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Cyclo-oxygenase isozymes in mucosal ulcerogenic and functional responses following barrier disruption in rat stomachs

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1 We examined the effects of selective and nonselective cyclo-oxygenase (COX) inhibitors on various functional changes in the rat stomach induced by topical application of taurocholate (TC) and investigated the preferential role of COX isozymes in these responses.

2 Rat stomachs mounted in *ex vivo* chambers were perfused with 50 mM HCl and transmucosal potential difference (p.d.), mucosal blood flow (GMBF), luminal acid loss and luminal levels of prostaglandin E_2 (PGE_2) were measured before, during and after exposure to 20 mM TC.

3 Mucosal application of TC in control rats caused a reduction in p.d., followed by an increase of luminal acid loss and GMBF, and produced only minimal damage in the mucosa 2 h later. Pretreatment with indomethacin (10 mg kg^{-1}, s.c.), a nonselective COX-1 and COX-2 inhibitor, attenuated the gastric hyperaemic response caused by TC without affecting p.d. and acid loss, resulting in haemorrhagic lesions in the mucosa. In contrast, selective COX-2 inhibitors, such as NS-398 and nimesulide (10 mg kg^{-1}, s.c.), had no effect on any of the responses induced by TC and did not cause gross damage in the mucosa.

4 Luminal PGE_2 levels were markedly increased during and after exposure to TC and this response was significantly inhibited by indomethacin but not by either NS-398 or nimesulide. The expression of COX-1-mRNA was consistently detected in the gastric mucosa before and after TC treatment, while a faint expression of COX-2-mRNA was detected only 2 h after TC treatment.

5 Both NS-398 and nimesulide significantly suppressed carrageenan-induced rat paw oedema, similar to indomethacin.

6 These results confirmed a mediator role for prostaglandins in the gastric hyperaemic response following TC-induced barrier disruption, and suggest that COX-1 but not COX-2 is a key enzyme in maintaining 'housekeeping' functions in the gastric mucosa under both normal and adverse conditions.

Keywords: COX-1; COX-2; taurocholate-induced gastric lesion; gastric hyperaemia; indomethacin; COX-2-selective inhibitor

Introduction

Cyclo-oxygenases 1 and 2 (COX-1 and COX-2), key enzymes in the biosynthesis of prostaglandins, are implicated as important mediators of various physiological processes. However, these isozymes mediate different biological actions (Murakami *et al*., 1994; Morita *et al*., 1995). COX-1 appears to be constitutively synthesized in many tissues (Simmons *et al*., 1991; O'Neill & Ford-Hutchinson, 1993), while COX-2 message and protein are normally undetectable in most tissues and its expression can be rapidly induced by proinflammatory or mitogenic agents (Xie *et al*., 1992; O'Banion *et al*., 1991; Kennedy *et al*., 1993). Important roles for endogenous prostaglandins have also been well documented in the stomach, i.e., involvement in regulation of various functions such as mucosal blood flow, mucus secretion and bicarbonate secretion and in modulation of gastric mucosal integrity (Robert, 1979). Indeed, exogenous prostaglandins afford gastric cytoprotection against noxious stimuli (Robert & Ruwart, 1982), while nonsteroidal anti-inflammatory drugs (NSAIDs) cause damage in the stomach by inhibiting COX activity (Whittle *et al*., 1983). Recent studies with selective COX-2 inhibitors suggest that theulcerogenic property of NSAIDs is brought about by the inhibition of COX-1 but not COX-2 activity, suggesting a 'housekeeping' role for COX-1 in the stomach (Futaki *et al*., 1993; 1994).

The application of mild irritants to the stomach causes an increased gastric mucosal blood flow (GMBF)(Whittle, 1983; Takeuchi *et al*., 1994). These agents damage the surface epithelium of the gastric mucosa, resulting in acid back-diffusion, yet they rarely cause macroscopically visible damage and rather protect the stomach against necrotizing agents (Holzer *et al*., 1991; Takeuchi *et al*., 1993). Such hyperaemic responses subside in the presence of NSAIDs, suggesting a role for endogenous prostaglandins in this phenomenon (Whittle, 1983; Takeuchi *et al*., 1986; 1987). However, the relative contribution of the COX-1 and COX-2 isoforms in the maintenance of these functional responses in the stomach following barrier disruption is not entirely clear.

In the present study, we examined the effects of selective COX-2 inhibitors on various functional changes of the rat stomach following exposure to taurocholate, compared them with the effect of indomethacin, a nonselective COX inhibitor, and investigated the role of COX isoforms in adaptive responses of the stomach observed under adverse conditions, i.e., after barrier disruption.

Methods

Male Sprague-Dawley rats (IGS), weighing 200–230 g (Charles River, Shizuoka, Japan), were used in all experiments. The animals were kept in individual cages with raised mesh bottoms and deprived of food but allowed free access to tap water for 18 h before the experiments. Studies were carried out with 4–6 rats per group.

Experimental protocol

The experiments were performed in four groups of rats; each group was pretreated with saline, indomethacin, NS-398 and nimesulide, respectively. We used indomethacin as a nonselective COX-1- and COX-2-inhibitor, and either NS-398 or

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nimesulide as a COX-2-selective inhibitor (Futaki *et al*., 1994; Tavares *et al*., 1995; Kargman *et al*., 1996; Nakatsugi *et al*., 1996). In these groups of rats, the effects of mucosal application of sodium taurocholate (TC) plus HCl on gastric transmucosal potential difference (p.d.), gastric mucosal blood flow (GMBF), luminal acid loss (acid back-diffusion), luminal contents of prostaglandin E_2 (PGE_2, and the gastric mucosa were examined under urethane anaesthesia. In some rats, the expression of mRNA for COX-1 and COX-2 was examined by use of reverse transcription-polymerase chain reaction (RT-PCR) in the gastric mucosa before and after exposure to TC. In a separate study, we also examined the effects of the above NSAIDs on carrageenan-induced paw oedema in rats.

Determination of p.d., GMBF and acid back-diffusion

Animals were anaesthetized with urethane (1.25 g kg^{-1}, i.p.), and the trachea was cannulated to ensure a patent airway. Acid secretion was completely inhibited by pretreatment with omeprazole (60 mg kg^{-1}, i.p.). Simultaneous measurement of p.d., GMBF and acid back-diffusion was performed in a chambered stomach as previously described (Takeuchi *et al*., 1994; Miyake *et al*., 1996). Briefly, the abdomen was incised, and the stomach was exposed and mounted on an *ex vivo* chamber (area exposed, 3.14 cm^2). At the beginning of each experiment, the mucosa was rinsed several times with a solution of 50 mM HCl plus 100 mM NaCl. When the gastric exudate became clear, 2 ml of the acid solution was instilled in the chamber and 15 min later the gastric contents were recovered from the chamber. This procedure was repeated every 15 min, three times before and 6 times after exposure of the mucosa to 20 mM TC for 30 min. The p.d. was determined by means of two agar bridges, one positioned in the chamber and the other in the abdominal cavity. GMBF was measured by a laser Doppler flowmeter (Advance Model ALF 21, Tokyo, Japan), the probe being placed gently on the corpus mucosa by use of a balancer, and changes in GMBF were continuously monitored on a two-channel recorder (U-228, Tokai-Irika, Tokyo, Japan) simultaneously with those of p.d. (Matsumoto *et al*., 1992). Acid back-diffusion (luminal acid loss) was determined from analyses of the collected acid solution. Each sample was analysed for volume and acid concentration, which was determined by automatic titration of an aliquot with 0.1 N NaOH to pH 7.0 (Autoburette, Comtite-7, Hiranuma, Tokyo, Japan). The amount of acid back-diffusion was calculated as the difference between the product of the final volume and concentration and the product of the initial volume and concentration. Positive values indicate that the net flux of H^+ was from the mucosa to the lumen, and the results are expressed as microequivalents per 15 min. Indomethacin (10 mg kg^{-1}), NS-398 (10 mg kg^{-1}) or nimesulide (10 mg kg^{-1}) was given s.c. 30 min before TC treatment. Control animals received saline as the vehicle.

Ninety minutes after exposure to TC, the mucosa was examined for haemorrhagic damage under a dissecting microscope with a square grid ($\times 10$); the area (mm^2) of each lesion was measured, summed per stomach and used as a lesion score. The person measuring the lesions did not know the groups to which test drugs were given. Tissue samples were then immersed into 10% formalin for histological observation, processed for routine light microscopy, sectioned at 5 μm and stained with haematoxylin and eosin.

Determination of prostaglandin E_2

The amount of PGE_2 that appeared in the lumen of the stomach was measured before, during and after exposure to 20 mM TC. In the chambered stomach, gastric contents were collected every 30 min, before, during and after exposure to TC, and stored at $-80°C$ until the assay. The PGE_2 content of each sample was measured by ELISA using PGE_2-kit (Cayman Chemical Co., Ann Arbour, MI, U.S.A.). Indomethacin (10 mg kg^{-1}), NS-398 (10 mg kg^{-1}) or nimesulide (10 mg kg^{-1}) was given s.c. 30 min before TC treatment.

Analyses of COX-1- and COX-2-mRNA by RT-PCR

The stomachs were exposed to 20 mM TC for 30 min and quickly removed from the chamber at various time intervals (0, 30, 90 and 120 min) after the exposure. Each tissue was then frozen in liquid nitrogen and stored at $-80°C$ until use. Stomach tissue samples were pooled from 2–3 rats for extraction of total RNA, which was prepared by a single-step acid phenol-chloroform extraction procedure by use of ISOGEN (Nippon gene, Tokyo, Japan). Total RNA primed by random hexadeoxyribonucleotide was reverse-transcribed with MMLV reverse-transcriptase. The sequences of sense and antisense primers for the rat COX-1 were 5′-AACC GTGTGTGTGACTTGCTGAA-3′ and 5′-AGAAGGAG CCCCTCAGAG CTCAGTG-3′, respectively, giving rise to a 887 bp PCR product (Feng *et al*., 1993). For the rat COX-2, those of sense and antisense primers were 5′-TGAT-GACTGCCCAACTCCCATG-3′ and 5′-AATGTTGAAG GTGTCCGGCAGC-3′, respectively, giving rise to a 702 bp PCR product (Iso *et al*., 1995). In addition, for the rat glyceraldehyde-3-phosphate dehydrogenase (G3PDH), a constitutively expressed gene, the sequences were 5′-GAACGG GAAGCTCACTGGCATGGC-3′ for sense primer and 5′-TGAGGTCCACCACCC TGTTGCTG-3′ for antisense primer, giving rise to a 310 bp PCR product. An aliquot of the RT reaction product served as a template in 35 cycles of PCR with 0.5 min of denaturation at 94°C, 0.7 min of annealing at 64°C for both COX-2 and G3PDH or at 60°C for COX-1, and 1 min of extension at 72°C on a thermal cycler. A portion of the PCR mixture was electrophoresed in 1.5% agarose gel in TAE buffer (Tris buffer 40 mM, EDTA 2 mM and acetic acid 20 mM; pH 8.1), and the gel was stained with ethidium bromide and photographed.

Formation of paw oedema by carrageenan

Paw oedema was induced in unanaesthetized rats by subplantar injection of carrageenan (0.1 ml of 1% carrageenan-saline solution) into the right hind paw (Salvemini *et al*., 1996). Paw volume was measured with a plethysmometer immediately before the injection of carrageenan and thereafter at 2 h intervals for 6 h. Oedema was expressed as the increase in paw volume (ml) after carrageenan injection relative to the pre-injection value for each animal. Indomethacin (10 mg kg^{-1}), NS-398 (10 mg kg^{-1}) or nimesulide (10 mg kg^{-1}) was given s.c. 30 min before carrageenan injection.

Preparation of drugs

Drugs used in this study were urethane (Tokyo Kasei, Tokyo, Japan), taurocholate Na (Difco Lab., Detroit, Michigan, U.S.A.), indomethacin, nimesulide, carrageenan (Sigma Chemicals, St. Louis, Montana, U.S.A.), NS-398 (N-[2 cyclohexyloxy-4-nitrophenyl] methanesulfonamide, Taisho Pharmaceutical Co., Tokyo, Japan) and omeprazole (Hassle, Mondale, Sweden). Indomethacin, NS-398 and nimesulide were suspended in saline with a drop of Tween 80, while omeprazole was suspended in 0.5% carboxylmethylcellulose solution. Carrageenan was dissolved in saline. Each agent was prepared immediately before use and administered i.p. or s.c. in a volume of 0.5 ml per 100 g body weight, or applied topically to the chamber in a volume of 2 ml per rat.

Statistics

Data are presented as the means ± s.e. from 4–6 rats per group. Statistical analyses were performed by a two-tailed Dunnett's multiple comparison test, and values of $P<0.05$ were regarded as significant.

Results

Effects of various NSAIDs on gastric functional and ulcerogenic responses induced by TC

p.d. responses Under chambered conditions in the presence of omeprazole (inhibition of acid secretion) and exogenous acid (50 mM HCl plus 100 mM NaCl), the rat stomach generated a p.d. of −59 to −64 mV (mucosa negative) and maintained relatively constant GMBF (100–140 mV: arbitrary units) during a 2 h experimental period. In control rats, the mucosal application of 20 mM TC for 30 min caused a marked reduction of p.d. from -60.3 ± 1.8 mV to -33.6 ± 2.6 mV, but after exposure the reduced p.d. was gradually normalized to basal values, the degree of p.d. recovery being $46.6 \pm 6.6\%$ at 60 min after treatment (Figures 1 and 2). Pretreatment of the animals with indomethacin (10 mg kg^{-1}, s.c.), a nonselective COX-1 and COX-2 inhibitor, did not affect the reduction in p.d. caused by TC but significantly delayed the recovery of p.d. after exposure to TC, the degree of p.d. recovery being $18.4 \pm 3.8\%$ at 90 min post treatment. On the other hand, COX-2 selective inhibitors such as NS-398 and nimesulide at a dose of 10 mg kg^{-1} had no effect on such p.d. responses after exposure to TC; the degree of p.d. recovery at 90 min post treatment was $59.2 \pm 3.9\%$ and $59.4 \pm 3.7\%$, respectively, which was not statistically significant when compared to control rats.

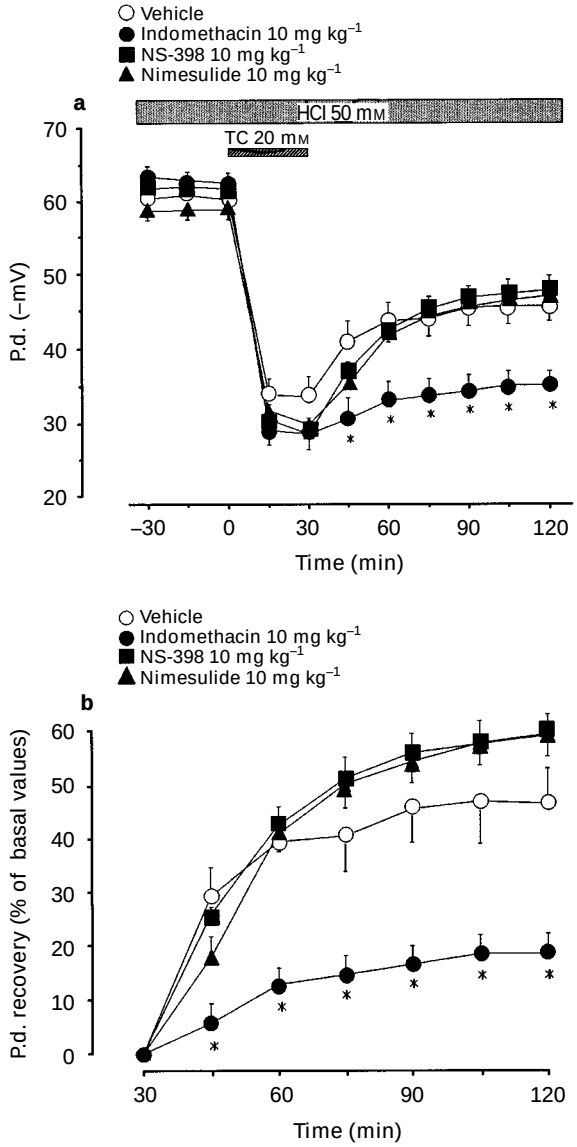

Figure 1 (a) Effects of indomethacin, NS-398 and nimesulide on changes in the potential difference (p.d) after exposure of the stomach to taurocholate (TC) in anaesthetized rats. The stomach was exposed to 50 mM HCl (2 ml) every 15 min before and after exposure to 20 mM taurocholate (in 50 mM HCl) for 30 min. Indomethacin, NS-398 or nimesulide was given subcutaneously at a dose of 10 mg kg^{-1} 30 min before exposure of the stomach to taurocholate. (b) Effects of these agents on the p.d. recovery after exposure of the stomach to 20 mM taurocholate for 30 min. Data are presented as the means of values determined every 15 min from 6 rats per group; vertical lines show s.e.mean *Significantly different from vehicle, at $P<0.05$.

GMBF responses The GMBF was significantly elevated during exposure to 20 mM TC (plus 50 mM HCl), reaching a peak increase of $101.7 \pm 19.4\%$, and remained significantly elevated even after removal of TC from the chamber (Figures 2 and 3). Even at 60 min after treatment, the GMBF showed a significant increase (~30%) as compared to pre-exposure values. This hyperaemic response caused by TC was all but totally attenuated in rats pretreated with indomethacin and the GMBF remained in a similar range before and after exposure to TC, the peak increase being only $12.6 \pm 7.2\%$ above basal levels. On the other hand, neither NS-398 nor nimesulide had any effect on the gastric hyperaemic response seen after exposure to TC, and the GMBF was significantly and persistently increased after TC treatment; the peak increase was $91.3 \pm 11.3\%$ and $104.7 \pm 13.7\%$, respectively, which was not significantly different from that observed in control animals ($101.7 \pm 19.4\%$).

Acid back-diffusion When the gastric mucosa was exposed to 50 mM HCl in the absence of acid secretion induced by omeprazole, a small but significant loss of luminal H^+ was consistently observed in control rats under normal conditions; acid loss (H^+) was less than 25 μEq 15 min^{-1}. Following the mucosal application of 20 mM TC, the loss of H^+ was significantly increased, reaching a maximal value (54.0 ± 3.0 μEq 15 min^{-1}) immediately after the exposure, then gradually decreasing to pre-exposure levels 90 min later (Figure 4). Pretreatment with either indomethacin, NS-398 or nimesulide did not significantly affect the increased mucosal permeability to H^+ in response to TC; the magnitude of H+ observed immediately after TC treatment was 58.7 ± 2.6 μEq 15 min^{-1}, 57.2 ± 6.1 μEq 15 min^{-1} and 55.5 ± 4.2 μEq 15 min^{-1}, respectively, which was not significantly different from that in control rats.

Mucosal injury Mucosal application of 20 mM TC or acid solution (50 mM HCl) by itself did not induce gross damage in the gastric mucosa, but these treatments given together produced a few haemorrhagic lesions in the gastric mucosa of control rats, the lesion score being 2.3 ± 0.7 mm^2 (Figure 5). When animals were pretreated with indomethacin, the lesions induced by TC plus HCl were significantly worsened; the lesion score was 36.7 ± 6.1 mm^2, which was about 10 times greater than that in the control group. Histologically, the stomachs of control rats exhibited widespread exfoliation of epithelial cells without deep damage beyond the basement membrane, whereas in those pretreated with indomethacin the damage was deep into the mucosa with haemorrhagic changes (not shown). In contrast, neither NS-398 nor nimesulide had any effect on the mucosal ulcerogenic response to TC plus HCl; the lesion score was 3.1 ± 1.1 mm^2 and 3.7 ± 1.3 mm^2, respectively, which was not significantly different when compared to control rats.

Effects of various NSAIDs on luminal PGE_2 release induced by TC

Luminal release of PGE_2 in control stomachs was very low, ranging between 0.05–0.2 ng 30 min^{-1} under normal conditions. Exposure of the stomach to 20 mM TC for 30 min caused a marked increase of PGE_2 release into the lumen, reaching 7.6 ± 2.0 ng 30 min^{-1} during exposure, and these

values remained at significantly high levels for 90 min even after removal of TC from the chamber (Figure 6). The luminal release of PGE_2 induced by TC treatment was significantly inhibited during the whole test period by prior administration of indomethacin. In these rats, the release of PGE_2 in the lumen was reduced to 2.3 ± 0.2 ng 30 min^{-1} during exposure and almost totally inhibited after exposure (less than 0.2 ng 30 min^{-1}). Neither NS-398 nor nimesulide affected the luminal release of PGE_2 during and after exposure to TC; a marked increase of PGE_2 was observed in response to TC, and the values were not significantly different from controls at any time points.

Changes in COX-1- and COX-2-mRNA expression induced by TC

Under normal conditions, only COX-1 gene expression was detected on agarose gels stained with ethidium bromide. After

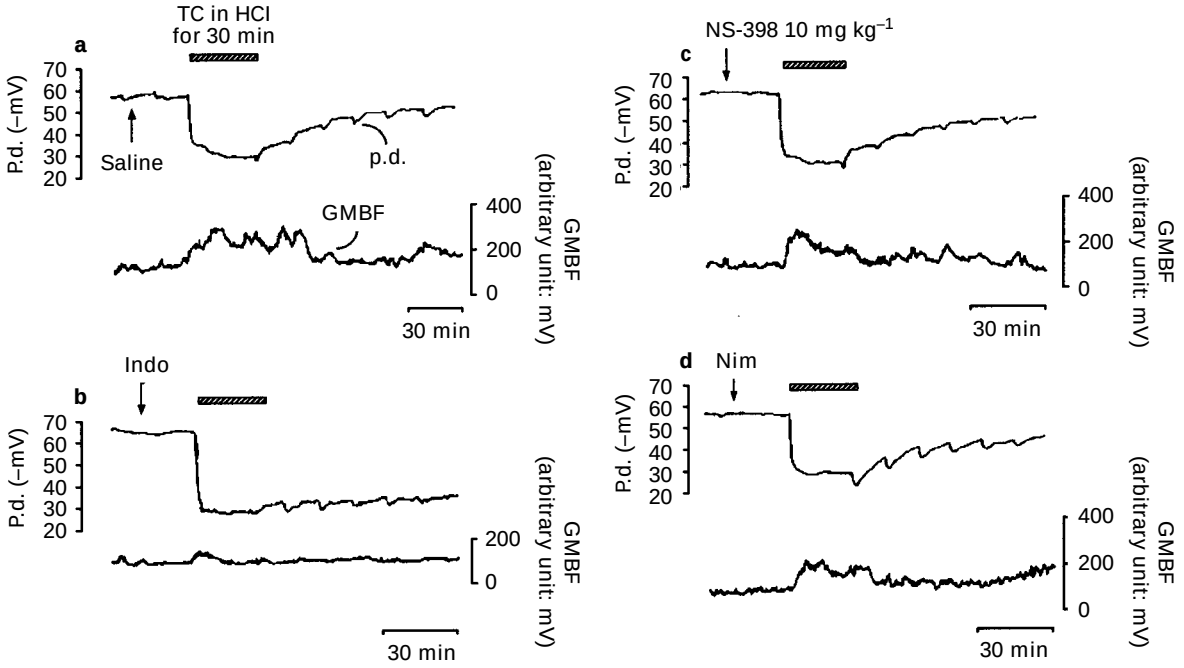

Figure 2 Representative recordings showing changes in the potential difference (p.d.) and mucosal blood flow (GMBF) before, during and after exposure of the stomach to 20 mM taurocholate (TC) plus 50 mM HCl in anaesthetized rats. Note that indomethacin (Indo; 10 mg kg^{-1}) totally attenuated the gastric hyperaemic response induced by taurocholate plus HCl and prevented the recovery of p.d. following the barrier disruption, while the selective COX-2 inhibitors such as NS-398 and nimesulide (Nim; 10 mg kg^{-1}) had no effect on either PD or GMBF responses to TC.

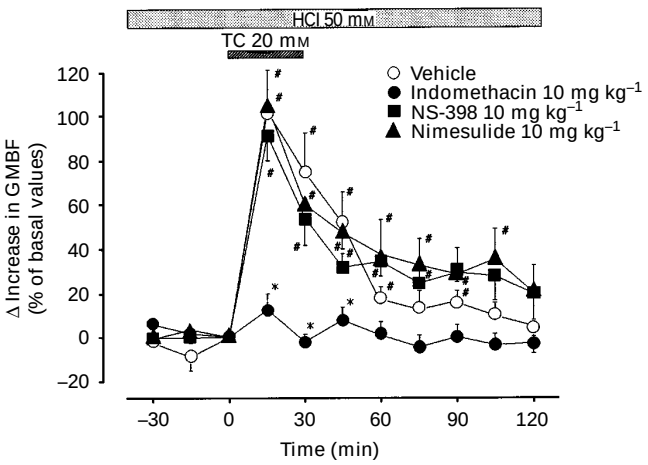

Figure 3 Effects of indomethacin, NS-398 and nimesulide on changes in mucosal blood flow (GMBF) caused by taurocholate (TC) in anaesthetized rats. The stomach was exposed to 50 mM HCl (2 ml) every 15 min before and after exposure to 20 mM taurocholate (in 50 mM HCl) for 30 min. Indomethacin, NS-398 or nimesulide was given subcutaneously at a dose of 10 mg kg^{-1} 30 min before TC treatment. Data are expressed as increase in GMBF (% of basal values) and represent the means of values determined every 15 min from 6 rats per group; vertical lines show s.e.mean. Significantly different at $P<0.05$: #from basal values (time 0) in the corresponding group; *from the vehicle group.

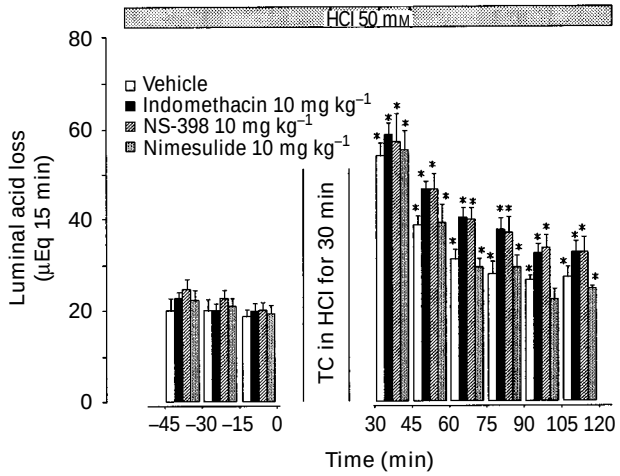

Figure 4 Effects of indomethacin, NS-398 and nimesulide on changes in luminal acid loss in the stomach caused by taurocholate (TC) in anaesthetized rats. The stomach was exposed to 50 mM HCl (2 ml) every 15 min before and after exposure to 20 mM taurocholate (in 50 mM HCl) for 30 min. Indomethacin, NS-398 or nimesulide was given subcutaneously at a dose of 10 mg kg^{-1} 30 min before TC treatment. Data are presented as the means ± s.e. of values determined every 15 min from 6 rats per group. *Significantly different from basal values (time 0) in the corresponding group, at $P<0.05$. No significant difference was observed between the four different groups.

exposure of the stomach to 20 mM TC for 30 min, the expression of COX-1-mRNA remained unchanged in the gastric mucosa during the 2 h test period, and a faint expression of COX-2-mRNA was observed only 2 h after exposure (Figure 7).

Effects of various NSAIDs on carrageenan-induced paw oedema

The intraplantar injection of carrageenan elicited acute hind-paw inflammation and caused a time-dependent increase in paw oedema, a peak response being observed 4 h after the injection (Figure 8). Treatment of rats with indomethacin 10 mg kg^{-1} before carrageenan administration significantly

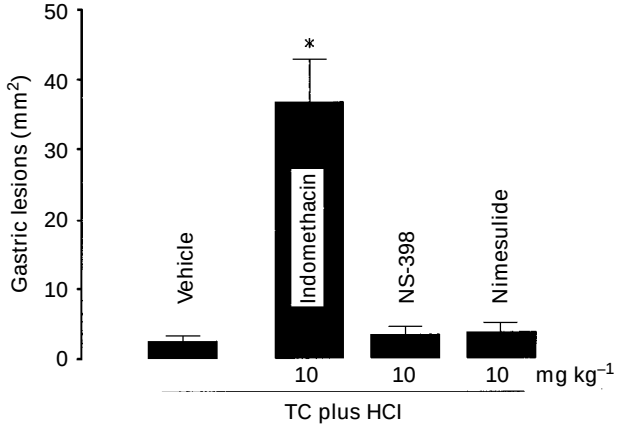

Figure 5 Development of gastric lesions in the anaesthetized rat stomach after exposure to taurocholate, in the presence of indomethacin, NS-398 or nimesulide. The stomach was exposed to 50 mM HCl (2 ml) every 15 min before and after exposure to 20 mM taurocholate (in 50 mM HCl) for 30 min, and examined for lesions 2 h after TC treatment. Indomethacin, NS-398 or nimesulide was given subcutaneously at a dose of 10 mg kg^{-1} 30 min before TC treatment. Data are presented as the means ± s.e. from 6 rats per group. *Significantly different from vehicle, at $P<0.05$.

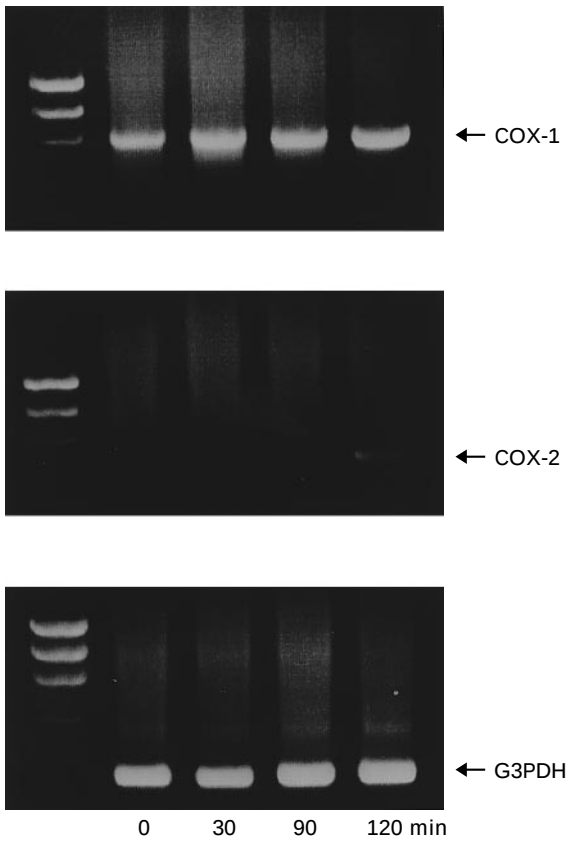

Figure 7 Mucosal expression of COX-1 and COX-2-mRNA in the stomach before and after exposure to taurocholate in anaesthetized rats. The stomach was exposed to 50 mM HCl (2 ml) every 15 min before and after exposure to 20 mM taurocholate (in 50 mM HCl) for 30 min. Figures show that the expression of COX-1-mRNA was consistently observed in the mucosa before and after exposure of the stomach to TC, while COX-2-mRNA was faintly expressed in the mucosa at 120 min but did not appear up to 90 min after TC treatment.

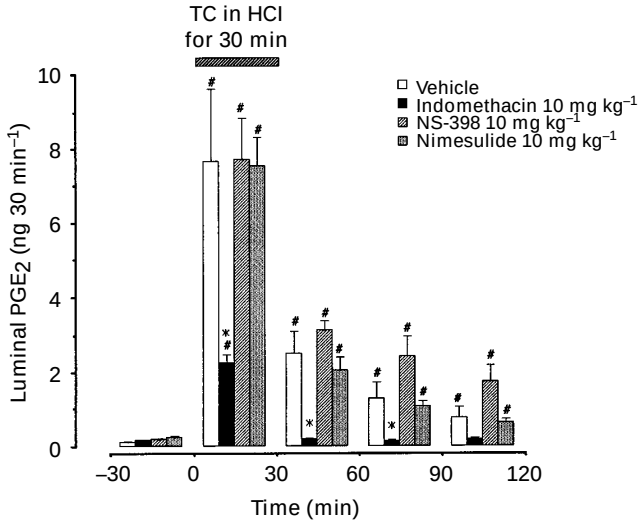

Figure 6 Effects of indomethacin, NS-398 or nimesulide on the luminal release of PGE_2 in the stomach after exposure to taurocholate (TC) in anaesthetized rats. The stomach was exposed to 50 mM HCl (2 ml) every 15 min before and after exposure to 20 mM taurocholate (in 50 mM HCl) for 30 min. Indomethacin, NS-398 or nimesulide was given subcutaneously at a dose of 10 mg kg^{-1} 30 min before TC treatment. Data are presented as the means ± s.e. of values determined every 30 min from 4–6 rats per group. Significantly different at $P<0.05$: #from basal values (time 0) in the corresponding group; *from the vehicle group.

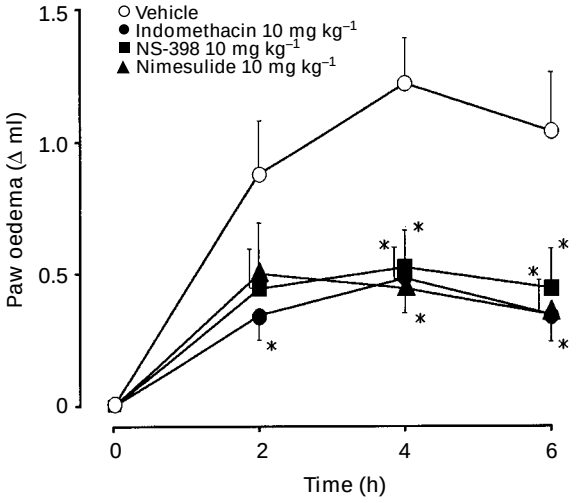

Figure 8 Anti-inflammatory effects of indomethacin, NS-398 or nimesulide on oedema formation induced in rat paws by carrageenan. The animals received a subplantar injection of carrageenan (0.1 ml of a 1% suspension in saline) into the right hind paw. Indomethacin, NS-398 or nimesulide was given subcutaneously at a dose of 10 mg kg^{-1} 30 min before carrageenan injection. Data are presented as the means from 6 rats per group; vertical lines show s.e.mean. *Significantly different from vehicle, at $P<0.05$.

suppressed paw volume, the inhibition being over 60% at all time points. Both NS-398 and nimesulide at 10 mg kg^{-1} significantly reduced paw volume 4 and 6 h after carrageenan injection. These agents also suppressed paw volume 2 h after administration of carrageenan, although this effect did not reach statistical significance.

Discussion

The present study confirmed a mediator role for endogenous prostaglandins in the gastric hyperaemic response in the rat stomach following barrier disruption by TC, and demonstrated that the gastric mucosa was ulcerated when the animals were pretreated with indomethacin, a non-selective COX-1 and COX-2 inhibitor, but not when pretreated with NS-398 or nimesulide, selective COX-2 inhibitors. These results suggest that the COX-1 but not COX-2 isoform is involved in the gastric hyperaemic response after barrier disruption and plays a role in protecting the gastric mucosa against acid injury.

In gastrointestinal tract tissues, prostaglandins are involved in numerous physiological processes, including control of gastric secretion, mucus production, mucosal blood flow and maintenance of mucosal integrity (Robert & Ruwart, 1982). The first enzyme in the pathway for prostaglandin synthesis, COX, has been shown to be present in the rat and human stomach (Mikkelsen *et al*., 1991). The two COX isoforms, referred to as COX-1 and COX-2 have similar catalytic activities; these enzymes catalyze the two-step conversion of arachidonic acid to PGH_2, the required precursor for the formation of all prostanoids. COX-1 is constitutively expressed in normal gastric mucosa and has been proposed to generate prostaglandins involved in the maintenance of essential physiological functions, such as gastric cytoprotection (Wallace, 1992; Feng *et al*., 1993; Vane & Bolting, 1995; Nakatsugi *et al*., 1996). The second isoform COX-2 is characterized by its ability to be rapidly induced in response to various proinflammatory stimuli such as mitogens, hormones and cytokines, and has been thought to be responsible for pathological prostaglandin production at inflammatory sites (Masferrer *et al*., 1994; Chan *et al*., 1995). On the other hand, the stomach responds to mucosal damaging agents by altering various functions such as mucosal blood flow (Whittle, 1983; Takeuchi *et al*., 1986; 1987; Lippe & Holzer, 1992; Miyake *et al*., 1996). Although the role of prostaglandins may vary with different types of mucosal irritants used to break the gastric mucosal barrier in the presence of luminal acid, this process is partly mediated by endogenous prostaglandins especially when the barrier is disrupted by TC or hyperosmolar NaCl (Whittle, 1983; Takeuchi *et al*., 1986; 1987). These agents damage surface epithelial cells and increase prostaglandin biosynthesis in the gastric mucosa, yet it remains unclear whether this prostaglandin response is mediated by the enzymatic activity of COX-1 or COX-2. If gastric lesions occurred in response to NSAIDs by causing functional disturbances related to COX-1 inhibition, then selective COX-2 inhibitors might be used safely without inducing gastric functional alteration and mucosal damage.

In the present study, we observed that the mucosal application of TC caused a reduction in p.d. followed by an increase in acid back-diffusion and GMBF, without extension to gross damage, in agreement with previous findings of others and ourselves (Whittle, 1983; Takeuchi *et al*., 1993). This treatment also enhanced prostaglandin biosynthesis in the stomach, inasmuch as the release of PGE_2 in the lumen was increased 10 fold the basal value after exposure to TC. Whittle (1983) showed that gastric hyperaemia following acid back-diffusion caused by TC is attenuated by indomethacin, suggesting the involvement of endogenous prostaglandins in this phenomenon. Others showed that this hyperaemic response is partly mediated by nitric oxide (NO) and sensory neurones, in addition to prostaglandins. However, since these factors interact with each other in maintaining gastric function and in modulating mucosal integrity (Holzer *et al*., 1991; Takeuchi *et al*., 1994), the lack of any one factor may lead to failure of full expression of these functions. As expected, we observed in this study that indomethacin, a non-selective COX inhibitor, attenuated the hyperaemic response to TC, without affecting PD reduction and acid back-diffusion, and caused haemorrhagic lesions in the mucosa. In contrast, the selective COX-2 inhibitors NS-398 and nimesulide had no effect on the gastric hyperaemic response following barrier disruption by TC and did not cause any gross damage in the stomach. Futaki *et al*., (1993; 1994) showed that NS-398 did not affect the activity of COX-1 purified from ram seminal vesicles even at 10^{-4} M but showed a potent inhibition against the activity of COX-2 isolated from sheep placenta in a concentration-dependent manner (IC_{50} value: 3.8×10^{-6} M). Likewise, nimesulide showed a preferential inhibition against the COX-2 activity rather than the COX-1 activity (Tavares *et al*., 1995; Nakatsugi *et al*., 1996). Of interest, these NSAIDs did not have a significant effect on the luminal release of PGE_2 during and after exposure of the stomach to TC, suggesting that the enhanced prostaglandin biosynthesis following barrier disruption is associated with COX-1 activity. These findings also indicate that the gastric hyperaemic response following acid back-diffusion is mediated by prostaglandins and is dependent on COX-1 enzymatic activity. We have previously shown that prednisolone, which is known to inhibit phospholipase A_2 activity, also inhibited functional changes in the stomach induced by hyperosmolar NaCl (Nobuhara *et al*., 1985). These findings together suggest that local application of mild irritants such as TC or hyperosmolar NaCl first releases arachidonic acid from membrane phospholipids in association with the surface epithelial injury and then increases prostaglandin production, resulting in adaptive cytoprotection in the stomach.

Kargman *et al*. (1996) recently showed that COX-1 protein was ubiquitously expressed in microsomes derived from various tissues in the gastrointestinal tract, including the stomach, while the expression of COX-2 protein was absent from most gastrointestinal tissues. However, recent immunohistochemical study showed that COX-2 is also expressed in surface mucous cells of normal gastric mucosa (Iseki, 1995). We observed that the COX-1-mRNA was expressed in the gastric mucosa, irrespective of whether or not the stomach was exposed to TC. In contrast, the expression of COX-2-mRNA was not detected in the normal gastric tissue, and only a faint expression was observed in the mucosa 2 h after exposure to TC. Since the increase of GMBF as well as PGE_2 release was most marked during exposure to TC and subsided gradually in later periods, it is unlikely that such a faint expression of COX-2 contributes to the events that occur in the stomach immediately after barrier disruption. However, TC irritates the mucosa by damaging the surface epithelial cells, and the tissue would be subsequently repaired by multiple processes such as restitution (Silen, 1987). It is possible that the expression of COX-2 in the irritated mucosa plays a role in events associated with tissue repair. Indeed, Mizuno *et al*., (1997) recently showed that both COX-2-mRNA and protein are expressed in the gastric mucosa after induction of acute and chronic ulcers in mice and play an important role in the healing of these ulcers.

A potent anti-inflamamtory action against carrageenan-induced paw oedema was observed by NS-398 and nimesulide as well as indomethacin. It is known that intrapleural injection of carrageenan produces an increased production of PGE_2 and induction of *de novo* synthesis of COX-2 in pleural exudate cells (Masferrer *et al*., 1994). COX-1 was also detected in these inflammatory exudate cells but remained at control levels, suggesting that the increased production of PGE_2 by inflammatory stimuli was mediated by newly synthesized COX-2 protein (Nakatsugi *et al*., 1996). Thus, the present results that both NS-398 and nimesulide suppressed carrageenan-induced paw oedema may be explained by inhibition of COX-2 enzymatic activity in inflammatory cells.

Inhibition of COX-1 enzymatic activity and subsequent reduction of PGE_2 production have been considered to be associated with the ulcerogenic potential of NSAIDs (Mas-

ferrer *et al*., 1994; Seibert *et al*., 1994). The present study demonstrated a differential sensitivity of COX isoforms to inhibition by different NSAIDs and supports the concept that anti-inflammatory effects and unwanted side effects of NSAIDs may be related to their ability to inhibit COX-2 and COX-1 activity, respectively (Vane & Bolting, 1995). Futaki *et al*. (1993) found that NS-398, although able to inhibit prostaglandin production in inflammatory tissues, had no effect on gastric prostaglandin content and did not modify stress-induced gastric ulcerations, while indomethacin decreased gastric prostaglandin levels and worsened gastric lesions in response to stress. Similar findings were obtained by Nakatsugi *et al*., (1996), who showed that nimesulide did not enhance stress-induced gastric lesions even at 30 times the anti-inflammatory dose, while both indomethacin and ibuprofen enhanced a decrease in gastric prostaglandin content during stress, resulting in aggravation of stress-induced gastric lesions. In the present study, TC plus HCl caused gastric lesions in the animals pretreated with indomethacin but not with either NS-398 or nimesulide. These results together with previous observations support the hypothesis that inhibition of COX-1 causes unwanted side effects and inhibition of COX-2 produces anti-inflammatory effects.

Taken together, the present study showed for the first time that the COX-1 isoform is involved in the gastric hyperaemic response after TC-induced barrier disruption in the stomach. The increase of GMBF following barrier disruption is one of the adaptive responses of the stomach and plays an important role in protecting the mucosa against acid injury by disposing of H^+. Since the stomach is continuously exposed to a variety of noxious stimuli such as acid, bile acids and food-related chemicals, it seems that endogenous prostaglandins derived from COX-1 enzymatic activity may be crucial in maintaining 'housekeeping' functions in the gastric mucosa under both normal and adverse conditions.

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