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Role of prostaglandins generated by cyclooxygenase-1 and cyclooxygenase-2 in healing of ischemia–reperfusion-induced gastric lesions

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Abstract

In this study, ischemia–reperfusion produced in rats by clamping the celiac artery for 0.5 h followed by 1 h of reperfusion was used to develop a new model of superficial gastric erosions progressing to deeper ulcers. Ischemia alone resulted in an immediate fall in gastric blood flow but no gross mucosal lesions were observed. When ischemia was followed by reperfusion, gastric erosive lesions occurred, reached a maximum at 12 h and then declined after 24 h. These acute erosions progressed into deeper lesions 24 h after ischemia–reperfusion and reached a peak after 3 days. Gastric blood flow and the mucosal generation of prostaglandin E_2 were significantly suppressed immediately following ischemia–reperfusion, but with the healing of deeper gastric ulcers, both gastric blood flow and prostaglandin E_2 generation were gradually restored. Cyclooxygenase-1 mRNA was detected by reverse transcription-polymerase chain reaction in intact gastric mucosa and throughout the recovery of the mucosa from acute ischemia–reperfusion lesions, whereas cyclooxygenase-2 mRNA, was recorded only after ischemia–reperfusion. NS-398 and rofecoxib, selective inhibitors of cyclooxyganase-2, failed to affect prostaglandin E_2 generation in the non-ulcerated gastric mucosa but inhibited it significantly in the ulcer area. The two cyclooxygenase-2 inhibitors as well as resveratrol, a specific cyclooxygenase-1 inhibitor and indomethacin and meloxicam, non-specific inhibitors of cyclooxygenase, augmented acute gastric erosions induced by ischemia–reperfusion and delayed significantly the progression of these lesions into deeper ulcers at each time interval after ischemia–reperfusion. We conclude that prostaglandins generated by both cyclooxygenase-1 and cyclooxygenase-2 contribute to the healing of gastric lesions induced by ischemia–reperfusion.

Keywords: Ischemia–reperfusion; Cyclooxygenase-1; Cyclooxygenase-2; Ulcer healing; Gastric blood flow; Interleukin-1β; Cyclooxygenase inhibitor; Gastrin

1. Introduction

Ischemia followed by reperfusion is known to induce gastric lesions predominantly due to excessive formation of reactive oxygen species, neutrophil activation and microvascular dysfunction (Itoh and Guth, 1985; Yoshikawa et al., 1992), but the involvement of endogenous prostaglandins, which are an important component of the gastric mucosal defense (Robert, 1979), in the mechanism of the healing of these lesions has been little examined. Previous studies documented that ischemia weakens the gastric mucosal barrier and increases the acid back diffusion, thus predisposing the mucosa to damage (Itoh and Guth, 1985; Kawai et al., 1994). After reperfusion, the generation of reactive oxygen species and the activation of neutrophils were found to increase the lipid peroxidation, which in combination with the aggressive action of gastric acid resulted in cellular death and mucosal damage (Andrews et al., 1994; Wada et al., 1996).

It is generally accepted that there are two isoforms of cyclooxygenase: constitutive cyclooxygenase-1, which produces PG for physiological reactions including the maintenance of mucosal integrity, gastric microcirculation, secretory activity and motor functions, and inducible cyclooxygenase-2, which is triggered by various cytokines, growth factors and endotoxins (Xie et al., 1991; Eberhart and Dubois, 1995; Feng et al., 1995). While prostaglandins

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produced by cyclooxygenase-1 appear to contribute to the physiological control of mucosal integrity, cyclooxygenase-2 products have been implicated in inflammatory reactions (Xie et al., 1991; Feng et al., 1995). Recently, an increased cyclooxygenase-2 expression was shown in gastric mucosa exposed to ischemia–reperfusion (Kishimoto et al., 1998a,b) but no attempt has been made to compare the effects of specific cyclooxygenase-1 and cyclooxygenase-2 inhibitors with the effects of classic non-specific inhibitors of cyclooxygenase such as indomethacin on the healing of ischemia–reperfusion-induced damage and to determine which cyclooxygenase isoform contributes to spontaneous recovery of gastric mucosa from lesions induced by ischemia–reperfusion.

Using the model of gastric lesions induced by ischemia–reperfusion as proposed recently by Wada et al. (1996), we examined the involvement of prostaglandins in the appearance of acute gastric erosions and their progression to deeper ulcers and their role in gastric blood flow induced by ischemia–reperfusion in rats treated with non-selective and selective cyclooxygenase-1 and cyclooxygenase-2 inhibitors. Among the cyclooxygenase inhibitors tested, we used resveratol, which is a phenolic product derived from grapes and which has been shown to inhibit selectively cyclooxygenase-1 and hydroxyperoxidase of cyclooxygenase-1 with an ED_{50} of 3.7 μm (Jang et al.,1997). We also determined whether addition of minute doses of exogenous PG to these inhibitors could influence the healing of gastric lesions and gastric blood flow in rats subjected to ischemia–reperfusion. In addition, we attempted to assess the expression of cyclooxygenase-1 and cyclooxygenase-2 mRNA, as determined with the reverse transcription-polymerase chain reaction, and to measure the changes in plasma gastrin and interleukin-1β levels in intact gastric mucosa and in mucosa exposed to ischemia–reperfusion and showing gastric lesions. Since proinflammatory cytokines such as interleukin-1β influence gastric secretion and ulcer healing (Robert et al., 1991), our rationale was to study the expression of interleukin-1β mRNA and plasma levels of this cytokine along with the time course of recovery from ischemia–reperfusion damage. Lastly, we attempted to determine the involvement of gastrin in the healing of ischemia–reperfusion damage since this hormone exhibits protective activity against lesions induced by corrosive substances such as absolute ethanol (Konturek et al., 1995). Moreover, prolonged hypergastrinemia contributes to the healing of preexisting ulcers in the rat stomach (Li and Helander, 1996).

2. Material and methods

2.1. Production of lesions induced by ischemia–reperfusion

Ischemia–reperfusion erosions were produced in 120 rats by the method originally described by Wada et al. (1996). Briefly, under pentobarbital anesthesia (50 mg/kg i.p.), the abdomen was opened and the celiac artery was identified and clamped with a small device for 30 min, followed by removal of the clamp to obtain reperfusion. Erosions were measured immediately after 30 min of ischemia, after 60 min of reperfusion (time 0) and then after 3, 12 or 24 h after the termination of ischemia–reperfusion. In separate groups of rats, the longer observation periods after ischemia–reperfusion, starting from day 3 up to day 15 after ischemia–reperfusion, were investigated to determine whether these erosions progressed into gastric ulcers as reported previously (Wada et al., 1996) and to examine to what extent spontaneous healing of these ulcers is affected by cyclooxygenase-1 and cyclooxygenase-2 inhibitors.

Several groups of rats (6–8 animals in each group), were pretreated 30 min before the ischemia–reperfusion with (1) vehicle (saline); (2) resveratrol (1,3-benzenediol,5-[2-(4-hydroxyphenyl) ethenyl], 10 mg/kg i.g.), a selective cyclooxygenase-1 inhibitor (Jang et al., 1997); (3) NS-398 (*N*-(2,cyclohexyloxy-4-nitrophenyl)methane sulfonamide, 10 mg/kg i.g.) and rofecoxib (MK-0106), highly selective cyclooxygenase-2 inhibitors (Futaki et al., 1993; Ehrich et al., 1999); (4) meloxicam (5 mg/kg i.g.), an agent that preferentially inhibits cyclooxygenase-2 (Vane and Botting, 1995; Engelhardt et al., 1996) or (5) indomethacin (5 mg/kg i.p.), a non-selective cyclooxygenase inhibitor (Masferrer et al., 1996). To determine the effects of cyclooxygenase inhibitors on the healing of superficial erosions that progressed into deeper gastric ulcers, the treatment with each cyclooxygenase inhibitor or vehicle was repeated daily, from day 1 to day 5 after the termination of ischemia–reperfusion. At the dose used in the present study, indomethacin has been shown to inhibit gastric prostaglandin E_2 generation by ~90% without causing any mucosal damage (Konturek et al., 1998). The dose of NS-398 was selected on the basis of previous studies showing that this agent suppressed almost completely the prostaglandin E_2 generation in exudate of air-pouch inflammation and inhibited gastric prostaglandin E_2 production under these inflammatory conditions (Futaki et al., 1993; Masferrer et al., 1994). NS-398 (Cayman Chemical, Ann Arbor, MI, USA) was first prepared as a stock solution by dissolving it in 10% ethanol to obtain a concentration of 5 mg/ml and then diluted to the desired concentration with saline according to manufacturer's recommendation, and finally administered in a total volume of 1 ml as described in our previous study (Konturek et al., 1998). Resveratrol (Cayman Chemical) was first dissolved in absolute ethanol to obtain a stock solution of 50 mg/ml and then diluted to the desired concentration with isotonic saline. Rofecoxib (a generous gift from Dr. W. Radziszewski, Merck, Sharp & Dohme, Warsaw, Poland) was first dissolved in methanol to obtain a stock solution of 50 mg/ml and then diluted to the desired concentration with isotonic saline. Control rats received the correspond-

ing vehicle. Our preliminary studies (data not included) showed that none of the cyclooxygenase inhibitors used in this study produced any gastric lesions at the doses tested.

In another group of animals subjected to ischemia–reperfusion and treated with cyclooxygenase-1 and cyclooxygenase-2 inhibitors, the effect of prostaglandin replacement therapy using 16,16 dimethyl prostaglandin E_2 (Upjohn, Kalamazoo, MI, USA) applied in a dose of 1 μg/kg (i.g.) was examined. This dose was found in our preliminary study to be without any influence on gastric lesions caused by ischemia–reperfusion or on the accompanying fall in gastric blood flow. For this purpose, synthetic prostaglandin E_2 analog was administered together with each cyclooxygenase-1 or cyclooxygenase-2 inhibitor, starting 30 min before ischemia–reperfusion and then applied daily up to 15 days after ischemia–reperfusion.

2.2. Determination of gastric blood flow, plasma interleukin-1β and gastrin concentrations

To evaluate the effect of ischemia–reperfusion with or without administration of cyclooxygenase inhibitors or vehicle on gastric blood flow, the groups of animals were anesthetized with ether and the abdomen was opened by midline incision. The stomach was exposed to assess blood flow, using the H_2-gas clearance technique as described previously (Brzozowski et al., 1997a, 1997b). Briefly, the gastric blood flow was measured immediately after 30 min ischemia and at 0, 3, 12, 24 h and then at day 3, 5, 10 and 15 after the end of ischemia–reperfusion using the two electrodes of an electrolytic regional blood flowmeter (Biotechnical Science, Model RBF-2, Osaka, Japan) inserted through the serosa into the mucosa. One of these electrodes was used for the local generation in the mucosa of H_2 and the other was used for measurement of this H_2. With this method, the H_2 generated locally is carried out by the flow of blood, while the polarographic current detector measures the decreasing tissue content of H_2. The clearance curve of tissue H_2 was used to calculate an absolute blood flow rate (ml/min/100 g) in the oxyntic gland area as described previously (Brzozowski et al., 1997a; Konturek et al., 1998). Measurements were taken 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 animals not exposed to ischemia–reperfusion.

Immediately after measurement of blood flow, a venous blood sample was withdrawn from the vena cava into EDTA-containing vials and used for the determination of plasma gastrin concentrations by RIA as described previously (Konturek et al., 1995) and the plasma interleukin-1β concentrations by specific ELISA assay according to the manufacturer's recommendations (Endogen, Cambridge, MA, USA).

2.3. Determination of prostaglandin E_2 generation in the gastric mucosa

For the prostaglandin E_2 measurement, samples of the oxyntic gland area were taken by biopsy (about 100 mg) from non-ulcerated and ulcerated gastric mucosa immediately after the animals had been killed to determine the mucosal generation of prostaglandin E_2 by specific RIA as described previously (Konturek et al., 1992). Briefly, samples taken from the damaged mucosa or taken 3 mm away from the damaged area were placed in pre-weighed Eppendorf vials, and 1 ml of Tris buffer (50 mM, pH 9.6) was added to each vial. The samples were finely minced (about 15 s) with scissors, then washed and centrifuged for 10 s and the pellet was resuspended in 1 ml of Tris. Then each sample was mixed on a Vortex mixer (Unipan, Warsaw, Poland) for 1 min and centrifuged for 15 s. The pellet was weighed, and the supernatant was transferred to a second Eppendorf vial containing indomethacin (10 mM) and kept at −20°C until RIA. prostaglandin E_2 was measured in duplicate using RIA kits (New England Nuclear, Munich, Germany). The ability of the mucosa to generate prostaglandin E_2 was expressed in nanograms per gram of wet tissue weight.

The stomachs were then quickly removed and pinned open for the determination of the area of gastric erosions by computerized planimetry (Morphomat, Carl Zeiss, Berlin, Germany) by two investigators under blinded conditions (Brzozowski et al., 1997a).

2.4. Histological and immunohistochemical evaluation of gastric mucosa in rats exposed to ischemia–reperfusion

The samples of gastric mucosa with or without ischemia–reperfusion were excised for histological examination at various time intervals after ischemia–reperfusion. The samples were fixed in 10% buffered formalin and embedded in paraffin. The paraffin sections were cut at a thickness of 5 μm and stained with hematoxylin and eosin. Histological examination was performed on coded slides by two experienced pathologists unaware of the treatments given.

2.5. Expression of cyclooxygenase-1 and cyclooxygenase-2 mRNA transcripts in the gastric mucosa determined by reverse transcription-polymerase chain reaction

Cyclooxygenase-1 and cyclooxygenase-2 mRNA were determined by reverse transcription-polymerase chain reaction in the gastric mucosa of intact rats and in the ulcerated mucosa of rats after various time intervals ranging from 0 to 24 h and then from 3 to 15 days following exposure to ischemia–reperfusion. Samples of the gastric oxyntic mucosa (about 500 mg) were scraped off onto ice using a 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 (1987), using a rapid guanidinum isothiocyanate/phenol chloroform single step extraction kit from Stratagene (Stratagene, Heidelberg, Germany). Following precipitation, the RNA was resuspended in RNase-free TE buffer and the concentration was estimated by measuring absorbance at 260 nm. Samples were frozen at −80°C until analysis.

First strand cDNA was synthesized from total cellular RNA (5 μg) using 200 U Strata Script™ reverse transcriptase (Stratagene) and oligo (dt) primers (Stratagene). After reverse transcription, transcriptase activity was destroyed by heating, and the cDNA was stored at −20°C until reverse transcription-polymerase chain reaction. The primer sequences were designed according to the published cDNA sequence for the rat cyclooxygenases (Nudel et al., 1983; Xie et al., 1991; Kennedy et al., 1993; Masferrer et al., 1996). The cyclooxygenase-1 primer sequences were as follows: up-stream, 5′-AGC CCC TCA TTC ACC CAT CAT TT and downstream, 5′-CAG GGA CGC CTG TTC TAC GG. The expected length of this PCR product was 561 bp. The cyclooxygenase-2 primer sequences were as follows: upstream, 5′-ACA ACA TTC CTT CCT TC and downstream, 5′-CCT TAT TTC CTT TCA CAC C. The expected length of this PCR product was 201 bp. A 543-base pair (bp) fragment of interleukin-1β was amplified from single-stranded DNA by PCR using two oligonucleotide primers for interleukin-1β. The interleukin-1β sense primer was 5′GCTACCTATGTCTTGCCCGT3′ and the antisense primer was 5′GACCATTGCTGTTTCCTAGG3′. The nucleotide sequences of the primers were chosen on the basis of published cDNA sequences encoding interleukin-1β (Reuter et al., 1996; Konturek et al., 1998). The primers for cyclooxygenase-1, cyclooxygenase-2 and interleukin-1β were synthesized by Biometra (Göttingen, Germany). Concomitantly, amplification of control rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Clon Tech, Palo Alto, CA) (983 bp) was performed on the same samples to verify RNA integrity.

The primer sequences for GAPDH were purchased from Clontech (Heidelberg, Germany). Reaction mixtures for PCR contained cDNA templates, 50 pmol of each primer, and 2.5 U of Taq DNA polymerase (Serva, Heidelberg) in 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dNTPs in a volume of 50 μl. RT blanks (no RNA included) and PCR blanks (no cDNA products included) were included in each analysis. DNA amplification was carried out under the following conditions: denaturation at 94°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 cyclooxygenase-1, cyclooxygenase-2 and interleukin-1β genes were amplified for 30 cycles. Each polymerase chain reaction product (8 μl) was electrophorezed on 1.5% agarose gels stained with ethidium bromide, and then visualized under UV light. The location of the predicted polymerase chain reaction product was confirmed by using a 100-bp ladder (Gibco BRL/Life Technologies, Eggenstein, Germany) as standard marker. To avoid polymerase chain reaction contamination, polymerase chain reactions were carried out in a dedicated area and the polymerase chain recation products were determined in a laminar flow hood separated from the polymerase chain reaction preparation area.

The intensity of bands was quantified by densitometry (LKB Ultrascan, Pharmacia, Sweden) as described in detail in our previous studies (Konturek et al., 1998). The cyclooxygenase-1, cyclooxygenase-2 and interleukin-1β signals were standardized against the GAPDH mRNA signal for each sample and results are expressed as cyclooxygenase-1, cyclooxygenase-2 and interleukin-1β mRNA/GAPDH mRNA ratio.

3. Results

3.1. Gastric lesions produced by ischemia–reperfusion and mucosal recovery from these lesions

Immediately after 30 min clamping of the celiac artery (ischemia), no gastric lesions were observed, but after 60 min of reperfusion, superficial bleeding gastric erosions were found in all tested stomachs. The area of these lesions was significantly increased at 3 h, peaked at 12 h after ischemia–reperfusion but then declined at 24 h after ischemia–reperfusion (Fig. 1). These superficial erosions progressed into smaller but deeper ulcerations whose area reached a maximum at day 3 after ischemia–reperfusion (Fig. 2). Then a gradual decrease in the area of these ulcers was observed at day 5 and day 10, with almost complete disappearance of these ulcers at day 15 following ischemia–reperfusion (Fig. 2). The typical gross and histological appearance of acute gastric lesions observed at 3 h and at day 3 following ischemia–reperfusion can be seen in Fig. 3A and B. At 3 h after the termination of ischemia–reperfusion desquamation of the surface epithelium occurred and acute necrotic and bleeding erosions developed in all stomachs, the lesions being localized mostly in the oxyntic gland area (Fig. 3A). With longer observation times, these acute lesions progressed into chronic ulcerated lesions, with some regeneration at the margin being recorded both macroscopically and microscopically at day 3 after ischemia–reperfusion (Fig. 3B).

The gastric blood flow in intact mucosa of vehicle-treated rats averaged 48 ± 6 ml/min per 100 g (taken as 100%) and clamping of the celiac artery caused an immediate and almost complete arrest of blood flow (Fig. 1). One hour after removal of the clamp (to provoke gastric reperfusion), the flow was increased but still remained about 45% lower than to the value recorded in the intact gastric mucosa (Fig. 1). At 3, 12 and 24 h after the termination of ischemia–reperfusion, a further significant

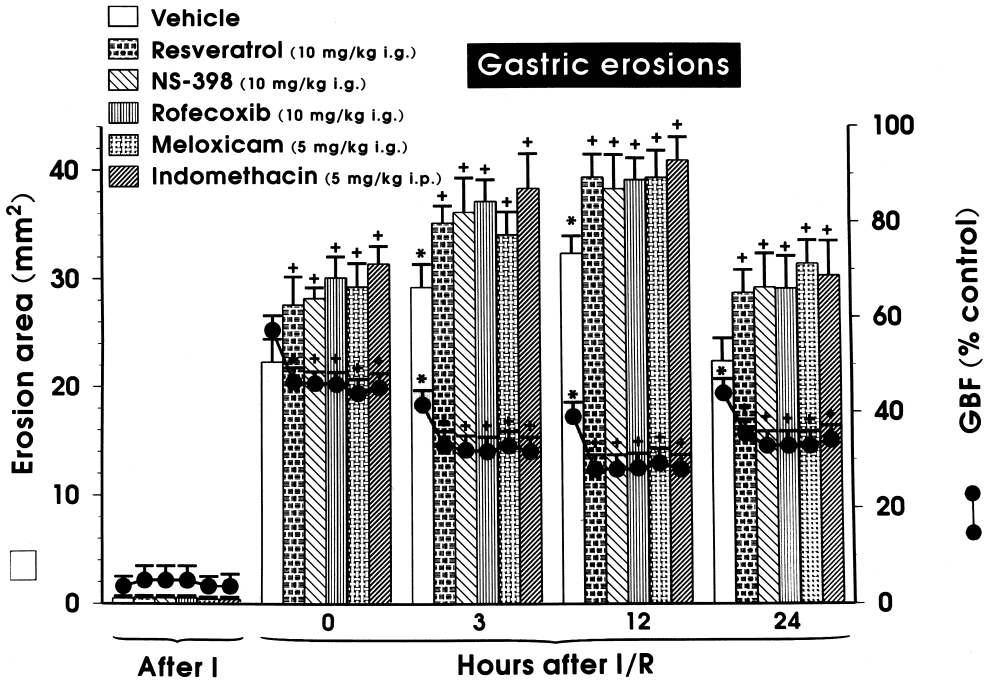

Fig. 1. Area of acute lesions and blood flow (GBF) in the gastric mucosa of rats exposed to 30 min of ischemia followed by 60 min of reperfusion and treated with vehicle, resveratrol (10 mg/kg i.g.), NS-398 (10 mg/kg i.g.), rofecoxib (10 mg/kg i.g.), meloxicam (5 mg/kg i.g.), or indomethacin (5 mg/kg i.p.). Values were measured immediately (time 0) or 3, 12 and 24 h after ischemia–reperfusion. Results are means ± S.E.M. for 6–8 rats. Asterisk indicates a significant change as compared with the value obtained at day 0. Cross indicates a significant change as compared with the value obtained in vehicle-control rats.

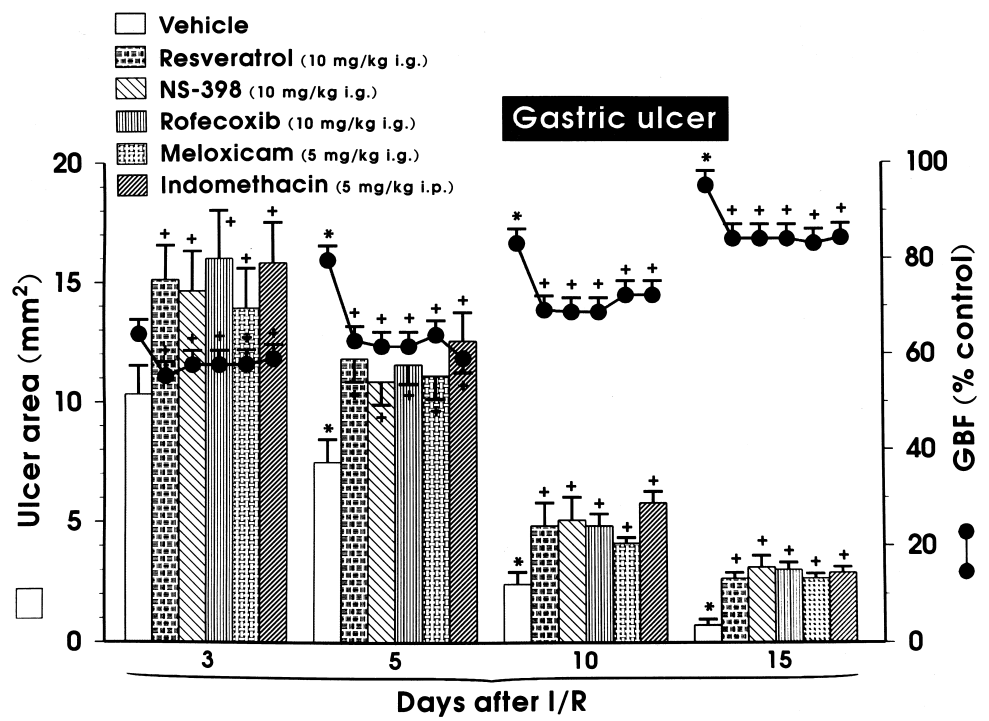

Fig. 2. Area of deep ulcers and blood flow (GBF) in the gastric mucosa of rats exposed to 30 min of ischemia followed by 60 min of reperfusion and treated with vehicle, resveratrol (10 mg/kg i.g.), NS-398 (10 mg/kg i.g.), rofecoxib (10 mg/kg i.g.), meloxicam (5 mg/kg i.g.), or indomethacin (5 mg/kg i.p.). Values were measured at days 3, 5, 10 and 15 after the end of ischemia–reperfusion. Results are means ± S.E.M. for 6–8 rats. Asterisk indicates a significant change as compared with the value obtained at day 3. Cross indicates a significant change as compared with the value obtained in vehicle-control rats at each day after the termination of ischemia–reperfusion.

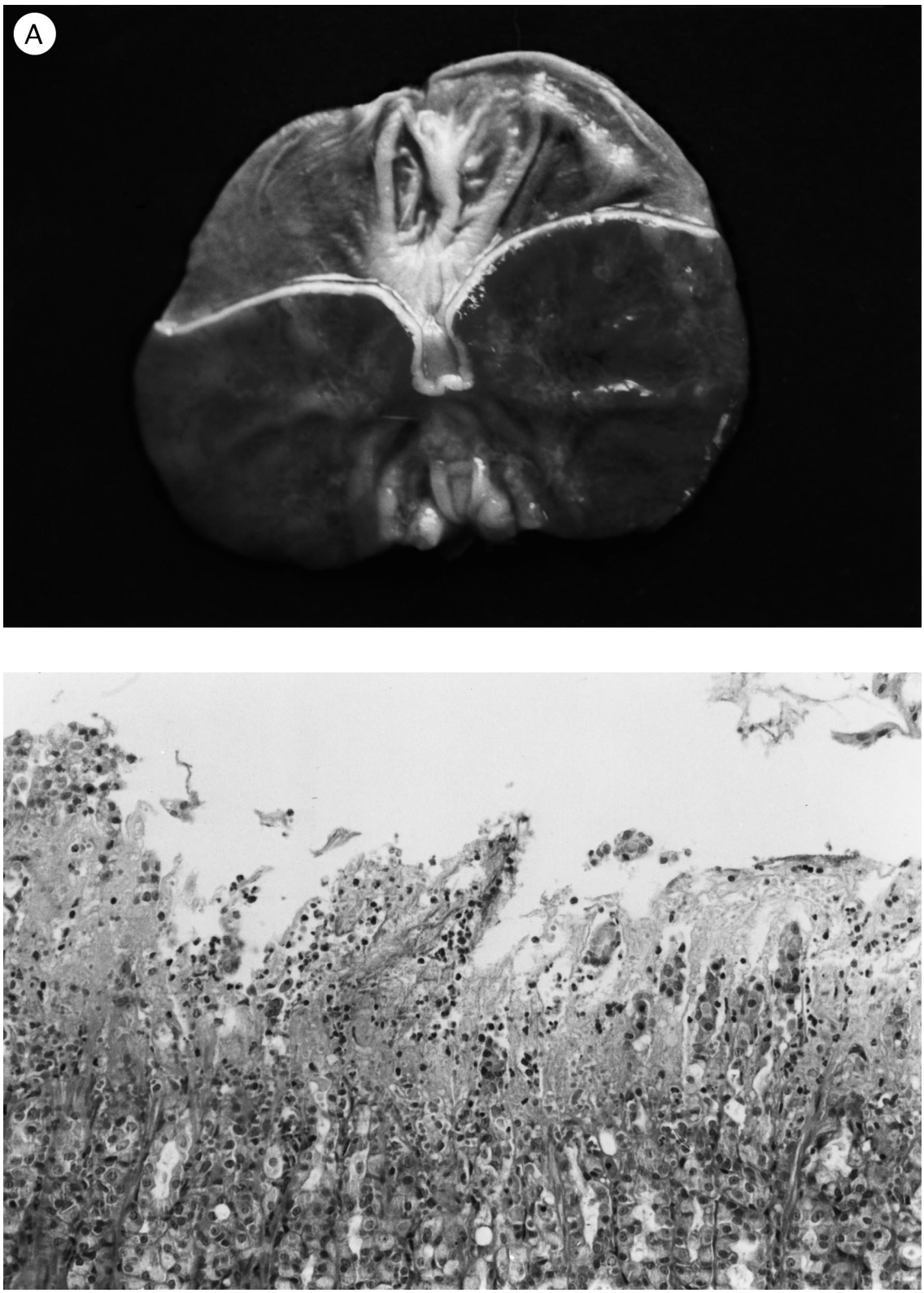

Fig. 3. Macroscopic and microscopic appearance of gastric mucosa in vehicle-treated animals exposed to standard ischemia–reperfusion and killed at 3 h (A) and at day 5 after the termination of ischemia–reperfusion (B). Note that at 3 h following exposure of the gastric mucosa to ischemia–reperfusion there was massive acute necrosis of the gastric oxyntic mucosa involving the upper half of the mucosal thickness. Necrotic tissue was denuded of surface epithelium, H and E, magn. 260× (A). At day 5 after the end of ischemia–reperfusion, chronic mucosal ulceration was observed. Necrotic area is well demarcated and early regeneration is present at the margin, H and E, magn. 150× (B).

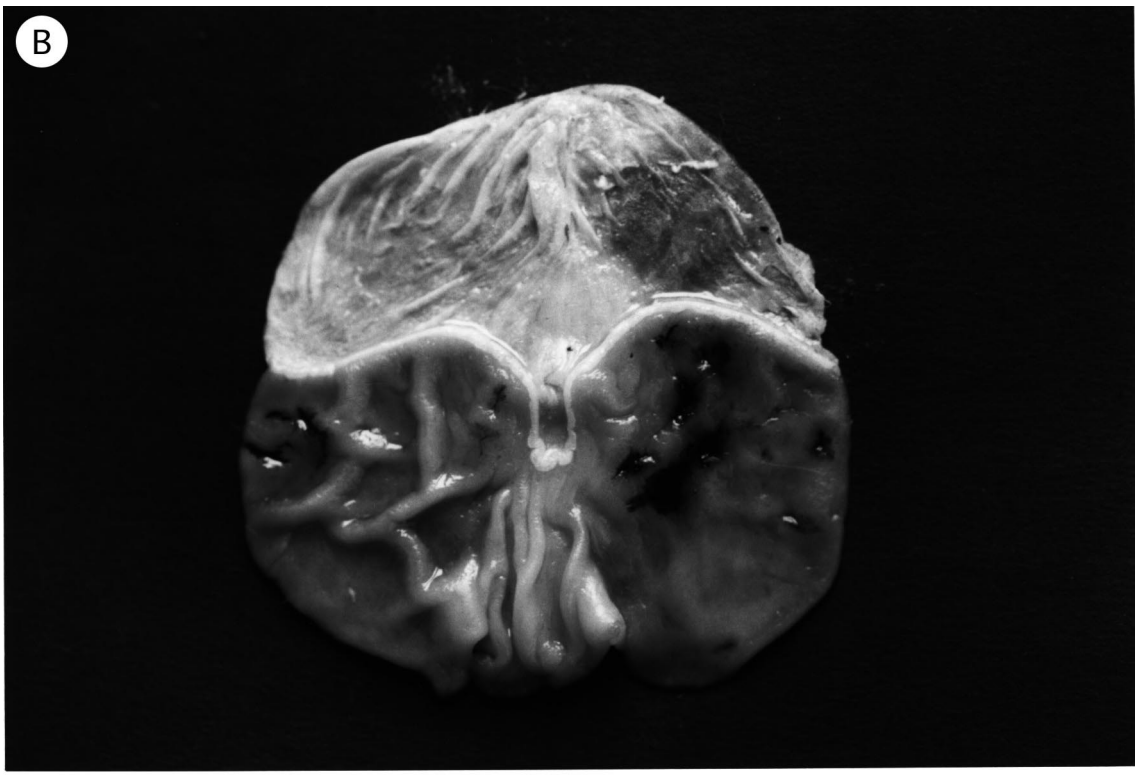

Fig. 3 (*continued*).

fall in the blood flow was observed as compared to the flow recorded immediately after the end of ischemia–reperfusion (Fig. 1). With the progression of these superficial lesions into deeper ulcers at day 3 after ischemia–reperfusion, the flow was still about 30% lower than that recorded in intact gastric mucosa, but at 5, 10 and 15 days, a gradual increase in blood flow was observed. At day 15 after ischemia–reperfusion, when these ulcers almost com-

pletely disappeared, blood flow returned to a value not significantly different from that recorded in intact gastric mucosa (Fig. 2).

3.2. Effect of non-selective and selective inhibitors of cyclooxygenase-1 and cyclooxygenase-2 on the time course of mucosal recovery from ischemia–reperfusion damage and gastric blood flow

As shown in Fig. 1, in rats treated with resveratrol or selective cyclooxygenase-2 inhibitors NS-398 and rofecoxib, the initial area of erosions caused by ischemia–reperfusion increased significantly above that recorded in vehicle-treated animals at all time periods after ischemia–reperfusion. At day 3 after ischemia–reperfusion, when erosions had progressed into ulcers, the area of these lesions in resveratrol- or NS-398- and rofecoxib-treated rats remained significantly larger than those measured in vehicle-treated controls (Fig. 2). This increase in the area of gastric lesions and gastric ulcers of rats treated with resveratrol or the cyclooxygenase-2 inhibitors was observed at all time intervals after ischemia–reperfusion including day 15 (Figs. 1 and 2). Also gastric blood flow, which stopped completely during ischemia, was significantly lower at all tested time intervals after ischemia–reperfusion than the respective values obtained in vehicle-treated control rats. This reduction in blood flow was still observed in animals treated with resveratrol and in those treated with the specific cyclooxygenase-2 inhibitors at 3, 5 and 10 days when the acute erosions had already progressed into deeper lesions, and even at day 15 after ischemia–reperfusion the blood flow in these rats had failed to return to the value recorded in vehicle-treated rats (Fig. 2).

Fig. 1 shows also the effects of pretreatment with meloxicam and indomethacin on the area of gastric lesions recorded from time 0 to 24 h after the end of ischemia–reperfusion. The administration of meloxicam resulted in a significant increase in the area of gastric lesions and this effect was accompanied by a marked fall in gastric blood flow at each time interval after ischemia–reperfusion (Fig. 1). Treatment with indomethacin also increased the area of gastric lesions above that measured in vehicle control rats and produced a significant fall in blood flow rate similar to that attained in animals treated with meloxicam. With the progression of acute erosions into deeper gastric lesions, the treatment with meloxicam or indomethacin delayed significantly the healing of these lesions and this was accompanied by a similar attenuation of blood flow to that observed in rats treated with selective cyclooxygenase inhibitors (Fig. 2).

3.3. Mucosal generation of prostaglandin E_2 and plasma interleukin-1β and gastrin levels during recovery from ischemia–reperfusion lesions and healing of ischemia–reperfusion gastric ulcers

The generation of prostaglandin E_2 in the vehicle-control gastric mucosa not exposed to ischemia–reperfusion averaged 128 ng/g ± 12 ng/g of wet tissue weight (Table 1). The exposure of the stomach to ischemia–reperfusion, which caused acute gastric erosions and a significant attenuation of gastric blood flow, resulted in a significant decrease in prostaglandin E_2 generation in both non-ulcerated and ulcerated gastric mucosa as compared to that recorded in intact gastric mucosa. This decrease in prostaglandin E_2 generation in non-ulcerated gastric mucosa persisted at 3, 12 and 24 h after ischemia–reperfusion, but at day 3 prostaglandin E_2 generation started to increase to reach at day 10 and 15 day after ischemia–reperfusion a value similar to that recorded in intact mucosa. A similar pattern of mucosal generation of prostaglandin E_2 was recorded in ulcerated gastric mucosa but the levels of prostaglandin E_2 were significantly higher in ulcerated gastric mucosa than in non-ulcerated gastric mucosa at the same times after the end of ischemia–reperfusion.

Table 1
Prostaglandin E_2 (PGE_2) generation in ulcerated and non-ulcerated mucosa, plasma interleukin-1β (IL-1β) and gastrin levels in vehicle-control rats and in those exposed to ischemia–reperfusion. Levels were measured immediately after the end of ischemia–reperfusion (0 h) and at various times after this procedure. Means ± S.E.M. for eight rats

Type of test	PGE_2 in non-ulcerated mucosa (ng/g)	PGE_2 in ulcerated mucosa (ng/g)	IL-1β (pg/ml)	Gastrin (pmol/l)
Vehicle (control)	128 ± 12	–	3 ± 0.5	32 ± 3
Time after ischemia–reperfusion				
0 h	65 ± 3[a]	68 ± 4	5 ± 1.3	35 ± 6
3 h	8 ± 6[a]	116 ± 3[b]	26 ± 1.8[a]	44 ± 5[a]
12 h	70 ± 5[a]	129 ± 4[b]	31 ± 6[a]	58 ± 6[a]
24 h	74 ± 6[a]	131 ± 3[b]	49 ± 3[a]	65 ± 7[a]
3 days	89 ± [a]	145 ± 4[b]	43 ± 12[a]	49 ± 5[a]
5 days	102 ± 9[a]	184 ± 6[b]	39 ± 10[a]	41 ± 4[a]
10 days	118 ± 9	192 ± 5[b]	22 ± 0.5[a]	36 ± 6
15 days	135 ± 12	153 ± 8	6 ± 0.3	28 ± 5

[a] Indicates a significant change as compared to vehicle (control).
[b] Indicates a significant change as compared to the values obtained in non-ulcerated gastric mucosa.

As shown in Table 1, the plasma interleukin-1β concentration in intact animals was negligible, but in animals exposed to ischemia–reperfusion the plasma interleukin-1β level gradually increased, starting 3 h after ischemia–reperfusion. This rise in plasma interleukin-1β concentration peaked at 24 h but subsequently decreased at day 3 after ischemia–reperfusion, falling at day 15 after ischemia–reperfusion to a value not significantly different from that in vehicle-treated control animals. The plasma gastrin concentration was significantly increased already at 3 h following the end of ischemia–reperfusion, the peak level was observed at 24 h but then the plasma gastrin level tended to decline at day 3, and at day 10 it was not significantly different from the value obtained in control animals not exposed to ischemia–reperfusion.

The generation of prostaglandin E_2 was significantly higher in ulcerated gastric mucosa than in non-ulcerated gastric mucosa at 3 h after the end of ischemia–reperfusion (Table 2). The administration of indomethacin (5 mg/kg i.p.), which suppressed the mucosal generation of prostaglandin E_2 in non-ulcerated and ulcerated gastric mucosa by about 90%, increased significantly the area of ischemia–reperfusion erosions and this effect was accompanied by a significant fall in gastric blood flow at 3 h after the end of ischemia–reperfusion (Table 2, Fig. 1).

Table 2
Effect of resveratrol (10 mg/kg i.g), NS-398 (10 mg/kg i.g.), rofecoxib (10 mg/kg i.g.), meloxicam (5 mg/kg i.g.) and indomethacin (5 mg/kg i.p.) on the generation of prostaglandin E_2 (PGE_2) in gastric mucosa at 3 h and 5 days after the end of ischemia–reperfusion, respectively. Results are means ± S.E.M. for 8–10 rats

Type of test	PGE_2 generation in non-ulcerated gastric mucosa (ng/g)	PGE_2 generation in ulcerated gastric mucosa (ng/g)
At 3 h after ischemia–reperfusion		
Vehicle	68 ± 6	116 ± 8[a]
Resveratrol	55 ± 4[b]	75 ± 6[c]
NS-398	62 ± 4	94 ± 3[c]
Rofecoxib	64 ± 5	85 ± 4[c]
Meloxicam	47 ± 3[b]	58 ± 5[c]
Indo	11 ± 2[b]	18 ± 3[c]
At day 5 after ischemia–reperfusion		
Vehicle	106 ± 9	135 ± 9[a]
Resveratrol	72 ± 4[b]	68 ± 5[c]
NS-398	102 ± 5	74 ± 5[c]
Rofecoxib	98 ± 6	65 ± 6[c]
Meloxicam	79 ± 4[b]	59 ± 4[c]
Indo	18 ± 2[b]	22 ± 3[c]

[a] Indicates a significant change as compared to the value obtained in non-ulcerated gastric mucosa.

[b] Indicates a significant change as compared to the value obtained in vehicle-treated non-ulcerated gastric mucosa at 3 h and at day 5 after the end of ischemia–reperfusion.

[c] Indicates a significant change as compared to the value obtained in vehicle-treated gastric mucosa at 3 h and day 5 after the termination of ischemia–reperfusion.

When NS-398 or rofecoxib was applied i.g. in a dose of 10 mg/kg, a significant increase in the area of gastric erosions was observed without there being a significant alteration in prostaglandin E_2 generation in the non-ulcerated gastric mucosa (Table 2, Fig. 1). In contrast, the generation of prostaglandin E_2 in the ulcerated gastric mucosa was significantly inhibited by the treatment with NS-398 or rofecoxib. Pretreatment with meloxicam, a non-specific cyclooxygenase-2 inhibitor applied i.g. in a dose of 5 mg/kg, aggravated ischemia–reperfusion-induced gastric erosions, while decreasing significantly blood flow and prostaglandin E_2 generation, in both non-ulcerated and ulcerated gastric mucosa as compared to those obtained in control animals. Resveratrol, which also augmented significantly the area of gastric erosions induced by ischemia–reperfusion, produced a significant fall in prostaglandin E_2 generation in both, non-ulcerated and ulcerated gastric mucosa (Table 2, Fig. 1).

3.4. Effect of the replacement therapy with exogenous prostaglandin E_2 on the delay in healing of ischemia–reperfusion-induced gastric lesions by the inhibition of cyclooxygenase

As shown in Fig. 4, the area of ischemia–reperfusion-induced erosions measured at 3 h after the end of ischemia–reperfusion was significantly larger and the gastric blood flow was significantly lower in rats treated with resveratrol (10 mg/kg i.g.), NS-398 and rofecoxib (10 mg/kg i.g.), meloxicam (5 mg/kg i.g.), and indomethacin (5 mg/kg i.p.), than in vehicle-treated animals. Addition of prostaglandin E_2 (1 μg/kg i.g.), which by itself failed to affect the area of erosions or blood flow caused by ischemia–reperfusion, to either resveratrol or cyclooxygenase-2 inhibitors, such as NS-398 or rofecoxib, abolished completely the increase in the area of gastric erosions and the accompanying fall in blood flow induced by the administration of these specific cyclooxygenase-1 or cyclooxygenase-2 inhibitors and significantly accelerated the healing of these lesions at various time intervals after ischemia–reperfusion in comparison to the effect of each selective cyclooxygenase inhibitor alone. Prostaglandin E_2, applied at a dose of 1 μg/kg i.g., also attenuated significantly the area of these lesions and the accompanying alterations in gastric blood flow induced by pretreatment with indomethacin or meloxicam (Fig. 4).

Fig. 5 shows the area of deep gastric lesions measured at day 5 after ischemia–reperfusion in series of experiments with animals treated with resveratrol (10 mg/kg i.g.), NS-398 and rofecoxib (10 mg/kg i.g.), meloxicam (5 mg/kg i.g.) and indomethacin (5 mg/kg i.p.), without or with the pretreatment with 16,16 dimethyl prostaglandin E_2 (1 μg/kg i.g.). The area of gastric lesions was significantly larger and the gastric blood flow was significantly lower in rats treated with resveratrol, NS-398, rofecoxib, meloxicam or indomethacin than in vehicle-treated ani-

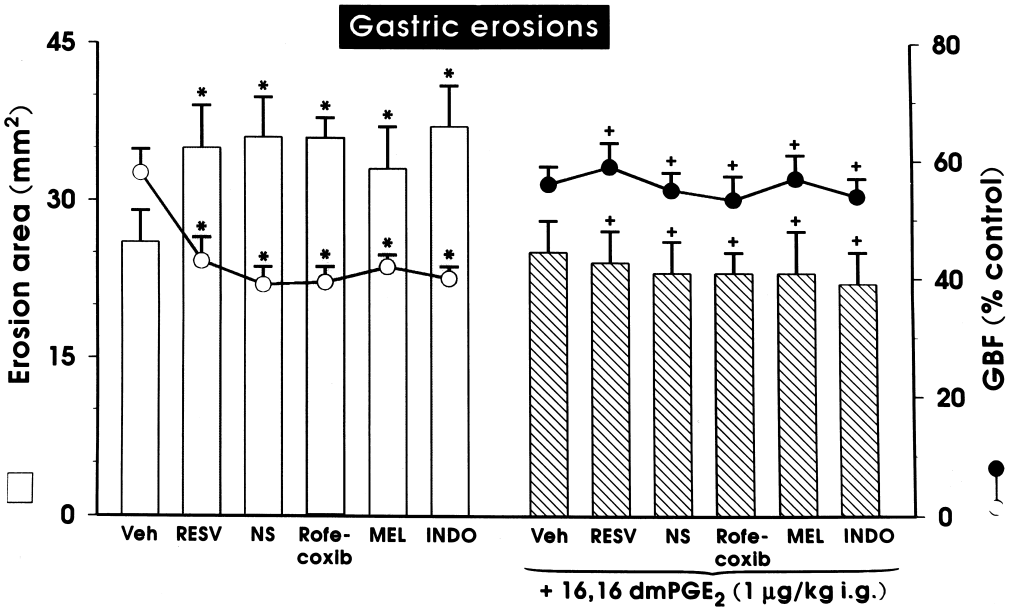

Fig. 4. Effect of resveratrol (10 mg/kg i.g.), NS-398 (10 mg/kg i.g.), rofecoxib (10 mg/kg i.g.), meloxicam (5 mg/kg i.g.), or indomethacin (5 mg/kg i.p.) with or without addition of 16,16 dimethyl PGE_2 (1 μg/kg i.g.) on the area of gastric lesions and accompanying changes in gastric blood flow induced by exposure to standard ischemia–reperfusion as determined 3 h later. Results are means ± S.E.M. for 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in vehicle-control gastric mucosa. Cross indicates a significant change as compared with the value obtained in rats without PGE_2 administration.

mals. Addition of prostaglandin E_2 at a dose of 1 μg/kg, which failed to affect significantly the area of ischemia–reperfusion-induced gastric ulcers and gastric blood flow, did not affect significantly these parameters as compared to those recorded in vehicle-treated animals. Prostaglandin E_2 analog also attenuated significantly the area of ischemia–reperfusion-induced gastric ulcers and the accompanying fall in gastric blood flow induced by meloxicam and indomethacin (Fig. 5). Prostaglandin E_2 added to resveratrol or specific cyclooxygenase-2 inhibitors counteracted

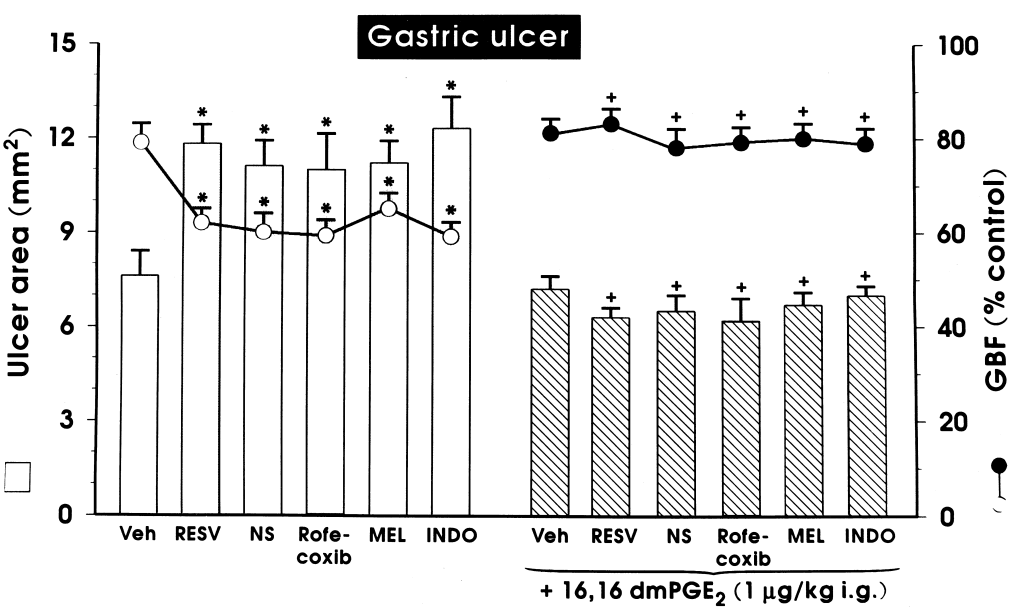

Fig. 5. Effect of resveratrol (10 mg/kg i.g.), NS-398 (10 mg/kg i.g.), rofecoxib (10 mg/kg i.g.), meloxicam (5 mg/kg i.g.), or indomethacin (5 mg/kg i.p.), with or without addition of 16,16 dimethyl PGE_2 (1 μg/kg i.g.) on the area of gastric ulcers and on gastric blood flow in rats exposed to standard ischemia–reperfusion as determined at day 5 after the end of ischemia–reperfusion. Results are means ± S.E.M. for 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in vehicle control gastric mucosa. Cross indicates a significant change as compared with the value obtained in rats without addition of PGE_2.

significantly the increase in area of gastric lesions and the accompanying fall in the gastric blood flow induced by administration of these cyclooxygenase inhibitors without the addition of prostaglandin E_2 (Fig. 5).

3.5. Expression of cyclooxygenase-1 and cyclooxygenase-2 mRNA by reverse transcription-polymerase chain reaction in gastric mucosa during the recovery from ischemia–reperfusion lesions

Fig. 6A–D shows the expression of GAPDH, cyclooxygenase-1, cyclooxygenase-2 and interleukin-1β mRNA in the gastric mucosa of intact rats treated with vehicle and not exposed to ischemia–reperfusion or exposed to ischemia–reperfusion and killed immediately (time 0) thereafter or during mucosal recovery from ischemia–reperfusion lesions at 3, 12 and 24 h or at 3, 10 and 15 days. The expression of GAPDH mRNA was well preserved in the mucosal biopsy samples taken both from control rats treated with vehicle and from rats exposed to ischemia–reperfusion and tested at various time intervals afterwards (Fig. 6A). As shown in Fig. 6B, cyclooxygenase-1 mRNA was detectable in the vehicle-treated gastric mucosa as well as

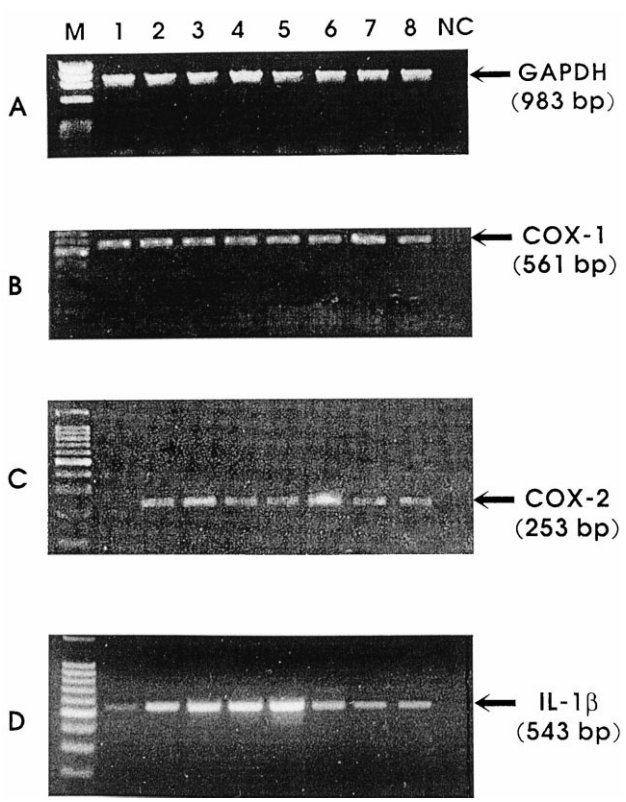

Fig. 6. Messenger RNA expression for GAPDH (A), cyclooxygenase-1 (B), cyclooxygenase-2 (C) and interleukin-1β mRNA (D) in intact gastric mucosa (lane 1) and at 0, 3, 12 and 24 h or 3, 10 and 15 days after the end of ischemia–reperfusion in rats treated with vehicle and exposed to a standard period of ischemia–reperfusion (lanes 2–8). NC is negative control (water). M-size marker DNA; Arrow — expected PCR product (bp).

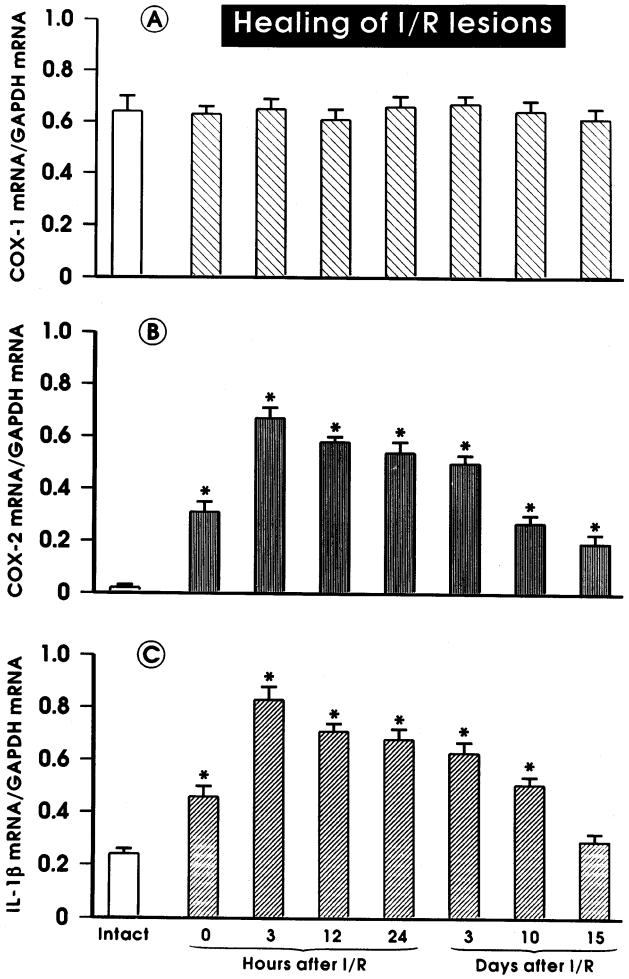

Fig. 7. Assessment of gene expression for cyclooxygenase-1 (A), cyclooxygenase-2 (B) and interleukin-1β (C) by the cyclooxygenase-1, cyclooxygenase-2 and interleukin-1β mRNA/GAPDH mRNA ratio in the gastric mucosa of intact rats and in those exposed to standard ischemia–reperfusion as measured 0, 3, 12 and 24 h or at 3, 10 and 15 days after the end of ischemia–reperfusion. The asterisk indicates a significant increase above the value obtained in intact gastric mucosa.

in the mucosa immediately after ischemia and after ischemia–reperfusion at each time interval tested. Then ratio of cyclooxygenase-1 mRNA to GAPDH mRNA revealed that the expression of cyclooxygenase-1 mRNA was similar at all time intervals after ischemia and ischemia–reperfusion (Fig. 7A). In contrast, the signal for cyclooxygenase-2 mRNA was negligible in vehicle-treated controls but was detectable in rats exposed to ischemia–reperfusion at 0, 3, 12 and 24 h or at 3, 10 and 15 days, with the strongest signal being detected at 3 h following the end of ischemia–reperfusion (Fig. 6C). The ratio of cyclooxygenase-2 mRNA to GAPDH mRNA confirmed that the expression of cyclooxygenase-2 peaked at 3 h after ischemia–reperfusion and then declined; however, it was still significantly stronger at all time periods after ischemia–reperfusion than that recorded in control animals (Fig. 7B). Fig. 6D shows that interleukin-1β mRNA was

detectable in intact gastric mucosa and that its expression was markedly increased after ischemia–reperfusion. The ratio of interleukin-1β mRNA to GAPDH mRNA showed that this expression was maximal at 3 h and was sustained over 12 and 24 h after ischemia–reperfusion. The ratio then declined at day 3 through day 15 to reach a similar value to that recorded in intact gastric mucosa (Fig. 7C).

4. Discussion

In this study a novel model was used to induce gastric lesions, namely, ischemia–reperfusion. Initially, acute erosions were produced that progressed into deeper chronic ulcers which, like naturally occurring ulcers in humans, appeared spontaneously and healed spontaneously within 15 days after ischemia–reperfusion. We confirmed the original observation of Wada et al. (1996) that the exposure of the gastric mucosa to ischemia induced by clamping of the celiac artery followed by reperfusion produces initially widespread acute gastric erosions that progress 24 h later into deeper chronic gastric lesions. Our histological investigations confirmed that these lesions were initially superficial bleeding erosions which then progressed into chronic ulcerated lesions that differed, however, from typical chronic gastric ulcers such as those induced by acetic acid (Konturek et al., 1987) because they never reached the muscularis mucosa. These chronic ulcerated lesions reached a maximum at day 3 after ischemia–reperfusion and healed progressively with time, the healing process being completed in vehicle-treated controls within 10–15 days after ischemia–reperfusion. As expected, these early ischemia–reperfusion lesions were accompanied by a marked fall in gastric blood flow with no significant alteration in plasma gastrin and interleukin-1β levels, but 3 h after ischemia–reperfusion, a significant rise in both plasma gastrin and interleukin-1β concentrations occurred. Following progression of the acute gastric lesions into chronic lesions, the blood flow and the elevated plasma interleukin-1β and gastrin levels gradually decreased and at 15 day reached values similar to those recorded in control rats without ischemia–reperfusion. The hypergastrinemia observed during the early period of recovery from these lesions could be attributed to the marked suppression of gastric acidity caused by exposure of the mucosa to ischemia–reperfusion, as demonstrated previously (Brzozowski et al., 1998; Nakamoto et al., 1998).

Previous studies have established that prostaglandin synthesis depends upon the activity of cyclooxygenase, a rate-limiting enzyme in the synthesis of eicosanoids (Eberhart and Dubois, 1995). Two isoforms of cyclooxygenase have been identified in many cells: a constitutive enzyme designated cyclooxygenase-1 and an inducible isoform known as cyclooxygenase-2 (Masferrer et al., 1994). cyclooxygenase-1 appears to be responsible for the production of prostaglandins that are physiologically important for homeostatic functions, such as maintenance of the mucosal integrity and mucosal blood flow (Vane and Botting, 1995). Under physiological conditions, prostanoid synthesis depends upon the availability of arachidonic acid and cyclooxygenase-1 activity. The latter is a major target of nonsteroidal anti-inflammatory agents causing mucosal damage in the stomach (Kargman et al., 1996). In contrast, cyclooxygenase-2 is not constitutively expressed in most tissues but is dramatically upregulated during inflammation (Kennedy et al., 1993; Kargman et al., 1996; Masferrer et al., 1996). The overexpression of the gene for cyclooxygenase-2 was demonstrated in vitro after the stimulation of cyclooxygenase-2 mRNA by proinflammatory cytokines such as interleukin-1β or tumor necrosis factor alpha (TNFα) (Ristimaki et al., 1994).

In the present study, the exposure to ischemia–reperfusion produced a significant fall in PGE_2 generation in the gastric mucosa but this reduced PGE_2 generation was gradually restored during mucosal recovery from gastric lesions, suggesting that endogenous prostaglandin may be involved in the spontaneous healing of these lesions. This is supported by the fact that prostaglandin E_2 generation reached higher values during the course of healing of ulcerated gastric mucosa than it did in non-ulcerated mucosa. This observation is in keeping with the recent finding of Lesch et al. (1998) that higher amounts of prostaglandin E_2 are detected at the site of ulceration than in non-ulcerated mucosa. Furthermore, pretreatment with indomethacin or meloxicam, both non-selective inhibitors of cyclooxygenase which suppress significantly prostaglandin E_2 generation, markedly delayed the healing of these lesions. The administration of resveratrol, a specific cyclooxygenase-1 inhibitor (Jang et al., 1997), caused only a small reduction in prostaglandin E_2 formation and prolonged significantly the healing time of these lesions to an extent similar to that seen with potent cyclooxygenase inhibitors such as indomethacin or meloxicam. These data are in agreement with the recent observation that resveratrol inhibits prostaglandin E_2 production in human mammary and oral epithelial cells stimulated by phorbol ester in vitro, an effect attributed to the selective inhibition of cyclooxygenase-1 (Jang et al., 1997). The data for this cyclooxygenase-1 inhibitor are, however, difficult to interpret because resveratrol was found to suppress phorbol ester-induced cyclooxygenase-2 mRNA whereas another study in vitro revealed that resveratrol exerted a potent inhibitory action on cyclooxygenase-1 and also stimulated prostaglandin H syntase-2 activity (Johnson and Maddipati, 1998). Further detailed studies in vivo are needed to clarify the inhibitory action of resveratrol in the gastrointestinal tract and to confirm its selectivity on cyclooxygenase-1 enzyme activity in the stomach.

Previous studies revealed that selective cyclooxygenase-2 inhibitors such as NS-398 and L-745,337 failed

to cause gastric ulcerations and to inhibit prostaglandin synthesis (Schmassmann et al., 1997; Lesch et al., 1998). However, other cyclooxygenase-2 inhibitors, such as PD 138387, which besides being selective cyclooxygenase-2 inhibitor was also found to suppress 5-lipooxygenase activity, failed to delay ulcer healing (Lesch et al., 1998). These authors proposed that this suppression of 5-lipooxygenase activity by this agent may counteract the impairment in healing caused by cyclooxygenase-2 inhibition. Moreover, it was suggested that the impairment of healing observed with NS-398 might be due to the chemical structure of this compound rather than to its inhibition of cyclooxygenase-2 (Lesch et al., 1998). This is why we used another cyclooxygenase-2 inhibitor, rofecoxib, which has been shown to inhibit selectively cyclooxygenase-2 and to exhibit analgesic and inflammatory activity similar to that of ibuprofen. We found that both NS-398 and rofecoxib failed to affect the generation of prostaglandin E_2 in the intact or non-ulcerated mucosa but significantly reduced that in ulcerated mucosa, and this was probably responsible for the marked delay in the healing of ischemia–reperfusion lesions. This finding is in agreement with the recent observation that cyclooxygenase-2 inhibitors suppress prostaglandin E_2 generation by cyclooxygenase-2 only at the site of pre-existing ulcers (Lesch et al., 1998).

These effects of both specific or nonspecific cyclooxygenase inhibitors were fully restored by the addition to these inhibitors of a minute dose of exogenous prostaglandin E_2 that by itself did not affect the alterations of gastric mucosal integrity and gastric blood flow provoked by ischemia–reperfusion. Moreover, the strong signals for cyclooxygenase-1 mRNA were detected in the gastric mucosa of vehicle control rats as well as those exposed to ischemia–reperfusion at each time interval tested, while cyclooxygenase-2 mRNA was not detectable in the vehicle-treated mucosa but was recorded in the mucosa immediately after the termination of the ischemia–reperfusion procedure and throughout the period of lesion recovery. This suggests that the upregulation of cyclooxygenase-2 mRNA with subsequent, probably local, production of protective prostaglandin contributes to mucosal recovery from lesions. Our finding is in keeping with a recent report by Kishimoto et al. (1998a,b), who demonstrated that the upregulation of cyclooxygenase-2 mRNA occurs in gastric mucosa during recovery from lesions induced by exposure of this mucosa to ischemia–reperfusion. The importance of cyclooxygenase-2 expression to mucosal integrity was emphasized in other reports by the demonstration that strong cyclooxygenase-2 expression was detected following colonic inflammation (Reuter et al., 1996) and during healing of chronic ulcers in mice (Mizuno et al., 1997). In our present study, the expression of cyclooxygenase-2 appeared almost immediately after ischemia–reperfusion and reached a maximum at 3 h. Cyclooxygenase-2 expression was still significantly elevated at 12 and 24 h but then with the progression of acute mucosal erosions into deeper chronic lesions there was a gradual decline in its expression, with almost complete disappearance at 15 day after ischemia–reperfusion. Therefore, it is reasonable to assume that excessive production by cyclooxygenase-2 of prostaglandin was probably limited to the mucosal lesion area and contributed to mucosal repair and to acceleration of lesion healing. It is of interest that in another report (Davies et al., 1997) aspirin applied i.g. in an ulcerogenic dose which suppressed almost completely cyclooxygenase activity and abolished the prostaglandin biosynthesis in the stomach resulted in an upregulation of cyclooxygenase-2 mRNA in the gastric mucosa. Moreover, this increased expression of cyclooxygenase-2 mRNA induced by aspirin in a dose that suppressed cyclooxygenase-1 activity was significantly diminished by the administration of exogenous prostaglandin E_2 (Davies et al., 1997). It was concluded (Davies et al., 1997) that this enhancement of cyclooxygenase-2 expression was not just a specific response of mucosa to injury because topically applied irritants, such as ethanol, indomethacin or salicylate, failed to alter significantly both cyclooxygenase-2 mRNA and cyclooxygenase-2 protein expression (Davies et al., 1997). It is possible, therefore, that the upregulation of cyclooxygenase-2 mRNA observed in our present study was triggered, at least in part, by the depletion of endogenous prostaglandin induced by ischemia–reperfusion. This is supported by our finding showing a decrease in prostaglandin biosynthesis after ischemia–reperfusion. Furthermore, replacement therapy with exogenous prostaglandin E_2, added to the specific inhibitor of cyclooxygenase-2 to supplement the deficiency of prostaglandin in the mucosa, attenuated significantly the deleterious effect of the tested cyclooxygenase-inhibitor on mucosal healing. Thus, our study demonstrates that prostaglandins generated by both cyclooxygenase-1 and cyclooxygenase-2 enzymes contribute to the healing of mucosa injured by ischemia–reperfusion. Cyclooxygenase-1 is found in the mucosa not involved in the injury, while cyclooxygenase-2 is found in the close vicinity of lesions, where marked inflammation occurs. This remains in agreement with the fact that a non-specific and very potent inhibitor of cyclooxygenase, such as indomethacin, at a dose that suppressed prostaglandin E_2 generation by about 90%, caused the strongest delay in healing of these lesions and impaired the microcirculatory response following healing. It is of interest that meloxicam, which is known to inhibit preferentially cyclooxygenase-2 but which also possesses some affinity for cyclooxygenase-1 (Engelhardt et al., 1996), antagonized completely these responses in a manner similar to that observed with indomethacin. Specific cyclooxygenase-2 blockers, such as rofecoxib, or NS-398, which failed to alter prostaglandin E_2 generation in macroscopically intact gastric mucosa but reduced that in the ulcerated mucosa, significantly attenuated the healing of these lesions and the accompanying increase in gastric blood flow to an extent similar to that obtained in animals pretreated with resvera-

trol, the specific cyclooxygenase-1 inhibitor. An explanation for this is that these specific cyclooxygenase-2 blockers attenuate prostaglandin formation in the ulcer area only, as demonstrated in this study, so that their effect on prostaglandins is not detected when biopsy samples for estimation of prostaglandin generation are taken from the grossly intact mucosa. Our notion that the expression of cyclooxygenase-2 plays an important role in the healing of gastric ulcers is also consistent with the recent observation of Gretzer et al. (1998) that prostaglandin generated by cyclooxygenase-2, and not only by cyclooxygenase-1, may be involved in adaptive cytoprotection induced by topically applied mild irritant, when a large area of mucosa is injured. Based on the previous evidence that cyclooxygenase-2 mRNA and cyclooxygenase-2 protein were identified in endothelia of gastric mucosal microvessels, it was concluded (Tarnawski et al., 1996; Gretzer et al., 1998) that cyclooxygenase-2 may be constitutively expressed and that it produces prostaglandin in similar way as cyclooxygenase-1 and is involved in the mechanism of adaptive cytoprotection. Another possibility to explain the contribution of endogenous prostaglandin to the healing of these lesions is that cyclooxygenase-1, which is generally regarded to be a constitutively expressed enzyme, produces excessive amounts of prostaglandins in the intact mucosa as well as in vitro isolated mucosal cells (Ferraz et al., 1997). The down-regulation of cyclooxygenase-1, which was associated with a decrease in biosynthesis of cytoprotective prostanoids, was recently implicated in the exacerbation of liver injury by ethanol (Nanji et al., 1997). The upregulation of cyclooxygenase-2 due to ethanol treatment in this study (Nanji et al., 1997) resulted in increased synthesis of inflammatory and vasoactive eicosanoids. This was, however, not the case in our present study because cyclooxygenase-1 mRNA was detectable in gastric mucosa at each time interval during recovery from ischemia–reperfusion and failed to showed any significant increase after this procedure. In contrast, the cyclooxygenase-2 transcript was undetectable in intact animals but was strongly upregulated following recovery from lesions induced by ischemia–reperfusion. The mechanism of this upregulation is unknown but the release of proinflammatory and antisecretory cytokines might contribute to the activation of the genes for cyclooxygenase-2, as described previously (Ristimaki et al., 1994). Indeed, we observed the upregulation of interleukin-1β transcript followed by an elevation of plasma interleukin-1β concentration especially during the early phase of mucosal recovery from lesions induced by ischemia–reperfusion at a time when overexpression of cyclooxygenase-2 transcript was also detected. With healing of mucosal lesions, the decrease in expression of these factors was evident, indicating that the upregulation of interleukin-1β, which probably activated the expression of cyclooxygenase-2 in our study, is an important event in the process of mucosal repair after damage induced by ischemia–reperfusion.

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