Levels of Malondialdehyde-Deoxyguanosine in the Gastric Mucosa: Relationship with Lipid Peroxidation, Ascorbic Acid, and *Helicobacter pylori*

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Abstract

***Helicobacter pylori* infection is associated with elevated gastric mucosal concentrations of the lipid peroxidation product malondialdehyde and reduced gastric juice vitamin C concentrations. Malondialdehyde can react with DNA bases to form the mutagenic adduct malondialdehyde-deoxyguanosine (M_1-dG). We aimed to determine gastric mucosal levels of M_1-dG in relation to *H. pylori* infection and malondialdehyde and vitamin C concentrations. Patients ($n = 124$) attending for endoscopy were studied. Levels of antral mucosal M_1-dG were determined using a sensitive immunoslot-blot technique; antral mucosal malondialdehyde was determined by thiobarbituric acid extraction, and gastric juice and antral mucosal ascorbic acid and total vitamin C were determined by high-performance liquid chromatography. Sixty-four *H. pylori*-positive patients received eradication therapy, and endoscopy was repeated at 6 and 12 months. Levels of M_1-dG did not differ between subjects with *H. pylori* gastritis ($n = 85$) and those with normal mucosa without *H. pylori* infection ($n = 39$; 56.6 *versus* 60.1 adducts/10^8 bases) and were unaffected by age or smoking habits. Malondialdehyde levels were higher (123.7 *versus* 82.5 pmol/g; $P < 0.001$), gastric juice ascorbic acid was lower (5.7 *versus* 15.0 μmol/ml; $P < 0.001$), and antral mucosal ascorbic acid was unchanged (48.0 *versus* 42.7 μmol/g) in *H. pylori* gastritis compared with normal mucosa. Multiple regression analysis revealed that M_1-dG increased significantly with increasing levels of malondialdehyde, antral ascorbic acid, and total antral vitamin C. M_1-dG levels were unchanged 6 months (63.3 *versus* 87.0 adducts/10^8 bases; $P = 0.24$; $n = 38$) and 12 months (66.7 *versus* 77.5 adducts/10^8 bases; $P = 0.8$; $n = 13$) after successful eradication of *H. pylori*. M_1-dG thus is detectable in gastric mucosa, but is not affected directly by *H. pylori*.**

Introduction

Although the incidence of gastric cancer is declining, it remains an important disease worldwide. The role of *Helicobacter pylori* in gastric carcinogenesis has been established in epidemiological studies and, more recently, in animal models, but the precise means through which this occurs have not yet been established (1–4). One possible mechanism for *H. pylori*-mediated carcinogenesis is through induction of DNA damage and mutations as a result of increased activity of reactive oxygen species in the gastric mucosa (5). These compounds can damage DNA directly by causing strand breaks, apurinic sites, or DNA adducts (6). In addition, reactions with the lipid bilayer result in the accumulation of degradation products, such as MDA,[2] a compound that has been shown to be present in increased concentrations in *H. pylori* gastritis (7, 8). Vitamin C is believed to be one of the major defenses against oxidative stress in the stomach and is concentrated several-fold from plasma to the gastric mucosa. Vitamin C exists in two forms, dehydroascorbic acid and the potent antioxidant ascorbic acid. Concentrations of the latter are diminished in gastric juice of *H. pylori*-infected patients, a process that may serve to exacerbate oxidative damage (9, 10).

Accumulation of MDA in *H. pylori*-infected gastric mucosa not only provides evidence of increased oxidative stress and lipid peroxidation, but may also have a carcinogenic role. MDA can react with DNA to form adducts and cross-links and has been shown to be mutagenic in bacterial and mammalian systems (11–13) and carcinogenic to rats (14). MDA induces a diverse spectrum of mutations, such as frameshift mutations and base pair substitutions in *Escherichia coli* (15). In humans, increased concentrations of lipid peroxidation products have been found in the serum of gastric cancer patients (16). The main DNA adduct is M_1-dG, which has been shown in further *E. coli* studies to result in a mutation frequency, after correction for strand bias, of 18% (a 500-fold increase over unmodified DNA; Ref. 17). M_1-dG has been detected in human liver, WBCs, and recently, in gastric tissue (18–21), and levels of M_1-dG and other MDA adducts were greater in breast tissue from patients with breast cancer than from those without breast cancer (22).

Received 11/10/00; revised 1/24/01; accepted 2/1/01.
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[2] The abbreviations used are: MDA, malondialdehyde; M_1-dG, malondialdehyde-deoxyguanosine; NSAID, nonsteroidal anti-inflammatory drug; 95% CI, 95% confidence interval.

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Detection of M_1-dG in human tissue has previously required gas chromatography/mass spectrometry or ^{32}P-postlabeling techniques (19, 21). However, these techniques require relatively large amounts of DNA, are time consuming, and are not easily applied to studies of small tissue samples from large numbers of subjects. More recently, a monoclonal antibody against M_1-dG has been developed and characterized (23), which has allowed validation of a sensitive immunoslot-blot assay for the adduct with a limit of detection of 2.5 adducts/10^8 normal bases (18, 24).

The purpose of this study was to determine the effect of *H. pylori* and lipid peroxidation on levels of M_1-dG in gastric biopsies and to examine whether ascorbic acid protects against this process. We measured levels of M_1-dG using the immunoslot-blot technique alongside mucosal MDA and plasma, mucosal, and gastric juice ascorbic acid and total vitamin C. We studied patients with normal and *H. pylori*-infected mucosa and followed *H. pylori* infected patients for up to 12 months after eradication of the organism.

Materials and Methods

Patients and Study Design. One hundred twenty-four patients attending for routine outpatient endoscopic investigation of dyspepsia were studied (72 males and 52 females; mean age, 49.1 years; range, 25–77 years). Patients were excluded if they had been taking antibiotics in the previous month, had had previous attempts at *H. pylori* eradication, or had previously had gastric surgery.

H. pylori status was determined by [^{13}C]urea breath test, antral biopsy for urease testing, and histological assessment of antral and corpus biopsies. Further antral biopsies were taken from all patients as follows: two for determination of M_1-dG, one for MDA assessment, and one for ascorbic acid and vitamin C determination. Prior to endoscopy, a 10-ml sample of venous blood was withdrawn into a lithium heparin tube; immediately after intubation, a sterile Teflon catheter was passed, and 5 ml of gastric juice were collected, both for assessment of ascorbic acid and vitamin C.

Of the 124 patients, 39 had all tests negative for *H. pylori* and histologically normal gastric mucosa, and 85 patients were deemed *H. pylori* positive on the basis of positive histology, urease, and [^{13}C]urea breath tests. Sixty-four *H. pylori*-positive patients received eradication therapy and were asked to return at 6 months for repeat biopsy and breath test. Of these 64 patients, 38 received open eradication therapy and 26 were in the treatment arm of a randomized double blind study of *H. pylori* eradication in nonulcer dyspepsia (H2BB3009) conducted by Glaxo Wellcome. All 64 patients received identical eradication therapy, which included ranitidine bismuth citrate (400 mg), clarithromycin (500 mg), and metronidazole (400 mg), all twice daily for 1 week. The remaining 21 patients received triple therapy placebo as part of H2BB3009. In addition to the above exclusion criteria, the patients in H2BB3009 all had normal endoscopies and had not been using proton pump inhibitors or NSAIDs in the preceding month. H2BB3009 had a 12-month follow-up, so a urease test was not performed at the 6-month endoscopy in these patients to aid investigator blinding. In these patients, therefore, *H. pylori* status at 6 months relied on histology and breath test, but they were asked to return at 12 months for further assessment, biopsy, and breath test. In all other ways the patients in the randomized trial and those receiving open eradication therapy were treated identically. This study was approved by the local research ethics committee, and all patients gave written informed consent.

Histology. Biopsies were fixed in 10% buffered formalin. Sequential 3-μm-thick sections were cut and stained with H&E and modified Giemsa stain. Gastritis was scored according to the modified Sydney classification, such that sections were graded between 0 and 3 (from absent to severe) for activity, chronic inflammation, atrophy, intestinal metaplasia, and *H. pylori* density by a single histopathologist who was blinded to patient details (25).

^{13}C Breath Test. The breath test was performed largely as described by Logan *et al.* (26). Subjects fasted for 4 h. Breath samples were collected after subjects drank 150 ml of a solution containing 4 g of citric acid in 200 ml of water and 30 min after subjects drank the remaining 50 ml of citric acid containing 100 mg of [^{13}C]urea. Samples were analyzed on a Europa Scientific ABCA mass spectrometer to give a ratio of $^{13}CO_2$ to $^{12}CO_2$. A delta difference between pre and post ratios of >5.0 was taken as a positive result for *H. pylori* infection. The procedure was similar for the patients in the Glaxo Wellcome study, but the test meal contained 2.4 g of citric acid in 200 ml of orange juice and the 100 mg of [^{13}C]urea were dissolved in 25 ml of water followed by a 25-ml water wash.

MDA. For each patient, one antral biopsy was snap frozen in liquid nitrogen at the time of endoscopy, stored at -70°C, and assayed within 2 weeks by the procedure of Yagi (27), as modified by Drake *et al.* (7). Each biopsy was immersed in 4 ml of water to which was added 1 ml of thiobarbituric acid solution (0.167 g thiobarbituric acid in 25 ml of water and 25 ml of glacial acetic acid). Mixtures were heated at 100°C for 60 min. After cooling on ice, 5 ml of butan-1-ol were added to extract the MDA equivalents. The tubes were centrifuged at 3000 rpm for 10 min to separate the aqueous and butan-1-ol phases. Fluorescence of the butan-1-ol phase at 555 nm was determined using an excitation wavelength of 515 nm. Values from the tissue specimens were compared against the freshly prepared standard solutions. This technique has an interassay coefficient of variation of 14.2% (based on paired biopsies from 142 patients).

Ascorbic Acid and Total Vitamin C Measurements. Venous blood samples were centrifuged, and 0.5-ml aliquots of plasma were added to 1.0 ml of 2% metaphosphoric acid alone and 1.0 ml of 2% metaphosphoric acid containing 9 mg of DTT (for reduction of dehydroascorbic acid to ascorbic acid to give a value for total vitamin C). Likewise, 0.5-ml aliquots of gastric juice were added to 0.5 ml of 2% metaphosphoric acid containing 0.5% sulfamic acid, alone and with 6 mg of DTT. All samples were snap frozen in liquid nitrogen and stored at -70°C. Prior to analysis, samples were thawed and centrifuged at $1000 \times g$. The supernatant solution was analyzed by high-performance liquid chromatography using reversed-phase ion-pair chromatography on a C_{18} column (28). Ascorbic acid was selectively measured using an electrochemical detector set at 350 mV. Total vitamin C was determined from the solutions initially prepared with DTT after incubation at 45°C for 120 min.

Biopsy samples were frozen immediately after endoscopy in liquid nitrogen, stored at -70°C, and assayed within 2 weeks. After thawing, biopsies were homogenized in 0.5–1.0 ml of metaphosphoric acid and divided into two parts. For total vitamin C determination, DTT was added to one part to a final concentration of 6 mg/ml and incubated at 45°C for 120 min prior to analysis. High-performance liquid chromatography was performed for both parts as above. This technique previously

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has been shown to be highly specific for ascorbic acid and results in 90% extraction of ascorbic acid and 75% extraction of dehydroascorbic acid from tissue (29). The interassay coefficient of variation for both ascorbic acid and total vitamin C is 5%.

M_1-dG Immunoslot-Blot Technique. Biopsies were snap frozen in liquid nitrogen and stored at −80°C until assay. Following homogenization, the gastric tissue was incubated with RNase A and proteinase K at 45°C for 2 h. The DNA was isolated using the Qiagen genomic DNA extraction kit (Qiagen Ltd). The amount and purity of the DNA was established by determining the UV absorbance at 260 and 280 nm (Kontron Uvikon 860 spectrophotometer). Adduct recovery after extraction had been determined previously as 96%.

The method has been described previously by Leuratti *et al.* (18). Briefly, DNA samples were sonicated and then heat denatured; the resulting single-stranded DNA was immobilized (1 μg) in triplicate onto a nitrocellulose filter using the Minifold II blotting apparatus (Schleicher and Schuell). The filter was then heated at 80°C for 1.5 h and blocked for nonspecific binding using nonfat milk powder dissolved in PBS containing 0.1% Tween 20 for an additional 1 h. After blocking, the filter was incubated overnight with the primary antibody specific for M_1-dG (23) at 4°C and then incubated with horseradish peroxidase-conjugated secondary antibody (goat antimouse) for 2 h at room temperature. After the filter was bathed with chemiluminescent reagents (Super Signal Ultra; Pierce and Warriner), it was exposed to chemiluminescence-sensitive Hyperfilm. An image of the filter was acquired using a Fluor-S MultiImager (Bio-Rad), which was used for quantitation of adduct levels. The level of M_1-dG adduct in the samples was determined from a calibration curve generated by the dilution (with control calf thymus DNA) of standard calf thymus DNA (treated with 2 mM MDA) containing known amounts of the adduct, blotted onto the same filter. A positive control of genomic human blood DNA (from Boehringer Mannheim) was run with each filter. The M_1-dG concentration in the control DNA from 12 consecutive filters was 90.4 ± 21.2 (SD) adducts/10^8 nucleotides, giving a coefficient of variation of 23.5%.

Statistics. For calculating the required sample size, we assumed a SD of log-transformed M_1-dG of ~0.25 adducts/10^8 normal bases, that *H. pylori* increases M_1-dG levels by 40% (*e.g.*, from 50 to 70 adducts/10^8 normal bases), and a ratio of *H. pylori*-positive to -negative patients in the sample of 2:1. The number of patients required is then ~120 patients in total to achieve statistical significance at the 5% level, with 80% power.

For skewed data, medians with interquartile ranges are presented. Subsequently, measures of MDA, ascorbic acid, total vitamin C, and M_1-dG were log-transformed to meet the normality and constant variance requirements of parametric statistical methods. Paired and unpaired *t* tests were performed on the transformed data. Spearman's correlation was used for assessment of the effect of gastritis, and Fisher's exact test was used for categorical data. To determine the relationship between M_1-dG and other continuous variables, univariate linear regression was performed. To account for the effect of *H. pylori* on MDA and ascorbic acid, each regression analysis was repeated with *H. pylori* as a second variable. Associations are presented as regression slopes with 95% CIs. The regression slopes for log-transformed data were then back-transformed and the percentage of increase in the dependent variable (either M_1-dG or MDA) was calculated for a 2-fold increase (doubling) of the independent variable. Two-tailed *P*s were determined in all cases. Data were analyzed using SPSS for Windows, version 8.0.

Table 1 Age, sex, smoking habits, drug intake, and endoscopic findings for all patients, according to *H. pylori* status

All analyses are Fisher's exact test except where indicated.

	H. pylori positive (n = 85)	*H. pylori* negative (n = 39)	*P*
Mean age (SD), years	51 (13)	45 (12)	0.02[a]
Sex, M:F (% male)	47:38 (55%)	25:14 (64%)	0.4
Smokers (%)	50	25	0.02
NSAID (%)	2	6	0.6
H2RA[b] (%)	23	28	0.6
PPI (%)	6	0	0.3
Endoscopy (%)			
Normal	66	78	
PUD	14	0	0.02[c]
Other	20	22	

[a] *t* test.
[b] H2RA, histamine receptor antagonist; PPI, proton pump inhibitor; PUD, peptic ulcer disease.
[c] Peptic ulcer disease *versus* all other diagnoses.

Results

One hundred twenty-four patients were studied, of whom 85 were *H. pylori* positive and 39 had normal gastric histology and negative *H. pylori* tests. Age, sex, smoking habits, endoscopic findings, use of NSAIDs, and acid-suppressive therapy in the month prior to endoscopy are all detailed in Table 1. *H. pylori*-positive patients were older and more likely to smoke than *H. pylori*-negative patients, but the groups were otherwise comparable.

As expected, and reported previously, ascorbic acid and vitamin C levels in gastric juice and plasma were significantly lower in *H. pylori*-positive patients than negative controls, whereas MDA concentrations were significantly higher (7, 10, 30). Concentrations of M_1-dG were, however, similar in *H. pylori*-positive and -negative patients (Table 2; Fig. 1). In addition, levels of M_1-dG were unaffected by age, smoking history, sex, the presence of peptic ulcer disease, and the use of histamine receptor antagonists, proton pump inhibitors, or NSAIDs. Sydney gastritis scores were available for 43 *H. pylori*-positive patients (all in the randomized trial), and no significant associations existed between M_1-dG and level of antral polymorph infiltrate, chronic inflammation, atrophy, intestinal metaplasia, or *H. pylori* infection density. The MDA concentration was positively related to antral polymorph infiltrate with borderline significance (Spearman's $\rho = 0.32$; $P = 0.05$), but did not correlate with any other of the parameters of gastritis. Total gastric juice vitamin C was significantly positively associated with antral chronic inflammatory infiltrate ($\rho = 0.42$; $P = 0.01$), but there were no other significant correlations between plasma, antral, or gastric juice ascorbic acid or total vitamin C and any of the gastritis scores.

After logarithmic conversion, univariate linear regression revealed a statistically significant positive relationship between MDA and total antral vitamin C. In addition, there were significant inverse relationships between MDA and total gastric juice vitamin C, plasma ascorbic acid, and total plasma vitamin C (Table 3). However, after adjustment for the presence or absence of *H. pylori*, the associations of total gastric juice vitamin C, plasma ascorbic acid, and total plasma vitamin C

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Table 2 Levels of antral M_1-dG, MDA and antral, gastric juice, and plasma ascorbic acid, and total vitamin C concentrations according to *H. pylori* infection

	H. pylori positive[a] (n = 85)	*H. pylori* negative[a] (n = 39)	P[b]
M_1-dG (adducts/10^8 normal bases)	56.6 (35.9–81.9)	60.1 (38.1–101)	0.42
MDA (nmol/g)	123.7 (93.5–157.2)	82.5 (59.5–104.7)	<0.001
Gastric juice AA[c] (μg/ml)	5.7 (2.7–10.3)	15.0 (7.3–35.0)	<0.001
Gastric juice TVC (μg/ml)	8.8 (5.1–12.3)	19.4 (9.9–40.8)	<0.001
Antral AA (μg/mg)	48.0 (25.8–62.0)	42.7 (22.8–73.4)	0.82
Antral TVC (μg/mg)	64.7 (45.6–82.5)	57.6 (39.0–79.2)	0.50
Plasma AA (μg/ml)	5.2 (2.8–9.7)	8.9 (5.8–11.4)	0.002
Plasma TVC (μg/ml)	7.2 (3.6–10.6)	9.8 (6.4–12.5)	0.008

[a] Values are medians and interquartile ranges.
[b] Unpaired t test of log-transformed data.
[c] AA, ascorbic acid; TVC, total vitamin C.

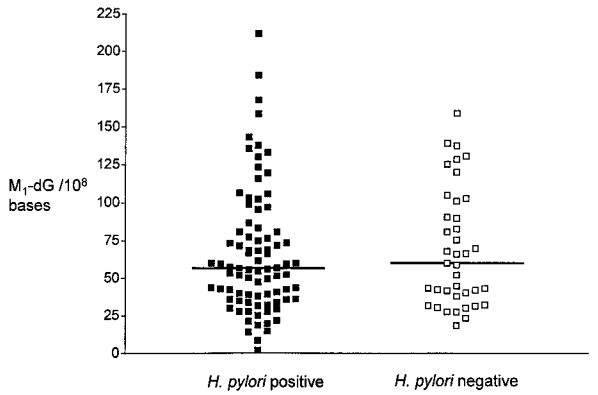

Fig. 1. Levels of M_1-dG in *H. pylori*-positive and -negative patients. *Horizontal bars* are medians.

with MDA were all nonsignificant, whereas there was a consistently significant positive relationship between presence of *H. pylori* and increasing MDA concentration ($P < 0.001$), suggesting that the aforementioned inverse relationships were the result of confounding by *H. pylori* infection. On the other hand, the positive relationship between total antral vitamin C and MDA remained significant ($P = 0.02$), and that between antral ascorbic acid and MDA became conventionally significant ($P = 0.04$). Thus, doubling of antral ascorbic acid and total antral vitamin C was reflected in 6% (95% CI, 0.2–11%) and 8% (95% CI, 1–15%) increases in MDA, respectively.

Application of the same analysis with M_1-dG as the dependent variable revealed a statistically significant positive relationship between M_1-dG and both antral and gastric juice ascorbic acid and total vitamin C (Table 4). The relationships between plasma ascorbic acid and total plasma vitamin C with M_1-dG were nonsignificant. After adjustment for the presence of *H. pylori*, gastric juice ascorbic acid and total gastric juice vitamin C ceased to be significantly associated, whereas antral ascorbic acid and total antral vitamin C remained significantly positively related to M_1-dG. Thus, doubling of antral ascorbic acid and total antral vitamin C was reflected in 14% (95% CI, 4–27%) and 18% (95% CI, 4–34%) increases in M_1-dG, respectively.

Likewise, univariate analysis demonstrated a weak positive relationship between MDA and M_1-dG, which became statistically significant after adjustment for the presence of *H. pylori* [a doubling of MDA reflected in a 36% (95% CI, 6–75%) increase in M_1-dG; $P = 0.02$]. This suggests an effect modification by *H. pylori* infection. Indeed, the relationship between MDA and M_1-dG was greater for *H. pylori*-positive cases [a doubling of MDA reflected in a 40% (95% CI, 3–93%) increase in M_1-dG; $P = 0.04$] than for *H. pylori*-negative controls [a doubling of MDA reflected in a 23% (95% CI, −23 to 87%) increase in M_1-dG; $P = 0.3$], although the difference in regression slopes was not statistically significant ($P = 0.6$; Fig. 2).

Forty-five of 64 *H. pylori*-positive patients who received eradication therapy returned at 6 months. Forty-two of these were negative, 1 was positive, and 2 were indeterminate (breath test positive and histology negative) for *H. pylori*. For patients with successful eradication, mean antral and corpus chronic inflammation scores fell significantly between 0 and 6 months [from 1.9 to 1.2 (95% CI of difference, 0.5–1.0; $P < 0.001$), and from 1.4 to 0.8 (95% CI, 0.1–1.2; $P = 0.03$), respectively], as did antral and corpus polymorph infiltration [from 1.25 to 0.33 (95% CI of difference, 0.2–1.7; $P = 0.02$), and from 0.8 to 0.1 (95% CI, 0.3–1.1; $P = 0.005$), respectively], but no significant changes were seen in atrophy or intestinal metaplasia scores. As expected, MDA concentrations fell (from 134.9 to 106.2 nmol/g; $P = 0.007$), and gastric juice ascorbic acid and total vitamin C both increased significantly 6 months after successful eradication of *H. pylori* (from 6.7 to 13.0 μg/ml for ascorbic acid; from 9.4 to 17.0 μg/ml for total vitamin C; $P < 0.001$ for both). No changes were seen in plasma or antral ascorbic acid or total vitamin C. Despite these potentially favorable changes, there was no change in M_1-dG concentration 6 months after successful eradication of *H. pylori* (Table 5).

Thirteen evaluable patients who had received eradication therapy returned at 12 months, all of whom were *H. pylori* negative on urease, histology, and breath test. On paired analysis, there was a borderline increase in M_1-dG from 6 to 12 months, (from a median of 60.8 to 77.5 adducts/10^8 bases; $P = 0.05$), but no difference between pretreatment and 12-month levels of M_1-dG (66.7 and 77.5 adducts/10^8 bases, respectively; $P = 0.8$). No significant changes were seen between 6 and 12 months for gastric juice, antral and plasma ascorbic acid, and total vitamin C or for MDA.

Twenty-one patients received placebo eradication therapy. Data were available for 12 of these patients at 6 months and for 8 patients at 12 months, all of whom remained *H. pylori* positive. MDA levels did not change significantly between pretreatment and 6 months (from 95.9 to 103.6 nmol/g; $P = 0.5$) but, surprisingly, increased significantly by 12 months (from 95.9 nmol/g at pretreatment to 178.7 nmol/g at 12 months; $P < 0.001$). In addition, M_1-dG did not change significantly between pretreatment and 6 months (from 38.9 to 70.6 adducts/10^8 bases; $P = 0.3$) but increased significantly between pretreatment and 12 months (from 38.9 to 74.5 ad-

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Table 3 Linear regression analysis of antral, plasma, and gastric juice ascorbic acid and total vitamin C, with MDA as the dependent variable, before and after adjustment for *H. pylori* infection

	Univariate analysis		Adjusted for *H. pylori* infection	
	Regression slope[a] (95% CI)	*P*	Regression slope[a] (95% CI)	*P*
Log antral AA[b]	0.08 (−0.002 to 0.17)	0.06	0.08 (0.003 to 0.15)	0.04
Log antral TVC	0.13 (0.03 to 0.23)	0.02	0.11 (0.02 to 0.20)	0.02
Log gastric juice AA	−0.04 (−0.10 to 0.02)	0.2	0.03 (−0.03 to 0.08)	0.4
Log gastric juice TVC	−0.09 (−0.16 to −0.01)	0.02	0.005 (−0.08 to 0.07)	0.9
Log plasma AA	−0.17 (−0.20 to −0.04)	0.005	−0.07 (−0.14 to 0.008)	0.08
Log plasma TVC	−0.14 (−0.24 to −0.04)	0.005	−0.08 (−0.17 to 0.01)	0.09

[a] The regression slope represents the increase in log MDA for each unit increase in the log-transformed variable.
[b] AA, ascorbic acid; TVC, total vitamin C.

Table 4 Linear regression analysis of MDA and antral, plasma, and gastric juice ascorbic acid and total vitamin C, with M_1-dG as the dependent variable, before and after adjustment for *H. pylori* infection

	Univariate analysis		Adjusted for *H. pylori* infection	
	Regression slope[a] (95% CI)	*P*	Regression slope[a] (95% CI)	*P*
Log MDA	0.28 (−0.04 to 0.59)	0.09	0.44 (0.08 to 0.81)	0.02
Log antral AA[b]	0.19 (0.05 to 0.34)	0.009	0.19 (0.05 to 0.34)	0.009
Log antral TVC	0.24 (0.06 to 0.42)	0.009	0.24 (0.06 to 0.42)	0.008
Log gastric Juice AA	0.11 (0.002 to 0.21)	0.05	−0.10 (−0.02 to 0.21)	0.09
Log gastric Juice TVC	0.11 (−0.03 to 0.24)	0.1	−0.10 (−0.06 to 0.25)	0.2
Log plasma AA	−0.05 (−0.19 to 0.08)	0.5	−0.06 (−0.20 to 0.08)	0.4
Log plasma TVC	−0.04 (−0.22 to 0.13)	0.6	−0.06 (−0.25 to 0.12)	0.5

[a] The regression slope represents the increase in log M_1-dG for each unit increase in the log-transformed variable.
[b] AA, ascorbic acid; TVC, total vitamin C.

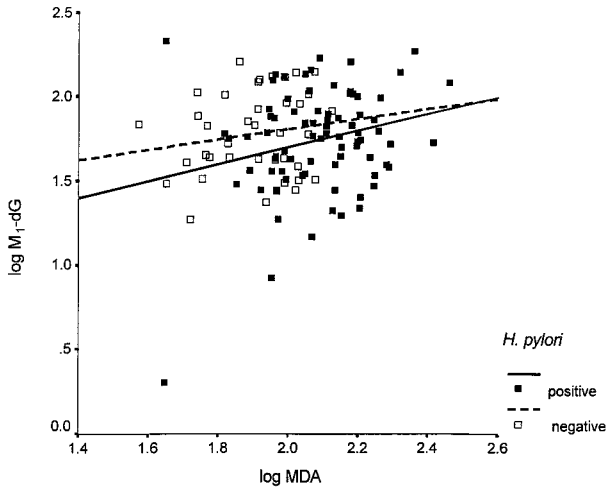

Fig. 2. Effect of MDA concentration on levels of M_1-dG in gastric mucosa and impact of *H. pylori* infection. Data are log converted. *Squares* represent individual data points stratified according to *H. pylori* infection: ■, *H. pylori* positive; □, *H. pylori* negative. *Lines* represent univariate linear regression slopes for *H. pylori*-positive (*solid line*) and -negative (*dashed line*) subjects.

ducts/10^8 bases; $P = 0.02$). No significant changes from pretreatment to 6 and 12 months were seen for plasma, antral, and gastric juice ascorbic acid and total vitamin C. Caution should be used, however, when interpreting these secondary analyses because of multiple testing and low power for these comparisons.

Discussion

H. pylori infection of the gastric mucosa stimulates influx of polymorphonuclear leukocytes, leading to the generation of reactive oxygen and nitrogen species. Cell membranes, which are rich in polyunsaturated fatty acids, are readily attacked by these compounds, producing fatty acid radicals and lipid hydroperoxides, which can decompose in complex ways, yielding more radical species and a wide range of compounds, notably aldehydes. Of these, MDA and 4-hydroxynonenal are the most common (31). MDA, which is also formed by the breakdown of prostaglandin endoperoxides (32), is a strongly genotoxic carbonyl compound that can react directly with DNA to produce a variety of adducts. The most common, formed after reaction of MDA with deoxyguanosine, is the mutagenic pyrimidopurinone compound M_1-dG (33). Recently, it has been demonstrated that M_1-dG can also be formed independently of lipid peroxidation through DNA oxidation and base propenal intermediates (34).

We sought to identify the relationship between M_1-dG and ascorbic acid, MDA, and *H. pylori* in the human stomach and studied a large number of patients before and after eradication of *H. pylori*. The immunoslot-blot technique used in this study has been carefully validated and has a detection limit of 2.5 adducts/10^8 normal bases, a level well below those detected in this study (18, 24). Our results are in keeping with the generation of M_1-dG in gastric mucosa at levels that are similar to those seen in healthy liver and are significantly higher than those recorded in leukocytes, pancreas, and breast (33). We found that *H. pylori* leads to increased concentrations of MDA and that, to a lesser extent, increased MDA is associated with an increase in M_1-dG. This is consistent with the anticipated role of lipid peroxidation and MDA as the major precursors of this adduct. The fact that the relationship between MDA and M_1-dG is relatively weak may be because MDA is generated predominantly at the cell membrane, some distance from cellular DNA.

Both ascorbic acid and total vitamin C are highly concentrated from plasma into gastric mucosa, but concentrations are

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Table 5 Levels of M_1-dG, MDA, and gastric juice, plasma, and antral ascorbic acid pretreatment and 6 and 12 months after successful eradication of *H. pylori* from 42 patients

Values are medians and interquartile ranges for the 42 initially *H. pylori*-positive patients in whom the infection had been successfully eradicated.

	H. pylori positive, pretreatment (n = 42)	6 months after eradication (n = 42)	12 months after eradication (n = 13)	P^a
M_1-dG (adducts/10^8 normal bases)	67.4 (39.3–108.2)	87.0 (62.3–106.6)	77.5 (47.2–107.0)	0.7
MDA (nmol/g)	134.9 (111.5–159.9)	106.2 (87.4–142.6)	111.4 (97.1–131.2)	0.007
Gastric juice AA[b] (μg/ml)	6.7 (2.9–11.5)	12.7 (6.6–30.8)	11.0 (3.4–24.8)	<0.001
Plasma AA (μg/ml)	5.1 (2.8–10.3)	6.8 (3.2–10.0)	8.4 (3.6–12.5)	0.4
Antral AA (μg/mg)	52.8 (36.7–79.3)	45.5 (24.6–65.5)	26.7 (10.0–57.0)	0.3

[a] Paired t tests for log-transformed data before and 6 months after eradication of *H. pylori*.
[b] AA, ascorbic acid.

reduced in the gastric juice of patients with *H. pylori* gastritis, a finding that was once again confirmed in our study. Ascorbic acid (the reduced form of vitamin C) is a potent antioxidant, able to scavenge reactive oxygen species in the gastric mucosa (35). However, we have shown that higher mucosal ascorbic acid and total vitamin C levels are associated with increases in both MDA and M_1-dG concentrations. This is consistent with the finding of a positive correlation between total antral vitamin C and MDA levels in a separate population of patients from our department and suggests that ascorbic acid and vitamin C may be promoting lipid peroxidation in the gastric mucosa (7). This may result from the ability of ascorbic acid to reduce iron to the ferrous form, which is a more potent catalyst of lipid peroxidation than ferric iron (36). The ability of ascorbic acid to promote formation of M_1-dG has also been demonstrated in liver homogenates, in which levels of M_1-dG were doubled after addition of ascorbic acid (33).

We hypothesized that increased oxidative stress and MDA concentrations in *H. pylori*-infected mucosa would lead to a concomitant increase in concentrations of M_1-dG. However, levels of M_1-dG were similar in *H. pylori*-infected and normal gastric mucosa and were not affected by any of the parameters of gastritis. Eradication of *H. pylori* resulted in expected reductions in mucosal inflammatory scores and MDA levels, but no significant changes were seen in M_1-dG. Although there was a small increase in M_1-dG levels from 6 to 12 months after eradication therapy, the level at 12 months was not different to pretreatment values, so this is unlikely to be a consequence of *H. pylori* eradication and, given the small numbers analyzed, could be the result of chance.

The lack of a direct relationship between *H. pylori* and M_1-dG may be explained by other effects of infection that compensate for increased MDA levels, such as induction of defenses against reactive oxygen species, lipid peroxidation, and DNA damage. Glutathione peroxidase is an important antioxidant that protects against lipid peroxidation by reducing lipid hydroperoxides to fatty acids. Concentrations are increased in Mongolian gerbils infected with *H. pylori* and in humans with gastritis and *H. pylori* infection (8, 37, 38). The other major protection against lipid peroxidation is the lipid-soluble chain-breaking antioxidant α-tocopherol. Although this compound is not concentrated in the gastric mucosa from plasma (39), levels are sustained by redox regeneration of tocopherol by ascorbic acid (which may indeed be one of its major functions in the gastric mucosa). Because proliferating cells have been shown to be resistant to the effects of lipid peroxidation, it may also be that the increased proliferative rate in *H. pylori* gastritis protects against formation of M_1-dG (36). Finally, repair of DNA adducts (which in this case is by nucleotide excision repair) is likely to be induced in inflamed, rapidly proliferating tissue (40). These factors may combine to counteract the elevated levels of MDA in *H. pylori* gastritis.

One difficulty with biopsy-based studies is that it is not possible to differentiate between the important cells in carcinogenesis (proliferating epithelial cells) and all other cells from the biopsy, including inflammatory and connective tissue cells. The concentration of these cells will differ between inflamed and normal biopsies, diluting any genuine differences in epithelial cell adduct concentrations. As a consequence, these data do not completely exclude an effect of *H. pylori* on levels of specific epithelial cell M_1-dG in the gastric mucosa.

Other studies of the effect of *H. pylori* on DNA damage are conflicting. Two studies found only small (but significant) increases in the oxidative DNA adduct 8-hydroxy-2-deoxyguanosine in *H. pylori* infection (41, 42). In a study of 281 subjects, urinary levels of 8-hydroxy-2-deoxyguanosine were found to be higher in persons without *H. pylori* infection than infected persons (43). O^6-Methylguanine, an alkyl adduct formed after reactions of DNA with N-nitroso compounds, has been detected in variable amounts in gastric mucosa (44, 45). The Eurogast study group found this adduct more frequently in leukocytes of individuals from areas with a high incidence of gastric carcinoma, but there was no link with *H. pylori* (46). In an earlier work, levels of DNA adducts measured by ^{32}P-postlabeling were similar in normal mucosa and atrophic gastritis or intestinal metaplasia (47), whereas others have shown abnormal DNA content in *H. pylori*-associated atrophic gastritis associated with abnormal c-myc and p53 expression (48). Finally, in our own studies of epithelial cells isolated from gastric biopsies, we found lower levels of DNA strand breaks in *H. pylori*-infected than normal mucosa (49).

At present, therefore, the relationship between *H. pylori* and DNA damage in the gastric mucosa remains elusive. What is perhaps of greater importance are the location of DNA damage and the rate at which damage is converted into significant mutations. The increase in size of the proliferative zone in *H. pylori* gastritis makes any damage more likely to occur in a proliferating cell, and the more rapid turnover means that DNA damage occurring in replicating cells is more likely to be incorrectly repaired (50, 51). Both of these factors could result in an increased mutational rate in *H. pylori* gastritis, such that even a very small increase in DNA damage may be amplified many times in terms of cancer risk.

In conclusion, we have demonstrated significant background levels of M_1-dG in gastric biopsies, raising the possibility that, despite a lack of association with *H. pylori* infection in this study, it could be an important carcinogen in the human stomach. However, future work on the mechanisms of gastric carcinogenesis should be aimed at characterizing DNA damage in proliferating epithelial cells.

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Acknowledgments

We thank Glaxo Wellcome for funding the M_1-dG analyses.

References

1. Forman, D., Newell, D. G., Fullerton, F., Yarnell, J. W. G., Stacey, A. R., Wald, N., and Sitas, F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. Br. Med. J., *302:* 1302–1305, 1991.

2. Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelman, J. H., Orentreich, N., and Sibley, R. K. *Helicobacter pylori* infection and the risk of gastric carcinoma. N. Engl. J. Med., *325:* 1127–1131, 1991.

3. Watanabe, T., Tada, M., Nagai, H., Sakaki, S., and Nakao, M. *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. Gastroenterology, *115:* 642–648, 1998.

4. Honda, S., Fujioka, T., Tokieda, M., Satoh, R., Nishizono, A., and Nasu, M. Development of *Helicobacter pylori* induced gastric carcinoma in Mongolian gerbils. Cancer Res., *58:* 4255–4259, 1998.

5. Davies, G. R., Simmonds, N. J., Stevens, T. R. J., Sheaff, M. T., Banatvala, N., Laurenson, I. F., Blake, D. R., and Rampton, D. S. *Helicobacter pylori* stimulates antral mucosal reactive oxygen metabolite production *in vivo*. Gut, *35:* 179–185, 1994.

6. Halliwell, B., and Aruoma, O. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett., *281:* 9–19, 1991.

7. Drake, I., Mapstone, N., Schorah, C., White, K., Chalmers, D., Dixon, M., and Axon, A. Reactive oxygen species activity and lipid peroxidation in *Helicobacter pylori* associated gastritis: relation to gastric mucosal ascorbic acid concentrations and effect of *H. pylori* eradication. Gut, *42:* 768–771, 1998.

8. Farinati, F., Della Libera, G., Cardin, R., Molari, A., Plebani, M., Rugge, M., Di Mario, F., and Naccarato, R. Gastric antioxidant, nitrites, and mucosal lipoperoxidation in chronic gastritis and *Helicobacter pylori* infection. J. Clin. Gastroenterol., *22:* 275–281, 1996.

9. Sobala, G., Schorah, C., Sanderson, M., Dixon, M., Tompkins, D., Godwin, P., and Axon, A. Ascorbic acid in the human stomach. Gastroenterology, *97:* 357–363, 1989.

10. Banerjee, S., Hawksby, C., Miller, S., Dahill, S., Beattie, A., and McColl, K. Effect of *Helicobacter pylori* and its eradication on gastric juice ascorbic acid. Gut, *35:* 317–322, 1994.

11. Basu, A. K., and Marnett, L. J. Unequivocal demonstration that malondialdehyde is a mutagen. Carcinogenesis (Lond.), *4:* 331–333, 1983.

12. Marnett, L. J. DNA adducts of α,β-unsaturated aldehydes, and dicarbonyl compounds. *In:* K. Hemminki, A. Dipple, D. E. G. Shuker, F. F. Kadlubar, D. Segerback, and H. Bartsch (eds.), DNA Adducts: Identification and Biological Significance, Vol. 125, pp. 151–163. Lyon: IARC Scientific Publications, 1994.

13. Mukai, F. H., and Goldstein, B. D. Mutagenicity of malondialdehyde, a decomposition product of peroxidised polyunsaturated fatty acids. Science (Washington DC), *191:* 868–869, 1976.

14. Spalding, J. W. Toxicology and carcinogenesis studies of malondialdehyde sodium salt (3-hydroxy-2-propenal, sodium salt) in F344/N rats and B6C3F1 mice. NTP Tech. Rep., *331:* 5–13, 1988.

15. Benamira, M., Johnson, K., Chaudary, A., Bruner, K., Tibbetts, C., and Marnett, L. J. Induction of mutations by replication of malondialdehyde-modified M13 DNA in *Escherichia coli*: determination of the extent of DNA modification, genetic requirements for mutagenesis, and types of mutations induced. Carcinogenesis (Lond.), *16:* 93–99, 1995.

16. Choi, M. A., Kim, B. S., and Yu, R. Serum antioxidant vitamin levels and lipid peroxidation in gastric carcinoma patients. Cancer Lett., *136:* 89–93, 1999.

17. Fink, S. P., Reddy, G. R., and Marnett, L. J. Mutagenicity in *Escherichia coli* of the major DNA adduct derived from the endogenous mutagen malondialdehyde. Proc. Natl. Acad. Sci. USA, *94:* 8652–8657, 1997.

18. Leuratti, C., Singh, R., Lagneau, C., Farmer, P. B., Plastaras, J. P., Marnett, L. J., and Shuker, D. E. G. Determination of malondialdehyde-induced DNA damage in human tissues using an immunoslot blot assay. Carcinogenesis (Lond.), *19:* 1919–1924, 1998.

19. Chaudhary, A. K., Nokubo, M., Reddy, G. R., Yeola, S. N., Morrow, J. D., Blair, I. A., and Marnett, L. J. Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science (Washington DC), *265:* 1580–1582, 1994.

20. Vaca, C. E., Fang, J., Mutanen, M., and Valsta, L. ^{32}P-Postlabelling determination of DNA adducts of malondialdehyde in humans: total white blood cells and breast tissue. Carcinogenesis (Lond.), *16:* 1847–1851, 1995.

21. Leuratti, C., Bingham, S., Hughes, R., Axon, A. T. R., Everett, S., Farmer, P. B., and Shuker, D. E. G. Detection by HPLC/^{32}P-postlabelling of malondialdehyde-deoxyguanosine monophosphate in human blood and tissue DNA in relation to diet and *H. pylori* infection. Proc. Am. Assoc. Cancer Res., *39:* 353, 1997.

22. Wang, M., Dhingra, K., Hittleman, W. N., Liehr, J. G., de Andrade, M., and Li, D. Lipid peroxidation induced putative malondialdehyde-DNA adducts in human breast tissues. Cancer Epidemiol. Biomarkers Prev., *5:* 705–710, 1996.

23. Sevilla, C. L., Mahle, N. H., Eliezer, N., Uzieblo, A., O'Hara, S. M., Nokubo, M., Miller, R., Rouzer, C. A., and Marnett, L. J. Development of monoclonal antibodies to the malondialdehyde-deoxyguanosine adduct, pyrimidopurinone. Chem. Res. Toxicol., *10:* 172–180, 1997.

24. Leuratti, C., Singh, R., Deag, E. J., Griech, E., Hughes, R., Bingham, S. A., Plastaras, J. P., Marnett, L. J., and Shuker, D. E. G. A sensitive immunoslot-blot assay for detection of malondialdehyde-deoxyguanosine in human DNA. *In:* B. Singer and H. Bartsch (eds.), Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis, Vol. 150, pp. 197–204. Lyon: IARC Scientific Publications, 1999.

25. Dixon, M. F., Genta, R. M., Yardley, J. H., Correa, P., and the participants of the International Workshop on the Histopathology of Gastritis. Classification and grading of gastritis. The updated Sydney system. Am. J. Surg. Pathol., *20:* 1161–1181, 1996.

26. Logan, R. P. H., Dill, S., Bauer, F. E., Walker, M. M., Hirschl, A. M., Gummett, P. A., Good, D., and Mossi, S. The European ^{13}C-urea breath test for the detection of *Helicobacter pylori*. Eur. J. Gastroenterol. Hepatol., *3:* 915–921, 1991.

27. Yagi, K. A simple fluorimetric assay for lipoperoxide in blood plasma. Biochem. Med., *15:* 212–216, 1976.

28. Sobala, G. M., Pignatelli, B., Schorah, C. J., Bartsch, H., Sanderson, M., Dixon, M. F., Shire, S., King, R. F. G., and Axon, A. T. R. 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 (Lond.), *12:* 193–198, 1991.

29. Waring, A. J., Drake, I. M., Schorah, C. J., White, K. L. M., Lynch, D. A. F., Axon, A. T. R., and Dixon, M. F. Ascorbic acid and total vitamin C concentrations in plasma, gastric juice, and gastrointestinal mucosa: effects of gastritis and oral supplementation. Gut, *38:* 171–176, 1996.

30. Everett, S., White, K., Drake, K., Schorah, C., and Axon, A. *H. pylori* is associated with reduced plasma ascorbic acid concentrations. Gastroenterology, *116:* A399, 1999.

31. Bartsch, H. Exocyclic adducts as new risk markers for DNA damage in man. *In:* B. Singer and H. Bartsch (eds.), Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis, Vol. 150, pp. 1–16. Lyon: IARC Scientific Publications, 1999.

32. Marnett, L. J. Generation of mutagens during arachidonic acid metabolism. Cancer Metastasis Rev., *13:* 303–308, 1994.

33. Marnett, L. J. Chemistry and biology of DNA damage by malondialdehyde. *In:* B. Singer and H. Bartsch (eds.), Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis, Vol. 150, pp. 17–25. Lyon: IARC Scientific Publications, 1999.

34. Dedon, P. C., Plastaras, J. P., Rouzer, C. A., and Marnett, L. J. Indirect mutagenesis by oxidative DNA damage: formation of the pyrimidopurinone adduct of deoxyguanosine by base propenal. Proc. Natl. Acad. Sci. USA, *95:* 11113–11116, 1998.

35. Drake, I., Davies, M., Mapstone, N., Dixon, M., Schorah, C., White, K., Chalmers, D., and Axon, A. Ascorbic acid may protect against human gastric cancer by scavenging mucosal oxygen radicals. Carcinogenesis (Lond.), *17:* 559–562, 1996.

36. Cheeseman, K. H. Lipid peroxidation and cancer. *In:* B. Halliwell and O. Aruoma (eds.), DNA and Free Radicals, pp. 109–144. New York: Ellis Horwood Limited, 1993.

37. Suzuki, H., Mori, M., Seto, K., Kai, A., Kawaguchi, C., Suzuki, M., Suematsu, M., Yoneta, T., Miura, S., and Ishii, H. *Helicobacter pylori*-associated gastric pro- and antioxidant formation in Mongolian gerbils. Free Radic. Biol. Med., *26:* 678–684, 1999.

38. Beno, I., Volkovova, K., Bakovsky, M., and Staruchova, M. Increased mucosal antioxidant enzyme activities in chronic gastritis and benign gastric polyps. Eur. J. Cancer Prev., *2:* 461–465, 1993.

39. Sanderson, M., White, K. L. M., Drake, I. M., and Schorah, C. J. Vitamin E and carotenoids in gastric biopsies: the relation to plasma concentrations in patients with and without *Helicobacter pylori* gastritis. Am. J. Clin. Nutr., *65:* 101–106, 1997.

40. Ames, B. N., Shigenaga, M. K., and Swirsky Gold, L. DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. Environ. Health Perspect., *101* (Suppl. 5)*:* 35–44, 1993.

41. Farinati, F., Cardin, R., Degan, P., Rugge, M., Mario, F. D., Bonvicini, P., and Naccarato, R. Oxidative DNA damage accumulation in gastric carcinogenesis. Gut, *42:* 351–356, 1998.

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42. Baik, S-C., Youn, H-S., Chung, M-H., Lee, W-K., Cho, M-J., Ko, G-H., Park, C-K., Kasai, H., and Rhee, K-H. Increased oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa. Cancer Res., *56:* 1279–1282, 1996.

43. Witherell, H. L., Hiatt, R. A., Replogle, M., and Parsonnet, J. *Helicobacter pylori* infection and urinary excretion of 8-hydroxy-2deoxyguanosine, an oxidative DNA adduct. Cancer Epidemiol., Biomark. Prev., *7:* 91–96, 1998.

44. Hall, C. N., Badawi, A. F., O'Connor, P. J., and Saffhill, R. The detection of alkylation damage in the DNA of human gastrointestinal tissues. Br. J. Cancer, *64:* 59–63, 1991.

45. Kyrtopoulos, S. A., Davaris, G., Haritopoulos, N., and Ampatzi, P. Studies in gastric carcinogenesis IV. O^6-Methylguanine and its repair in normal and atrophic biopsy samples of human gastric mucosa. Correlation O^6-alkylguanine-DNA-alkyltransferase activities in gastric mucosa and circulating lymphocytes. Carcinogenesis (Lond.), *11:* 431–436, 1990.

46. The Eurogast Study Group. O^6-Methylguanine in blood leucocyte DNA: an association with the geographic prevalence of gastric cancer and with low levels of serum pepsinogen A, a marker of severe chronic atrophic gastritis. Carcinogenesis (Lond.), *15:* 1815–1820, 1994.

47. Dyke, G. W., Craven, J. L., Hall, R., and Garner, R. C. DNA damage as measured by ^{32}P-postlabelling in normal and pre-malignant gastric mucosa. Cancer Lett., *77:* 45–50, 1994.

48. Nardone, G., Staibano, S., Rocco, A., Mezza, E., D'armiento, F. P., Insabato, L., Coppola, A., Salvatore, G., Lucariello, A., Figura, N., De Rosa, G., and Budillon, G. Effect of *Helicobacter pylori* infection and its eradication on cell proliferation, DNA status, and oncogene expression in patients with chronic gastritis. Gut, *44:* 789–799, 1999.

49. Everett, S. M., White, K., Schorah, C., Dixon, M., and Axon, A. The effect of *Helicobacter pylori* eradication on DNA single strand breaks in gastric epithelial cells. Gastroenterology, *12:* A113, 1997.

50. Lynch, D. A. F., Mapstone, N. P., Clarke, A. M. T., Sobala, G., Jackson, P., Morrison, L., Dixon, M., Quirre, P., and Axon, A. Cell proliferation in *Helicobacter pylori* associated gastritis and the effect of eradication therapy. Gut, *36:* 346–350, 1995.

51. Havard, T. J., Sarsfield, P., Wotherspoon, A. C., and Steer, H. W. Increased gastric epithelial cell proliferation in *Helicobacter pylori* associated follicular gastritis. J. Clin. Pathol., *49:* 68–71, 1996.

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Cancer Epidemiol Biomarkers Prev 2001;10:369-376.

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