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ROLE OF p38 MITOGEN-ACTIVATED PROTEIN KINASE IN THE HEALING OF GASTRIC ULCERS IN RATS

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p38 belongs to the mitogen-activated protein kinase family and plays a crucial role in cellular responses to both cytokines and various stresses. We investigated the role of p38 in the healing of experimental gastric ulcers. Gastric ulcers were induced by submucosal injection of acetic acid solution into male rats. Western blotting and a kinase assay examined the p38 activity and phosphorylation state in ulcerated tisue. After orally administering FR167653 (p38 kinase inhibitor) for 3 to 14 days, the production level of cytokines and the protein-level expression of cyclooxygenase and inducible nitric oxide synthase were examined by enzyme-linked immunosorbent assay and Western blotting. Only in fibroblasts and macrophages/monocytes in ulcerated tissue, p38 was found to be phosphorylated with an elevated kinase activity level. FR167653 inhibited the activity of p38, yet had no effect on its phosphorylation state. The drug significantly impaired ulcer healing (without affecting acid secretion) and angiogenesis in the ulcer base. The production of interleukin-1β and tumor necrosis factor-α were significantly reduced after FR167653 treatment. In addition the expression of cyclooxygenase-2 and inducible nitric oxide synthase proteins increased PGE_2 generation and NO_x secretion in the ulcerated stomach were suppressed by FR167653. From these findings, we conclude that p38, activated by gastric ulceration, might play some role in the healing of gastic ulcers in rats.

Key words: *FR167653, p38 kinase inhibitor, acetic acid ulcer, angiogenesis.*

INTRODUCTION

It is known that gastric acid is the one of the most important factors influencing gastric ulcer healing, as changes in gastric acid output considerably affect the rate of ulcer healing (1—3). As a matter of fact, the suppression of gastric acid by histamine H_2 blockers and proton pump inhibitors has achieved marked improvement in ulcer healing. Another significant factor affecting gastric ulcer healing is the inflammatory response that occurs regardless of the presence or absence of *Helicobacter pylori* infection.

Interleukin (IL)-1 and tumor necrosis factor (TNF)-α are potent inducers of such inflammatory responses, and the mRNA expression of these cytokines is induced by gastric ulceration in rats (4, 5). Furthermore, it was revealed that ERK, a mitogen-activated protein kinase (MAP kinase) family, was activated upon the onset of gastric ulceration (6, 7). The inhibition of this ERK-signaling pathway resulted in a significant delay in ulcer healing, suggesting that ERK plays an instrumental role in ulcer healing.

As reviewed by Garrington and Johnson (8), p38 MAP kinase is activated by both cytokines and various cellular stresses, ultimately resulting in a stress response and apoptosis. Interestingly, recent studies demonstrate that the expression of IL-1, TNF-α, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) are extensively regulated by p38 (9—14). Among the above molecules, IL-1, COX and iNOS are considered to result in a beneficial effect on ulcer healing (15—18). FR167653 is a known inhibitor for the production of IL-1 and TNF-a (5, 19—21). As this study demonstrates, FR167653 is also potent inhibitor of p38. Using FR167653, this study was attempted to discern whether or not p38 exerts an effect on ulcer healing.

MATERIALS AND METHODS

Animals

Male Donryu rats (200—280 g; Nihon SLC, Shizuoka) were used for this study. The animals were kept in room under regulated temperature (approximately 20—22°C) and humidity (approximately 55%), with a 12/12-h light/dark cycle. Prior to ulceration, the animals were deprived of food for 5 h to allow for easy injection of the acetic acid solution into the gastric wall.

Induction of gastric ulcers

Under ether annesthesia, gastric ulcers were induced by submucosal injection of 20% acetic acid (v/v, 0.4 ml) into the border between the antrum and fundus on the anterior wall of the stomach (22). After closure of the abdomen, the rats were maintained in a normal manner. Since deep and well-defined ulcers were observed 5 days after injection of the acid, the 5th daywas defined as the day of ulceration. On the indicated days, the rat stomachs were incised along the greater curvature, and the ulcerated area (mm^2) was determined under a dissecting microscope without knowledge of the treatment administered (×10; Olympus, Tokyo).

Immunohistochemical staining of phosphorylated p38

Gastric specimens were extracted from normal and ulcerated tissues 3 days after ulceration. After the samples had been fixed with 4% paraformaldehyde in phosphate-buffered saline, 8 μm-frozen sections were prepared. The sections were incubated with anti-phosphorylated p38

antibody (Promega, Madison, WI) following deactivation of endogenous peroxidase with 0.3% H_2O_2 and blockage of nonspecific binding sites. Activated p38 was visualized by the avidin-biotin-peroxidase complex method using a Vectastain ABC-peroxidase kit (Vector Laboratories, Burlingame, CA) and 3',3'-diaminobenzidine tetrahydrochloride (Dojindo Laboratories, Kumamoto). The sections were successively stained with hematoxylin.

Western blot analysis

Gastric specimens (100 mg) obtained from the normal and ulcerated tissues were homogenized in 2 ml of lysis buffer consisting of 50 mmol/l HEPES-NaOH (pH 7.6), 300 mmol/l NaCl, 0.5% Triton X-100, 0.2% EDTA, 1.5 mmol/l $MgCl_2$, 20 mmol/l β-glycerophosphate, 1 mmol/l phenylmethylsulfonyl fluoride, 10 mmol/l sodium orthovanadate, 10 mmol/l sodium fluoride, 10 μg/ml leupeptin, 2 μg/ml aprotinin and 1 mmol/l dithiothreitol. The homogenates were centrifuged at 10,000 g for 15 min, and the resulting supernatants were used as gastric extracts. The protein extracts (10 μg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Hybond P membranes (Amersham Pharmacia, Backinghamshire, England). The membranes were incubated with anti-p38 antibody (New England Biolabs, Beverly, MA) overnight at 4°C after nonspecific binding sites had been blocked. Both inactive and active forms of p38 were detected on x-ray films (Fuji Film, Tokyo) by means of an enhanced chemiluminescence system (New Englabd Biolabs).

In another series of experiments, the levels of protein expression of cyclooxygenase (COX)-1, COX-2, inducible nitric oxide synthase (iNOS) and β-actin in the normal tissue and ulcer base were also determined using anti-COX-1 rabbit polyclonal (Cayman, Chemicals, Ann Arbor, MI), anti-COX-2 rabbit polyclonal (Cayman Chemicals, Ann Arbor, MI), anti-iNOS rabbit polyclonal (SIGMA, st. Louis, MO) and anti-β-atin mouse monoclonal (SIGMA, St. Louis, MO) antibodies, respectively.

p38 kinase assay

Following centrifugation, the immunoprecipitates were washed with 500 μl of lysis buffer and kinase buffer, which consisted of 50 mmol/l HEPES-NaOH (pH 7.6), 50 mmol/l $MgCl_2$, 20 mmol/l β-glycerophosphate, 10 mmol/l sodium orthovanadate, 10 mmol/l sodium fluoride and 1 mmol/l dithiothreitol. The resulting pellets were resuspended with 50 μl of kinase buffer, which contained 5 μg of myelin basic protein (Sigma Chemicals, St. Louis, MO), 200 μmol/l adenosine 5'-triphosphate (ATP) and 1 μCi of [γ^{-32}P]ATP (Amersham, Backinghamshire). The mixtures were incubated for 30 min at 30°C. After centrifugation at 10,000 g for 20 min, the reaction was terminated by adding 10 μl of 100% trichloroacetic acid to the supernatants. After centrifuging the supernatants at 10,000 g for 20 min, the pellets were subjected to SDS-PAGE. The radioactivity was detected by an image analyzer (BAS-5000 Mac; Fuji Film).

Determination of gastric acid secretion

Gastric acid secretion was determined by pylorus-ligation. FR167653 (60, 90 mg/kg) or saline was orally administered twice daily for 7 and 14 days after ulceration to the ulcerated rats. After the final treatment, the animals were fasted for 18 h and the pylorus was ligated under ether anesthesia. One h prior to the ligation, and additional administration of FR167653 or vehicle was administered at the same dosage level. Three h later, the animals were killed and the stomachs were excised. Subsequently, the gastric contents were collected and analyzed as to volume and acidity.

Acidity was determined by automatic titration of the contents against 0.1 mol/l NaOH to pH 7.0 (Comtite 5; Hiranuma, Tokyo). Gastric acid output was calculated as volume x acidity and expressed as microequivalents (μEq) per h.

Histological evaluation of ulcer healing

In addition to gross observation, the status of the ulcer healing was also histologically assessed, as previously described by our group (17). In brief, gastric specimens were taken from ulcerated tissues and fixed in 10% formalin and embedded in paraffin. 5 μm sections were prepared and subsequently stained with hematoxylin and eosin. The length (mm) of the regenerated mucosa on the ulcer base and the thickness (mm) of the base were measured under a light microscope. For evaluation of angiogenesis in the ulcer base, gastric specimens were fixed in 4% paraformaldehyde in phosphate-buffered saline, and 8 μm-frozen sections were prepared. Microvessels were immunostained with an antibody against von Willebrand factor (factor VIII-related endothelial antigen) (DAKO, Glostrop). The number of microvessels was determined in 3 randomly chosen 1 mm^2 fields. The microvessel density was expressed as the number of microvessels per mm^2 of the ulcer base. In order to detect the number of neutrophils that had infiltrated into the ulcer base, myeloperoxidase (MPO) activity-dependent staining was performed. The frozen sections were incubated in 50 mmol/l Tris-HCl (pH 7.6), which contained 0.2 mg/ml 3', 3'-diaminobenzidine tetrahydrochloride, in the presence of 0.005% H_2O_2 at room temperature. Neutrophil infiltration was expressed as the number of neutrophils per mm^2 of the ulcer base.

Determined of IL-1β and TNF-α production

Gastric specimens (100 mg) were taken from normal and ulcerated tissues, and washed with Dulbecco's modified Eagles medium (GIBCO BRL, Gaithersburg, MD) supplemented with 1 mg/ml bovine serum albumin, 200 units/ml penicillin, 200 units/ml streptomycin and 0.5 μg/ml amphotericin B. The samples were subsequently placed onto culture plates and incubated in 0.5 ml of the above medium for 3 h at 37°C under 5% CO_2 in air. An aliqout of the conditioned medium was recovered and the level of IL-1β and TNF-α was determined. The amounts of IL-1β and TNF-α were quantified by the enzyme-linked immunosorbent assay (rat IL-1β and rat TNF-α ELISA kits; Bio Source International, Camarillo, CA). The cytokine production was expressed as pg IL-1β or TNF-α per g tissue per h.

Determination of PGE_2 generation in the gastric tissue

PGE_2 generation in the gastric tissue was determined by the method from Lee and Feldman (23). The animals with ulcers were treated with vehicle or FR167653 (90 mg/kg × 2) for 3 days from the day of ulceration. One hour after the final administration, the normal tissue (glandular portion of the stomach) and ulcer base were collected. These samples were then placed in 50 mM Tris-HCl (pH 8.4) buffer, and minced with scissors. After washing, the samples were resuspended in 1 ml of buffer. Subsequently, the tissue samples were subjected to vortex mixing for 1 min at room temperature to stimulate PGE_2 generation, followed by centrifugation at 10,000 × for 15 min. The amounts of PGE_2 in the resulting supernatants were determined by enzyme-immunoassay (PGE_2 EIA kit; Cayman Chemicals, Ann Arbor, MI). PGE_2 generation was expressed as pg PGE_2/mg tissue /min.

Determination of NO_x contents in the gastric juice

The animals with ulcers were treated with vehicle or FR167653 (90 mg/kg × 2) for 3 days from the day of ulceration. One hour after the final administration, the pylorus was ligated. Three hours later, the gastric juice was collected. The NO_x contents in the gastric juice were determined by the Griess reaction-dependent method (24). After reduction of NO_3^- to NO_2^- with nitrate reductase, nitrites were incubated with Griess reagent (0.1% naphthalene diamine dihydrochloride and 1% sulfanilamide in H_2PO_4) for 10 min at 25°C, and measured the absorbance at 545 nm.

Drugs

FR167653 (1-[7-(4-fluorophenyl)-1,2,3,4-tetrahydro-8-pyridyl-pyrazolo[5,1-c][1,2,4]triazin-2--yl]-2-phenylethanedione sulfate monohydrate) was kindly supplied by Fujisawa Pharmaceutical Co. (Osaka). The drug, dissolved in saline, was administered orally (by gastric intubation) twice daily for 3, 7 or 14 days after ulceration in a volume of 5 ml/kg body weight. Control animals received saline alone.

Statistical analysis

The data are presented as means ± SEM. Statistical differences in the dose-response studies were evaluated by Dunnett's multiple comparison test. Student's t-test was used for the comparison between any two groups. A P value of < 0.05 was regarded as significant.

RESULTS

Activation of p38 by gastric ulceration

The cellular localization of phosphorylated p38 in ulcerated tissue was defined by immunohistochemical staining (*Fig. 1-A*). Strong immunoreactivity for phosphorylated p38 was abundant only in the ulcer base. Immunoreactive signals were found in spindle-shaped cells and mononuclear cells. Considering the presence of granulation tissue, these cell types morphologically appeared to be fibroblasts and macrophages/monocytes. On the other hand, immunoreactivity for phosphorylated p38 was not detected in the normal mucosa. The phosphorylation state of p38 was examined by Western blotting (*Fig. 1-B*). In normal mucosa, phosphorylated p38 was not detected. Nonetheless, the phosphorylation of p38 was induced by gastric ulceration. In ulcerated tissues, p38 was phosphorylated at the time of ulceration. The level of the phosphorylation detected time-dependently increased up to 7 days after ulceration, the activation of p38 decreased thereafter. It should be noted, however, that the amount of p38 was not affected at the time of ulceration or during the period of ulcer healing. FR167653 was not found to affect the phosphorylation state of p38, but the kinase assay revealed that FR167653 inhibits the p38 activity (*Fig. 1-C*).

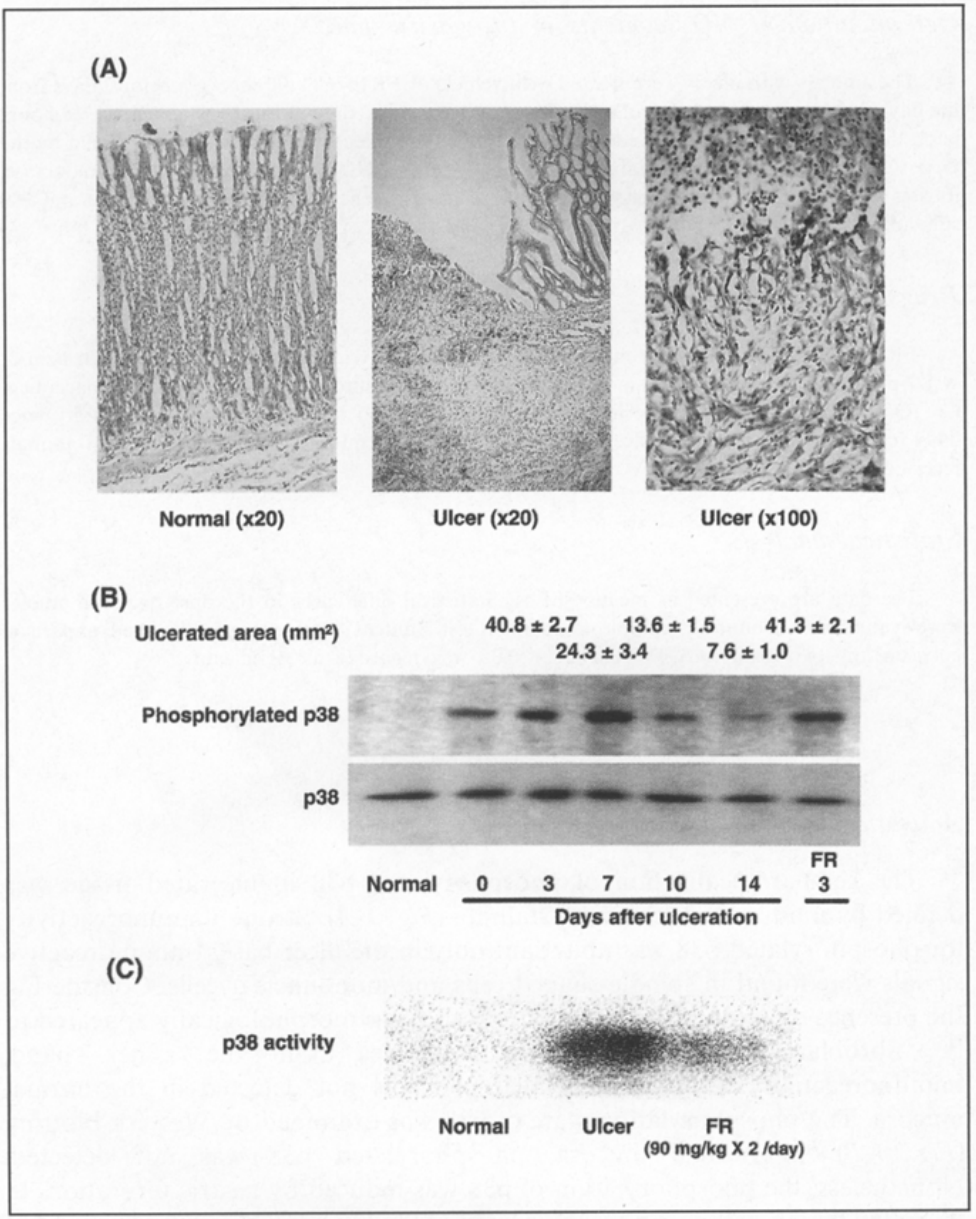

Fig. 1. Determination of p38 activation in the normal and ulcerated tissue in rats. (A): Note that the immunoreactivity of phosphorylated p38 was found in the base of 3-day old ulcers. (B): Time course changes of the ulcerated area and the phosphorylation of p38: FR (FR167653) was orally administered twice daily for 3 days after ulceration. Phosphorylated p38 and p38 in the gastric tissues were detected by Western blot analysis. Data are presented as means ± SEM (n = 6—8). (C): p38 activity, measured by kinase assay, in the normal gastric tissues with 3-day-old ulcers with or without FR167653 treatment.

Effects of FR167653 on ulcer healing and gastric acid secretion

Repeated administration of FR167653 (90 mg/kg) orally twice daily for 14 days had no effect on the gastric mucosa of normal rats (data not shown). In addition, FR167653 (90 mg/kg) did not affect the healing of gastric ulcers 3 days after ulceration. In contrast, the drug (60 and 90 mg/kg) was found to significantly impair ulcer healing after 7- and 14-day treatments (*Fig. 2, 3*). It was also determined that the gastric acid secretion of these animals was not affected by treatment with FR167653 for 7 and 14 days. It was of interest that FR167653, administered for 14 days beginning 7 days after ulceration, had little or no effect on ulcer healing (data not shown).

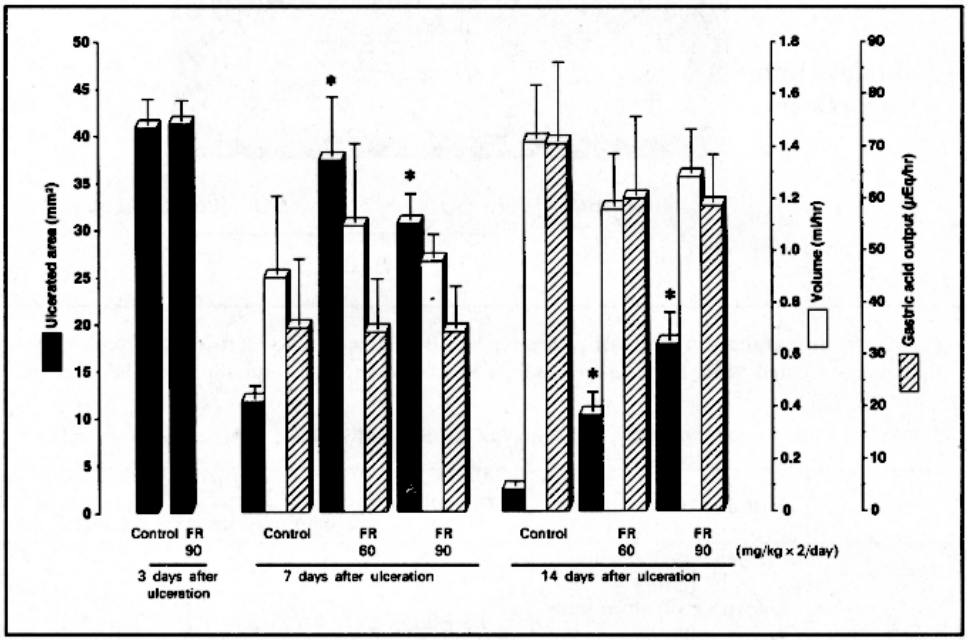

Fig. 2. Effect of FR (FR167653) on the gastric ulcer healing and basal gastric acid secretion in rats. The drug was orally administered twice daily for 3, 7 and 14 days. Secretory analysis was performed by pylorus ligation (3 hr). The data are presented as means ± SEM (n = 5—8). * Significantly different from the corresponding control, $P < 0.05$.

Histological analysis of ulcerated mucosa treated with FR167653 confirmed the changes apparent upon gross observation, i. e., the length of the regenerated mucosa was significantly shorter than observed in the control group (*Table 1*). After 7- and 14-day treatments, there was a significant increase in the thickness of the ulcer base and a significant reduction in microvessel density in the ulcer base, compared with the controls. Neutrophil infiltration was hardly observed in the ulcer base 7 days after ulceration. In addition, FR167653, administered

for 7 days, did not affect neutrophil infiltration. In contrast, a high concentration of neutrophils infiltrated into the ulcer base 14 days after ulceration. It should be noted that FR167653 significantly suppressed neutrophil infiltration into the ulcer base.

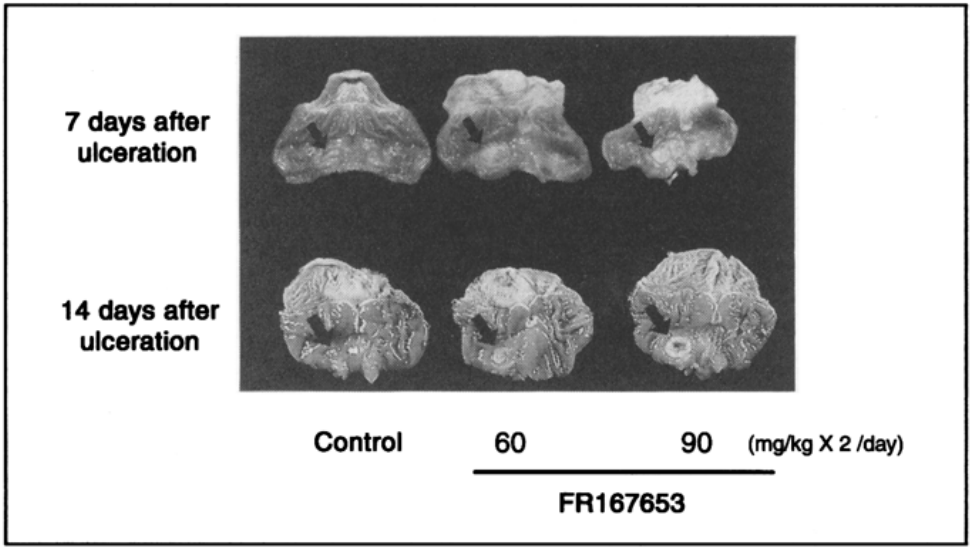

Fig. 3. Gross appearances of the gastric ulcers in rats treated with FR (FR167653) or vehicle. Note that ulcers treated with FR was markedly extended compared with those in the control.

Table 1. Effect of FR167653 on healing of acetic acid-induced gastric ulcers in rats.

	Histological evaluation	Control	FR167653	
			60 mg/kg × 2/day	90 mg/kg × 2/day
7 days after ulceration	Regenerated mucosa (mm)	1.4 ± 0.1	0.7 ± 0.1 *	0.7 ± 0.1 *
	Thickness of ulcer base (mm)	1.3 ± 0.1	1.6 ± 0.2 *	1.6 ± 0.1 *
	Microvessel density (counts/mm^2)	47.1 ± 3.9	18.7 ± 2.4 *	18.7 ± 4.1 *
	Infiltrated neutrophils (cells/mm^2)	188 ± 104	106 ± 29	152 ± 102
14 days after ulceration	Regenerated mucosa (mm)	2.0 ± 0.2	1.0 ± 0.1 *	0.9 ± 0.02 *
	Thickness of ulcer base (mm)	1.0 ± 0.1	1.3 ± 0.1	1.6 ± 0.1 *
	Microvessel density (counts/mm^2)	50.2 ± 6.6	34.2 ± 4.0	29.8 ± 5.6 *
	Infiltrated neutrophils (cells/mm^2)	4450 ± 459	776 ± 199 *	138 ± 27 *

FR167653 was orally administered twice daily for 7 and 14 days after ulceration. Data are presented as means ± SEM (n = 5—7).

* Significantly different from the corresponding control, $P < 0.05$.

Effect of FR167653 on IL-1β and TNF-α production

Although IL-1β and TNF-α levels were low in the normal mucosa, these levels were significantly increased by approximately 8 fold in the ulcerated tissue 3 days after ulceration (*Fig. 4*). At 7 and 14 days, the increased TNF-α production was slightly reduced, while the level of IL-1b had returned to near normal values. The increases in IL-1β and TNF-α production were markedly prevented by treatment with FR167653 (90 mg/kg) for 3 days by 81.4% and 69.8%, respectively.

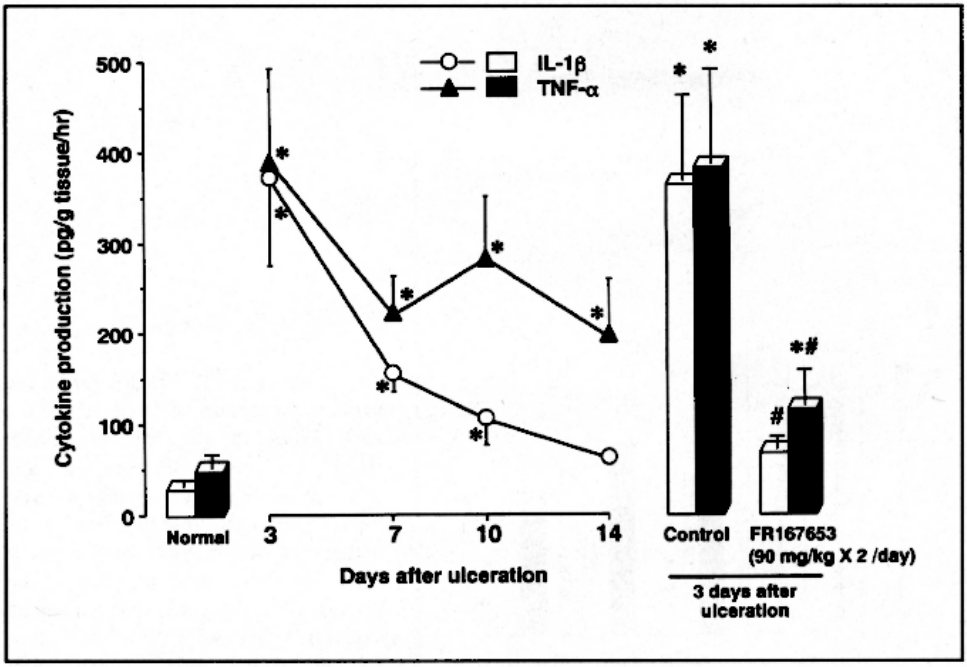

Fig. 4. Time-course changes in IL-1β and TNF-α production in the ulcerated gastric tissue in rats and the effect of FR167653 on these cytokines. The drug was orally administered twice daily for 3 days after ulceration. Data are presented as means ± SEM (n = 6). *, # Significantly different from the normal and control, respectively; $P < 0.05$.

Effect of FR167653 on expression of COX-1, COX-2 and iNOS protein

COX-1 expression was measured in the normal and ulcerated tissues and found to be present to the same degree (*Fig. 5*). Accordingly, COX-1 expression was not affected by treatment with FR167653 (90 mg/kg) for 3 days. COX-2

and iNOS proteins were not detected in the normal tissues, yet these proteins were present at increased levels in the ulcerated tissues. Such increased levels of expression were suppressed in the FR167653-treated ulcerated tissues.

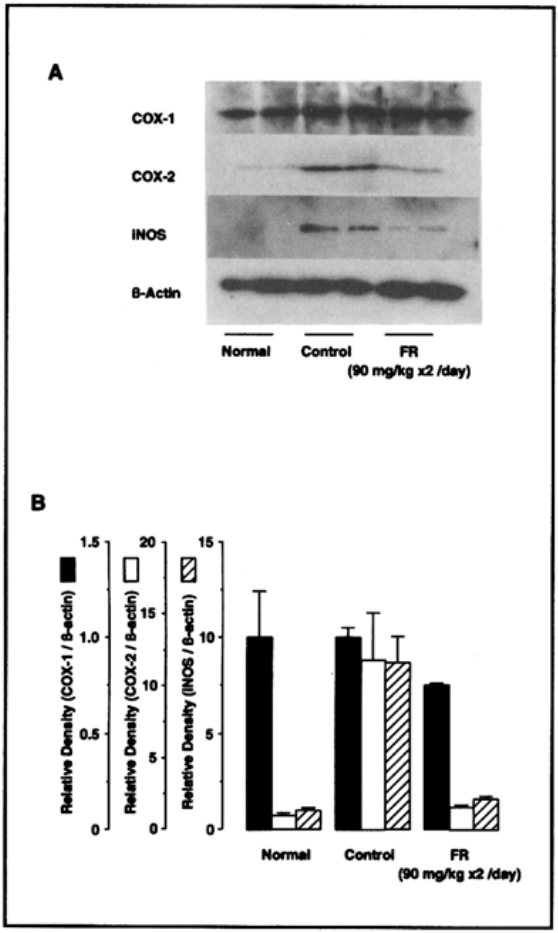

Fig. 5. Effect of FR (FR167653) on the protein expression of COX-1, COX-2 and iNOS in the normal gastric tissues and ulcerated tissue in rats. (A): Western blot pattern, (B): Densitometrical quantification. The drug was orally administered twice daily for 3 days after ulceration. The protein expression was determined by Western blot analysis. Note that COX-2 and iNOS expression increased in the ulcerated tissue were clearly suppressed by FR treatment.

Effect of FR167653 on PGE_2 generation and NO_x secretion

PGE_2 generation in the normal gastric tissue was about 50 pg/mg tissue/min (*Fig. 6-A*). The amount of PGE_2 generation in the ulcerated tissue was significantly increased. However, FR167653 inhibited the overproduction of PGE_2 in the ulcerated tissue. The same tendency was observed in the case of NO_x contents in the gastric juice (*Fig. 6-B*).

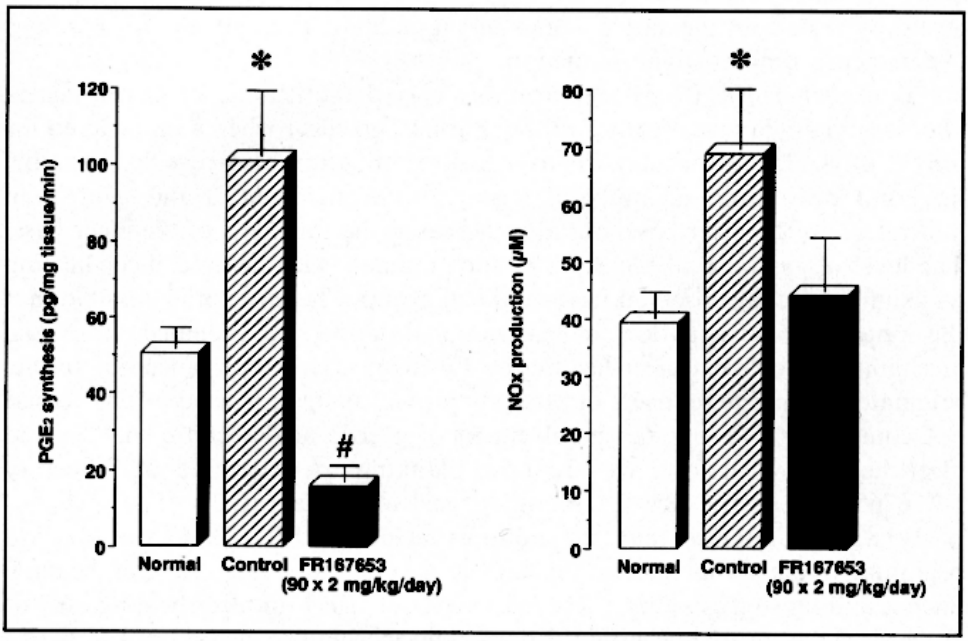

Fig. 6. Effect of FR167653 on PGE_2 generation in the ulcerated tissue (A) and NO_x secretion into the gastric juice in the rats with or without ulcers. The drug was orally administered twice daily for 3 days after ulceration. Data are presented as the mean ± SEM (n = 6). * Significantly different from vehicle treatment group, $P < 0.05$.

DISCUSSION

This study is the first to indicate both that p38 MAP kinase is activated by gastric ulceration and that phosphorylated p38 is only abundant in fibroblasts and macrophages/monocytes morphologically confirmed in the ulcer base. It is well known that fibroblasts and macrophages/monocytes produce various cytokines and/or growth factors in the stomach (25—27). The results of the present study indicate that such cells play a key role as regulators of inflammatory responses in ulcerated tissues, suggesting their potential involvement in ulcer healing.

It was found that the activation of p38 was observed as early as the day of ulceration, reaching peak activation 7 days after ulceration. Such a finding might be due to the fact that mature granulation tissue formed on the day of ulceration contains many fibroblasts. Our previous studies showed that both IL-1β and TNF-α mRNA are expressed in ulcerated tissues (5). In this study, it was confirmed that the production of IL-1β and TNF-α protein was significantly increased in the ulcerated tissues by approximately 8 fold 3 days after ulceration. While the level of the former gradually returned to control

levels by 14 days, the latter remained at a high level for up to 14 days, experiencing only a slight reduction.

As expected, FR167653 significantly delayed the healing of gastric ulcers after 7- and 14-day treatments, although it had no effect when administered for only 3 days after ulceration. Histological examination also revealed that the drug not only inhibited mucosal regeneration, angiogenesis and neutrophil infiltration in the ulcer base, but also increased the thickness of the ulcer base. The level of gastric acid secretion of such animals with delayed ulcer healing was similar to that observed in the control group. Therefore, it is possible that the potential participation of gastric acid could be excluded from the mechanism of delayed ulcer healing by FR167653. However, since our results originated from different sets of rats (using pylorus ligation method), we could not completely rule out the involvement of gastric acid secretion in delayed ulcer healing. Therefore, we are now planning to determine the effect of FR167653 on gastric acid secretion in gastric fistula rats.

It was of interest that the administration of FR167653 for 14 days beginning 7 days after ulceration had no deleterious effect on ulcer healing. Such a finding suggests that FR167653 exerts its effect on ulcer healing only at an early stage, probably within 7 days. The mechanism by which FR167653 delays ulcer healing is explained as follows.

As expected from previous studies (5, 19—21), we confirmed that FR167653 significantly inhibited the production of IL-1β and TNF-α protein in ulcerated tissues after 3 days treatment. While we did not actually determine whether or not FR167653 inhibited such cytokines for 7 or 14 days, it is reasonable to expect that the drug continued its inhibition, as it was administered twice daily until the end of the experiments.

Mizuno *et al.* (16) and ourselves (17) previously reported both that NS-398, a COX-2 selective inhibitor, significantly impaired the healing of acetic acid ulcers in mice and rats, respectively. In addition, these two groups demonstrated that PGE_2, induced by COX-2, plays an important role in ulcer healing. In addition, Konturek *et al.* (27) reported that both N^G-nitro-L-arginine and N^G-monomethyl-L-arginine administered for 7 days significantly delayed the healing of acetic acid ulcers in rats. They suggested that endogenous NO promotes ulcer healing by maintaining mucosal blood flow around the ulcers, leading to increased angiogenesis in the granulation tissues. Furthermore, Akiba *et al.* (18) revealed both that iNOS expression was increased in ulcerated tissues and that a selective iNOS inhibitor, aminoguanidine, significantly inhibited the ulcer healing. Based on such results, Akiba *et al.* suggested that iNOS might play a beneficial role in ulcer healing. We also confirmed the increased expression of not only iNOS protein, but also COX-2 protein, in the gastric tissues of 3-day old ulcers.

In this study, it was found that FR167653 inhibited the protein expression of COX-2 and iNOS, which were increased by gastric ulceration. Furthermore we confirmed that FR167653 significantly inhibited the over production of PGE_2 and NO_x caused by ulceration. As such proteins are regulated by p38 (12—14), the above findings might result from inhibition of the increased activity of p38 in ulcreated tissue. Yet, our group previously demonstrated in rats that since an IL-1 receptor antagonist inhibited the expression of COX-2, iNOS, cytokine-induced neutrophil chemoattractant (CINC)-1 and basic fibroblast growth factor (bFGF) mRNA in ulcerated tissue, IL-1 appears to regulate the mRNA expression of these molecules (15). Accordingly, it is conceivable that the mechanism by which FR167653 suppresses COX-2 and iNOS protein might be at least partly mediated by inhibited IL-1β production.

Based on histological studies, indomethacin and NS-398 were found to suppress the regeneration of the gastric mucosa, the maturation of the ulcer base and angiogenesis in the ulcer base (17). Similar to indomethacin and NS-398, FR167653 resulted in such changes in the ulcerated gastric mucosa, most probably through the inhibition of the COX-2 protein. In addition to the inhibited COX-2 protein, it remains possible that FR167653 inhibited angiogenesis through other two pathways: 1) inhibition of p38 was followed by inhibited expression of IL-1β, leading to inhibited expression of iNOS; and 2) inhibition of p38 results in the inhibition of iNOS expression. The results of this study are consistent with results reported by Jackson *et al.* (28). They found that SB 220025, a selective p38 inhibitor, inhibited the production of IL-1β and TNF-α protein in a mice model, resulting in inhibited inflammatory angiogenesis. As previously mentioned, we found that IL-1 stimulates the expression of bFGF, a potent stimulant for angiogenesis (29—31), in ulcerated tissues (15). Accordingly, one potential explanation for inhibited angiogenesis resulting from FR167653 is that the drug might reduce the expression of bFGF in ulcerated tissue, due to inhibition of IL-1β and p38. Further study is warranted to pursue this postulation.

CINC belongs to the IL-8 chemokine family and has potent chemotactic activity in vitro (32, 33). IL-1 and TNF-α increased the migration of neutrophils via induction of mRNA expression of CINC (34). Our group has also previously reported significant increases of CINC production, chemotactic activity and MPO activity in ulcerated tissue (35). Furthermore, it has been reported that IL-1β (36) and TNF-α (37) induce the expression of several adhesion molecules and elevate neutrophil infiltration. Taken altogether, it is possible that the mechanism by which FR167653 inhibits neutrophil infiltration involves inhibition of CINC production and coupled with expression of adhesion proteins. An alternative explanation is that such inhibition of infiltration might result from suppression of both angiogenesis and maturation of the ulcer base.

We are now planning to examine the effect of another structurally unrelated inhibitor of p38 MAP kinase on ulcer healing and its mechanism. Since p38 has been shown to regulate many factors or molecules which are important for restoration and regeneration, the involvement of growth factors such as EGF and HGF need to be evaluated. The mechanism of p38 kinase-inactivation by FR167653 appears to be similar to the case of SB202190 which compete with ATP for binding to p38 MAP kinase and inhibits its activity (38).

It is concluded that gastric ulceration activates p38 MAP kinase in fibroblasts and macrophages/monocytes in ulcerated tissues, which in turn induces COX-2 and iNOS production, eventually leading to the production of PGE^2 and NO, and these molecules contribute the healing of the ulcer in rats.

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