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CYCLOOXYGENASE-2 SELECTIVE AND NITRIC OXIDE-RELEASING NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND GASTRIC MUCOSAL RESPONSES

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Occurrence of gastrointestinal damage and delayed healing of pre-existing ulcer are commonly observed in association with clinical use of nonsteroidal antiinflammatory drugs (NSAIDs). We examined the effects of NS-398, the cyclooxygenase (COX)-2 selective inhibitor, and nitric oxide (NO)- releasing aspirin (NCX-4016) on gastric mucosal ulcerogenic and healing responses in experimental animals, in comparison with those of nonselective COX inhibitors such as indomethacin and aspirin. Indomethacin and aspirin given orally were ulcerogenic by themselves in rat stomachs, while either NS-398 or NCX-4016 was not ulcerogenic at the doses which exert the equipotent antiinflammatory action with indomethacin or aspirin. Among these NSAIDs, only NCX-4016 showed a dose-dependent protection against gastric lesions induced by HCl/ethanol in rats. On the other hand, the healing of gastric ulcers induced in mice by thermal-cauterization was significantly delayed by repeated administration of these NSAIDs for more than 7 days, except NCX-4016. Gastric mucosal prostaglandin contents were reduced by indomethacin, aspirin and NCX-4016 in both normal and ulcerated mucosa, while NS-398 significantly decreased prostaglandin generation only in the ulcerated mucosa. Oral administration of NCX-4016 in pylorus-ligated rats and mice increased the levels of NO metabolites in the gastric contents. In addition, both NS-398 and NCX-4016 showed an equipotent anti-inflammatory effect against carrageenan-induced paw edema in rats as compared with indomethacin and aspirin. These results suggest that both indomethacin and aspirin are ulcerogenic by themselves and impair the healing of pre-existing gastric ulcers as well. The former action is due to inhibition of COX-1, while the latter effect may be accounted for by inhibition of COX-2 and mimicked by NS-398, the COX-2 selective NSAID. NCX-4016, despite inhibiting both COX-1 and COX-2, protects the stomach against damage and preserves the healing response of gastric ulcers, probably because of the beneficial action of NO.

Key words: NSAID, COX-2 selective inhibitor, NO-releasing aspirin, gastric mucosa, lesion, healing

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INTRODUCTION

Nonsteroidal antiinflammatry drugs (NSAIDs) are among the most frequently used drugs world wide. The major limitation to their use is gastrointestinal side effects, including the formation of gastric lesions, the potentiation of ulcerogenic response to stress, and interference with the healing of pre-existing gastric ulcers (1—4). A number of strategies have been recently used to develop new NSAIDs that spare the gastrointestinal tract. One of them is to develop NSAIDs that only inhibit the inducible isoform of cyclooxygenase (COX)-2, thereby exerting anti-inflammatory activity but sparing gastrointestinal prostaglandin (PG) synthesis (5—7), because PGs derived from COX-1 are believed to be responsible for maintaining housekeeping function and mucosal integrity of the stomach (8, 9). Another strategy for developing gastrointestinal-sparing NSAIDs is the coupling of a nitric oxide (NO)-releasing moiety to standard NSAIDs (10—12). The rational behing this strategy is that the NO released from these derivatives will exert beneficial effects on the gastric mucosa by enhancing the mucosal defensive ability (13). Although these approaches have been successfully demonstrated to lessen the incidence of damage in the gastrointestinal tract (10—12), only a few studies deal with their effects on healing of pre-existing ulcers (14, 15).

In the present study, we examined the effects of NS-398, the COX-2 selective NSAID, and NO-releasing aspirin derivative (NCX-4016) on various aspects of gastric mucosal ulcerogenic response, including the mucosal irritating action in the presence or absence of other ulcerogenic stimuli in rats as well as the influence on healing of chronic gastric ulcers in mice, and compared those with the effects of nonselective COX inhibitors such as indomethacin and aspirin.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing $200 \sim 230$ g (Charles River, GS, Yokohama, Japan), and male ddy mice, weighing $30 \sim 35$ g (SLC, Kyoto, Japan), were used in all experiments. The animals were kept in individual cages with raised mesh bottoms and deprived of food but allowed free access to tap water for 18 hr prior to the experiments. Studies were carried out using $5 \sim 7$ animals under unanesthetized conditions, unless otherwise specified.

Induction of Gastric Lesions

The effects of various NSAIDs on gastric ulcerogenic responses were examined in the following four sets (A~D) of studies; A: the ulcerogenic effects on the gastric mucosa; B: the effect on

stress-induced gastric lesions; C: the effects on HCl/ethanol-induced gastric lesions; D: the effects on the healing of chronic gastric ulcers.

Study A: The rats were given indomethacin (5~30 mg/kg), NS-398 (10~30 mg/kg), aspirin (20~100 mg/kg) or NCX-4016 (38~190 mg/kg) orally, and killed 4 hr later. Then, the stomachs were removed, inflated by injecting 10 ml of 2% formalin for 10 min to fix the tissue walls, and opened along the greater curvature of the stomach. The area (mm^2) of hemorrhagic lesions developed in the stomach was measured under a dissecting microscope with a square grid ($\times 10$), summed per stomach, and used as a lesion score. This procedure was applied to Studies B, C and D.

Study B: Under urethane anesthetized conditions (1.25 g/kg, i.p.), the rats were exposed to cold without any surgical manipulation. In brief, an animal was placed in a styrofoam box, and the body temperature was lowered to 28~30°C with a refrigerant pack (16). Rectal temperature was continuously monitored with a rectal thermometer (Nihon Koden, MGA-3, Tokyo, Japan). At the end of 4-hr experiments, the stomaches were removed, and the area (mm^2) of each of the lesions that developed in the glandular mucosa was measured under a dissecting microscope. Indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) or NCX-4016 (38 mg/kg) was administered s.c. 30 min before the onset of hypothermia.

Study C: The rats were given 1 ml of HCl/ethanol (60% ethanol plus 10 mM HCl) orally through esophageal intubation. One hour later, the animals were killed under deep ether anesthesia, the stomachs removed, treated with formalin, and gastric lesions were measured. Indomethacin (5~10 mg/kg), NS~398 (10~30 mg/kg), aspirin (20~100 mg/kg) or NCX-4016 (38~190 mg/kg) was administered p.o. 30 min before HCl/ethanol treatment. In some cases, the effect of FK409 (the NO donor) (17) on HCl/ethanol-induced gastric lesions was examined. FK409 (1 mg/kg) was given p.o. 30 min before administration of HCl/ethanol.

Study D: Chronic gastric ulcers were induced in mice by thermal cauterization, according to a method described previously (18). Under ether anesthesia, the stomach was exposed through a midline incision, the electric probe (Fuchigami, Kyoto, Japan: diameter, 4 mm^2) was attached to the mid-corpus mucosa, and a gastric ulcer was induced by heating the tip at 70°C for 15 sec. The animals were killed on various days (3, 7, 10, and 14) after the operation, and the stomachs removed, treated with formalin, and opened along the greater curvature. The area (square millimeters) and depth (millimeters) of ulcer crater were measured under a dissecting microscope ($\times 20$), and the ulcer score (cubic millimeters) was calculated as the product of the area and depth of the ulcer crater. Indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) or NCX-4016 (38 mg/kg) was administered s.c. once daily for 7 days, starting from three days after ulcer induction. Control animals received the vehicle alone.

Formation of Paw Edema by Carrageenan

Paw edema was induced in unanesthetized rats by subplantar injection of carrageenan (0.1 ml of 1% carrageenan-saline solution) into the right hind paw (19). Paw volume was measured using a plethysmometer, immediately before and every 2 hr for 6 hr after the injection of carrageenan. Edema was expressed as the increase in paw volume (Δml) after carrageenan injection relative to the pre-injection value for each animal. Indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) or NCX-4016 (38 mg/kg) was given s.c. 30 min before carrageenan injection.

Determination of Prostaglandin E_2

The effects of various NSAIDs on PGE$_2$ contents in both the intact and ulcerated mucosa of the mouse stomach were determined. Gastric ulcers were induced by thermal-cauterization (70°C for 15 sec), as described above. Indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) or

NCX-4016 (38 mg/kg) was administered s.c. once daily for 5 days, starting 3 days after ulcer induction, and the animals killed 2 hr after final administration. The stomachs were removed, and the corpus mucosa of both the intact and ulcerated portion were excised, weighed, and put in a tube containing 100% ethanol plus 0.1 M indomethacin (20). Then, the samples were minced with scissors, homogenized, and centrifuged for 10 min at 12 000 r.p.m. at 4°C. The supernatant of each sample was used for determination of PGE_2 by ELISA using PGE_2-kit (Cayman Chemical Co., Ann Arbour, MI, USA).

Measurement of NOx in Gastric Contents

No_x levels were determined in the gastric contents of pylorus-ligated rats and mice after s.c. administration of aspirin and NCX-4016. Under ether anesthesia, the abdomen was incised, and the pylorus was ligated. Two hours after the ligation, the gastric contents were recovered. Samples were centrifuged for 15 min at 3000 r.p.m. and stored at $-80°C$ until the assay. No_x was measured in aliquots of the samples by the Griess method after reduction of nitrate to nitrite with reductase (from Aspergillus; Sigma). Nitrites were incubated with Griess reagent (0.1% naphthylene diamine dihydrochloride and 1% sulfanilamide in 2.5% H_3PO_4) for 10 min at room temperature, and the absorbance at 550 nm was measured (21). Aspirin (20 mg/kg) or NCX-4016 (38 mg/kg) was given s.c. immediately after the pylorus ligation.

Preparation of Drugs

Drugs used in this study were urethane (Tokyo Kasei, Tokyo, Japan), indomethacin, aspirin (Sigma Chemical, St. Louis, Montana, USA), NS-398 (Taisho Pharmaceutical Co. Ltd., Tokyo, Japan), and NO-releasing aspirin (NCX-4016; NiCox, Paris, France). Indomethacin, NS-398, aspirin or NCX-4016 was suspended in 1% carboxymethylcelluloe solution (CMC), while othe drugs were dissolved in saline. Each agent was prepared immediately before use and administered i.p., p.o. or s.c. in a volume of 0.5 ml per 100 g body weight in rats, or 0.25 ml per 100 g body weight in mice.

Statistics

Data are presented as the means $\pm$ SE from $5 \sim 7$ rats or mice per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of $P < 0.05$ were regarded as significant.

RESULTS

Effects of Various NSAIDs on Gastric Mucosa

Oral administration of indomethacin ($5 \sim 30$ mg/kg) caused hemorrhagic damage in the gastric mucosa, in a dose-dependent manner, and the lesion score at highest dose was 14.5 ± 2.1 mm^2 (*Fig. 1*). Aspirin given p.o. did not cause any damage in the stomach at lower doses (20 and 60 mg/kg), but produced

Fig. 1. Mucosal ulcerogenic effects of indomethacin (5～30 mg/kg), NS-398 (10～30 mg/kg), aspirin (20～100 mg/kg) and NCX-4016 (38～190 mg/kg) in the rat stomach. Each drug was administered orally, and the animals were killed 4 hr later. Data are presented as the means ± SE from 5～8 rats. Statistically significant difference at P < 0.05; * from control.
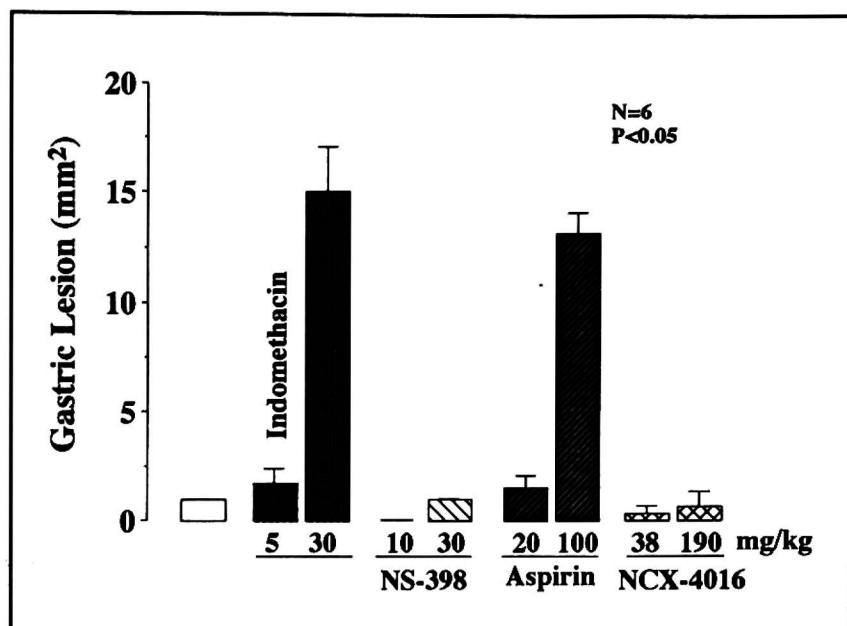
hemorrhagic lesions at 100 mg/kg, the lesion score being 12.8 ± 0.9 mm^2. However, neither NS-398 (10～30 mg/kg) nor NCX-4016 (38～190 mg/kg) produced gross damage in the stomach at any doses used in the present study.

Effects of Various NSAIDs on Gastric Ulcerogenic Response to Stress

Lowering of the body temperature (28～30°C) for 4 hr caused hemorrhagic damage in the stomach, the lesion score being 18.4 ± 4.1 mm^2 (Fig. 2). Pretreament of the rats with indomethacin (2 mg/kg, s.c.) or aspirin (20 mg/kg, s.c.) significantly worsened the severity of gastric lesions, and the lesions score was 43.6 ± 9.5 mm^2 or 39.3 ± 7.5 mm^2, respectively. On the other hand, prior administration of NS-398 (10 mg/kg, s.c.) did not significantly affect the
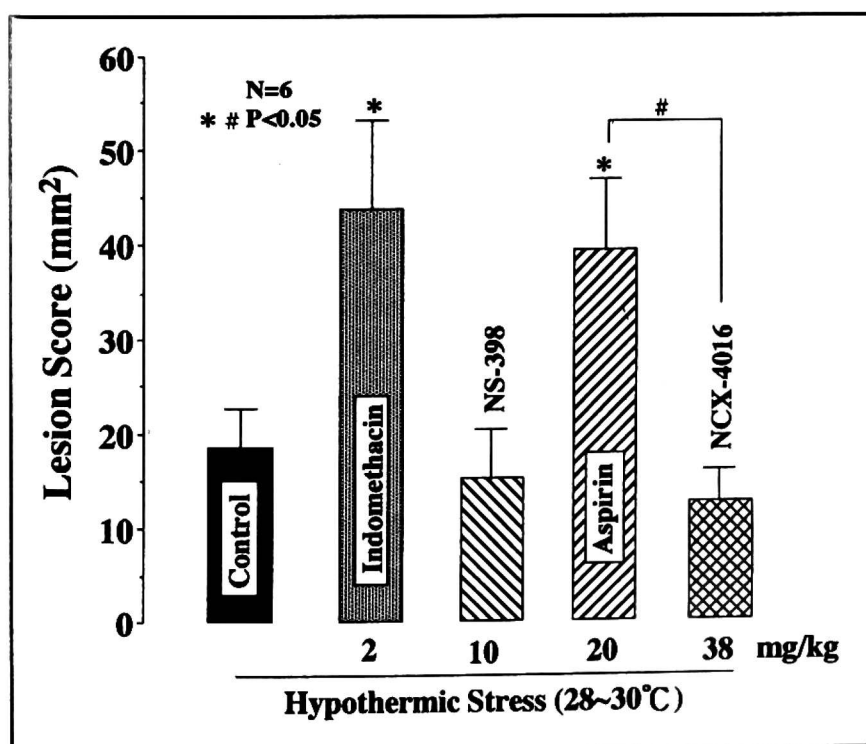

Fig. 2. Effects of indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) and NCX-4016 (38 mg/kg) on gastric ulcerogenic response induced by hypothermic stress in anesthetized rats. The animals were placed in a styrene foam box, and the body temperature was maintained between 28～30°C for 4 hr by exposing the animals to a refrigerant pack. Each drug was given s.c. 1 hr before the onset of hypothermic stress. Data are presented as the means ± SE from 6 rats. Statistically significant difference ar P < 0.05; *from control; #from aspirin alone.

ulcerogenic response to stress; the lesion score being 15.2 ± 5.3 mm^2. Likewise, NCX-4016 (38 mg/kg, s.c.) had no effect on gastric ulcerogenic response to hypothermic stress, and the lesion score was 12.6 ± 3.4 mm^2, which was significantly less than that observed in the presence of aspirin.

Effects of Various NSAIDs on Ethanol-Induced gastric Lesions

Intragastric administration of HCl/ethanol (60% ethanol plus 150 mM HCl) caused multiple band-like lesions in the gastric mucosa, the lesion score being 128.2 ± 19.6 mm^2. The severity of these lesions was dose-dependently reduced by prior p.o. administration of NCX-4016 (38 ~ 190 mg/kg), and a significant effect was observed at 38 mg/kg or greater, the inhibition at 137 mg/kg being 59.3% (*Fig. 3*). Although indomethacin (5 ~ 10 mg/kg) and NS-398 (10 ~ 30 mg/kg) did not have any effects on the development of gastric lesions in response to HCl/ethanol, aspirin given p.o. 30 min before ethanol significantly reduced the severity of gastric lesions only at the highest dose100 mg/kg. On the other hand, gastric ulcerogenic response induced by HCl/ethanol was potently prevented by p.o. administration FK409 (1 mg/kg), the NO donor, the inhibition being 91.2%.
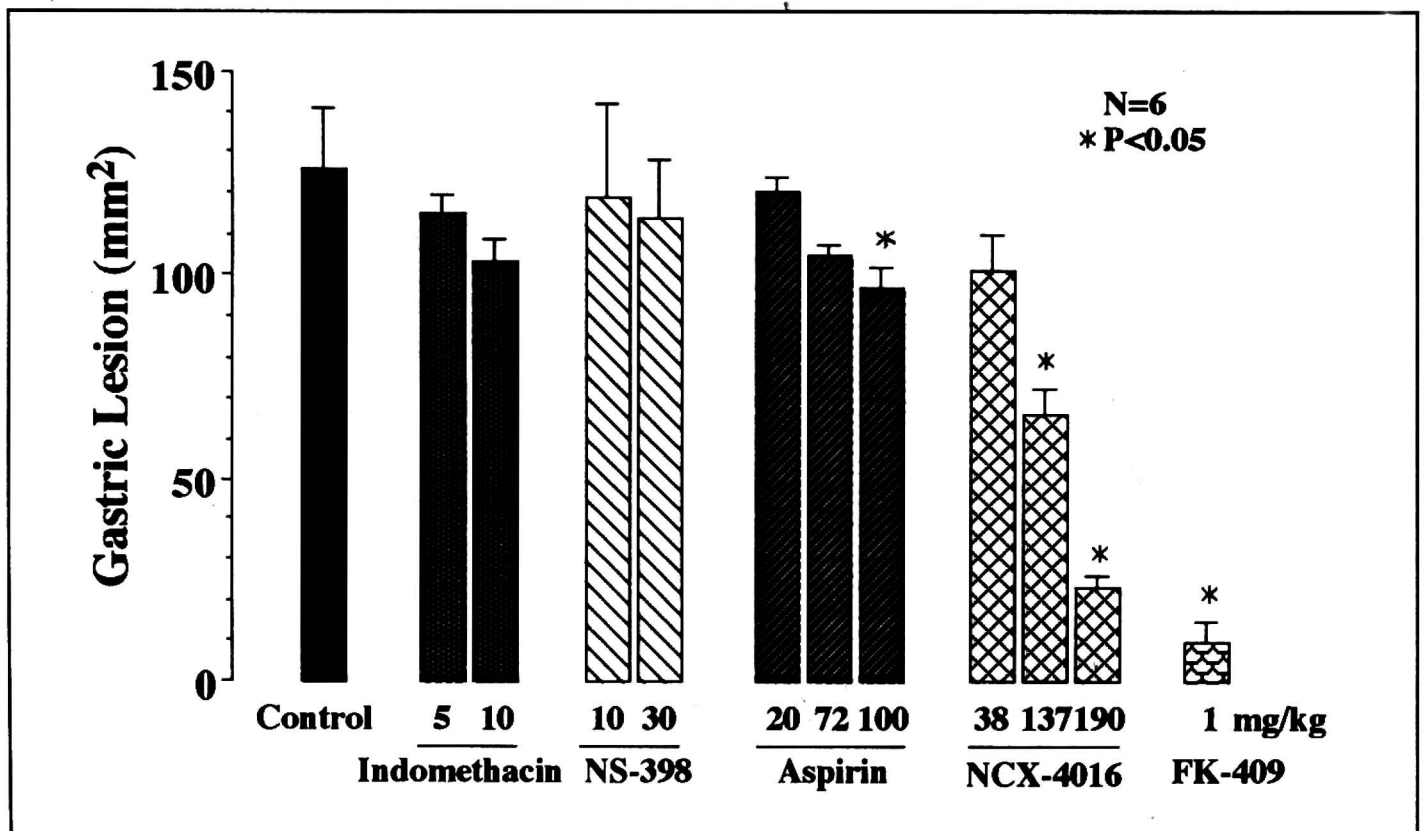

Fig. 3. Effects of indomethacin, NS-398, aspirin and NCX-4016 on HCl/ethanol-induced gastric lesions in rats. The animals were administered with 1 ml of HCl/ethanol (60% in 150 mM HCl), and killed 1 hr later. Indomethacin (5 ~ 10 mg/kg), NS-398 (10 ~ 30 mg/kg),.. aspirin (20 ~ 100 mg/kg), NCX-4016 (38 ~ 190 mg/kg) or FK409 (1 mg/kg) was administred p.o. 30 min before HCl/ethanol treatment. Data are presented as the means $\pm$ SE from 5 ~ 7 rats. *Statistically significant difference from controls, at $P < 0.05$.

Effects of Various NSAIDs on Healing of Gastric Ulcers

Three days after subjecting the mouse stomach to thermal-cauterization (70°C, 15 sec), a welldefined ulcer developed in the corpus mucosa, the ulcer score being 27.1 ± 1.8 mm^3. The ulcers healed gradually within 14 days, and the ulcer score on day 7, 10 and 14 days was 12.5 ± 3.3 mm^3, 4.1 ± 1.0 mm^3 and 2.2 ± 0.2 mm^3, respectively. The healing of the ulcers was markedly impaired when the animals were given indomethacin (2 mg/kg, s.c.) once daily for 7 days starting from 3 days after ulcer induction; the ulcer score observed on day 10 was 17.4 ± 1.7 mm^3, which was significantly greater than that of control (12.5 ± 3.5 mm^3) (*Fig. 4*). Significantly delayed healing was also observed on day 10 (25.4 ± 5.0 mm^3) in the animals treated with s.c. administration of asipirin (20 mg/kg). Likewise, the repeated s.c. administration of NS-398 (10 mg/kg) delayed the healing of gastric ulcers; the ulcer score was 24.8 ± 2.8 mm^3. On the other hand, administration of NCX-4016 (38 mg/kg, s.c.) for 7 days did not affect the healing of gastric ulcers; the ulcer score on day 10 was 6.1 ± 0.8 mm^3, which was significantly smaller than the values (25.4 ± 5.0 mm^3) observed in the animals treated with aspirin.
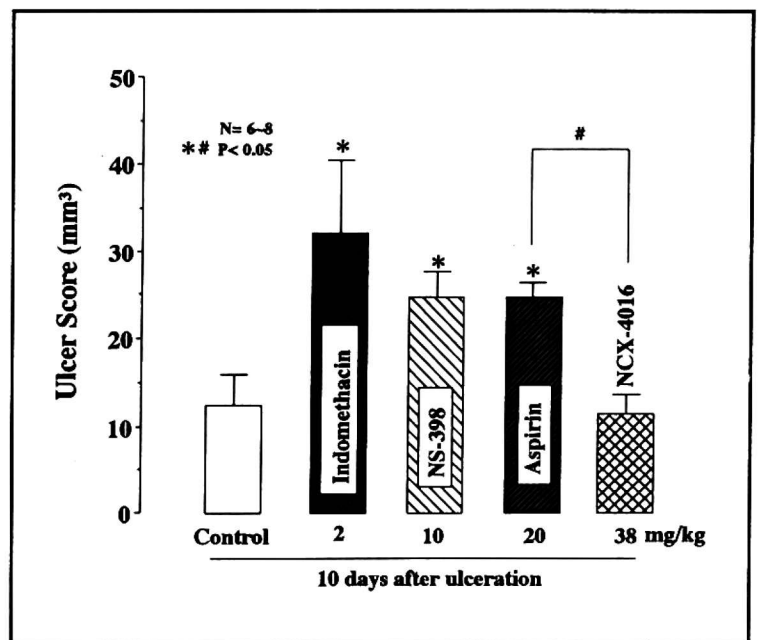

Fig. 4. Effects of indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) and NCX-4016 (38 mg/kg) on healing of gastric ulcers in mice. Gastric ulcers were induced by thermal-cauterization (70°C for 15 seconds). Each drug was given s.c. once daily for 7 days, starting 3 days after ulcer induction. Data are presented as the means $\pm$ SE from 5~8 mice. Statistically significant difference at $P < 0.05$; *from control; #from aspirin alone.

Effects of Various NSAIDs on PGE$_2$ Levels in Gastric Mucosa

The level of PGE$_2$ in the normal mouse gastric mucosa was 233.1 ± 32.8 ng/g tissue. The PGE$_2$ generation was significantly increased in the ulcerated mucosa (day 7), the value being 906.4 ± 128.8 ng/g tissue. The increased PGE$_2$ contents in the ulcerated mucosa was significantly reduced when the animals were treated with indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) as well as NCX-4016 (38 mg/kg), the inhibition being 72.3%, 48.3%,

54.2% and 42.1%, respectively (*Fig. 5A*). On the other hand, both in-domethacin, aspirin and NCX-4016 had a significant reducing effects on the PGE_2 contents in the intact gastric mucosa of the same animals, whereas NS-398 had no effect on PGE_2 generation in the intact mucosa without ulceration (*Fig. 5B*). The inhibition by indomethacin, aspirin and NCX-4016 of PGE_2 generation in the intact mucosa was 84.6%, 73.2% and 50.1% respectively.
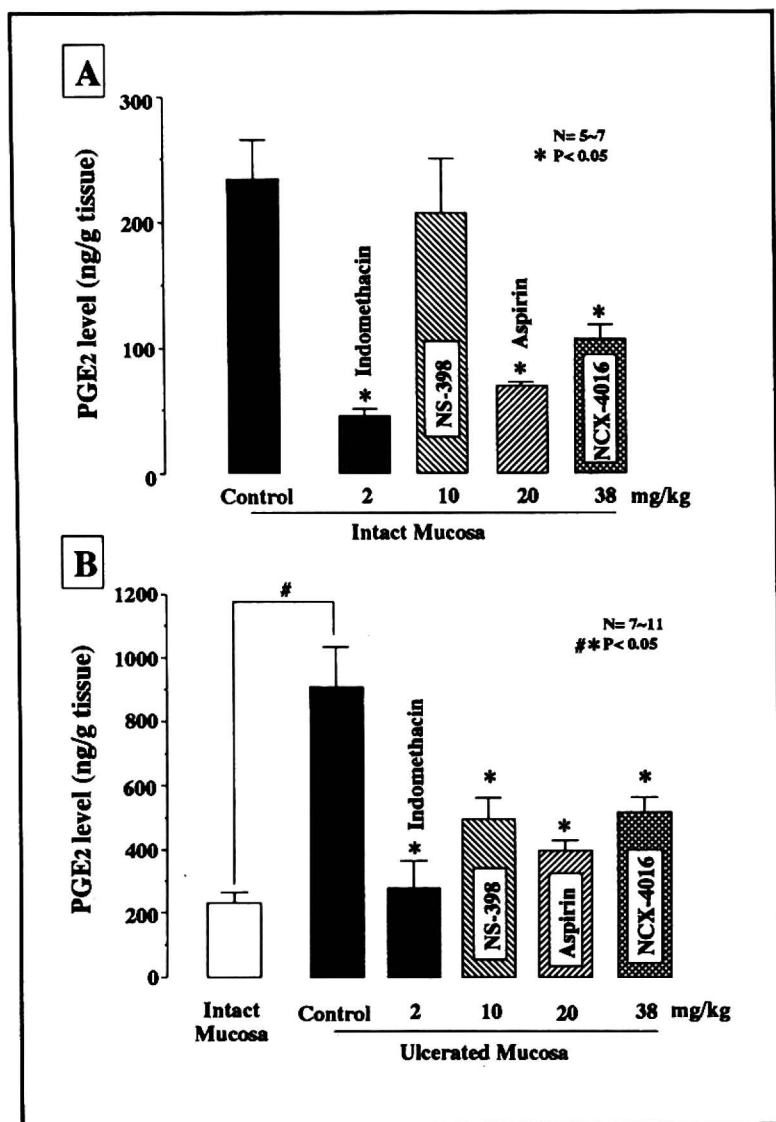

Fig. 5. Effects of indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) and NCX-4016 (38 mg/kg) on mucosal PGE_2 contents in the ulcerated (A) and intact (B) mucosa of mouse stomachs. Gastric ulcers were induced by thermal-cauterization (70°C for 15 seconds). Each drug was given s.c. once daily for 4 days starting 3 days after ulcer induction. The animals were killed 2 hr after the final administration, and PGE_2 contents were determined in the corpus mucosa by ELISA. Data are presented as the means $\pm$ SE from 5~11 mice. *Statistically significant difference from controls, at P<0.05.

Effect of NCX-4016 on NOx Levels in Gastric Contents

In pylorus-ligated animals, the values in the gastric contents were about 100 nmol/2 hr in rats and 20 nmol/2 hr in mice, respectively (*Fig. 6*). However, a significantly greater amount of NO was found in the gastric contents when the animals were given NCX-4016 (38 mg/kg) s.c. after pylorus ligation; the values were 1066.7 ± 109.5 nmol/2 hr in rats and 194.3 ± 44.1 nmol/2 hr in mice, respectively. Subcutaneous administration of aspirin (20 mg/kg) did not have any effects on NOx levels in gastric contents.

Fig. 6. NOx levels in gastric juice after administration of aspirin or NCX-4016 in pylorus-ligated rats and mice. Aspirin (20 mg/kg) or NCX-4016 (38 mg/kg) was administered s.c. immediately after the pylorus ligation. NOx levels in the gastric juice were determined 2 hr later. Data are presented as the means ± SE from 5 animals. *Statistically significant difference from controls, at $P < 0.05$.
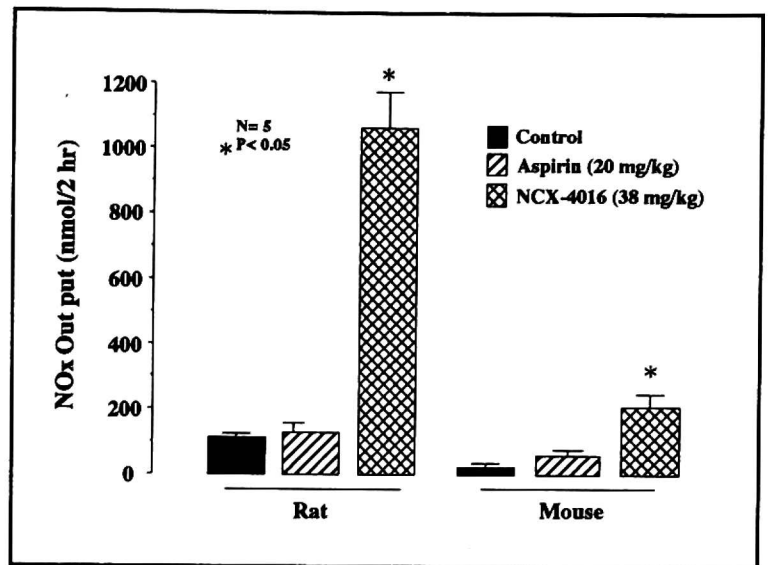
Effect of Various NSAIDs on Carrageenan-Induced Paw Edema

The intraplantar injection of carrageenan elicited acute hindpaw inflammation and caused a time-dependent increase in paw edema, a peak response being observed at 4 hr after the injection. Treatment of rats with indomethacin (2 mg/kg) and aspirin (20 mg/kg) before carrageenan administration significantly suppressed paw volume during a 6-hr test period, the inhibition being over 65% at 4 and 6 hr (*Fig. 7*). Likewise, NS-398 (10 mg/kg) and NCX-4016 (38 mg/kg) significantly decreased the paw volume; the inhibition at 4 hr after carrageenan injection was 60% and 68.8%, respectively.
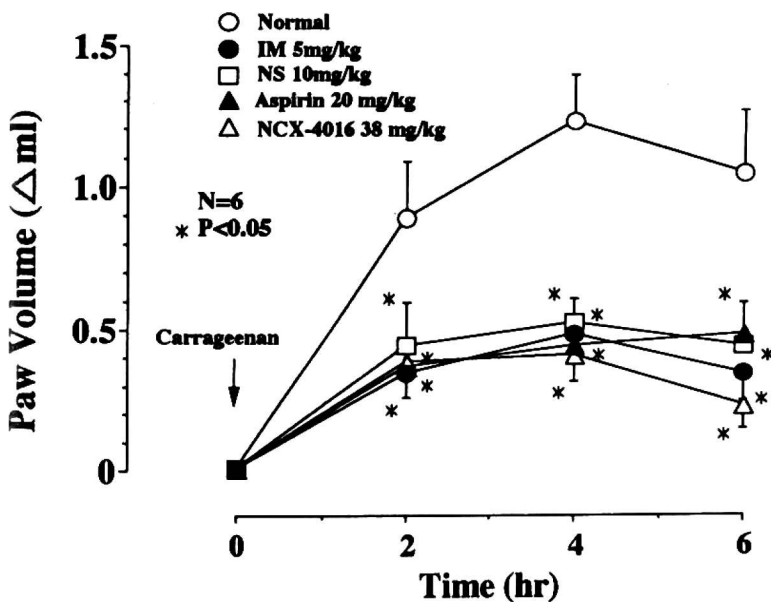

Fig. 7. Antiinflammatory effects of indomethacin (2 mg/kg), NS-398 (10 mg/kg), aspirin (20 mg/kg) and NCX-4016 (38 mg/kg) on edema formation induced in rat paws by carrageenan. The animals received a subplantar injection of carrageenan (0.1 ml of a 1% suspension in saline) into the right hind paw. Each drug was given s.c. 30 min before carrageenan injection. Data are presented as the means ± SE of values determined every 2 hr after carrageenan injection from 6 rats. *Statistically significant difference from controls, at $P < 0.05$.

DISCUSSION

The untoward effects of NSAIDs include the formation of gastric lesions, aggravation of the ulcerogenic response to stress and the impairment of healing of pre-existing ulcers. Newly developed selective COX-2 inhibitors and

NO-releasing NSAIDs are reported to reduce side effects of NSAIDs. Both COX-2 selective and NO-releasing NSAIDs exhibit less ulcerogenic properties in the gastrointestinal tract, despite showing a potent anti-inflamatory and analgestic action, similar to conventional NSAIDs (6—8, 10—12, 22). In the present study, we showed that NS-398 and NCX-4016 had different effects on gastric cytoprotection against ethanol injury and healing of gastric ulcer, although both drugs did not have either a direct irritating action or a worsening effect on the ulcerogenic response to stress.

In general, NSAIDs are by themselves ulcerogenic in the stomach and also potentiate the ulcerogenic response to various stimuli including stress (3, 5). Multiple elements have been postulated in the mechanism of gastric ulcerogenic response to NSAIDs, such as suppression of PG biosynthesis, topical, irritant action, activation of neutrophil, and increase of gastric motility (5, 23—25). Among NSAIDs examined in the present study, both indomethacin and aspirin were ulcerogenic by themselves in the stomach and potentiated stress-induced gastric lesions. Because aspirin produces gastric lesions when administered orally but not parenterally, the topical irritant action is more crucial in causing gastic mucosal damage (23). Since NCX-4016 by itself was devoid of ulcerogenic property and did not potentiate stress-induced gastric lesions, despite inhibiting PG generation in the stomach, it is assumed that the addition of a NO-releasing moiety to aspirin markedly reduced its short-term ulcerogenic property without altering its effectiveness as a COX inhibitor. The mechanism responsible for the lack of ulcerogenic property of NO-releasing aspirin derivatives is not yet clear. One possibility is that the topical irritant property was reduced by adding an nitroxy-butyl moiety to aspirin, therefore reducing the ulcerogenic effects. It is conceivable that the NO-releasing aspirin derivative, by releasing NO, exerted a protective effect that counteracted the potential damaging effects of COX inhibition. Indeed, we found a considerable amount of NOx, the metabolites of NO, in the lumen of the stomach after s.c. administration of NCX-4016. Of interest, NCX-4016 exhibited a dose-dependent protection against HCl/ethanol-induced gastric damage. Since a potent inhibition of ethanol-induced lesions was observed by an exogenous NO donor FK409 (17), the protective effects of NO-releasing aspirin derivative may be attributable to NO released from this compound.

Similar to the NO-releasing aspirin derivative, the COX-2 selective NSAID NS-398 did not cause gross damage in the gastric mucosa and modify the ulcerogenic response to stress. Since NS-398 did not suppress PG biosynthetic activity in the normal mucosa, in contrast to NCX-4016, the reason for reducing the ulcerogenic property of these compounds may be different. Indeed, NS-398 did not have any effects on HCl/ethanol-induced gastric lesions, which were potently prevented by NCX-4016. These results are consistent with previous observations that NS-398, although inhibiting PG

production in inflammatory tissues (6, 16), had no effect on gastric PG contents and did not modify stress-induced gastric ulcerations, while indomethacin decreased gastric PG levels and worsened gastric lesions in response to stress (5, 6). These results demonstrated a differential sensitivity of COX isoforms to inhibition by different NSAIDs and support the concept that antiinflammatory effects and unwanted side effects of NSAIDs are related to their ability to inhibit COX-2 and COX-1 activity, respectively (12).

On the other hand, the retarding effects of NSAIDs on gastric ulcer healing are thought to be attributable to the suppression of gastric PG synthesis by these agents (4). Therefore, better therapies for accelerating ulcer healing or new NSAIDs that spare the gastric mucosa would be valuable tools in the therapy of inflammatory conditions, particularly in patients prone to ulceration. We confirmed that both indomethacin and aspirin not only increased the mucosal ulcerogenic responses but also impaired the healing of gastric ulcers as well. The latter effect may be accounted for by inhibition of COX-2 activity, since the healing of gastric ulcers was also impaired by NS-398, the selective COX-2 inhibitor. Indeed, Mizuno *et al* (15) recently reported that both COX-2-mRNA and protein are expressed in the gastric mucosa after induction of acute and chronic ulcers in mice and play an important role in the healing of these ulcers. These results suggest that COX-2 is an important source of PGs when an ulcer is present, and that the PGs derived from the COX-2 may be crucial in maintaining the healing response of gastric ulcer. In the present study, NCX-4016 did not affect the healing response of gastric ulcers, despite the fact that the compound inhibited COX-1 and COX-2 as effectively as the parent NSAID, aspirin. Konturek *et al* (26) reported that the healing of gastric ulcers induced by acetic acid was delayed or promoted by administration of NO synthase inhibitors or L-arginine, respectively. We also reported that the inhibition of NO production by N^G-nitro-L-arginine methyl ester impaired the healing of acute gastric injury (27). It is now well established that NO is an important mediator of gastric mucosal defence, modulating mucosal blood flow and mucus secretion (13, 28). Thus, it is possible that NO released from NO-releasing aspirin derivative accelerates ulcer healing by elevating the resistance of the mucosa at the ulcer margin to further damage and by counteracting the potential worsening effects of COX inhibition.

NS-398 and NCX-4016 showed a potent anti-inflamatory action against carrageenan-induced paw edema, as effectively as indomethacin and aspirin, in agreement with previous studies (29). Intrapleural injection of carrageenan produce an increase of PGE_2 production and induction of *de novo* synthesis of COX-2 in pleural exudate cells (30), suggesting that the increased production of PGE_2 by inflammatory stimuli was mediated by newly synthesized COX-2 protein (7). It is assumed that NS-398 suppressed carrageenan-induced paw edema may be explained by inhibition of COX-2 enzymatic activity in

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inflammatory cells. These findings also suggest that the addition of the NO-releasing moiety did not interfere with the ability of this compound to inhibit COX activity at a peripheral inflammatory site (10—12).

Taken together, the present study suggests that nonselective COX inhibitors such as indomethacin and aspirin are ulcerogenic by themselves and impaired the healing response of gastric ulcers. NS-398 the selective COX-2 inhibitor by itself was not ulcerogenic but impaired the healing of gastric ulcers, similar to indomethacin. Thus, it is conceivable that the former action is due to inhibition of COX-1, while the latter effect may be accounted for by inhibition of COX-2 and is mimicked by the COX-2 selective inhibitor. NO-releasing aspirin derivative, NCX-4016, while inhibiting both COX-1 and COX-2, was not ulcerogenic, did not impair the ulcer healing, and rather showed gastroprotective activity against noxious stimulus, suggesting a beneficial influence of NO released from this compound on the gastric mucosa.

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