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Mucosal acid causes gastric mucosal microcirculatory disturbance in nonsteroidal anti-inflammatory drug-treated rats

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Abstract

The mechanism by which nonsteroidal anti-inflammatory drugs (NSAIDs) suppress gastric mucosal blood flow is not fully understood, although the depletion of mucosal prostaglandin E_2 has been proposed as one possible explanation. We investigated the role of gastric acid on gastric mucosal blood flow in NSAID-treated rats. A rat stomach was mounted in an *ex vivo* chamber, and gastric mucosal blood flow was measured sequentially in a 5-mm^2 area of the gastric corpus using a scanning laser Doppler perfusion image system. Results showed that diclofenac (5 mg/kg s.c.) and indomethacin (10 mg/kg s.c.) did not affect gastric mucosal blood flow, although both strongly decreased mucosal prostaglandin E_2 when saline was instilled into the gastric chamber. On replacement of the saline in the chamber with 100 mM hydrochloric acid, these drugs caused a decrease in gastric mucosal blood flow levels within 30 min. The specific cyclooxygenase (COX)-2 inhibitors celecoxib (50 mg/kg s.c.) and rofecoxib (25 mg/kg s.c.) did not affect mucosal prostaglandin E_2 level, nor did they decrease gastric mucosal blood flow, even when hydrochloric acid was added to the chamber. Furthermore, measurement of vasoconstrictive factors present in the mucosa showed that endothelin-1 levels increased after administration of diclofenac s.c. in the presence of intragastric hydrochloric acid. This indicates that the presence of mucosal hydrochloric acid plays an important role in the NSAID-induced decrease in gastric mucosal blood flow, while the COX-1-derived basal prostaglandin E_2, which is unlikely to control gastric mucosal blood flow itself, protects microcirculatory systems from mucosal hydrochloric acid.

Keywords: Hydrochloric acid; Prostaglandin; Nonsteroidal anti-inflammatory drug-induced gastric ulcer; Gastric mucosal blood flow; Endothelin

1. Introduction

It is known that nonsteroidal anti-inflammatory drugs (NSAIDs) administration leads to a high incidence of inflammation and ulcers in the digestive tract, especially in the stomach. Furthermore, treatment with NSAIDs is suspended in 5% to 15% of patients with rheumatoid arthritis because of gastrointestinal disturbance (Awtry and Loscalzo, 2000; Singh et al., 1996). Therefore, it is considered important to prevent NSAIDs-induced gastric mucosal lesions to avoid any deterioration in quality of life.

A close relationship between NSAID-induced gastric mucosal lesions and gastric microcirculatory disturbance has been suggested in previous papers. Kawano et al. reported that administration of indomethacin to healthy subjects caused a marked reduction in the amount of hemoglobin in the gastric mucosa as well as oxygen saturation of the hemoglobin in the gastric mucosa immediately after administration, while erosive lesions and petechial hemorrhages developed in the ischemic mucosa (Kawano et al., 1996). These authors also suggested that the primary pathology of NSAID-induced acute gastric mucosal damage might be mucosal lesions caused by ischemia.

For the most part, NSAIDs-induced reduction in gastric mucosal blood flow and damage to the gastric mucosa seem to be caused by suppression of the production of mucosal prostaglandin E_2 due to an inhibition of cyclooxygenase (COX)-1. However, given that NSAIDs inhibited prostaglandin biosynthesis by more than 95% without causing gastric mucosal damage in rats (Ligumsky et al., 1983), and that NSAIDs do not always cause gastric mucosal damage even when administered

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at doses sufficient to inhibit prostaglandin biosynthesis in humans (Frezza et al., 2001), the mechanisms of NSAIDs-induced damage cannot be fully explained by the inhibition of prostaglandin E_2 biosynthesis alone. In addition, anti-secretory agents such as histamine H_2-receptor antagonists may slow the decrease in gastric mucosal blood flow caused by gastric mucosal damaging factors, such as NSAIDs and ethanol (Hata et al., 2005; Kontrurek et al., 1991; Miyata et al., 1991; Segawa et al., 1991). However, although some histamine H_2-receptor antagonists have been shown to be likely stimulants of prostaglandin E_2 production and to increase blood flow, much about this phenomenon remains unclear, as the these agents have no affinity for the COX enzymes and capsaicin receptors that are responsible for the increase of mucosal prostaglandin E_2 and nitric oxide (Nishihara et al., 2002).

Here, to examine the involvement of gastric acid in NSAID-induced decreases in gastric mucosal blood flow, we investigated the effects of 100 mM hydrochloric acid, nearly the concentration of gastric juice, applied to the surface of the gastric mucosa in NSAID-treated rats using a scanning laser Doppler perfusion image system that was able to measure the extensive blood flow in the gastric mucosa.

It is known that, when the mucosa is exposed to ethanol, which is a gastric mucosal damaging factor like gastric acid, vasoconstrictive factors, such as leukotrienes and endothelins, are produced, which in turn cause the gastric mucosal blood flow to decrease drastically (Kawano and Tsuji, 2000; Masuda et al., 1993; Peskar, 1991). For this reason, we also assessed the effect of gastric acid on the amount of vasoconstrictive factors present in the mucosa. Diclofenac and indomethacin were used as typical NSAIDs and celecoxib and rofecoxib as specific COX-2 inhibitors. The potential effect of the differences in COX selectivity among these agents on the effect of gastric acid on gastric mucosal blood flow was tested.

2. Materials and methods

2.1. Animals

Eight- to ten-week-old male Sprague-Dawley rats (Charles River Japan, Inc., Yokohama, Japan) were used. The animals were fasted for 18 h prior to the experiments. Water was supplied *ad libitum*. All animal experimental procedures were performed in accordance with the guidelines of the Animal Experiment Committee of Astellas Pharma Inc.

2.2. Drugs

Diclofenac sodium and indomethacin, typical non-specific COX inhibitors, were purchased from Sigma-Aldrich Japan (Tokyo, Japan) and dissolved in physiological saline (0.9% w/v sodium chloride) and 4% (w/v) $NaHCO_3$/saline, respectively. Celecoxib was provided by Pfizer Pharmaceuticals (New York, NY, USA). Rofecoxib was synthesized by Astellas Pharma Inc. (Ibaraki, Japan). These specific COX-2 inhibitors were dissolved in Polyethylene Glycol #400:saline (2:1) for use in the experiments. The vehicles used in this study were confirmed to have no influence on the amount of mucosal prostaglandin E_2 and gastric mucosal blood flow. All other chemicals were of reagent grade.

2.3. Determination of gastric mucosal blood flow using an ex vivo chamber system

Subcutaneously administered diclofenac (5 mg/kg), indomethacin (10 mg/kg), celecoxib (50 mg/kg), and rofecoxib (25 mg/kg) were used as test drugs in this study. Individual animals received one compound only. After anesthesia with urethane (1.25 g/kg i.p.), the animals underwent laparotomy, and the stomach body of each rat was mounted in an *ex vivo* chamber (Hirata et al., 1997), and instilled with physiological saline (2 ml) onto the mucosal surface by gentle bolus injection. The solution in the chamber was replaced at least every 30 min during the following study. Blood flow was then allowed to stabilize for at least 1.5 h. The surface of the gastric mucosa was then washed and refilled with saline, and gastric mucosal blood flow was recorded sequentially in a 5-mm^2 area in the lower part of the gastric corpus using a scanning laser Doppler perfusion image system (Peri Scan PIM KK, Perimed, Sweden) (Jakobsson and Nilsson, 1993). After the gastric mucosal blood flow was stabilized, a test drug was subcutaneously administered under the ventrolateral layer of the skin. Thirty minutes later, physiological saline at the mucosal surface was replaced with either 100 mM hydrochloric acid or physiological saline. The gastric mucosal blood flow was then measured for 30 min (acid application period). Subsequently, to determine the reaction after acid wash-out, physiological saline was instilled onto the mucosal surface and gastric mucosal blood flow was measured for a further 30 min. As a control, normal rats injected with physiological saline under the ventrolateral layer of the skin were examined using the same procedure. Gastric mucosal blood flow was determined as the percent change over time, with the value obtained immediately prior to drug injection taken as 100%.

2.4. Mucosal sampling

To investigate whether mucosal acid influences the quantities of mucosal vasoactive factors found *in vivo*, exogenous application of hydrochloric acid to the mucosa of pylorus-ligated rats was maintained over the treatment period. Fasted rats were subcutaneously injected with famotidine (3 mg/kg) to suppress endogenous acid production. Fifteen minutes later, the animals were subcutaneously injected with test drug, then subject to laparotomy under diethyl ether anesthesia and the pylorus was ligated. Thirty minutes after drug injection, either 100 mM hydrochloric acid or physiological saline (2 ml/kg) was administered by oral gavage using a gastric tube. The stomach was excised 1 h later, an incision was made along the greater curvature, and the gastrointestinal mucosal surface was washed lightly with saline solution. Mucosal samples from the gastric corpus were divided into three parts and immediately frozen in liquid nitrogen at −80 °C until the extraction of the vasoactive factors, as described below.

2.5. Mucosal prostaglandin E_2

The gastric mucosa tissue was cut finely, added to 1 mM indomethacin/ethanol (5 ml), and homogenized on ice. The suspension was then left to rest at 4 °C for 1 h to extract prostaglandin E_2 from the gastric tissue. After centrifuging the suspension (10,000 ×*g*, 20 min, 4 °C), 1 ml of the supernatant was collected and dried using an evaporator. The dried residue was dissolved in 500 μl of buffer solution to determine the concentration of prostaglandin E_2 using a commercially available prostaglandin E_2 measuring kit (Cayman Chemical). The amount of prostaglandin E_2 in the wet tissue was then calculated.

2.6. Extraction and measurement of mucosal endothelin-1 and the cysteinyl-leukotorienes

Endothelin-1 was extracted from the gastric mucosal tissue according to the method of Matsumoto et al. (1989) with a minor modification. In brief, the sample was homogenized in 10 (w/v) volumes of 1 M acetic acid −20 mM hydrochloric acid and immediately heated at 100 °C for 10 min to inactivate the proteases. The homogenate was then centrifuged at 25,400 ×*g* for 30 min. The supernatant was applied to an Oasis® HLB cartridge (Waters Corporation, Milford, MA, USA), and the absorbed peptide was eluted with 0.1% trifluoroacetic acid plus 60% acetonitrile in distilled water. The eluate was lyophilized to dryness and subjected to immunoassay using an endothelin-1 measuring kit (IBL Japan, Gunma, Japan). Cysteinyl-leukotorienes were also extracted from the tissue according to the manufacturer's protocol included in the Cysteinyl-leukotoriene measuring kit (Cayman Chemical, Ann Arbor, MI, USA),

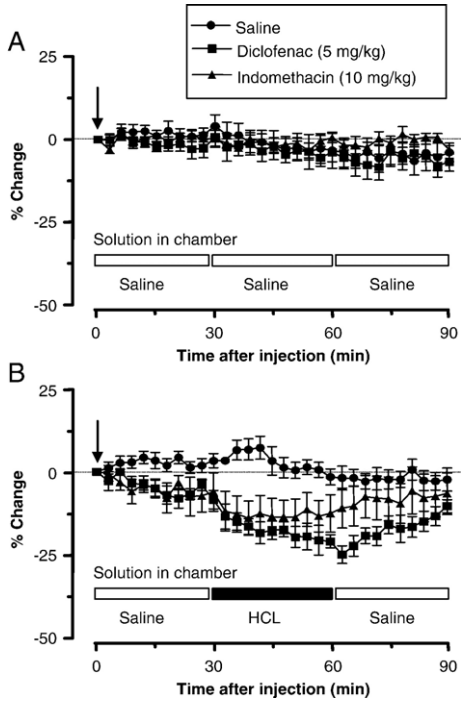

Fig. 1. Time course of changes in gastric mucosal blood flow after exposure of the mucosa to physiological saline (A) or 100 mM hydrochloric acid (HCL) solution (B) in saline-, diclofenac- or indomethacin-injected rats. Thirty minutes after drug injection, either saline or 100 mM hydrochloric acid was applied to the chambered stomach for 30 min (acid application period). Gastric mucosal blood flow was monitored before, during, and after the acid application period. Results are expressed as the mean±S.E.M. of five or six rats.

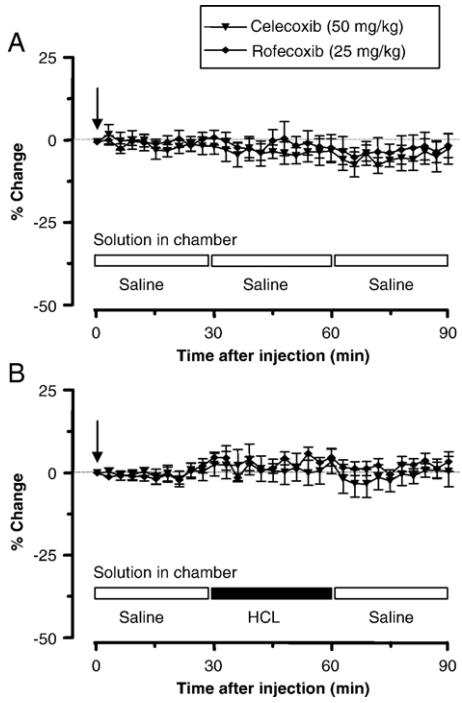

Fig. 2. Time course of changes in gastric mucosal blood flow after exposure of the mucosa to physiological saline (A) or 100 mM hydrochloric acid (HCL) solution (B) in celecoxib- or rofecoxib-injected rats. Thirty minutes after drug injection, either saline or 100 mM hydrochloric acid was applied to the chambered stomach for 30 min (acid application period). Gastric mucosal blood flow was monitored before, during, and after the acid application period. Results are expressed as the mean±S.E.M. of five or six rats.

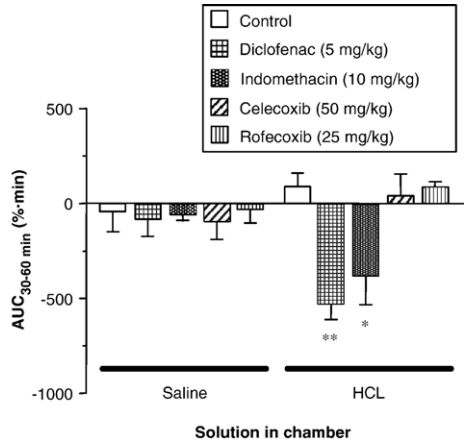

Fig. 3. Summary of changes in gastric mucosal blood flow after exposure of the mucosa to 100 mM of hydrochloric acid (HCL) solution in saline-injected control, or diclofenac-, indomethacin-, celecoxib- or rofecoxib-injected rats. The area under the time-percent change in mucosal blood flow curve (AUC) was calculated for the time during the acid application period using the trapezoidal rule. Results are expressed as the mean±S.E.M. of five or six rats. *$P<0.05$ and **$P<0.01$ versus each intragastric saline group using Student's *t*-test.

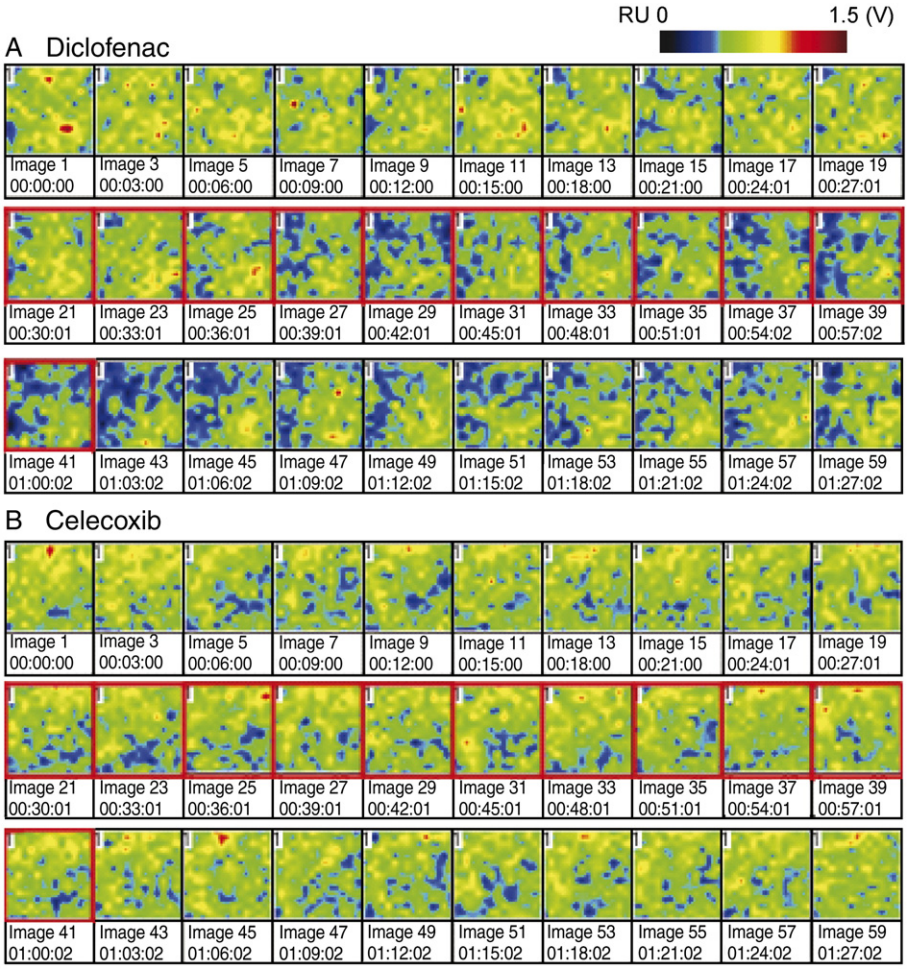

Fig. 4. Representative laser Doppler perfusion images of gastric mucosal blood flow in diclofenac or celecoxib-injected rats. In the color-coded images, normal perfusion is depicted in green and marked blood flow reductions are indicated in blue. Time values under the images indicate time after drug injection. Images 21 to 41, which are outlined in red, were recorded during the acid application period. RU (V) indicates relative units.

except that an Oasis® HLB cartridge was used instead of a C-18 SPE cartridge. Antibody in the kit was 100% cross-reactive to leukotoriene C_4 and D_4.

2.7. Statistical analysis

Results are expressed as the mean±S.E.M. The area under the curve (AUC) of percent changes in gastric mucosal blood flow (%min) during the acid application period was calculated using the trapezoidal rule. Statistical differences between the groups were analyzed using either Student's *t*-test (two-tailed) or Dunnett's multiple range test. A difference of $P<0.05$ was considered significant.

3. Results

3.1. Changes in gastric mucosal blood flow of rats injected with NSAIDs

The time course of changes in blood flow in rats that received saline, diclofenac or indomethacin by subcutaneous injection is shown in Fig. 1. Changes associated with the presence or absence of hydrochloric acid during the acid application period are compared in Fig. 3.

When physiological saline was filled in the chamber, the gastric mucosal blood flow was maintained at a nearly constant

Table 1
Changes in gastric mucosal prostaglandin E_2 levels after exposure of the mucosa to 100 mM hydrochloric acid solution in saline-injected control, and diclofenac-, indomethacin-, celecoxib- or rofecoxib-injected rats

Intragastric solution	Control	Diclofenac (5 mg/kg)	Indomethacin (10 mg/kg)	Celecoxib (50 mg/kg)	Rofecoxib (25 mg/kg)
Prostaglandin E_2 (ng/g wet tissue)					
Saline	164±40	40±5[a]	n.t.	168±28	n.t.
HCL	173±28	38±6[b]	6±1[b]	182±27	152±18

Fasted rats were subcutaneously injected with a test drug, and then their pylori were ligated. Thirty minutes after drug injection, 100 mM of either hydrochloric acid (HCL) or physiological saline was administered orally. Gastric mucosal samples were collected 1 h later and immediately frozen in liquid nitrogen. All samples were assayed using competition ELISA. Results are expressed as the mean±S.E.M. of five to eight rats. [a]$P<0.05$ and [b]$P<0.01$ versus each control group using Dunnett's test. n.t.: not tested.

level throughout the 90-min measurement period regardless of which drug was injected (Fig. 1A). Blood flow momentarily increased in control rats when 100 mM hydrochloric acid was applied to the gastric mucosal surface, although the change was not significant (Figs. 1B and 3).

In diclofenac (5 mg/kg s.c.)-treated rats, in contrast, gastric mucosal blood flow gradually decreased when 100 mM hydrochloric acid was applied to the mucosal surface (Fig. 1B). The changes seen during the acid application period were significantly greater than those in the saline-injected control group ($P<0.01$, Fig. 3). Blood flow was gradually restored to near-basal levels when the hydrochloric acid in the gastric chamber was replaced with physiological saline (Fig. 1B).

In indomethacin (10 mg/kg s.c.)-treated rats, the gastric mucosal blood flow also gradually decreased when 100 mM hydrochloric acid was applied to the mucosal surface (Fig. 1B). As with diclofenac, the changes during the acid application period were significantly greater than those in the saline-injected control group ($P<0.05$, Fig. 3).

3.2. Changes in the gastric mucosal blood flow of rats injected with specific COX-2 inhibitors

In celecoxib (50 mg/kg s.c.)-treated rats, gastric mucosal blood flow was maintained at a nearly constant level throughout the measurement period when the chamber was filled with saline (Fig. 2A). In contrast to the results for NSAIDs, no significant changes in the gastric mucosal blood flow were detected, even when 100 mM hydrochloric acid was applied (Figs. 2B and 3). Similar results were obtained with rofecoxib at a dose of 25 mg/kg.

3.3. Laser Doppler perfusion images in rats injected with diclofenac and celecoxib

Fig. 4 shows representative computer-generated color graphic images of the distribution of gastric mucosal blood flow in the lower part of the gastric corpus before and after the acid application period in rats injected with diclofenac or celecoxib. Regardless of the drug injected, color pattern did not change during the first 30 min that the chamber contained physiological saline, indicating that the gastric mucosal blood flow was stable. In the diclofenac-injected rats, however, application of 100 mM hydrochloric acid to the chamber resulted in an area of low blood flow area (blue) spread topically along the longitudinal axis of the gastric corpus (Fig. 4A). Furthermore, subsequent replacement of the 100 mM hydrochloric acid with physiological saline resulted in a return of blood flow in the reduced blood flow area to the basal flow level (green). In contrast to these results with diclofenac, almost no change in color pattern was seen in celecoxib-injected rats during or after the acid application period (Fig. 4B).

Table 2
Changes in gastric mucosal vasoactive factors after exposure of the mucosa to 100 mM hydrochloric acid solution in saline-injected control, and diclofenac- or celecoxib-injected rats

Intragastric solution	Control	Diclofenac (5 mg/kg)	Celecoxib (50 mg/kg)
Endothelin-1 (pg/g wet tissue)			
Saline	453±71	630±51	534±66
HCL	592±39	1004±108[a]	558±47
Cysteinyl-leukotorienes (ng/g wet tissue)			
Saline	2.1±0.4	2.3±0.4	1.9±0.3
HCL	2.5±0.9	2.9±0.7	2.3±0.3

Fasted rats were subcutaneously injected with a test drug, and then their pylori were ligated. Thirty minutes after the drug injection, 100 mM of either hydrochloric acid (HCL) or physiological saline was administered orally. Gastric mucosal samples were collected 1 h later, divided into two or three parts, and immediately frozen in liquid nitrogen. All samples were assayed using competition ELISA. Results are expressed as the mean±S.E.M. of six to eight rats. [a]$P<0.01$ versus each control group using Dunnett's test.

3.4. Changes in gastric mucosal prostaglandin E_2 in pylorus-ligated rats

Gastric mucosal prostaglandin E_2 levels in the saline-injected control group 1 h after intragastric administration of saline or 100 mM hydrochloric acid were 164±40 and 173±28 ng/g wet tissue, respectively. The difference between these values was not significant (Table 1). Diclofenac (5 mg/kg s.c.) and indomethacin (10 mg/kg s.c.) significantly decreased the amount of mucosal prostaglandin E_2 compared with the saline-injected control groups. In contrast, celecoxib (50 mg/kg s.c.) and rofecoxib (25 mg/kg s.c.) had no effect on the amount of mucosal prostaglandin E_2.

3.5. Changes in gastric mucosal vasoactive factors in pylorus-ligated rats

Endothelin-1 levels in the gastric mucosa of the saline-injected control group 1 h after intragastric administration of saline or 100 mM hydrochloric acid were 453±71 and 592±39 pg/g wet tissue, respectively. The difference between these values was not significant (Table 2). Diclofenac increased the amount of mucosal endothelin-1 to a significant degree only when intragastric acid was present. Under these conditions, the amount of mucosal endothelin-1 was 1,004±108 pg/g wet tissue, which was significantly greater (>70%, $P<0.01$) than that in the intragastric hydrochloric acid control group. Celecoxib did not affect the amount of mucosal endothelin-1, even after intragastric administration of 100 mM hydrochloric acid. In contrast, no significant change in the amount of cysteinyl-leukotorienes could be detected in the gastric mucosa regardless of the type of drug or intragastric solution used (Table 2).

4. Discussion

We examined the involvement of mucosal acid in the NSAID-induced decrease in gastric mucosal blood flow by applying acid to the gastric mucosal surface of rats pretreated with typical NSAID or specific COX-2 inhibitors. Our findings indicated that the key factor in NSAID-induced gastric mucosal microcirculatory disturbance is gastric acid itself, and not

prostaglandin E_2. This disturbance did not occur in the absence of intragastric acid, even when mucosal prostaglandin E_2 was depressed by NSAID injection.

It has been reported that the NSAID-induced decrease in gastric mucosal blood flow in humans is notable around the gastric antrum. NSAID-induced gastric mucosal lesions frequently develop in this area as well (Taha et al., 1993). Accordingly, the primary pathology of NSAID-induced acute gastric mucosal damage is likely to be mucosal lesions due to ischemia. The main mechanism by which NSAIDs reduce gastric mucosal blood flow is thought to be their inhibition of COX-1. This inhibition leads to a deficiency in endogenous prostaglandin E_2, which in turn causes vasodilation resulting in a microcirculatory disturbance in the gastric mucosa (Shorrock and Rees, 1989). However, recent clinical and non-clinical studies have demonstrated that the mechanism of the NSAID-induced disturbance can not be fully explained by the inhibition of prostaglandin biosynthesis alone (Frezza et al., 2001; Ligumsky et al., 1983; Shorrock and Rees, 1989).

Here, gastric mucosal blood flow showed almost no change on subcutaneous injection of high doses of the nonselective NSAIDs diclofenac and indomethacin in anesthetized rats. This was true even when the amount of prostaglandin E_2 had been reduced by approximately more than 80% and mucosal acid had been washed off the mucosal surface (Fig. 1A). However, on application of hydrochloric acid at 100 mM, an acidity level almost equivalent to that of gastric juice, to the mucosal surface following the injection of NSAIDs, gastric mucosal blood flow decreased significantly (Fig. 1B). This finding suggests that acid on the gastric mucosal surface is likely involved in the NSAIDs-induced decrease in gastric mucosal blood flow. Furthermore, animal and human studies using a video endoscope-equipped computer graphics system have shown that the injured area corresponds to the area most likely to experience a decrease in gastric mucosal blood flow (Kawano and Tsuji, 2000). In this study, a scanning laser Doppler perfusion image system revealed that acid-induced areas of decreased blood flow in the mucosa of the stomach also varied. Areas of acid-induced decreases in gastric mucosal blood flow were oriented longitudinally along the gastric corpus (Fig. 4A). This finding agreed well with those of a previous study in which linear ulcers developed in a similar fashion in NSAID-injected rats (Keto et al., 2003).

In contrast to these findings, it is known that mild irritants such as high concentrations of hydrochloric acid increase gastric mucosal blood flow (Pique et al., 1988; Takeuchi et al., 1994). In this study also, gastric mucosal blood flow tended to increase when 100 mM of hydrochloric acid was applied to the mucosal surface in saline-injected control rats (Fig. 1B). A number of studies on the mechanism of mild irritant-induced blood flow increases have suggested the involvement of a nitrous oxide synthesizing enzyme (iNOS)-stimulating action (Phillipson et al., 2003) and an endogenous prostaglandin E_2-increasing action (Kato et al., 1993; Takeuchi et al., 1994). However, given that exposure of the stomach to a mild irritant produced a marked 80-fold increase in mucosal prostaglandin E_2 production but only a 2-fold increase in gastric mucosal blood flow (Hirata et al., 1997), it is unlikely that the basal concentration of mucosal prostaglandin E_2 contributes to the control of gastric mucosal blood flow, even if its production is strongly inhibited by NSAIDs. However, prostaglandin E_2 in the basal mucosa is indispensable to gastric mucosal defense and to the prevention of the adverse influence of back-diffused acid. Furthermore, prostaglandin E_2 stimulates bicarbonate secretion and increases mucus gel thickness, thus enhancing the integrity of the mucus barrier (Shorrock and Rees, 1989; Takezono et al., 2004). Consistent with these results, the specific COX-2 inhibitors celecoxib and rofecoxib, which unlike diclofenac and indomethacin did not affect the amount of basal prostaglandin E_2, had no effect on gastric mucosal blood flow even when hydrochloric acid was added to the chamber (Fig. 2B). Taken together, the present and previous findings support the hypothesis that gastric acid is the key factor in NSAID-induced gastric mucosal microcirculatory disturbance, and that COX-1-induced prostaglandin E_2 protects against this disturbance by preventing the acid reaction to microcirculatory systems, and not via any direct vasodilatory effect on the submucosal veins.

The mechanism of the acid-induced decreases in gastric mucosal blood flow following NSAID injection observed in this study are not well understood. However, it is known that the degree of gastric mucosal blood flow is markedly reduced when vasoconstrictive factors, such as leukotrienes and endothelins, are produced by exposing the submucosal microcirculatory system to ethanol (Kawano and Tsuji, 2000; Masuda et al., 1993; Peskar, 1991). A similar acute reaction may be caused by the back-diffusion of intragastric acid when the mucosal protective response is suppressed by NSAIDs. In fact, we found that intragastric administration of 100 mM hydrochloric acid along with diclofenac injection increased the amount of mucosal endothelin-1 (Table 2). Further studies are required to determine whether the increase in the amount of endothelin-1 in the mucosa was derived from the direct effect of acid on the submucosal microcirculatory system or if it was a secondary effect caused by acid-induced gastric damage. The second possibility is less likely because the decrease in gastric mucosal blood flow induced by 100 mM hydrochloric acid appeared to be reversible (Fig. 1B). Consequently, the acid-induced decrease in gastric mucosal blood flow is likely attributable to mechanisms able to cause a blood flow disturbance within a relatively short time, such as through a microcirculation modifier like endothelin-1, rather than to irreversible tissue damage to the capillary system.

It has been reported that anti-secretory agents such as histamine H_2-receptor antagonists ameliorate the decrease in gastric mucosal blood flow caused by factors that disturb the gastric mucosa, such as NSAIDs and ethanol (Hata et al., 2005; Kontrurek et al., 1991; Miyata et al., 1991; Segawa et al., 1991). Although some histamine H_2-receptor antagonists have been shown to be likely stimulants of prostaglandin E_2 production and to increase blood flow (Kontrurek et al., 1991; Nishihara et al., 2002; Someya et al., 2003), much about this phenomenon remains unclear, as these agents have no affinity for COX enzymes or capsaicin receptors (Nishihara et al., 2002). The results of this study suggest the likelihood that the acid

suppressed gastric mucosal blood flow. This in turn suggests the possibility that anti-secretory agents can ameliorate a decrease in gastric mucosal blood flow through their inherent acid-reducing effect.

Part of this study included the use of 100 mM hydrochloric acid to estimate the effect of mucosal gastric acid on the gastric mucosal blood flow when mucosal prostaglandin is depleted. However, there is no evidence that the exogenous application of acid after saline reflects physiological conditions, in which blood flow within the gastrointestinal tract is also influenced by extrinsic neurohumoral factors that are responsible for gastric acid secretion. This difference may be a limitation of this study.

In conclusion, these results clearly demonstrate that the presence of mucosal hydrochloric acid plays an important role in the NSAID-induced decrease in gastric mucosal blood flow. This acid-induced disturbance in blood flow occurs within a relatively short time, such as via a microcirculation modifier like endothelin-1, rather than via any irreversible tissue damage to the capillary system. COX-1-derivered basal prostaglandin E_2 does not appear to control gastric mucosal blood flow itself, but prevents NSAID-induced decreases in gastric mucosal blood flow by preventing acid-induced mucosal microcirculatory disturbance.

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