Ornithine decarboxylase activity is a marker of premalignancy in longstanding *Helicobacter pylori* infection

S E Patchett, P H Katerlasis, Z W Zhang, E M Alstead, P Domizio, M J G Farthing

Abstract

**Background**—Longstanding *Helicobacter pylori* infection may increase the risk of developing gastric adenocarcinoma. The sequence of chronic active gastritis leading to atrophy and subsequent intestinal metaplasia is thought to be a key step in gastric carcinogenesis. Ornithine decarboxylase (ODC) activity is increased in some pre-malignant gastrointestinal conditions and is essential for malignant transformation in vitro.

**Aims**—To measure ODC activity in the antrum of *H pylori* infected and non-infected subjects and to relate this to histological abnormalities associated with recent and longstanding *H pylori* infection.

**Methods**—Six antral mucosal biopsy specimens were obtained from 75 patients for detailed histological assessment and measurement of ODC activity. Samples were measured in duplicate and results expressed as median, interquartile range in pmol/mg protein/h.

**Results**—ODC activity was significantly higher in *H pylori* positive (164, 88–259 pmol/mg/h) than *H pylori* negative subjects (99–8, 55–158 pmol/mg/h, p=0.003). However the presence of gastritis, irrespective of the severity of inflammation or activity had no influence on ODC activity. Gastritis with atrophy was associated with increased ODC activity, which was closely related to the severity of the atrophy (p=0.01). Similarly, ODC activity was significantly increased in subjects with intestinal metaplasia (196, 83–25) compared with those without intestinal metaplasia (1117, 65–175, p<0.04).

**Conclusions**—These results indicate that the histological changes associated with longstanding *H pylori* infection rather than inflammation alone are associated with increased polyamine biosynthetic activity. This may be relevant to *H pylori* associated gastric carcinogenesis.

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Keywords: ornithine decarboxylase, *Helicobacter pylori*, intestinal metaplasia, gastric atrophy.

Although the rates of gastric cancer have been declining in recent years, it remains the fourth most common cause of cancer death in the United Kingdom, accounting for almost 10 000 deaths each year.¹ The reasons for the decline in gastric malignancy are unclear but is probably because of a reduction in distal gastric cancers of the intestinal type.² This is in contrast with tumours of the proximal stomach, which seem to be increasing in frequency in developed countries.³

*Helicobacter pylori* causes chronic active gastritis and its presence is strongly associated with peptic ulcer disease.⁴ ⁵ More recently, several studies strongly support its role as an aetiological factor in the development of gastric cancer.⁶ ⁷ *H pylori* is a chronic infection that typically remains active for life. It is thus a probable source of active inflammation lasting for decades, which is thought to progress to atrophy with time. This is in accordance with Correa’s hypothesis of gastric carcinogenesis in which gastric mucosa progresses from chronic gastritis through atrophy to metaplasia, dysplasia, and finally cancer.⁸

Ornithine decarboxylase (ODC) is the first and rate limiting step in the biosynthesis of polyamines, which are ubiquitous intracellular bases required for normal and neoplastic growth.⁹ This enzyme has been implicated as an essential promoter of cellular proliferation and tumour development.¹⁰ ¹¹ In this study, we have measured ODC activity in gastric mucosa of subjects with *H pylori* infection and have related this to the presence and severity of inflammatory and putative premalignant mucosal changes known to be associated with longstanding *H pylori* infection.

**Methods**

**Patients**

Patients with dyspepsia attending for upper gastrointestinal endoscopy were recruited for the study. Patients taking non-steroidal anti-inflammatory agents, antibiotics, bismuth preparations, or proton pump inhibitors within a month of endoscopy were excluded from the study as were patients who had undergone previous gastric resection.

**Study design**

Using standard endoscopy forceps, six gastric mucosal biopsy specimens were obtained from within 2 cm of the pylorus. Three specimens were obtained for measurement of ODC activity and three were used for histological assessment of antral mucosa.

Specimens were fixed in formal saline, processed routinely, and embedded in paraffin wax. Four μm sections were cut from each
specimen and stained using haematoxylin and eosin, a combined alcian blue/periodic acid-Schiff stain and a modified Giemsa method. The degree of histological change in the gastric antral biopsy specimens was classified in accordance with the Sydney system. The severity (mononuclear cell infiltrate) and activity (polymorph infiltrate) of inflammation, glandular atrophy, and intestinal metaplasia was graded by a single pathologist (PD) for each variable on a 4 point scale from 0 (absent) to 3 (severe).

Mucosal ODC activity was assayed using a modification of the method described by Beaven et al. After collection, samples were immediately snap frozen in liquid nitrogen and stored at –70°C before analysis. Samples were subsequently homogenised using an Ultra-Turrac homogeniser in 300 μl of buffer containing 0·1 M TRIS (pH 7·2), 5 mM dithiothreitol (DTT), and 0·25 mM pyridoxal phosphate. The reaction mixture consisted of 15 μl of homogenate, 0·25 mM pyridoxal phosphate, 5 mM DTT with 0·15 μCi of L-[14C]-ornithine hydrochloride (Amersham International) in a total volume of 30 μl. The reaction tube was sealed with a rubber cap in which was embedded a 0·5 cm square of Whatman No 1 filter paper soaked with 50 μl methylbenzenethionium hydroxide. Tubes were incubated at 37°C for 60 minutes and the reaction then stopped by injecting 100 μl 20% trichloroacetic acid. After an additional 30 minutes incubation for equilibration, the filter paper was removed and placed into 5 ml of scintillation fluid and counted in LKB Wallac Ultra-beta 1210 scintillation counter. The protein content of the homogenate was estimated using the bicinchoninic acid microtitre protein assay (Pierce). Samples were measured in duplicate and expressed in pmol/mg protein/h.

Statistical analyses
Data are presented as group medians with interquartile ranges. Differences in ODC activity between histological groups were analysed using the Kruskal-Wallis test and differences between pairs were analysed using the Wilcoxon test for unpaired data. Data regarding ODC activity and severity of gastritis and atrophy were analysed using a non-parametric test for trend (Cuzick’s test). Values of less than 0·05 were considered significant in all analyses.

Results
ODC activity, H pylori status, and antral histology was assessed in 75 patients (45 male) with a mean age of 54±8 years (range 18–82). Thirty five patients (46·6%) were H pylori positive. Median ODC activity was significantly higher in patients with H pylori infection (164, 88–259) compared with those who were H pylori negative (99·8, 55–158), p<0·003. The Table shows antral histological findings in all 75 patients and median ODC activity for each group. ODC activity in patients with chronic active gastritis alone was not significantly different from those with normal antral histology. Indeed in the group as a whole, neither the severity of gastritis or activity of gastritis was significantly related to the level of ODC activity suggesting that the degree of antral gastritis in itself was not a determinant of ODC activity (Fig 1). More advanced antral histological changes were however associated with increased polyamine biosynthetic activity. Figure 2 shows the relation between the presence and severity of gastric atrophy. A statistically significant stepwise increase in median ODC activity is evident as the presence and degree of atrophy increases (p<0·001). Furthermore, patients with severe atrophy had significantly higher ODC activity than either those with mild atrophy or those without atrophy (p<0·01). Of the 75 patients studied, 15 (20%) were found to have evidence of intestinal metaplasia. The median ODC
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Figure 3: ODC activity in patients with and without intestinal metaplasia (median (interquartile range)).

activity in this group of patients was significantly higher than in those without intestinal metaplasia (p<0.04) (Fig 3).

Discussion

*H pylori* infection is the cause of chronic active gastritis and is a major factor in the aetiology-pathogenesis of peptic ulcer disease. There is now substantial evidence to support a link between chronic *H pylori* infection and subsequent development of gastric cancer. *H pylori* infection is more common in patients with gastric carcinoma, than in healthy controls and gastric cancer is many times more prevalent in populations with very high rates of *H pylori* infection. Accordingly a WHO working group have recently concluded that sufficient evidence exists to support a causal role between *H pylori* and gastric cancer. Gastric carcinogenesis is however a multi-step process progressing through the stages of gastritis, atrophy, metaplasia, and dysplasia. Recent studies have also confirmed that longterm infection with *H pylori* is a significant risk factor for the development of atrophy and intestinal metaplasia in the stomach.

Several studies have shown that diseases predisposing to gastrointestinal cancer are associated with modifications of epithelial cell proliferation and differentiation. In the human stomach, expansion of the proliferative compartment of epithelial cells and a true hyper-proliferative state is associated with histological abnormalities associated with gastric cancer. Several groups have now shown that infection with *H pylori* is associated with an increase in mucosal proliferation assessed immunohistochemically, and that this increase in mucosal proliferation can be reversed through eradication of the organism. Other studies have reported increased mucosal proliferation in gastric atrophy but not in superficial gastritis. Increased cell turnover associated with chronic *H pylori* infection is thought to be the first pre-morphological change in the pathway leading to frank cancer formation.

ODC activity is considered to be a biochemical marker of cell turnover and plays a key part in the onset of cell proliferation. ODC is expressed at low activity in quiescent cells but activity rapidly increases when cells enter a proliferative phase. Chemical promoters of malignancy increase ODC activity in rats and cancer cells invariably contain high concentrations of polyamines probably as a manifestation of this increased proliferative rate. This central role of ODC in the carcinogenic process has suggested that it might be of value as a screening tool for those at risk of developing pre-cancer or cancer. In patients with familial adenomatous polyposis (FAP) for example, ODC activity is relatively low in morphologically normal mucosa but increases with the development of adenomas and with dysplasia. Furthermore, phenotypically normal relatives of FAP patients who possess the FAP genotype have increased colonic ODC activity, which precludes the development of morphological changes.

Increased ODC activity has also been reported in the morphologically normal mucosa of patients with sporadic colorectal adenomas and in dysplastic Barrett’s oesophageal mucosa and in the postoperative stomach. Moreover, in vitro studies suggest that polyamine synthesis is required for malignant transformation of epithelial cell lines. In this study, we have examined the relation between polyamine biosynthetic activity and the associated antral histological change that results from *H pylori* infection. The results indicate that longterm *H pylori* infection is associated with an increase in ODC activity when compared with uninfected patients. Our findings are in accord with previous immunohistochemical studies relating *H pylori* infection to changes in mucosal proliferation. The presence of inflammation in itself however, was not an important determinant of ODC activity as the severity of activity or inflammation was not significantly related to levels of ODC activity. The presence of atrophy and intestinal metaplasia was strongly associated with increased levels of ODC activity, with more severe atrophy being associated with higher ODC levels. These data are entirely consistent with the views of Kuipers and others who have suggested that atrophy and metaplasia are the result of longterm *H pylori* infection, which then predispose to the development of gastric cancer.

That the highest levels of ODC activity are associated with atrophy and intestinal metaplasia, suggests that it is the consequence of *H pylori* infection, rather than the presence of the organism itself, which is responsible for increased polyamine biosynthesis. Although we have not examined the effect of *H pylori* eradication on ODC activity, immunohistochemical data suggest that eradication of the organism is associated with a decrease in mucosal proliferation. There is no convincing evidence however that established atrophy or metaplasia can be reversed by treatment, though chronic inflammation is readily cured with eradication treatment. Our findings indicate therefore that increased polyamine biosynthetic activity is increased in antral lesions associated with longterm *H pylori* infection and support the concept that these histological changes are associated
with a predisposition to gastric neoplasia. Eradication of *H. pylori* infection in all patients regardless of symptoms may therefore be a reasonable aim to reduce the likelihood of developing gastric cancer later in life.

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