ELSEVIER

European Journal of Pharmacology 451 (2002) 191–202

www.elsevier.com/locate/ejphar

Effect of *Helicobacter pylori* on delay in ulcer healing induced by aspirin in rats

Peter C. Konturek [a], Tomasz Brzozowski [b], Slawomir Kwiecień [b], Danuta Drozdowicz [b], Igor A. Harsch [a], Holger Meixner [a], Jerzy Stachura [c], Eckhart G. Hahn [a], Stanislaw J. Konturek [b,*]

[a] *First Department of Medicine, University Erlangen-Nuremberg, Erlangen, Germany*
[b] *Department of Physiology, Jagiellonian University School of Medicine, 16 Grzegorzecka Street, 31-531 Krakow, Poland*
[c] *Department of Pathomorphology, Jagiellonian University Medical School, Krakow, Poland*

Received 14 February 2002; received in revised form 17 July 2002; accepted 9 August 2002

Abstract

Helicobacter pylori and nonsteroidal anti-inflammatory drugs (NSAIDs) are major pathogenic factors in peptic ulcer disease but whether these two factors exert synergistic or antagonistic effects on ulcer healing has been a subject of controversy. We compared the effect of aspirin alone with that of aspirin combined with *H. pylori* on gastric acid secretion and healing of acetic acid gastric ulcers in rats. The *H. pylori* colonization of gastric mucosa was determined by viable *H. pylori* culture, histology and assessment of bacterial DNA using polymerase chain reaction (PCR). The area of ulcers, gastric blood flow, mucosal generation of prostaglandin E_2 and plasma gastrin levels and expression of cyclooxygenase-1, cyclooxygenase-2 and growth factors was determined. Aspirin delayed significantly the healing of chronic gastric ulcers, decreased the gastric blood flow at the ulcer margin and gastric mucosal prostaglandin E_2 generation being without significant influence on gastric acid output. *H. pylori* acquisition that produced moderate gastric inflammation at the ulcer margin delayed significantly the healing of gastric ulcers, decreased significantly both the gastric blood flow at the ulcer margin and the gastric secretion while raising significantly the gastric mucosal prostaglandin E_2 generation and plasma gastrin levels. *H. pylori* infection attenuated the aspirin-induced inhibition of ulcer healing and accompanying fall in the gastric blood flow. Both aspirin and *H. pylori* up-regulated significantly cyclooxygenase-2 messenger RNA (mRNA) and protein but not that of cyclooxygenase-1 at the ulcer margin. Aspirin reduced significantly the transforming growth factor alpha- and vascular endothelial growth factor mRNAs, but these effects were significantly attenuated by *H. pylori*. We conclude that *H. pylori* antagonizes, in part, aspirin-induced delay of ulcer healing due to suppression of acid secretion, the enhancement in prostaglandin E_2 possibly derived from cyclooxygenase-2 and the overexpression of transforming growth factor alpha and vascular endothelial growth factor in the ulcer area.

Keywords: Aspirin; *Helicobacter pylori*; Ulcer healing; Gastric secretion; Gastric blood flow; Cyclooxygenase; Growth factor

1. Introduction

Helicobacter pylori and nonsteroidal anti-inflammatory drugs (NSAID) are major pathogenic factors in peptic ulcer disease (Tytgat, 2000). The results of studies on the interaction between NSAID and *H. pylori* are controversial and confused (Fendrick and Scheiman, 2001). Generally, it is expected that these two well-established risk factors for peptic ulcer disease should exert a synergistic effect on the risk of this disease (Chan et al., 1998). However, this issue was not studied carefully, since peptic ulcer disease in NSAID users infected with *H. pylori* was less frequently diagnosed than in those taking NSAID without *H. pylori* infection (Loeb et al., 1992). Furthermore, conflicting results were obtained from controlled randomized trials that examined whether *H. pylori* eradication could influence ulcer healing in individuals subjected to NSAID therapy (Bianchi Porro et al., 1999; Chan et al., 1998) and whether this eradication could reduce the development of peptic ulcer disease in NSAID takers (Chan et al., 1997; Hawkey et al., 1998). As a broad generalization, *H. pylori*-positive healthy individuals without ulcer history benefit from *H.*

* Corresponding author. Tel.: +48-12-4211006; fax: +48-12-4211578.
E-mail address: mpkontur@cyf-kr.edu.pl (S.J. Konturek).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02250-1

pylori eradication therapy at the start of NSAID therapy (Chan et al., 1997). In contrast, the eradication of *H. pylori* in some clinical trials failed to affect the healing of gastric ulcers in NSAID users who were treated with omeprazole (Hawkey et al., 1998). This implies that *H. pylori* acts to increase risk at the start of NSAID treatment (Chan, 2002) but with prolonged NSAID therapy, *H. pylori* exerts protective influence against NSAID-induced ulcerogenesis, possibly by stimulating mucosal prostaglandins and other protective factors.

The healing of chronic ulceration is a complex process that includes filling of the mucosal defect with granulation tissue, cell proliferation at the ulcer margin, and an adequate blood supply delivering oxygen and nutrients to the ulcer area (Tarnawski, 1993). The ulcer healing is accelerated by various growth factors, including transforming growth factor alpha and vascular endothelial growth factor (Konturek et al., 1997; Szabo and Vincze, 2000). Transforming growth factor alpha is normally expressed in the gastric mucosa to maintain the physiological functions of gastric mucosa, but its expression is significantly increased in the ulcerated gastric mucosa. Transforming growth factor alpha, like epidermal growth factor, acts via epidermal growth factor-receptor accelerating the cell proliferation, migration and inhibition of gastric acid secretion (Coffey et al., 1995). Vascular endothelial growth factor is a heparin-binding glycoprotein that occurs in five isoforms, which are generated as a result of alternative splicing from a single vascular endothelial growth factor gene. Vascular endothelial growth factor acts specifically on vascular endothelial cells to increase vascular permeability and to stimulate endothelial cell proliferation, migration and tube formation (angiogenesis). Vascular endothelial growth factor also increases blood flow and prevents endothelial cell apoptosis, acting as the major angiogenic factor that was proposed to contribute to the ulcer healing (Neufeld et al., 1999; Takahashi et al., 1998) and showing enhanced expression at the ulcer margin (Takahashi et al., 1997), however, its interaction with *H. pylori* or its products remains unknown.

Aspirin delays ulcer healing through various mechanisms including: (1) significant inhibition of biosynthesis of prostaglandins (Wang et al., 1989), (2) reduction in cell regeneration and inhibition of ulcer contraction (Penney et al., 1994) and (3) decrease in mucosal blood flow in the ulcer margin (Hirose et al., 1991). Previous studies have demonstrated that the deleterious effect of NSAID on ulcer healing in *H. pylori*-positive healthy subjects treated with omeprazole could not be attenuated by *H. pylori* eradication (Hawkey et al., 1998), but the results of this study are difficult to interpret because of maintenance treatment with antisecretory agents such as omeprazole with greatly enhanced activity in *H. pylori*-infected subjects. Recent review based on 16 studies of 2625 NSAID takers without secretory treatment shows a clear synergism for the development of mucosal ulcerations by *H. pylori* infection and NSAID use (Huang et al., 2002).

In the present study, we investigated whether inoculation of rats with live *H. pylori* affects the aspirin-induced delay of the healing of preexisting gastric ulcers induced in rats by acetic acid technique. An attempt was made to determine whether *H. pylori* colonizes non-ulcerated and ulcerated rat gastric mucosa and whether the effect of these two major ulcer risk factors, aspirin and *H. pylori* applied alone or in combination, can influence gastric acid secretion and gastric blood flow at the ulcer margin as well as gene expression for cyclooxygenase-1, cyclooxygenase-2 and growth factors, such as transforming growth factor alpha and vascular endothelial growth factor in animals with preexisting gastric ulcers.

2. Material and methods

2.1. Production of gastric ulcers and determination of gastric acid secretion and gastric blood flow at ulcer margin

Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and approved by the local animal care committee of the Jagiellonian University in Cracow. Male Wistar rats, weighing 180–220 g and fasted for 24 h before the study were employed in all studies. Gastric ulcers were produced in 24 rats using our modification of acetic acid method originally proposed by Okabe et al. (1971). Animals were anesthetized with ether, the stomach was exposed and a round plastic mold (6 mm in diameter) was placed tightly on the anterior serosal surface of the stomach at the antro-oxyntic border. Seventy-five microliters of 100% acetic acid was poured into the mold and allowed to remain on the gastric wall for 25 s. This produced immediate necrosis of the entire mucosa and submucosa (but not serosa) within the area where the acetic acid was applied, i.e. 28 mm^2. The excess of acetic acid was then removed and the serosa was gently washed out with saline. Our previous studies documented that these ulcers became chronic within 2–3 days and healed completely within 2–3 weeks (Konturek et al., 1997). After the application of acetic ulcers, the animals were allowed to recover from anesthesia and received only water at the day of operation.

The alterations of gastric secretion during ulcer healing in rats treated with vehicle (saline) and aspirin applied with or without the inoculation of *H. pylori* were tested in a separate group of 60 starved rats with acetic acid ulcers, surgically equipped with chronic gastric fistulas as described previously (Konturek et al., 1997). Control sham-operated rats with gastric fistulas were also included, but instead of acetic acid, 70 μl of saline was applied to the serosal surface of the stomach for 20 s.

The rats with acetic acid ulcers with or without gastric fistula were randomly assigned to the following four major treatment groups A, B, C and D. In group A, rats were

inoculated intragastrically (i.g.) using a gavage with live *H. pylori* suspension ($CagA^+$; 3×10^9 CFU/ml) at day 2 upon ulcer induction and this inoculation was repeated at day 4 and day 6 upon ulcer induction followed on day 7 with saline (vehicle) treatment that was continued for 10 days. *H. pylori* used in this study was originally obtained from the gastric antrum of the patient with duodenal ulcer, passed in the mice and reisolated in order to achieve better colonization in this species as proposed by Li et al. (1998). Group B received instead of *H. pylori inoculum*, the treatment with vehicle (saline) starting at day 2, that was repeated at days 4 and 6 after ulcer induction followed from the day 7 by i.g. treatment with aspirin at a dose of 50 mg/kg dissolved in 0.15 N HCl. Treatment with aspirin in these animals was continued subsequently throughout 10 consecutive days. Group C, similarly to group A, received at days 2, 4 and 6 upon ulcer induction, live *H. pylori* suspension, followed by i.g. treatment with aspirin (50 mg/kg/day i.g.) that started at day 7 following ulcer induction and was continued throughout 10 consecutive days. Group D served as a control and received only vehicle (saline) without *H. pylori* or aspirin treatment. Four hours before each subsequent *H. pylori* inoculation, gastric acid secretion in rats was suppressed by a single dose of omeprazole (20 mg/kg subcutaneously) suspended in 0.5% carboxy methylocelulose (CMC). The animals in all groups were sacrificed 16 days upon ulcer induction.

For determination of gastric acid secretion, non-inoculated rats equipped with gastric fistulas without ulcer and those with gastric ulcers inoculated with *H. pylori* with or without aspirin treatment, were placed at days 8 and 16 upon ulcer induction in the individual Bollman cages to prevent coprophagy. Each fistula was then opened, and the stomach was rinsed gently with 5–8 ml of tap water at 37 °C. Basal gastric secretion was collected for 120 min, during which time all animals received saline at a rate of 4 ml/h subcutaneously. The gastric juice was collected every 30 min, the volume was measured, and then the acid concentration and output were determined and expressed as the output per 30 min as described previously (Brzozowski et al., 2001). After completing the secretory studies, the rats were sacrificed and gastric mucosal samples were taken for an assessment of the presence of *H. pylori* using the method described below.

For evaluation of the involvement of *H. pylori* applied alone or combined with aspirin on ulcer healing and gastric blood flow, the groups of animals were anesthetized with ether at day 16 upon ulcer induction, the abdomen was opened and the stomach was exposed to determine the gastric blood flow at the ulcer margin and the contralateral intact mucosa using H_2-gas clearance technique as described before (Konturek et al., 1998a,b). The gastric blood flow was expressed as the percentage of the basal flow recorded in the gastric mucosa of control rats with saline applied to the serosa through the plastic mold.

The stomachs were then quickly removed and pinned open for the determination of the area of gastric ulcers by planimetry (Morphomat, Carl Zeiss, Berlin, German) by two investigators under blinded conditions.

2.2. Measurement of prostaglandin E_2 in the gastric mucosa, quantification of viable H. pylori and histological evaluation of gastric mucosa

The samples of the oxyntic gland area from the ulcer margin were taken by biopsy (about 100 mg) immediately after the gastric blood flow had been measured to determine the mucosal generation of prostaglandin E_2 by specific radioimmunoassay (RIA) as described previously (Konturek et al., 1998a,b). Prostaglandin E_2 was measured in duplicate using RIA kits (New England Nuclear, Munich, Germany). The capability of the mucosa to generate prostaglandin E_2 was expressed in nanograms per gram of wet tissue weight.

Following assessment of the area of gastric ulcers, the half of the stomach that did not contain an ulcer or its scar was excised and homogenized in 1 ml of phosphate-buffered saline with a homogenizer (Ultra Turax) followed by dilution with the same buffer as described in detail in our previous studies in mice (Brzozowski et al., 1998; Konturek et al., 1999). Briefly, the aliquots (100 μl) of the dilutions were applied to Helicobacter agar plates (Becton-Dickinson, UK) that were incubated at 37 °C under microaereophilic atmosphere for 7 days. The colonies were identified as *H. pylori* as determined from their shape (spiral) under a microscope (× 2000; Olympus, Tokyo, Japan). In addition, positive test for oxidase, urease and catalase was performed to indicate specificity for *H. pylori*. The number of colonies was determined and the viable *H. pylori* was expressed as CFU/mg of gastric tissue weight.

The half of the stomach from the ulcer area and the grossly unchanged mucosa in rats inoculated with *H. pylori* with or without aspirin or vehicle administration was excised during autopsy and immediately fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin for the histological assessment of mucosal inflammation and the quality of ulcer healing. Coded specimens of mucosa stained with hematoxylin and eosin were evaluated at 260 × magnification by two experienced pathologists unaware of the treatment given.

2.3. Determination of plasma gastrin levels

At the termination of some experiments with i.g. administration of vehicle, aspirin, *H. pylori*, and *H. pylori* combined with aspirin, the animals were anesthetized with ether and the blood samples (about 3 ml) were taken from the vena cava for the RIA of plasma gastrin levels as described previously (Brzozowski et al., 1998). For comparison, intact rats fasted overnight and were given only vehicle saline i.p., were anaesthetized with ether and the blood samples were collected for the determination of control values of gastrin in plasma. The blood samples collected in heparin-coated

polypropylene tubes were centrifuged at 3000 rpm for 20 min at 4 °C, and the supernatant clear plasma was then stored at −80 °C until measurement of plasma gastrin levels using RIA. Gastrin antibody No. 4562 (final dilution, 1:100,000) was a gift of Professor J. Rehfeld from Copenhagen, Denmark (Konturek et al., 2001). The limit of assay sensitivity was 0.5 mM of sample and the interassay and intra-assay coefficients of variation were 7% and 9%, respectively.

2.4. Detection of H. pylori 16S rRNA by polymerase chain reaction (PCR)

For DNA extraction, Trizol Reagent was used according to the manufacturer's instructions (Gibco BRL/Life Technology, Eggenstein, Germany). Briefly, the samples from gastric mucosa were homogenized in 250 μl of Trizol Reagent for 15–20 s. After 5 min incubation, 50 μl of chloroform was added, followed by centrifugation at 12,000 × *g* for 15 min, which led to the separation of the sample solution in an aqueous and an organic phase. After removal of the aqueous phase, the DNA was extracted from the samples by precipitation with 75 μl of 100% ethanol. Following the precipitation and series of washes, the DNA was solubilized in 8 mM NaOH. The sample was precipitated again with ethanol and resolved in TE-buffer. The concentration of DNA was estimated by absorbance at 260 nm. DNA samples were stored at −80 °C.

The polymerase chain reaction was performed as described in our previous studies (Konturek et al., 1998a,b). Briefly, 2 μl of DNA was amplified in a 50-μl reaction volume containing 2 U Taq polymerase, dNTP (200 μM each) (Pharmacia, Germany), 1.5 mM $MgCl_2$, 5 μl 10 × polymerase chain reaction buffer (50 mM KCl, 10 mM Tris–HCl, pH = 8.3) and primers for *H. pylori* 16S RNA used at a final concentration of 1 mM (all reagents from Takara, Shiga, Japan). The polymerase chain reaction mixture was amplified in a DNA thermal cycler (MJ Research, PTC 200, Watertown, MA). Amplification consisted of initial denaturation at 94 °C for 2 min, followed by denaturation at 94 °C for 45 s, primer annealing at 61 °C for 45 s, and extension for 2 min at 72 °C. The samples were amplified for 35 cycles, with the final extension step at 72 °C for 7 min. The nucleotide sequence of the primers was based on the sequences of the published cDNAs (Drazek et al., 1994). The sense primer 16S rRNA 5′-TCA GCC TAT GTC CTA TCA GC-3′ and the antisense primer 16S rRNA 5′-CAG TAA TGT TCC AGC AGG TC-3′ yield the 500 bp product. The primers were synthesized by Gibco BRL/Life Technologies. As a positive control for *H. pylori* primers, the DNA extracted from pure *H. pylori* culture was amplified with the same primer set.

Polymerase chain reaction products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Location of predicted products was confirmed by using 100-bp ladder (Takara) as a standard size marker.

2.5. Reverse transcription-polymerase chain reaction for detection of messenger RNA (mRNA) for transforming growth factor alpha, vascular endothelial growth factor, cyclooxygenase-1 and cyclooxygenase-2

The extraction of total RNA from gastric ulcerated tissues and control tissues was carried out as previously described. Briefly, total RNA was extracted from mucosal samples using a guanidinum isothiocyanate/phenol chloroform single-step extraction kit from Stratagene (Heidelberg, Germany) based on the method described by Chomczynski and Sacchi (1987). Following precipitation, RNA was resuspended in RNase-free TE buffer and its concentration was estimated by absorbance at 260 nm wavelengths. Furthermore, the quality of each RNA sample was determined by running the agarose-formaldehyde electrophoresis. RNA samples were stored at −80 °C until analysis.

Single-stranded cDNA was generated from 5 μg of total cellular RNA using Moloney murine leukemia virus reverse transcriptase and oligo-(dT)-primers as described before (Konturek et al., 1998a,b). Briefly, 5 μg of total RNA was uncoiled by heating (65 °C for 5 min) and then reverse transcribed (at 37 °C for 1 h) into complementary DNA (cDNA) in a 50-μl reaction mixture that contained 50 U MMLV-RT, 0.3 μg oligo-(dT)-primer, 40 U RNase Block Ribonuclease Inhibitor, 2 μl of a 100-mM mixture of deoxyadenosine triphosphate (dATP), deoxythymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP), 5 μl of 10 × first-strand buffer (all reagents provided by Stratagene). The resultant cDNA (2 μl) was amplified in a 50-μl reaction volume containing 2 U Taq polymerase, dNTP (200 μM each) (Pharmacia, Germany), 1.5 mM $MgCl_2$, 5 μl 10 × polymerase chain reaction buffer (100 mM KCl, 20 mM Tris–HCl, pH = 8.3) and specific primers for β-actin, cyclooxygenase-1, cyclooxygenase-2, vascular endothelial growth factor and transforming growth factor alpha used at a final concentration of 1 mM (all reagents purchased from Takara). The mixture was overlaid with 25 μl of mineral oil to prevent evaporation. The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT) and the incubation and thermal cycling conditions were as follows: denaturation at 94 °C for 1 min, annealing at 60 °C for 45 s and extension 72 °C for 2 min. The number of cycles was 30 for β-actin, 33 for cyclooxygenase-1, cyclooxygenase-2, transforming growth factor alpha and vascular endothelial growth factor. The nucleotide sequence of the primers was as follows: β-actin, sense 5′-GAT CTT GAT CTT CAT GGT GCT AGG-3′, antisense 5′-TTG TAA CCA ACT GGG ACG ATA TGG-3′; cyclooxygenase-1, sense 5′-AGC CCC TCA TTC ACC CAT CAT TT-3′, antisense 5′-CAG GGA CGC CTG TTC TAC GG-3′; cyclooxygenase-2, sense 5′-ACA ACA TTC CTT CCT TC-3′, antisense 5′-CCT TAT TTC CTT TCA CAC C-3′; vascular endothelial growth factor, sense 5′-TTG AAC ACC GAG CAG T-3′, antisense 5′-GGT CCT

CTG CCA TTC T-3′; and transforming growth factor alpha, sense 5′-ATG GTC CCC GCG GCC GGA CA 3′, antisense 5′-ATG GTC CCC GCG GCC GGA CA-3′. The primer sequences for β-actin, cyclooxygenase-1, cyclooxygenase-2, vascular endothelial growth factor and transforming growth factor alpha were based on the sequences of the published cDNAs and were synthesized by Gibco BRL/Life Technologies.

Polymerase chain reaction products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Location of a predicted product was confirmed by using 100-bp ladder (Takara) as a standard size marker. The gel was then photographed under UV transillumination. The intensity of PCR products was measured using video image analysis system (Kodak Digital Science) according to the method described elsewhere (Konturek et al., 1998a,b). The signal for analyzed mRNA was standardized semi-quantitatively against that of the β-actin mRNA from each sample and the results were expressed as cyclooxygenase-1–, cyclooxygenase-2–, vascular endothelial growth factor– or transforming growth factor alpha–β-actin mRNA ratio.

2.6. Western blot analysis for cyclooxygenase-1 and cyclooxygenase-2

Shock frozen tissue from rat stomach was homogenized in lysis buffer (100 mmol Tris–HCl, pH 7.4, 15% glycerol, 2 mmol EDTA, 2% SDS, 100 mmol DDT) by the addition of 1:20 dilution of aprotinin and 1:50 dilution of 100 mmol PMSF. Insoluble material was removed by centrifugation at 12,000 × *g* for 15 min. Approximately 100 μg of cellular protein extract were loaded into a well, separated electrophoretically through a 10% SDS–polyacrylamide gel and transferred onto Sequi-Blot™ PVDF membrane (BioRad, NY, USA) by electroblotting. 0.4% I-Block (Tropix, Bedford, USA) in TBS–Tween-20 buffer (137 mmol NaCl, 20 mmol Tris–HCl, pH 7.4, 0.1% Tween-20) was used to block filters for at least 1 h at room temperature. Specific primary antibody against cyclooxygenase-1 (mouse monoclonal, H-3; dilution 1:500; Santa Cruz, USA) or cyclooxygenase-2 (goat polyclonal, C-20; dilution 1:200; Santa Cruz, USA) or β-actin (mouse monoclonal, A5441, dilution 1:1000; Sigma Aldrich, Germany) was added to the membrane, followed by an anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (1:2 000, Santa Cruz, USA). Incubation of primary antibody was followed by three washes with TBS–Tween-20 buffer for 10 min. Incubation of the secondary antibody was followed by four washes for 10 min. Non-isotopic visualization of immunocomplexes was achieved by chemiluminescence using chemiluminescence blotting substrate (Western-Star-System from Tropix). Thereafter, the developed membrane was exposed to an X-ray film (Kodak, Wiesbaden, Germany). Comparison between different treatment groups was made by determining the cyclooxygenase-1–, cyclooxygenase-2–, vascular endothelial growth factor– and transforming growth factor alpha–β-actin ratio of the immunoreactive area by densitometry.

2.7. Statistical analysis

Data were expressed as means ± S.E.M. from 8 to 10 animals per group and comparisons between groups were made by Student's *t*-test for unpaired data or by analysis of variance and Duncan's multiple range tests, with $p < 0.05$ taken as significant.

3. Results

3.1. Effect of vehicle, H. pylori and aspirin alone or H. pylori applied in combination with aspirin on the area of gastric ulcers and accompanying changes in gastric blood flow at ulcer margin, histology of the gastric mucosa and expression of 16S rRNA

Fig. 1 shows the changes in the area of gastric ulcer and gastric blood flow at ulcer margin in rats inoculated intragastrically with vehicle, *H. pylori*, aspirin (50 mg/kg/day) or in those treated with the combination of *H. pylori* and aspirin (50 mg/kg/day) that was given from day 7 after ulcer induction and was continued for 10 consecutive days. In rats treated with vehicle, a significant reduction in the area of these ulcers was observed from initial size of about 28 mm^2 at day 0 to 7.6 ± 0.4 mm^2 at day 16 upon ulcer induction. In rats treated with aspirin or *H. pylori*, a significant delay in ulcer healing was observed and the ulcer area at day 16 reached the values of 17.4 ± 1.2 and 15.9 ± 1.3 mm^2, respectively. In contrast, inoculation with live *H. pylori* applied before exposure to aspirin led to the significant decrease in the ulcer area at day 16 upon ulcer induction as compared to that in rats treated with *H. pylori*

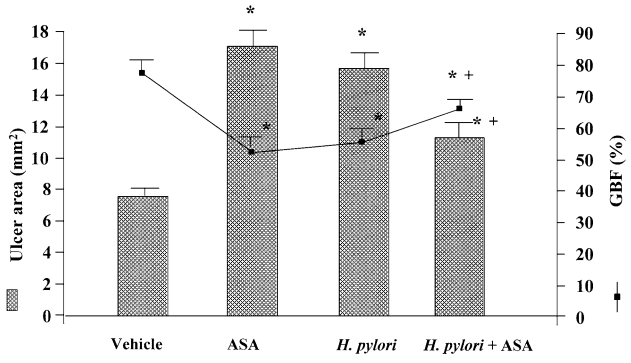

Fig. 1. Mean area of gastric ulcers and changes in gastric blood flow (GBF) at day 16 in rats treated intragastrically with vehicle, live *H. pylori*, acidified aspirin (aspirin, 50 mg/kg/day), and aspirin combined with *H. pylori* administered at days 2, 4 and 6 before aspirin treatment. Mean ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared to the value obtained in vehicle-treated rats. Cross indicates significant change as compared to the value obtained in aspirin-treated rats.

Table 1
Colonization levels of *H. pylori* in the antral gastric mucosa, the prostaglandin E_2 generation and plasma gastrin levels in rats with gastric ulcers inoculated with vehicle, *H. pylori* with or without the combination with aspirin (50 mg/kg/day i.g.) at day 16 upon ulcer induction

Type of test	Bacterial culture (CFU/mg mucosa)	PGE_2 generation (ng/g tissue)	Plasma gastrin (pmol/l)
Intact (control)	0	184 ± 15	62 ± 8
Vehicle	0	215 ± 8 *	72 ± 4
H. pylori	1234 ± 74	249 ± 12 $^{+}$	96 ± 8 *
Aspirin	0	43 ± 4 $^{+}$	69 ± 5
H. pylori + aspirin	1148 ± 63	138 ± 6 $^{++}$	92 ± 6 *

Results are means ± S.E.M. of 8–10 rats.
* Indicates a significant change as compared to the value obtained in intact animals.
$^{+}$ Indicates a significant change as compared to the value recorded in vehicle-treated rats.
$^{++}$ Indicates a significant change as compared to the value recorded in animals treated with aspirin alone.

or aspirin alone, though it was still significantly higher than that obtained in vehicle-treated control animals (Fig. 1).

The gastric blood flow in the non-ulcerated mucosa of rats treated with vehicle averaged 46 ml/min/100 g (taken as 100%) being significantly reduced by about 13% at the ulcer margin at day 16, when compared to that in the contralateral intact mucosa. In rats treated with aspirin or *H. pylori*, a significant reduction in the gastric blood flow at the ulcer margin was observed as compared to that in vehicle-treated rats (52 ± 3% and 56 ± 4% vs. 77 ± 4%) (Fig. 1). When rats inoculated with *H. pylori* were combined with aspirin treatment, the gastric blood flow rose significantly at ulcer margin as compared to the respective values obtained in aspirin-treated or *H. pylori* inoculated rats, though the gastric blood flow in these animals failed to reach the value obtained in vehicle-treated gastric mucosa (Fig. 1).

As shown in Table 1, *H. pylori* was detected in antrum of *H. pylori*-inoculated rats but was undetectable by bacterial culture in uninoculated rats and those treated with aspirin alone. Addition of aspirin to *H. pylori*-inoculated tended to decrease, though not significantly, the number of viable bacterial colonies as compared to the value of CFUs/mg mucosa in *H. pylori*-inoculated animals without aspirin treatment.

Histologically, the gastric ulcers were healed almost completely in all rats treated with vehicle at day 16 upon ulcer induction as reflected by the ulcer scar in majority of these animals (Fig. 2A). In the ulcer margin of uninfected rats, the infiltration of both mononuclear and polymononu-

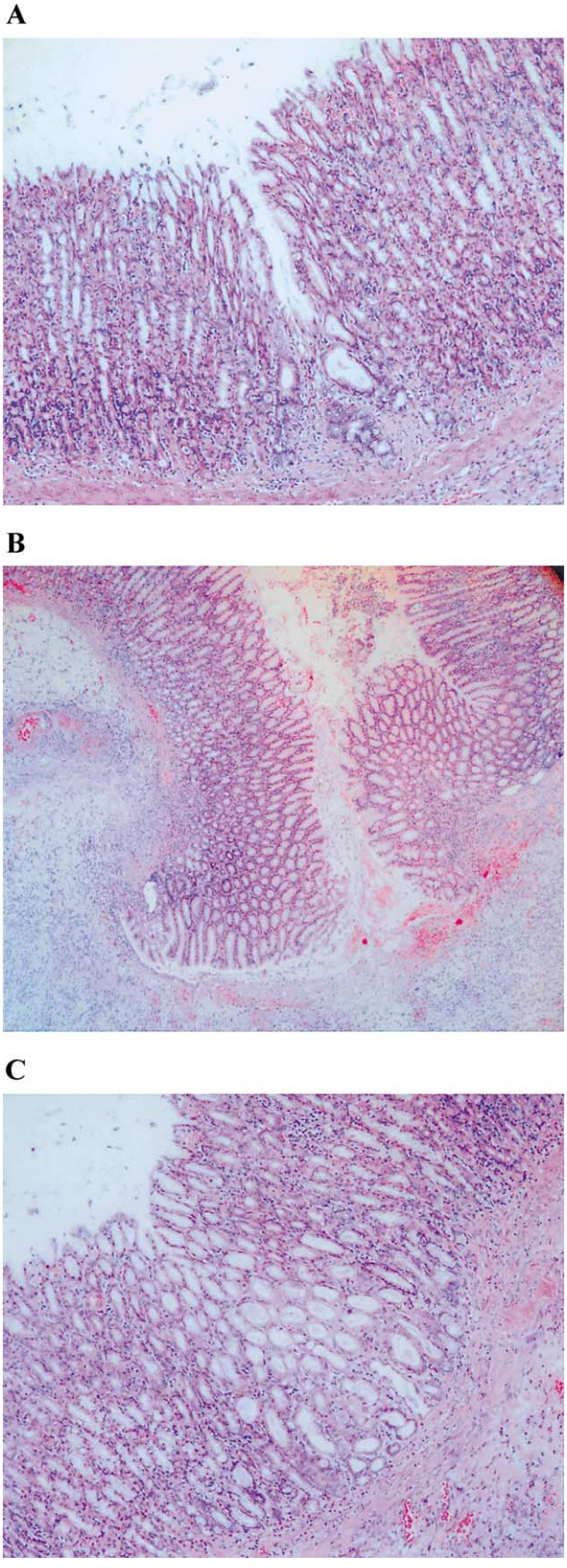

Fig. 2. Histological appearance of gastric ulcer at day 16 upon ulcer induction in rat treated with vehicle (control) (A). The gastric ulcer is healed almost completely and the infiltration of neutrophils is negligible. In *H. pylori*-infected rat, the unhealed gastric ulcer with clearly defined ulcer crater and a poor development of the mucosa at ulcer margin is evident (B). In rat treated with *H. pylori* plus aspirin, the more advanced healing and the small neutrophil infiltration of the mucosa are observed microscopically but the ulcer scar in these animals exhibits a marked gland dilatation and an incomplete restoration of mucosal cells (C).

clear neutrophils was negligible and no inflammation in the mucosa distant to the ulcer was observed. In contrast, unhealed gastric ulcers with clearly defined ulcer crater and a poor restoration of the mucosa at ulcer margin accompanied by cystic dilatation of the glandular structure and incomplete restoration of surface epithelium were observed in *H. pylori*-inoculated animals (Fig. 2B). In the ulcer margin of *H. pylori*-inoculated rats, infiltration of both mononuclear and polymorphonuclear leukocytes as well as lymphocytes was more obvious than in the non-infected animals. In rats treated with *H. pylori* plus aspirin, the more advanced healing and significant reduction in neutrophil infiltration of the mucosa were observed microscopically but the ulcer scar in these animals exhibited a marked gland dilatation and an incomplete restoration of mucosal cells.

As shown in Fig. 3, the expression of *H. pylori*-16S rRNA determined by PCR at day 16 upon ulcer induction was undetectable in animals treated with vehicle or exposed to aspirin but appeared as a strong signal in those inoculated with *H. pylori* with or without aspirin treatment.

3.2. Effect of vehicle, H. pylori alone and H. pylori combined with aspirin on gastric acid secretion, mucosal generation of prostaglandin E_2 and plasma gastrin levels

The results of gastric secretory studies in conscious rats equipped with gastric fistula with or without induction of gastric ulcers are presented in Table 2. In control rats without gastric ulcers, the basal acid output averaged 144 ± 12 μmol/30 min. Immediately after induction of gastric ulcers, the gastric acid output was significantly reduced by about 55% as compared with the respective values in control rats without ulcer induction (data not shown). In vehicle-controls at day 8, the gastric secretion tended to increase but still significant inhibition of gastric acid output was observed in vehicle-treated animals as compared to those in rats without gastric ulcers. Inoculation with *H. pylori* of rats with gastric ulcer resulted in a further significant decrease in gastric acid output at day 8 as compared to that treated with vehicle (saline). In contrast, aspirin alone (50 mg/kg/day i.g.) increased, though not significantly, gastric acid output as compared to vehicle-treated rats and failed to influence the inhibition of this secretion caused by *H. pylori*. At day 16 upon ulcer induction, the values of gastric acid output in vehicle-control rats were still significantly lower as compared to those obtained in rats without ulcers. In rats inoculated with *H. pylori*, a significant decrease in the gastric acid output was still observed at day 16 as compared to respective vehicle-controls. The treatment with aspirin alone failed to affect the gastric acid output at day 16 in rats without *H. pylori* inoculation and had no influence on the suppression of this secretion caused by *H. pylori*.

Table 2
Effect of vehicle (saline), alive *H. pylori* (three times inoculation at days 2, 4 and 6 upon ulcer induction) applied alone or combined with aspirin (50 mg/kg/day) and on gastric acid secretion in rats with gastric ulcers equipped with gastric fistula

Type of test	Gastric acid output (μmol/30 min)
Intact (Control)	144 ± 12
Day 8	
Vehicle	68 ± 6 *
H. pylori	45 ± 5 ++
Aspirin	87 ± 4 +
H. pylori + aspirin	48 ± 5 ++
Day 16	
Vehicle	86 ± 8 *
H. pylori	66 ± 5 ++
Aspirin	109 ± 7 +
H. pylori + aspirin	69 ± 8 ++

Results are means ± S.E.M. of 8–10 rats.
* Indicates a significant change as compared with the value obtained in vehicle-treated animals.
+ Indicates significant change as compared with the value obtained in animals treated with *H. pylori*.
++ Indicates a significant decrease as compared to the value obtained in vehicle control animals at respective days upon ulcer induction.

The results of treatment with vehicle, aspirin and *H. pylori* applied alone or in combination with aspirin, on the generation of prostaglandin E_2 in the gastric mucosa, are presented in Table 1. The prostaglandin E_2 generation in the intact gastric mucosa averaged 184 ± 15 ng/g wet tissues weight and this significantly increased at ulcer margin of vehicle-treated controls. In *H. pylori* inoculated rats, a further significant increase in the prostaglandin E_2 generation was observed as compared to the value of this generation recorded in vehicle-treated animals. Aspirin (50 mg/kg/day i.g.), which by itself increased significantly the area of gastric ulcers (Fig. 1), suppressed prostaglandin E_2 generation in the gastric mucosa by ~85%. Addition of aspirin to *H. pylori* attenuated significantly the rise in the prostaglandin E_2 generation induced by *H. pylori*.

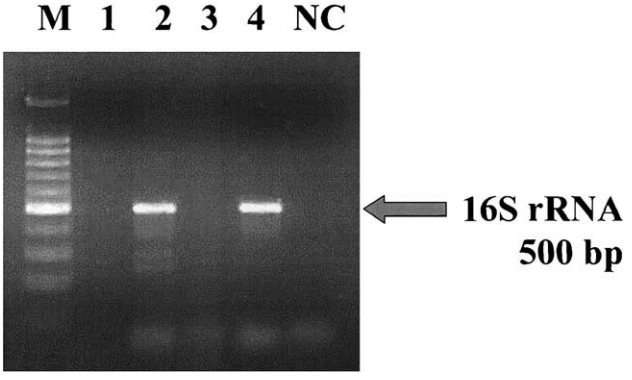

Fig. 3. Representative analysis of 16S rRNA expression at day 16 upon ulcer induction in the gastric mucosa excised from ulcer margin of rat treated with vehicle (lane 1), *H. pylori* (lane 2), aspirin (lane 3) and *H. pylori* followed by a 10-day treatment with aspirin (lane 4). M—DNA molecular weight marker, NC—negative control (lane 5).

As shown in Table 1, the fasting plasma gastrin levels at day 16 upon ulcer induction averaged 62 ± 8 pM/l and this was significantly elevated in animals inoculated with *H. pylori*. The concurrent treatment with aspirin, which significantly prolonged the ulcer healing, failed to influence significantly the plasma gastrin levels in vehicle-treated gastric mucosa. When aspirin was administered to rats inoculated three times with *H. pylori* prior to aspirin treatment, no significant alterations in the plasma gastrin levels were observed as compared to the values recorded in animals treated with *H. pylori* alone (Table 1).

3.3. Effect of vehicle, aspirin with or without combination with H. pylori on the mRNA expression of cyclooxygenase-1, cyclooxygenase-2, transforming growth factor alpha and vascular endothelial growth factor in the gastric mucosa

As shown in Fig. 4, the expression of cyclooxygenase-1 was not significantly altered in rats with gastric ulcers treated with vehicle, *H. pylori* or aspirin alone or in those treated with the combination of aspirin with *H. pylori*, as compared to intact mucosa. On the contrary, cyclooxygenase-2 expression was significantly elevated in vehicle-treated ulcerated gastric mucosa as compared to the intact gastric mucosa. The cyclooxygenase-2 expression also remained at the increased level in the rats treated with aspirin or *H. pylori* alone or in those treated with the combination of aspirin and bacteria as compared to that detected in intact gastric mucosa. However, there were no significant changes in the mucosal expression of cyclooxygenase-2 in rats with gastric ulcer treated with vehicle, aspirin, *H. pylori* or *H. pylori* combined with aspirin.

The expression of transforming growth factor alpha and vascular endothelial growth factor mRNA was detectable as a faint signal in the intact gastric mucosa (Fig. 5). In the ulcerated mucosa, a significant increase in the levels of mRNA expression for transforming growth factor alpha and vascular endothelial growth factor was observed. The treatment with aspirin resulted in a significant reduction in the expression of transforming growth factor alpha and vascular endothelial growth factor mRNAs as compared to those observed in vehicle-treated animals. In contrast, in rats treated with *H. pylori*, the expression of transforming growth factor alpha and vascular endothelial growth factor remained at the increased level as compared to those recorded in intact mucosa. In rats inoculated with *H. pylori* followed by aspirin, the expression of the messenger RNA for these two growth factors was significantly up-regulated toward the level observed in the vehicle-treated rats (Fig. 5).

3.4. Immunoblot analysis of cyclooxygenase-1 and cyclooxygenase-2 protein expression in the gastric mucosa of rats treated with vehicle, H. pylori or aspirin alone or aspirin applied in combination with live H. pylori

As shown in Fig. 6, the expression of cyclooxygenase-1 protein was detectable in the intact gastric mucosa and has not been altered in rats with gastric ulcer treated with vehicle aspirin and *H. pylori* alone or in those treated with the combination of *H. pylori* and aspirin. On the contrary,

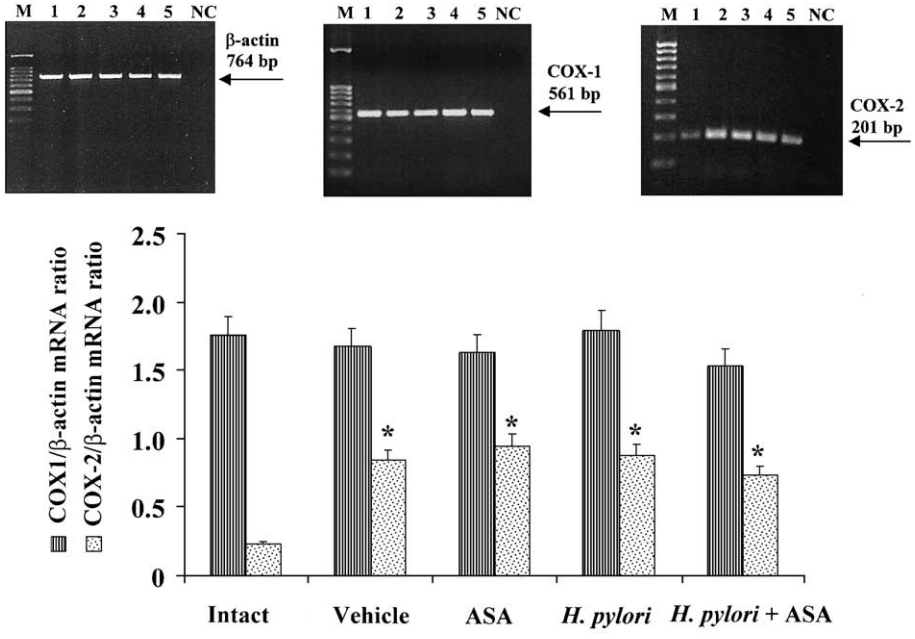

Fig. 4. Representative analysis of mRNA expression for cyclooxygenase-1 and cyclooxygenase-2 in the intact gastric mucosa (lane 1), and in those with acetic acid ulcer treated with vehicle (lane 2), aspirin (lane 3), *H. pylori* (lane 4) and *H. pylori* followed by a 10-day treatment with aspirin (lane 5). M—DNA molecular weight marker, NC—negative control. Bottom—results of densitometry analysis. Asterisk indicates a significant change as compared to the value obtained in intact gastric mucosa.

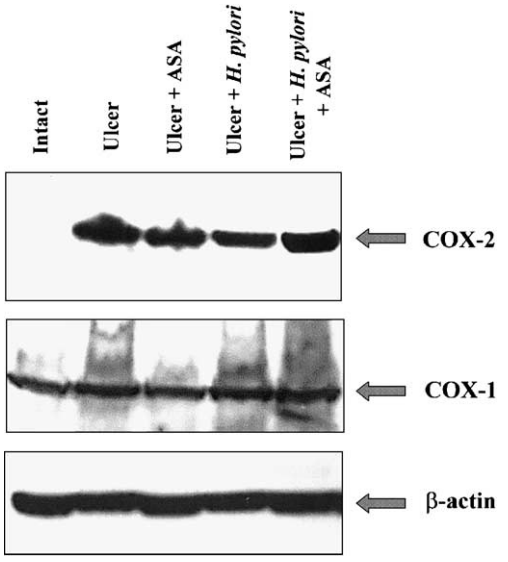

Fig. 5. Representative Western blot analysis of protein expression for cyclooxygenase-1 and cyclooxygenase-2 in the gastric mucosa of rat without ulcer (intact) (lane 1), ulcer and treated with vehicle (lane 2), ulcer and treated with aspirin (lane 3), ulcer and treated with *H. pylori* (lane 4) and ulcer with administration of *H. pylori* followed by a 10-day treatment with aspirin (lane 5).

the cyclooxygenase-2 expression was undetectable at the level of protein in the intact gastric mucosa, but increased significantly in vehicle-treated animals with chronic acetic acid ulcers. The treatment with aspirin, *H. pylori* alone or the combination of aspirin with live *H. pylori* did not result in the further significant change in cyclooxygenase-2 expression, which remained at the increased level similar to that observed in vehicle-control gastric mucosa (Fig. 6).

4. Discussion

This study demonstrates that gastric inoculation of rats with *H. pylori* at the start of the treatment with aspirin, partly reduced the delay in healing caused by this agent. The successful colonization of the rat gastric mucosa by *H. pylori* was confirmed in our study by quantitative *H. pylori* culture and by detection of bacterial DNA by PCR method as well as by histological examination of neutrophil infiltration reflecting the inflammatory changes in gastric mucosa, especially in the area of gastric ulcer. The colonization by *H. pylori* of rat gastric mucosa was mild to moderate and comparable to that in *H. pylori*-infected mice as shown in our previous studies (Brzozowski et al., 1998; Konturek et al., 1999) but was definitively less pronounced than that reported in humans (Dixon et al., 1996). It is of interest that the *H. pylori*-infected mucosa not involved in gastric ulcer failed to show significant inflammation, confirming the observations by other investigators (Li et al., 1997) that mild or moderate mucosal inflammation in rats infected with *H. pylori* is limited predominantly to the ulcer area in this species, thus providing a useful model for studying the

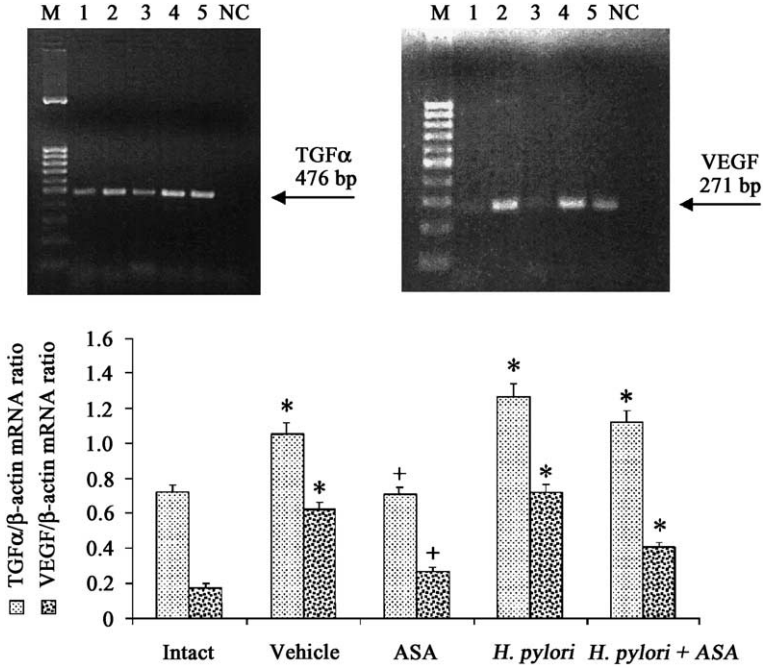

Fig. 6. Representative analysis of mRNA expression for transforming growth factor alpha and vascular endothelial growth factor in the intact gastric mucosa (lane 1) and in those with gastric ulcer treated with vehicle (lane 2), aspirin (lane 3), *H. pylori* (lane 4) and *H. pylori* followed by a 10-day treatment with aspirin (lane 5). M—DNA molecular weight marker, NC—negative control. Bottom—results of densitometry analysis. Asterisk indicates a significant change as compared to the value obtained in intact gastric mucosa. Cross indicates a significant change as compared to the value obtained in vehicle-treated control animals.

pathogenesis of ulcerogenesis by *H. pylori* infection, which, however, may not exactly reflect that occurring in humans. This notion is supported by the fact that we used *H. pylori* isolated from duodenal ulcer patients who show antrum-predominant gastritis and acid hypersecretion. However, in our present study, rats infected with *H. pylori* exhibited hypochlorhydria rather that acid hypersecretion indicating that our animal data at least in terms of secretory changes represent the effects of acute gastric inflammation and, therefore, could not be extrapolated into the human scenario of chronic *H. pylori* infection. Both *H. pylori* and aspirin separately delayed healing of preexisting gastric ulcers in our present study but their combination failed to delay the ulcer healing more than that achieved by application of aspirin or *H. pylori* alone. This could be due to the suppression of acid secretion by bacteria itself or to the antisecretory activity of *H. pylori* derived lipopolysaccharide (LPS) endotoxins (Konturek et al., 1998a,b) that may result in the reduction in acid secretion and in the limitation of action of acid-dependent ulcerogens such as aspirin on ulcer healing. This *H. pylori*-induced gastric hypochlorhydria, which was not seen in animals treated with aspirin alone, could result in marked hypergastrinemia observed in *H. pylori*-infected animals suggesting that gastrin, originally recognized for its trophic effect on gastric mucosa, may also contribute to the acceleration of ulcer healing observed in rats treated concomitantly with *H. pylori* and aspirin.

Our observation is in keeping with the notion that the action of *H. pylori* and aspirin may not be synergistic on ulcer healing but actually seems to be antagonistic, at least in our model with preexisting ulcers induced by acetic acid. This is in keeping with recent clinical observation by Hawkey et al. (1998) that in NSAID users with peptic ulcer treated by conventional acid suppressors such as omeprazole, *H. pylori* eradication was not associated with a significant enhancement in healing of gastric ulcers as compared to that in long-term NSAID users who were *H. pylori*-infected. Apparently, both these pathogenic factors, namely, *H. pylori* and aspirin, each acting independently, prolonged ulcer healing in human subjects (Hawkey et al., 1998) and also delayed healing of preexisting experimental ulcerations in our present study.

Another explanation for the mechanism of *H. pylori*-induced acceleration of ulcer healing in rats exposed to aspirin could be the pronounced enhancement in prostaglandin E_2 production possibly due to overexpression of cyclooxygenase-2 and the up-regulation of the growth factors such as transforming growth factor alpha and vascular endothelial growth factor in the ulcer area observed in animals inoculated with these bacteria. Our data indicate that exposure of rat gastric mucosa to live *H. pylori* triggers mucosal defense mechanisms that appear to attenuate, in part, the deleterious effect of aspirin on ulcer healing. One possible explanation for the antagonistic effects of the combined treatment with aspirin and *H. pylori* on the ulcer healing as compared to *H. pylori* or aspirin applied alone could be the overexpression of cyclooxygenase-2 by *H. pylori* with consecutive elevation of prostaglandin E_2 production in the gastric mucosa. This mechanism could, at least in part, counteract the inhibition of mucosal prostaglandins generation induced by aspirin, since prostaglandin E_2 is known to be an important mucosal defensive factor leading to increased mucosal protection and to a rise in gastric blood flow and secretion of mucus and bicarbonates (Wallace, 1996).

The gastric inoculation with *H. pylori* combined with aspirin treatment failed to produce further increase in the mRNA and protein expression of cyclooxygenase-2 as compared to those detected in *H. pylori* or aspirin administered separately to rats with acetic acid ulcers. This rapid up-regulation of cyclooxygenase-2 mRNA in response to aspirin has been recognized before and seems to represent a compensatory response to inhibition of gastric prostaglandin synthesis (Davies et al., 1997). In agreement to this hypothesis, we have shown in this report that the dose (50 mg/kg/day) of nonselective cyclooxygenase inhibitor such as aspirin induced an overexpression of cyclooxygenases-2 at the level of mRNA and protein but suppressed the cyclooxygenase-1 and cyclooxygenases-2 enzyme activities as documented by the profound decrease in the generation of prostaglandin E_2 in the gastric mucosa in aspirin-treated animals. Thus, we provided an experimental evidence that aspirin and *H. pylori* differ from each other in their ability to influence the mucosal prostaglandin generation because aspirin suppressed while *H. pylori* actually enhanced this generation. This did not necessarily correspond to another protective parameter such as gastric blood flow at ulcer margin that was actually diminished despite the fact that prostaglandin E_2 generation was increased in *H. pylori*-inoculated stomach. This fall in the gastric blood flow in *H. pylori*-infected mucosa paralleled with hyposecretion in these animals, both being possibly attributed to *H. pylori*-cytotoxins such as LPS and ammonia released from these bacteria (Brzozowski et al., 1995). When *H. pylori* was combined with aspirin, the prostaglandin E_2 generation remained somewhat higher than that in aspirin alone-treated animals suggesting that *H. pylori*, when applied prior to aspirin, counteracted, in part, the inhibition of endogenous prostaglandin E_2 caused by aspirin. Thus, we assume that the limited effect of aspirin on ulcer healing in *H. pylori*-infected stomach is mediated by the rise in the endogenous prostaglandins derived from *H. pylori*-induced up-regulation of cyclooxygenase-2 mRNA and protein. This notion is supported by our finding that under the same experimental conditions, *H. pylori* up-regulated both cyclooxygenase-2 mRNA and protein but remained without significant influence on cyclooxygenase-1 expression. Further studies are needed to explain whether cyclooxygenase inhibition by aspirin has beneficial influence on the pathogenic action of *H. pylori* in rats with healing of preexisting gastric ulcers. It is of interest that the presence of aspirin in the stomach tended to reduce the number of bacteria possibly due to

direct action of this drug on the growth of this germ (see Table 1).

On the other hand, recent study showed that *H. pylori* impaired the gastric adaptation to aspirin in humans as evidenced by persistent microbleeding, suggesting that *H. pylori* enhances the gastric toxicity of this NSAID (Konturek et al., 1998a,b). In all subjects, aspirin-induced gastric damage reached its maximum on day 3, while in those infected with *H. pylori*, this damage was maintained at a similar level up to day 14. After *H. pylori* eradication, the damage was significantly lessened both in endoscopy and histology at day 14 and was accompanied by increased mucosal expression and luminal release of transforming growth factor alpha (Konturek et al., 1998a,b). Similarly, as in our present animal study, the prostaglandin E_2 generation was significantly greater in *H. pylori*-positive subjects than after *H. pylori* eradication, but aspirin treatment resulted in >90% reduction of this generation independent of *H. pylori* status (Konturek et al., 1998a,b). They concluded that gastric adaptation to aspirin was impaired in *H. pylori*-positive subjects, but eradication of this bacterium restored this adaptation process (Konturek et al., 1998a,b). Our current animal findings appeared to be contradictory to this observation in humans but the difference could be easily explained by the difference in experimental conditions and the fact that we used rats with chronic gastric ulcers, while their study recruited human volunteers without previous ulcer history (Konturek et al., 1998a,b). Moreover, the dose of 50 mg/kg of aspirin applied intragastrically by itself failed to cause gross mucosal damage in the rat stomach but prolonged significantly healing of preexisting gastric ulcerations. Since gastric adaptation in experimental animals is triggered by the direct contact of the gastric mucosa with ulcerogen such as aspirin applied in injurious dose (Brzozowski et al., 1996), it is apparent that we could not address the question as to whether *H. pylori* infection influences the phenomenon of gastric adaptation to aspirin in our rat model with chronic gastric ulcers.

Another attempt in this study was to determine the interaction of two major mucosal damaging factors such as *H. pylori* and aspirin on the expression of mucosal growth factors such as transforming growth factor alpha and vascular endothelial growth factor. We found that aspirin significantly reduced the expression of messenger RNA for transforming growth factor alpha and vascular endothelial growth factor in rats with gastric ulcers. The mechanism of this action is not fully explained but could be attributed to aspirin-induced depletion of gastric mucosal prostaglandin synthesis that are known to stimulate growth factors in the gastric mucosa (Fig. 7). This notion is in keeping with the observation by Takahashi et al. (1996) that prostaglandins stimulate vascular endothelial growth factor and vascular endothelial growth factor expression in gastric fibroblasts. Aspirin has also been shown to reduce transforming growth factor alpha expression in human rectal mucosa from individuals with a history of adenomatous polyps of the colon (Barnes et al., 1999). All these observations may indicate that prostaglandins are important inducers of expression of growth factors in gastric mucosa. On the other hand, there is also evidence that the increased expression of growth factors, including transforming growth factor alpha, vascular endothelial growth factor and gastrin, contribute to the increased cyclooxygenase-2 expression in *H. pylori*-infected mucosa resulting in the increased prostaglandin synthesis (Konturek et al., 2001). On the basis of previous observations and our present findings, we conclude that suppression of growth factor expression by aspirin, especially in the area of gastric ulcer, can contribute significantly to the delay in ulcer healing observed in these animals. This is supported by the fact that the inoculation with *H. pylori* that reversed, in part, the aspirin-induced suppression of growth factor expression, attenuated the delay in healing with an accompanying fall in the microcirculatory response at the ulcer margin induced by aspirin.

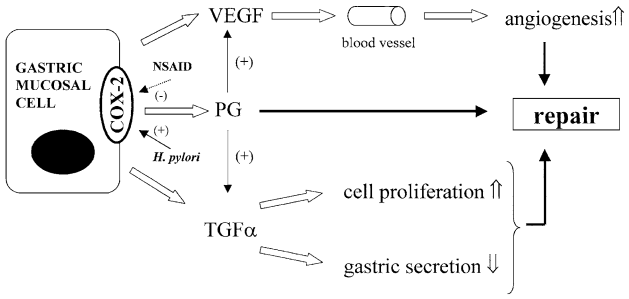

Fig. 7. Schematic presentation of the possible interaction between aspirin and *H. pylori* in ulcer healing.

In conclusion, *H. pylori* attenuates the aspirin-induced delay in gastric ulcer healing in the rat model and this effect may be due to the inhibition of gastric acid secretion, hypergastrinemia, known to exert trophic influence on the gastric mucosa, and to the up-regulation mRNA and the activity of cyclooxygenase-2, leading to enhancement of endogenous prostaglandins and overexpression of mucosal growth factors, including transforming growth factor alpha and vascular endothelial growth factor.

Acknowledgements

This study was supported by the interdisciplinary Center for Clinical Investigation (Project A3) at the University Erlangen-Nuremberg.

References

Barnes, C.J., Hamby-Mason, R.L., Hardman, W.E., Cameron, I.L., Speeg, K.V., Lee, M., 1999. Effect of aspirin on prostaglandin E_2 formation and transforming growth factor alpha expression in human rectal mucosa from individuals with a history of adenomatous polyps of the colon. Cancer Epidemiol. Biomark. Prev. 8, 311–315.

Bianchi Porro, G., Parente, F., Imbesi, V., Montrone, F., Caruso, I., 1999.

Role of Helicobacter in ulcer healing and recurrence of gastric and duodenal ulcers in long term NSAID users; response to omeprazole dual therapy. Gut 39, 22–26.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Ernst, H., Stachura, J., Hahn, E.G., 1995. Gastric adaptation to injury by repeated doses of aspirin strengthens mucosal defense against subsequent exposure to various strong irritants in rats. Gut 37, 749–757.

Brzozowski, T., Konturek, P.C., Konturek, J., Ernst, H., Sliwowski, Z., Hahn, E.G., 1996. Mucosal irritation, adaptive cytoprotection and adaptation to topical ammonia in the rat stomach. Scand. J. Gastroenterol. 31, 837–846.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Karczewska, E., Pajdo, R., Stachura, J., Ghiara, P., Hahn, E.G., 1998. Gastric secretion and ulcer healing in mouse stomach infected with cytotoxin expressing strain of *Helicobacter pylori*. J. Physiol. Pharmacol. 49, 387–403.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Schuppan, D., Drozdowicz, D., Kwiecien, S., Majka, J., Nakamura, T., Hahn, E.G., 2001. Effect of local application of growth factors on gastric ulcer healing and mucosal expression of cyclooxygenase-1 and cyclooxygenase-2. Digestion 64, 15–29.

Chan, F.K.L., 2002. *Helicobacter pylori*, NSAIDs and gastrointestinal hemorrhage. Eur. J. Gastroenterol. Hepatol. l4, 1–3.

Chan, F.K.L., Sung, J.J., Chung, S.C., To, K.F., Yung, M.Y., Leung, V.K., Lee, Y.T., Chan, C.S., Li, E.K., Woo, J., 1997. Randomized trial of eradication of *H. pylori* before non-steroidal anti-inflammatory drug therapy to prevent peptic ulcers. Lancet 350, 975–979.

Chan, F.K., Sung, J.J., Suen, R., Lee, Y.T., Wu, J.C., Leung, W.K., Chan, H.L., Lai, A.C., Lau, J.Y., Ng, E.K., Chung, S.C., 1998. Does eradication of *Helicobacter pylori* impair healing of nonsteroidal anti-inflammatory drug associated bleeding peptic ulcers? A prospective randomized study. Aliment. Pharmacol. Ther. 12, 1201–1205.

Chomczynski, P., Sacchi, N., 1987. Single step method of RNA isolation by acid guanidinum thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Coffey, R.J., Romano, M., Goldenring, J., 1995. Roles for transforming growth factor-α in the stomach. J. Clin. Gastroenterol. 21 (Suppl. 1), S36–S39.

Davies, N.M., Sharkey, K.A., Asfaha, S., MacNaughton, W.K., Wallace, J.L., 1997. Aspirin causes rapid up-regulation of cyclooxygenase-2 expression in the stomach of the rats. Aliment. Pharmacol. Ther. 11, 1101–1108.

Dixon, M.F., Genta, R.M., Yardley, J.H., Correa, P., 1996. Classification and grading of gastritis. The updated Sydney system. International Workshop on the Histopathology of Gastritis, Houston 1994. Am. J. Surg. Pathol. 20, 1161–1181.

Drazek, E.S., Dubois, A., Holmes, R.K., 1994. Characterization and presumptive identification of *Helicobacter pylori* isolates from rhesus monkeys. J. Clin. Microbiol. 32, 1799–1804.

Fendrick, A.M., Scheiman, J.M., 2001. *Helicobacter pylori* and NSAID gastropathy: an ambiguous association. Curr. Rheumatol. Rep. 1 3 (2), 107–111.

Hawkey, C.J., Tulassay, Z., Szczepanski, L., van Rensburg, C.J., Filipowicz-Sosnowska, A., Lanas, A., Wason, C.M., Peacock, R.A., Gillon, K.R., 1998. Randomized controlled trial of *Helicobacter pylori* eradication in patients on nonsteroidal anti-inflammatory drugs: the HELP NSAIDs study. Helicobacter Eradication for Lesion Prevention. Lancet 352, 1016–1021.

Hirose, H., Takeuchi, K., Okabe, S., 1991. Effect of indomethacin on gastric mucosal blood flow around acetic acid-induced gastric ulcers in rats. Gastroenterology 100, 1259–1265.

Huang, J.-Q., Sridhar, S., Hunt, R., 2002. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic ulcer disease: a meta-analysis. Lancet 359, 14–20.

Konturek, P.C., Brzozowski, T., Konturek, S.J., Ernst, H., Drozdowicz, D., Pajdo, R., 1997. Expression of epidermal growth factor and transforming growth factor alpha during ulcer healing. Scand. J. Gastroenterol. 32, 6–15.

Konturek, J.W., Dembinski, A., Konturek, S.J., Stachura, J., Domschke, W., 1998a. Infection of *Helicobacter pylori* in gastric adaptation to continued administration of aspirin in humans. Gastroenterology 114, 245–255.

Konturek, P.C., Brzozowski, T., Konturek, S.J., Taut, A., Sliwowski, Z., Stachura, J., Hahn, E.G., 1998b. Activation of genes for growth factors and cyclooxygenase in rat gastric mucosa during recovery from stress damage. Eur. J. Pharmacol. 342, 55–65.

Konturek, P.Ch., Brzozowski, T., Konturek, S.J., Stachura, J., Karczewska, E., Pajdo, R., Ghiara, P., Hahn, E.G., 1999. Mouse model of *Helicobacter pylori* infection: studies of gastric function and ulcer healing. Aliment. Pharmacol. Ther. 13, 333–346.

Konturek, S.J., Konturek, P.C., Plonka, A., Duda, A., Sito, E., Zuchowicz, M., Hahn, E.G., 2001. Implication of gastrin in cyclooxygenase-2 expression in *Helicobacter pylori* infected gastric ulceration. Prostaglandins Other Lipid Mediat. 66, 39–51.

Li, H., Mellgard, B., Helander, H., 1997. Inoculation of VacA- and CagA-*Helicobacter pylori* delays gastric ulcer healing in rat. Scand. J. Gastroenterol. 32, 439–444.

Li, H., Kalies, I., Mellegard, B., Helander, H.F., 1998. A rat model of chronic Helicobacter infection. Studies on epithelial cell turnover and gastric ulcer healing. Scand. J. Gastroenterol. 33, 370–378.

Loeb, D.S., Talley, N.J., Alquist, D.A., Carpenter, H.A., Zinsmeister, A.R., 1992. Long term nonsteroidal anti-inflammatory drug use and gastroduodenal injury: the role of *Helicobacter pylori*. Gastroenterology 102, 1899–1905.

Neufeld, G., Tzafra, C., Gengrinovitch, S., Poltorak, Z., 1999. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 13, 9–22.

Okabe, S., Pfeiffer, C.J., Roth, J.L.A., 1971. A method for experimental, penetrating gastric and duodenal ulcers in rats. Am. J. Dig. Dis. 16, 277–284.

Penney, A.G., Andrews, F.J., O B'rien, P.E., 1994. Effects of misoprostol on delayed ulcer healing induced by aspirin. Dig. Dis. Sci. 39, 934–939.

Szabo, S., Vincze, A., 2000. Growth factors in ulcer healing: lessons from recent studies. J. Physiol. (Paris) 94 (2), 77–81.

Takahashi, M., Ota, S., Hata, Y., Mikami, Y., Azuma, N., Nakamura, T., Terano, A., Omata, M., 1996. Hepatocyte growth factor as a key to modulate anti-ulcer action of prostaglandins in stomach. J. Clin. Invest. 98, 2604–2611.

Takahashi, M., Kawabe, T., Ogura, K., Maeda, S., Mikami, Y., Kaneko, N., Terano, A., Omata, M., 1997. Expression of vascular endothelial growth factor at the human gastric margin and in cultured gastric fibroblasts: a new angiogenic factor for gastric ulcer healing. Biochem. Biophys. Res. Commun. 234, 493–498.

Takahashi, M., Maeda, S., Ogura, K., Terano, A., Omata, M., 1998. The possible role of vascular endothelial growth factor (VEGF) in gastric ulcer healing: effect of sofalcone on VEGF release in vitro. J. Clin. Gastroenterol. 27 (Suppl. 1), S178–S182.

Tarnawski, A., 1993. Cellular mechanism of gastric ulcer healing. In: Domschke, W., Konturek, S.J. (Eds.), The Stomach. Springer-Verlag, Berlin, pp. 177–192.

Tytgat, G.N.J., 2000. Ulcers and gastritis. Endoscopy 32, 108–117.

Wallace, J.L., 1996. Cooperative modulation of gastrointestinal mucosal defense by prostaglandins and nitric oxide. Clin. Invest. Med. 19, 346–351.

Wang, J.Y., Yamasaki, S., Takeuchi, K., Okabe, S., 1989. Delayed healing of acetic acid-induced gastric ulcers in rats by indomethacin. Gastroenterology 96, 393–402.