Effect of Gefarnate on Acute Gastric Mucosal Lesion Progression in Rats Treated with Compound 48/80, a Mast Cell Degranulator, in Comparison with That of Teprenone

Yoshiji OHTA,*[,a] Takashi KOBAYASHI,[b] Yoichiro IMAI,[c] Kazuo INUI,[b] Junji YOSHINO,[b] and Saburo NAKAZAWA[c]

[a] *Department of Chemistry, Fujita Health University School of Medicine; Toyoake, Aichi 470–1192, Japan:* [b] *Department Intrnal Medicine, Second Teaching Hospital, Fujita Health University School of Medicine; Nagoya, Aichi 454–8509, Japan: and* [c] *Department of Clinical Biochemistry, Fujita Health University College; Toyoake, Aichi 470–1192, Japan.*
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We have reported that teprenone (geranylgeranylacetone), an anti-ulcer drug, prevents acute gastric mucosal lesion progression in rats treated once with compound 48/80 (C48/80), a mast cell degranulator, possibly by suppressing mucus depletion, neutrophil infiltration, and oxidative stress in the gastric mucosa. Herein, we examined the preventive effect of gefarnate (geranyl farnesylacetate), an anti-ulcer drug, on acute gastric mucosal lesion progression in rats treated once with C48/80 (0.75 mg/kg, i.p.) in comparison with that of teprenone, because the chemical structure and anti-ulcer action of gefarnate are similar to those of teprenone. Gefarnate (50, 100 or 200 mg/kg) administered orally at 0.5 h after C48/80 treatment, at which time gastric mucosal lesions appeared, reduced progressive gastric mucosal lesions at 3 h dose-dependently. At 3 h after C48/80 treatment, the gastric mucosa had decreased adherent mucus and hexosamine contents and increased myeloperoxdiase (an index of neutrophil infiltration) and xanthine oxidase activities and thiobarbituric acid reactive substances (an index of lipid peroxidation) content. Post-administered gefarnate attenuated all these changes dose-dependently. These preventive effects of gefarnate were similar to those of teprenone at a dose of 200 mg/kg. Post-administered gefarnate did not affect the increases in serum serotonin and histamine concentrations and the decrease in gastric mucosal blood flow at 3 h after C48/80 treatment like teprenone. These results indicate that orally administered gefarnate prevents acute gastric mucosal lesion progression in C48/80-treated rats possibly by suppressing mucus depletion, neutrophil infiltration, and oxidative stress in the gastric mucosa like teprenone.

Key words compound 48/80; gastric mucosal lesion (rat); gefarnate; gastric mucus; neutrophil infiltration; oxidative stress

Gefarnate (geranyl farnesylacetate), an anti-ulcer drug, is clinically used for the treatment of gastric ulcers and gastritis.[1,2] Takagi and Yano[3] reported that when gefarnate was orally administered to fasted rats for 5 d, the level of hexosamine, an index of gastric mucus, in the pyloric tissue of the stomach increased and that the reduction of hexosamine content in the gastric pyloric tissue of rats with 3 d of water immersion restraint stress (WIRS) was prevented by daily simultaneous administration of gefarnate. There are several reports showing that gefarnate exerts a protective effect against acute gastric mucosal lesions in various *in vivo* experimental models such as gastric mucosal lesions induced by histamine–antihistamine, immobilization, reserpine, fasting, ethanol, asprin, HCl–asprin, HCl–ethanol, HCl–taurocholate, and WIRS.[3—10] Hara *et al.*[6] showed that when pylorus-ligated rats with oral HCl–asprin treatment received a single oral pre-administration of gefarnate, this drug exerted a protective effect against gastric mucosal lesions with attenuation of decreased gastric mucosal hexosamine content. In addition, Kobayashi *et al.*[10] reported that gefarnate prevented WIRS-induced ulcer formation by inhibiting the reduction of endogenous prostaglandin E_2 and prostacyclin (prostaglandin I_2) levels.

Teprenone (geranylgeranylacetone), of which chemical structure is similar to that of gefarnate, is an anti-ulcer drug developed in Japan. This drug is clinically used for the treatment of gastric ulcers and gastritis.[11,12] Teprenone is known to stimulate gastric mucus synthesis and secretion in rat gastric cultured cells[13—15] and in the gastric tissue of rats.[16,17] There are several reports showing that teprenone exerts a protective effect against acute gastric mucosal lesions in various *in vivo* experimental models, such as gastric mucosal lesions induced by asprin, cold restraint stress, WIRS, and HCl–taurocholate, through preservation of gastric mucus synthesis and secretion.[8,18—20] It has been shown that teprenone protects cultured rat gastric mucosal cells against reactive oxygen species (ROS) such as superoxide radical (O_2^-) by increasing the production of mucus.[21] In addition, it has been shown *in vitro* that teprenone inhibits the adhesion of neutrophils to endothelial cells and the expression of CD11b/CD18a, an adhesion molecule, on neutrophils when the neutrophils are activated by *Helicobacter pylori* water extract.[22] We reported that teprenone exerted protective and preventive effects against acute gastric mucosal lesions in rats with WIRS not only by preservation of gastric mucus synthesis and secretion but also by inhibition of neutrophil infiltration and enhanced lipid peroxidation in the gastric mucosa.[20]

Compound 48/80 (C48/80) is known to cause degranulation of connective tissue mast cells, but not mucosal mast cells, with release of serotonin and histamine from the cells.[23,24] We have shown in rats with a single treatment of C48/80 that the development of gastric mucosal lesions occurs with decreases in Se-glutathione peroxidase activity and vitamin E and hexosamine contents and increases in neutrophil infiltration, xanthine oxidase (XO) activity, and lipid peroxide content in the gastric mucosal tissue and that gastric mucosal blood flow is reduced with gastric mucosal lesion formation, while the decreased blood flow is recovered with the lesion progression.[25] We have also shown in rats treated

* To whom correspondence should be addressed. e-mail: yohta@fujita-hu.ac.jp
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once with C48/80 that neutrophils infiltrating into the gastric mucosal tissue participate in gastric mucosal lesion formation and progression, while the xanthine–XO system in the gastric mucosal tissue takes part mainly in the lesion progression.[26] Furthermore, it has been shown in rats treated once with C48/80 that acutely released endogenous serotonin contributes to gastric mucosal lesion formation, while released endogenous histamine mainly contributes to the lesion progression, although gastric acid plays little role in the pathogenesis of C48/80-induced gastric mucosal lesions.[27] Our previous reports have shown that teprenone exerts protective and preventive effects against acute gastric mucosal lesions in rats treated once with C48/80 possibly by inhibiting mucus depletion, neutrophil infiltration, and oxidative stress in the gastric mucosa.[28,29]

In the present study, we examined the effect of orally administered gefarnate on acute gastric mucosal lesion progression and the changes in the gastric mucosal activities of myeloperoxidase (MPO), an index of tissue neutrophil infiltration,[30] and XO, the gastric mucosal contents of thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation, and hexosamine, and gastric adherent mucus content with the lesion progression in rats with a single C48/80 treatment in comparison that of teprenone. We further examined the effect of the post-administered gefarnate on the changes in serum serotonin and histamine concentrations and gastric mucosal blood flow with gastric mucosal lesion progression in the C48/80-treated rats in comparison with that of teprenone.

MATERIALS AND METHODS

Materials C48/80, dioctyl sodium sulfossucinate (DSS), methyl serotonin, 3,3′,5,5′-tetramethylbenzidne and xanthine was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); alcin blue GX8, *N*,*N*-dimethylformamide, ethylenediaminetetraacetic acid (EDTA), glucosamine, 2-thiobarbituric acid, and other chemicals from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Gefarnate was obtained from Hamari Drug Ind., Co., (Osaka, Japan). Teprenone without any additive was kindly provided by Eisai Co. (Tokyo, Japan).

Animals Male Wistar rats aged six weeks were purchased from Japan SLC Co. (Hamamatsu, Japan). The animals were housed in cages in a ventilated animal room with controlled temperature (23±2 °C) and relative humidity (55±5%) with 12 h of light (7:00 to 19:00). The animals were maintained with free access to rat chow, Oriental MF (Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum for one week. All animals received humane care in compliance with the Guidelines for the Management of Laboratory Animals in Fujita Health University.

Gastric Mucosal Lesion Induction C48/80 (0.75 mg/kg), dissolved in distilled water, was intraperitoneally injected to 7-week-old rats fasted for 24 h, as described previously.[25—29] The control rats received an intraperitoneal (i.p.) injection of an equal volume of distilled water. All animals were maintained with free access to water and without food during the experiment. The animals were sacrificed under ether anesthesia 0.5 or 3 h after C48/80 injection. The stomachs were removed, inflated with 10 ml of 0.9% NaCl, and put into 10% formalin for 10 min. The stomachs were then opened along the greater curvature and examined for lesions in the glandular part under a dissecting microscope (×10). The severity of gastric mucosal lesions was estimated using the index of the following eight grades of lesions as described in our previous reports:[25—29] grade 0, no lesion (normal); grade I, edema only; grade II, damaged area of 1—10 mm^2; grade III, damaged area of 11—20 mm^2; grade IV, damaged area of 21—30 mm^2; grade V, damaged area of 31—40 mm^2; grade VI, damaged area of 41—50 mm^2; grade VII, damaged area of >51 mm^2.

Administration of Gefarnate and Teprenone Gefarnate or teprenone was suspended in 0.5% arabic gum at a constant dosing volume of 5 ml/kg. Gefarnate (50, 100 or 200 mg/kg) or teprenone (200 mg/kg) was orally administered to fasted rats with a stomach tube at 0.5 h after C48/80 treatment. Rats untreated with either gefarnate or teprenone received an equal volume of 0.5% arabic gum used as a vehicle at the same time point.

Determinations of Gastric Mucosal MPO, XO, TBARS, Adherent Mucus, and Hexosamine Gastric mucosal MPO was assayed by the method of Suzuki *et al*.[31] For this enzyme assay, gastric mucosal tissues were homogenized in 9 volumes of ice-cold 0.05 M Tris–HCl buffer (pH 7.4). After sonication on ice for 20 s using a Handy Sonic model UR-20P (Tomy Seiko Co., Tokyo, Japan), the homogenate was centrifuged at 4 °C (10000×***g***, 20 min), and the resultant supernatant was dialyzed against 100 volumes of the same buffer at 4 °C for 24 h. MPO activity was assessed by measuring the hydrogen peroxide (H_2O_2)-dependent oxidation of tetramethylbenzidine at 37 °C. One unit (U) of this enzyme is defined as the amount of enzyme causing a change in absorbance of 1.0 per min at 655 nm. Gastric mucosal XO was assayed by the method of Hashimoto.[32] For this enzyme assay, gastric mucosal tissues were homogenized in 9 volumes of ice-cold 0.25 M sucrose. The homogenate was sonicated as described above. The sonicated homogenate was centrifuged at 4 °C (10000×***g***, 20 min), and the resultant supernatant was dialyzed against 100 volumes of the same solution at 4 °C for 24 h. XO activity was assessed by measuring the increase in absorbance at 292 nm following the formation of uric acid at 30 °C. One unit (U) of this enzyme is defined as the amount of enzyme forming 1 μmol uric acid per min. Gastric mucosal TBARS was spectrophotometrically determined by the thiobarbituric acid method of Ohkawa *et al*.[33] except that 1.0 mM EDTA was added to the reaction medium. For this determination, gastric mucosal tissues were homogenized in 9 volumes of ice-cold 20 mM EDTA. The amount of TBARS is expressed as that of malondialdehyde (MDA) equivalents. Gastric adherent mucus was assayed by the method of Kitagawa *et al*.[34] as follows: the removed stomach was cut open along with the greater curvature and rinsed with 10 ml of ice-cold 0.25 M sucrose. Then, 50 mm^2 (approx. 8 mm in diameter) of the glandular portion of the stomach was excised with a scalpel and the excised part was weighed. The excised stomach was soaked in 2 ml of 0.1% alcian blue, which was dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate (pH 5.8), for 2 h. Uncomplexed dye was removed by two successive washes in 2 ml of 0.25 M sucrose for 15 and 45 min, and then dye complex with mucus was extracted with 30% DSS for 2 h. After centrifugation (3000 rpm for 10 min), the optical density of

the solution of alcian blue extracted with DSS was read at 620 nm and the concentration of the extracted alcian blue was calculated in comparison with a calibration curve obtained from known concentrations of alcian blue solutions. The concentration of gastric mucosal adherent mucus is expressed as that of alcian blue adhered to the gastric mucosal surface (mg/g tissue). Gastric mucosal hexosamine was assayed as follows: gastric mucosal mucin was extracted with Triton X-100 and then hydrolyzed with hydrochloric acid. Hexosamine obtained form the hydrolyzed mucin was assayed by the method of Neuhaus and Letzring[35] using acetylacetone, Ehrlich's reagent, and glucosamine as a standard.

Determinations of Serum Serotonin and Histamine For serum serotonin and histamine determinations, blood was collected from the inferior vena cava of rats upon sacrifice and then serum was obtained from the collected blood by centrifugation. Serum samples were deproteinized by adding perchloric acid at a final concentration of 3% and then centrifuged at 4 °C for 10 min (10000×***g***). Serum serotonin was measured by the method of Shibata *et al*.[36] using high-performance liquid chromatography with electrochemical detection except that 40 mM sodium dihydrogenphosphate used for the mobile phase was replaced by 0.1 M citric acid–0.1 M sodium acetate (0.7 : 1.0, v/v). Methyl serotonin was used as an internal standard. Serum histamine was measured by the methods of Lorenz *et al*.[37] and Shore *et al*.[38] Histamine was reacted with *o*-phthalaldehyde and the intensity of the resultant fluorescence was measured using a spectrofluorophotometer (the excitation wavelength, 360 nm; the emission wavelength, 450 nm).

Measurement of Gastric Mucosal Blood Flow Gastric mucosal blood flow was measured using a laser Doppler flowmeter, Laser Flow BRL-100 (Bio Research Center Co., Nagoya), as described in our previous reports.[25—29] Rats used for this measurement were anesthetized with pentobarbital sodium 10 min before the onset of the measurement and the abdomen was opened on an operation mat. The mat was heated at 37 °C during the operation and blood flow measurement. The laser probe was attached to the serosal side of the corpus mucosa by aid of a cyanoacrylate-typed instantaneous adhesive, Aron Alpha (Toha Gosei Co., Tokyo), and the blood flow changes were monitored on a recorder for at least 5 min after the onset of the measurement. Gastric mucosal blood flow in C48/80-treated rats is expressed as a relative percentage toward the mean value of gastric mucosal blood flow determined in control rats without C48/80 treatment. The values of gastric mucosal blood flow measured in C48/80-untreated rats were constant within at least 5% in standard deviation.

Determinations of MPO and XO Activities in Gastric Mucosal Tissues Treated with and without Gefarnate MPO and XO activities were measured in gastric mucosal tissues treated with and without gefarnate as follows: gastric mucosal tissues were collected from three different rats sacrificed at 3 h after C48/80 treatment. Gastric mucosal tissue samples for the assays of MPO and XO were prepared by the same methods as described above. MPO and XO activities in gastric mucosal tissue samples were measured in the corresponding reaction mixture containing either a solution of gefarnate dissolved in 0.01% Tween 80 or 0.01% Tween 80 (vehicle) by the same MPO and XO assay methods as described above, respectively.

Analysis of Data Results obtained for gastric mucosal and serum components and enzymes and gastric mucosal blood flow are expressed as the mean±S.D. The results were analyzed by computerized statistical package (StatView). Each mean value was compared by one-way analysis of variance (one-way ANOVA) and Fisher's PLSD (Protected Least Significance Difference) for multiple comparisons as the post hoc test. Statistical analyses of the severity of mucosal lesions were carried out using the Kruskal–Wallis test. Values of significance were set at $p<0.05$ for both tests.

RESULTS

Effects of Post-Administered Gefarnate and Teprenone on Gastric Mucosal Lesion Development As shown in Fig. 1, apparent gastric mucosal lesions were observed 0.5 h after a single treatment of C48/80 (0.75 mg/kg) and progressive gastric mucosal lesions were found at 3 h when the severity of gastric mucosal lesions was estimated using the lesion gradation. Oral post-administration of gefarnate (50, 100 or 200 mg/kg) significantly reduced progressive gastric mucosal lesions at 3 h after C48/80 treatment, and this preventive effect of gefarnate occurred in a dose-dependent manner (Fig. 1). The same post-administration of teprenone (200 mg/kg) reduced progressive C48/80-induced gastric mucosal lesions significantly (Fig. 1). There was no significant difference in the reduced severity of gastric mucosal lesions between the C48/80-treatred groups post-administered with of teprenone and gefarnate at a dose of 200 mg/kg (Fig. 1). No gastric mucosal lesion was found in untreated rats with and without drug administration (data not shown).

Effects of Post-Administered Gefarnate and Teprenone on Serum Serotonin and Histamine Concentrations and Gastric Mucosal Blood Flow Rats treated with C48/80 alone had 4.4- and 59.8-fold higher serum serotonin and histamine concentrations, respectively, than untreated control rats at 0.5 h after the treatment and the C48/80-treated group had 2.3- and 5.5-fold higher serum serotonin and histamine concentrations, respectively, than the control group at 3 h (Figs. 2A, B). Post-administration of gefarnate (50, 100 or 200 mg/kg) or teprenone (200 mg/kg) did not affect the increases in serum serotonin and histamine concentrations at 3 h after C48/80 treatment (Figs. 2A, B). The C48/80-treated group had 25.1 and 75.8% of gastric mucosal blood flow level in the control group at 0.5 and 3 h after the treatment, respectively (Fig. 2C). Post-administration of gefarnate (50, 100 or 200 mg/kg) or teprenone (200 mg/kg) did not affect the decrease in gastric mucosal blood flow at 3 h after C48/80 treatment (Fig. 2C).

Effects of Post-Administered Gefarnate and Teprenone on Gastric Adherent Mucus and Gastric Mucosal Hexosamine Contents Gastric adherent mucus and gastric mucosal hexosamine contents in rats treated with C48/80 alone were not different from those in untreated control rats at 0.5 h after the treatment but the C48/80-treated group had significantly lower gastric adherent mucus and gastric mucosal hexosamine contents than the control group at 3 h: the adherent mucus and hexosamine contents in the C48/80-treated group were 23.1 and 74.1% of those in the control group at 3 h (Fig. 3). Post-administered gefarnate (50, 100 or 200 mg/kg) sig-

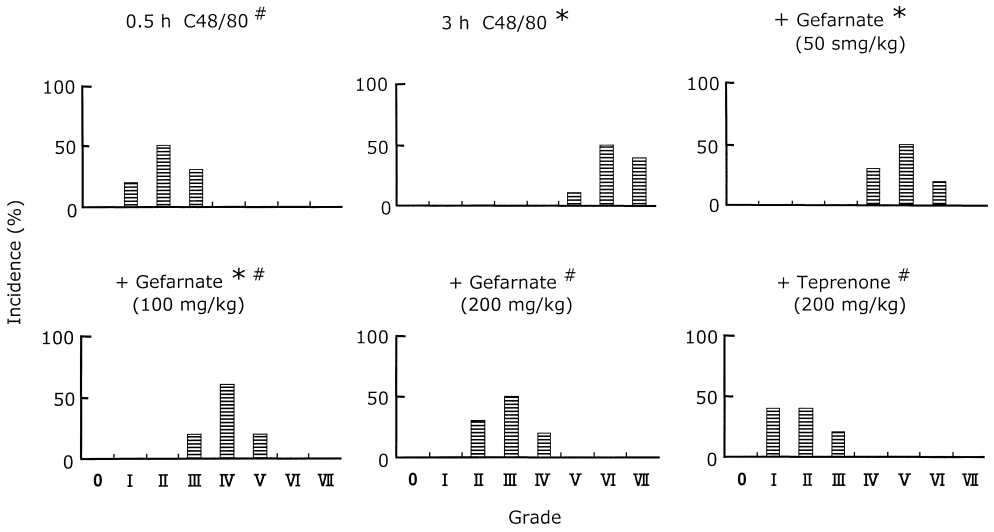

Fig. 1. Effects of Post-Administered Gefarnate and Teprenone on Acute Gastric Mucosal Lesion Development in Rats Treated Once with C48/80

Rats received oral administration of either gefarnate (50, 100 or 250 mg/kg), teprenone (250 mg/kg) or arabic gum (vehicle) at 0.5 h after a single i.p. injection of C48/80 (0.75 mg/kg). The severity of gastric mucosal lesions was estimated 0.5 and 3 h after the C48/80 injection using the lesion gradation. The number of rats used is 10 for each group. * Significantly different from the C48/80-treated group at 0.5 h; $p<0.05$; # significantly different from the C48/80-treated group at 3 h, $p<0.05$.

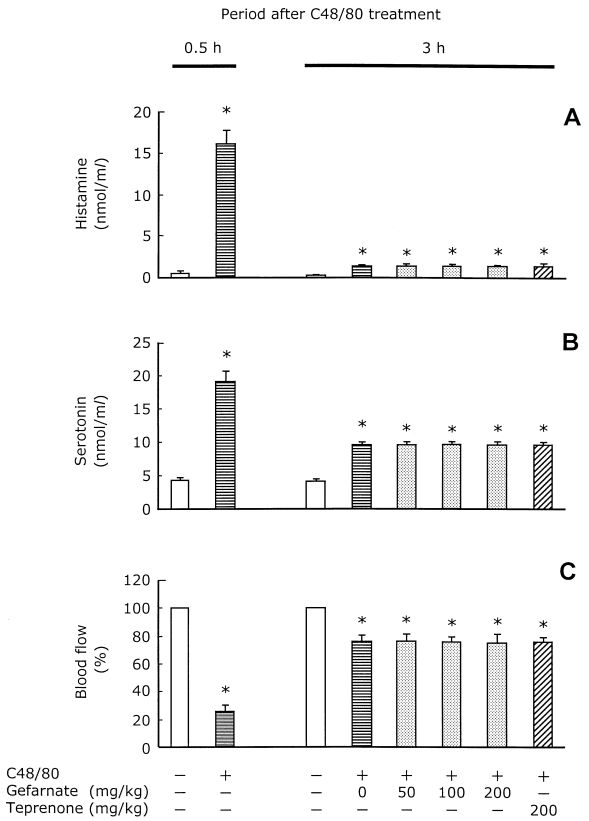

Fig. 2. Effects of Post-Administered Gefarnate and Teprenone on Serum Serotonin (A) and Histamine (B) Concentrations and Gastric Mucosal Blood Flow (C) in Rats Treated Once with C48/80

Rats received oral administration of either gefarnate (50, 100 or 250 mg/kg), teprenone (250 mg/kg) or arabic gum (vehicle) at 0.5 h after a single i.p. injection of C48/80 (0.75 mg/kg). Serum serotonin and histamine and gastric mucosal blood flow were measured 0.5 and 3 h after the C48/80 injection. Each value is a mean±S.D. ($n=5$—10). * Significantly different from the untreated control group at 0.5 or 3 h; $p<0.05$; # significantly different from the C48/80-treated group at 3 h, $p<0.05$.

nificantly attenuated the decreases in gastric adherent mucus and gastric mucosal hexosamine contents at 3 h after C48/80 treatment dose-dependently (Fig. 3). Post-administered

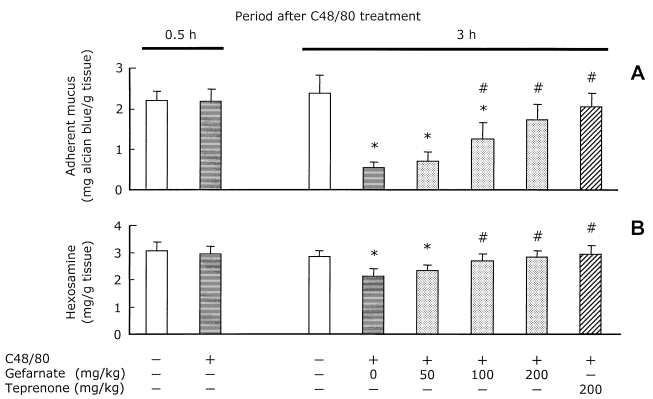

Fig. 3. Effects of Post-Administered Gefarnate and Teprenone on Gastric Adherent Mucus (A) and Gastric Mucosal Hexosamine (B) Contents in Rats Treated Once with C48/80

Rats received oral administration of either gefarnate (50, 100 or 250 mg/kg), teprenone (250 mg/kg) or arabic gum (vehicle) at 0.5 h after a single i.p. injection of C48/80 (0.75 mg/kg). Gastric adherent mucus (A) and gastric mucosal hexosamine (B) were assayed 0.5 and 3 h after the C48/80 injection. Each value is a mean±S.D. ($n=5$—10). * Significantly different from the corresponding control group at 0.5 or 3 h, $p<0.05$; # significantly different from the C48/80-treated group at 3 h, $p<0.05$.

teprenone (200 mg/kg) significantly attenuated the decreases in gastric adherent mucus and gastric mucosal hexosamine contents at 3 h after C48/80 treatment significantly (Fig. 3). These levels of the C48/80-treatred group post-administered with teprenone (200 mg/kg) were not significantly from those of C48/80-treatred group post-administered with the same dose of gefarnate (Fig. 3).

Effects of Post-Administered Gefarnate and Teprenone on Gastric Mucosal TBARS Content and MPO and XO Activities Gastric mucosal TBARS content and MPO and XO activities in rats treated with C48/80 alone were significantly higher than those in untreated control rats at 0.5 h after the treatment and the C48/80-treated group had further increases in gastric mucosal TBARS content and MPO and XO activities at 3 h; the TBARS content and MPO and XO activities in the C48/80-treated group were 1.9-, 3.1-, and 3.7-fold, respectively, higher than those in the control group at

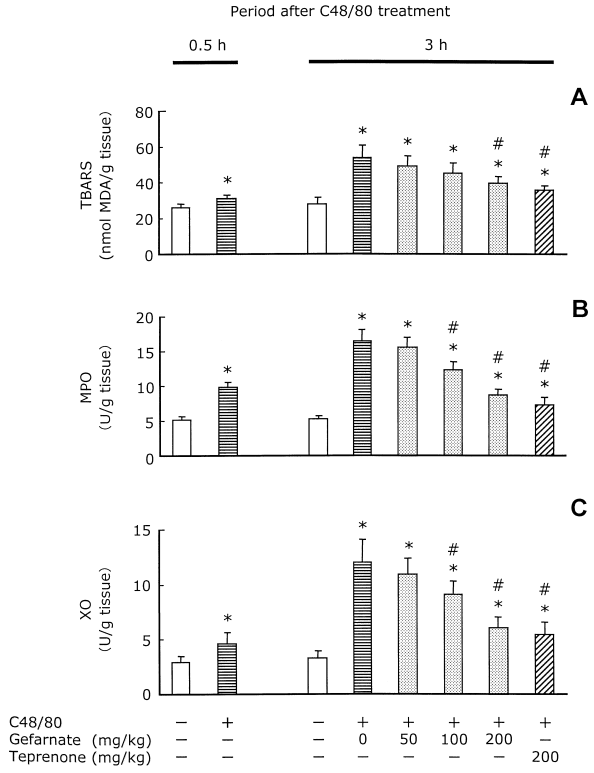

Fig. 4. Effects of Post-Administered Gefarnate and Teprenone on Gastric Mucosal TBARS Content (A) and MPO (B) and XO (C) Activities in Rats Treated Once with C48/80

Rats received oral administration of either gefarnate (50, 100 or 250 mg/kg), teprenone (250 mg/kg) or arabic gum (vehicle) at 0.5 h after a single i.p. injection of C48/80 (0.85 mg/kg). Gastric mucosal TBARS, MPO, and XO were assayed 0.5 and 3 h after the C48/80 injection. Each value is a mean±S.D. (n=5—10). * Significantly different from the corresponding control group at 0.5 or 3 h, $p<0.05$; # significantly different from the C48/80-treated group at 3 h, $p<0.05$.

3 h (Fig. 4). Post-administered gefarnate (50, 100 or 200 mg/kg) significantly attenuated the increases in gastric mucosal TBARS content, MPO and XO activities found at 3 h after C48/80 treatment in a dose-dependent manner (Fig. 4). Post-administered teprenone (200 mg/kg) also attenuated the increases in gastric mucosal TBARS content and MPO and XO activities at 3 h after C48/80 treatment significantly (Fig. 4). These levels of the C48/80-treatred group post-administered with teprenone (200 mg/kg) were not significantly different from those of the C48/80-treatred group post-administered with the same dose of gefarnate (Fig. 4).

Effect of Geranate on *in Vitro* XO and MPO Activities in Gastric Mucosal Tissues When the effect of gefarnate on XO and MPO activities in gastric mucosal tissue preparations from three different C48/80-treated rats was examined at its concentration of 10, 50, and 100 μg/ml, the drug had little effect at any concentrations used (Fig. 5).

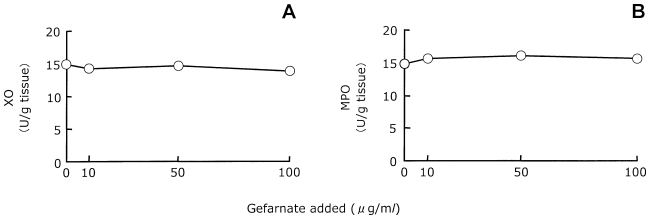

Fig. 5. Effects of Gefarnate on *in Vitro* Gastric Mucosal XO (A) and MPO (B) Activities in Rats Treated Once with C48/80

XO and MPO activities were measured in gastric mucosal tissues from rats treated once with C48/80 in the presence or absence of either gefarnate (10, 50 or 100 μg/l) or Tween 80 (vehicle). Each value is a mean with three different gastric mucosal preparations.

DISCUSSION

The model of acute gastric mucosal lesions in rats treated once with C48/80, a mast cell degranulator, has been thought to be important for clarifying the roles of ischemia-reperfusion, oxidative stress, and inflammation in the pathogenesis of gastritis in humans.[25—27] In addition, it has been shown that *Helicobacter pylori* might cause gastric mucosal inflammation, the reduction of gastric mucosal blood flow, and gastric mucosal microcirculatory disturbances through mast cell degranulation.[39—41] Teprenone, of which chemical structure is similar to that of gefarnate, is known to prevent the progression of C48/80-induced acute gastric mucosal lesions in rats in a dose-dependent manner.[29] The present study has clearly shown that gefarnate administered orally to rats with a single C48/80 treatment after the appearance of acute gastric mucosal lesions prevents the gastric mucosal lesion progression in a dose-dependent manner. This preventive effect of gefarnate (200 mg/kg) was almost equal to that of the same dose of teprenone.

In the present study, marked increases in serum serotonin and histamine concentrations following mast cell degranulation occurred at 0.5 h after C48/80 treatment, at which time gastric mucosal lesions appeared, and less increased serum serotonin and histamine concentrations remained at 3 h, at which time gastric mucosal lesions progressed, as reported previously.[25—29] It is not known that gefarnate affects directly mast cell degranulation. However, oral post-administration of gefarnate (50, 100, 200 mg/kg) had no effect on the increases in serum serotonin and histamine concentrations found at 3 h after C48/80 treatment like post-administered teprenone (200 mg/kg). Teprenone is known to have no effect on increases in serum serotonin and histamine concentrations following mast cell degranualtion in C48/80-treated rats.[28,29] These results suggest that administered gefarnate prevents the progression of C48/80-induced gastric mucosal lesions in rats without affecting the blood levels of histamine and serotonin released from the connective tissue mast cells like teprenone.

In the present study, C48/80-treated rats showed a marked decrease in gastric mucosal blood flow at 0.5 h after the treatment and a partial recovery of the decreased gastric mucosal blood flow at 3 h, as reported previously.[25—29] Thus, a single C48/80 treatment causes an ischemia-reperfusion-like change in gastric mucosal blood flow in rats. Oral post-administration of gefarnate at a dose of 50, 100 or 200 mg/kg did not affect the recovery of decreased gastric mucosal blood flow found at 3 h after C48/80 treatment. Post-administration of teprenone (200 mg/kg) had no effect on the recovery of decreased gastric mucosal blood flow, as reported previously.[29] These results indicate that orally administered gefarnate prevents the progression of C48/80-induced acute gastric mucosal lesions in rats without altering the change in gastric mucosal blood flow like teprenone.

It has been shown that neutrophil infiltration, an increase in XO activity, an enhancement of lipid peroxidation, and a

decrease in mucus level in gastric mucosal tissues contribute to the progression of C48/80-induced acute gastric mucosal lesions in rats.[25,26] We have reported that orally administered teprenone exerts protective and preventive actions against C48/80-induced acute gastric mucosal lesions in rats by preservation of gastric mucus synthesis and secretion and also by inhibition of neutrophil infiltration, an increase in XO activity, and an enhancement of lipid peroxidation in the gastric mucosa.[28,29] Gastric mucus plays a critical role in the primary defense of the gastric mucosa and provides a protective barrier in the gastric epithelium.[42] It is known that mucin interacts with ROS *in vitro*.[43] It is also known that gastric mucus plays an important role in protecting the gastric mucosa of rats against ischemia-reperfusion stress.[44] In the present study, apparent decreases in gastric adherent mucus and gastric mucosal hexosamine contents were found 3 h after C48/80 treatment, as shown in our previous reports.[25,28,29] Post-administered gefarnate (50, 100 or 200 mg/kg) attenuated the decreases in gastric adherent mucus and gastric mucosal hexosamine contents found at 3 h after C48/80 treatment in a dose-dependent manner. These attenuating effects of gefarnate (200 mg/kg) were almost equal to those of the same dose of teprenone. Teprenone is known to protect cultured rat gastric mucosal cells against ROS by increasing mucus production.[21] NADPH oxidoreductase in activated neutrophils generates O_2^-, one of ROS.[45] XO also generates ROS such as O_2^- and H_2O_2 during the oxidation of hypoxanthine or xanthine.[46,47] From these findings, it seems likely that orally administered gefarnate exerts a preventive effect on the progression of C48/80-induced acute gastric mucosal lesions in rats by protecting the gastric mucosal barrier and tissue against the attack of ROS derived from infiltrated neutrophils and the xanthine–XO system through preservation of gastric mucus like teprenone. It has been reported that fasted rats administered orally with gefarnate (100 mg/kg/d) for 5 d have increased hexosamine levels in the pyloric tissue of the stomach.[3] However, the mechanism by which orally administered gefarnate causes an increase in gastric mucus levels in rats has not been clarified yet.

In the present study, gefarnate (50, 100 or 200 mg/kg) administered at 0.5 h after C48/80 treatment attenuated the further increases in the activities of gastric mucosal MPO, an index of tissue neutrophil infiltration,[30] and XO and the content of TBARS, an index of lipid peroxidation, found at 3 h after C48/80 treatment in a dose-dependent manner. These attenuating effects of gefarnate (200 mg/kg) were almost equal to those of the same dose of teprenone. Accordingly, it is conceivable that gefarnate prevents the progression of C48/80-induced acute gastric mucosal lesions in rats by inhibition of neutrophil infiltration, an increase in XO activity, and an enhancement of lipid peroxidation in the gastric mucosa like teprenone. Lipid peroxidation is known to occur *via* ROS generated not only by the xanthine–XO system but also by NADPH oxidoreductase in activated neutrophils.[48,49] It is also known that MPO mediates lipid peroxidation in the presence of H_2O_2 with halide ions.[50] Therefore, we examined whether gefarnate inhibits XO and MPO activities in gastric mucosal tissues prepared from C48/80-treated rats. As a result, gefarnate at various concentrations up to 100 μg/ml had no inhibitory action on *in vitro* gastric mucosal XO and MPO activities. This result may allow us to assume that post-administered gefarnate does not attenuate increased gastric mucosal XO and MPO activities at a progressed stage of C48/80-induced acute gastric mucosal lesions in rats through its direct inhibition of both enzyme activities in the gastric mucosal tissue. It has been indicated that an increase in gastric mucosal XO activity is closely related to neutrophil infiltration into gastric mucosal tissues during the progression of C48/80-induced acute gastric mucosal lesions in rats.[26] Teprenone is known to have no inhibitory action on *in vitro* gastric mucosal MPO and XO activities.[20,29] However, teprenone is known to inhibit the adhesion of activated neutrophils to endothelial cells and the expression of CD11b/CD18a on the activated neutrophils.[22] Therefore, it seems likely that post-administered gefarnate inhibits neutrophil infiltration into the gastric mucosal tissue of C48/80-treated rats and thereby reduces an increase in gastric mucosal XO activity in the tissue like teprenone. It is known that gefarnate and teprenone inhibit lipid peroxidation induced by 2,2′-azobis(2,4-dimehtlylvaleronitrile), a lipid-soluble radical initiator, in linoleic acid, although these drugs have no activity to scavenge O_2^- *in vitro*.[51,52] However, teprenone is known to have no inhibitory action on lipid peroxidation induced by 2,2′-azobis(2-amidinopropane), a water-soluble radical initiator, in gastric mucosal tissues.[53] Therefore, it is unclear at present whether post-administered gefarnate reduces an increase in gastric mucosal lipid peroxidation during the progression of C48/80-induced acute gastric mucosal lesions in rats by its direct inhibition of enhanced lipid peroxidation in the tissue. However, there seems to be a possibility that post-administered gefarnate reduces an increase in gastric mucosal lipid peroxidation during the progression of C48/80-induced acute gastric mucosal lesions in rats by its indirect inhibition of enhanced lipid peroxidation in the tissue through its inhibitory action on neutrophil infiltration into the tissue like teprenone.

It has been reported that gefarnate (100 mg/kg/d) pre-administered to rats for 7 d protects against WIRS-induced gastric mucosal lesions by attenuating decreased endogenous prostaglandin E_2 and prostacyclin levels.[10] However, it has been shown that when gefarnate (100 mg/kg/d) is subcutaneously administered to normal rats for 7 d, no significant increases in prostaglandin E_2 and prostacyclin levels occur in the gastric mucosa.[54] We have reported that pretreatment with a low dose of indomethacin (5 mg/kg), which is known to inhibit prostaglandin production, but not to induce gastric mucosal lesions, had no effect on the formation and progression of C48/80-induced gastric mucosal lesions in rats.[55] Accordingly, it seems unlikely that orally administered gefarnate prevents the progression of C48/80-induced acute gastric mucosal lesions in rats by maintaining prostaglandin levels in the gastric mucosa.

In conclusion, the results of the present study indicate that orally administered gefarnate exerts a preventive effect on acute gastric mucosal lesion progression in C48/80-treated rats and that this preventive effect of gefarnate is similar to that of teprenone. These results also suggest that the administered gefarnate could exert this preventive effect by suppressing mucus depletion, neutrophil infiltration, and oxidative stress in the gastric mucosa like teprenone.

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