NO-flurbiprofen maintains duodenal blood flow, enhances mucus secretion contributing to lower mucosal injury

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NO-flurbiprofen maintains duodenal blood flow, enhances mucus secretion contributing to lower mucosal injury. Am J Physiol Gastrointest Liver Physiol 283: G1090–G1097, 2002. First published August 14, 2002; 10.1152/ajpgi.00480.2001.—This study investigates possible mechanisms behind the reduced gastrointestinal ulcerogenicity of nitric oxide (NO)-flurbiprofen compared with flurbiprofen. The duodenal mucosa of Inactin-anaesthetised rats was exteriorized for intravital microscopy. Blood flow was measured with laser-Doppler flowmetry (LDF), mucus thickness with micropipettes, and P-selectin expression with dual-labeled antibody technique, and mucosal integrity by 51Cr-EDTA permeability. Exposure of the duodenum to flurbiprofen (1.0 mg/ml) for 90 min significantly reduced LDF to 75 ± 4%, whereas NO-flurbiprofen (1.3 mg/ml) had no significant effect. Mucosal permeability to 51Cr-EDTA was unchanged in the control and NO-flurbiprofen groups but increased significantly from 1.0 ± 0.2 to 3.7 ± 0.7 μl/min·g−1 after 90-min exposure to flurbiprofen. Expression of ICAM-1 was significantly increased after oral flurbiprofen but not by NO-flurbiprofen. Positive effects of NO-flurbiprofen compared with flurbiprofen on mucus formation, blood flow, and adhesion molecule expression likely contribute to the reduced mucosal injury observed with NO-flurbiprofen.

laser-Doppler flowmetry; nonsteroidal anti-inflammatory drugs; nitric oxide; mucosal permeability; adhesion molecules

NONSTEROIDAL ANTI-INFLAMMATORY drugs (NSAIDs) are capable of causing severe mucosal injury to the stomach and small bowel. This is a result of direct NSAID injury to the mucosal epithelium and indirectly from the drug’s ability to inhibit prostaglandin synthesis and attenuate the mucosal protective mechanisms normally elicited in response to injurious agents. Two of the protective mechanisms that maintain the gastrointestinal mucosal barrier against the hostile environment of the gut lumen are mucus secretion and blood flow. Mucus contributes to mucosal defense by providing a physical barrier to bacteria and other toxic agents and by creating a pH gradient with the secreted bicarbonate. Blood flow has to meet the relatively high metabolic needs of the gastrointestinal tract as well as provide both valuable buffering and a pathway for removal of toxins that may have entered the tissue. Mechanisms by which NSAIDs induce injury may well differ in different parts of the gastrointestinal tract (41). In the stomach, there is conflicting evidence on the extent to which NSAID-induced ischemia initiates or amplifies the mucosal injury, with some studies suggesting that NSAIDs cause mucosal ischemia (6, 19, 26) and others reporting an increase in blood flow or an increase in ulcer-site flow (11, 13, 29). In the dog small bowel, there is evidence that indomethacin given either intravenously or rectally reduces blood flow (15, 17, 18) and that the reduction in flow is due to an increase in vascular resistance accompanied by a rise in blood pressure. Ibuprofen, however, caused no change in canine intestinal blood flow or bowel pressure (17), indicating that the response may vary depending on the NSAID used. A study of patients on chronic NSAID treatment revealed a 40% reduction in the laser-Doppler signal recorded from the stomach and duodenal mucosa compared with values recorded from control patients (38). These data suggest that NSAID treatment reduces gastrointestinal mucosal blood flow in humans.

The barrier function of mucus is very important in protecting the mucosa (3), and the thickness of the mucus layer in the stomach and duodenum of the rat is dramatically reduced by indomethacin (2, 24, 36), thereby increasing the susceptibility of these organs to injury. Conversely, mucus secretion and gel thickness in the stomach and duodenum are increased by prostaglandin stimulation (2, 24, 32).

Another possible mechanism for NSAIDs to induce damage is by promoting leukocyte-endothelial adhesion. Evidence for this involves increased synthesis of TNF-α and leukotriene B4 and upregulation of ICAM-1 on NSAID treatment (5, 42).
Recently, NSAIDs have been produced coupled to a nitric oxide (NO) moiety. These NO-NSAIDs appear to be far less injurious to the gastrointestinal tract without reducing their efficacy (33, 43–45). NO has been shown to be an important regulator of mucus secretion in the stomach (9, 10, 22), and the NO synthase inhibitor N-nitro-L-arginine reduced mucus accumulation in the rat duodenum (36). NO is also an important mediator of vascular tone in the gastrointestinal tract, as well as in neutrophils (7) and P-selectin on the vascular endothelium. Inhibition of NO synthesis enhances the adhesion of leukocytes to the endothelium. Thus possible mechanisms by which the NO-NSAIDs may reduce mucosal injury is by an NO-induced increase in mucosal blood flow, by increasing mucous production, and/or by the action of NO in reducing leukocyte adhesion within the mucosal microcirculation. Therefore, the aim of the present study was to investigate whether NO-flurbiprofen (HCT 1026) maintained duodenal mucosal integrity by enhanced mucosal blood flow and mucus secretion and/or reduced leukocyte-endothelial interactions. This was achieved by comparing the effects of flurbiprofen and NO-flurbiprofen on laser-Doppler flow, mucus accumulation measured by means of microelectrodes, and P-selectin and ICAM-1 expression using the dual-labeled antibody technique (35) in the duodenal mucosa of the rat.

**MATERIALS AND METHODS**

**Animal Preparation**

Male rats (Sprague Dawley, Møllegård, Denmark, or F1 hybrids of Lewis and DA Animal Department, Biomedical Center, Uppsala, Sweden) weighing 170–230 g were kept under standardized conditions of temperature (21–22°C) and illumination (12:12-h light-dark) and had free access to water and pelleted food (Ewos, Södertälje, Sweden). Rats were fasted for 20 h before experiments but had free access to water. They were anesthetized with thiobutabarbital-Na [120 mg/kg ip (Inactin); Research Biochemicals International, Natick, MA]. Body temperature was maintained at 37 ± 0.5°C by a heating pad controlled by a rectal thermistor probe. Spontaneous breathing was facilitated by a cannula inserted into the trachea, and systemic arterial blood pressure was continuously monitored through a femoral artery. A femoral vein was cannulated for continuous infusion of a Ringer solution containing (in mM) 25 NaHCO3, 120 NaCl, 2.5 KCl, and 0.75 CaCl2 at 0.8–1.0 ml/h (not performed in the antibody experiments).

**Ethics Approval**

All experimental procedures in this study were approved by the Swedish Laboratory Animal Ethical Committee in Uppsala and were conducted in accordance with the Guidelines of the Swedish National Board for Laboratory Animals.

**Blood Flow and Mucus Measurements**

Preparation of the duodenal mucosa for blood flow and mucus measurements has been described previously (36). Briefly, after a midline abdominal incision, the common bile duct was cannulated close (2–3 mm) to its entrance into the duodenum and drained outside the body to avoid leakage of pancreaticobiliary secretion into the preparation. The rat was placed on its left side on a heating pad on a lucite microscope stage. A segment of proximal duodenum (15 mm) was opened along the antimesenteric border and was draped of the duodenal mucosa. To avoid impairment of the blood flow to the duodenum, a gap was left between the bottom of the chamber and the mucosa and was sealed with silicone grease (Dow Corning). A stereomicroscope (model MZ12; Leica, Heerbrugg, Switzerland) was used to observe the mucosa, which was transilluminated with light guided by fiber optics from a 150-W light source (model FOT 150; Fiber Optic, Spreitenbach, Switzerland). The water-jacketed chamber was filled with 5 ml of 0.9% unbuffered sodium chloride and kept at 37–38°C by heated water pumped through the hollow walls.

**Blood Flow Measurements**

Laser-Doppler flowmetry (LDF) was used for blood flow measurements as described earlier (21). The flowmeter (Periflux model Pf 4001; Perimed, Stockholm, Sweden) was connected to a computer and MacLab data-sampling unit (ADInstruments). The laser probe (633-nm wavelength and 0.5-mm probe fiber separation) was held in a fixed position at a distance of 1–2 mm above the mucosa by a micromanipulator (Leitz, Stuttgart, Germany). Blood flow was monitored continuously throughout the experiment. Evaluation of the accuracy of the LDF technique in measuring gastrointestinal blood flow was performed in earlier studies (1, 21, 28).

**Experimental Protocol (Blood Flow)**

Animals were given at least 1 h to achieve a steady state after the operation before the experiment started. After systemic blood pressure and blood flow (LDF signal) were stable, control values where the mucosa was exposed to 0.9% NaCl only were recorded for 20 min. This was followed by 90-min exposure to either the vehicle, flurbiprofen, or NO-flurbiprofen (HCT 1026, kind gift from Nicox, Milano, Italy). In control animals (n = 5), the duodenal mucosa was exposed to the vehicle (1.4% Tween-80 in 0.9% saline). This vehicle was used to dissolve both the drug one would expect to see in the duodenum, if a therapeutic dose was dissolved in gut contents.

Blood flow (LDF) is expressed as percentage of control where the control value is the mean ± SE during the 20-min period of exposure to saline before exposure to the vehicle, flurbiprofen, or NO-flurbiprofen. Vascular resistance was calculated as the mean arterial blood pressure divided by the mean blood flow calculated for each 10-min period. Resistance, like blood flow, is presented as a percentage of the first 20-min control value.
Mucus Measurements

Mucus thickness was measured with micropipettes (36) connected to a micromanipulator (Leitz) with a digimatic indicator (IDC series model 543; Mitutoyo, Tokyo, Japan). Glass tubing (borosilicate tubing with 1.2-mm outer diameter and 0.6-mm inner diameter; Frederick Haer, Brunswick, ME) was pulled with a pipette puller (model pp-83; Narishige Scientific Instrument Laboratories, Tokyo, Japan) to a tip diameter of 1–3 μm. Pipettes were siliconized to prevent mucus adhering to the glass. The tip was dipped into a silicone solution, MS-1107 in 25% acetone, dried at 100°C for 30 min, and stored at 100°C for up to 1 wk. The luminal surface of the mucus gel was visualized by graphite particles (activated charcoal, extra pure; Merck) placed on the gel, and the epithelial cell surface was visible through the microscope. The micropipette was inserted into the mucus gel at an angle (a) of ~30° to the surface. The angle was measured with a protractor, and the same angle was maintained throughout an experiment. The distances (D) traveled by the micropipette from the luminal surface of the mucus gel to the tips of five different villi was measured, and a mean ± SE value was calculated. The mucus thickness (T), which is the vertical distance between the cell surface and the luminal mucus surface, was then calculated from the formula T = D × sin a. The mean ± SE value of measurements made on the tops of five different villi was treated as one observation.

Experimental Protocol (Mucosal Permeability)

Observations of mucus thickness were not begun for at least 1 h after surgery. Mucus was then removed by gentle suction with a catheter coupled to a suction pump. Mucus thickness was recorded 20, 40, 60, and 80 min after the suction. Mucus was again removed, and mucus levels were recorded at 15 and 30 min.

During the first 20 min, the mucosa was covered with saline. Animals were then divided into three groups. In the control group (n = 6), the vehicle was applied topically and changed every 30 min throughout the experiment. In the second group (n = 6), flurbiprofen (1 mg/ml) was applied to the mucosa, and in a third group (n = 6), NO-flurbiprofen (1.3 mg/ml) was applied and the solution was changed as described for controls.

Mucus accumulation was calculated by subtracting the residual mucus thickness measured immediately after removal by suction (~20 μm) from the mean ± SE mucus thickness recorded at each time point.

Measurements of Mucosal Permeability to 51Cr-EDTA

Clearance of 51Cr-EDTA from tissue to duodenal lumen was used as an index of mucosal permeability. Briefly, the bile duct was cannulated, and the bile was drained outside the body. Kidneys were tied to prevent loss of tracer, and a cannula was introduced via the esophagus and stomach into the proximal duodenum and tied in place by a ligature around the pyloric groove. A draining cannula was introduced through the wall of the duodenum 5 cm distal to the pylorus, and the lumen of the duodenum was perfused with 0.9% saline (pH 7.4) at a rate of 0.5 ml/min. 51Cr-EDTA (100 μCi) was injected via a cannula in the femoral vein, and blood samples were taken from a cannula in the femoral artery to determine 51Cr activity in the plasma using an LKB gamma spectrometer (model 1282 Compgamma CS; LKB Pharmacia Diagnostic, Uppsala, Sweden).

Experimental Protocol (Mucosal Permeability)

After injection of the 51Cr-EDTA, the duodenum was given 30 min to equilibrate followed by 30-min perfusion of the lumen with saline alone (basal permeability). This was followed by 90-min perfusion with either vehicle (control; n = 3), flurbiprofen (1.0 mg/ml; n = 6), or NO-flurbiprofen (1.3 mg/ml; n = 4). Plasma samples were taken every 30 min, and samples of luminal perfusate were taken every 10 min and assayed for 51Cr activity. Mucosal permeability was calculated as blood-to-lumen clearance of 51Cr-EDTA as follows (4):

\[
\text{plasma 51Cr-activity (cpm/ml) \times perfusate flow rate (ml/min) } \\
\text{perfusate 51Cr-activity (cpm/ml) \times tissue weight (g)}
\]

ICAM-1 and P-Selectin Expression

Labeling of ICAM-1 and P-selectin with 125I and 131I. Monoclonal antibodies directed against ICAM-1 (IA29) (39) or P-selectin (RMP-1) (46) were labeled with 125I (DuPont NEN, Boston, MA), whereas isotype-matched nonbinding antibodies (P29) (31) were labeled with 131I. (Antibodies were kindly supplied by Dr. D. Neil Granger, Louisiana State University, Shreveport, LA.)

Radioiodination was performed using the iodogen method as previously described (34) in which 250 μg of protein is incubated with 250 μCi of 125I or 131I for 5 min on ice in an iodogen-coated (125 μg) test tube. After incubation, labeled antibodies were separated from the free 125I or 131I by gel filtration on a Sephadex PD-10 column (LKB Pharmacia). The column was equilibrated with 50-ml phosphate buffer containing 1% bovine serum albumin. The labeled antibody was applied to the column and eluted with two fractions of 2.5 ml. The labeled antibody was contained in the second fraction. Absence of free 125I or 131I was ensured by dialysis, with <1% of the total activity appearing in the dialysate. SDS-PAGE showed normal heavy- and light-chain moieties of expected molecular weight (35). Labeled antibodies were stored in 0.5-ml aliquots at 4°C for a maximum of 3 wk and were dialysed immediately before being used in each experiment.

Measurement of ICAM-1 and P-selectin expression. The duodenum was exposed to flurbiprofen (20 mg/kg) or NO-flurbiprofen (30 mg/kg) by gavage, in carboxymethyl cellulose [oral doses taken from the study of Wallace et al. (44)]. 90 min before injection of labeled antibodies. Control rats received vehicle alone.

To measure ICAM-1 expression, a mixture of 10-μg of 125I-ICAM-1 MAb (IA29), 230-μg unlabeled IA29, and 5-μg isotype-matched nonbinding antibody (P-23) labeled with 131I were injected into the jugular vein catheter. These doses were determined by procedures described in a previous article (35). To measure P-selectin expression, a mixture of 10 μg of 125I-P-selectin MAb (RMP-1) and 5 μg of 131I-nonbinding MAb (P-23) were injected into a different group of rats. After tracer injection, the animals were heparinized (3,000 IU/kg), and blood samples were taken via the carotid artery catheter at 2.5 and 5.0 min. At 5.0 min, the animal was exsanguinated via the carotid artery with simultaneous infusion of bicarbonate buffer via the jugular vein. The vena cava was then severed, and the circulation was flushed via the carotid artery with ~60 ml buffer. Five centimeters of the duodenum were then harvested, blotted dry, and weighed, and radioactivity was measured as described in Expression of ICAM-1 and P-selectin activity.

Expression of ICAM-1 and P-selectin activity. Activity of 125I and 131I was determined by using an LKB 1282 Compu-
gamma. Samples were counted for sufficient time to obtain accuracy of ±1%. Total activity injected (and total ng of antibody injected) in each experiment was calculated by counting a 5-μl sample of the injectate. Activity remaining in the injection syringe was subtracted from the total injected counts. Accumulated activity in the tissue was expressed as percent antibody bound per gram of tissue and was calculated as: % antibody bound/g = (%ID/g for 125I) – (%ID/g for 131I) × (%ID 125I in plasma)/(%ID 131I in plasma), where ID is injected dose (per gram). The nanogram of antibody bound per gram was calculated as: ng antibody bound/g = (corrected % injected 125I bound/g) × total ng 125I – antibody injected × (100)–1.

**Statistical Methods**

Results are expressed as means ± SE. Statistical evaluations performed were ANOVA followed by the Fishers protected least significant difference test or the Bonferroni t-test for multiple comparisons. Differences were regarded as significant at \( P < 0.05 \).

**RESULTS**

**Blood Flow**

Mean arterial blood pressure, duodenal mucosal blood flow, and mucosal vascular resistance did not change significantly during the control experiments when the vehicle alone was placed on the mucosa for a period of 90 min (Fig. 1). Exposure of the duodenum to flurbiprofen (1.0 mg/ml) for 90 min significantly (\( P < 0.001 \)) reduced LDF to 70 ± 4% of control, increased vascular resistance to 120 ± 6% (\( P < 0.001 \)), and reduced mean arterial blood pressure to 75 ± 5 mmHg or 84 ± 4% of the control value of 91 ± 8 mmHg (\( P < 0.001 \)). NO-flurbiprofen (1.3 mg/ml) had no significant effect on arterial blood pressure, mucosal blood flow, or mucosal vascular resistance.

**Mucus Accumulation**

The initial (control) rate of mucus accumulation observed in the first 20 min before drug administration and immediately after the removal of loosely adherent mucus was similar in all groups (Fig. 2). In the control group of rats, this was 72 ± 20 μm over the 20-min period or 3.6 ± 1 μm/min. The vehicle (Twee dilute in saline) was then applied in the control group, and mucus accumulation continued at 2.7 ± 1 μm/min during the 20- to 40-min period but at a much lower rate during the 40- to 80-min period (0.5 ± 0.3 μm/min). After a second removal of the mucus layer, accumulation occurred at a similar rate (2.7 ± 0.5 μm/min) to that recorded after the first mucus removal. Mean arterial blood pressure was initially 108 ± 3 mmHg and 99 ± 4 at the end of the experiment.

In the flurbiprofen-treated animals, the initial 20-min predrug (saline in the lumen) rate of mucus formation was 4.0 ± 1 μm/min. The addition of flurbiprofen (1 mg/ml) to the mucosa reduced mucus accumulation (0.3 ± 0.6 μm/min) during the 20- to 40-min period and then caused decreased mucus production (−0.2 ± 0.7 μm/min) during the 40- to 80-min period.

Eighty minutes after the first mucus removal, the mucus depth was significantly less than in control animals. After a second removal of the mucus layer, accumulation occurred again at a slower rate (1.6 ± 0.4 μm/min) than in the control. Mean arterial blood pressure was 102 ± 2 mmHg before the experiment and decreased significantly to 75 ± 1 mmHg after 90 min of flurbiprofen treatment.

In the NO-flurbiprofen-treated group, the initial 20-min predrug rate of mucus formation was 4.4 ± 1 μm/min, similar to that observed in the other groups. During NO-flurbiprofen administration, mucus accumulation was 3.2 ± 1.6 μm/min over the 20- to 40-min period and 1 ± 0.4 μm/min during the 40- to 80-min period. That is, NO-flurbiprofen tended to increase mucus production, and mucus accumulation was significantly greater than control at 80 min and significantly greater than flurbiprofen at 80-, 100-, and 110-min time points. Mean arterial blood pressure was 105 ± 4 mmHg before the experiment and 92 ± 5 mmHg after 90 min of NO-flurbiprofen treatment.

The pH of the solution bathing the gastric mucosa varied between 6.3 and 7.5 in all groups (unbuffered saline pH 5.8), indicating an alkaline secretion.
urbiprofen enhanced mucus production. Flurbiprofen significantly reduced mucus accumulation (*P < 0.05 compared with NO-flurbiprofen), whereas NO-flurbiprofen enhanced mucus production.

51Cr-EDTA Permeability

Three groups of animals were all exposed to saline alone for the first 30 min and in the control group. 51Cr-EDTA clearance measured during this saline period was 0.6 to 1.2 μl·min⁻¹·g⁻¹ and was not significantly different from the control values recorded for the other groups (Fig. 3). Control animals were exposed to the vehicle for a further 90 min, and the 51Cr-EDTA clearance remained unchanged. The addition of flurbiprofen caused a progressive increase in 51Cr-EDTA clearance to 3.7 ± 0.7 μl·min⁻¹·g⁻¹ after 90-min exposure, which was significantly higher than control and NO-flurbiprofen. NO-flurbiprofen had no significant effect on permeability, and after 90-min exposure, 51Cr-EDTA clearance was 1.1 ± 0.2 μl·min⁻¹·g⁻¹, similar to the value of 1.2 ± 0.1 μl·min⁻¹·g⁻¹ recorded during the first 10 min of the experiment.

Over the 90-min period of the experiment, blood pressure remained constant in control animals at 86 ± 6 mmHg (t = 0) to 81 ± 5 mmHg (t = 90 min) and in animals where the duodenum was exposed to flurbiprofen at 83 ± 11 mmHg (t = 0) to 74 ± 9 mmHg (t = 90 min). When the duodenum was exposed to NO-flurbiprofen, blood pressure fell from 103 ± 1 mmHg (t = 0) to 84 ± 2 mmHg (t = 90). The absolute value of blood pressure was not significantly different after 90 min in all three groups studied.

ICAM-1 and P-Selectin Expression

Expression of ICAM-1 or P-selectin (ng antibody bound/g tissue) was measured in the duodenal wall of control rats and in rats pretreated with flurbiprofen or NO-flurbiprofen (Fig. 4). In control animals, expression of ICAM-1 was 366 ± 23 ng/g (n = 6). This increased significantly to 471 ± 22 (n = 9) after oral flurbiprofen but did not increase after NO-flurbiprofen (386 ± 34, n = 5).

In control animals, there was almost no binding of P-selectin antibody indicating very low constitutive expression of P-selectin (1.0 ± 0.2, n = 5). Neither flurbiprofen (1.1 ± 0.4, n = 5) nor NO-flurbiprofen (1.1 ± 0.5, n = 5) had any affect on P-selectin expression 90 min after oral administration (Fig. 4).

DISCUSSION

Exposure of the duodenal mucosa to flurbiprofen caused a significant (350%) increase in 51Cr-EDTA clearance. Rat duodenum behaves in a similar manner to the jejunum, where we have previously shown that another NSAID (indomethacin, 1 mg/ml) applied to the lumen caused a progressive increase in both 51Cr-EDTA clearance and in histological injury (4). In the present study, there was no change in 51Cr-EDTA clearance across the duodenal mucosa in control animals over the 90-min period of exposure to the vehicle nor in animals exposed to NO-flurbiprofen.

Although we have not measured injury directly, we interpret the lack of change in mucosal permeability to 51Cr-EDTA with NO-flurbiprofen to indicate a lack of disruption to mucosal integrity.

Reduced mucosal toxicity of NO-NSAIDs was first established by Wallace and colleagues (33, 44, 45), who...
Reported effects of NSAIDs on gastrointestinal blood flow are varied, particularly in the stomach, where the findings of NSAID-induced reduction (6, 19, 26) or increase (11, 13, 29, 34) in blood flow may depend on factors such as route of drug administration, presence or absence of acid in the stomach, method of measurement of flow, and presence of hemorrhagic injury influencing measurement. In the small bowel, findings are more consistent with NSAIDs generally found to induce a reduction in flow. Indomethacin is known to reduce intestinal blood flow in cats and dogs (15, 17, 18, 30); however, in the rat, intestinal blood flow showed a modest increase 18 h after subcutaneous injection of indomethacin (8). Patients receiving oral NSAID therapy had reduced laser-Doppler signals from the duodenal mucosa compared with controls, suggesting an NSAID-induced reduction in human duodenal mucosal blood flow (38). The present finding that flurbiprofen reduced duodenal mucosal blood flow in the rat is consistent with these studies. However, the reduction in flow was modest (30%) and unlikely to cause mucosal injury per se over the 90-min period of the experiment. In the dog small intestine, for example, reduction in blood flow to <50% of control for 2 h was required to produce detectable mucosal injury (12).

In this study, flurbiprofen caused a fall in mucosal blood flow and an increase in vascular resistance without affecting blood pressure, suggesting a direct effect of the drug on the duodenal mucosal circulation. Forty minutes after the blood flow reduction was measured, blood pressure was also slightly but significantly reduced. The mechanism responsible for the reduced blood pressure remains uncertain. The fall in mucosal blood flow may be due to inhibition of synthesis of dilator prostaglandins in the duodenal mucosa. Although NO-flurbiprofen is known to be equally as effective in reducing prostaglandin synthesis as the parent drug (44), it did not reduce duodenal mucosal blood flow or increase vascular resistance. Mucosal blood flow remained unchanged over the 90-min period of exposure to NO-flurbiprofen. It is possible that the vasoconstriction induced by prostaglandin synthesis inhibition is offset by the dilator action of the NO moiety of the NO-flurbiprofen. NO might be donated from the NO-flurbiprofen within the tissue, but some cleavage may occur in the luminal solution. Takeuchi et al. (37) showed that instillation of NO-aspirin into the rat stomach resulted in significant intraluminal NO production. Furthermore, Keeble et al. (25) showed that NO-flurbiprofen relaxed blood vessels in vitro by an NO-dependent mechanism. The ability of NO-flurbiprofen to maintain blood flow may contribute to the nonulcerogenic nature of the drug.

Another mechanism by which NSAIDs may predispose the gastrointestinal tract to injury is by interfering with mucus formation. In the present study, exposure of the duodenal mucosa to flurbiprofen resulted in the inhibition of mucus production, whereas in control preparations and those exposed to NO-NSAID there was continual growth of the mucus layer. The greatest depth of mucus was consistently recorded in the pres-
ence of NO-flurbiprofen, and the mucus layer was significantly thicker than in control and flurbiprofen groups after 60-min exposure to the drug (Fig. 2, t = 80 min). NO has been shown to stimulate mucus production from gastric mucosal cells in vitro (10) and in the intact animal (9). It is likely that the ability of NO-flurbiprofen to maintain healthy mucus secretion is another factor contributing to its gastrointestinal sparing nature.

It is well known that NO modulated leukocyte adhesion to endothelium by altering the expression of adhesion molecules such as ICAM-1 and P-selectin (7, 16, 27). In the present study, flurbiprofen caused upregulation of ICAM-1, suggesting that ICAM-1-mediated leukocyte adhesion may contribute to flurbiprofen-induced injury. In a previous study (14), we showed that injury to the small bowel caused by another NSAID, indomethacin, was reduced by depletion of circulating neutrophils, again suggesting that leukocytes play a role in NSAID injury. NO-flurbiprofen did not increase ICAM-1 expression in our study. NO is known to inhibit the expression of the β2 adhesion molecules on neutrophils and P-selectin on the vascular endothelium (43), and this may contribute to the reduced ulcerogenicity of this drug.

It is surprising that flurbiprofen increased ICAM-1 without increasing P-selectin expression. Morise et al. (34) found rapid expression of P-selectin that peaked 60 min after exposure of the rat stomach to indomethacin, whereas ICAM-1 expression remained elevated for 3 h after exposure. It is possible that flurbiprofen caused P-selectin to increase at an earlier time point than the 90 min chosen in this study.

In conclusion, the present data indicate that duodenal mucosal blood flow and mucus production are reduced by flurbiprofen but were maintained or increased above control levels by NO-flurbiprofen. Furthermore, NO-flurbiprofen prevented the increase in adhesion molecule expression observed with flurbiprofen and did not increase duodenal mucosal permeability. It is likely that these mechanisms, together with other possible protective mechanisms associated with the NO form of the drug, such as its capacity to prevent the proapoptotic activity of flurbiprofen (23), all contribute to its reduced gastrointestinal toxicity.

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REFERENCES

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