

Low prevalence of *Helicobacter pylori* in inflammatory bowel disease: association with sulphasalazine

E El-Omar, I Penman, G Cruikshank, S Dover, S Banerjee, C Williams, K E L McColl

Abstract

The prevalence of IgG antibodies to *Helicobacter pylori* was examined in 110 patients with inflammatory bowel disease (IBD) (63 ulcerative colitis, 47 Crohn's disease) and compared with 100 age and sex matched control patients. The overall prevalence of *H pylori* seropositivity in the IBD patients was 22%, which was significantly less than that of 52% in the controls ($p < 0.002$). There was no difference in prevalence between ulcerative colitis and Crohn's patients. The low seropositivity in the IBD patients resulted from a very low prevalence of 10% in those currently receiving sulphasalazine ($n=40$) and similarly low prevalence of 7% in those previously receiving sulphasalazine ($n=30$). In those receiving olsalazine or mesalazine and who had never had sulphasalazine, the prevalence of seropositivity was 45%. Further studies using ^{14}C urea breath test and microscopy of antral biopsy specimens confirmed that the negative serology in patients receiving sulphasalazine resulted from absence of the infection rather than absence of humoral immune response to it. In six control patients with *H pylori* infection, a two week course of sulphasalazine (500 mg four times daily) only caused slight suppression of the ^{14}C urea breath test. In vitro studies failed to show any direct antibacterial effect of sulphasalazine on *H pylori*. These findings indicate that longterm treatment with sulphasalazine leads to eradication of *H pylori* infection and that this does not result from a direct antibacterial effect. It may be caused by the drug treating the gastritis and thereby depriving the bacterium of essential nutrients exuded by the inflamed mucosa.

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Helicobacter pylori infection is now acknowledged to be the main cause of chronic antral gastritis.^{1,2} Colonisation of the gastric antrum by the bacterium results in an intense inflammatory reaction by both the humoral and cell mediated arms of the immune response.³ Despite this mucosal response the infection persists indefinitely and the factors sustaining the chronic colonisation are poorly understood. Though *H pylori* infection is largely confined to the mucosa of the antrum and body of

the stomach it has been clearly shown to predispose to ulceration of the more distal duodenal mucosa.^{4,5} This study was originally undertaken to find out if colonisation of the upper gastrointestinal tract by *H pylori* might also play a part in the pathogenesis of chronic ulcerative diseases further down the gastrointestinal tract, in particular Crohn's disease and ulcerative colitis.

One of the major mechanisms proposed for inflammatory bowel disease is that the mucosal immune system mounts an inappropriate response against normal luminal constituents such as bacteria, components of the diet or other ingested material.^{6,7} In *H pylori* infection there is acquisition of mucosa associated lymphoid tissue within the gastric mucosa⁸ and a B cell and T cell mediated immune response against surface proteins on the bacterium.^{9,10} This immune process could cross react with other bacteria in the small or large bowel with antigenically similar surface proteins. *H pylori* related antral gastritis also causes leakiness of the gastric epithelium to large molecular weight proteins.¹¹ Ability of ingested proteins to penetrate the inflamed mucosa and come into contact with mucosa associated lymphoid tissue could also initiate an immune response against them.

To find out if *H pylori* infection might participate in the pathogenesis of inflammatory bowel diseases, we examined the prevalence of the infection in patients with and without inflammatory bowel disease. In view of the finding of a very low prevalence of *H pylori* infection in patients exposed to sulphasalazine further studies were done to investigate the effect of this drug on the organism.

Patients and methods

Venous blood samples for *H pylori* serology were obtained from 110 randomly selected patients with inflammatory bowel disease (IBD) attending the gastrointestinal outpatient clinic. Sixty three had ulcerative colitis and 47 had Crohn's disease. Details were recorded concerning the duration, extent, and activity of their IBD, current and previous medical treatment, and previous surgery.

Control serum samples were also obtained from 100 age and sex matched patients from whom venous blood was taken for cross-matching by the hospital's blood transfusion service. These patients were from the same catchment area and were admitted for a variety of surgical procedures. Patients admitted with

University Department
of Medicine and
Therapeutics
E El-Omar
I Penman
S Dover
S Banerjee
K E L McColl

Departments of
Immunology
G Cruikshank
and Bacteriology,
Western Infirmary,
Glasgow
C Williams

Correspondence to:
Professor K E L McColl,
University Department of
Medicine and Therapeutics,
Western Infirmary, Glasgow
G11 6NT.

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TABLE I Percentage seropositivity to *H pylori* in patients with ulcerative colitis v Crohn's disease and in those with active v inactive disease

	Type of IBD		Current activity of IBD	
	Ulcerative colitis	Crohn's disease	Active	Inactive
Number	63	47	22	88
Mean age (y)	47.3	38.7	41.3	43.9
Percentage seropositive	27	15	18	23

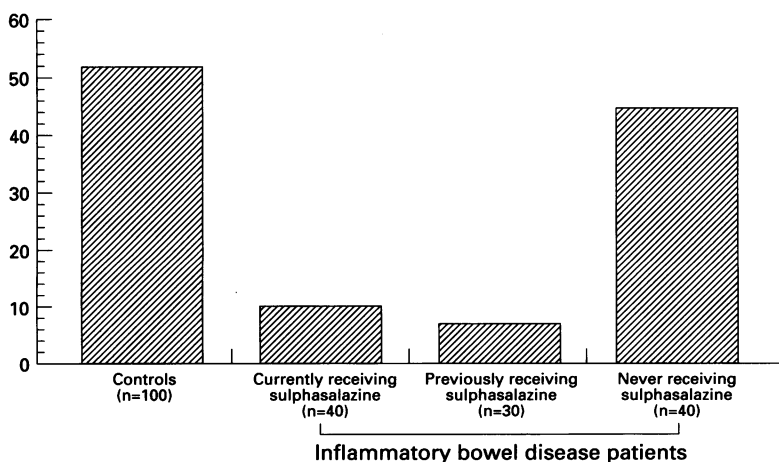
upper gastrointestinal bleeding or perforation were excluded from the control group.

H PYLORI SEROLOGY

H pylori IgG serology was performed on all specimens using the Helico-G enzyme linked immunosorbent assay (ELISA) kit (Porton, Cambridge). Preliminary studies were performed on serum samples from 44 subjects of known *H pylori* status from our hospital's catchment area to determine the sensitivity and specificity of the serological test. The *H pylori* status of these patients had been confirmed by urea breath test and microscopic examination of endoscopic antral biopsy specimens. These preliminary studies showed that using a value of 15 U/ml or above as positive, the serological test had a sensitivity of 96% and specificity of 84%. In the actual study the serum samples from the IBD patients and controls were assayed in the same batches.

CONFIRMATION TESTS OF H PYLORI STATUS

In 24 of the IBD patients who were taking sulphasalazine and who had negative *H pylori* serology, further tests were performed to confirm whether the negative serology resulted from absence of the infection or from lack of an IgG response to the infection. These patients had either a ¹⁴C urea breath test performed alone (n=18) as previously described¹² or in addition to endoscopic antral biopsy (n=6). The antral biopsy specimen was fixed in formalin and the sections stained with haematoxylin and eosin.



Percentage seropositivity to *H pylori* in control subjects and in IBD patients divided on the basis of exposure to sulphasalazine.

IN VIVO AND IN VITRO EFFECT OF SULPHASALAZINE ON H PYLORI

Six healthy volunteers with *H pylori* infection had ¹⁴C breath tests performed before and on the final day of a two week course of sulphasalazine 500 mg four times daily. The sulphasalazine was taken two hours before their second breath test. Serum samples for *H pylori* serology were obtained before and on the final day of sulphasalazine treatment. The urea breath test has been shown to be a useful means of assessing the in vivo effects of antibacterial agents on *H pylori*.^{13 14}

The direct antibacterial effect of sulphasalazine and sulphapyridine against *H pylori* was assessed by standard methods.^{15 16} Five recent isolates of *H pylori* were incubated in tryptone soya broth supplemented with 10% horse blood for 72 hours in a microaerophilic atmosphere (BBL Campypak). One μ l of this culture was transferred to the surface of an antibiotic containing isosensitest agar plate giving a final concentration of about 10^6 colony forming units. Control organisms *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (NCTC 10418) were included on each plate and plates were incubated for 72 hours at 37°C in a microaerophilic atmosphere. The concentration that inhibited at least 80% of growth was interpreted as the minimum inhibitory concentration (MIC).¹⁷ The pure drugs were obtained from Sigma Chemical Company Ltd (Dorset, UK) and their in vitro antibacterial effect assessed at concentrations up to 256 mg/l ($MIC_{90} > 256$ mg/l).

STATISTICAL ANALYSIS

Assessment of differences between groups was performed using the Mann-Whitney U test. Assessment of changes after sulphasalazine treatment was performed using the Wilcoxon test for paired samples. Patients gave informed consent to participate in the study and the project was approved by the Western Infirmary ethical committee.

Results

The mean age of the 110 patients with IBD was 42 years (range 14–83) and 52% were females. The mean age of the 100 control patients was 42 years (range 19–71) and 48% were females. Using our standard value of 15 U/ml or above as seropositive, only 22% of the IBD patients were positive for *H pylori* compared with 52% of the controls. The median anti-*H pylori* IgG titre in the controls was 17 U/ml compared with ≤ 6.25 U/ml in the IBD patients ($p < 0.002$). The percentage seropositivity for *H pylori* was similar in ulcerative colitis v Crohn's disease patients and in patients with active v inactive disease (Table I).

The low prevalence of seropositivity to *H pylori* in the IBD patients as a whole resulted from the very low prevalence of 10% in the 40 patients currently receiving sulphasalazine and of 7% in the 30 patients previously receiving

TABLE II Percentage of seropositivity of *H pylori* in IBD patients on the basis of exposure to sulphasalazine treatment

	Currently receiving sulphasalazine	Previously receiving sulphasalazine	Never receiving sulphasalazine		
			Receiving mesalazine	Receiving olsalazine	Neither
Number	40	30	24	5	11
Mean age	46	46.6	38.5	40.6	38.7
Mean duration of disease (y)	11.4	13	3	8.2	5.9
Percentage seropositive	10	7	46	40	45

sulphasalazine (Figure). Patients who had never received sulphasalazine but who were receiving olsalazine (n=5), mesalazine (n=24) or neither (n=11) had a prevalence of 40%, which was similar to that in the control group without IBD (Table II).

CONFIRMATORY TESTS OF *H PYLORI* STATUS

The ¹⁴C urea test was negative in 22 of 24 patients receiving sulphasalazine with negative serology examined. In the six patients who had a ¹⁴C urea breath test and microscopic examination of antral biopsy specimens, there was no evidence of *H pylori* by either test.

EFFECT OF SULPHASALAZINE ON *H PYLORI*

In vitro – sulphasalazine and sulphapyridine had no bacteriostatic or bactericidal effect against any of the *H pylori* isolates tested. This included both clinical isolates and NCTC strain of *H pylori* (11638).

In vivo – in the six *H pylori* positive healthy volunteers examined their ¹⁴C urea breath test remained positive after their two week course of sulphasalazine. The mean 20 minute ¹⁴C urea breath test values fell, however, from 246 (range: 180–422) to 196 (range: 135–225) (p<0.04).

Discussion

This study shows that the prevalence of *H pylori* seropositivity is much lower in patients with chronic IBD than in an age and sex matched control group. The post hoc analysis of the data collected in the prospective study showed that the low prevalence of *H pylori* seropositivity in IBD is related to current or previous exposure to sulphasalazine. Several findings in our study confirm that the low *H pylori* seropositivity associated with sulphasalazine treatment results from the absence of the infection rather than from the drug interfering with the IgG response to the organism. The confirmatory studies of *H pylori* status performed in the subgroup of seronegative patients receiving sulphasalazine confirmed absence of the infection. The fact that patients who had stopped receiving sulphasalazine months or years previously also had a low prevalence of *H pylori* seropositivity provides further evidence against it resulting from the drug suppressing the IgG response.

The explanation for the absence of *H pylori* in the patients currently or previously receiving sulphasalazine needs to be considered. The prevalence of *H pylori* infection increases with age and in the Western world roughly

equals the age of the patient.¹⁸ This is shown by our hospital control group with a mean age of 42 years having a prevalence of 52%. The increasing prevalence of *H pylori* with age is thought to result from the fact that the infection can be contracted at any age and then becomes chronic and persists indefinitely. The very low prevalence of *H pylori* infection in the patients exposed to sulphasalazine probably result from the infection having been eradicated. If those receiving sulphasalazine had merely been protected from developing the infection then the prevalence would be reduced only by a percentage equivalent to the number of years taking the drug, which was a mean of 12 years. The fact that the patients who had previously received sulphasalazine but were not currently taking it had a very low prevalence of *H pylori* also shows that their infection had been eradicated rather than merely suppressed.

The mechanism by which sulphasalazine treatment could result in eradication of *H pylori* is unclear. The *in vitro* studies using sulphasalazine and its metabolite sulphapyridine failed to show any direct bacterial or bacteriostatic effect. In addition, 14 days of treatment with sulphasalazine only minimally suppressed the ¹⁴C urea breath test in the infected healthy volunteers, again showing little evidence of a direct effect of the drug on the bacterium.

One indirect mechanism by which sulphasalazine could lead to eradication of the infection is by its anti-inflammatory properties suppressing the antral gastritis and thus making the gastric mucosa a less hospitable environment for the organism. Exudation of plasma through the inflamed mucosa could provide *H pylori* with essential nutrients. The mucosal anti-inflammatory properties of sulphasalazine may with time result in suppression of the antral gastritis and thereby lead to eradication of the infection. The small but significant fall in the breath test value at the end of two weeks of sulphasalazine treatment would be consistent with a reduction in density of infection because of some resolution of gastritis. A lowered prevalence of *H pylori* was not apparent in patients taking pure 5-aminosalicylic acid preparations. Sulphasalazine, however, has been shown to have *in vivo* anti-inflammatory properties not possessed by 5-aminosalicylic acid including inhibition of B lymphocytes¹⁹ and suppression of natural killer cell activity.²⁰ Sulphasalazine also exerts its anti-inflammatory effects outwith the large bowel as utilised in the treatment of rheumatoid arthritis.

Another indirect method by which sulphala-

salazine could eradicate *H pylori* is by interfering with the adherence of the organism to the gastric mucosa. *H pylori* is known to bind to specific glycerolipid receptors on the gastric epithelial cells.²¹ Interestingly, sulphasalazine at concentrations present in human plasma has been found to block receptors for bacteria derived peptides on human neutrophils.²²

In addition to its use in IBD, sulphasalazine is also used as a second line agent in patients with rheumatoid arthritis. In these patients the prevalence of *H pylori* has been seen to be not so noticeably reduced²³ and this may be explained by the fact that such patients do not receive it for so long and also all receive the enteric coated preparation, which may lack the local gastric effect. The patients attending our gastroenterology clinic were all prescribed the non-enteric coated preparation. Studies in arthritis patients, however, have noted that those receiving gold treatment have a very low prevalence of *H pylori*.²³ The finding that these two main second time anti-inflammatory agents both eradicate *H pylori* may provide a clue to their mechanism of action against the organism or even a clue to the mechanisms by which they produce their beneficial effect in arthritis. An association between gastrointestinal infection and arthritis has been recognised for many years.^{24 25}

The original aim of this study was to find out if there might be an increased prevalence of *H pylori* infection in patients with IBD and thus evidence supporting a possible role of the infection in the pathogenesis of the bowel disease. Though the reduced prevalence of the infection does not support such a role it also does not refute it. The well established beneficial effects of sulphasalazine in IBD could partly result from it eradicating *H pylori* and thus preventing the chronic stimulation of the mucosal immune system caused by the infection. The fact that the patients receiving olsalazine or mesalazine who had never had sulphasalazine had a prevalence of *H pylori* infection similar to the normal controls does not completely exclude a role for *H pylori* either. It is possible that our patients with IBD all had evidence of *H pylori* infection on initial presentation and that sulphasalazine has led to its eradication in nearly all patients treated whereas olsalazine and mesalazine lead to its eradication in only 50%. It will be necessary to examine *H pylori* status in patients presenting with IBD before commencing any such drug treatment.

In addition to the implications for the immunological basis of IBD and rheumatoid arthritis, our findings are relevant to the treatment of *H pylori*. Eradication of the infection is an important challenge and it is possible that sulphasalazine might provide a new approach to it, possibly by being given in conjunction with another antibiotic.

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