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Effects of specific inhibition of cyclo-oxygenase-1 and cyclo-oxygenase-2 in the rat stomach with normal mucosa and after acid challenge

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1 Effects of the cyclo-oxygenase (COX)-1 inhibitor SC-560 and the COX-2 inhibitors rofecoxib and DFU were investigated in the normal stomach and after acid challenge.

2 In healthy rats, neither SC-560 nor rofecoxib (20 mg kg^{-1} each) given alone damaged the mucosa. Co-treatment with SC-560 and rofecoxib, however, induced severe lesions comparable to indomethacin (20 mg kg^{-1}) whereas co-administration of SC-560 and DFU (20 mg kg^{-1} each) had no comparable ulcerogenic effect 5 h after dosing.

3 SC-560 (20 mg kg^{-1}) inhibited gastric 6-keto-prostaglandin (PG) $F_{1\alpha}$ by $86 \pm 5\%$ and platelet thromboxane (TX) B_2 formation by $89 \pm 4\%$ comparable to indomethacin (20 mg kg^{-1}). Rofecoxib (20 mg kg^{-1}) did not inhibit gastric and platelet eicosanoids.

4 Intragastric HCl elevated mucosal mRNA levels of COX-2 but not COX-1. Dexamethasone (2 mg kg^{-1}) prevented the up-regulation of COX-2.

5 After acid challenge, SC-560 (5 and 20 mg kg^{-1}) induced dose-dependent injury. Rofecoxib (20 mg kg^{-1}), DFU (5 mg kg^{-1}) and dexamethasone (2 mg kg^{-1}) given alone were not ulcerogenic but aggravated SC-560-induced damage. DFU augmented SC-560 damage 1 but not 5 h after administration whereas rofecoxib increased injury after both treatment periods suggesting different time courses.

6 Gastric injurious effects of rofecoxib and DFU correlated with inhibition of inflammatory PGE_2.

7 The findings show that in the normal stomach lesions only develop when both COX-1 and COX-2 are inhibited. In contrast, during acid challenge inhibition of COX-1 renders the mucosa more vulnerable suggesting an important role of COX-1 in mucosal defence in the presence of a potentially noxious agent. In this function COX-1 is supported by COX-2. In the face of pending injury, however, COX-2 cannot maintain mucosal integrity when the activity of COX-1 is suppressed.

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Abbreviations: COX, cyclo-oxygenase; DNA, deoxyribonucleic acid; GAPDH, glyceraldehyde-3 phosphate dehydrogenase; mRNA, messenger ribonucleic acid; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; RIA, radioimmunossay; RT–PCR, reverse transcription-polymerase chain reaction; TX, thromboxane

Introduction

Two isoforms of cyclo-oxygenase (COX), the key enzyme in prostaglandin (PG) and thromboxane (TX) biosynthesis, referred to as COX-1 and COX-2, have been identified. Each enzyme is encoded by a separate gene (Hla & Neilson, 1992) and has a distinct pattern of expression and biological function. COX-1 is expressed constitutively and high levels can be detected in most tissues (O'Neill & Ford-Hutchinson, 1993). In contrast, levels of COX-2 mRNA and protein are usually low or undetectable under basal conditions but are rapidly elevated during inflammation or mitogenic stimulation (Raz *et al*., 1989; Kujubu *et al*., 1991). The COX isoforms are the primary target enzymes for non-steroidal anti-inflammatory drugs (NSAIDs). It was postulated that inhibition of COX-2 mediates the anti-inflammatory and chemopreventive effects of NSAIDs without relevant influence on homeostasis reactions, whereas inhibition of COX-1 is responsible for the NSAID-associated side effects in the gastrointestinal tract, cardiovascular system and kidney (Vane & Botting, 1995). This concept was corroborated by studies showing that e.g. the severity of gastrointestinal side effects that occur during NSAID therapy correlates with the COX-1 selectivity of the drugs (Warner *et al*., 1999). Furthermore, it was demonstrated that selective COX-2 inhibitors do not induce gastric mucosal damage in experimental animals (Futaki *et al*., 1993; Masferrer *et al*., 1994; Riendeau *et al*., 1997; Schmassmann *et al*., 1998; Chan *et al*., 1999) and cause significantly less gastrointestinal side effects in humans than standard NSAIDs that inhibit both COX-1 and COX-2 (Laine *et al*., 1999; Langman *et al*., 1999; Simon *et al*., 1999).

In contrast to the initial hypothesis, recent findings suggest that COX-2 contributes to prostaglandin biosynth-

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esis in physiological situations. Thus, COX-2 is expressed constitutively in the brain (Lukiw & Bazan, 1997), kidney (Harris, 1996) and pancreatic islets (Robertson, 1998). A physiological role of COX-2 is supported by the finding that COX-2 deficient mice have defects in renal function (Morham *et al*., 1995), female reproductive physiology (Lim *et al*., 1997), and regulation of bone resorption (Raisz, 1999). Moreover, in healthy humans, urinary excretion of 6-keto-$PGF_{1\alpha}$ and the metabolite 2,3 dinor 6-keto-$PGF_{1\alpha}$ was significantly suppressed by the COX-2 inhibitor celecoxib implicating a major role for COX-2 in the biosynthesis of renal and systemic PGI_2 under physiological conditions (McAdam *et al*., 1999). In rats, COX-2 inhibitors counteracted the gastroprotective effects of a mild irritant (Gretzer *et al*., 1998) or luminal perfusion with peptone (Ehrlich *et al*., 1998) and markedly aggravated gastric damage induced by ischaemia-reperfusion (Maricic *et al*., 1999). COX-2 inhibitors delayed the healing of gastric ulcers in mice (Mizuno *et al*., 1997) and rats (Schmassmann *et al*., 1998) and inhibited angiogenesis, epithelial cell proliferation and maturation of the granulation tissue in chronic gastric ulcers (Schmassmann *et al*., 1998). Furthermore, in a rat colitis model, COX-2 inhibitors were demonstrated to cause exacerbation of colonic inflammation (Reuter *et al*., 1996). On the other hand, in the rat stomach chronic administration of endotoxin increased the expression of both COX-1 and COX-2 mRNA but only the non-selective COX inhibitor indomethacin and not a selective COX-2 inhibitor counteracted the protective effect of endotoxin (Ferraz *et al*., 1997). Hence, both COX-1 and COX-2 were found to mediate gastrointestinal defence reactions in certain pathophysiological situations.

Standard NSAIDs cause profound inhibition of gastric mucosal prostaglandin formation and induce gastric mucosal injury in experimental animals and humans. As selective COX-2 inhibitors have a markedly reduced capacity to exert gastrotoxic effects, it was postulated that in the healthy stomach COX-1 is the exclusive COX isoform responsible for the maintenance of mucosal integrity (Vane & Botting, 1995; Warner *et al*., 1999) with COX-2 contributing to mucosal defence only under pathophysiological conditions. This hypothesis seemed to be supported by the finding that a selective COX-1 inhibitor (Smith *et al*., 1998) but not selective COX-2 inhibitors (Mizuno *et al*., 1997; Schmassmann *et al*., 1998; Gretzer *et al*., 1998; Ehrlich *et al*., 1998; Maricic *et al*., 1999) cause measurable inhibition of gastric mucosal prostaglandin formation. The present study investigates in rats the effects of the selective COX-1 inhibitor [5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole] (SC-560) (Smith *et al*., 1998) and the selective COX-2 inhibitors 4-[4-(methylsulphonyl)phenyl]-3-phenyl-2(5*H*)-furanone (rofecoxib) (Chan *et al*., 1999) and 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5II)-furanone (DFU) (Riendeau *et al*., 1997) on gastric mucosal integrity in the normal rat stomach and after intraluminal acid instillation. The doses of the COX-2 inhibitors used in our experiments were selected with respect to the reported anti-inflammatory effects of rofecoxib (Chan *et al*., 1999) and DFU (Riendeau *et al*., 1997) in various models of inflammation in rats. The findings of this study show that in the normal stomach mucosal damage only develops when both COX-1 and COX-2 are inhibited. A similar conclusion has recently been reached using other COX-2 inhibitors in combination with SC-560 by Wallace *et al*. (2000). In contrast to normal gastric mucosa, in acid-challenged gastric mucosa selective inhibition of COX-1 is sufficient to produce mucosal injury that is further increased by simultaneous COX-2 inhibition. Some of this work has been previously published in abstract form (Gretzer *et al*., 2000).

Methods

Animal models

Male Wistar rats (180–220 g) were fasted overnight with free access to tap water. All experimental protocols were approved by the Animal Care Committee of the Ruhr-University of Bochum.

Normal gastric mucosa Rats were treated orally with indomethacin (5 or 20 mg kg^{-1}), SC-560 (20 mg kg^{-1}), or rofecoxib (20 mg kg^{-1}). Additional groups of rats received concurrent oral treatment with SC-560 (20 mg kg^{-1}) and rofecoxib (1–20 mg kg^{-1}) or DFU (20 mg kg^{-1}). Controls received the vehicle (2.5 ml kg^{-1} of 0.25% methylcellulose). Five hours after drug administration, rats were killed by cervical dislocation. The stomach was removed and gross mucosal damage was assessed in a blinded manner by calculation of a lesion index (LI) using a 0–3 scoring system based on the number and severity factor of lesions. The severity factor was defined according to the length of the lesions. Severity factor 0 is = no lesions visible; I = lesions <2 mm; II = lesions 2–4 mm; III = lesions >4 mm. The lesion index was calculated as the total number of lesions multiplied by their respective severity factor.

Acid-challenged gastric mucosa Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (50 mg kg^{-1}) and tracheotomized. After ligation of the oesophagus and pylorus, 1 ml of HCl (200 or 300 mM) or saline was instilled into the gastric lumen through the forestomach. Sixty minutes later, the stomach was excised and gross mucosal damage was assessed in a blinded manner by calculation of a lesion index as described above.

Groups of rats were treated orally with indomethacin (5 mg kg^{-1}), SC-560 (5 or 20 mg kg^{-1}), rofecoxib (20 mg kg^{-1}), DFU (5 mg kg^{-1}) or dexamethasone (2 mg kg^{-1}). Further groups of rats were treated orally with rofecoxib (20 mg kg^{-1}), DFU (5 mg kg^{-1}) or dexamethasone (2 mg kg^{-1}) concurrent with SC-560 (5 mg or 20 mg kg^{-1}). Sixty minutes after drug administration, 1 ml of 200 mM HCl was instilled intragastrically. For dexamethasone, the pretreatment period was 2 h. Additional rats were treated orally with indomethacin (5 mg kg^{-1}), SC-560 (5 mg kg^{-1}) or DFU (5 mg kg^{-1}) concurrent with SC-560 (5 mg kg^{-1}) 60 min before intragastric instillation of 1 ml of 300 mM HCl. To assess the duration of action of the COX-2 inhibitors, other groups of rats received combined treatments of SC-560 (5 mg kg^{-1}) and DFU (5 mg kg^{-1}) or rofecoxib (20 mg kg^{-1}) 5 h before acid instillation. Vehicle (0.25% methylcellulose)-treated and acid-challenged rats were included as controls in all experiments.

Histology

For histological study, a strip of the stomach wall parallel to the limiting ridge was processed using routine methods, stained with H&E and examined under a light microscope in a blinded randomized fashion by a pathologist who was not aware of the treatment protocol. Histological injury was assessed as surface mucus cell damage showing vacuolated cells with pyknotic nuclei and hyalinized cytoplasm, and surface cell damage plus disruption of cells lining the gastric pits with the parietal cells having pyknotic nuclei and hyalinized cytoplasm, loss of normal glandular architecture, and cellular dropout. The length of mucosal areas showing histological damage was determined and expressed as per cent of the total section length studied.

Reverse transcription-polymerase chain reaction (RT–PCR)

Total RNA from gastric mucosa was extracted using Trizol. After treatment with RNase-free DNase I to remove contaminating DNA, RNA was purified using a Nucleo-Spin Kit. RT–PCR was performed as described previously using primers for COX-1, COX-2 and GAPDH as described (Maricic *et al*., 1999). Three μl for COX-2 and 1 μl for COX-1 and GAPDH of the reverse transcribed product were amplified. Cycling parameters were: 95°C for 1 min; specific annealing temperature 59°C for 1 min; final extension 7 min at 72°C. Thirty cycles were performed for COX-2 and for COX-1 and 26 for GAPDH amplification. The PCR products were run on an agarose gel containing ethidium-bromide.

Carrageenan-soaked sponge model of inflammation

Release of PGE_2 into inflammatory exudates was determined to ascertain that rofecoxib and DFU at the doses that interfered with gastric mucosal integrity inhibit COX-2 catalyzed prostaglandin formation. Sterile polyester sponges ($1.3 \times 0.7 \times 0.4$ cm), soaked in 2% carrageenan (w v^{-1}) in saline, were implanted s.c. to produce an inflammatory response as described previously (Gretzer *et al*., 1998). Groups of 5–6 rats were treated orally with rofecoxib (20 mg kg^{-1}) or DFU (5 mg kg^{-1}) 30 min before sponges were implanted for 5 h. To reveal a short-lasting effect, additional experiments were performed with DFU (5 mg kg^{-1}) administered orally 10 min before implantation of sponges for 2.5 h. After removal, sponges were immersed in 2 ml of phosphate (0.01 M, pH 7.4) – buffered saline containing 5 u ml^{-1} heparin. They were then squeezed, the exudates were immediately centrifuged at 4°C for 10 min and the supernatants were kept frozen at −80°C until analysis of PGE_2.

Assessment of eicosanoid formation

Formation of gastric mucosal 6-keto-$PGF_{1\alpha}$ After assessment of gross mucosal damage, fragments of the mucosa were excised, blotted, weighed and aliquots (40 mg) were incubated in triplicate in oxygenated Tyrode solution at 37°C for 10 min. Release of 6-keto-$PGF_{1\alpha}$ into the medium was determined using radioimmunoassay (RIA).

Formation of platelet TXB_2 Release of TXB_2 from platelets was determined during clotting of whole blood. Blood was obtained by cardiac puncture after cervical dislocation. The sample was divided into three aliquots and incubated at 37°C for 60 min. After separation of serum from cellular elements concentrations of TXB_2 were measured by RIA.

Formation of inflammatory PGE_2 The amounts of PGE_2 in the exudates accumulating in the carrageenan-soaked sponges during 2.5 and 5 h were determined using RIA.

Statistical analysis

Results are expressed as mean ± s.e.mean of n values. Comparisons between groups were made using Student's t-test for unpaired data or the Wilcoxon rank test for non-parametric data. A P value of <0.05 was considered to be significant.

Materials

SC-560 was kindly provided by Dr R.A. Marks (Searle, Skokie, IL, U.S.A.). DFU was a generous gift from Dr R. Young (Merck-Frosst Canada, Montreal, Canada). Rofecoxib (Vioxx®) was purchased at the pharmacy. Trizol was obtained from Life Technologies, Lofer, Austria, DNase I from Roche, Vienna, Austria and Nucleo-Spin Kit from Machery and Nagel, Düren, Germany. COX-1 and COX-2 primers were synthesized at the Department of Biotechnology, Technical University of Graz, and primers for GAPDH were purchased from Clontech (Palo Alto, CA, U.S.A.). Reverse Transcription System and PCR Core kit were obtained from Promega, Mannheim, Germany. All other chemicals were purchased from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). ^{3}[H]-TXB_2 and ^{3}[H]-6-keto-$PGF_{1\alpha}$ were from New England Nuclear Co. (Dreieich, Germany).

Results

Normal gastric mucosa

In the normal stomach, indomethacin (5 and 20 mg kg^{-1}) induced dose-dependent mucosal damage 5 h after oral administration. Whereas damage produced by indomethacin at 5 mg kg^{-1} was minor, substantial damage was observed at the 20 mg kg^{-1} dose. In contrast, mucosal injury was not observed 5 h after oral administration of SC-560 (20 mg kg^{-1}). Rofecoxib (20 mg kg^{-1}) given alone did not cause mucosal injury but addition of rofecoxib (1, 5 and 20 mg kg^{-1}) to the SC-560 treatment (20 mg kg^{-1}) damaged the gastric mucosa in a dose-dependent manner. The injurious effect of the combined treatment was also dose-dependent for SC-560 (5–20 mg kg^{-1}, p.o., data not shown). Co-administration of DFU (20 mg kg^{-1}) and SC-560 (20 mg kg^{-1}) did not cause substantially more injury than SC-560 alone. Results are shown in Figure 1.

Rats treated with indomethacin (20 mg kg^{-1}) showed significant histological mucosal injury 5 h after drug administration. In contrast, histological damage was negligible in

rats treated with SC-560 (20 mg kg^{-1}) alone or rofecoxib (20 mg kg^{-1}) alone. However, substantial histological injury was found in rats after concurrent administration of SC-560 (20 mg kg^{-1}) and rofecoxib (20 mg kg^{-1}). No histological damage occurred in rats after co-treatment with SC-560 (20 mg kg^{-1}) and DFU (20 mg kg^{-1}). Results are shown in Figure 2.

Indomethacin (5 and 20 mg kg^{-1}) significantly inhibited gastric mucosal formation of 6-keto-PGF$_{1\alpha}$ by 76±4 and 88±3% and platelet TXB$_2$ release by 81±9 and 97±2%, respectively, 5 h after drug administration. Similarly, SC-560 (20 mg kg^{-1}) induced near-maximal inhibition of gastric mucosal 6-keto-PGF$_{1\alpha}$ by 86±5% and platelet TXB$_2$ release by 89±4%. Rofecoxib (20 mg kg^{-1}) did not inhibit gastric mucosal 6-keto-PGF$_{1\alpha}$ and platelet TXB$_2$ formation. Furthermore, the SC-560-induced inhibition of gastric mucosal 6-keto-PGF$_{1\alpha}$ formation and platelet TXB$_2$ release was not enhanced by concurrent administration of rofecoxib or DFU. Results are shown in Figure 3.

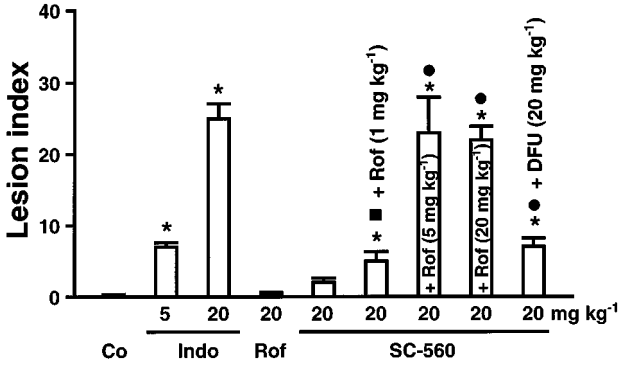

Figure 1 Lesion formation in healthy rats 5 h after oral administration of the non-selective COX inhibitor indomethacin, the COX-1 inhibitor SC-560 and the COX-2 inhibitors rofecoxib and DFU. Values are mean±s.e.mean of 4–12 rats. *$P<0.001$ vs vehicle-treated controls; ●$P<0.001$, ■$P<0.05$ vs SC-560 alone.

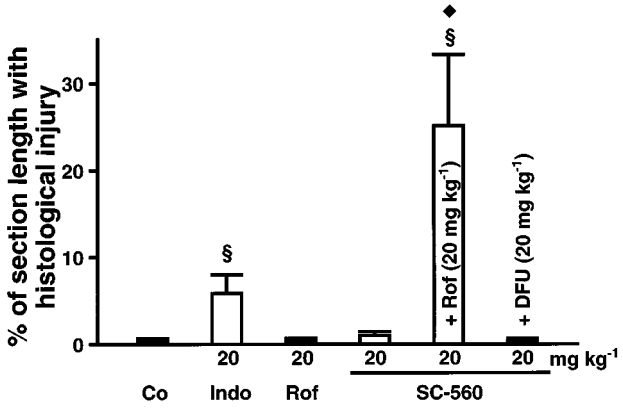

Figure 2 Effects of indomethacin, SC-560, rofecoxib and DFU on mucosal histology in normal mucosa. Healthy rats were treated with drugs and histological injury was assessed 5 h later. The length of mucosal areas with histological injury was determined and expressed as per cent of the total section length studied per stomach. Rats treated with COX inhibitors were compared with vehicle-treated control rats. Values are mean±s.e.mean of 4–7 rats. §$P<0.05$ vs controls; ◆$P<0.02$ vs SC-560 alone.

Acid-challenged gastric mucosa

Gastric mucosal COX-1 mRNA as assessed by RT–PCR was readily detectable 60 min after instillation of saline and was not modified by instillation of HCl (300 mM). In contrast, COX-2 mRNA was just above the detection limit in the gastric mucosa of rats instilled with saline but was substantially up-regulated 60 min after acid instillation. Pretreatment with dexamethasone (2 mg kg^{-1}) prevented the acid-induced induction of COX-2. Representative agarose gels and the COX/GAPDH ratios as assessed by densitometry are shown in Figure 4.

Negligible mucosal damage was observed 60 min after instillation of 200 or 300 mM HCl. Pretreatment with indomethacin (5 mg kg^{-1}) significantly damaged the mucosa challenged with 200 or 300 mM HCl. Pretreatment with SC-560 (5 and 20 mg kg^{-1}) induced dose-dependent damage of the mucosa exposed to 200 or 300 mM HCl. Rofecoxib (20 mg kg^{-1}) and DFU (5 mg kg^{-1}) given alone 60 min before acid instillation had no significant effect on gastric mucosal integrity but markedly aggravated the injury induced by SC-560. Lower doses of rofecoxib had

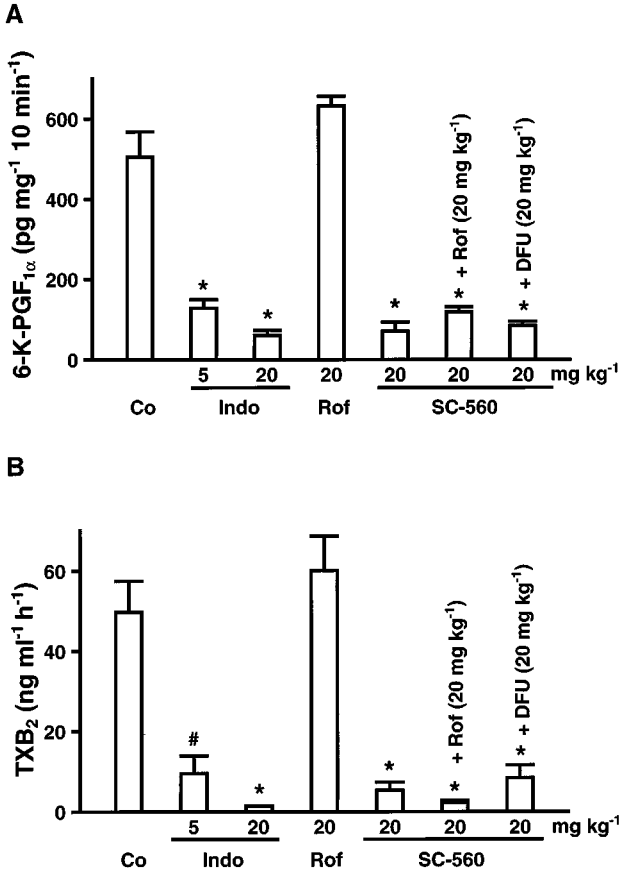

Figure 3 Effects of indomethacin, SC-560, rofecoxib and DFU on gastric mucosal 6-keto-PGF$_{1\alpha}$ (A) and platelet TXB$_2$ (B) formation in healthy rats. Gastric mucosal fragments were harvested 5 h after drug administration and incubated in Tyrode solution at 37°C for 10 min. Blood was collected by cardiac puncture 5 h after drug administration and was incubated at 37°C for 60 min. Release of 6-keto-PGF$_{1\alpha}$ and TXB$_2$ was measured using RIA. Effects of COX inhibitors were compared with vehicle-treated control rats. Values are mean± s.e.mean of 4–9 rats. #$P<0.01$, *$P<0.001$ vs controls.

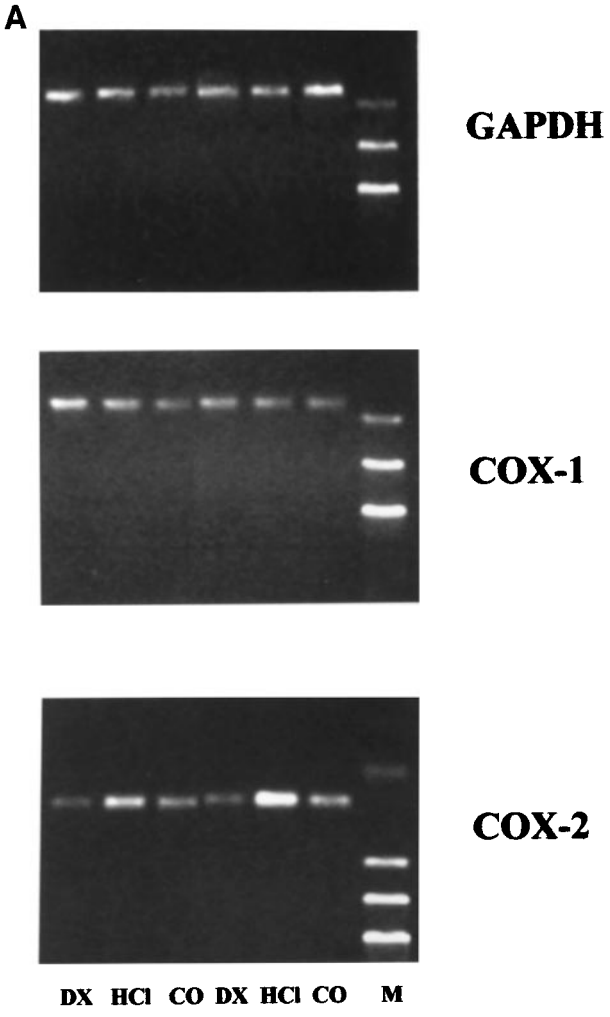

Figure 4 Effect of acid instillation on mRNA expression of COX-1 and COX-2. Total RNA was extracted from gastric mucosa of rats instilled with 1 ml of 300 mM HCl or 1 ml of saline. The agarose gels show representative RT–PCRs specific for COX-1, COX-2 and GAPDH (as internal standard). The lanes show samples obtained from saline-treated control rats, HCl-treated rats and HCl-treated rats after pretreatment with dexamethasone (2 mg kg^{-1}) (A) The relative signal intensities of COX-1 and COX-2 to that of GAPDH were quantified and the ratio of control rats was set to 100% (B). Values are mean±s.e.mean of four rats. #$P<0.01$ vs controls; ▲$P<0.01$ vs HCl alone.

no effect (data not shown). The results obtained after gastric challenge with 200 mM HCl are shown in Figure 5. Drug effects on the gastric mucosa after challenge with 300 mM HCl were qualitatively identical (data not shown). When the pretreatment period was prolonged to 5 h, co-administration of rofecoxib (20 mg kg^{-1}) still enhanced the injurious effect of SC-560 (5 mg kg^{-1}) in the stomach challenged with 200 mM HCl. In contrast, 5 h after drug administration the effect of DFU (5 mg kg^{-1}) on damage induced by SC-560 (5 mg kg^{-1}) in the mucosa challenged with 200 mM HCl was lost (Figure 6) indicating a shorter duration of action of DFU as compared with rofecoxib. As shown in Figure 7 dexamethasone (2 mg kg^{-1}) given alone did not increase acid-induced injury but significantly augmented the damage elicited by SC-560 (5 mg kg^{-1}).

Effect of rofecoxib and DFU on release of PGE_2 into inflammatory exudates

Five hours after implantation, the exudates accumulated in the carrageenan-soaked sponges in vehicle-treated control rats contained large amounts of PGE_2 (7.4±0.6 ng ml^{-1}, $n=5$). Treatment with rofecoxib (20 mg kg^{-1}) reduced PGE_2 concentrations in the exudates 5 h after implantation by 73±3% ($P<0.001$, $n=5$). Treatment with DFU (5 mg kg^{-1}) had no effect on release of inflammatory PGE_2 during the 5 h period of sponge implantation. When the duration of sponge implantation was shortened to 2.5 h, accumulation of PGE_2 in the exudates of vehicle-treated control rats was 2.6±0.3 ng ml^{-1} ($n=6$). During the 2.5 h implantation period DFU (5 mg kg^{-1}) significantly reduced accumulation of PGE_2 in the exudates by 47±8% ($P<0.02$, $n=6$) indicating that DFU inhibits COX-2 in the rat but has a limited duration of action. Work published previously has demonstrated that dexamethasone near-maximally inhibits release of PGE_2 into inflammatory exudates during a 5 h sponge implantation period (Schmassmann *et al*., 1998). Results are shown in Figure 8.

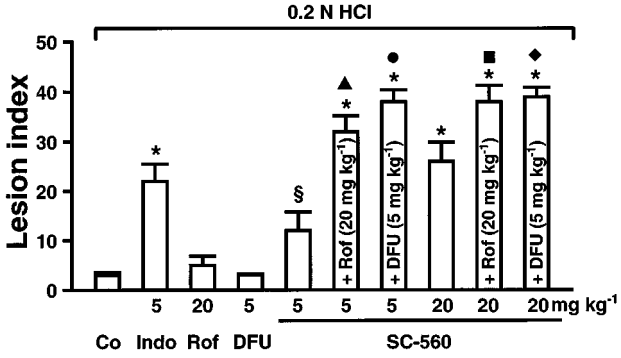

Figure 5 Lesion formation in rats after acid challenge. Rats were treated orally with indomethacin, SC-560, rofecoxib or DFU 60 min before instillation of 1 ml of 200 mM HCl and gastric mucosal damage was assessed 60 min later. Controls received the vehicle before 1 ml of 200 mM HCl. Values are mean±s.e.mean of 4–7 rats. §$P<0.05$, *$P<0.001$ vs controls; ■$P<0.05$. ◆$P<0.02$, ▲$P<0.01$, ●$P<0.001$ vs the corresponding dose of SC-560 alone.

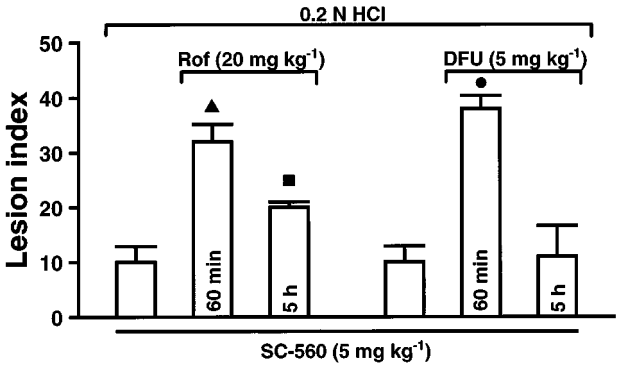

Figure 6 Comparison of the effects of rofecoxib and DFU on mucosal damage induced by SC-560 in the acid-challenged stomach using different pretreatment periods. Rats were treated orally with SC-560, rofecoxib or DFU 60 min or 5 h before instillation of 1 ml of 200 mM HCl and gastric mucosal damage was assessed after a further 60 min period. Values are mean±s.e.mean of 4–7 rats. ■$P<0.05$, ▲$P<0.01$, ●$P<0.001$ vs SC-560 alone.

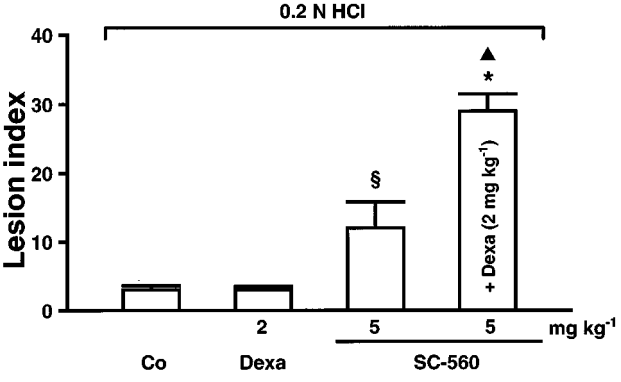

Figure 7 Effect of dexamethasone on lesion formation in rats after acid challenge. Rats were treated orally with SC-560 (60 min) or dexamethasone (120 min) before instillation of 1 ml of 200 mM HCl and gastric mucosal damage was assessed 60 min later. Controls received the vehicle before 1 ml of 200 mM HCl. Values are mean±s.e.mean of 4–6 rats. §$P<0.05$, *$P<0.001$ vs controls; ▲$P<0.01$ vs SC-560 alone.

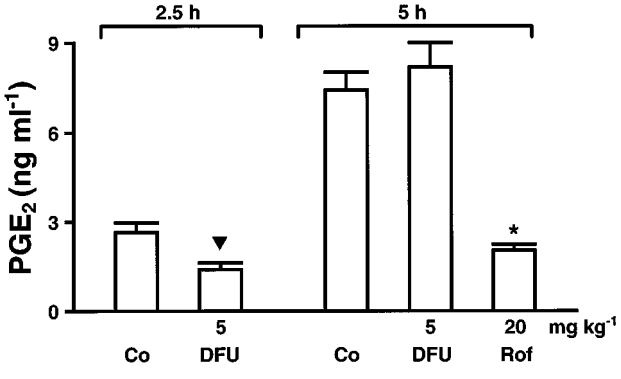

Figure 8 Effect of rofecoxib and DFU on release of PGE_2 into inflammatory exudates elicited by s.c. implantation of carrageenan-soaked sponges. Concentrations of PGE_2 in the inflammatory exudates were measured 2.5 or 5 h after sponge implantation. Columns represent the mean±s.e.mean of 5–6 experiments. ▼$P<0.02$, *$P<0.001$ vs vehicle controls.

Discussion

A non-selective COX-inhibitor suppressed gastric mucosal prostaglandin formation and simultaneously induced gastric mucosal lesions in a dose-dependent manner. In contrast, in healthy rats the selective COX-1 inhibitor SC-560 did not cause mucosal injury, although formation of gastric 6-keto-$PGF_{1\alpha}$ and platelet TXB_2 was substantially inhibited. A previous study has shown that SC-560 did not reduce the release of COX-2-derived PGE_2 into the inflammatory exudate in the carrageenan-airpouch rat model (Wallace *et al*., 2000) indicating that SC-560 selectively inhibits COX-1. In our study the inhibitory effect of SC-560 on gastric prostaglandin and platelet thromboxane formation was equivalent to that observed with the 20 mg kg^{-1} dose of indomethacin which caused severe gastric mucosal injury. Rofecoxib given alone did not damage the gastric mucosa. However, combined treatment with SC-560 and rofecoxib produced severe mucosal damage 5 h after drug administration comparable to the injury observed with indomethacin at 20 mg kg^{-1}. The injurious effect of the combined treatment was dose-dependent for both compounds.

In contrast to rofecoxib, addition of DFU to SC-560 did not result in a substantial increase of the ulcerogenicity of the COX-1 inhibitor in normal gastric mucosa. Both rofecoxib (Chan *et al*., 1999) and DFU (Riendeau *et al*., 1997) at the doses used in the present investigation have previously been found to exert potent and dose-dependent anti-inflammatory effects in various models of inflammation in rats. Furthermore, our results show that both compounds inhibit the release of PGE_2 into inflammatory exudates elicited by s.c. implantation of carrageenan-soaked sponges. Prostaglandins generated during carrageenan-induced inflammation are supposed to be synthesized primarily *via* the COX-2 pathway and their production was found to be suppressed by various selective COX-2 inhibitors and dexamethasone but not by SC-560 (Masferrer *et al*., 1994; Seibert *et al*., 1994; Schmassmann *et al*., 1998; Wallace *et al*., 2000). Neither rofecoxib nor DFU inhibited the COX-1-derived TXB_2 formation in platelets indicating that they act as selective COX-2 inhibitors under the experimental conditions used. However, whereas rofecoxib reduced inflammatory PGE_2 formation during a 5 h sponge implantation period, DFU inhibited inflammatory PGE_2 release only during 2.5 h but not during 5 h of sponge implantation. The short duration of action of DFU was confirmed by the observations made in the acid-challenged stomach. Thus, DFU aggravated the injurious effect of SC-560 when administered 60 min before acid instillation, whereas no aggravation was observed when the pretreatment interval was prolonged to 5 h. This is in contrast to the effect of rofecoxib which augmented the SC-560-induced damage in the acid-challenged stomach after a 60 min as well as after a 5 h pretreatment interval. This could explain the lack of a substantial effect of DFU during co-administration with SC-560 in the normal rat stomach as this model uses a 5 h treatment period. Whether other factors in addition to the duration of biological activity contribute to the difference in the ulcerogenic potency of DFU and rofecoxib in the normal stomach in the presence of COX-1 suppression remains to be investigated.

Our results indicate that simultaneous inhibition of both COX-1 and COX-2 is necessary to produce damage in the healthy stomach. During preparation of this manuscript a similar conclusion has been published by Wallace *et al.* (2000). The findings are in line with observations made in mice with COX-1 and COX-2 gene disruption. Thus, COX-1-deficient mice had no gastric pathology even though their gastric PGE_2 levels were about 1% of the levels observed in wild-type animals (Langenbach *et al*., 1995). Similarly, mice lacking COX-2 did not develop spontaneous gastric lesions (Morham *et al*., 1995).

Although indomethacin and SC-560 caused comparable near-maximal inhibition of gastric mucosal formation of 6-keto-$PGF_{1\alpha}$, only indomethacin induced severe gastric lesions. This indicates that reduction of total gastric prostaglandin biosynthesis as measured by the techniques used in the present study does not correlate with NSAID-induced gastrotoxicity. This study and previous investigations (Schmassmann *et al*., 1998; Gretzer *et al*., 1998; Ehrlich *et al*., 1998; Maricic *et al*., 1999) could not demonstrate suppression of gastric mucosal prostaglandin production after isolated administration of COX-2 inhibitors. Likewise, neither DFU nor rofecoxib caused a significant increase in the inhibitory effect of SC-560 on eicosanoid formation during combined treatment. This may be due to the fact that in the gastric mucosa the majority of prostaglandins is generated *via* the COX-1 pathway and COX-2 derived prostaglandins represent only such a small part of the total prostaglandin pool that isolated inhibition of the COX-2 isoenzyme does not result in a substantial and therefore measurable reduction of the total amount of prostaglandins generated. COX-1 and COX-2 differ in cellular source and distribution of intracellular activity. Thus, in normal human and/or rat gastric mucosa COX-1 expression was found mainly in epithelial cells, glandular cells, and mucous neck cells (Iseki, 1995; Tarnawski *et al*., 1996; Donnelly *et al*., 1997) whereas COX-2 was expressed predominantly in endothelial cells, myofibroblasts, surface mucous cells, and mononuclear cells (Iseki, 1995; Tarnawski *et al*., 1996; Donnelly *et al*., 1997) in addition to surface epithelial cells and parietal cells (McCarthy *et al*., 1999). Although COX-1 and COX-2 are present in the same subcellular locations, the endoplasmic reticulum and nuclear envelope, the activity of COX-1 occurs primarily in the endoplasmic reticulum whereas that of COX-2 is concentrated over the surface of the nucleus (Morita *et al*., 1995). Furthermore, COX-1 and COX-2 utilize different arachidonate substrate pools coupled to different extracellular stimuli and different phospholipase systems (Smith *et al*., 1996). The specific location and substrate access of the COX isoforms may lead to a compartmentalization of the eicosanoids synthesized. A dissociation between inhibition of prostaglandin formation and biological effect was also reported for SC-560. Thus, in a standard model of inflammation and pain, the carrageenan foot paw oedema, SC-560 reduced PGE_2 levels to the same extent as was observed after treatment with the COX-2 inhibitor celecoxib, but did not affect established hyperalgesia (Smith *et al*., 1998). Taken together, these results suggest that not tissue levels of prostaglandins but effects on specific biosynthetic pathways in specific cell types and intracellular locations determine the biological response to COX inhibition.

The finding that in the normal stomach even marked suppression of total gastric mucosal prostaglandin formation is not ulcerogenic when the COX-2 pathway is preserved may open the possibility to develop potent anti-aggregatory drugs without relevant gastrointestinal side effects. Formation of thromboxane in platelets occurs predominantly or even exclusively *via* the COX-1 pathway. Our study shows that SC-560 causes near-maximal inhibition of platelet TXB_2 biosynthesis without relevant production of gastric mucosal lesions. Thus, similar to the development of selective COX-2 inhibitors as anti-inflammatory and analgesic drugs with reduced gastrointestinal toxicity, selective COX-1 inhibitors could possibly represent a new class of anti-platelet drugs with low incidence of gastrointestinal side effects.

Intragastric instillation of acid significantly elevated gastric mucosal levels of COX-2 mRNA which were close to the detection limit in normal gastric mucosa. Levels of COX-1 mRNA were high in normal gastric mucosa and were not modified by intragastric acid instillation. Pretreatment with dexamethasone prevented the acid-induced increase in mucosal expression of COX-2 mRNA without affecting levels of COX-1 mRNA. Recently, it was reported that in organ culture experiments of mucosal explants of Barrett's oesophagus acid added *in vitro* to the incubation medium induced expression of COX-2 and decreased expression of COX-1 protein (Shirvani *et al*., 2000). The results of our study show that acid-induced up-regulation of COX-2 mRNA also occurs in normal gastric mucosa *in vivo*. COX-2 mRNA levels increased rapidly and a significant elevation was observed already 60 min after acid instillation. Whether the elevation of COX-2 mRNA levels is associated with increased expression of COX-2 protein and/or enzyme activity remains to be established. In the rat stomach *in vivo*, acid challenge did neither increase nor decrease mucosal mRNA levels of COX-1.

The role of COX-1 in mucosal defence is different in the stomach challenged with exogenous acid. In this situation inhibition of COX-1 has ulcerogenic potential even without concurrent administration of a COX-2 inhibitor. Thus, whereas instillation of acid alone did not produce overt gastric injury, pretreatment with SC-560 damaged the acid-challenged mucosa in a dose-dependent manner. In contrast, neither rofecoxib nor DFU given alone augmented injury of the gastric mucosa after acid exposure. Both compounds, however, significantly aggravated the severity of mucosal lesions after acid challenge induced by SC-560. Similarly, suppression of acid-induced up-regulation of COX-2 mRNA by dexamethasone markedly increased mucosal injury associated with COX-1 inhibition.

In conclusion, the results of our study show that in the healthy rat stomach neither isolated inhibition of COX-1 nor of COX-2 is ulcerogenic. Gastric mucosal lesions only develop when both isoenzymes are inhibited. In contrast, during acid challenge specific inhibition of COX-1 alone renders the mucosa more vulnerable suggesting an important role of COX-1 in mucosal resistance responsible for minimizing damage in the presence of a potentially noxious agent. In this function COX-1 is supported by COX-2 but, in contrast to normal gastric mucosa, COX-2 alone is not sufficient to maintain full mucosal integrity in the face of pending injury.

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