JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2000, 51, 4, 751—773

T. BRZOZOWSKI, P. CH. KONTUREK *, S. J. KONTUREK, R. PAJDO, D. SCHUPPAN, D. DROZDOWICZ, A. PTAK, M. PAWLIK, T. NAKAMURA **, E. G. HAHN*

INVOLVEMENT OF CYCLOOXYGENASE (COX)-2 PRODUCTS IN ACCELERATION OF ULCER HEALING BY GASTRIN AND HEPATOCYTE GROWTH FACTOR

Department of Physiology, Jagiellonian University School of Medicine, Cracow, Poland
* Department of Medicine I, University of Medicine Erlangen-Nuremberg, Erlangen, Germany and
** Biomedical Research Center, Osaka University Medical School, Japan

Ulcer healing involves expression of various growth factors including hepatocyte growth factor (HGF) at the ulcer margin and the rise in plasma gastrin but the effects of locally applied HGF and gastrin, which are known to act as trophic factors for the gastric mucosa, with or without neutralizing antibodies against HGF and gastrin or COX-1 and COX-2 inhibitors on ulcer healing and the expression of cyclooxygenase (COX)-1 and COX-2 during this healing have been little studied. Rats with gastric ulcers induced by serosal application of acetic acid (ulcer area 28 mm^2) received a submucosal injection of either: 1)vehicle (saline), 2) HGF and 3) gastrin with or without neutralizing antibodies against HGF and gastrin or treatment with indomethacin (2 mg/kg-d i.p.), a non-specific inhibitor of COX, or NS-398 (5 mg/kg-d i.g.) and Vioxx (10 mg/kg-d i.g.), both highly specific COX-2 inhibitors. Each growth factor and specific antibodies against HGF and gastrin (100 ng/100 µl each) were injected just around the ulcer immediately after ulcer induction and this local application was repeated at day 2 following anesthesia and laparotomy. At day 13 and 21, the area of ulcers was determined by planimetry, the gastric blood flow (GBF) at ulcer margin was examined by H_2-gas clearance technique and mucosal generation of PGE_2 and the expression of COX-1 and COX-2 mRNA in the non-ulcerated and ulcerated gastric mucosa was analyzed using RT-PCR. The gastric ulcers healed progressively within 21 days and this effect was accompanied by significant increase in the GBF at the ulcer margin and expression of COX-2 mRNA and COX-2 protein at the ulcer area. Treatment with HGF and gastrin significantly accelerated the rate of ulcer healing and raised GBF at ulcer margin causing further significant upregulation of COX-2 mRNA and COX-2 protein (but not of COX-1 mRNA) in the ulcerated mucosa. The upregulation of COX-2 mRNA induced by HGF was significantly attenuated by the concurrent local treatment with antibody against this growth peptide. Indomethacin and both COX-2 inhibitors significantly prolonged the ulcer healing, while suppressing the generation of PGE_2 in non-ulcerated and ulcerated gastric mucosa and the GBF at ulcer margin. The acceleration of ulcer healing by HGF and gastrin and accompanying rise in the GBF at ulcer margin were significantly attenuated by the concurrent treatment with indomethacin or NS-398 and Vioxx. HGF injections produced a significant rise in the plasma gastrin levels and this was significantly attenuated by the cotreatment with NS-398. We conclude that 1) neutralization of HGF and gastrin by their specific

antibodies delays ulcer healing due fall in the microcirculation around the ulcer and a decrease in the COX-2 expression, 2) COX-2 derived prostaglandins may play an important role in acceleration of the ulcer healing by various growth factors including HGF and gastrin, 3) enhancement of the local pool for growth factors such as HGF and gastrin at the ulcer site could offer a new modality for treatment of gastric ulcer.

Key words: *ulcer healing, hepatocyte growth factor, gastrin, prostaglandin, gastric blood flow, cyclooxygenase-1, cyclooxygenase-2.*

INTRODUCTION

It is known that ulcer healing involves expression of various growth factors and their receptors at the ulcer margin (1—5). Administration of growth factors have been shown to accelerate ulcer healing by increasing epithelial cell proliferation and angiogenesis in the ulcer bed (6—8). Hepatocyte growth factor (HGF), also identified as a scatter factor, is a mesenchymally derived pleiotropic factor that mediates epithelial-mesenchymal interactions (9—11). Studies *in vivo* and *in vitro* have demonstrated that HGF binds to upregulated c-met/HGF receptor on epithelial cells in the gastric ulcer margin to enhance cell scattering and migration and to stimulate cell proliferation (12, 13). Recent evidence indicates that mRNA for both, HGF and c-met/HGF receptors is rapidly increased following acute gastric mucosal injury as compared to non-injured gastric mucosa suggesting that this peptide plays an important role in the gastric defense and mucosal repair (12).

Gastrin is known to be a potent stimulant of gastric acid secretion due to the release of histamine from enterochromatoffin-like cells (ECL) that acts on specific H_2-receptors on the parietal cells (14). Gastrin was originally reported to increase the cell proliferation and to exert the trophic action on the oxyntic mucosa (15) but its contribution to the mechanism of gastric mucosal integrity and ulcer healing has been little studied. We have demonstrated previously (16) that gastrin-17 (G-17) prevents ethanol-induced gastric lesions with the extent similar to that exhibited by peptone meal, known to be a potent releaser of this hormone . It was proposed that the protective activity exhibited by endogenous and exogenous gastrin involves an activation of specific gastrin receptors (CCK_B-R) and depends upon the enhancement of gastric microcirculation through the nitric oxide (NO), a potent vasodilatator released from sensory nerves (16). Although the gastroprotective activity of antral hormone is well-established, little is known about the role of this hormone in ulcer healing and in the microcirculatory response at the ulcer margin and about the implication of products of cyclooxygnease (COX) in these processes.

Among two isoforms of COX, constitutive COX-1 provides prostaglandins (PG) that control physiological functions such as mucosal integrity, gastric

secretion, motility and mucosal blood flow, whereas inducible isoform, COX-2 that is promoted by mitogens, endotoxins and inflammatory mediators, is primarily overexpressed at the site of inflammation (17—19). Recently, COX-2 mRNA expression and upregulation of c-met/HGF was demonstrated at the ulcer edge during healing of experimental gastric ulcers (20, 21) suggesting that PG-derived from COX-2 that is coexpressed with HGF, may play an important role in healing of these chronic ulcers. Furthermore, HGF accelerated ulcer healing *via* an increase in cell proliferation involving activation of COX-2 and specific MAP kinases, particularly, ERK-2 signaling pathway (21). No attempts have been made to determine whether local application of HGF or gastrin simulating their paracrine release at or near the area of gastric ulcer, can accelerate the healing of pre-existing ulcerations and whether COX isoforms, especially COX-2, contributes to the mechanism of ulcer healing by both, HGF and gastrin applied directly to the ulcer area.

In the present study, we determined the effect of local application of HGF and gastrin by submucosal injections of these peptides into the margin of gastric ulcers (22), on the healing of chronic gastric ulcerations and accompanying changes in of the gastric blood flow (GBF) and generation of PGE_2 in the intact and ulcerated gastric mucosa. In addition, the effect of specific antibodies against HGF and gastrin applied submucosally, with or without the combination with exogenous HGF and gastrin, was employed to determine whether neutralization of these peptides by antibodies can influence their effects on ulcer healing and the GBF at ulcer margin. Attempts have been made to elucidate the influence of the suppression of COX activity by non-selective COX as well as selective COX-2 inhibitors (20, 23) on ulcer healing and the expression of COX-1 and COX-2 mRNA in the gastric mucosa of rats with or without the submucosal injection with gastrin and HGF.

MATERIAL AND METHODS

Male Wistar rats weighing 180—220 g were used in all studies. Rats were fasted 18 h before the experiment without any limitation of their free access the drinking water.

Production of gastric ulcers

Gastric ulcers were produced in 120 fasted rats using our modification (24) of acetic acid method originally proposed by Okabe *et al.* (25). With the animals under ether anesthesia, the stomach was exposed and 75 μl of acetic acid was poured through the plastic mold (6 mm diameter) onto serosal surface of anterior wall of the stomach just proximal to the antral gland area for 25 s. This produced an immediate necrosis of the entire mucosa and submucosa within the area where the acetic acid was applied, i.e., about 28 mm^2 and resulted in the formation within 2—3 days of chronic ulcers without tendency of perforation or penetration to the surrounding organs

occurring in original Okabe's technique (25, 26). After the application of acetic acid, the animals were allowed to recover from anesthesia and then they were divided into various groups and received normal chow and water ad libitum for the next 13 and 21 days.

Experimental groups and treatments

Two major series of Wistar rats (A&B), each consisting of 60 animals, were used. Rats with gastric ulcers (series A) received a submucosal injection of either: 1) vehicle (saline); 2) HGF or 3) gastrin. Each peptide (100 ng in 100 µl of phosphate buffered saline) was injected just around the ulcer immediately after ulcer induction with acetic acid (day 0) and the local application of these growth peptides was repeated after laparotomy at day 2 following ulcer induction according to the method described in detail elsewhere (22). Rats of series B received the submucosal injection with neutralizing antibodies against HGF and gastrin (100 ng in 100 µl of PBS) alone or in the combination with HGF and gastrin at day 0 and day 2 in the manner similar to that described for rats treated with the each growth factor applied alone. The human recombinant HGF and rabbit anti-human HGF antibody were kindly provided by Dr. T. Nakamura from Biomedical Research Center, Osaka University Medical School, Japan. This anti-human HGF antibody does not cross react with rat HGF and is suitable to induce complete neutralization of human HGF. The gastrin antibody was a generous gift from Prof. J. Rehfeld (University Hospital, Copenhagen, Denmark). In some tests, rats with gastric ulcers injected with or without vehicle, HGF and gastrin were pretreated with indomethacin (5 mg/kg-day i.p.), a non-selective COX inhibitor or with one of two highly selective COX-2 inhibitors, NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)-methansulfonamide; Taisho Pharmaceutical Co., Tokyo, Japan) applied i.g. in a dose of 10 mg/kg-d (20) and Vioxx (Merck Sharp and Dohme Inc., Whitehous Station, USA) administered in a dose of 5 mg/kg-d i.g. These doses of COX inhibitors were selected based on our previous studies to determine the effects of the suppression of COX-1 and COX-2 activity on ulcer healing and GBF at the ulcer area as described previously (23, 26, 27). The animals were killed at day 3, 9 and 15 after ulcer induction and the area of gastric ulcerations was determined by planimetry and the GBF was measured by H_2-gas clearance method as described below. Half of the stomach which was not involved in the GBF measurement was employed for determination of generation of PGE_2 by RIA and COX-1 and COX-2 mRNA by RT-PCR.

Determination of gastric blood flow

To evaluate the effect of vehicle, HGF and gastrin without or with concurrent treatment with HGF and gastrin antibodies on ulcer healing and gastric blood flow (GBF), the animals were anaesthetized with ether and the abdomen was opened and the stomach was exposed to assess the GBF at ulcer margin and in the adjacent intact mucosa using H_2-gas clearance technique as described before (26, 27). The H_2 clearance curve determined by one of electrodes of flowmeter was used to calculate an absolute flow rate (ml/min/100g) in the oxyntic gland area. The measurement was made in three areas of the gastric oxyntic mucosa and the mean values of these measurements were calculated and expressed as percent changes from those recorded in vehicle-treated control gastric mucosa.

Determination of plasma gastrin and PGE_2 generation in the gastric mucosa

Immediately after GBF measurement, a venous blood sample was withdrawn from the vena cava into the EDTA containing vials and used for the determination of plasma gastrin levels by radioimmunoassay (RIA) as described previously (28). The stomach was then 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.

The samples of the oxyntic gland area were taken by biopsy (about 100 mg) immediately after the animals had been killed to determine the mucosal generation of PGE_2 by specific radioimmunoassay (RIA) as described previously (23). PGE_2 was measured in duplicate using RIA kits (New England Nuclear, Munich, Germany). The capability of the mucosa to generate PGE_2 was expressed in nanograms per gram of wet tissue weight.

Expression of COX-1 and COX-2 mRNA in the gastric mucosa determined by RT-PCR

COX-1 and COX-2 mRNA were determined by RT-PCR in the oxyntic mucosa of intact rats or in those with gastric ulcer treated with HGF and gastrin with or without antibody against these growth factors at day 13 and 21 upon ulcer induction. Samples of the mucosa (about 500 mg) were scraped off on ice using glass slide and then immediately snap frozen in liquid nitrogen, and stored at −80°C. Total RNA was isolated from the gastric oxyntic mucosa according to Chomczynski and Sacchi (29) using a rapid guanidinum isothiocyanate/phenol chloroform single step extraction kit from Stratagene (Stratagene GmbH, Heidelberg, Germany). Following precipitation, the RNA was resuspended in RNase-free TE buffer and the concentration was estimated by absorbance at 260 nm wavelength. Samples were frozen at −80°C until analysis.

First strand cDNA was synthesized from total cellular RNA (5 µg) using 200 U Strata Script TM reverse transcriptase (Stratagene GmbH, Heidelberg, Germany) and oligo (dt) primers (Stratagene GmbH, Heidelberg, Germany). After the reverse transcription, the transcriptase activity was destroyed by heating, and the cDNA were stored at −20°C until PCR. The primer sequences were designed according to the published cDNA sequence for the rat cyclooxygenases (30—32). The COX-1 primer sequences were as follows: up-stream, 5'-AGC CCC TCA TTC ACC CAT CAT TT; downstream, 5'-CAG GGA CGC CTG TTC TAC GG. The expected length of this PCR product was 561 bp. The COX-2 primer sequences were as follows: upstream, 5'-ACA ACA TTC CTT CCT TC; downstream, 5'-CCT TAT TTC CTT TCA CAC C. The expected length of this PCR product was 201 bp. Concomitantly, amplification of control rat β-actin was performed on the same samples to verify RNA integrity.

DNA amplification was carried out under the following conditions; denaturation at 90°C for 1 min, annealing at 60°C for 45 s, and extension at 72°C for 45 s. To maximize amplification specificity, Taq DNA polymerase was added to the PCR mixture during the hot start of cycle 1. The amplification of PCR was performed at 30 cycles. Each PCR-product (8 µl) was electrophoresed on 1.5% agarose gel stained with ethidium bromide, and then visualized under UV light. Location of predicted PCR product was confirmed by using a 100-base pair ladder (Gibco BRL/Life Technologies, Eggenstein, Germany) as standard marker. To avoid PCR contamination, PCR reactions were prepared in a dedicated area used only for PCR and the PCR product were opened in a laminar flow hood separated from the PCR preparation area.

The intensity of bands was quantified using densitometry (LKB Ultrascan, Pharmacia, Sweden) as described in details in our previous studies (33). The COX-1 and COX-2 signals were standardized against the β-actin mRNA signal for each sample and results expressed as COX-1 and COX-2/β-actin mRNA ratio.

Protein extraction and analysis of COX-2 expression in the gastric mucosa by Western Blot

Shock frozen tissue excised from ulcer margin of rat stomach injected submucosally with vehicle and HGF, was homogenized in lysis buffer (100 mM Tris-HCl, pH 7.4, 15% glycerol, 2 mM EDTA, 2% SDS, 100 mM DDT) by addition of 1:20 dilution of aprotinin and 1:50 dilution of 100 mM PMSF similarly as in our previous studies (34). Insoluble material was removed by centrifugation at 12 000 g for 15 min. Approximately 100 μg of protein cellular extract were loaded into a well, separated electrophoretically through a 13.5% % SDS-polyacrylamide gel and transferred onto Sequi-Blot TM PVDF membrane (BioRad, USA)) by electroblotting. Skim fast milk powder (5% w/v) in TBS/Tween-20 buffer (137 mM NaCl, 20 mM Tris-HCl, pH 7.4, 0.1% Tween-20) was used to block filters for at least 1 hr at room temperature. The dilution of 1:1000 of specific primary rabbit polyclonal antibody against COX-2 (Santa Cruz, USA) or 1:1000 dilution of rabbit polyclonal anti-β-actin (Sigma, Aldrich, Germany) antibody was added to the membrane, followed by an anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (1:2000, Santa Cruz, USA). Incubation of primary antibody was followed by 4 washes for 10 min. Non-isotopic visualization of immune complexes was achieved by chemiluminescence using BM Chemiluminescence Blotting Substrate (Boehringer, Mannheim, Germany). Thereafter, the developed membrane was exposed to an X-ray film (Kodak, Wiesbaden, Germany). Comparisons between different treatment groups were made by determining the COX-1- and COX-2 mRNA/β-actin ratio of the immunoreactive area by densitometry.

Statistical analysis

The data are expressed as means S.E.M. Comparisons between groups of parametric data 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.

Experiments were carried out in accordance with recommendations from the Declaration of Helsinki.

RESULTS

Effect of HGF and gastrin with or without the combination with their antibodies on the area of gastric ulcer and the GBF at ulcer margin

The time course of gastric ulcer healing in rats injected submucosally with vehicle, HGF and gastrin starting from the initial size recorded at day 0 to day 13 and day 21 is shown in *Fig. 1*. The area of the application of acetic acid (day 0) in all three groups of rats was 28 mm^2. The area of gastric ulcers in animals injected submucosally with vehicle (saline) was significantly reduced from 23 ± 3 mm^2 at day 3 to 12.5 ± 2.4 mm^2 and 8.5 ± 1.8 mm^2 at day 13 and day 21, respectively. In

contrast, the area of ulcers in rats treated with HGF and gastrin remained significantly smaller as compared to respective values in vehicle-treated control rats. Submucosal injection with HGF and gastrin failed to affect the area of gastric ulcers at day 3, but produced a significant decrease in the area of these ulcers by about 58% and 62%, at day 13 and by about 89% and 94% at day 21, respectively, as compared to respective values obtained in vehicle-treated control animals (*Fig. 1*).

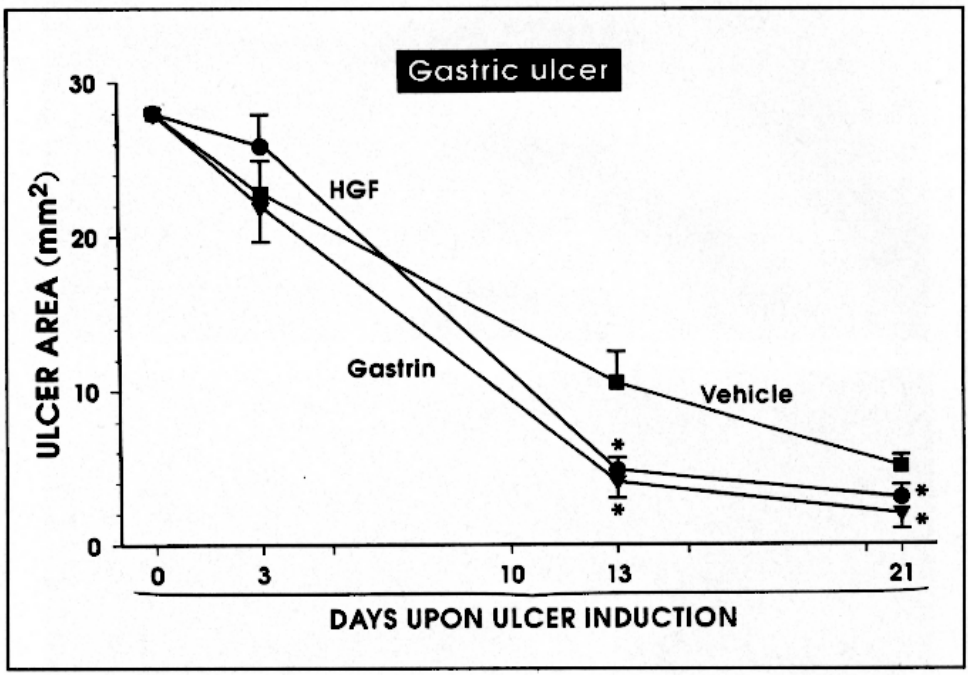

Fig. 1. Mean area of gastric ulcers in rats injected submucosally with vehicle, HGF or gastrin (100 ng/100 µl each) and determined at day 0, 3, 13 and 21 upon ulcer induction. Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated animals.

At day 21, about 65% of control vehicle-treated rats showed almost complete healing of gastric ulcer but in those with macroscopically healed ulcer, microscopically the ulcer scar exhibited a marked gland dilation and an incomplete reconstruction of mucosal cells (*Fig. 2A*). In contrast, in all rats treated with HGF, gastric ulcers were not observed both macroscopically and microscopically and the healing zone at the ulcer margin showed well developed restoration of surface epithelium (*Fig. 2B*).

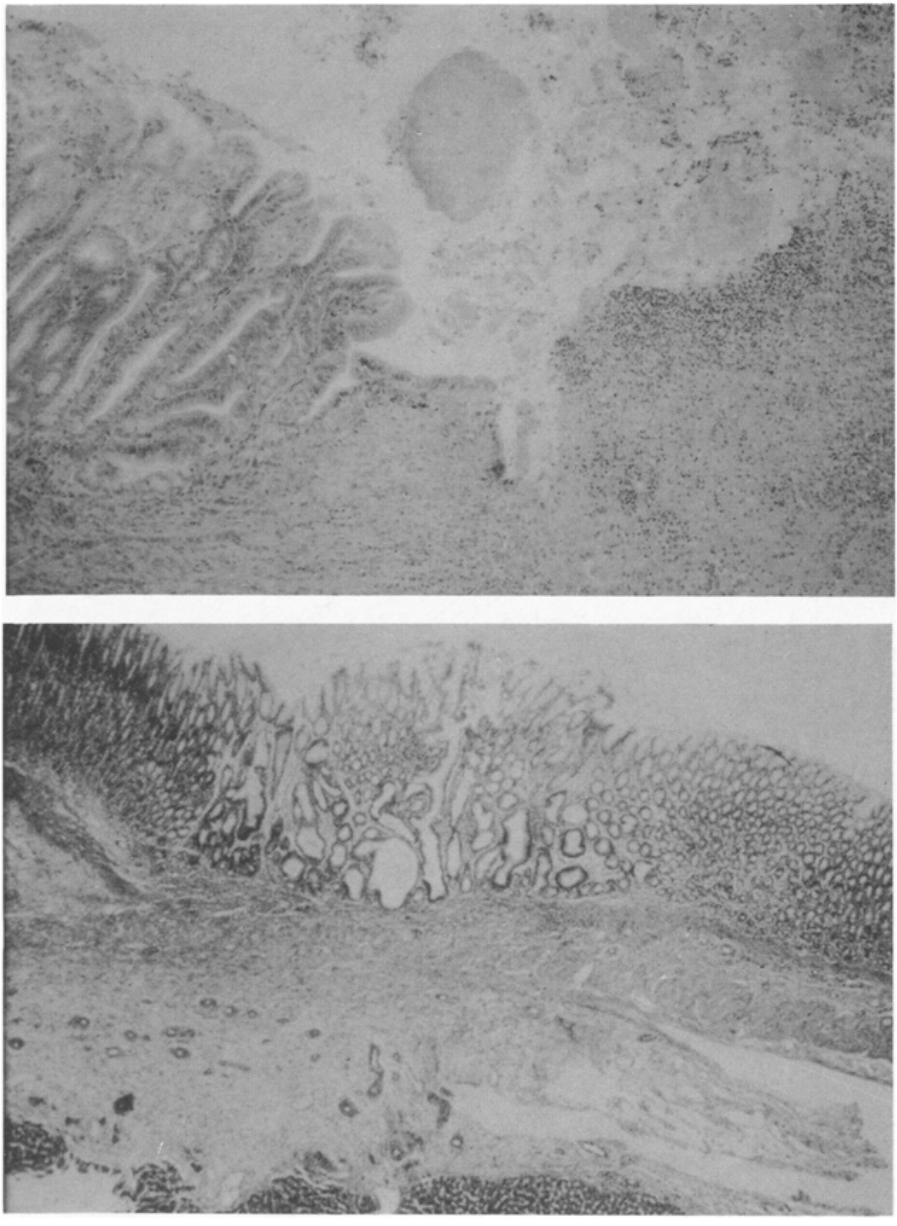

Fig. 2 A, B. Histological appearance of gastric ulcer in the rat injected submucosally with vehicle (control). (A) Gastric ulcer in vehicle-treated mucosa at day 13 after ulcer induction. Note, that healing zone is poorly developed and non-healed ulcer consists of inflammatory cell reach exudate; (B) Gastric ulcer in rat treated with HGF. Comparing to control rat, the gastric ulcer is almost completely healed but the ulcer scar is filled with poorly differentiated epithelium forming dilated irregular glands; H&E, magnification $\times 260$.

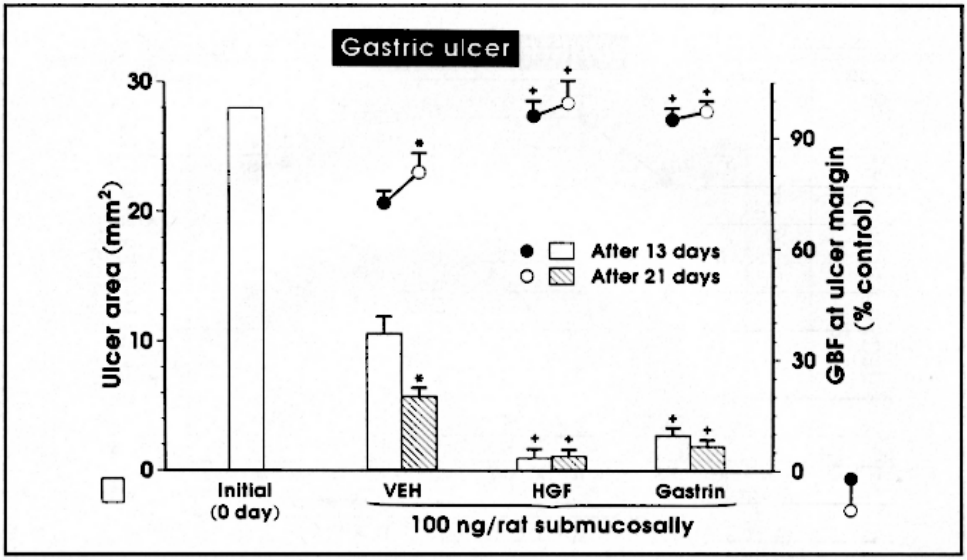

Fig. 3. Mean area of gastric ulcers and the gastric blood flow (GBF) at ulcer margin in rats injected submucosally with vehicle (VEH), HGF and gastrin and determined at day 13 and day 21 upon ulcer induction. Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated gastric mucosa at day 13 upon ulcer induction. Cross indicates significant change as compared to the values recorded in vehicle-control rats at day 21 upon ulcer induction.

Fig. 3 shows the effect of submucosal injection (100 ng in 100 µl each) with HGF and gastrin on the area of gastric ulcers and the alterations in the GBF at ulcer margin determined at day 13 and day 21 upon ulcer induction. Such submucosal injection with HGF and gastrin resulted in a significant decrease in the ulcer area and this was accompanied by a significant rise in the GBF at ulcer margin. The concurrent treatment with specific neutralizing antibodies against HGF and gastrin, which by themselves failed to significantly influence the area of gastric ulcers and the GBF at ulcer margin, completely reversed the acceleration of the ulcer healing and the accompanying rise in the GBF induced by these growth factors (*Fig. 4*).

Effect of non-selective and selective COX-2 inhibitors on the area of gastric ulcer GBF at ulcer margin and generation of PGE_2 in the gastric mucosa in rats with or without submucosal injection of HGF and gastrin

As shown in *Figs. 5, 6* and *7*, the treatment with HGF or gastrin applied submucosally in a dose 100 ng/100 µl, resulted in a similar acceleration of the ulcer healing and GBF at ulcer margin to those presented in *Fig. 3*. In contrast, the treatment with indomethacin (5 mg/kg-d i.p.), which by itself suppressed the

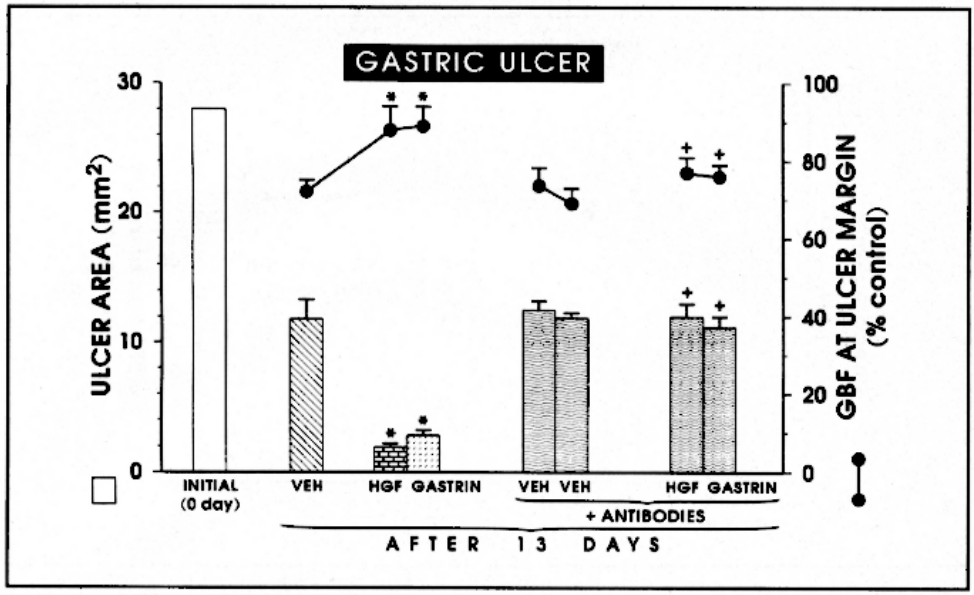

Fig. 4. Mean area of gastric ulcers and the GBF in treated with vehicle (VEH), HGF and gastrin with or without concurrent treatment with antibodies against HGF and gastrin. Mean ± SEM of 8—10 rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated gastric mucosa. Cross indicates significant change as compared to the values recorded in rats without treatment with antibodies.

mucosal generation of PGE_2 by 85% (20), produced a significant delay in ulcer healing and a marked fall in the GBF at ulcer margin in animals injected submucosally with vehicle. Such treatment with indomethacin abolished the acceleration of the ulcer healing and the rise in the GBF induced by HGF and gastrin applied submucosally (*Fig. 5*). Likewise, the treatment with highly selective COX-2 inhibitors, NS-398 and Vioxx, resulted in similar increase in the area of gastric ulcers and small, though not significant decrease in the GBF at ulcer margin as compared to the respective values at day 13 upon ulcer induction (*Figs. 6* and *7*). The acceleration of the ulcer healing and accompanying rise in the GBF at ulcer margin caused by HGF and gastrin applied submucosally were completely eliminated in these rats by the concurrent treatment with NS-398 and Vioxx.

The generation of PGE_2 averaged 157 ± 18 ng/g wet tissue weight in the non-ulcerated gastric mucosa and this reached significantly higher value in the ulcerated gastric mucosa (*Fig. 8*). The treatment with indomethacin, NS-398 and Vioxx significantly inhibited the generation of PGE_2 in the non-ulcerated and ulcerated gastric mucosa (*Fig. 8*). The generation of PGE_2 in the ulcerated gastric mucosa tended to increase, especially in animals treated with HGF but it failed to reach statistical significance as compared to the vehicle-treated

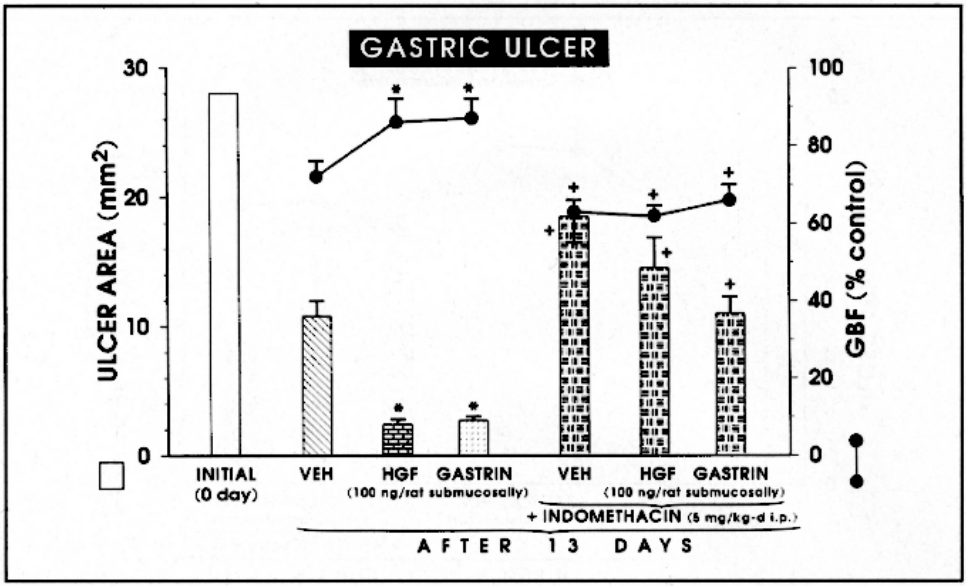

Fig. 5. Mean area of gastric ulcers, the GBF at ulcer margin in rats treated throughout the period of 13 days with vehicle, HGF and gastrin with or without the combination with indomethacin (5 mg/kg-d i.p.). Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated animals. Cross indicates significant change as compared to the value obtained in rats without indomethacin treatment.

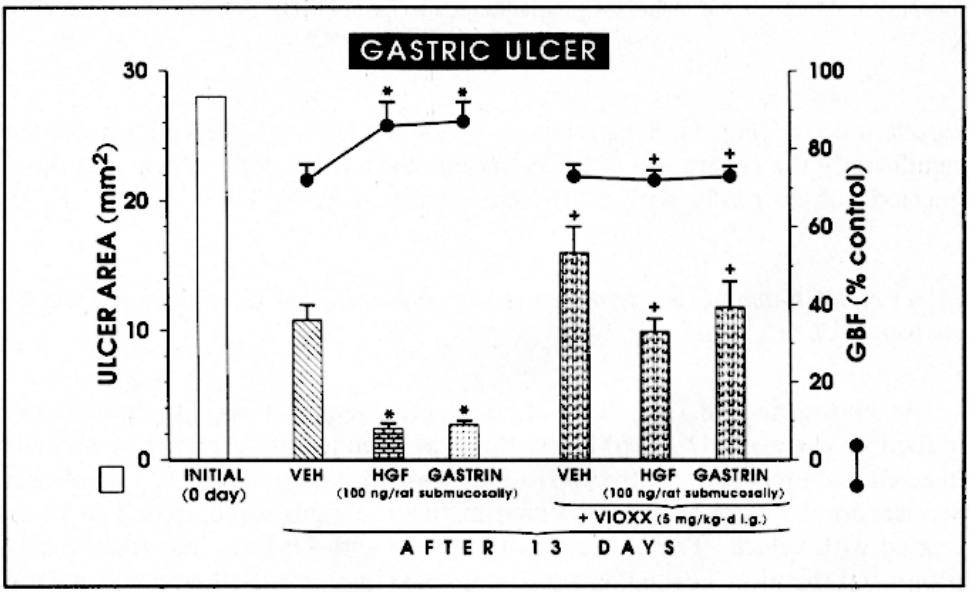

Fig. 6. Mean area of gastric ulcers, the GBF at ulcer margin in rats treated throughout the period of 13 days with vehicle, HGF and gastrin with or without the combination with NS-398 (10 mg/kg-d i.g.). Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated animals. Cross indicates significant change as compared to the value obtained in rats without NS-398 treatment.

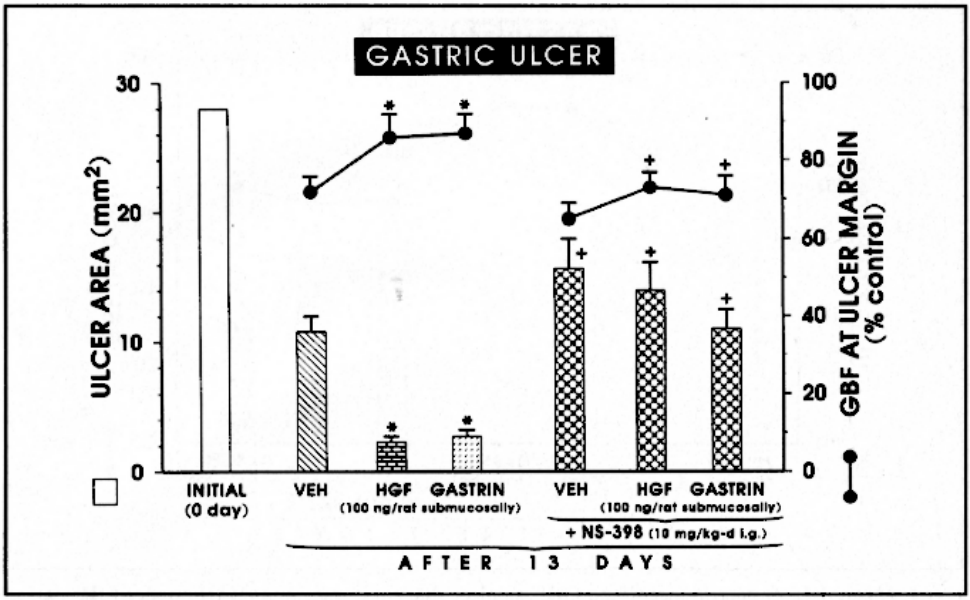

Fig. 7. Mean area of gastric ulcers, the GBF at ulcer margin in rats treated throughout the period of 13 days with vehicle, HGF and gastrin with or without the combination with Vioxx (5 mg/kg-d i.g.). Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated animals. Cross indicates significant change as compared to the value obtained in rats without Vioxx treatment.

gastric mucosa (*Fig. 9*). Treatment with NS-398 (10 mg/kg-d i.g.), attenuated significantly the generation of PGE_2 in animals treated with vehicle and those injected submucosally with HGF and gastrin (*Fig. 9*).

Effect of HGF and gastrin with or without suppression of COX-2 on the plasma gastrin levels

As shown in *Fig. 10*, the plasma gastrin levels at day 13 upon ulcer induction averaged 42 ± 8 pM and this was significantly elevated in animals injected submucosally with HGF and gastrin along with the significant acceleration in ulcer healing observed in these animals as compared to those treated with vehicle. The concurrent treatment with NS-398, that significantly prolonged the ulcer healing, failed to influence significantly the plasma gastrin levels in vehicle-treated gastric mucosa. When NS-398 was administered to rats injected submucosally with HGF or gastrin, the delay in ulcer healing was observed along with significant decline in the plasma gastrin levels observed in these animals (*Fig. 10*).

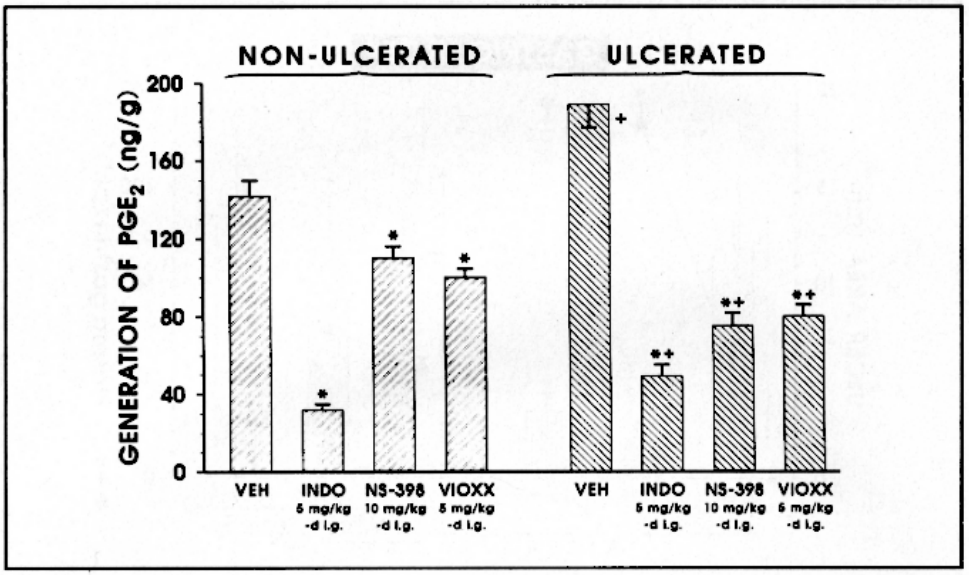

Fig. 8. Effect of vehicle (VEH), indomethacin (5 mg/kg-d i.p.), NS-398 (10 mg/kg-d i.g.) and Vioxx (5 mg/kg-d i.g.) on the generation of PGE2 in the non-ulcerated (intact) and ulcerated gastric mucosa at day 13 upon ulcer induction. Mean ± SEM of 6—8 rats. Single Asterisk indicates significant change as compared to the value obtained in vehicle-treated animals. Cross indicates significant change as compared to the value obtained in non-ulcerated gastric mucosa. Asterisk and cross indicate a significant change as compared to the value obtained in ulcerated gastric mucosa.

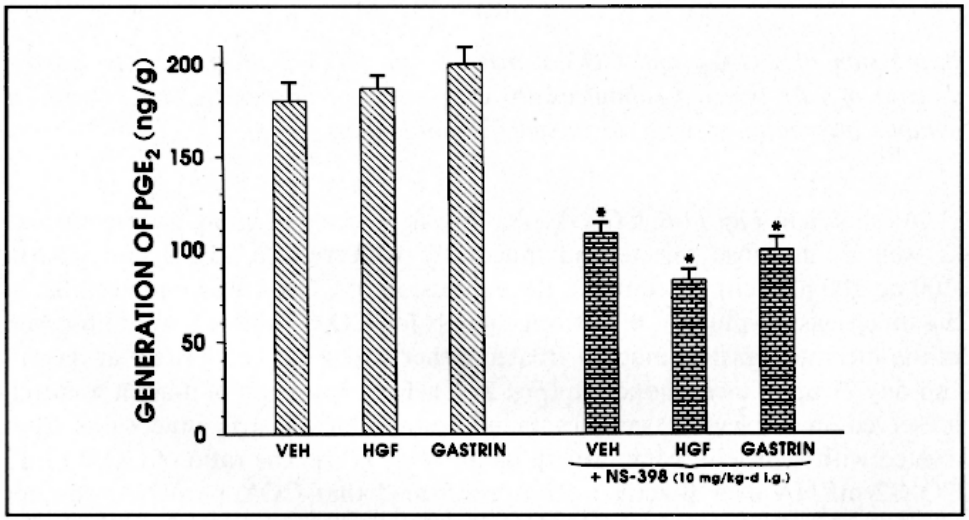

Fig. 9. Effect of vehicle (VEH), HGF and gastrin with or without treatment with NS-398 (10 mg/kg-d i.g.) on the generation of PGE_2 in the ulcerated gastric mucosa at day 13 upon ulcer induction. Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated animals.

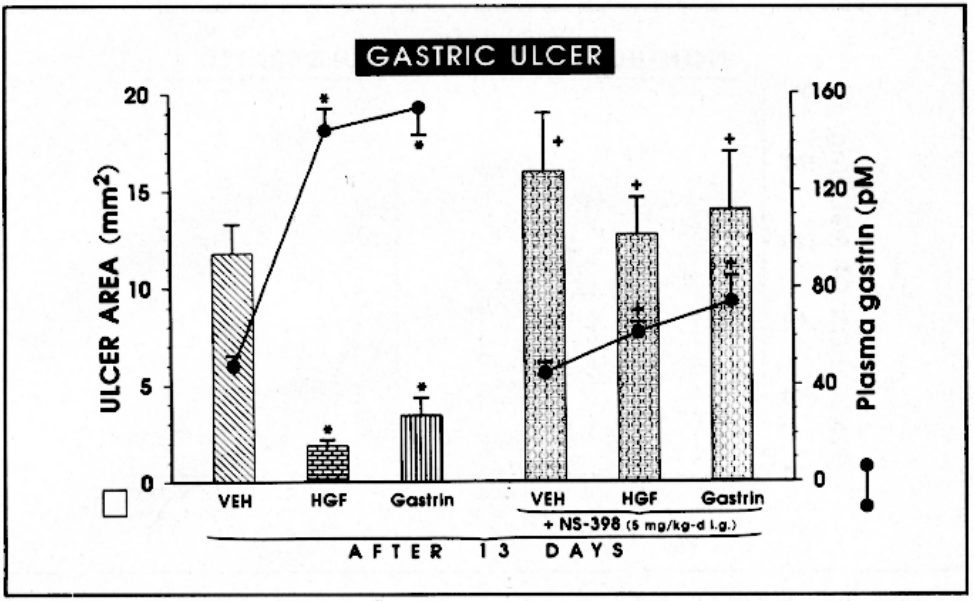

Fig. 10. Mean area of gastric ulcers and plasma gastrin levels in rats injected submucosally with vehicle (VEH), HGF and gastrin with or without the concurrent treatment with NS-398 (5 mg/kg-d i.g.). Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated animals. Cross indicates significant change as compared to the value obtained in rats without NS-398 treatment.

Expression of COX-1 and COX-2 mRNA and COX-2 protein in the gastric mucosa of rats injected submucosally with vehicle and growth factors with or without pretreatment with their specific antibodies

As shown in *Fig. 11 B*, COX-1 mRNA was detected in intact gastric mucosa as well as in those injected submucosally with vehicle, HGF and gastrin (100 ng/100 μl each). In contrast, the expression of COX-2 was undetectable in the intact gastric mucosa but strong signals for COX-2 mRNA were observed in the ulcerated gastric mucosa treated either with HGF or gastrin at day 13 and day 21 upon ulcer induction (*Fig. 11 C*). The expression of β-actin was well preserved in all gastric samples taken from intact gastric mucosa or that treated with vehicle or each growth factor (*Fig. 11 A*). The ratio of COX-1 and COX-2 mRNA over β-actin mRNA confirmed that COX-1 mRNA was not significantly affected by vehicle, HGF and gastrin but COX-2 mRNA was significantly elevated in the ulcerated gastric mucosa of vehicle- and HGF- or gastrin-treated animals over that observed in the intact gastric mucosa (*Figs. 12* and *13*).

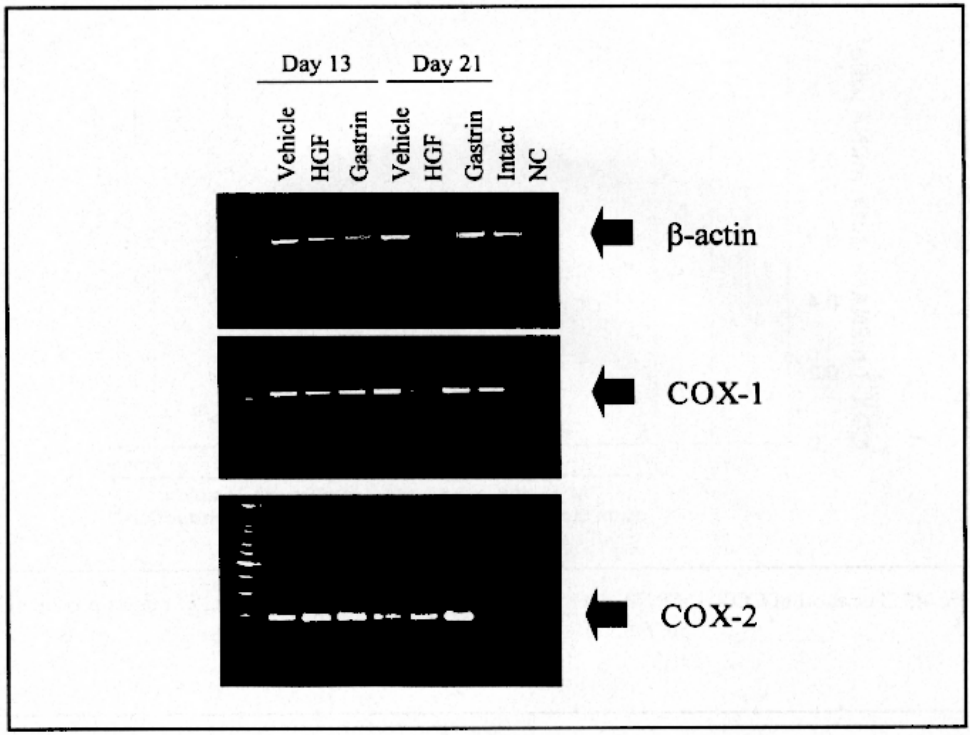

Fig. 11. Expression of β-actin mRNA, COX-1 mRNA and COX-2 mRNA in the ulcerated gastric mucosa treated with vehicle (lane 1), HGF (lane 2) and gastrin (lane 3) at day 13 upon ulcer induction and with vehicle (lane 4), HGF (lane 5) and gastrin (lane 6) at day 21 upon ulcer induction. Lanes 7 and 8 are intact gastric mucosa and negative control (water), respectively. The arrow indicates PCR product size for COX-1 (501 bp), COX-2 (201 bp) or β-actin (764 bp), respectively. M is PCR size marker.

In the intact rats, COX-2 mRNA and COX-2 protein was not detected while in the ulcerated mucosa at day 13 and day 21, the strong signals COX-2 mRNA and the expected 72 kD protein were observed (Figs. 14 and 15). The ratio of COX-2 mRNA over β-actin mRNA reached significantly higher value in the mucosal sample taken from the gastric mucosa injected submucosally with HGF than that of vehicle-control and this was significantly attenuated by addition of HGF antibody to this growth peptide (Fig. 14). In contrast , the ratio of COX-1 mRNA over β-actin remained unaffected significantly by the injection of HGF or its combination with specific antibody. The expression of β-actin was well preserved in the mucosal samples taken from intact rats and those treated with vehicle and HGF (*Fig. 14*).

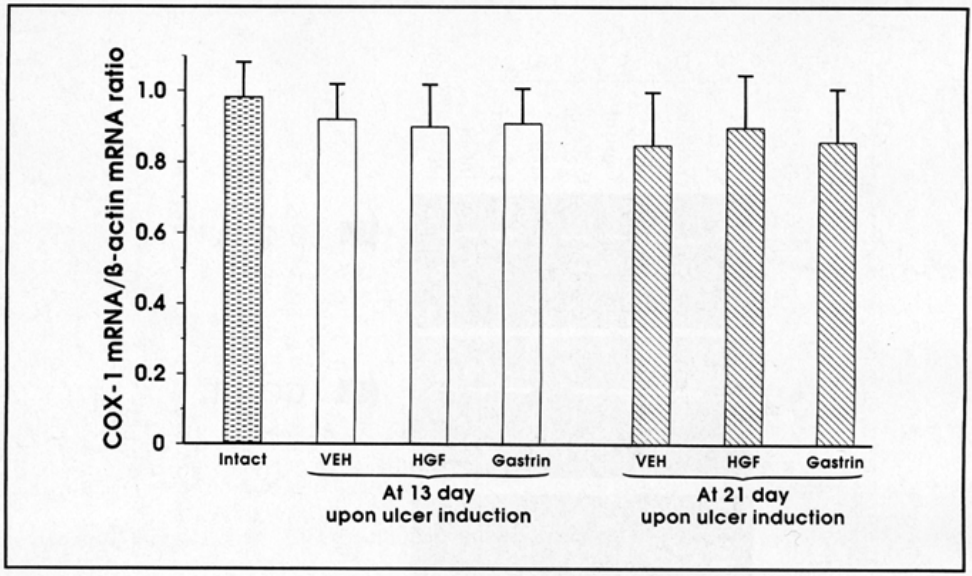

Fig. 12. The ratio of COX-1 mRNA over β-actin mRNA in the gastric mucosa of rats as presented in *Fig. 11.* Mean ± SEM of 4—6 rats.

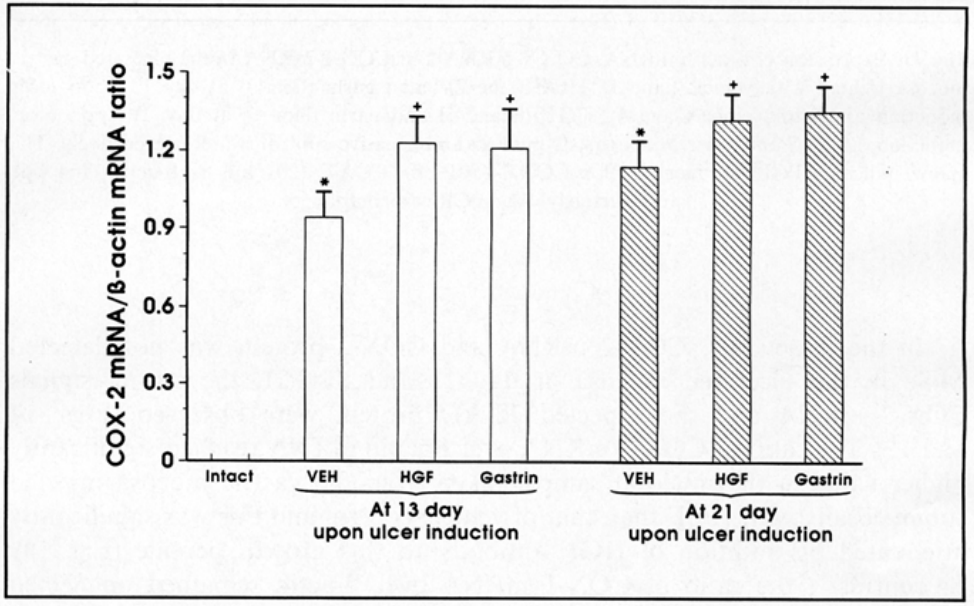

Fig. 13. The ratio of COX-2 mRNA over β-actin mRNA in the gastric mucosa of rats as presented in *Fig. 11.* Mean ± SEM of 4—6 rats. Asterisk indicates a significant change as compared to the value obtained in intact gastric mucosa. Cross indicates a significant change as compared to the value obtained in vehicle-control gastric mucosa.

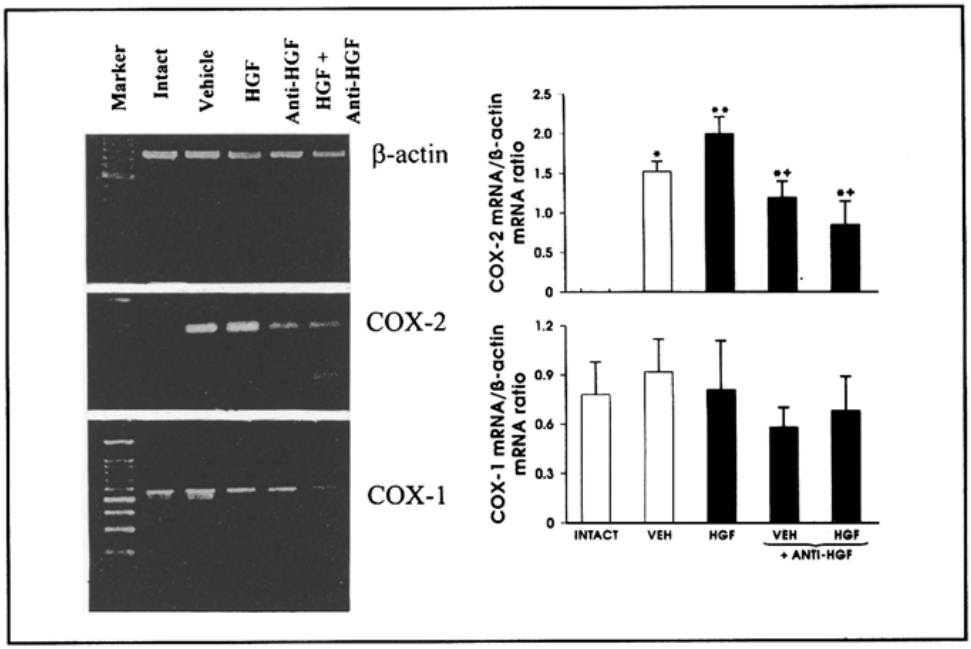

Fig. 14. Expression of -actin mRNA, COX-1 mRNA and COX-2 mRNA and the ratio COX-1 mRNA and COX-2 mRNA over β-actin in intact gastric mucosa (lane 1) and in the ulcerated gastric mucosa treated with vehicle (lane 2), HGF alone (lane 3), the combination of HGF with specific antibody against HGF (lane 4) and antibody against HGF applied alone (lane 5) at day 13 upon ulcer induction; M is DNA ladder. The arrow indicates PCR product size for COX-1 (501 bp), COX-2 (201 bp) or β-actin (764 bp), respectively. M is PCR size marker.

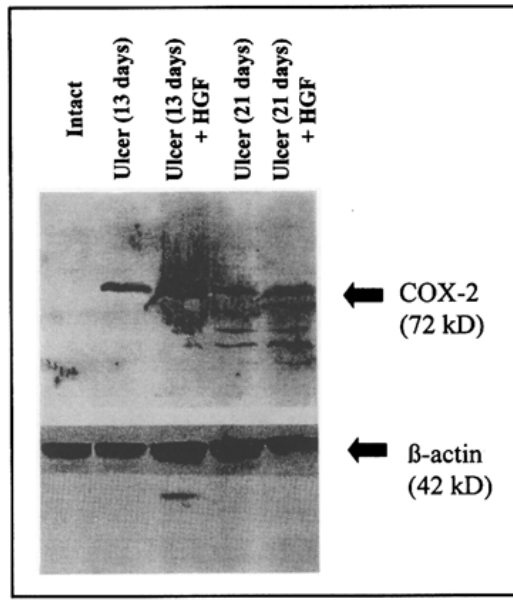

Fig. 15. Representative Western blot analysis of COX-2 protein and β-actin protein in intact rats and those injected submucosally with vehicle or HGF determined at day 13 and day 21 upon ulcer induction.

DISCUSSION

Our study demonstrates that local application of HGF and gastrin, that provided an enhancement of local pool for growth peptides and their accumulation in the ulcer area, significantly accelerated the healing of these ulcers. This increase in the healing rate induced by local application of HGF and gastrin was accompanied by a significant rise in the GBF at ulcer margin and plasma gastrin levels suggesting that hyperemia at the area margin is crucial for the ulcer healing and mucosal microcirculation and that the hypergastrinemia resulting in possible trophic effect due to an increase in the gastric cell proliferation (35), may contribute to the observed healing activity of these growth factors. Furthermore, we found that COX-2 mRNA is overexpressed at the margin of gastric ulcer during the process of spontaneous healing and that COX-2 mRNA but not that of COX-1, is significantly upregulated by the treatment with HGF and gastrin. This beneficial effect of both peptides on ulcer healing and accompanying hyperemia at ulcer margin were significantly attenuated by co-administration of neutralizing antibodies of these growth factors emphasizing the specificity of healing effects exhibited by both, HGF and gastrin. It is of interest that an overexpression of COX-2 mRNA and COX-2 protein by HGF was significantly attenuated by specific antibody against this growth peptide. Our study suggests that COX-2 enzyme and PG derived from this enzyme, play an important role in the mechanism of ulcer healing and in the acceleration of this healing by HGF and gastrin. This notion is supported by the fact that the suppression of the PGE_2 generation by indomethacin or by highly selective COX-2 inhibitors such as NS-398 or Vioxx, especially in the area surrounding the gastric ulcer, resulted in a marked prolongation of ulcer healing with concomitant impairment of the GBF at the ulcer margin.

Our present study indicates that growth peptides injected to the margin of gastric ulcer, which is considered as an area crucial for mucosal regeneration, restoration of mucosal glandular structure and cell proliferation, accelerated healing of chronic ulcers and that these effects are, at least in part, mediated by PG derived from COX-2. To our best knowledge there is a first demonstration that HGF and gastrin applied directly to ulcer area by a means of repeated submucosal injection to the ulcer margin, are effective in speeding up the process of ulcer healing, thus offering the new modality for the treatment of gastric ulcers. The importance of availability of growth factors in the ulcer area and the subsequent acceleration of ulcer healing was documented by the fact that injection of specific neutralizing antibodies that interact with each of these peptides tested, completely abolished the increase in ulcer healing and accompanying microcirculatory response at the margin of gastric ulcer. It is of interest that local injection with gastrin that was shown before to exhibit the

protective activity in the stomach (16), also resulted in the acceleration of ulcer healing similar to that attained with HGF that is recognized as a potent mitogen in the gastric mucosa. In similar study by Li and Helander (35), parentaral administration of G-17 significantly accelerated the rate of ulcer healing and this was attributed to the increase in the cell proliferation induced by this peptide without any significant influence of G-17 on the apoptotic cell index in the ulcerated gastric mucosa.

Previous studies documented that PG, a major metabolites of arachidonic acid generated by COX-1 and COX-2, contribute to the mechanism of gastric mucosal integrity and gastric mucosal defense mechanisms (23, 36, 37). We and others have demonstrated that suppression of endogenous PG by indomethacin, which is a non-selective COX-inhibitor, delayed ulcer healing and attenuated the increase in the GBF at the ulcer area (26, 38, 39). PG, the products of COX-1 enzyme, are known to exhibit housekeeping functions in the gastrointestinal tract, while COX-2 derived metabolites are produced and released at the site of inflammation (40, 41). It was suggested that COX-2 enzyme is involved in gastric epithelial restitution induced by HGF and in the proliferate response of gastrointestinal tract to certain growth factors (42). Upregulation of COX-2 mRNA and HGF mRNA at the ulcer edge could be due to local overexpression of various cytokines such as IL-1, that is expressed in macrophage/monocytes and fibroblasts in the ulcer base as reported previously (43). Recent evidence indicates that HGF which possessed mitogenic and motogenic activities, upregulated COX-2 *in vitro* and the activation of ERK-2 signaling pathway was proposed to explain this phenomenon (21). Our observation is in keeping with these studies by demonstration that COX-2 mRNA was upregulated in gastric mucosa treated with HGF and gastrin and that at the same experimental conditions, the mRNA for COX-1 remained unaffected. Moreover, expression of COX-2 at the level of protein was detected in the gastric mucosa injected locally with HGF and this was significantly mitigated by the concurrent treatment with anti-HGF antibody, suggesting that, indeed, COX-2 derived PG play an important role in the mechanism of ulcer healing by this growth peptide. This notion is supported by observation that mucosal generation of PGE_2 reached significantly higher values in the ulcerated gastric mucosa than that obtained from non-ulcerated gastric mucosa suggesting that COX-2 derived PG can contribute to the ulcer healing by mediating of the hyperemic response in the ulcer margin. This remains in agreement with the recent reports in mice and rats that COX-2 mRNA is expressed at the area of gastric ulcer and that the suppression of COX-2 by new class of selective COX-2 inhibitors, delayed significantly the ulcer healing (20, 23, 44—46). In our study, indomethacin, a non-selective COX inhibitor that suppressed PGE_2 generation in ulcerated and non-ulcerated gastric mucosa, delayed ulcer healing and significantly attenuated the rise in the GBF

at ulcer margin evoked by HGF and gastrin. This remains in apparent disagreement with our previous observation that gastrin afforded protection against ethanol-induced gastric lesions and accompanying rise in GBF were not significantly influenced by indomethacin, thus, militating against a major role of endogenous PG in this gastroprotection (16). The reason for this discrepancy could be that acute mucosal injury induced by a corrosive agents such as 100% ethanol may have very little in common with healing of pre-existing ulcers such as those induced by acetic acid method. Moreover, the induction of COX-2 mRNA, which in our hands was undetectable in intact gastric mucosa but was expressed at day 13 and 21 upon ulcer induction, suggesting that ulcer healing effects of COX-2 may require much longer time to occur, than 1 hour which is the standard time necessary to examine gastric lesions after topical application of ethanol. Similarly to indomethacin, Vioxx and NS-398, both highly selective COX-2 inhibitors (47—49) that by themselves, prolonged significantly the ulcer healing and eliminated the rise in GBF caused by gastrin and HGF. Furthermore, we found for the first time that plasma gastrin levels were significantly elevated in animals injected locally with HGF to the extent similar to that obtained with gastrin and these effects were significantly attenuated by the treatment with NS-398. This suggests that COX-2 derived PG may be involved in healing action of HGF *via* an increase in plasma level of gastrin, possibly due to inhibition of gastric secretion by these COX-2 derivatives but this hypothesis remains to be further elucidated.

In summary, we demonstrated that enrichment of HGF and gastrin in the ulcer area by their local application exerts beneficial effect on ulcer healing and offers new opportunity for the treatment of gastric ulcers. The acceleration of the ulcer healing and the rise in GBF at ulcer margin induced by HGF and gastrin were completely eliminated by the co-treatment with antibodies against both peptides reinforcing the idea that local availability of these growth factors in the ulcer area is essential for ulcer healing and microcirculation at the ulcer area. Moreover, we found that the ulcer healing effect of HGF involves an increase in the microcirculation and plasma gastrin levels as well as overexpression of COX-2. The healing of pre-existing ulcers by HGF and gastrin was significantly impaired in our study by the specific inhibition of COX-2 suggesting that COX-2 derived PG play an important role in the mechanism of ulcer healing by these growth peptides.

REFERENCES

1. Konturek SJ. Role of growth factors in gastroduodenal protection and healing of peptic ulcers. *Gastroenterology* 1990, 1, 40—65.
2. Lee H, Hansson HA, Norstrom E, Helander HF. Immunoreactivities for epidermal growth factor (EGF) and for receptors in rats with gastric ulcers. *Cell Tissue Res* 1991, 265, 211—218.

3. Wright NA, Pike C, Elia G. Induction of a novel epidermal growth factor — secreting cell lineage by mucosal ulceration in human gastrointestinal stem cells. *Nature* 1990, 343, 82—85.
4. Tarnawski A, Stachura J, Durbin T, Sarfeh IJ, Gergely H. Increased expression of epidermal growth factor receptor during gastric ulcer healing in rats. *Gastroenterology* 1992, 102, 695—698.
5. Konturek PCh, Brzozowski T, Konturek SJ, Ernst H, Drozdowicz D, Pajdo R. Expression of epidermal growth factor and transforming growth factor alpha during ulcer healing. *Scand J Gastroenterol* 1997, 32, 6—15.
6. Schmassmann A, Tarnawski A, Peskar BM, Varga L, Flogerzi B, Halter F. Influence of acid secretion and angiogenesis on kinetics of gastric ulcer healing in rats; interaction with indomethacin. *Am J Physiol* 1995, 268, G276-G285.
7. Szabo S, Folkman J, Vattay P, Morales RE, Pinkus GS, Kato K. Accelerated healing of duodenal ulcers by oral administration of a mutein of basic fibroblast growth factor in rats. *Gastroenterology* 1994, 106, 1106—1111.
8. Konturek SJ, Brzozowski T, Majka J, Pawlik W, Stachura J. Omentum and basic fibroblast growth factor in healing of chronic gastric ulcerations in rats. *Dig Dis Sci* 1994, 39, 1064—71.
9. Nakamura T, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. *Biochem Biophys Res Commun* 1984, 122, 1250—59.
10. Weidner KM, Arakaki N, Hartmann G, Vandekerckhove J, Weingart S, Reider H, Fonatsch C, Tsubouchi H, Hishida T, Daikuhara Y, Birchmeier W. Evidence for the identity of human scatter factor and human hepatocyte growth factor. *Proc Natl Acad Sci USA* 1991, 88, 7001—7005.
11. Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande-Woude GF, Aaronson SA. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science* 1991, 251, 802—804.
12. Kinoshita Y, Nakata H, Hassan S, Ashara M, Kawanami CH, Matsushima Y, Inamoto Y, Ping CY, Min D, Nakamwa A, Chiba T. Gene expression of keratinocyte and hepatocyte growth factors during the healing of rat gastric mucosal lesions. *Gastroenterology* 1995, 109, 1068—1077.
13. Schmassmann A, Stettler Ch, Poulson R, Tarasova N, Hirschi C, Flogerzi B, Matsumo K, Nakamwa T, Halter F. Roles of hepatocyte growth factor and its receptor during gastric ulcer healing in rats. *Gastroenterology* 1997, 113, 1858—1872.
14. Sandvik AK, Waldum HL, Kleveland PM, Schulze Sognen B. Gastrin produces an immediate and dose-dependent histamine release preceding acid secretion in the totally isolated, vascularly perfused rat stomach. *Scand J Gastroenterol* 1987, 22, 803—808.
15. Hakanson R, Bloom H, Carlsson E, Larsson H, Ryberg B, Sundler F. Hypergastrinemia produces trophic effects in stomach but not in pancreas and intestines. *Reg Pept* 1986, 13, 225—33.
16. Konturek SJ, Brzozowski T, Bielanski W, Schally AV. Role of endogenous gastrin in gastroprotection. *Eur J Pharmacol* 1995, 278, 203—212.
17. Eberhart CE, Dubois RN. Eicosanoids and the gastrointestinal tract. *Gastroenterology* 1995, 109, 258—301.
18. Masferrer IL, Zweifel BS, Maming PT, Hauser SD, Eaky KM, Smith WG, Isakson PC, Siebert HK. Selective inhibition of inducible cyclooxygenase-2 *in vivo* is anti-inflammatory and ulcerogenic. *Proc Natl Acad Sci USA* 1994, 9, 3228—3232.
19. Vane JR, Botting RM . New insights into the mode of action of anti-inflammatory drugs. *Inflamm Res* 1995, 44, 1—10.
20. Mizuno H, Sakamoto Ch, Matsuda K, Wada K, Kchida T, Noguchi H, Akamatsu T, Kasuga M. Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. *Gastroenterology* 1997, 12, 387—397.

21. Jones MK, Sasaki E, Halter F, Pai R, Nakamura T, Arakawa T, Kuroki T, Tarnawski AS. HGF triggers activation of the COX-2 gene in rat gastric epithelial cells: action mediated through the ERK2 signaling pathway. *FASEB-J* 1999, 13, 2186—2194.
22. Ernst H, Konturek P, Hahn EG, Brzozowski T, Konturek SJ. Acceleration of wound healing in gastric ulcers by local injection of neutralizing antibody to transforming growth factor 1. *Gut* 1996, 39, 172—175.
23. Brzozowski T, Konturek PCh, Konturek SJ, Sliwowski Z, Drozdowicz D *et al.* Role of prostaglandin-generated by cyclooxygenase-1 and cyclooxygenase-2 in healing of ischemia-reperfusion induced gastric lesions. *Eur J Pharmacol* 1999, 385, 47—61.
24. Konturek SJ, Brzozowski T, Garlicki J, Majka J, Stachura J, Nauert CM. Intragastric pH in the gastroprotective and ulcer healing activity of aluminium-containing antacids. *Digestion* 1991, 49, 140—141.
25. Okabe S, Pfeiffer CJ, Roth ILA. A method for experimental penetrating gastric and duodenal ulcers in rats. Digestion 1987, 38, 103—113.
26. Konturek SJ, Stachura J, Radecki T, Drozdowicz D, Brzozowski T. Cytoprotective and ulcer healing properties of prostaglandin E2, colloidal bismuth and sucralfate in rats. *Digestion* 1987, 38, 102—113.
27. Konturek SJ, Brzozowski T, Majka J, Pytko-Polonczyk J, Stachura J. Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. *Eur J Pharmacol* 1993, 239, 215—217.
28. Brzozowski T, Konturek P Ch, Konturek SJ, Pajdo R, Drozdowicz D, Kwiecien S *et al.* Acceleration of ulcer healing by cholecystokinin (CCK): role of CCK-A receptors, somatostatin, nitric oxide and sensory nerves. *Reg Pept* 1999, 82, 19—33.
29. Chomczynski J, Sacchi N. A single step method of RNA isolation by acid guanidinum thiocyanate. *Anal Biol Chem* 1987, 162, 156—159.
30. Kennedy BP, Chan CC, Culp SA, Cronlish WA. Cloning and expression of rat prostaglandin endoperoxide synthase (cyclooxygenase)-2 cDNA. *Biochem Biophys Res Commun* 1993, 197, 494—500.
31. O'Banion MK, Winn VD, Young DA. Cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. *Proc Natl Acad Sci* 1992, 89, 4888—4892.
32. Xie W, Chipman JG, Robertson DL, Erikson DL, Simmons DL. Expression of a mitogen responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci USA* 1991, 88, 2692—2696.
33. Konturek PCh, Brzozowski T, Konturek SJ, Taut A, Kwiecien S, Pajdo R, Sliwowski Z, Hahn EG. Bacterial lipopolysaccharide protects gastric mucosa against acute injury in rats by activation of genes for cyclooxygenase and endogenous prostaglandins. *Digestion* 1998, 59, 284—297.
34. Konturek PC, Brzozowski T, Sulekova Z, Meixner H, Hahn EG, Konturek SJ. Enhanced expression of leptin following acute gastric injury in rat. *J Physiol Pharmacol* 1999, 50, 587—96.
35. Li H, Helander H. Hypergastrinemia increases proliferation of gastroduodenal epithelium during gastric ulcer healing in rats. *Dig Dis Sci* 1996, 41, 40—48.
36. Nakano O, Sakamoto Ch, Matsuda K, Konda Y, Matozaki T, Nishisaki H, Wada K, Suzuki T, Uchida T, Nagao M, Kasyga M. Induction of cyclooxygenase protein and stimulation of prostaglandin E2 release by epidermal growth factor in cultured guinea pig gastric mucous cells. *Dig Dis Sci* 1995, 40, 1679—1686.
37. Brzozowski T, Drozdowicz D. Szlachcic A, Pytko-Polonczyk J, Majka J *et al.* Role of nitric oxide and prostaglandins in gastroprotection induced by capsaicin and papaverine. *Digestion* 1993, 54, 24—34.
38. Wang JY, Yamasaki S, Takeuchi K, Okabe S. Delayed healing of acetic acid-induced gastric ulcer in rats indomethacin. *Gastroenterology* 1989, 96, 393—402.

39. Konturek SJ, Brzozowski T, Majka J, Dembinski A, Slomiany A, Slomiany BL. Transforming growth factor and epidermal growth factor in protection and healing of gastric mucosal injury. *Scand J Gastroenterol* 1992, 27, 649—655.
40. Feng L, Sun W, Xia Y, Tang WW, Chanmugan P, Soyoda E. Cloning two isoforms of rat cyclooxygenase; differential regulation of their expression. *Arch Biochem Biophys* 1992, 307, 361—368.
41. Kargman S, Charleson S, Cartwright M, Frank J, Rindean D, Mancini J, Evans J, O'Neill G. Characterization of prostaglandin G/H synthase 1 and 2 in rat, dog, monkey and human gastrointestinal tracts. *Gastroenterology* 1996, 111, 445—454.
42. Horie-Sakata K, Shimada T, Hiraishi H, Terano A. Role of cyclooxygenase-2 in hepatocyte growth factor-mediated gastric epithelial restitution. *J Clin Gastroenterol* 1998, 27 (Suppl 1), S40—46.
43. Takahashi S, Kobayashi N, Okabe S. Regulation by endogenous interleukin-1 of mRNA expression of healing-related factors in gastric ulcers in rats. J Pharmacol Exp Ther 1999, 291, 634—641.
44. Lesch CA, Gilbertsen RB, Song Y, Dyer RD, Sehrier D, Kraus ER, Sanchez B, Guglietta A. Effect of novel anti-inflammatory compounds on healing of acetic acid-induced gastric ulcer in rats. *J Pharm Exp Ther* 1998, 287, 301—306.
45. Takeuchi K, Suzuki K, Yammamoto H, Araki H, Mizoguchi H, Ukawa H. Cyclooxygenase-2 selective and nitric oxide-releasing nonsteroidal anti-inflammatory drugs and gastric mucosal responses. *J Physiol Pharmacol* 1998, 49, 501—505.
46. Schmassmann A, Peskar BM, Stettler C, Netzer P, Stroff T, Flogerzi, Halter F. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastrointestinal ulcer models in rats. Br J Pharmacol 1997, 123, 795—804.
47. Furst DE. Pharmacology and efficacy of cyclooxygenase (COX) inhibitors. *Am J Med* 1999, 107, 185—228.
48. Futaki N, Yoshikawa K, Hamasaka Y, Arai I, Higuchi S, Izuka H, Otomo S. NS-398 a novel non-steroidal anti-inflammatory drug with potent analgesic and antipyretic effects which causes minimal stomach lesions. *Gen Pharmacol* 1993, 24, 105—110.
49. Ehrich EW, Dallob A, De Lepeleire I, Van Hecken A, Riendeau D, Yuan W, Porras A, Wittereich J, Seibold JR, De Schepper P, Mehlisch DR, Gertz BJ. Characterization of rofecoxib as a cyclooxygenase-2 isoform inhibitor and demonstration of analgesia in the dental pain model. *Clin Pharmacol Ther* 1999, 65, 336—347.

Received: October 3, 2000
Accepted: October 18, 2000

Author's address: Prof. S. J. Konturek, Department of Physiology, Jagiellonian University School of Medicine, 16 Grzegorzecka Str., 31-531 Cracow, Poland.