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***HELICOBACTER PYLORI* AND GASTRIC ADAPTATION TO REPEATED ASPIRIN ADMINISTRATION IN HUMANS**Department of Medicine B, University of Munster, Munster, Germany and
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The gastric irritant properties of nonsteroidal anti-inflammatory drugs (NSAID) are well established but the pathogenic mechanisms by which these agents damage the mucosa or delay its repair are poorly understood. The phenomenon of gastric adaptation after repeated exposures to ASA is well documented but the involvement of *Helicobacter pylori* (*H. pylori*) in NSAID-induced gastropathy and adaptation has not been elucidated. The aim of this study was 1) to compare the gastric damage in response to repeated exposures to ASA in the same subjects before and after eradication of *H. pylori* and 2) to examine the morphological and functional changes of gastric mucosa during the 14 day treatment with ASA in *H. pylori*-infected subjects before and after eradication of this bacteria. Eight healthy volunteers (age 19—28) with *H. pylori* infection were given ASA 1g bd during 14 days before and after *H. pylori* eradication. Mucosal damage was evaluated by endoscopy before and at 3, 7 and 14 days of ASA administration using modified Lanza score. During endoscopy mucosal biopsies were obtained for determination of DNA synthesis, by measuring ³H-thymidine incorporation into DNA. Prior to each endoscopy gastric microbleeding was determined in three consecutive gastric washings. Three months after successful eradication of *H. pylori* confirmed by ¹³C-urea breath test and mucosal rapid urease test, the same subjects received again 14 day treatment with ASA and underwent the same examinations as prior to the therapy. In all subjects, ASA administration induced acute gastric damage with endoscopic Lanza score reaching maximum at 3rd day. In *H. pylori*-positive subjects, this damage was maintained at similar level up to day 14th, whereas in *H. pylori*-eradicated subjects, this damage was lessened at day 14th by about 60—75%. Gastric microbleeding also reached its maximum at 3rd day of ASA treatment being significantly higher in *H. pylori*-eradicated subjects than in those with *H. pylori* infection. This microbleeding decreased to almost normal values by the end of the study in all *H. pylori*-negative subjects but remained significantly elevated in *H. pylori*-infected subjects. DNA synthesis before and following ASA administration was significantly higher in subjects after *H. pylori* eradication than in those with *H. pylori* infection. Moreover, this DNA synthesis showed significant increase at day 7 of ASA administration only in *H. pylori*-eradicated subjects. We conclude that: 1) gastric adaptation to ASA is impaired in *H. pylori*-positive subjects but eradication of *H. pylori* restores this adaptation, 2) the DNA synthesis and possibly also mucosal cell turnover in response to ASA are suppressed in *H. pylori* infection and this can be reversed by eradication of *H. pylori*.

Key words: *stomach, adaptation, Helicobacter pylori, aspirin, gastric blood flow, epithelial cell proliferation.*

INTRODUCTION

Helicobacter pylori (*H. pylori*) and nonsteroidal antiinflammatory drugs (NSAIDs) are the commonest etiologic factors associated with gastritis and peptic ulceration. How aspirin (ASA) and other NSAIDs damage the gastroduodenal mucosa or delay its regeneration is not clearly understood. The deleterious effects of ASA on gastroduodenal mucosa are mainly attributed to direct damage of mucosal cells and its ability to reduce the formation of prostaglandins (1—4). Although prostaglandins (PG) exhibit a potent protective effect on gastrointestinal mucosa (5) and the inhibition of cyclooxygenase activity increases its susceptibility to injury by other irritants (6), the precise mechanisms of the ulcerogenic action of NSAIDs and the protective activity of PG are not clear.

Studies of Eastwood and Quimby (7) revealed that continued exposure to ASA increases proliferative activity of mucosal cells as measured by ³H-thymidine uptake. Other studies, showing elevated DNA synthesis in gastric mucosa during chronic indomethacin administration (8) and increased mitotic activity of mucosal cells after chronic ingestion of other NSAIDs (9), support the suggestion that higher turnover rate of gastric mucosa could be one of the mechanisms underlying gastric adaptation to these drugs (10).

H. pylori is now widely accepted as an etiologic agent inducing gastric inflammation and a risk factor in peptic ulcer disease (11—13). Furthermore, some link between *H. pylori*-infection and gastric carcinoma and B cell MALT lymphoma has been postulated (14, 15). Epidemiological studies have shown that the highest *H. pylori*-infection rate occurs in the elderly and in this age group the NSAID-related damage to the gastrointestinal tract reaches the highest level (16). As previously suggested, the *H. pylori* infection may contribute to NSAID-induced mucosal damage but the understanding of mechanisms underlying mucosal injury as brought about by both, NSAIDs and *H. pylori* is emerging only slowly. Studies on gastric adaptation to NSAIDs have mainly been performed on *H. pylori*-negative subjects (10, 17—20) and only recently the influence of *H. pylori* on gastric adaptation to naproxen has been examined (20). The data collected so far are, however, heterogeneous and controversial.

In this study we compared the ability of gastric mucosa to adapt to repeated exposures to ASA in *H. pylori*-infected subjects, with that after eradication of *H. pylori*.

METHODS

Subjects

Eight healthy volunteers of both sexes (4 males and 4 females), between 19 and 28 years of age and weighing 67—80 kg entered the study. All subjects were *H. pylori*-positive and the presence of *H. pylori* infection was determined by ¹³C-urea breath test (21) and endoscopy with mucosal biopsies and rapid urease test (HUT-test, Astra, Wedel, Germany). Subjects were requested to

refrain from the use of alcohol and any medications for two weeks prior to and during the study. All of them were in good health without any previous or present gastrointestinal disease and with normal laboratory values for blood chemistry and hematology. This study was approved by the appropriate Institutional Review Committee, and all subjects gave written informed consent to participate.

Study design

After the pre-study assessments, each subject underwent ASA treatment. Oral ASA was taken twice daily; two tablets of 500 mg unbuffered aspirin (Bayer, Leverkusen, Germany) were taken after breakfast at 0800 h and two tablets before bed time summing up to a daily total dose of 2 g. This treatment was continued for 14 consecutive days. After the termination of ASA treatment all subjects underwent eradication using triple therapy including amoxicillin (Amoxypen, Grünenthal, Stolberg, Germany) 1 g bd plus clarithromycin (Klacid, Abbott, Wiesbaden, Germany) 500 mg bd plus lansoprazole (Agopton, Takeda, Aachen, Germany) 30 mg bd given for 10 days. All 8 subjects were successfully eradicated following this therapy regimen as assessed by ^{13}C -urea breath test. Three months later these *H. pylori*-eradicated subjects entered the second 14 day treatment with ASA according to the same protocol as before the eradication of *H. pylori*.

Assessments

Before and after *H. pylori* eradication gastric microbleeding, endoscopy with the determination of gastric mucosal damage and collection of gastric mucosal biopsies were performed 24 h prior to ASA treatment. The above tests were repeated at day 3rd, 7th, and 14th of ASA course.

The rate of gastric microbleeding was determined as follows: each volunteer swallowed 16 French gauge orogastric tube. The stomach was rinsed of debris with 100 ml of distilled water, then 100 ml of test solution was instilled into the stomach for 10 min washing period as described before (22). After 5 min of each period 2 ml of phenol red as a marker in 10 ml of water was introduced *via* the orogastric tube and dispersed in the stomach. After 10 min of each washing period the gastric content was gently siphoned out and collected. Three successive washouts were performed at 10 min intervals. Test solution or phenol red was introduced into the stomach and the subjects performed a standard series of manoeuvres designed to ensure contact of washing with the whole gastric mucosa to allow the blood to accumulate in this washing. Blood rinsed from the stomach and accumulated in the gastric washing was quantified by the peroxidase activity of hemoglobin. Gastric microbleeding during each 10 min period was calculated after the correction for phenol red recovery. Mean gastric microbleeding for three 10 min washing periods was calculated and expressed as mean bleeding rate in ml/day. Phenol red was measured spectrophotometrically at pH 10.5 and wave length of 560 nm.

Thirty minutes after the completion of gastric washing standard, unседated, upper gastrointestinal endoscopy was performed by one investigator using an Olympus GIF 100 endoscope and recorded on video tape that was evaluated for mucosal damage using the Lanza score system (23) by the second investigator, being unaware of the ASA treatment and the *H. pylori*-status. Grading score was from 0 — normal to 4 — large area of submucosal hemorrhage with active bleeding or widespread involvement of the stomach.

During endoscopy two mucosal pinch biopsy specimens were taken from the gastric antrum and the corpus for estimation of DNA synthesis as well as RNA and DNA concentration in the gastric mucosa as described previously (10). Rates of DNA synthesis in mucosal biopsies were measured by incubating the tissue at 37°C for 30 min in Eagles Minimal Essential Culture Medium

containing 2 $\mu\text{Ci/ml}$ ^3H -thymidine (5 $\mu\text{Ci/mmol}$ Amersham, England). Tissue was gassed continuously with 95% oxygen/5% carbon dioxide during the incubation. The action was stopped with 0.4 N perchloric acid containing carrier thymidine at 5 mM. Following this procedure samples were hydrolyzed in 0.3 N KOH for 90 min at 37°C. DNA was reprecipitated with 10% perchloric acid. RNA content of the supernatant was determined using the orcinol reaction (24). After standing on ice for 10 min, the DNA-containing tubes were centrifuged, and the supernatant was discarded. DNA in the residual pellet was solubilized in 10% perchloric acid and heated to 70°C for 20 min. DNA content of the samples was determined by the Burton procedure (25), as modified by Giles and Myers (26). Incorporation of ^3H -thymidine into DNA was determined by counting 0.5 ml DNA-containing filtrate in a Beckman liquid scintillation system. DNA and RNA contents were expressed as micrograms per 100 mg mucosa and DNA synthesis was expressed as disintegrations per minute (DPM) per μg DNA.

Results are expressed as means \pm SEMs. The significance of the difference between means was evaluated using analysis of variance followed by Duncan's test with a level of confidence at $P < 0.05$.

RESULTS

Gastric microbleeding

All subjects before and after *H. pylori* eradication completed ASA treatment. In *H. pylori*-infected subjects, the pretreatment value of gastric microbleeding was 1.2 ± 0.6 ml/day and it was not significantly different from the value recorded in *H. pylori*-eradicated subjects (Fig. 1). After 3 days of ASA

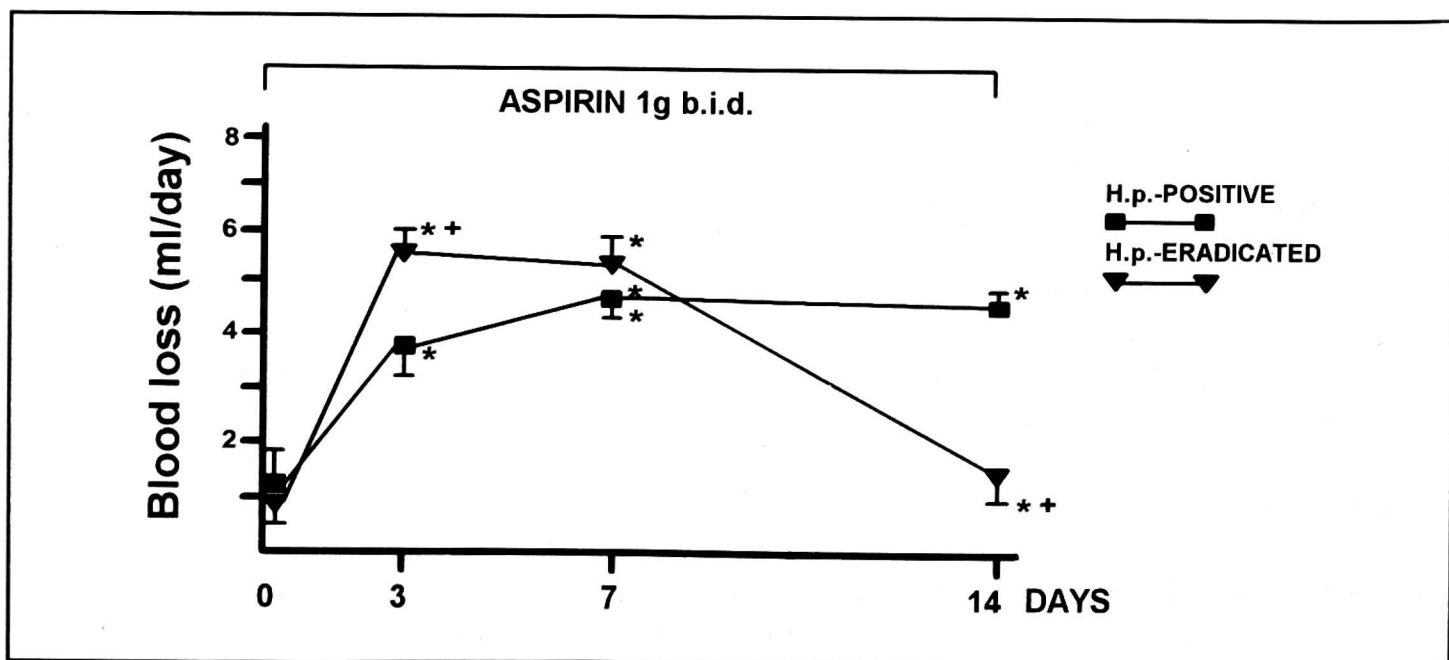


Fig. 1. Gastric microbleeding in 8 subjects with *H. pylori* infection before and after eradication treated with ASA for 14 days. Means \pm SEM. Asterisk indicates significant ($P < 0.01$) changes as compared to initial values before ASA treatment, cross indicates significant change compared to the value obtained in *H. pylori*-infected subjects.

treatment, the gastric microbleeding increased significantly ($P < 0.001$) in all subjects tested reaching peak that in *H. pylori*-infected subjects was significantly lower (3.9 ± 0.6 ml/day) than that obtained in ASA course in *H. pylori*-eradicated (5.5 ± 0.4 ml/day). At 7th and 14th day of ASA treatment, *H. pylori*-infected subjects showed enhanced microbleeding that remained at the level not significantly different from that recorded at 3rd day of this treatment. In contrast, the microbleeding rate in *H. pylori*-eradicated subjects decreased in the course of ASA treatment to fall at 14th day to the value that was significantly lower than that in *H. pylori*-positive subjects. Thus, the continuation of ASA treatment for 14 days resulted in a significant ($P < 0.05$) attenuation of the rate of gastric microbleeding in *H. pylori*-eradicated subjects contrasting with the elevated rate of bleeding in *H. pylori*-positive subjects.

Gastroscopy

In *H. pylori*-negative and *H. pylori*-positive subjects, the initial gastroscopy, did not show any visible mucosal abnormalities in any of the subjects tested and, thus, Lanza score was 0 (Fig. 2). By contrast, gastroscopy performed at 3rd

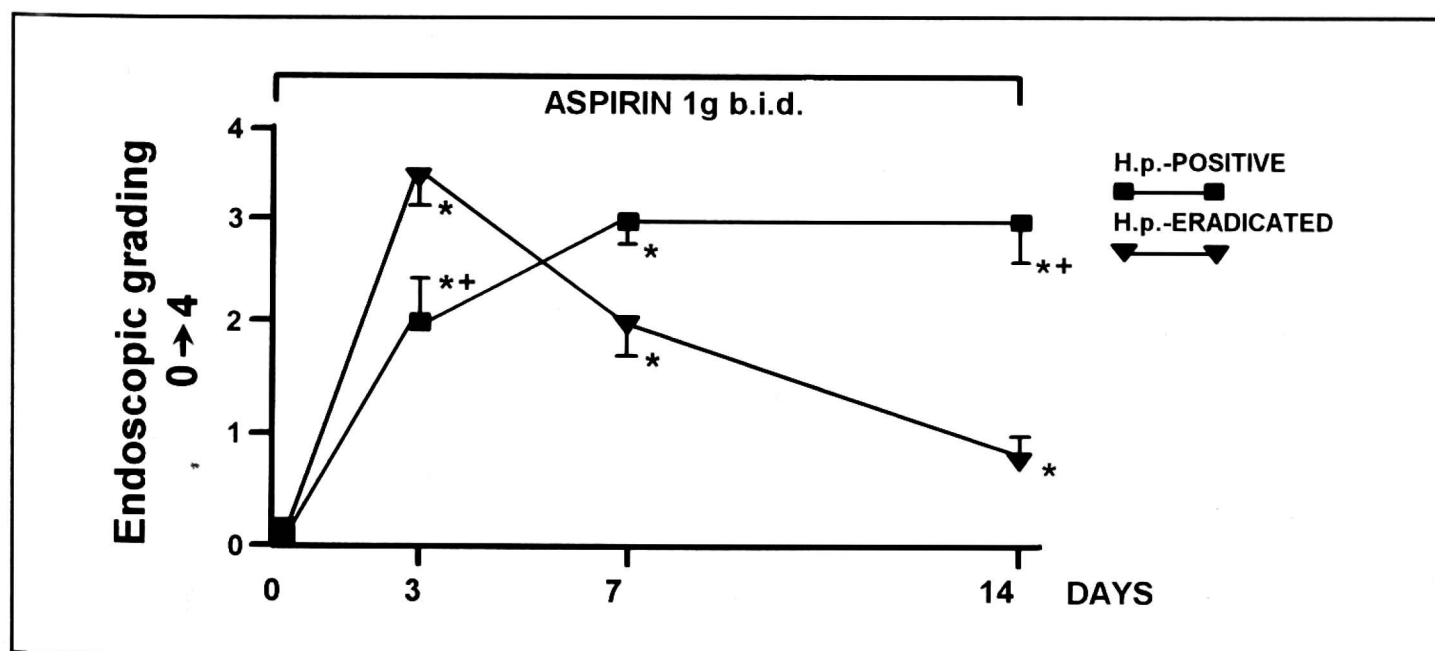


Fig. 2. Endoscopic score of gastric mucosa in 8 *H. pylori* infected subjects before and after eradication treated with ASA for 14 days. Means \pm SEM. Asterisk indicates significant changes ($P < 0.01$) as compared to initial values at day 0, cross indicates significant change compared to the values obtained in *H. pylori*-infected subjects.

day of ASA treatment showed numerous and large areas of hemorrhages located mainly in the oxyntic mucosa and to a lesser degree in antral mucosa both in *H. pylori*-negative and *H. pylori*-positive subjects. In *H. pylori*-infected subjects the Lanza score reached at the 3rd day an average of 2.0 ± 0.5 and remained at similar level at 7th (3.0 ± 0.2) and 14th day (3.0 ± 0.5) of ASA administration. In *H. pylori*-eradicated subjects during the ASA treatment the

Lanza score at the 3rd day reached significantly higher value than that observed after the same period of ASA treatment in the same subjects before the eradication of *H. pylori*. Continuation of ASA treatment in *H. pylori*-eradicated subjects led to significant resolution of mucosal damage partly on day 7th and almost completely (only single submucosal hemorrhages were observed) at 14th day of this treatment. This normalised Lanza score was significantly lower than that recorded at 14th day of ASA treatment in *H. pylori*-positive subjects.

DNA synthesis ✓

In *H. pylori*-infected subjects, the DNA synthesis in gastric corpus and antrum showed a significant fall after ASA administration and this fall was observed throughout the course of ASA-treatment (Fig. 3). In *H. pylori*-eradicated subjects, the DNA synthesis at 3rd day of ASA treatment tended to decrease but not significantly in oxyntic and antral mucosa but then significantly increased at 7th day of treatment. At day 14th, the rate of DNA synthesis in *H. pylori*-eradicated subjects returned to the values similar to that before the ASA treatment.

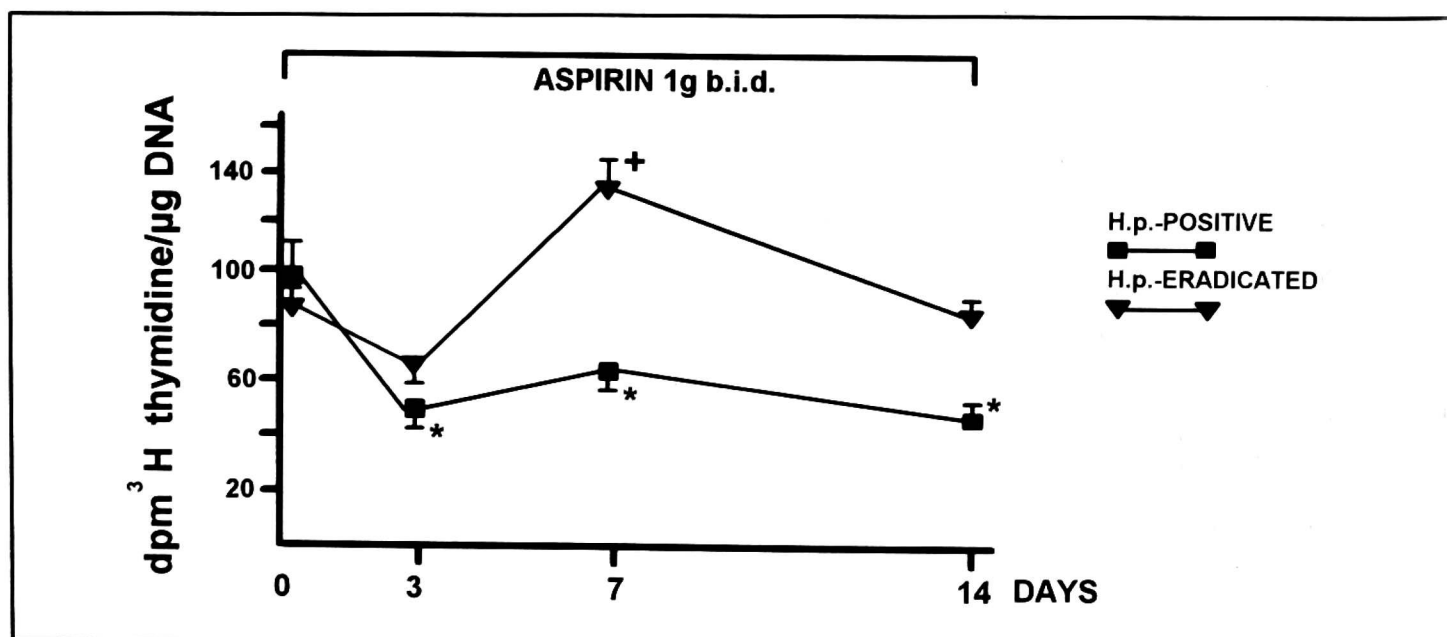


Fig. 3. Mucosal DNA synthesis in the corpus of the stomach during 14 days of continuous ASA treatment before and after eradication of *H. pylori*. Values are means \pm SEM of determinations in 8 subjects. Asterisk indicates significant changes as compared with pre-aspirin values, cross indicates significant change compared to the tests before eradication of *H. pylori*.

Plasma salicylate

In all subjects before and after eradication of *H. pylori*, after 3 days treatment with ASA, plasma salicylate concentration was 11.03 ± 0.87 μ g/ml and remained similar to this value in all subjects up to the end of ASA ingestion.

DISCUSSION

This study confirms earlier findings that aspirin has a deleterious effect on the gastric mucosa (1—4, 6, 16, 18—20) and that this occurs independent of *H. pylori* status (9). The dramatic increase in macroscopic damage of gastric mucosa reached its maximum on the 3rd day of ASA treatment and this was accompanied by a marked mucosal blood loss (10,22). These mucosal lesions caused by ASA and other NSAIDs were reported to resolve along with the course of treatment but little is known whether mucosal infection by *H. pylori* affects the gastric damage by NSAID and gastric adaptation to these drugs (20).

The major finding of this study is that in subjects with *H. pylori* infection, confirmed by ^{13}C -urea breath test and rapid mucosal urease test, the ASA treatment caused initially smaller mucosal damage than in *H. pylori*-negative subjects but then the damage (elevated Lanza score and microbleeding) was sustained indicating that gastric adaptation to ASA was virtually lost. The eradication of *H. pylori* restored the ability of the mucosa to adapt to ASA as indicated by the resolution of mucosal damage and the fall in microbleeding despite the continuation of drug administration. In contrast to *H. pylori*-infected subjects, the *H. pylori*-eradicated subjects showed a remarkable ability to adapt to repeated exposures to injurious action of ASA so that macroscopic damage inflicted on the first contact with ASA was minimised on repeated drug challenge (10, 17—20). Recently Lipscomb *et al.* (20) observed that adaptation to naproxen occurs after 28 days independent of *H. pylori* status. The gastric adaptation was however, studied in two different groups of subjects, one with *H. pylori* infection and another without such infection so the intraindividual analysis was not possible in this study. Additionally, naproxen used in this study has pharmacokinetic properties different from those of ASA, which is especially abnoxious for the gastric mucosa at lower intragastric pH. In the present study, in subjects infected with *H. pylori* we observed substantially elevated gastric blood loss that closely correlated to the mucosal damage expressed by Lanza score. Following eradication of *H. pylori* in our subjects the ability of gastric mucosa to adapt to ASA challenge was fully restored. Our finding is supported by recent studies of Kordecki *et al.* (27) and by Lee *et al.* (28) who have shown that eradication of *H. pylori* significantly decreased the number of recurrences of gastric and duodenal mucosal lesions and reduced the occurrence of peptic ulcer in patients taking NSAIDs.

As shown previously, one of the most common factors accompanying the exposure of mucosa to NSAIDs and the development of gastric adaptation is an increased mucosal cell proliferation (7,9,10). We observed an initial fall in DNA synthesis at 3rd day and this was followed by a significant increase of this

synthesis at 7th and 14th days of ASA treatment in *H. pylori*-eradicated subjects while this rate was substantially decreased in *H. pylori*-infected subjects. This might suggest that *H. pylori* infection impairs the ability of mucosal cells to increase their proliferating activity in order to adapt to ASA and that the removal of *H. pylori* restores the adaptation possibly *via* enhancement of mucosal cell proliferation.

This study shows that at 3rd day upon the administration of ASA, the extent of mucosal damage, as evaluated by Lanza score and the gastric microbleeding, was significantly smaller in *H. pylori*-infected subjects than those without *H. pylori* in the stomach. This indicates that the *H. pylori*-infected gastric mucosa exhibits enhanced mucosal defence and is more resistant to ASA-induced damage than the intact non-infected mucosa. This could be interpreted that the infected mucosa is adapted to the presence of *H. pylori* and that this adaptation may have some impact on the noxious action of NSAID on the mucosa. The mechanism of this adaptation to *H. pylori* may be related to initially higher mucosal generation of PG in *H. pylori* infected stomach (29) and represents an overall symbiosis between the bacteria and the stomach (30).

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