Cell proliferation in the post-surgical stomach, dietary salt, and the effect of *H pylori* eradication

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Abstract

Aims—To study the epithelial kinetics of the post-surgical stomach with reference to dietary salt intake and *H pylori*.

Methods—Endoscopic biopsies of the antrum/anastomosis and corpus were taken for histology and MIB-1 immunostaining. The labelling index (LI%) was determined in the three zones of the gastric glands (zone 1 = surface + gastric pit; zone 2 = isthmus; zone 3 = gland base) in patients with vagotomy and pyloroplasty (n = 12), gastroenterostomy + vagotomy (n = 4), partial gastrectomy (n = 3), and Billroth I operation (n = 3). Dietary salt was determined by urinary sodium/creatinine ratio. Twelve patients were *H pylori* positive (10 vagotomy and pyloroplasty; 2 partial gastrectomy) and had a repeat biopsy three months after antihelicobacter treatment (10 were *H pylori* negative after treatment).

Results—There was no correlation between salt intake and antrum/anastomosis ($r = -0.34$; $p = 0.2$) or corpus ($r = -0.16$; $p = 0.2$) labelling indices. Gastric mucosal proliferation is increased in the antrum/anastomosis compared to the corpus in *H pylori* positive ($p = 0.014$) but not *H pylori* negative subjects ($p = 0.084$). This may reflect the different types of post-surgical stomach in each group. Gastric mucosal proliferation is reduced in antrum/anastomosis ($p = 0.002$) and corpus ($p = 0.016$) following *H pylori* eradication.

Conclusions—Dietary salt does not influence gastric mucosal proliferation in the post-surgical stomach but *H pylori* may have a role in gastric stump carcinogenesis.

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Keywords: epithelial kinetics; post-surgical stomach; *H pylori*

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cation of the organism returns epithelial proliferation to normal levels in the intact stomach.¹⁴⁻¹⁸ Dietary salt has been shown to correlate with gastric epithelial proliferation in the intact stomach.¹⁹

Recent work on gastric epithelial kinetics in the post-surgical stomach suggests that *H pylori* and bile also have a synergistic effect on mucosal proliferation in the gastric remnant, in that gastric mucosal proliferation is increased in the post-surgical stomach compared with the intact stomach, and *H pylori* infection of the gastric remnant is associated with greater mucosal proliferation than when this organism is absent.²⁰

Our aims in this investigation were to study further the epithelial kinetics of the post-surgical stomach with reference to *H pylori* and dietary salt intake.

Methods

Patients undergoing routine diagnostic endoscopy were recruited with their informed consent. Those taking histamine receptor antagonists, proton pump inhibitors, non-steroidal anti-inflammatory drugs, antibiotics, or bismuth salts were excluded. The study was approved by the hospital ethics committee.

Twenty two patients were recruited (table 1) (16 men and six women; median age 61 years, range 40 to 74). None had previously undergone biliary tract surgery. Twelve subjects had undergone vagotomy and pyloroplasty, four gastroenterostomy and vagotomy, three Polya gastrectomy, and three Billroth I partial gastrectomy.

URINARY Na/CREATININE

Dietary salt was measured by determining the urinary sodium to creatinine ratio.²¹ A sample of urine, passed between 8 am and midday, was collected in a universal container. Urinary sodium and creatinine concentrations were measured using a Vitros analyser (Johnson and Johnson Clinical Diagnostics Inc).

ENDOSCOPIC BIOPSIES

At endoscopy, biopsies were taken from within 5 cm of the anastomosis (4) or the antrum (4), and from the corpus (4), using standard forceps. Biopsies from each site were placed in 10% formalin, routinely processed, and stained with haematoxylin and eosin. All endoscopies were performed by one endoscopist.

H PYLORI

A modified Giemsa stain was used to detect *H pylori* in the antrum/anastomosis and corpus biopsies. All sections were examined by one

The post-surgical stomach is at increased risk of cancer.¹⁻⁴ Bile reflux, which is an invariable consequence of operations that remove or bypass the pylorus,^{5,6} is thought to be a major factor in carcinogenesis. Bile and *Helicobacter pylori* have a synergistic effect on the development of intestinal metaplasia in the gastric mucosa,⁷ an important step in the development of gastric cancer.

Dietary salt intake⁸ and *H pylori* infection⁹⁻¹² are risk factors for the development of gastric cancer in the intact stomach. Epithelial hyperproliferation is important in gastric carcinogenesis.¹³ Gastric epithelial proliferation is increased in *H pylori* gastritis, and eradication of the organism returns epithelial proliferation to normal levels in the intact stomach.

Table 1 The age, sex, urinary sodium (Na) to creatinine (Creat) ratio, type of gastric surgery, and *H pylori* status of the patients studied

Patient	Age (years)	Sex	Urinary Na:Creat	Surgery	<i>H pylori</i>
1	51	M	0.04	PG	POS
2	59	M	0.05	V+P	POS
3	41	M	0.03	V+P	POS
4	61	M	—	V+P	POS
5	53	M	0.03	V+P	POS
6	66	F	0.03	V+P	POS
7	49	M	0.04	V+P	POS
8	67	M	0.01	V+P	POS
9	65	F	0.04	V+P	POS
10	61	M	0.02	V+P	POS
11	69	M	—	PG	POS
12	48	F	0.01	V+P	POS
13	71	F	—	PG	NEG
14	61	F	0.04	GE	NEG
15	68	M	0.02	B-I	NEG
16	69	M	0.03	B-I	NEG
17	74	M	—	GE	NEG
18	54	M	0.07	V+P	NEG
19	64	M	—	V+P	NEG
20	47	M	0.02	GE	NEG
21	71	M	0.05	GE	NEG
22	47	M	0.08	B-I	NEG

B-I, Billroth I operation; GE, vagotomy and gastroenterostomy; PG, Polya gastrectomy; V+P, vagotomy and pyloroplasty.

histopathologist with a special interest in gastrointestinal disease who was unaware of endoscopic diagnosis. Eradication of the microorganism was assumed to have occurred only if both antrum/anastomosis and corpus biopsies were negative.

MIB-1 IMMUNOSTAINING

Sections for immunohistochemical labelling with MIB-1 monoclonal antibody (1 in 100 dilution; Coulter Electronics) were placed in citrate buffer (pH 6.0) and brought to boiling in a microwave oven at 650 W. This procedure was repeated twice with five minute resting intervals between boiling. After cooling, the sections were labelled with MIB-1 using a three step avidin-biotin complex immunoperoxidase technique.

LABELLING INDEX

We counted only those sections with a full thickness of mucosa (epithelium to muscularis mucosae) and oriented perpendicularly to the epithelial surface. For the purpose of counting, the gastric mucosa was divided into three zones: zone 1 = surface and gastric pit; zone 2 = isthmus; zone 3 = gland base. The number of cells to be counted was determined by counting consecutive high power fields until the continuous mean varied by less than 5%. The number of positively staining nuclei per 500 epithelial cell nuclei (or whole section when less than 500 cells were present) was counted in each zone and expressed as a percentage. This value corresponds to the labelling index (LI%). Only unequivocally stained cells were counted as positive. All sections were counted by one person who was unaware of the endoscopic diagnosis and *H pylori* status.

ERADICATION THERAPY

Antihelicobacter treatment consisted of a two week course of tetracycline 500 mg four times daily, metronidazole 400 mg three times daily,

Table 2 Details the total and zone 2 median (range) labelling indices (LI%) in the antrum/anastomosis and corpus of the *H pylori* positive (HP pos) and negative (HP neg) post-surgical stomachs

Patient group/ remnant site	HP pos (n=12)	HP neg (n=10)
Antrum/anastomosis		
Total LI%	33.6 (13.7 to 51.4)	31.1 (12.7 to 47.3)
Zone 2 LI%	60.9 (33.1 to 83.4)	49.9 (34.8 to 8.4)
Corpus		
Total LI%	17.7 (3.4 to 32.5)	20.7 (9.6 to 38.9)
Zone 2 LI%	42.8 (8.0 to 65.8)	40.75 (33.6 to 56.8)

and tripotassium dicitrate-bismuthate 120 mg four times daily. Patients underwent repeat endoscopy and biopsy three months after completion of treatment.

STATISTICAL ANALYSIS

The Mann-Whitney U test was used for analysis of non-parametric data and Wilcoxon rank sum test was employed when analysing the pre- and post-*H pylori* eradication data. Spearman rank analysis was used for the correlation studies. A probability (p) value of less than 0.05 was regarded as significant.

Results

Twelve patients were positive for *H pylori* (eight male and four female; median age 60 years, range 41 to 69). Ten subjects had vagotomy and pyloroplasty and two had a partial gastrectomy. Twelve of the antrum/anastomosis specimens and 10 of the corpus biopsy specimens were suitable for counting. In the 10 *H pylori* negative subjects, all the antrum/anastomotic and corpus biopsies were suitable for counting.

CELL PROLIFERATION IN THE POST-SURGICAL STOMACH

Positive staining for MIB-1 varied in the same direction for all three zones. Most were situated in zone 2. For the purposes of this study total and zone 2 LI% are presented.

There was no difference in antrum/anastomosis or corpus (total and zone 2) LI% when comparing *H pylori* positive patients with a post-surgical stomach to *H pylori* negative subjects (table 2).

In *H pylori* positive patients, antrum/anastomosis total and zone 2 LI% were significantly increased compared with the corpus ($p = 0.004$ and $p = 0.014$, respectively). Those subjects with an *H pylori* negative post-surgical stomach had higher total ($p = 0.01$), but not zone 2 ($p = 0.08$), LI% in the antrum/anastomosis compared with the corpus (table 2).

EFFECT OF *H PYLORI* ERADICATION

The effects of *H pylori* eradication are shown in figs 1 and 2 and table 3. The 12 patients who were positive for *H pylori* were given antihelicobacter treatment and had further biopsies approximately three months later. Ten were negative for *H pylori* following treatment. Of these, 10 biopsy specimens from antrum/anastomosis and seven from the corpus were

Figure 1 Total and zone 2 labelling indices in antrum before and after *H pylori* eradication.

Figure 2 Total and zone 2 labelling indices in corpus before and after *H pylori* eradication.

suitable for counting. Eradication of *H pylori* resulted in a significant reduction in total and zone 2 LI% in the antrum/anastomosis and corpus biopsies.

CELL PROLIFERATION AND DIETARY SALT

There was no correlation between cell proliferation (total LI%) in antrum/anastomosis (17 patients) or corpus (16 patients) and urinary sodium to creatinine ratio ($r = -0.3$; $p = 0.24$; and $r = -0.1$; $p = 0.62$, respectively).

Table 3 Eradication of *H pylori* from the antrum/anastomosis and corpus of the gastric remnant leading to a reduction in total and zone 2 labelling indices

Remnant site LI%/treatment group	Pre-eradication	Post-eradication
Antrum/anastomosis (n=10)		
Total LI%	35.6 (13.7 to 51.4)	21.8 (11.1 to 55.2)a
Zone 2 LI%	64.8 (47.6 to 83.4)	47.3 (28.1 to 79.6)b
Corpus (n=7)		
Total LI%	18.8 (13.1 to 32.5)	12.8 (6.2 to 31.2)c
Zone 2 LI%	51.1 (36.1 to 65.8)	31.4 (20.3 to 48.6)d

Values are median (range).

a, $p=0.01$; b, $p=0.002$; c, $p=0.08$; d, $p=0.02$.

Discussion

The risk of gastric cancer developing in patients with peptic ulcer disease is lower than expected.^{22, 23} However, following surgery for benign gastroduodenal disease there is an increased likelihood of developing gastric carcinoma.^{1-4, 24} Animal studies have shown that after gastric surgery, duodenogastric reflux and denervation play an important role in the pathogenesis of cancer of the remnant.^{25, 26} The risk of developing cancer is related to the degree of duodenogastric reflux in both the intact and post-surgical stomach without the administration of exogenous carcinogens.^{27, 28}

The normal mucosal response to damage is inflammation and regeneration, in which increased cell proliferation plays an important role. A persistent increase in cell proliferation and the presence of carcinogens are both thought to be of importance in carcinogenesis.^{13, 29, 30} Increased labelling indices are present in gastric mucosal foveolae adjacent to gastrojejunostomy compared with mucosa near a gastrostomy, indicating that surgically induced reflux of duodenogastric contents increases mucosal proliferation.³¹ Studies of mucosal proliferation in the gastric remnant after partial gastrectomy show an upward shift of the proliferative compartment at the anastomosis³² compared with other areas of the gastric stump,³³ while surgical diversion of bile leads to a reduction in gastric remnant epithelial proliferation.^{34, 35}

In this study we used MIB-1 as a marker of gastric epithelial proliferation. This immuno-histochemical technique stains in an identical manner to the Ki67 antigen,³⁶ which it represents, and correlates well with in vitro bromodeoxyuridine labelling in gastric³⁷ and other tissue.³⁸ We employed a modified Giemsa stain to detect *H pylori*. It could be argued that a more sensitive technique for detecting *H pylori*, namely immunohistochemical staining, should have been used. However, detection of *H pylori* using the modified Giemsa stain has a very high sensitivity and specificity (98.8% and 99.2%, respectively), and in a large comparative study has been shown to be equivalent to the gold standard carbon-13 urea breath test (sensitivity 98.7% and specificity 98.4%).³⁹ Given these figures, the false negative rate using the modified Giemsa stain is approximately 1% and would not, therefore, significantly affect the results of this study.

We found no difference in gastric remnant epithelial proliferation between subjects with *H pylori* infection and those subjects where the organism was absent. This finding contrasts with the results from previous work we have undertaken on gastric epithelial kinetics in the post-surgical stomach.²⁰ The reason for the differing results is found in the types of gastric surgery present in patients who took part in the different studies. In our original study the *H pylori* positive and negative groups were comparable in the types of gastric surgery represented. In this study they are not. Ten of the 12 *H pylori* positive subjects had had vagotomy and pyloroplasty, in contrast to the *H pylori* negative group which comprised patients with gastric surgery where the pylorus had been removed or bypassed. These types of surgery are associated with a greater degree of bile reflux and associated proliferative mucosal response than vagotomy and pyloroplasty surgery.³⁴ *H pylori* infection of the post-surgical stomach is found commonly in subjects with vagotomy and pyloroplasty, while bile reflux is strongly associated with Billroth I, Billroth II, and gastroenterostomy operations.⁴⁰⁻⁴¹ Bile acids inhibit the adherence of *H pylori* in vitro⁴² but the two can coexist.⁷ The greater degree of bile reflux in the *H pylori* negative group may cause epithelial hyperproliferation comparable to that found in *H pylori* infected subjects after vagotomy and pyloroplasty, where bile reflux is less marked.

The finding that cell proliferation is greater in the antrum/anastomosis than in the more proximal remnant is in keeping with the distal portion of the remnant being in greater contact with enterogastric reflux contents. The increased exposure to bile results in more gastric mucosal damage and therefore greater inflammatory and proliferative responses. In the *H pylori* positive subjects the combination of the organism and bile reflux has been shown to have a synergistic effect on cell proliferation in the post-surgical stomach.²⁰ The observation that in *H pylori* negative subjects there is no difference in zone 2 cell proliferation between the distal and proximal gastric remnant is interesting. Damage to gastric mucosa by bile leads to a characteristic histological appearance, with foveolar hyperplasia.⁴³ This may result in an upward expansion of the proliferative compartment to involve zone 1. In this way the total proliferation of the gastric glands may be increased in the distal remnant in response to a greater degree of exposure to bile, but this effect is not seen in zone 2 because of the shift in the proliferative compartment. Little is known about epithelial kinetics in reflux gastritis affecting the intact stomach.

We used standard bismuth based triple therapy as our antihelicobacter regime. In previous work using this regime, the eradication rate was approximately 50%.¹⁴⁻¹⁷ By using this treatment we had hoped to acquire an equal number of *H pylori* positive and negative subjects post-treatment for the purpose of comparison. However, the treatment success rate was higher than expected.

Because we used the bismuth based triple therapy, follow up endoscopy and biopsy was delayed for at least three months. Bismuth is known to have an anti-inflammatory effect and may persist in the gastric mucosa for weeks.⁴⁴ The delay in clearing bismuth has been highlighted by previous studies on gastric epithelial kinetics,¹⁴⁻¹⁸ leading to a reduction in gastric mucosal cell proliferation whether the organism has been eradicated or not.

We have shown that eradication of *H pylori* from the post-surgical stomach reduces epithelial proliferation. Though the study group comprised a heterogeneous collection of different types of gastric surgery, bile reflux is probably a common denominator for these patients. The effect of bile reflux was not specifically addressed in this study. Accurate measurement of bile reflux is technically difficult.⁴⁵ One measurement of gastric bile acid concentration does not reflect the extent or duration of enterogastric reflux.⁴⁶ In order to address this, the patients in the treatment group acted as their own controls. However, it is possible that eradication of *H pylori* from the post-surgical stomach may in some way alter the degree of bile reflux and influence the labelling indices.

Previous studies on the effect of *H pylori* eradication on gastric mucosal cell proliferation have concentrated on the intact stomach.¹⁴⁻¹⁷⁻⁴⁷ The role of bile reflux on gastric remnant epithelial kinetics has also been studied.³³⁻³⁴ Bile and *H pylori* appear to have a synergistic effect on gastric stump epithelial kinetics.²⁰ This is the first report of the effect of *H pylori* eradication on cell proliferation in the post-surgical stomach and provides evidence for the possible role of *H pylori* as a promoter for gastric remnant carcinogenesis. It is possible that a subgroup of patients who have undergone gastric surgery for benign disease and are thereby exposed to duodenogastric reflux, and who also have persistent *H pylori* infection, are at increased risk of developing gastric carcinoma of the remnant. Gastric stump epithelial proliferation may increase in response to *H pylori* infection in a similar manner to the intact stomach, namely through compensatory hyperproliferation following cell damage, through the mucosal inflammatory response,⁴⁷⁻⁴⁸ and through a direct effect by the organism on the epithelial cells.⁴⁹

The dietary factor most consistently associated with gastric cancer is a high salt intake.⁸ Concentrated salt solution damages gastric mucosa, leading to inflammatory reparative changes, and is associated with mucosal atrophy. This damage increases cell proliferation which may amplify the action of carcinogens,⁵⁰ perhaps by increasing the mutagenicity of nitrosated food.⁵¹ Increased cell proliferation and tumorigenesis occurs in rats fed a high salt diet after one dose of a N-nitroso compound.⁵² Given the importance of salt ingestion in the pathogenesis of gastric cancer we have sought to determine whether there is any relation between dietary salt intake and the degree of epithelial proliferation in the gastric remnant. We did not find any such correlation. This may have reflected the small sample size

studied. Alternatively, the immunostaining technique used may not be sensitive enough to accurately reflect the mucosal state of proliferation. This explanation is unlikely. MIB-1 labelling in gastric mucosa correlates well with bromodeoxyuridine immunostaining.³⁷ Dietary salt has been shown to correlate with epithelial cell proliferation in *H pylori* gastritis of the intact stomach.¹⁹ However, in this study PCNA was used as a marker of epithelial proliferation. Concerns have been expressed about the accuracy of this technique in gastric biopsy material.³⁷ It is possible that other factors, such as bile and *H pylori*, have a greater effect on gastric remnant epithelial kinetics.

In conclusion dietary salt does not appear to affect cell proliferation in the post-surgical stomach. *H pylori* increases cell proliferation in the gastric remnant and may play a role as a promoter of gastric stump carcinoma.

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