Helicobacter pylori gastritis and epithelial cell proliferation in patients with reflux oesophagitis after treatment with lansoprazole

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Abstract
Background—Helicobacter pylori gastritis may spread proximally in the stomach during profound acid inhibition.
Aims—To examine histological gastric body changes and epithelial cell proliferation before and after treatment with lansoprazole.
Patients and methods—Patients diagnosed as having reflux oesophagitis grade 1 or 2 were enrolled and treated for 12 weeks with lansoprazole (30 mg every morning). After 12 weeks, 103 of the 118 patients appeared endoscopically healed and were asymptomatic; they then received maintenance treatment with 15 or 30 mg lansoprazole daily. Biopsy specimens obtained from similar sites before and after treatment, were available from 90 patients after a median of 64 weeks (range 15–73 weeks). Epithelial cell proliferation was determined by the number of Ki-67 antigen positive cells per gland.
Results—Of these 90 patients, 44 (49%) were found to be infected with *H pylori*. Their median inflammation score had increased from grade 1 before to grade 2 after treatment (p<0.0001). Initially, the number of Ki-67 antigen positive cells per gland was significantly higher in the *H pylori* infected than in the uninfected group and increased further after treatment (p<0.0001). In uninfected patients, no significant change in inflammation or proliferation occurred during treatment.
Conclusions—A marked increase in body gastritis was observed in *H pylori* infected individuals during long term treatment with the proton pump inhibitor lansoprazole. Epithelial cell proliferation and atrophy also increased in infected but not in uninfected patients.
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The intragastric distribution of *Helicobacter pylori* may be altered by proton pump inhibitor treatment,[1–3] and several studies have reported that acid suppression increases *H pylori* gastritis of the corpus.[2 4–6] Generally, increased body gastritis and a decrease in the activity of antral gastritis has been regarded as a consequence of long term treatment. However, a substantial increase in gastritis in the corpus after only 14 days of treatment with omeprazole (20 mg daily) has been reported.[7] In addition, long term treatment with proton pump inhibitors may accelerate the development of atrophic changes of the gastric mucosa in *H pylori* infected individuals.[2 8]

Interest in this phenomenon has been boosted by the possibility that the widespread and indiscriminate use of proton pump inhibitors may expand the infected population at risk of developing gastric cancer. Chronic *H pylori* gastritis predisposes significantly to the development of gastric atrophy and intestinal metaplasia.[9 10] Importantly, atrophic body gastritis as well as multifocal atrophic gastritis have been reported to be associated with an increased incidence of gastric cancer.[11 12] Studies in rats have suggested that hypergastrinaemia induced during omeprazole treatment causes persistent gastric epithelial cell proliferation.[13] Furthermore, epithelial hyperproliferation has been reported in *H pylori* infected patients[14 15]; this can be reversed when the bacterium is eradicated.[16–18] Epithelial cell proliferation is likewise increased in subjects with precancerous lesions or gastric carcinoma.[19] An inverse relationship apparently exists between the acid secretory capacity of the gastric mucosa and the rate of cell proliferation in both antral and fundic mucosa.[20] Increased cell division may lead to accumulation of genetic errors and to neoplastic transformation.[21]

The aim of this study was to characterise histologically the gastric body mucosa and epithelial cell proliferation before and after long term treatment with lansoprazole (15 or 30 mg per day). This proton pump inhibitor is increasingly used as an alternative to omeprazole and studies are needed to evaluate its local effects in the stomach. In addition to ordinary histology, we used immunohistochemistry with monoclonal antibodies to the Ki-67 defined nuclear antigen that is expressed in all phases of the cell cycle except for G_0 and early G_1.[22] This protein has a very short half life at the end of mitosis,[23] which makes it a robust marker of cell proliferation.

Materials and Methods

PATIENTS
Patients were enrolled during a long term clinical study of lansoprazole (Lederle Läkemedel, Stockholm, Sweden) treatment of reflux oesophagitis. The clinical results have been

reported elsewhere.[24] Eligibility for the study was based on the presence of moderate or severe reflux oesophagitis.[25] Patients needing, or having recently used, H_2 receptor antagonists, prokinetics, sucralphate, or drugs with anticholinergic effects were excluded. The study was approved by the Regional Ethics Committee for Medical Research in Western Norway. Patients were requested to sign an informed consent to participate.

The initial healing phase lasted for 12 weeks with 30 mg lansoprazole per day. If healed and asymptomatic at that time, patients were randomised to maintenance treatment, 15 or 30 mg lansoprazole every morning for up to 12 months or until symptomatic or endoscopic relapse. Medication in the maintenance phase was given as either two 15 mg lansoprazole hard gelatin capsules (Lederle) or one lansoprazole 15 mg capsule combined with one visually identical placebo capsule. Endoscopy was performed after three, six, and 12 months as well as on clinical relapse. Patients returned for a follow up visit and endoscopy four weeks after completing medication with lansoprazole. A blood sample was drawn from every patient before and after treatment for the measurement of serum gastrin levels.

SPECIMENS AND TISSUE PREPARATION
Four to six biopsy specimens were obtained from the major curvature of the stomach, approximately 10 cm distal to the gastric cardia. The forceps were large and equipped with a needle, and the cups were fenestrated to make the specimens as large as possible (length 5 mm). Endoscopy was performed between 8 am and 11 am to control for diurnal variation. The specimens were routinely fixed in formalin (pH 7.0) overnight and then embedded in paraffin wax.

One tissue section cut at 4 μm was stained with haematoxylin and eosin (H&E). Histological slides from various visits were scrambled and coded before being examined independently by the chief investigator (AEB) and an experienced pathologist (PB). The grading was compared, and in case of discrepant results (approximately 8%), agreement was reached by joint re-evaluation. The variables for grading of gastritis were based on a four point scale (0, absent; 1, mild; 2, moderate; and 3, marked) as schematically described in the updated Sydney system.[26] Inflammation (chronic gastritis) was scored according to the density of mononuclear cells in the lamina propria, inflammatory activity was based on the numbers of neutrophils in the lamina propria and epithelium, and metaplasia depended on the presence of intestinal epithelial elements. Gastric atrophy was scored on a similar scale according to the reduction in glandular elements accompanied by increased interglandular connective tissue. Care was taken to avoid confusion between true atrophy and inflammation induced separation of glands. The density of *H pylori* organisms was graded by in situ immunofluorescence and Giemsa staining according to their number and distribution by the chief investigator in a blinded manner.

Adjacent serial tissue sections were used for the detection of *H pylori* and for immunohistochemical staining of the Ki-67 defined antigen (see below).

IMMUNOHISTOCHEMICAL DETECTION OF *H PYLORI*
The presence of *H pylori* on gastric surface epithelium was shown immunohistochemically by incubation for 20 hours at room temperature with a specific rabbit antiserum diluted 1/10 (Dako, Glostrup, Denmark) after antigenic retrieval by tissue section digestion (10 g/l trypsin, 10 minutes, 37°C). This method distinguished *H pylori* from other curved bacteria present in the stomach and has been reported to have a sensitivity of 100% and a specificity of 94% compared with cultivation results.[27] Fluorescein isothiocyanate (FITC) conjugated swine antirabbit IgG diluted 1/160 (Dako) was applied for three hours as the secondary reagent. Omission of the primary antiserum abolished the immunofluorescence completely. After mounting, the tissue sections were examined blind by the chief investigator in a Leitz DMR-DXE microscope camera equipped with a Ploem-type vertical illuminator system (Leica, Wetzlar, Germany). In addition, adjacent Giemsa stained sections were studied by light microscopy. In the case of discrepant results (approximately 5%), the immunohistochemical finding was considered definitive. A ^{14}C-urea breath test[28] was performed in 69 patients after the conclusion of the study following two weeks without acid suppressive treatment.

IMMUNOHISTOCHEMISTRY FOR THE Ki-67 DEFINED ANTIGEN
The immunohistochemical method was performed as described by McCormick *et al*.[29] Briefly, sections were cut at 4 μm, mounted on glass slides, air dried for two days at 4°C, dewaxed in xylene, and rehydrated in a series of graded ethanol. To retrieve the nuclear antigen, the slides were placed in plastic jars filled with sodium citrate buffer (10 mM, pH 6.0) and heated in a Philips microwave oven at 850 W until boiling, then at 350 W five times (five minutes each). Contact between air and the sections was avoided. The sections were finally rinsed in phosphate buffered (pH 7.4) isotonic saline for 10 minutes prior to immunostaining.

The primary monoclonal antibodies were a mixture of supernatants (diluted 1/50) derived from clones MIB1 and MIB5 (courtesy of Dr J Gerdes) directed against recombinant parts of the Ki-67 defined antigen[30]; incubation was performed for 20 hours at room temperature. Secondary reagent was biotinylated horse antimouse IgG (5 mg/l; Vector Laboratories, Burlingame, California) applied for three hours, followed for 30 minutes by complexes of streptavidin and biotinylated horseradish peroxidase (prepared according to instructions of the manufacturer, Dako). The sections were rinsed in 0.05 M Tris-HCl, pH 7.6, for 10 minutes. The chromogen was 0.05% (w/v) diaminobenzidine substrate (Aldrich Chemical Co., Wisconsin) and 0.015% H_2O_2 in Tris-HCl, pH 7.6. Omission of the primary
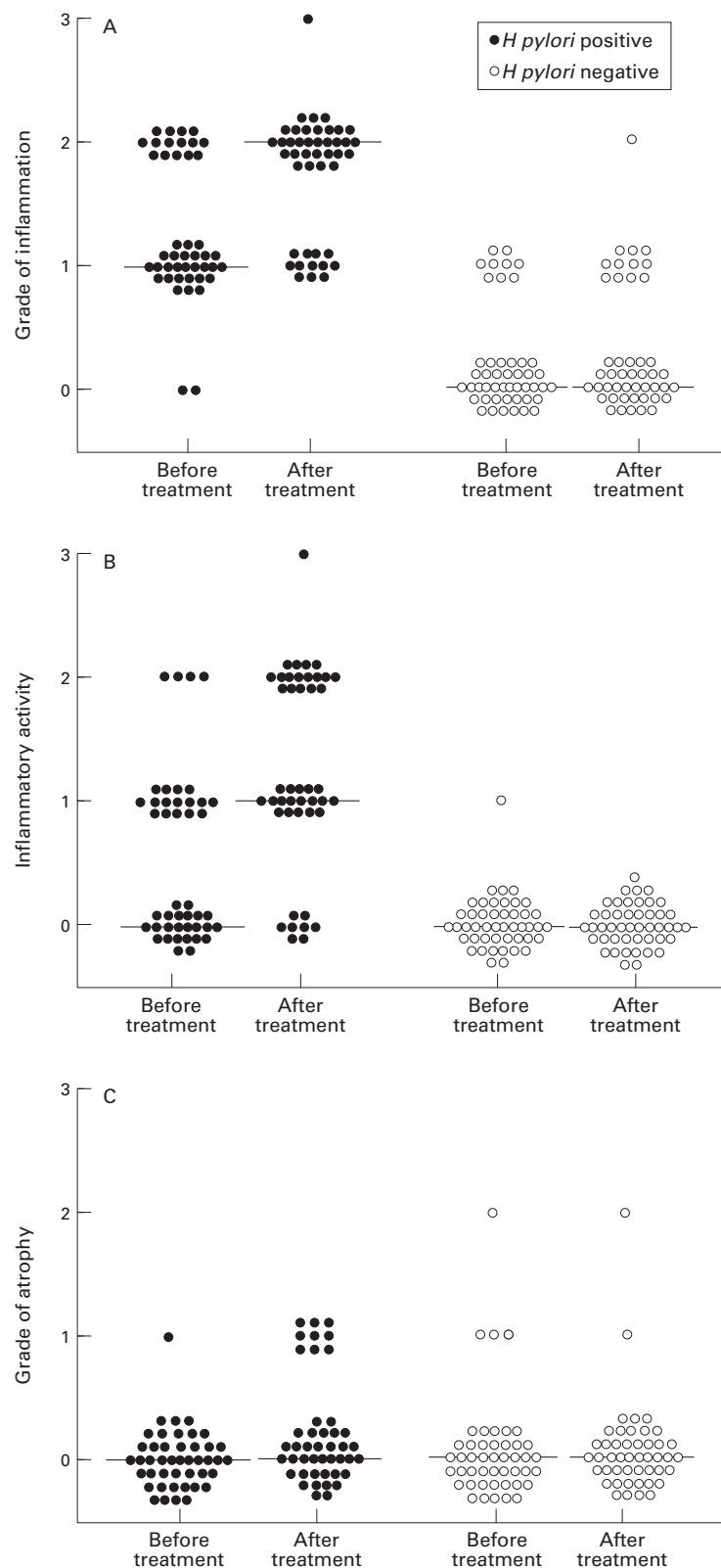
Figure 1:   Scatter diagrams depicting (A) grade of corpus inflammation, (B) inflammatory activity, and (C) atrophy in H pylori infected and uninfected patients before and after treatment. Median scores indicated by horizontal lines.

monoclonal antibodies abolished all nuclear staining. Sections were counterstained with haematoxylin and mounted in Eukitt (O. Kindler GmbH & Co., Freiburg, Germany).

EPITHELIAL CELL PROLIFERATION

Only well oriented tissue sections containing gastric pits and glands cut longitudinally along the entire isthmus zone were used for enumeration of Ki-67 antigen positive cells. The number of cells to be included was determined by counting Ki-67 antigen positive cells in consecutive proliferation zones until the accumulated mean varied by less than 5% (usually 6–10 isthmus zones). The mean number of positive cells was calculated per gland.

The chief investigator performed the immunohistochemical counting blinded to patient data, stage of treatment, and *H pylori* status. Six weeks after completion of the study, 30 immunostained sections were randomly selected for blind re-evaluation.

SERUM GASTRIN

Serum gastrin was analysed by a commercially available iodine-125 labelled radioimmunoassay kit (ICN Pharmaceuticals Inc., Costa Mesa, California).

STATISTICAL ANALYSIS

The Mann-Whitney test was applied to compare cell proliferation and serum gastrin in different groups of patients before and after treatment. Wilcoxon's matched pairs rank sum test was used for comparison of gastritis, cell proliferation, and gastrin serum levels before and after treatment. Analysis of frequency tables was performed with Fisher's exact test. Correlation between grades of inflammation and the number of Ki-67 antigen positive cells was based on Spearman's correlation test. Two-tailed p values less than 0.05 were considered statistically significant.

Results

A total of 118 patients diagnosed as having reflux oesophagitis grade 1 or 2 was enrolled and treated for 12 weeks with lansoprazole (30 mg every morning). At the end of this test period, 103 asymptomatic patients who appeared endoscopically healed were included in the maintenance study.

Biopsy specimens from the gastric body were available at enrolment from 102 of the 103 patients who entered the maintenance study (79 males and 23 females; median age 60 years, range 21–77). Biopsy specimens were not taken at follow up in 12 of these patients. Tissue samples before and after long term lansoprazole treatment were therefore available from a total of 90 patients (72 males and 18 females; median age 60.5 years, range 23–77). Their median duration of treatment was 64 weeks (range 15–73). Biopsy specimens were taken at completion of medication in 85 patients, and four weeks after treatment in the remaining five patients. As the density of bacteria might have changed after completion, data for these patients regarding bacterial density after treatment were not included in the statistical analyses.

Forty four (49%) of these 90 patients (median age 60 years, range 33–77) were found to be infected with *H pylori* based on immunohistochemistry, while 46 (51%) patients (median age
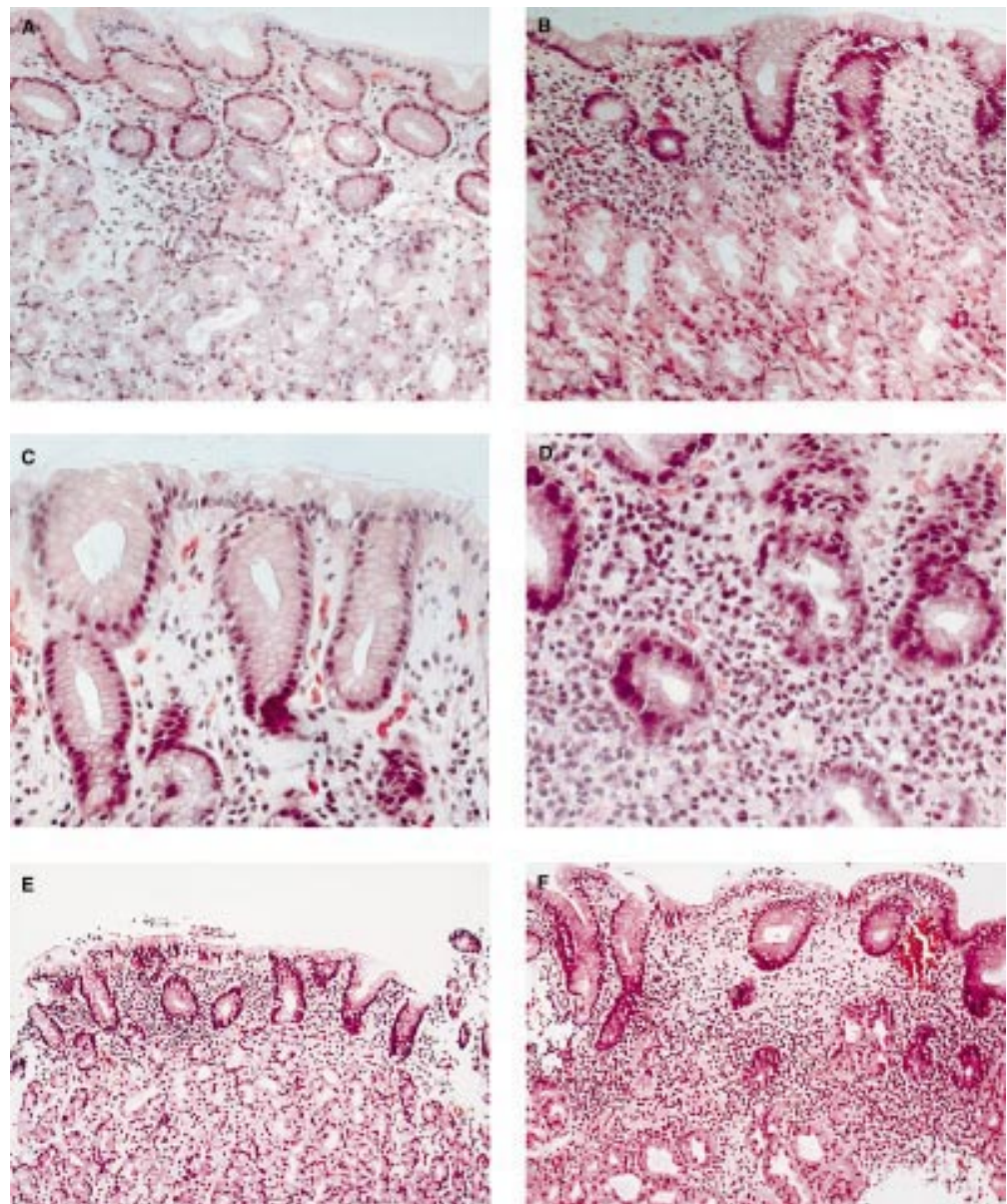
Figure 2: Effect of lansoprazole treatment on histology of gastric body mucosa (H&E staining) in H pylori infected patients. Few leucocytes are present within the lamina propria (grade 1 inflammation, grade 0 activity) in one patient before treatment (A); after 64 weeks of treatment, intensified inflammation (grade 2 inflammation, grade 1 activity) is evident (B). In another patient, neutrophils are absent before treatment (C); after 48 weeks of treatment, numerous neutrophils appear in the isthmus region and within the epithelium (D). In a third patient, moderate (grade 2) inflammation but no glandular atrophy is present before treatment (E); after 65 weeks of treatment, mild to moderate loss of glands (grade 1 atrophy) is evident together with persistent inflammation (F). Original magnifications: A and B, ×250; C and D, ×400; E and F, ×160.

60 years, range 23–73) were uninfected. There was agreement between the ^{14}C-urea breath test (performed after the study) and immunohistochemistry in 61 of 69 (88%) patients; six patients were deemed to be infected by immunohistochemistry but were negative by ^{14}C-urea breath test, whereas two patient were positive by ^{14}C-urea breath test but negative by immunohistochemistry. Exclusion of all patients with disagreement between the two tests did not change the conclusions of this study; the infection status was therefore based on immunohistochemistry for all patients.

GASTRITIS
In the *H pylori* infected group (n=44), the grade of inflammation (mononuclear cell infiltration) at the end of treatment had increased by one grade or more in 22 patients, remained unchanged in 18 patients, and decreased by one grade in four patients. The median inflammatory score increased from grade 1 before to grade 2 after treatment (p<0.0001; figs 1A and 2A). Significantly higher inflammatory activity (number of neutrophils) was also found after treatment: the activity increased by one grade or more in 29 patients, remained unchanged in eight patients, and decreased in seven patients (figs 1B and 2B). The median activity score increased from grade 0 before to grade 1 after treatment (p<0.0001, fig 1B). Accompanying this increase in inflammation and activity, the median density of *H pylori* organisms changed
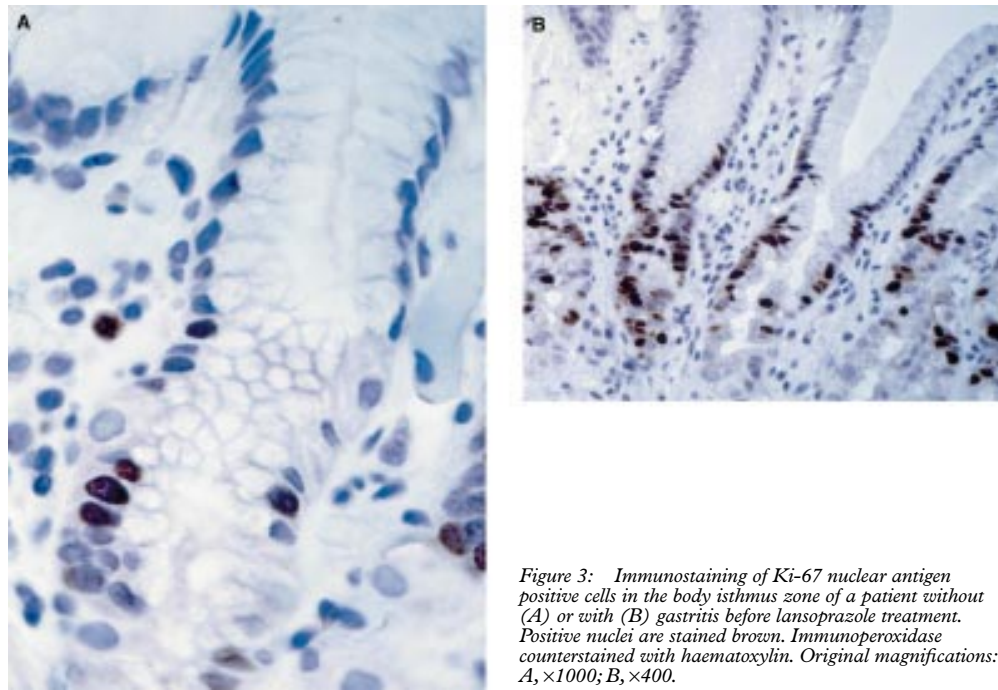
Figure 3: Immunostaining of Ki-67 nuclear antigen positive cells in the body isthmus zone of a patient without (A) or with (B) gastritis before lansoprazole treatment. Positive nuclei are stained brown. Immunoperoxidase counterstained with haematoxylin. Original magnifications: A, ×1000; B, ×400.

from grade 1 before to grade 2 after treatment but with no significant difference (p=0.24).

In the uninfected group (n=46), no inflammation was found at enrolment in 37 patients. Seven of these patients developed grade 1 inflammation during treatment. Nine patients had grade 1 inflammation at enrolment, but no inflammation was found in four of them after treatment. Four patients had grade 1 inflammation both before and after treatment, while inflammation increased from grade 1 to grade 2 in one patient. The grade of inflammation in this group as a whole did not change significantly during treatment (p=0.3, fig 1A). Inflammatory activity was observed in only one patient before, and in none after treatment (fig 1B).

ATROPHY AND INTESTINAL METAPLASIA

In the *H pylori* infected group (n=44), no gastric atrophy was found initially in 43 patients, nine (21%) of whom developed grade 1 atrophy during treatment (p=0.02, fig 1C). Patients who developed atrophy were generally younger than those who did not (median 46 versus 61 years, p=0.04). Eight of the nine infected patients who did, and 29 of the 35 infected patients who did not develop atrophy were males (p=1.0). There was no significant difference in duration of treatment (63 versus 64 weeks, p=0.4) or grade of inflammation at inclusion or after treatment in these two subgroups. At the initiation of the study, cell proliferation tended to be higher in the infected patients who later developed atrophy than in those who did not (median 23.8 versus 19.0 cells, p=0.12); the same was true at the end of treatment (median 33.5 versus 25.2 cells, p=0.05). Moderate or marked atrophy was not observed. In one infected patient, atrophy was present at enrolment, whereas no atrophic gastritis but mild intestinal metaplasia was seen

after treatment. The annual increase in the prevalence of atrophy in patients infected with *H pylori* was estimated to be 15%. Intestinal metaplasia was not observed in infected patients before treatment. Grade 1 intestinal metaplasia without atrophic gastritis was present in two patients after treatment.

In the uninfected group (n=46), no gastric atrophy was found initially in 42 patients, one (2%) of whom developed grade 1 atrophy during treatment (p=1.0, fig 1C). Grade 1 atrophy at inclusion progressed to grade 2 after treatment in another patient. Grade 1 atrophy was present at inclusion, but absent after treatment, in two patients. Grade 2 atrophy was present at inclusion in one patient but was absent after treatment. Intestinal metaplasia was observed in two patients: grade 1 metaplasia was found in one patient before treatment and remained unchanged after treatment; grade 1 metaplasia together with grade 2 atrophy (see above) was observed after treatment in the other patient.

SERUM GASTRIN

Of the 90 patients referred to above, 48 had been treated with 15 mg and 42 with 30 mg lansoprazole in the maintenance period. Serum gastrin levels were significantly elevated (p<0.0001) after treatment in both subgroups compared with the inclusion levels: in the 15 mg group, the gastrin concentration increased from a median of 49.0 ng/l (range 31.7–87.5) to 91.4 ng/l (range 40.0–544.9), and in the 30 mg group from 45.2 ng/l (range 25.5–174.6) to 95.0 ng/l (range 46.6–1000).

Median serum gastrin levels tended to be elevated in *H pylori* infected compared with uninfected patients both at inclusion in the study (median 50.7 ng/l, range 33.8–163.4 versus 46.3 ng/l, range 25.5–174.6; p=0.04) and after treatment (median 107.9 ng/l, range

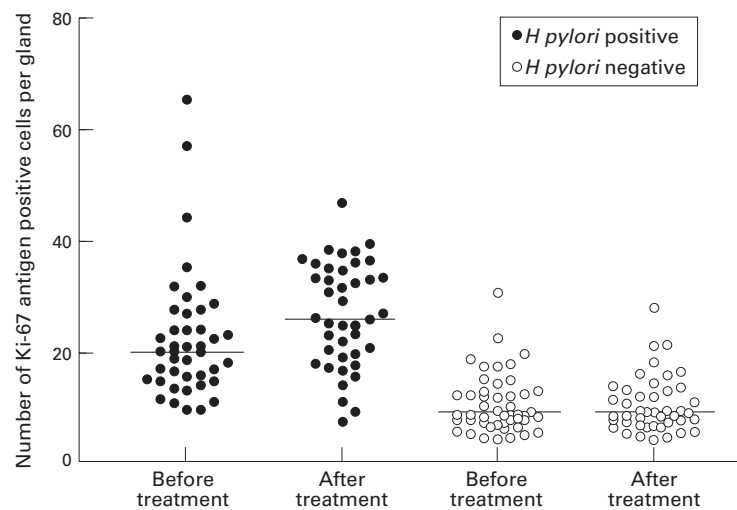*Figure 4:   Scatter diagram depicting gastric body epithelial cell proliferation in H pylori infected and uninfected patients before and after lansoprazole treatment. Data based on immunostaining for Ki-67 nuclear antigen. Median values indicated by horizontal lines.*
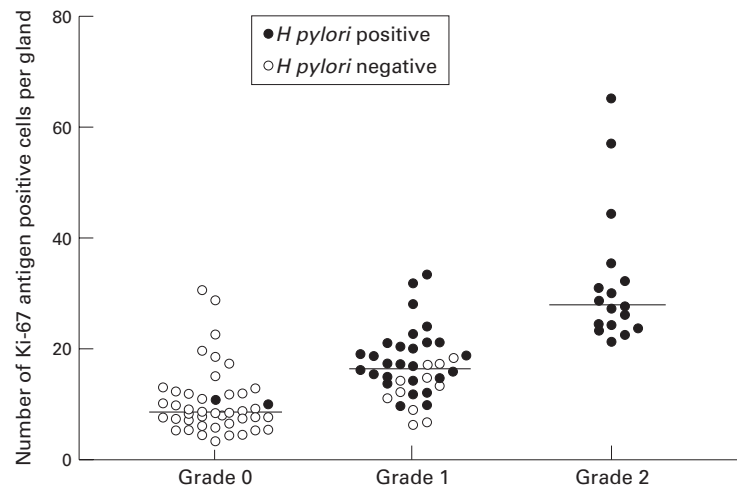

Figure 5:   Scatter diagram depicting Ki-67 nuclear antigen positive cells per gastric gland in relation to grade of inflammation at the start of the study in H pylori infected and uninfected patients as indicated (Spearman's r=0.72, p<0.0001). Median values indicated by horizontal lines.

42.9–544.9 versus 85.9 ng/l, range 40.0–1000; p=0.07).

CELL PROLIFERATION
The brown nuclei of Ki-67 antigen positive cells were easily distinguished from the blue nuclei of negative cells (fig 3). Omission of the primary antibodies abolished the positive staining, and blocking of endogenous peroxidase activity was unnecessary. The coefficient of variation of 30 pairs of repeated blind counts of positive nuclei was 15%, indicating satisfactory reproducibility.

The number of Ki-67 antigen positive cells per gland could be determined before and after treatment in 85 of 90 patients (94%). The proliferative isthmus zones were not adequately represented for evaluation in sections from five patients; this was most often the case in marked inflammation where the pits were so elongated that the entire isthmus zone had not been cut longitudinally.

The number of Ki-67 antigen positive cells per gland was increased more than twofold in 41 *H pylori* infected compared with 44 uninfected patients (median 20.3 versus 9.1 cells; p<0.0001, fig 4). The number increased significantly with increasing grade of inflammation at inclusion in the study (fig 5). Long term treatment with lansoprazole significantly increased cell proliferation in the infected (median 20.3 cells before and 26.3 cells after; p<0.0001, fig 4) but not in the uninfected (median 9.1 cells before and 8.9 cells after; p=1.0, fig 4) patients.

Discussion
Many patients with reflux oesophagitis receive maintenance treatment with proton pump inhibitors; the long term safety of these drugs has been extensively evaluated but is not entirely established. Concern has been raised about the development of focal hyperplasia of argyrophilic cells during treatment,[2 4 31] although no gastric carcinoid development has been reported in humans. However, several recent studies have reported altered distribution of *H pylori* and increased body gastritis during long and short term treatment with proton pump inhibitors.[1 2 4 6 7] Although these studies have evaluated changes in gastritis, information regarding the effects on gastric epithelial cell proliferation during proton pump inhibitor treatment has to our knowledge been lacking.

In this study we found significantly increased grade of inflammation, glandular atrophy, and epithelial proliferation in the corpus of patients infected with *H pylori* after treatment with lansoprazole over an average time of 64 weeks. Such changes were not observed in uninfected patients. Controversy exists as to whether *H pylori* colonisation is altered in the corpus during acid suppressive treatment. Decreased numbers of *H pylori* organisms in both antrum and corpus during omeprazole treatment has been reported.[3] Furthermore, Kuipers *et al*[6] reported that corpus inflammation was significantly intensified despite stable bacterial counts after eight weeks with 40 mg omeprazole daily, whereas a trend towards increased bacterial density after treatment was observed in our study. Although lansoprazole exhibits a bacteriostatic effect on *H pylori* in culture,[32] this may not be the case in vivo. We furthermore observed that with increasing grade of inflammation, the number of neutrophils (inflammatory activity) increased significantly in the lamina propria and epithelium; this was true in 66% of the *H pylori* infected patients after treatment. Intensified inflammation could be due to an effect of bacteria or their products and the epithelium; perhaps a reduced acid output promoted such interactions. It has been shown in vitro that epithelial cells infected with live *H pylori* secrete large amounts of interleukin 8, and this chemokine has a strong chemoattractant effect on neutrophils.[33] Continuous neutrophil activation may lead to tissue destruction.[34]

Development of gastric atrophy was found after the treatment in 21% of our *H pylori*

infected patients, but in only 2% of those without infection. However, only mild (grade 1) lesions were observed. Persistent inflammation might subsequently lead to more severe atrophy. Indeed, the atrophy score in the oxyntic mucosa of patients with *H pylori* infection has been reported to increase during the first two years of treatment with lansoprazole but then remain relatively stable.[2] Patients with *H pylori* infection and reflux oesophagitis treated with omeprazole for an average of five years, have been found to be at increased risk of atrophic gastritis compared with patients who underwent fundoplication.[8] The annual increase in the prevalence of atrophy during lansoprazole treatment in our *H pylori* infected patients (15%) was somewhat higher than a comparable figure (9.9%) determined in another study.[2] Notably, in the latter study the patients had been treated with ranitidine for three months or more before treatment, and 10.5% had partial atrophy before lansoprazole was administered. The effects of acid suppression on the body mucosa might therefore have been underestimated. The patients included in our study had received no treatment with H_2 receptor antagonists during at least two months immediately prior to the study, and only one (2%) of the infected patients showed mild atrophy on enrolment. It was interesting to note that infected patients who developed atrophy were younger than those who did not. A more vigorous immune response in young individuals could have accelerated tissue destruction when the host–bacterium interaction was altered by acid suppression.

The present study was controlled for *H pylori* infection but not for lansoprazole, which was used by both infected and uninfected patients alike. It is therefore not possible to draw definite conclusions about the role of this drug in explaining the intensified corpus inflammation, atrophy, and epithelial cell proliferation. However, the natural progression of *H pylori* gastritis appears to be slow, showing no or only mild one grade progression or even regression over a 12 year follow up.[35] It therefore seems justified to believe that the marked changes in gastritis observed in our study were a result of drug induced hypochlorhydria, rather that reflecting the natural course of *H pylori* infection.

Epithelial cell proliferation was twofold higher in infected than in uninfected patients at the start of the study. This observation was in accordance with earlier reports[14–18] and suggested a pathogenic link between *H pylori*, chronic gastritis, and increased epithelial cell proliferation. Such proliferation is regarded as one of the earliest mucosal changes in the development of adenocarcinoma because it is a key factor in the initiation of carcinogenesis, enhancing the effectiveness of mutagens by limiting the time for DNA repair and expanding the proliferative zone towards the gastric lumen.[36] We used an immunohistochemical method that permitted satisfactory determination of gastric epithelial cell proliferation in routinely formalin fixed archival tissue blocks by applying monoclonal antibodies to the Ki-67 defined nuclear antigen. Such staining has proved to be a reliable indicator of proliferating cells in gastric biopsy material compared with bromodeoxyuridine incorporation.[37] The highest number of positive nuclei was found in patients with moderate or marked inflammation, in agreement with in vitro observations of an indirect influence by the inflammatory response to *H pylori* on the rate of epithelial cell proliferation.[38] Recently, increased mRNA expression of hepatocyte stimulating factor has been found in the mucosa of *H pylori* infected individuals[39]; this could explain the link between *H pylori* and increased cell proliferation.

Gastrin stimulates the growth of human gastric cancer cells in vitro,[40] and epithelial cells of fundic mucosa are responsive to the trophic action of pentagastrin.[41] In our study, noninfected as well as infected patients had significantly elevated serum gastrin levels after long term treatment with lansoprazole, probably induced by profound acid suppression. However, epithelial cell proliferation increased during treatment only in those with *H pylori* infection. It is therefore unlikely that increased serum gastrin levels per se will cause persistently elevated epithelial cell proliferation in humans. Because such proliferation increased with increasing degree of inflammation in our infected patients, it probably reflected intensified inflammation; but hypergastrinaemia cannot be excluded as a cofactor in this development.

In conclusion, a marked increase in corpus gastritis was observed in *H pylori* infected but not in uninfected patients after long term treatment with lansoprazole. Glandular atrophy and epithelial cell proliferation were also increased in infected patients. These findings support the notion that *H pylori* eradication should be seriously considered before long term treatment with proton pump inhibitors is initiated.

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