Campylobacter pyloridis and acid induced gastric metaplasia in the pathogenesis of duodenitis

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SUMMARY Biopsy specimens of gastric and duodenal mucosa from 290 patients were examined histologically for metaplasia and *Campylobacter pyloridis*. Estimates of pH on samples of fasting gastric juice from 55 of the patients were performed, and mucosal biopsy specimens from 33 patients were also cultured for *C. pyloridis*. Active duodenitis was seen in 34 duodenal biopsy specimens. Thirty (88%) of the patients with active duodenitis had both >5% gastric metaplasia in the duodenal specimen and *C. pyloridis* associated gastritis. These two factors coexisted in only 0.43% of patients with no duodenal inflammation. When *C. pyloridis* were seen histologically in duodenal biopsy specimens they were confined to areas of gastric metaplasia and never occurred in the absence of a polymorph infiltrate. Of the 55 patients with measurements of gastric juice pH, gastric metaplasia was present in the duodenum in 20 of 42 with a pH of <2.5, and in 0 of 13 with a pH of >2.5.

These results suggest that acid induced gastric metaplasia in the duodenum and *C. pyloridis* associated gastritis may be synergistic in the pathogenesis of duodenitis; the metaplastic gastric epithelium allows *C. pyloridis* to colonise the duodenal mucosa, where it produces an acute inflammatory response.

Traditionally, the pathophysiology of duodenal ulcer is related to excess secretion of gastric acid,¹ and most current medical and surgical approaches to treatment are aimed, at least in part, at reducing acid output. The correlation between duodenal ulceration and high gastric acidity, however, is incomplete, implying the existence of other pathogenic factors.² Microscopy of mucosa bordering on duodenal ulcers usually shows active duodenitis, and the same histological appearances may be seen in symptomatic patients in the absence of an overt ulcer crater.³ This has led to the concept of duodenitis and duodenal ulceration as representing different points in a disease spectrum with a common underlying pathogenesis.³⁻⁵

A consistent histological finding in duodenitis is the presence of gastric metaplasia of the duodenal epithelium,³ and this has been associated, both in man^{6,7} and in experimental animals,⁸ with high acidity of the duodenal contents. Most patients with duodenal ulcer have antral gastritis⁹ which is associated with colonisation by *Campylobacter pyloridis*.^{10,11}

Although these bacteria do not colonise small intestinal mucosa, they are often present on areas of gastric metaplasia in the duodenum.^{10,12} This has led Marshall to postulate that duodenitis may be caused by secondary infection with *C. pyloridis* in areas of gastric epithelium in the duodenum.¹⁰

This study aimed to explore the relation between gastric acid, gastric metaplasia in the duodenum, and mucosal colonisation by *C. pyloridis* in dyspeptic patients with and without duodenitis or duodenal ulceration.

Material and methods

Single mucosal biopsy specimens from the duodenal bulb and gastric antrum and body were obtained from 254 consenting patients with non-ulcer dyspepsia (117 males, aged 16-80, mean age 47 years) during endoscopy. Similar sets of biopsy specimens from 23 preoperative duodenal ulcer patients and 13 patients who had had highly selective vagotomy (HSV) for duodenal ulcer were available from archive material.

The specimens were formalin fixed, routinely pro-

Table 1 Gastric histology of paired antral and body biopsy specimens from 254 patients with non-ulcer dyspepsia and related to presence of *C. pyloridis*

Gastric histology	<i>C. pyloridis</i>	
	Present	Absent
Normal	0	106
Antral gastritis, normal body	39	1
Body gastritis, normal antrum	0	4
Gastritis affecting antrum and body	94	10

cessed, and 3 µm sections stained with haematoxylin and eosin, either the Warthin-Starry or a modified Giesma technique,¹³ for identification of mucosal *C. pyloridis*, and by alcian blue periodic acid Schiff for identification of metaplasia.

The presence and degree of gastric inflammation in the antrum and body were graded according to Whitehead's classification.¹⁴ The duodenal histology was graded as follows:

Normal A normal duodenal architecture with villus: crypt ratio of >2:1 in most areas, with or without Brunner's glands above the muscularis mucosae, but with no increase in lamina propria cellularity or evidence of epithelial damage.

No significant duodenitis Architectural changes only, with blunted or distorted villi, but without a definite increase in mononuclear cells in the lamina propria or evidence of epithelial damage.

Chronic duodenitis A definite increase in mononuclear cells in the lamina propria, with evidence of epithelial damage.

Active duodenitis Intraepithelial neutrophils present; this was always a focal change, superimposed on a picture of chronic duodenitis.

In addition, the extent of gastric metaplasia in the duodenal biopsy specimen was subjectively assessed as nil, <5%, 5–20%, or >20% of the surface epithelium. Gastric heterotopia was diagnosed when specialised gastric glands of body type were present in the duodenum.

Additional antral biopsy specimens from 33 of the patients were homogenised and cultured on heated blood agar at 37°C under microaerophilic conditions for at least four days, as previously described for isolation of *C. pyloridis*.¹⁵

Samples of fasting gastric juice aspirated at the start of endoscopy were available from 55 of the dyspeptic patients for estimation of pH.

Statistical analysis was by the Mann-Whitney test for gastric juice pH in patients with and without gastric metaplasia in the duodenum, and elsewhere by the χ^2 test, with Yates's correction, where appropriate.

Results

BACTERIOLOGY

Colonies of campylobacters were isolated from 169 antral biopsy specimens; these were all identified as *C. pyloridis* by their characteristic biotyping,¹⁶ including oxidase, catalase, and urease positivity, and nalidixic acid resistance. Of the 33 cases cultured, *C. pyloridis* were obtained from 16 of 21 (76%) of patients in whom *C. pyloridis* were identified histologically, and from none of the 12 who were histologically *C. pyloridis* negative. This, therefore, confirmed the specificity of the histological technique in the identification of *C. pyloridis*, which, in our hands, provided the more sensitive method for detection of mucosal colonisation of the upper gastrointestinal tract.

COLONISATION WITH CAMPYLOBACTER PYLORIDIS

All patients who had duodenal ulcer or HSV had histological evidence of chronic gastritis associated with *C. pyloridis* colonisation. Table 1 shows the gastric histology of the patients with non-ulcer dyspepsia. One hundred and forty eight cases showed chronic gastritis, and in 133 (89.8%) this was histologically associated with *C. pyloridis*. One hundred and six pairs of gastric biopsy specimens showed no evidence of chronic gastritis, and no mucosal bacteria were detected in any of these.

Overall, 169 of the 290 patients studied (58.3%) were histologically *C. pyloridis* positive. Table 2 shows the relation between the duodenal histology and the presence of *C. pyloridis* in the stomach for all patients. Ninety seven per cent of patients with active duodenitis were *C. pyloridis* positive, compared with 53.1% of patients without active duodenal inflammation.

Table 2 Duodenal histology from all 290 patients studied and related to gastric colonisation by *C. pyloridis*

Duodenal histology	Normal stomach	Campylobacter positive gastritis	Campylobacter negative gastritis
Normal	86	107	11
No significant duodenitis	13	18	0
Chronic duodenitis	6	11	4
Active duodenitis	1	33	0

Table 3 Presence of gastric metaplasia related to duodenal histology in patients with duodenal ulcer after HSV and in those with non-ulcer dyspepsia

	Duodenal ulcer (n = 53)				HSV (n = 13)			Non-ulcer dyspepsia (n = 254)			
Duodenal histology	0%	< 5%	5–20%	> 20%	0%	< 5%	5–20%	0%	< 5%	5–20%	> 20%
Normal	2	2	0	0	6	0	0	162	24	7	1
No significant duodenitis	3	0	0	0	5	0	0	15	4	4	0
Chronic duodenitis	2	1	0	0	0	0	0	5	6	6	1
Active duodenitis	1	0	5	7	0	1	1	0	1	11	7

GASTRIC METAPLASIA IN THE DUODENUM

Gastric metaplasia was identified in 89 of 290 (30.7%) duodenal biopsy specimens; table 3 shows the prevalence of metaplasia in the different patient groups and its relation to duodenal histology. In 39 biopsy specimens there were small foci of gastric metaplasia, occupying less than 5% of the mucosal surface; these small groups of metaplastic cells showed a tendency to occur at the tips of villi, and were rarely associated with active duodenitis. More extensive gastric metaplasia, assessed as >5% of the epithelial surface, was present in 50 specimens; in 12 this was an isolated finding, not associated with features of duodenitis, although villous blunting was often present, while in 31 (62%) of the specimens there was active duodenitis. Thus although small foci of metaplasia often occur in otherwise normal duodenal mucosa, there was a significant association between more extensive gastric metaplasia (>5%) and active duodenitis ($p < 0.001$).

There was no significant difference in the preva-

lence of gastric metaplasia in the duodenum between the patients with gastric *C pyloridis* (33.5%) compared with those without (28.7%). Similarly, the presence of gastritis had no effect on the finding of gastric metaplasia in the duodenum, except that such metaplasia was not seen in patients in whom atrophic gastritis with intestinal or pyloric metaplasia was present in the body mucosa. The gastric mucosa was normal in the four cases with heterotopic gastric mucosa in the duodenum.

ACTIVE DUODENITIS

Active duodenitis was present in 34 of the total 290 duodenal biopsy specimens from all groups. This included 19 biopsy specimens from patients with non-ulcer dyspepsia patients, 13 from patients with duodenal ulcer, and two from the group who had had HSV. In 30 of these cases there was both >5% gastric metaplasia in the duodenum and gastric colonisation by *C pyloridis* (fig 1). A further four patients (all with non-ulcer dyspepsia) had both gastric metaplasia of >5% and gastric campylobacter but without active duodenitis; three had chronic duodenitis, and only one had a histologically normal duodenal biopsy specimen. Thus the combination of >5% gastric metaplasia and gastritis associated with *C pyloridis* occurred in 88% cases with active duodenitis and in only 0.43% (1 of 235) patients without duodenal inflammation.

In the Warthin-Starry or Giemsa stained sections, *C pyloridis* were observed in 19 of 36 (53%) of biopsy specimens showing active duodenitis; the bacteria were only found on areas of gastric metaplasia, never occurred in the duodenum in the absence of a polymorph infiltrate, and were seen as often in the patients with non-ulcer duodenitis as in those with duodenal ulcers. Polymorph infiltration of the epithelium occurred predominantly in areas of gastric metaplasia (fig 2) and was often closely associated with surface bacteria (figs 3 and 4).

In one case of active duodenitis the stomach was histologically normal and no *C pyloridis* were detected. In two cases there were only small foci of gastric metaplasia in the duodenal biopsy specimen; both of these patients showed *Campylobacter*

Fig 1 Distribution of active duodenitis of >5% gastric metaplasia, and gastritis associated with *C pyloridis* in 290 duodenal biopsy specimens.

Fig 2 Duodenal mucosa showing gastric metaplasia of surface epithelium. Intraepithelial neutrophil is present (arrow) in metaplastic area. (Alcian blue/periodic acid Schiff).

Fig 3 Mucosal colonisation by *C. pyloridis* restricted to areas of gastric epithelium.

Fig 4 (a) Duodenal biopsy specimen showing active duodenitis. *C. pyloridis* can be seen in gastric metaplasia (left of picture); (b) higher magnification of (a) to show proximity of intraepithelial neutrophil to surface *C. pyloridis*. (modified Giemsa.)

colonisation, both of the stomach and of the foci of gastric metaplasia. Only one patient positive for gastric *Campylobacter* with active duodenitis showed no gastric metaplasia in the duodenal biopsy specimen.

GASTRIC JUICE pH AND GASTRIC METAPLASIA IN THE DUODENUM

Fig 5 shows the relation between the fasting gastric juice pH estimates in 55 patients with non-ulcer dys-

Fig 5 Relation between fasting gastric juice pH and gastric metaplasia in duodenums of 55 patients with non-ulcer dyspepsia ($p < 0.0001$).

pepsia and the presence of gastric metaplasia. Gastric metaplasia was seen in 20 of 42 patients with a fasting pH of < 2.5 and in none of the 13 with a fasting pH of > 2.5 . Gastric metaplasia affecting more than 5% of the surface was associated with a pH of < 2.0 in 10 of 11 cases. The association between the presence of any gastric metaplasia in the duodenum and low fasting gastric juice pH was highly significant ($p < 0.0001$).

Discussion

As a group patients with duodenal ulcer tend to have a higher gastric acid output than the general population. These patients have an increased sensitivity to gastrin² and an increased rate of gastric emptying,¹ both abnormalities resulting in material of low pH entering the duodenum.² Duodenal ulcers are prone to recur, and duodenitis may persist between periods of ulceration, suggesting "a diathesis comprising hyperchlorhydria and duodenitis proceeding to duodenal ulcer".¹⁴ Our data suggest that the presence of *C. pyloridis* forms the missing link in the pathogenesis of this duodenal ulcer diathesis, acting synergistically with acid induced gastric metaplasia to cause mucosal damage in the duodenum.

Gastric metaplasia is closely associated with the presence of duodenitis, although it can occur in the

otherwise normal duodenum.^{17, 18} Previous workers have noted an apparent predilection of the polymorph infiltrate in active duodenitis for areas of gastric metaplasia, but were at a loss to explain this phenomenon.¹⁸ The common occurrence of *C. pyloridis* specifically in these inflamed sites suggests a causal relation. The number of polymorphs infiltrating the epithelium has been shown to correlate with the density of bacterial colonisation,¹⁹ and the observation of *C. pyloridis* within phagocytic vacuoles²⁰ suggests that the bacteria can attract polymorphs.

Gastric metaplasia has been viewed as a protective adaptation in duodenal epithelium exposed to high acidity,⁷ and may be present without duodenal inflammation. In patients who also have gastritis associated with *C. pyloridis*, however, the presence of gastric metaplasia allows these bacteria to colonise the duodenum. We observed *C. pyloridis* in the duodenum in 53% of biopsy specimens showing active duodenitis, but this may be an underestimate due to the sampling error of a single biopsy specimen. Steer, using scanning electron microscopy, detected the bacteria on mucosa adjacent to duodenal ulcers in 73% of cases, again restricted to sites of gastric metaplasia.¹²

It could be argued that our data reflect the independent association of duodenitis, gastric metaplasia, and *C. pyloridis* colonisation, with the common factor of type B antral gastritis. The low incidence of *C. pyloridis* in type A autoimmune gastritis has been reported.²¹ This type of gastritis, affecting predominantly the acid secreting body mucosa, is associated with hypochlorhydria, and thus duodenal gastric metaplasia is unlikely to be present in these patients. We found no instances of gastric metaplasia of $> 5\%$ in duodenal biopsy specimens from patients with intestinal or pyloric metaplasia in their gastric body biopsy specimen, a group that includes those patients with type A gastritis. Furthermore, only one patient exhibited concurrent gastric metaplasia and *Campylobacter* negative gastritis, so that we were unable to resolve the question of whether duodenitis was associated simply with the presence of antral inflammation, or specifically with *C. pyloridis*.

Gastric heterotopia was present in 1.4% of duodenal biopsy specimens; this is similar to the 0.9% prevalence reported by Shouska.¹⁸ In all of our cases the gastric mucosa was normal; thus we could not observe the effect of *C. pyloridis* colonisation on this heterotopic (presumed congenital) gastric epithelium not associated with inflammation.

If a combination of gastric metaplasia induced by excess acid and *C. pyloridis* infection are necessary for duodenal inflammation and ulceration to develop, then removal of either of these factors would be expected to result in healing. We have previously

reported that patients treated for duodenal ulcer with HSV remain colonised with gastric *C pyloridis*.²² In the present study the prevalence of gastric metaplasia in the patients who had had HSV (15%) is significantly less than the 65% prevalence seen in the preoperative patients with duodenal ulcer ($p = 0.01$). If gastric metaplasia is an adaptive response to high duodenal acidity then these results imply that it is reversed by treatment aimed at reducing acid output. This change is accompanied by a significantly lower prevalence of duodenitis than in patients with duodenal ulcer.

Healing of duodenal ulcer by bismuth compounds, which are bactericidal to *C pyloridis*, but do not affect acidity, is associated with eradication of *C pyloridis*, improvement in duodenitis,²³ and longer interval before ulcer relapse than is seen in patients treated with histamine H2 receptor antagonists.²⁴⁻²⁶ The mode of action of bismuth is uncertain as it may also protect the ulcer base by complexing with protein.²⁷ The success of ulcer healing by the antimicrobials furazolidone²⁸ and metronidazole,²⁹ however, suggests that the bactericidal effect of bismuth may, indeed, be its mode of action. Thus evidence from treatment of duodenal ulcer is consistent with a dual pathogenesis of high acidity plus *C pyloridis* infection.

Jenkins *et al.*, in a detailed study to define duodenitis using quantitative histology, concluded that the mucosal response to injury "suggests a dual pattern of response—that is, an immune response reflected in changes in plasma cell populations and an acute inflammatory response closely related to the indicators of epithelial damage, gastric metaplasia, and villous atrophy".³⁰ A combination of bacterial and chemical aggressive factors would account for this observation. Furthermore, they found an increase in all plasma cell types in duodenitis, but only the counts of IgG plasma cells correlated with polymorph infiltrate of the epithelium. We have previously shown that polymorph infiltration in active chronic gastritis is associated with the presence of *C pyloridis* coated with IgG on the epithelial surface.³¹

Although it is not yet established whether *C pyloridis* are causally related to duodenal ulceration, we have found that the combination of significant gastric metaplasia in the duodenum and gastritis associated with *C pyloridis* identifies those patients with active duodenitis, with a sensitivity of 88% and specificity of 98%. The association is equally strong whether or not an ulcer crater was present, consistent with a common pathogenesis for duodenitis and duodenal ulceration.

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