study (9.4%) agrees with that previously reported by us and others.[38, 39] In these patients, the administration of ascorbic acid failed to affect intestinal metaplasia regression and this would suggest that previous bacterial eradication is a strongly recommended criterion. It may be that in the presence of active gastritis, the administered ascorbic acid is 'consumed' by the oxygen free-radicals produced by polymorphonuclear cells.

In conclusion, we found that administration of ascorbic acid helps significantly in resolving intestinal metaplasia of the gastric mucosa following *H. pylori* eradication. Since this therapy is safe, well tolerated and inexpensive, its use as a chemoprevention treatment should be considered. Long-term studies will be needed to assess if this effect is maintained.

REFERENCES

1 Blaser MJ. Hypotheses on the pathogenesis and natural history of *Helicobacter pylori*-induced inflammation. Gastroenterology 1992; 102: 720–7.
2 Craanen ME, Dekker W, Block P, Ferwerda J, Tytgat GNJ. Intestinal metaplasia and *Helicobacter pylori*: an endoscopic bioptic study of the gastric antrum. Gut 1992; 33: 16–20.
3 Eidt S, Stolte M. Prevalence of intestinal metaplasia in *Helicobacter pylori* gastritis. Scand J Gastostroenterol 1994; 29: 607–10.
4 Filipe MI, Newell DG, Johnston BJ, Caygill C, Reed PI. *Helicobacter pylori* in patients with intestinal metaplasia and in controls: a serological and biopsy study in four UK centres. Eur J Cancer Prev 1995; 4: 175–80.
5 Fontham ETH, Ruiz B, Perez A, Hunter F, Correa P. Determinants of *Helicobacter pylori* infection and chronic gastritis. Am J Gastroenterol 1995; 90: 1094–101.
6 Kuipers EJ, Uyterlinde AM, Pena AS, *et al*. Long-term sequelae of *Helicobacter pylori* gastritis. Lancet 1995; 345: 1525–8.
7 Rugge M, Cassaro M, Leandro G, *et al*. *Helicobacter pylori* in promotion of gastric carcinogenesis. Dig Dis Sci 1996; 41: 950–5.
8 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process – First American cancer society award lecture on cancer epidemiology and prevention. Cancer Res 1992; 52: 6735–40.
9 Filipe MI, Potet F, Bogomoletz WV, *et al*. Incomplete sulphomucin-secreting intestinal metaplasia for gastric cancer. Preliminary data from prospective study from three centres. Gut 1985; 26: 1319–26.
10 Silva S, Filipe MI, Pinho A. Variants of intestinal metaplasia in the evolution of chronic atrophic gastritis and gastric ulcer. A follow up study. Gut 1990; 31: 1097–104.
11 Ierardi E, Francavilla R, Panella C. Effect of *Helicobacter pylori* eradication on intestinal metaplasia and gastric epithelium proliferation. Ital J Gastroenterol Hepatol 1997; 29: 470–5.
12 Niemela S, Karttunen T, Kerola T. Chronic gastritis in patients with gastric ulcer: a 10-year follow-up. Scand J Gastroenterol 1995; 30: 428–33.
13 Niemela S, Karttunen T, Kerola T. *Helicobacter pylori*-associated gastritis. Evolution of histologic changes over 10 years. Scand J Gastroenterol 1995; 30: 542–9.
14 Di Napoli A, Petrino R, Boero M, Bellis D, Chiandussi L. Quantitative assessment of histological changes in chronic gastritis after eradication of *Helicobacter pylori*. J Clin Pathol 1992; 45: 796–8.
15 Witteman EM, Mravunac M, Becx MJCM, *et al*. Improvement of gastric inflammation and resolution of epithelial damage one year after eradication of *Helicobacter pylori*. J Clin Pathol 1995; 48: 250–6.
16 Forbes GM, Warren JR, Glaser ME, Cullen DJ, Marshall BJ, Collins BJ. Long-term follow-up of gastric histology after *Helicobacter pylori* eradication. J Gastroenterol Hepatol 1996; 11: 670–3.
17 Filipe MI, Barbatis C, Sandey A, Ma J. Expression of intestinal mucin antigens in the gastric epithelium and its relationship with malignancy. Hum Pathol 1988; 19: 19–26.
18 Farinati F, Della Libera G, Cardin R, *et al*. Gastric antioxidant, nitrites, and mucosal lipoperoxidation in chronic gastritis and *Helicobacter pylori* infection. J Clin Gastroenterol 1996; 22: 275–81.
19 de Sanjosè S, Munoz N, Sobala G, *et al*. Antioxidants, *Helicobacter pylori* and stomach cancer in Venezuela. Eur J Cancer Prev 1996; 5: 57–62.
20 Buckley M, O'Morain C. *Helicobacter pylori* and gastric cancer. Eur J Cancer Prev 1995; 4: 139–44.
21 Caygill CPJ. Plasma vitamin concentration in patients with intestinal metaplasia and in controls. Eur J Cancer Prev 1992; 1: 177–86.
22 Sobala GM, Pignatelli B, Schorah CJ, *et al*. Levels of nitrite, nitrate, N-nitroso compounds, ascorbic acid and total bile acids in gastric juice of patients with and without precancerous conditions of the stomach. Carcinogenesis 1991; 12: 193–8.
23 Rokkas T, Papatheodorou G, Karameris A, Mavrogeogis A, Kalogeropoulos N, Giannikos N. *Helicobacter pylori* infection and gastric juice vitamin C level: impact of eradication. Dig Dis Sci 1995; 40: 615–21.
24 Sobala GM, Schorah CJ, Shires S, *et al*. Effect of eradication of *Helicobacter pylori* on gastric juice ascobic acid concentrations. Gut 1993; 34: 1038–41.
25 Zullo A, Rinaldi V, Pugliano F, Diana F, Attili AF. Omeprazole plus clarithromycin and either tinidazole or tetracycline for *Helicobacter pylori* infection: a randomized, prospective study. Am J Gastroenterol 1997; 92: 2029–31.
26 Rinaldi V, Zullo A, Pugliano F, Valente C, Diana F, Attili AF. The management of failed dual or triple therapy for *Helicobacter pylori* eradication. Aliment Pharmacol Ther 1997; 11: 929–33.
27 Rinaldi V, Zullo A, De Francesco V, *et al*. *Helicobacter pylori* eradication with proton pump inhibitor-based triple therapies and re-treatment with ranitidine bismuth citrate-based triple therapy. Aliment Pharmacol Ther 1999; 13: 163–8.
28 Dixon FM, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. Am J Surg Pathol 1996; 20: 1161–81.
29 Johnston CS. Biomarkers for establishing a tolerable upper intake level for vitamin C. Nutrition Rev 1999; 57: 71–7.
30 Valle J, Seppala K, Sipponen P, Kosunen T. Disappearance of gastritis after eradication of *Helicobacter pylori*. Scand J Gastroenterol 1991; 26: 1057–65.
31 Genta RM. Atrophy, metaplasia and dysplasia: are they reversible? Ital J Gastroenterol Hepatol 1998; 30: S324–5.
32 Ballanti P, Zullo A, Rinaldi V, *et al*. Measurement variability and diagnostic sensitivity of gastric mucosal inflammatory cell morphometry. Anal Quant Cytol Histol 1998; 21: 21–8.
33 Panella C, Ierardi E, Polimeno L, *et al*. Proliferative activity of gastric epithelium in progressive stages of *Helicobacter pylori* infection. Dig Dis Sci 1996; 41: 1132–8.

34 Cahill RJ, Kilgallen C, Beattie S, Hamilton H, O'Morain C. Gastric epithelial cell kinetics in the progression from normal mucosa to gastric carcinoma. Gut 1996; 38: 177–81.
35 Cahill RJ, O'Sullivan KR, Mathias PM, Beattie S, Hamilton H, O'Morain C. Effects of vitamin antioxidant supplementation on cell kinetics of patients with adenomatous polyps. Gut 1993; 34: 963–7.
36 Heller R, Munscher Paulig F, Grabner R, Till U. L-Ascorbic acid potentiates nitric oxide synthesis in endothelial cells. J Biol Chem 1999; 274: 8254–60.
37 Elliot SN. Nitric oxide: a regulator of mucosa defense and injuri. J Gastroenterol 1998; 33: 792–803.
38 Zullo A, Rinaldi V, Hassan C, *et al.* Clinical and histologic predictors of *Helicobacter pylori* infection recurrence. J Clin Gastroenterol 2000; 31: 38–41.
39 Penston JG. *Helicobacter pylori* eradication: understandable caution but no excuse for exertion. Aliment Pharmacol Ther 1994; 8: 369–79.