Protective Effect of Teprenone Against Acute Gastric Mucosal Lesions Induced by Compound 48/80, a Mast Cell Degranulator, in Rats

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Abstract. The protective effect of teprenone, an anti-ulcer drug, against acute gastric mucosal lesions was examined in rats with a single intraperitoneal injection of compound 48/80 (0.75 mg/kg). Teprenone (50, 100, or 200 mg/kg) was orally administered 0.5 h before compound 48/80 treatment. Administered teprenone prevented gastric mucosal lesion development found at 3 h after compound 48/80 treatment dose-dependently, although no dose of teprenone affected the decreased gastric mucosal blood flow and increased serum serotonin and histamine concentrations found at 3 h after the treatment. Increases in the activities of myeloperoxidase (an index of neutrophil infiltration) and xanthine oxidase and the content of thiobarbituric acid reactive substances (an index of lipid peroxidation) and decreases in the contents of hexosamine (a marker of gastric mucus) and adherent mucus occurred in gastric mucosal tissues at 3 h after compound 48/80 treatment. Administered teprenone dose-dependently attenuated all these changes found at 3 h after compound 48/80 treatment. These results indicate that orally administered teprenone protects against compound 48/80-induced acute gastric mucosal lesions in rats possibly through its stimulatory action on gastric mucus synthesis and secretion and its inhibitory action on neutrophil infiltration and enhanced lipid peroxidation in the gastric mucosal tissue.

Keywords: compound 48/80, gastric mucosal lesion (rat), teprenone, gastric mucus, neutrophil infiltration

Introduction

Teprenone (6,10,14,18-teramethyl-5,9,13,17-nonadecatetraene-2-one), an acyclic polyisoprenoid, that is known as tetraprenylacetone or geranylgeranylacetone, is an anti-ulcer drug developed in Japan. This drug is clinically used for treatments of gastric ulcers and gastritis (1–3). Teprenone is known to stimulate gastric mucous synthesis and secretion in rat gastric cultured cells (4–6) and in gastric tissues of rats (7, 8). This drug is also known to increase gastric mucus level in the ulcerated and intact regions of the stomach of patients (9). It has been shown that teprenone exerts a protective effect against acute gastric mucosal lesions in various in vivo experimental models through preservation of gastric mucus synthesis and secretion (10–14). It has also been shown that teprenone promotes the healing of acetic acid-induced chronic gastric ulcers in rats (10, 15).

It has been shown that teprenone protects cultured rat gastric mucosal cells against reactive oxygen species (ROS) such as superoxide radical by increasing the production of mucus (16). It is known that gastric mucus interacts with ROS, especially hydroxyl radical in vitro (17). We have reported that teprenone exerts protective and preventive effects against acute gastric mucosal lesions in rats with water immersion restraint stress 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 (13). We have also reported that teprenone promotes the healing of acetic acid-induced chronic gastric ulcers in...
rats by inhibiting neutrophil infiltration and enhanced lipid peroxidation as well as by stimulating gastric mucus secretion in ulcerated gastric tissues (15). 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 (18).

Compound 48/80 is known to cause degranulation of connective tissue mast cells, but not mucosal mast cells, with release of serotonin and histamine from the cells (19, 20). We have shown in rats with a single treatment of compound 48/80 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 (21). We have also shown in rats treated once with compound 48/80 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 (22). Furthermore, it has been shown in rats treated once with compound 48/80 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 no important role in the pathogenesis of compound 48/80-induced gastric mucosal lesions (21, 23). Our recent reports have shown that ebselen, which is known to possess not only glutathione peroxidase-like activity but also antioxidative and anti-inflammatory properties, exerts a protective effect against the formation and progression of compound 48/80-induced acute gastric mucosal lesions in rats (24, 25). Therefore, there is a possibility that teprenone exerts a protective effect against acute gastric mucosal lesions in rats treated once with compound 48/80 through its action to stimulate gastric mucus synthesis and secretion and/or to inhibit neutrophil infiltration and enhanced lipid peroxidation in the gastric mucosal tissue.

In the present study, we attempted to clarify whether teprenone protects against compound 48/80-induced acute gastric mucosal lesions in rats by examining the effect of orally pre-administered teprenone on the lesion development and changes in the gastric mucosal activities of myeloperoxidase (MPO), an index of tissue neutrophil infiltration (26), and XO and the gastric mucosal contents of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation; hexosamine, a marker of gastric mucus; and adherent mucus with the lesion development in rats with a single compound 48/80 treatment. We further examined the effect of the pre-administered teprenone on changes in gastric mucosal blood flow and serum serotonin and histamine concentrations with gastric mucosal lesion development in the compound 48/80-treated rats.

**Materials and Methods**

**Materials**

Compound 48/80, dioctyl sodium sulfosuccinate (DSS), methyl serotonin, 3,3',5,5'-tetramethylbenzidne, and xanthine was purchased from Sigma Chemical Co. (St. Louis, MO, USA); alcian blue GX8, N,N-dimethylformamide, ethylenediaminetaetraacetic acid (EDTA), glucosamine, N6-monomethyl-L-arginine monooacetate (L-NMMA), N6-monomethyl-D-arginine monooacetate (D-NMMA), 2-thiobarbituric acid, and other chemicals were from Wako Pure Chemical Ind., Ltd. (Osaka). Teprenone without any additive was kindly provided by Eisai Co. (Tokyo).

**Animals**

Male Wistar rats, aged six weeks, were purchased from Japan SLC Co. (Hamamatsu). 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) and tap water ad libitum for one week. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of Fujita Health University.

**Gastric mucosal lesion induction by compound 48/80**

Compound 48/80 (0.75 mg/kg body weight), dissolved in distilled water, was intraperitoneally injected to 7-week-old rats, which had been fasted for 24 h, as described previously (21–25). 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 3 h after compound 48/80 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...
Teprenone (50, 100, or 200 mg/kg body weight) was orally administered to fasted rats with a stomach tube injected with another experiment, rats were injected subcutaneously arabic gum used as a vehicle at the same time point. In untreated rats that received an equal volume of 0.5% arabic gum at a constant dosing volume of 5 ml/kg body weight. Teprenone (50, 100, or 200 mg/kg body weight) was therefore administered to fasted rats with a stomach tube at 0.5 h before compound 48/80 treatment. Teprenone-untreated rats that received an equal volume of 0.5% arabic gum used as a vehicle at the same time point. In another experiment, rats were injected subcutaneously injected with L-NMMA, a non-specific inhibitor of nitric oxide synthase (NOS) (100 mg/kg body weight), or its D-enantiomer D-NMMA (100 mg/kg body weight), which was dissolved in isotonic saline, immediately after oral administration of teprenone (200 mg/kg body weight) and then treated with compound 48/80. Rats without L-NMMA or D-NMMA injection were given the same volume of the vehicle isotonic saline in the same manner at the same time point.

Determinations of gastric mucosal MPO, XO, TBARS, hexosamine, and adherent mucus

Gastric mucosal MPO was assayed by the method of Suzuki et al. (27). For the assay of this enzyme, gastric mucosal tissues were homogenized in 9 vol 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), the homogenate was centrifuged at 4°C for 20 s. The resulting supernatant was dialyzed against 100 vol of the same buffer at 4°C for 24 h. MPO activity was assessed by measuring the H2O2-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 (28). For this enzyme assay, gastric mucosal tissues were homogenized in 9 vol of ice-cold 0.25 M sucrose. The homogenate was sonicated as described above. The sonicated homogenate was centrifuged at 4°C (10,000 × g, 20 min), and the resultant supernatant was dialyzed against 100 vol of the same buffer at 4°C for 24 h. MPO activity was assessed by measuring 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 (28). For this enzyme assay, gastric mucosal tissues were homogenized in 9 vol of ice-cold 0.25 M sucrose. The homogenate was sonicated as described above. The sonicated homogenate was centrifuged at 4°C (10,000 × g, 20 min), and the resultant supernatant was dialyzed against 100 vol 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 deter-

mined by the thiobarbituric acid method of Ohkawa et al. (29) except that 1.0 mM EDTA was added to the reaction medium. For this determination, gastric mucosal tissues were homogenized in 9 vol of ice-cold 20 mM EDTA. The amount of TBARS is expressed as that of malondialdehyde (MDA) equivalents. Gastric mucosal hexosamine was assayed by the method of Neuhaus and Letzring (30) Briefly, gastric mucosal mucin was extracted with Triton X-100 and then hydrolyzed with hydrochloric acid. Hexosamine obtained from the hydrolyzed mucin was assayed using acetylacetone and Ehrlich’s reagent. Gastric adherent mucus was assayed by the method of Kitagawa et al. (31) as follows: the removed stomach was cut open along the greater curvature and rinsed with 10 ml of ice-cold 0.25 M sucrose. Then, 50 mm2 (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 the dye complex with mucus was extracted with 30% DSS for 2 h. After centrifugation (3,000 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 with alcian blue solutions of known concentrations. The concentration of gastric mucosal adherent mucus is expressed as that of alcian blue adhered to the gastric mucosal surface (μg/g tissue).

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 (10,000 × g). Serum serotonin was measured by the method of Shibata et al. (32) using high-performance liquid chromatography with electrochemical detection except that 40 mM sodium dihydrogen phosphate 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 Shore et al. (34). Histamine was reacted with α-phthalaldehyde and the intensity of the resultant fluorescence was measured using a spectrophotometer (excitation wavelength, 360 nm; 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 (21 – 25). 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 compound 48/80-treated rats is expressed as a relative percentage toward the mean value of gastric mucosal blood flow determined in control rats without compound 48/80 treatment. The values of gastric mucosal blood flow measured in compound 48/80-untreated rats were constant within at least 5% in standard deviation.

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 a 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.

Table 1. Effect of teprenone administration on gastric mucosal lesion development in rats with a single compound 48/80 injection

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lesion index (%)</th>
<th>P-value (vs compound 48/80-treated group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 48/80</td>
<td>0 0 0 0 20 30 50</td>
<td>—</td>
</tr>
<tr>
<td>+ Teprenone (50 mg/kg)</td>
<td>0 0 0 20 50 30 0</td>
<td>0.05</td>
</tr>
<tr>
<td>+ Teprenone (100 mg/kg)</td>
<td>0 0 20 60 0 0 0</td>
<td>0.05</td>
</tr>
<tr>
<td>+ Teprenone (200 mg/kg)</td>
<td>70 50 10 0 0 0 0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Rats received oral administration of teprenone (50, 100, or 200 mg/kg) or arabic gum (vehicle) at 0.5 h before injection of compound 48/80 (0.75 mg/kg, i.p.) and the severity of gastric mucosal lesions was estimated 3 h after the compound 48/80 injection. The number of rats used in each group is 10.
and then the effect of the administered teprenone on gastric mucosal MPO and XO activities and TBARS content was examined 3 h after the treatment, the results shown in Fig. 2 were obtained. Rats treated with compound 48/80 alone had significantly higher gastric mucosal MPO activity than untreated control rats at 3 h after the treatment; the MPO activity in the compound 48/80-treated group was 3.1-fold higher than that in the control group. Pre-administered teprenone significantly attenuated the increase in gastric mucosal MPO activity at 3 h after compound 48/80 treatment dose-dependently. The compound 48/80-treated group had significantly higher TBARS content than the control group at 3 h after the treatment; the TBARS content in the compound 48/80-treated group was 2.1-fold higher than that in the control group. Pre-administered teprenone significantly attenuated the increase in gastric mucosal XO activity at 3 h after compound 48/80 treatment dose-dependently. The compound 48/80-treated group had significantly higher TBARS content than the control group at 3 h after the treatment; the TBARS content in the compound 48/80-treated group was 2.1-fold higher than that in the control group. Pre-administered teprenone significantly attenuated the increase in gastric mucosal XO activity at 3 h after compound 48/80 treatment dose-dependently.

**Fig. 1.** Effect of teprenone administration on serum serotonin (A) and histamine (B) concentrations and gastric mucosal blood flow (C) in rats with and without a single compound 48/80 injection. Rats with and without injection of compound 48/80 (0.75 mg/kg, i.p.) received oral administration of teprenone (50, 100, or 200 mg/kg) or arabic gum (vehicle) at 0.5 h before the compound 48/80 injection; and then serum serotonin and histamine concentrations and gastric mucosal blood flow were measured 3 h after the compound 48/80 injection as described in Materials and Methods. Each value is a mean ± S.D. *Significantly different from control rats untreated with both compound 48/80 and teprenone, P<0.05.

**Fig. 2.** Effect of teprenone administration on gastric mucosal MPO (A) and XO (B) activities and TBARS content (C) in rats with and without a single compound 48/80 injection. Rats with and without injection of compound 48/80 (0.75 mg/kg, i.p.) received oral administration of teprenone (50, 100, or 200 mg/kg) or arabic gum (vehicle) at 0.5 h before the compound 48/80 injection; and then gastric mucosal MPO, XO, and TBARS were assayed 3 h after the compound 48/80 injection as described in Materials and Methods. Each value is a mean ± S.D. *Significantly different from control rats untreated with both compound 48/80 and teprenone, P<0.05. #Significantly different from rats treated with compound 48/80 alone, P<0.05.

When teprenone at a dose of 50, 100, or 200 mg/kg...
was orally administered to rats with and without compound 48/80 treatment 0.5 h before the treatment and then the effect of the administered teprenone on gastric mucosal hexosamine and gastric adherent mucus content was examined 3 h after the treatment, the results shown in Fig. 3 were obtained. Gastric mucosal hexosamine content in rats treated with compound 48/80 alone was significantly lower than that in untreated control rats at 3 h after the treatment: the gastric mucosal hexosamine content in the compound 48/80-treated rats was 72.5% of that in the control group. Pre-administered teprenone significantly attenuated the decrease in gastric mucosal hexosamine content at 3 h after compound 48/80 treatment dose-dependently. The gastric mucosal hexosamine content in the compound 48/80-treated group pre-administered with teprenone (100 mg/kg) was similar to that in the control group, while the compound 48/80-treated group pre-administered with teprenone (200 mg/kg) had significantly higher gastric mucosal hexosamine content than the control group. The compound 48/80-treated group had significantly lower gastric adherent mucus content than the control group; the gastric adherent mucus content in the compound 48/80-treated group was 36.9% of that in the control group. Pre-administered teprenone significantly attenuated the decrease in gastric adherent mucus content at 3 h after compound 48/80 treatment dose-dependently. The gastric adherent mucus content in the compound 48/80-treated group pre-administered with teprenone (200 mg/kg) was similar to that in the control group. Teprenone administered to untreated rats increased the gastric mucosal hexosamine and gastric adherent mucus contents in a dose-dependent manner.

### Effect of co-administration of teprenone and L-NMMA on gastric mucosal TBARS and hexosamine and gastric adherent mucus contents

When teprenone (200 mg/kg) was pre-administered with L-NMMA (100 mg/kg), a NOS inhibitor, or D-NMMA (100 mg/kg) to compound 48/80-treated rats, co-administration of L-NMMA almost completely counteracted the preventive effect of pre-administered teprenone on the increase in gastric mucosal TBARS

### Table 2. Effect of co-administration of teprenone and L-NMMA on gastric mucosal TBARS, hexosamine, and gastric adherent mucus contents in rats with and without a single compound 48/80 injection

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TBARS (nmol MDA/g tissue)</th>
<th>Hexosamine (mg/g tissue)</th>
<th>Adherent mucus (mg alcian blue/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>25.8 ± 2.5</td>
<td>3.14 ± 0.23</td>
<td>2.21 ± 0.18</td>
</tr>
<tr>
<td>Compound 48/80</td>
<td>10</td>
<td>56.3 ± 3.7*</td>
<td>2.11 ± 0.33*</td>
<td>0.81 ± 0.24*</td>
</tr>
<tr>
<td>+ Teprenone</td>
<td>10</td>
<td>33.5 ± 3.1*</td>
<td>3.55 ± 0.28**</td>
<td>1.95 ± 0.27*</td>
</tr>
<tr>
<td>+ Teprenone and L-NMMA</td>
<td>10</td>
<td>55.8 ± 4.1*</td>
<td>2.18 ± 0.25*</td>
<td>0.83 ± 0.25*</td>
</tr>
<tr>
<td>+ Teprenone and D-NMMA</td>
<td>10</td>
<td>34.1 ± 2.8*</td>
<td>3.58 ± 0.31*</td>
<td>1.96 ± 0.32*</td>
</tr>
</tbody>
</table>

Rats treated with compound 48/80 (0.75 mg/kg, i.p.) were given L-NMMA (100 mg/kg, i.p.), D-NMMA (100 mg/kg, i.p.), or isotonic saline (vehicle, i.p.) immediately after oral administration of teprenone (200 mg/kg) or arabic gum (vehicle) at 0.5 h before the compound 48/80 injection. Control rats were given isotonic saline and arabic gum. Gastric mucosal TBARS and hexosamine and gastric mucus in each group were assayed 3 h after the compound 48/80 treatment as described in Materials and Methods. Each value is a mean ± S.D. *Significantly different from the control group, P<0.05. **Significantly different from the compound 48/80-treated group, P<0.05.
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Discussion

The present study has clearly shown that teprenone pre-administered orally at a dose of 50, 100, or 200 mg/kg exerts a protective effect against acute gastric mucosal lesions in rats with a single compound 48/80 treatment in a dose-dependent manner.

In the present study, increased serum serotonin and histamine concentrations were observed at 3 h after a single compound 48/80 treatment, at which time gastric mucosal lesions developed, as reported previously (21 – 25). The increases in serum serotonin and histamine concentrations were not altered by the pre-administration of teprenone. These results suggest that teprenone protects against acute gastric mucosal lesions in compound 48/80-treated rats without affecting the blood levels of histamine and serotonin released from the connective tissue mast cells.

It has been reported that teprenone (100 mg/kg per day, p.o.) pre-administered to rats for 3 consecutive days attenuates reduced gastric mucosal blood flow after hemorrhage and retransfusion in the corpus and antral regions (35), although there is a report showing no change in gastric mucosal blood flow in normal rats with a single administration of teprenone (200 mg/kg, p.o.) (36). We have shown in rats treated once with compound 48/80 (0.75 mg/kg, i.p.) that a marked decrease in gastric mucosal blood flow occurs 0.5 h after the treatment and the decreased gastric mucosal blood flow is partially recovered at 3 h (21 – 25).

Thus, gastric mucosal blood flow shows an ischemia-reperfusion-like change in compound 48/80-treated rats. In the present study, no dose of teprenone pre-administered to compound 48/80-treated rats affected the reduction of gastric mucosal blood flow found at 3 h after the treatment. In addition, there was no change in gastric mucosal blood flow in normal rats given teprenone. These results indicate that teprenone protects against acute gastric mucosal lesions in compound 48/80-treated rats without altering the change in gastric mucosal blood flow.

We have reported that orally administered teprenone exerts protective and preventive actions against stress-induced acute gastric mucosal lesions in rats by inhibition of neutrophil infiltration and enhanced lipid peroxidation in the gastric mucosa as well as by preservation of gastric mucus synthesis and secretion (13). Teprenone is known to inhibit the adhesion of activated neutrophils to endothelial cells (18). Teprenone is also known to protect against stress-induced gastric mucosal injury in rats by inhibiting increases in the concentrations of adenosine, which is metabolized to hypoxanthine and xanthine via XO, uric acid (a metabolite of the XO-catalyzed oxidation of xanthine) and lipid peroxide, and a decrease in hypoxanthine concentration in the gastric mucosal tissue (12). XO generates ROS such as superoxide radical and hydrogen peroxide during the oxidation of hypoxanthine or xanthine (37, 38). It has been shown that neutrophil infiltration, increased XO activity, and enhanced lipid peroxidation in gastric mucosal tissues contribute to the development of compound 48/80-induced acute gastric mucosal lesions (21, 22).

In the present study, teprenone pre-administered to compound 48/80-treated rats attenuated the increases in the activities of gastric mucosal MPO, an index of tissue neutrophil infiltration (26), and XO found at 3 h after the treatment in a dose-dependent manner. In addition, teprenone given to normal rats caused a dose-dependent reduction of gastric mucosal MPO activity, as reported previously (13). However, no dose of teprenone affected gastric mucosal XO activity in normal rats. It is known that teprenone does not inhibit gastric mucosal MPO activity in vitro (13). It has been suggested that the increase in gastric mucosal XO activity in compound 48/80-treated rats is related to neutrophil infiltration into the gastric mucosal tissue (22). We have observed that teprenone at a concentration of 10 to 100 µg/ml does not inhibit XO activity in gastric mucosal tissue preparations from compound 48/80-treated rats (unpublished data). Lipid peroxidation occurs via ROS generated not only by the xanthine-XO system but also by activated NADPH oxidoreductase in neutrophils (39, 40). It is also known that MPO mediates lipid peroxidation in the presence of hydrogen peroxide with halide ions (41). In the present study, teprenone pre-administered to compound 48/80-treated rats attenuated the increased content of gastric mucosal TBARS, an index of lipid peroxidation, found at 3 h after the treatment in a dose-dependent manner. Teprenone at a concentration of 10 to 100 µg/ml does not inhibit in vitro lipid peroxidation induced by 2,2′-azobis(2-amidinopropane), a water-soluble radical initiator, in gastric mucosal tissue preparations from compound 48/80-treated rats (15). Teprenone at various concentrations up to 100 µg/ml has no activity to scavenge superoxide radical and hydroxyl radical (13). In addition, teprenone administered to normal rats did not influence the gastric mucosal TBARS content. Therefore, these findings suggest that teprenone could exert a protective effect against acute gastric mucosal lesions in compound
48/80-treated rats through its inhibitory action on neutrophil infiltration into the gastric mucosal tissue.

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 (17). It is also known that gastric mucus plays an important role in protecting the gastric mucosa of rats from ischemia-reperfusion stress (43). In the present study, the decreased gastric mucosal content of hexosamine, a marker of gastric mucus, in compound 48/80-treated rats was found at 3 h after the treatment, as reported previously (21). A marked decrease in gastric adherent mucus content was also observed in the compound 48/80-treated rats at the same time point. Pre-administered teprenone attenuated the compound 48/80-induced reductions in gastric mucosal hexosamine and gastric adherent mucus contents in a dose-dependent manner. In addition, compound 48/80-treated rats given teprenone (200 mg/kg) had a little more gastric mucosal hexosamine content than normal rats not given teprenone. Teprenone administered to normal rats caused dose-dependent increases in gastric mucosal hexosamine and gastric adherent mucus contents, as reported previously (13). Teprenone is known to protect cultured rat gastric mucosal cells against ROS by increasing the mucus production (16).

However, the drug has no activity to scavenge ROS and to inhibit lipid peroxidation in vitro, as described above. Teprenone is known to stimulate gastric mucus synthesis and secretion through a nitric oxide (NO)-dependent pathway (7, 8). Therefore, we examined a possibility that the preventive effect of pre-administered teprenone on the increase in gastric mucosal TBARS content in compound 48/80-treated rats is due to the indirect antioxidant action of the drug, that is, the ROS scavenging action of gastric mucus increased through the NO-mediated stimulatory action of the drug on gastric mucus synthesis and secretion. The preventive effect of pre-administered teprenone on the increase in gastric mucosal TBARS content and the decreases in gastric mucosal hexosamine and gastric adherent mucus contents in compound 48/80-treated rats was found to be counteracted by co-treatment with D-NMMA, which is a non-selective NOS inhibitor, but not L-NMMA, which does not work as a NOS inhibitor. This result suggests the possibility that teprenone exerts an indirect antioxidant action in compound 48/80-treated rats by increasing gastric mucus through its NO-mediated stimulatory action on gastric mucus synthesis and secretion. Our previous report has indicated that pre-administered teprenone prevents decreases in gastric mucus synthesis and secretion in rats with water immersion restraint stress by maintaining constitutive NOS activity in the gastric mucosal tissue (44). Kim and Kim (45) have reported that gastric mucus depletion associated with decreases in gastric mucosal NOS activity, the expression of neuronal NOS (a constitutive NOS), and cyclic GMP content contributes, at least in part, to the development of ischemia-reperfusion-induced gastric mucosal injury, in which oxidative damage is involved, in rats. Accordingly, it is conceivable that teprenone protects against acute gastric mucosal lesions in compound 48/80-treated rats by protecting the gastric mucosal barrier and tissue from the attack of ROS derived from infiltrated neutrophils and the xanthine-XO system and/or lipid peroxidation mediated by the ROS through an indirect antioxidant action depending on its NO-mediated stimulatory action on gastric mucus synthesis and secretion.

Mikami et al. (46) have shown that endogenous prostaglandins in the gastric mucosa of rats may play a role, at least to some extent, in the teprenone-induced increase in the activity of UDP-galactosyltransferase, a key enzyme in the synthesis of mucus glycoprotein. In contrast, Terano et al. (4) have demonstrated that teprenone stimulates the synthesis and secretion of mucus by cultured gastric mucosal cells without increasing prostaglandin synthesis in the cells. It has been suggested that endogenous prostaglandins may be partially involved in the protective effect of teprenone against ethanol-induced gastric mucosal injury in rats (47), although there is a report suggesting that the protective effect of teprenone against ethanol-induced gastric mucosal injury is not mediated by endogenous prostaglandins (48). We have demonstrated that when compound 48/80-treated rats are pretreated with a low dose of indomethacin (5 mg/kg), which is known to inhibit prostaglandin production, but not to induce gastric mucosal lesions, the indomethacin pretreatment has no effect on gastric mucosal lesion development in the compound 48/80-treated rats (49). Therefore, it seems unlikely that teprenone exerts a protective effect against compound 48/80-induced acute gastric mucosal lesions through endogenous prostaglandins. Hsu et al. (50) have reported that in rats receiving a single compound 48/80 treatment, basal or histamine-stimulated gastric acid secretion is not stimulated but is rather inhibited by serotonin released from degranulated mast cells. Murakami et al. (10) have shown that pre-administration of teprenone at the antiulcer dose (200 mg/kg, p.o.), although this dose does not reduce gastric acid secretion, effectively prevents a reduction of gastric mucosal hexosamine content in rats subjected to cold-restraint stress. Therefore, it seems unlikely that teprenone stimulates gastric mucus synthesis and secretion in compound 48/80-treated rats by affecting gastric acid.
secretion.

In conclusion, the results of the present study indicate that in rats with a single compound 48\%/80 treatment, orally administered teprenone exerts a protective effect against acute gastric mucosal lesions. The results also suggest that the administered teprenone could exert this protective effect through its stimulatory action on gastric mucus synthesis and secretion and its inhibitory action on neutrophil infiltration and enhanced lipid peroxidation in the gastric mucosal tissue.

References

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