Campylobacter pylori, duodenal ulcer, and gastric metaplasia: possible role of functional heterotopic tissue in ulcerogenesis

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SUMMARY Multiple pinch biopsies were taken from the duodenum and antrum of 137 subjects (46 active duodenal ulceration; 44 healed ulcers; 47 'normals'), and examined for the presence and grade of gastritis, gastric metaplasia, and *Campylobacter pylori*. These factors, as well as age, sex, cigarette, and anti-inflammatory agent intake were evaluated as possible risk factors for duodenal ulceration. Pentagastrin induced Congo Red staining of the duodenal bulb was performed in an additional 43 cases, to determine the presence of functioning parietal cells in the duodenum. Ninety eight per cent of patients with duodenal infection with *C pylori* had active or healed duodenal ulcers. Bacteria were confined to areas of gastric metaplasia which was always infiltrated with inflammatory cells. The metaplastic tissue was usually superficial in type, although patients had *C pylori* associated with heterotopic tissue: this has not been previously described. Congo Red staining of the duodenal bulb showed that functioning endogenous acid producing tissue could be found most often at the edges of duodenal ulcers, but also in non-ulcer subjects. Cigarette smoking, age, sex, and ingestion of non-steroidal anti-inflammatory agents were not to be found to be significant risk factors for duodenal ulceration. In contrast, the presence of duodenal infection with *C pylori* proved to be a strong risk factor for duodenal ulceration (RR=51), together with gastric metaplasia (RR=6.2), and antral *C pylori* infection (RR=7.6). These data identify duodenal infection with *C pylori* as the strongest risk factor for development of duodenal ulceration. Our finding of endogenous acid production around the edges of duodenal ulcers suggests an active role for parietal cells in the duodenum. We postulate a synergistic role for duodenal *C pylori* and endogenous acid production in the development of duodenal ulceration.

The spiral bacterium *Campylobacter pyloridis* is now clearly established as the major causative agent of non autoimmune gastritis.^{1,2} The part played by this organism in peptic ulceration, however, remains controversial. Many authors have noted an extremely high association between *C pylori* infection in the antrum and duodenal ulceration, leading to suggestions that this bacterium may play an active role in ulcerogenesis.³

Steer⁴ initially observed that bacteria in the duodenum, now known to be *C pylori*, were always associated with gastric type cells. Wyatt^{5,6} in a study on pinch biopsies has recently confirmed that gastric metaplasia is an essential prerequisite for duodenal infection by *C pylori*. She noted that the organism was always seen in association with superficial gastric metaplasia – that is, mucus producing gastric epithelial cells.

Heterotopic tissue—that is, gastric metaplastic tissue containing chief cells and parietal cells—was only rarely seen in the duodenum by Wyatt. This is in contrast with two studies on wholly resected duo-

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Table 1 Patient profiles for subjects undergoing gastroduodenal biopsy

Ulcer status	Subjects n (%)	Mean age (range) (years)	M:F ratio (% male)	% smokers (range no. smoked per day)	Regular NSAID intake
No ulcer (no ulcer history)	47/137 (35)	41 (15–72)	3.6:1 (68)	58 (10–40)	17
Healed ulcer	44/137 (32)	41 (21–63)	2.8:1 (70)	59 (10–40)	9
Active ulcer	46/137 (34)	40 (19–63)	2.2:1 (78)	47 (15–50)	15

denal bulbs. In one necropsy study from Japan involving 118 individuals without gastric and duodenal disease, 27 subjects (22%) had clusters of parietal cells in the proximal duodenum.⁷ Johansen and Hansen⁸ while examining specimens of duodenum resected at operation found parietal cell heterotopia in 10% of those with gastric cancer compared with 52% with duodenal ulcer. It appeared important therefore to determine the pathology of duodenal ulceration particularly with respect to the presence of parietal cells, their potential for endogenous acid production and the association of this tissue with *C. pylori*.

The success of histamine 2 receptor antagonists (H₂ antagonists) and vagotomy in treating duodenal ulcer underlines the importance of acid in the pathogenesis of this condition.^{9,10} This appears to be in conflict with those authors who suggest *C. pylori* to be the cause of duodenal ulceration.³ A synergistic role for both *C. pylori* and acid in ulcer initiation and maintenance has not been investigated.

This study reports on the histology and microbiology of the duodenal bulb and stomach of 90 ulcer patients and 47 non-ulcer patients. A further 43 patients underwent an endoscopic Congo Red test. Using multivariate logistic regression analysis of these results, *C. pylori* infection of the duodenum is shown to be an extremely high risk factor for duodenal ulcer disease. Gastric metaplastic tissue is essential for infection of the duodenum and functional heterotopic tissue may be important in the pathogenesis of ulcer formation.

Methods

SUBJECTS

This study was approved by St Vincent's Hospital Ethics Committee, and written informed consent was obtained from 137 subjects, comprising 46 with active duodenal ulceration, 44 with healed duodenal ulcers and 47 with macroscopically normal duodenal bulbs

and no current or previous history of duodenal ulcer disease (Table 1). A minimum of two 'pinch' biopsies were taken from the duodenal bulb and gastric antrum of each subject at upper gastrointestinal endoscopy. All the subjects with a 'normal' duodenum had presented for endoscopy with indications other than ulcer symptoms – for example, dysphagia, symptoms of gastro-oesophageal reflux, or iron deficiency anaemia. The average age of the ulcer group was 42.9 years (range 19–66) and of the non-ulcer group 42.1 years (range 15–72). There were no significant differences with regard to sex, smoking or the ingestion of non-steroidal anti-inflammatory drugs.

HISTOLOGICAL STUDIES

Sections of biopsy tissue were stained with haematoxylin and eosin, examined to determine the number of foci of gastric metaplasia and graded for the presence of mononuclear cells ('chronic gastritis') and polymorphonuclear leucocytes ('active gastritis').¹¹ The grades were as follows:

Chronic gastritis

- 0 = nil mononuclear cells,
- I = only occasional inflammatory cells present in patchy distribution; Mononuclear cells not generally confluent;
- II = inflammatory cell infiltration intermediate between grades one and three;
- III = very dense infiltration of mononuclear cells throughout the entire section with the presence of lymphoid follicles.

Active gastritis

- 0 = nil polymorphonuclear leucocytes;
- I = only occasional inflammatory cells present in patchy distribution. Polymorphonuclear cells not seen in every high power field;
- II = inflammatory cell infiltration intermediate between grades one and three;
- III = very dense infiltration of inflammatory cells throughout the entire section and/or gland abscesses with polymorphonuclear cells.

Warthin-Starry stains were used to assess the presence and grade of *C. pylori* organisms (0-III)¹¹

- 0 = no bacteria seen;
- I = occasional bacteria present but not seen in every high power field;
- II = bacterial numbers intermediate between grades one and three;
- III = numerous bacteria in all fields, usually confluent and covering the entire mucosal surface.

The grades of gastritis and *C. pylori* infection were assessed by an experienced histopathologist (MR)

who was unaware of the origin of each specimen. Periodic acid Schiff (PAS) stains were used when necessary to assess the extent of gastric metaplasia more easily. Metaplastic tissue was graded as either 'superficial' or 'heterotopic'. Superficial gastric metaplasia was defined as ectopic gastric mucus producing epithelial cells, whilst heterotopic gastric metaplasia contained fully developed fundic tissue with chief cells and parietal cells. Rapid biopsy urease testing¹¹ of adjacent biopsy specimens was performed in all cases as confirmation of *C pylori* infection.

ACID SECRETING STUDIES

Acid production in the duodenal bulb was assessed in 43 subjects (36 with duodenal ulcer disease and seven with no history of peptic ulceration) by the method of Ikeda *et al*¹² as modified by Donoghue.¹³ Two hundred and fifty micrograms of pentagastrin (ICI, UK) were injected intramuscularly 30 minutes before the endoscopic examination. At endoscopy, 10 ml 0.3% Congo Red in 0.5% sodium bicarbonate was sprayed over the duodenal bulb. Acid production by duodenal mucosa was considered to be present if the solution changed from a bright red colour to blue/black.

STATISTICAL ANALYSIS

The variable of interest (response) was the presence or absence of duodenal ulcer disease. Risk variables evaluated were superficial and heterotopic gastric metaplasia, antral and duodenal *C pylori* infections, age, gender, non-steroidal anti-inflammatory drug, and cigarette consumption. The significance of the

various possible risk factors on the presence of duodenal ulcer disease was determined using step wise multivariate logistic regression by Dr V GebSKI of the Department of Biostatistics, Macquarie University, Sydney, Australia using the methods outlined by Cox.¹⁴

Results

Ninety eight per cent of subjects with duodenal *C pylori* infection had either active or healed duodenal ulcers. Eighty three per cent of those with active ulcers and 73% of those with healed ulcers had duodenal infection with *C pylori* (Table 2). The specificity of *C pylori* association for gastric metaplasia is shown in Figure 1. There is an area in this section of superficial type gastric cells adjacent to small intestinal surface cells. The bacteria are only associated with gastric type cells, and abruptly cease at the junction of gastric and small intestinal epithelial cells. The gastric metaplasia may be a single cell layer, sometimes mixed with intestinal cells. *C pylori* is clearly able to colonise these single cell layers. In this same section, the bacteria are also seen to colonise the full thickness gastric metaplasia in very large numbers.

The gastric metaplasia seen in biopsies was mostly superficial (Table 2b). In 11% of the ulcer patients, however, and in 2% of non-ulcer patients full thickness gastric metaplasia of the heterotopic type was observed. This heterotopia was always focal. Gastric metaplasia was detected commonly in both ulcer and non-ulcer patients (Table 2b), although the incidence

Table 2 (a) *Grades of inflammation and C pylori in the antrum and duodenum of 137 ulcer and non ulcer subjects.*

Grade	Ulcer patients (n=90)						Non-ulcer patients (n=47)					
	Antrum			Duodenum			Antrum			Duodenum		
	C pylori		Inflammation	C pylori		Inflammation	C pylori		Inflammation	C pylori		Inflammation
	Act	Chr		Act	Chr		Act	Chr		Act	Chr	
0	6*	2	1	2	4	2	77	68	64	98	79	85
I	1	10	18	29	29	15	4	11	2	2	17	6
II	59	46	35	20	38	41	11	13	28	0	4	9
III	34	42	46	48	29	42	8	8	6	0	0	0

*= %; Act=active; Chr=chronic.

(b) *Results of histopathological assessment of biopsy specimens*

Ulcer status	Superficial metaplasia	Heterotopic metaplasia	Duodenal C pylori	Antral C pylori
Active ulcer	42/46 (91%)	5/46 (11%)	38/46 (83%)	43/46 (93%)
Healed ulcer	41/44 (93%)	1 (2%)	32/44 (73%)	42/44 (95%)
No ulcer	15/47 (32%)	0/47 (0%)	1/47 (2%)	11/47 (23%)

Fig. 1 Warthin Starry stain of a duodenal biopsy from a patient with a duodenal ulcer. SGM=superficial gastric metaplasia. FTGM=full thickness gastric metaplasia (heterotopia). Inset (a) An area of superficial gastric metaplasia and intestinal tissue with *C. pylori* only associated with the surface of the single layer of gastric type epithelium. Inset (b) Heavy *C. pylori* infection within an area of full thickness gastric metaplasia.

was higher in those with active duodenal ulcer disease, 91% v 32% ($p<0.01$). Patients with healed ulcers also had a very high incidence of gastric metaplasia (95%).

The grades of gastritis and *C. pylori* infection of the duodenal and antral biopsies from the patients studied are shown in Table 2. Antral *C. pylori* infection was found in close association with duodenal ulceration. Ninety three per cent of those with active duodenal ulceration and 95% of those with healed ulceration had evidence of *C. pylori* infection. In contrast, only 23% of those with no history of duodenal ulceration had antral *C. pylori* infection ($p<0.001$ χ^2 analysis). Although our grading system for *C. pylori* was only semiquantitative it appeared that the actual concentration of *C. pylori* in the duodenum of ulcer patients was less than that seen in the antrum of the same patients. Only 71% of the ulcer patients had high grades (II and III) of *C. pylori* infection in the duodenum compared with 93% in the antrum.

The association of *C. pylori* and both active and

chronic inflammation is clearly demonstrated (Table 2a). In the antra of ulcer patients 93% had significant numbers of *C. pylori* (>grade II), 88% had significant polymorphonuclear cell accumulation and 81% significant mononuclear cells. Comparative figures in the duodenum for the ulcer patients were 68%, 67%, 83%.

INVESTIGATION OF FUNCTIONAL HETEROTOPIC TISSUE

Congo red staining of the duodenal bulb in a further 43 patients showed that functioning endogenous acid producing tissue can be present independently of the presence of *C. pylori* infection (Table 3). All three patients with gastric metaplasia but no *C. pylori* infection showed a colour change from red to black indicative of acid secretion. The majority of specimens showing gastric metaplasia and coexisting *C. pylori* infection changed from red to black (26/36). In patients with no areas of gastric metaplasia, there were no acid induced colour changes detected. The areas of acid secretion were focal and were distrib-

Table 3 Presence of acid secreting areas in duodenal tissue of ulcer and non-ulcer patients

Active ulcer	Status of patient			Patients (n)	Number showing acid secreting areas in the duodenum
	Healed ulcer	Metaplasia	C pylori		
+	—	+	+	23	20
—	+	+	+	13	6
—	—	+	—	3	3
—	—	—	—	4	0

*Separate biopsies were taken from all patients for histology.

uted around the ulcers. Because of the Congo red discoloration stained biopsies could not be satisfactorily examined histologically. Where biopsies could be taken from the same area at repeat endoscopy, however, acid secreting cells were seen in about a third of the specimens, a very high proportion given the small size of biopsies and the likely irregular distribution of heterotopic tissue. Examples of the distribution of acid secreting areas with respect to the ulcer site in the duodenal bulb are shown in Figures 2 and 3. The black areas always appeared concentrated to one side of the bulb. The ulcer site was always in this area. Because of the high incidence of colonisation of gastric metaplastic tissue by *C pylori* in ulcer patients compared with non-ulcer patients, results were analysed to determine the significance of gastric metaplasia and *C pylori* infection as risk factors in duodenal disease.

STATISTICAL ANALYSIS

The results of step wise logistic regression are shown in Table 4. Gastric metaplasia, antral *C pylori* infection, and duodenal *C pylori* infection were shown to be significant predictive factors for duodenal ulceration. By far the strongest risk factor for duodenal ulceration is duodenal *C pylori* infection (relative risk=51 for duodenal *C pylori* infection compared with eight for antral infection). Age, sex, cigarette smoking, and non-steroidal anti inflammatory drug intake were not found to be significant risk factors in the 90 ulcer patients studied (Table 1).

Discussion

The cause of duodenal ulceration remains unknown. Various contributory factors, however, have been identified. In our study, we examined age, sex, cigarette consumption, non-steroidal anti-inflammatory intake, gastric metaplasia, antral *C pylori* infection, and duodenal *C pylori* infection. The most striking association with duodenal ulceration was duodenal infection with *C pylori* (RR=51). We

Fig. 2 Diagram of the appearance of endoscopic fields from the duodenal bulb of six representative patients after pentagastrin induced Congo red staining.

found only one patient who had duodenal *C pylori* infection and no history of ulcer disease. If *C pylori* is demonstrated on duodenal biopsy, the likelihood of ulcer disease is 172:1. This extremely high risk factor strongly supports a role for *C pylori*, either active or passive, in ulcerogenesis. Thus there is clearly a need to understand more about the association of *C pylori* with the duodenal tissue.

The histological examination of the biopsies from 137 subjects reported here confirms the association described previously of *C pylori* and gastric metaplasia in the duodenum. This association is specific for the surface of the gastric type epithelial cell. Colonisation could be clearly seen on areas of a single

Fig. 3 Photograph of an endoscopic field of a patient with an active duodenal ulcer and biopsy proven *C pylori* infection and gastric metaplasia showing a large area of Congo red stained acid secreting tissue.

mucus producing cell layer. This bacterium appears to be adapted to the ecological niche of gastric mucus.¹⁵ Thus, the specificity of association could either be as a result of the type of mucus produced by these cells or to the presence of specific receptors on the cell surface at the intercellular junction. In all specimens where *C pylori* was found associated with metaplasia, an underlying infiltration of polymorphonuclear cells was noted. This is equivalent to the situation in the stomach, where the presence of *C pylori* is always associated with active gastritis, and is considered to be the cause of the inflammation. The histological gradings on the patients reported here confirmed the association of antral *C pylori* infection with active chronic gastritis.

Table 4 Statistical interpretation of biopsy data

(i) Using multivariate logistic regression, the significance of a given factor in duodenal ulcer disease is as follows:			
Gastric metaplasia	p=0.036		
Antral <i>C pylori</i> infection	p=0.063		
Duodenal <i>C pylori</i> infection	p=0.001		
(ii) Significant risk factors in duodenal ulcer disease			
Factor assessed	Relative risk	Odds ratio	Confidence limits
Gastric metaplasia	6.22 (0.82*)	21.2	1.1-27.8
Antral <i>C pylori</i> infection	7.67 (0.75)	55.6	0.93-17.3
Duodenal <i>C pylori</i> infection	51.0 (1.01)	171.9	4.8-341.2

*Standard error.

Gastric metaplasia has been found in all parts of the gastrointestinal and biliary tracts, from the oesophagus to the rectum, and even in the pancreas and gall bladder.¹⁶⁻¹⁸ According to Taylor and Perera,^{19,20} there are two types of gastric metaplasia, (i) superficial and (ii) heterotopic. Each of these types can be further classified as either congenital or acquired. Superficial gastric metaplasia contains no fundic cells and purely involves the epithelial lining cells. Heterotopic gastric metaplasia contains parietal and chief cells. The very well structured gastric heterotopic tissue described by James *et al*²¹ is probably congenital in origin. The more patchy, disorganised full thickness metaplasia described by Hoedemaker,²² and the superficial type described by Taylor²⁰ and Wyatt⁶ are more likely to be acquired later in life. It is these two latter types of gastric metaplasia that are found so frequently in association with duodenal ulceration, and which *C pylori* so commonly infects.

Consideration of the type and function of the metaplastic tissue associated with *C pylori* and the ulcer itself is important in consideration of the possible mechanisms of ulcerogenesis. Using single 'pinch' biopsies, Wyatt⁶ noted a very low incidence of gastric heterotopia, suggesting that functioning gastric tissue plays little role in ulcer formation. Studies looking at whole duodenal bulbs^{7,8} suggest, however, that heterotopia is very common, especially in duodenal ulcer disease (52%). Willis¹⁶ has shown that heterotopic tissue is often functional tissue. In the present study, at least two 'pinch' biopsies were taken from the edges of ulcers, thus increasing the frequency of detection of heterotopia. The pentagastrin induced Congo red studies support the concept that gastric heterotopia is more common than can be shown by 'pinch' biopsy alone, and that this tissue may contain functioning parietal cells. Ikeda¹² has previously validated this Congo red method which identifies acid secreting areas of heterotopia. Because acid is considered such an important ingredient for ulcer formation, it is important to determine how often duodenal ulceration is associated with functioning areas of metaplastic tissue. By definition, the tissue central to an ulcer is destroyed, and thus the very pathology which we are seeking may have eroded by the ulcer itself.

Other factors (as yet unidentified) may induce metaplasia, or it may be congenital. As it is such a common finding, even among the normal population - that is, Kreunig found superficial gastric metaplasia in 64% of a group of healthy volunteers,²³ it is possible that gastric metaplasia is simply a variation of normal.

One important factor in ulcerogenesis could be the transmission of the *C pylori* from the antrum to areas

of gastric metaplasia within the duodenum. It is important to note that four patients who had gastric metaplasia in the duodenum but *C. pylori* confined to the antrum had no history of duodenal ulceration – that is, some subjects can have a longstanding antral *C. pylori* infection and gastric metaplasia in the duodenum which for some reason does not become infected. Other factors to be considered could relate to blood flow or other anatomical differences at the ulcer site. Why is it that ulcers recur at the same site? Conversely, can duodenal ulceration arise in areas of strictly superficial type gastric metaplasia? Acid induced tissue damage is an essential path towards ulcer formation. The question is, does *C. pylori* catalyse this damage, and if so how? If the association is restricted to superficial metaplasia only, then one has to postulate that the presence of the organism changes the local environment in a way that facilitates damage by gastric acid – for example, by changing the properties of intestinal mucus at the site.³ If ulcerogenesis occurs at sites of full thickness metaplasia, then the hydrogen ion back diffusion hypothesis proposed previously could be relevant. According to this hypothesis, the high urease activity of the *C. pylori* on the surface of the tissue alters the local milieu such that the normal process of hydrogen ion transport to the lumen is disturbed.²⁴ This results in the back diffusion of hydrogen ions into the tissue. These submucosal hydrogen ions may initiate tissue damage. Thus, endogenous duodenal acid secretion may contribute to the pathology of duodenal ulceration. In other words, there may be a synergetic role for duodenal *C. pylori* infection and acid produced at the ulcer site.

Duodenal ulceration is a multifactorial disease.²⁵ Family history, blood type, cigarette consumption, non-steroidal anti-inflammatory agents, and hyperacidity have all been identified as possible contributory factors. Any discussion on the role of *C. pylori* in ulcerogenesis has to take these factors into consideration. One possible link is that a number of as yet undefined variables may influence the presence or degree of gastric metaplasia in the duodenum. Wyatt⁶ has suggested that a major factor in the induction of gastric metaplasia is gastric hyperacidity. This is controversial, as a number of studies have indicated that metaplasia is not influenced by hyperacidity.^{26,27} Bendtsen *et al.*²⁸ recently found no difference in the mean hydrogen ion activity of 24 hour duodenal pH for ulcer and on-ulcer subjects. The observation of gastric metaplasia in areas such as pancreas, colon, and gall bladder is further evidence that acid is not required for its development.

In a study of 2350 normal duodenal bulbs Terruzzi²⁹ found 13 cases of macroscopic nodules in the duodenal bulb that were found to be hetero-

topic areas of well developed gastric mucosa with fundic glands composed of parietal and chief cells in all cases. Significantly eight of 13 had had a previous diagnosis of duodenal ulcer. These findings are consistent with the above hypothesis. Unlike our study, however, Terruzzi claimed that the gastric metaplasia in the bulb disappeared on subsequent biopsy. He suggested that the metaplasia is a delayed type healing process of peptic ulcer. This is unlikely, as both Wyatt and ourselves frequently identified gastric metaplasia in subjects with no history of ulcer disease.

In conclusion, these data identify duodenal *C. pylori* infection as the strongest risk factor for duodenal ulceration. Our finding of endogenous acid production in the duodenum of some ulcer patients plus the accumulating evidence that ulcer relapse is significantly reduced in duodenal ulcer patients from whom *C. pylori* has been eradicated^{30,32} lend weight to the proposal that *C. pylori* is actively involved in the pathogenesis of duodenal ulceration. Current concepts of duodenal ulceration and factors involved in pathogenesis need to be reappraised in the light of the proposed synergy between *C. pylori* infection and locally secreted acid induced damage.

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