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Preventive effect of teprenone on acute gastric mucosal lesion progression in compound 48/80-treated rats

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Abstract

The preventive effect of teprenone (6,10,14,18-teramethyl-5,9,13,17-nonadecatetaene-2-one), an anti-ulcer drug, on acute gastric mucosal lesion progression was examined in rats with a single intraperitoneal (i.p.) injection of compound 48/80 (0.75 mg/kg). Teprenone (20, 100 or 200 mg/kg), which was orally administered 0.5 h after compound 48/80 treatment at which time gastric mucosal lesions appeared, prevented gastric mucosal lesion development at 3 h after the treatment dose-dependently. Gastric mucosal tissues of compound 48/80-treated rats showed increases in myeloperoxidase (an index of neutrophil infiltration) and xanthine oxidase activities and thiobarbituric acid reactive substances (an index of lipid peroxidation) content and decreases in Se-glutathione peroxidase activity and hexosamine and vitamin E contents at 3 h after the treatment. Post-administered teprenone attenuated all these changes dose-dependently. These results indicate that teprenone prevents acute gastric mucosal lesion progression in compound 48/80-treated rats possibly by suppressing gastric mucus depletion, neutrophil infiltration and oxidative stress in the gastric mucosal tissue.

Keywords: Compound 48/80; Gastric mucosal lesion, rat; Teprenone; Gastric mucus; Neutrophil infiltration; Oxidative stress

1. Introduction

Teprenone (6,10,14,18-teramethyl-5,9,13,17-nonadecatetaene-2-one), an acylic polyisopresnoid, which is known as tetraprenylacetone or geranylgeranylacetone, is an anti-ulcer drug developed in Japan. This drug is clinically used for the treatment of gastric ulcers and gastritis (Arakawa et al., 1984; Shirakabe et al., 1995; Nagasawa et al., 1998). Teprenone is known to stimulate gastric mucus synthesis and secretion in rat gastric cultured cells (Terano et al., 1986a; Hassan et al., 1998; Rokutan et al., 2000) and in gastric tissues of rats (Tatsuta et al., 1995; Nishida and Ohta, 1998). This drug is also known to increase gastric mucus level in the ulcerated and intact regions of the stomach of patients (Nakazawa et al., 1983). 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 (Murakami et al., 1981, 1982; Itoh et al., 1991; Nishida et al., 1998; Saita and Murakami, 2000, Ohta et al., 2003a). It has also been shown that teprenone promotes the healing of acetic acid-induced chronic gastric ulcers in rats (Murakami et al., 1981; Kobayashi et al., 2001).

It is known that teprenone protects cultured rat gastric mucosal cells against reactive oxygen species such as superoxide radical by increasing the production of mucus (Hiraishi et al., 1993). It is also known that gastric mucin interacts with reactive oxygen species, especially hydroxyl radical, in vitro (Grisham et al., 1987). It has been 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 (Nishida et al., 1998). It has also been

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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 (Kobayashi et al., 2001). 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 (Yoshikawa et al., 1999).

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 (Enerback and Lundin, 1974; Irman-Florjanc and Erjavec, 1983). We have shown in rats with a single compound 48/80 treatment 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 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 (Ohta et al., 1997). 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–xanthine oxidase system in the gastric mucosal tissue takes part mainly in the lesion progression (Ohta et al., 1999a). 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 little important role in the pathogenesis of compound 48/80-induced gastric mucosal lesions (Ohta et al., 1997, 1999b). Our recent report has shown that 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 (Ohta et al., 2003a). Therefore, there is a possibility that teprenone exerts a preventive effect on acute gastric mucosal lesion progression in rats treated once with compound 48/80 by attenuating gastric mucus depletion and/or by suppressing neutrophil infiltration and oxidative stress in the gastric mucosal tissue.

The purpose of the present study was to clarify whether teprenone exerts a preventive effect on acute gastric mucosal lesion progression in rats treated once with compound 48/80. In the present study, we examined the effect of orally administered teprenone on acute gastric mucosal lesion progression and changes in the gastric mucosal activities of Se-glutathione peroxidase, myeloperoxidase, an index of tissue neutrophil infiltration (Krawisz et al., 1984), and xanthine oxidase and the gastric mucosal contents of thiobarbituric acid reactive substances, an index of lipid peroxidation, hexosamine, a marker of gastric mucus, and vitamin E with the lesion progression in rats with a single compound 48/80 treatment. We further examined the effect of administered teprenone on changes in gastric mucosal blood flow and serum serotonin and histamine concentrations with gastric mucosal lesion progression in the compound 48/80-treated rats.

2. Materials and methods

2.1. Materials

Compound 48/80, methyl serotonin, 3,3′,5,5′-tetramethylbenzidine and xanthine were purchased from Sigma (St. Louis, MO, USA); *N*,*N*-dimethylformamide, ethylenediaminetetraacetic acid (EDTA), glucosamine, GSH, NADPH, 2-thiobarbituric acid, α-tocopherol, yeast glutathione reductase and other chemicals were from Wako (Osaka, Japan). Teprenone without any additive was kindly provided by Eisai (Tokyo, Japan).

2.2. Animals

Male Wistar rats aged 6 weeks were purchased from Japan SLC (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 (07:00 to 19:00 h). The animals were maintained with free access to rat chow, Oriental MF (Oriental Yeast, Tokyo, Japan) and tap water ad libitum for 1 week. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of Fujita Health University.

2.3. Gastric mucosal lesion induction by compound 48/80 and its estimation

Compound 48/80 (0.75 mg/kg body weight), dissolved in distilled water, was intraperitoneally injected to 7-week-old rats fasted for 24 h, as described previously (Ohta et al., 1997, 1999a,b, 2003a,b). 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 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 reports (Ohta et al., 1997, 1999a,b, 2003a,b): 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.

2.4. Administration of teprenone

Teprenone was suspended in 0.5% arabic gum at a constant dosing volume of 5 ml/kg body weight. Teprenone (20, 100 or 200 mg/kg body weight) was orally administered to fasted rats with a stomach tube at 0.5 h after compound 48/80 treatment. Teprenone-untreated rats received an equal volume of 0.5% arabic gum used as a vehicle at the same time point.

2.5. Determinations of gastric mucosal enzymes and components

Gastric mucosal Se-glutathione peroxidase and myeloperoxidase were assayed by the methods of Hochstein and Utley (1968) and Suzuki et al. (1983), respectively. For the assays of both enzymes, gastric mucosal tissues were homogenized in nine volume 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, Tokyo, Japan), the homogenate was centrifuged at 4 °C (10,000 × *g*, 20 min), and the resultant supernatant was dialyzed against 100 volumes of the same buffer at 4 °C for 24 h. Se-glutathione peroxidase activity was determined at 37 °C by recording the decrease in absorbance at 340 nm following the oxidation of NADPH in the presence of H_2O_2, GSH, and yeast glutathione reductase. One unit (U) of this enzyme is defined as the amount of enzyme oxidizing 1 μmol NADPH per min. Myeloperoxidase activity was assessed by measuring the hydrogen peroxide-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 xanthine oxidase was assayed by the method of Hashimoto (1974). 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. Xanthine oxidase 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 thiobarbituric acid reactive substances were spectrophotometrically determined by the thiobarbituric acid method of Ohkawa et al. (1979) 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 thiobarbituric acid reactive substances is expressed as that of malondialdehyde equivalents. Gastric mucosal hexosamine was assayed by the method of Neuhaus and Letzring (1957). Briefly, gastric mucosal mucin was extracted with Triton X-100 and then hydrolyzed with hydrochloric acid. Hexosamine obtained form the hydrolyzed mucin was measured using acetylacetone and Ehrlich's reagent. Gastric vitamin E was determined by the high-performance liquid chromatographic method of Abe et al. (1975) using fluorescence detection. Vitamin E in gastric mucosal tissues was extracted with *n*-hexane. Its content is expressed as the amount of α-tocopherol.

2.6. 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. (1988) 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. (1972) and Shore et al. (1959). Histamine was reacted with *o*-phthalaldehyde and the intensity of the resultant fluorescence was measured using a spectrophotometer (the excitation wavelength, 360 nm; the emission wavelength, 450 nm).

2.7. Measurement of gastric mucosal blood flow

Gastric mucosal blood flow was measured using a laser Doppler flowmeter, Laser Flow BRL-100 (Bio Research Center, Nagoya), as described in our previous reports (Ohta et al., 1997, 1999a,b, 2003a). 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, 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.

2.8. 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 (ANOVA) and Fisher's Protected Least Significance Difference (PLSD) 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.

3. Results

3.1. Effect of post-teprenone administration on gastric mucosal lesion development

As shown in Table 1, apparent gastric mucosal lesions were observed 0.5 h after a single treatment of compound 48/80 (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. When teprenone at a dose of 20, 100 or 200 mg/kg was orally administered to compound 48/80-treated rats at 0.5 h after the treatment, the drug at a dose of 100 or 200 mg/kg, but not 20 mg/kg, significantly prevented gastric mucosal lesion development found at 3 h after compound 48/80 treatment and this preventive effect of teprenone was dose-dependent (Table 1). The severity of gastric mucosal lesions in the group treated with teprenone (200 mg/kg) was similar to that found at 0.5 h after compound 48/80 treatment (Table 1). No gastric mucosal lesion was found in untreated rats with and without teprenone administration (data not shown).

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

Time after compound 48/80 injection and groups	Lesion index (%)								*P* value
	0	I	II	III	IV	V	VI	VII	
0.5 h									
Compound 48/80	0	20	40	40	0	0	0	0	–
3 h									
Compound 48/80	0	0	0	0	0	30	40	30	–
+ Teprenone (20 mg/kg)	0	0	0	10	20	40	30	0	NS
+ Teprenone (100 mg/kg)	0	0	10	30	40	20	0	0	0.05
+ Teprenone (200 mg/kg)	0	10	40	50	0	0	0	0	0.05

Rats received oral administration of teprenone (20, 100 or 200 mg/kg) or arabic gum (vehicle) at 0.5 h after injection of compound 48/80 (0.75 mg/kg, i.p.) and the severity of gastric mucosal lesions was estimated 0.5 and 3 h after the compound 48/80 injection. The number of rats used in each group is 10. NS indicates not significant.

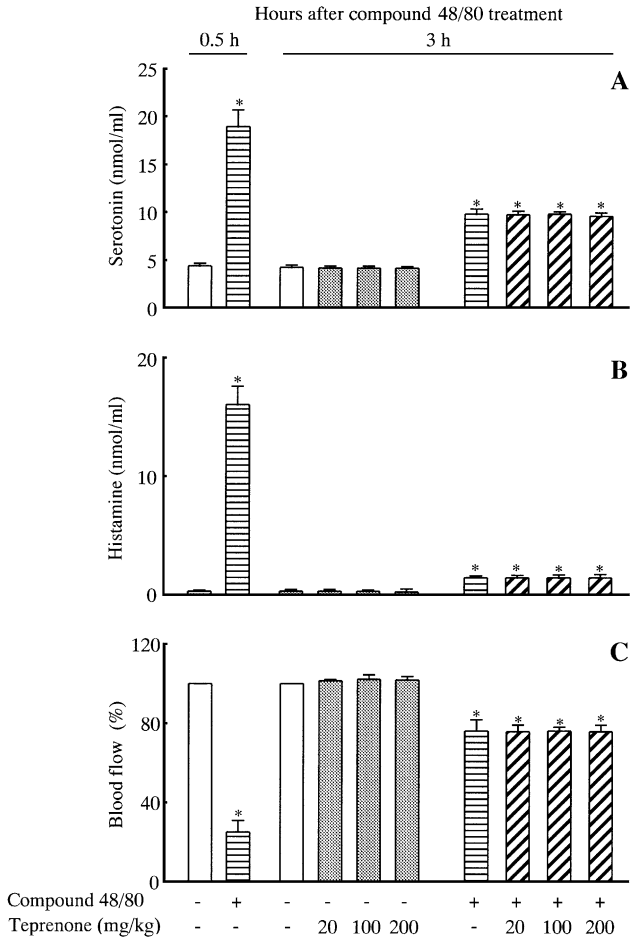

Fig. 1. Effect of post-teprenone administration on serum serotonin (A) and histamine (B) concentrations and gastric mucosal blood flow (C) in rats treated with compound 48/80. Rats with a single i.p. injection of compound 48/80 (0.75 mg/kg) or vehicle (distilled water) received a single p.o. administration of teprenone (20, 100 or 200 mg/kg) or vehicle (0.5% arabic gum) at 0.5 h after the compound 48/80 injection. Serum serotonin and histamine and gastric mucosal blood flow were determined 0.5 or 3 h after the compound 48/80 injection as described in Section 2. Each value is a mean ± S.D. ($n = 5$ for compound 48/80-untreated rats with and without teprenone administration; $n = 10$ for compound 48/80-treated rats with and without teprenone administration). *$P < 0.05$ (vs. control rats treated with vehicle alone).

3.2. Effect of post-teprenone administration on serum serotonin and histamine concentrations and gastric mucosal blood flow

Rats treated with compound 8/80 alone had 3.4- and 21.5-fold higher serum serotonin and histamine concentrations, respectively, than untreated control rats at 0.5 h after the treatment and the compound 48/80-treated group had 2.4- and 5.4-fold higher serum serotonin and histamine concentrations, respectively, than the control group at 3 h (Fig. 1A and B). Post-administration of teprenone (20, 100 or 200 mg/kg) did not affect the increases in serum serotonin and histamine concentrations at 3 h after compound 48/80 treatment (Fig. 1A and B). Gastric mucosal

blood flow in the compound 48/80-treated group was 25.2% and 72.5% of that in the control group at 0.5 and 3 h after the treatment, respectively (Fig. 1C). Post-administration of teprenone (20, 100 or 200 mg/kg) did not affect the decrease in gastric mucosal blood flow at 3 h after compound 48/80 treatment (Fig. 1C). Teprenone (20, 100 or 200 mg/kg) administered to untreated rats in the same manner did not affect the serum serotonin and histamine concentration and gastric mucosal blood flow (Fig. 1).

3.3. Effect of post-teprenone administration on gastric mucosal myeloperoxidase and xanthine oxidase activities

Rats treated with compound 48/80 alone had significantly higher gastric mucosal myeloperoxidase activity than untreated control rats at 0.5 h after the treatment and further increase in that activity in the compound 48/80-treated group was observed at 3 h; the compound 48/80-treated group had 3.1-fold higher gastric mucosal myeloperoxidase activity than the control group at 3 h (Fig. 2A). Post-administered teprenone at a dose of 100 or 200 mg/kg, but not 20 mg/kg, significantly attenuated the increase in gastric mucosal myeloperoxidase activity at 3 h after compound 48/80 treatment in a dose-dependent manner (Fig. 2A). The compound 48/80-treated group had significantly higher gastric mucosal xanthine oxidase activity than the control group at 0.5 h after the treatment and further increase in that activity in the compound 48/80-treated group was found at 3 h; the compound 48/80-treated group had 4.2-fold higher gastric mucosal xanthine oxidase activity than the control group (Fig. 2B). Post-administered teprenone at a dose of 100 or 200 mg/kg, but not 20 mg/kg, significantly attenuated the increase in gastric mucosal xanthine oxidase activity at 3 h after compound 48/80 treatment dose-dependently (Fig. 2B). Teprenone (20, 100 or 200 mg/kg) administered to untreated rats reduced the gastric mucosal myeloperoxidase activity in a dose-dependent manner but did not affect the gastric mucosal xanthine oxidase activity (Fig. 2).

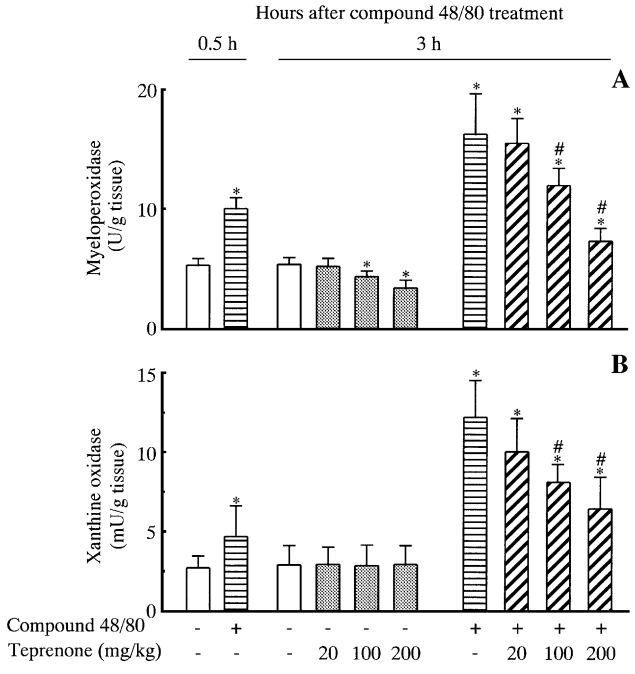

Fig. 2. Effect of post-teprenone administration on gastric mucosal myeloperoxidase (A) and xanthine oxidase (B) activities in rats treated with compound 48/80. Rats with a single i.p. injection of compound 48/80 (0.75 mg/kg) or vehicle (distilled water) received a single p.o. administration of teprenone (20, 100 or 200 mg/kg) or vehicle (0.5% arabic gum) at 0.5 h after the compound 48/80 injection. Gastric mucosal myeloperoxidase and xanthine oxidase were assayed 0.5 or 3 h after the compound 48/80 injection as described in Section 2. Each value is a mean ± S.D. ($n = 5$ for compound 48/80-untreated rats with and without teprenone administration; $n = 10$ for compound 48/80-treated rats with and without teprenone administration). $^{*}P < 0.05$ (vs. control rats treated with vehicle alone); $^{\#}P < 0.05$ (vs. rats treated with compound 48/80 alone).

3.4. Effect of post-teprenone administration on gastric mucosal thiobarbituric acid reactive substances content and Se-glutathione peroxidase activity

Gastric mucosal thiobarbituric acid reactive substances content in rats treated with compound 48/80 alone was significantly higher than that in untreated control rats at 0.5 h after the treatment and further increase in that content in the compound 48/80-treated group occurred at 3 h: The compound 48/80-treated group had 1.9-fold higher gastric mucosal thiobarbituric acid reactive substances content than the control group at 3 h (Fig. 3A). Post-administered teprenone at a dose of 100 or 200 mg/kg, but not 20 mg/kg, significantly attenuated the increase in gastric mucosal thiobarbituric acid reactive substances content at 3 h after compound 48/80 treatment in a dose-dependent manner (Fig. 3A). Gastric mucosal Se-glutathione peroxidase activity in the compound 48/80-treated group was significantly lower than that in the control group at 0.5 h after the treatment and further decrease in that activity was found in the compound 48/80-treated group at 3 h: The compound 48/80-treated group had a 40.3% of gastric mucosal Se-glutathione peroxidase activity in the control group at 3 h (Fig. 3B). Post-administered teprenone at a dose of 100 or 200 mg/kg, but not 20 mg/kg, significantly attenuated the decrease in gastric mucosal Se-glutathione peroxidase activity at 3 h after compound 48/80 treatment in a dose-dependent manner (Fig. 2B). None of doses of teprenone had any effect on gastric mucosal thiobarbituric acid reactive substances content and Se-glutathione peroxidase activity in untreated rats (Fig. 3).

3.5. Effect of teprenone on gastric mucosal hexosamine and vitamin E contents

Gastric mucosal hexosamine content in rats treated with compound 48/80 alone was not different from that in

untreated control rats at 0.5 h after the treatment but the compound 48/80-treated group had significantly lower gastric mucosal hexosamine content than the control group at 3 h: The gastric mucosal hexosamine content in the compound 48/80-treated rats was 63.6% of that in the control group at 3 h (Fig. 4A). Post-administered teprenone at a dose of 100 or 200 mg/kg, but not 20 mg/kg, significantly attenuated the decrease in gastric mucosal hexosamine content at 3 h after compound 48/80 treatment dose-dependently (Fig. 4A). There was no significant difference in gastric mucosal vitamin E content between the compound 48/80-treated and control groups at 0.5 after the treatment but the compound 48/80-treated group had significantly lower gastric mucosal vitamin E content than the control group at 3 h: The compound 48/80-treated group had 50.5% of gastric mucosal vitamin E content in the control group at 3 h (Fig. 4B). Post-administered teprenone at a dose of 100 or 200 mg/kg, but not 20 mg/kg, significantly attenuated the decrease in gastric mucosal vitamin E content at 3 h after compound 48/80 treatment dose-dependently (Fig. 4B). Teprenone (20, 100 or 200 mg/kg) administered to untreated rats increased the

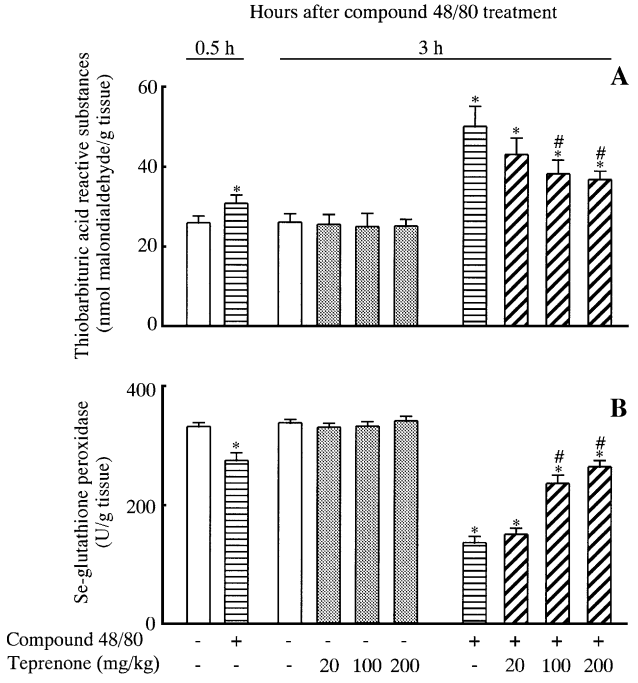

Fig. 3. Effect of post-teprenone administration on gastric mucosal thiobarbituric acid reactive substances (A) content and Se-glutathione peroxidase (B) activity in rats treated with compound 48/80. Rats with a single i.p. injection of compound 48/80 (0.75 mg/kg) or vehicle (distilled water) received a single p.o. administration of teprenone (20, 100 or 200 mg/kg) or vehicle (0.5% arabic gum) at 0.5 h after the compound 48/80 injection. Gastric mucosal thiobarbituric acid reactive substances and Se-glutathione peroxidase were assayed 0.5 or 3 h after the compound 48/80 injection as described in Section 2. Each value is a mean ± S.D. ($n=5$ for compound 48/80-untreated rats with and without teprenone administration; $n=10$ for compound 48/80-treated rats with and without teprenone administration). $^{*}P<0.05$ (vs. control rats treated with vehicle alone); $^{\#}P<0.05$ (vs. rats treated with compound 48/80 alone).

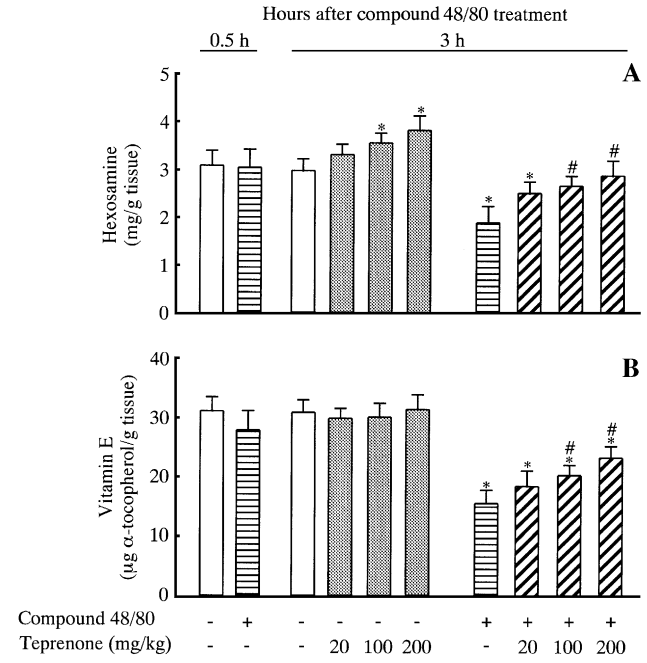

Fig. 4. Effect of post-teprenone administration on gastric mucosal hexosamine (A) and vitamin E (B) contents in rats treated with compound 48/80. Rats with a single i.p. injection of compound 48/80 (0.75 mg/kg) or vehicle (distilled water) received a single p.o. administration of teprenone (20, 100 or 200 mg/kg) or vehicle (0.5% arabic gum) at 0.5 h after the compound 48/80 injection. Gastric mucosal hexosamine and vitamin E were assayed 0.5 or 3 h after the compound 48/80 injection as described in Section 2. Each value is a mean ± S.D. ($n=5$ for compound 48/80-untreated rats with and without teprenone administration; $n=10$ for compound 48/80-treated rats with and without teprenone administration). $^{*}P<0.05$ (vs. control rats treated with vehicle alone); $^{\#}P<0.05$ (vs. rats treated with compound 48/80 alone).

gastric mucosal hexosamine content in a dose-dependent manner but did not affect the gastric mucosal vitamin E content (Fig. 4).

4. Discussion

The model of acute gastric mucosal lesions in rats treated once with compound 48/80, 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 (Ohta et al., 1997, 1999a,b). In addition, it has been shown that *H. pylori* might cause gastric mucosal inflammation, the reduction of gastric mucosal blood flow, and gastric mucosal microcirculatory disturbances through mast cell degranulation (Atuma et al., 1999; Yamamoto et al., 1999; Kalia et al., 2000). Teprenone is known to protect against gastric mucosal injury induced by *H. pylori* in rats (Saita and Murakami, 2000). The present study has clearly shown that orally administered teprenone prevents gastric mucosal lesion progression in rats with a single compound 48/80 treatment in a dose-dependent manner.

In the present study, marked increases in serum serotonin and histamine concentrations occurred at 0.5 h after a single compound 48/80 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 (Ohta et al., 1997, 1999a,b). Oral post-administration of teprenonse (20, 100 and 200 mg/kg) to compound 48/80-treated rats had no effect on the increases in serum serotonin and histamine concentrations found at 3 h after the treatment. Our recent report has shown that the protective effect of orally administered teprenone against compound 48/80-induced acute gastric mucosal lesions in rats is not due to its preventive action on mast cell degranulation caused by compound 48/80 (Ohta et al., 2003a). Takeuchi et al. (1986) have shown that a single i.p. injection of compound 48/80 (0.75 mg/kg) to rats causes continuous increases in serotonin and histamine levels in the serum, while this injection causes transient increases in serotonin and histamine levels in the gastric mucosal tissue which occurs around 0.5 h after the injection, and have suggested that compound 48/80-induced gastric mucosal lesions might be induced by the generalized release of serotonin and histamine from extragastric sources. From these findings, it can be thought that the preventive effect of orally administered teprenone on gastric mucosal lesion progression in rats with a single compound 48/80 treatment is not due to prevention of gastric mast cell degranulation caused by compound 48/80 and the release of serotonin and histamine from degranulated extragastric mast cells.

It has been reported that oral pre-administration of teprenone (100 mg/kg per day) for 3 consecutive days attenuates reduced gastric mucosal blood flow after hemorrhage and retransfusion in the corpus and antral regions of rats (Hachiya et al., 1996), although there is a report showing no change in gastric mucosal blood flow in normal rats with a single oral administration of teprenone (200 mg/kg) (Morimoto et al., 1991). In the present study, rats treated once with compound 48/80 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 (Ohta et al., 1997, 1999a,b). Thus, gastric mucosal blood flow exhibits an ischemia–reperfusion-like change in rats treated once with compound 48/80. Oral post-administration of teprenone to rats treated once with compound 48/80 at a dose of 20, 100 or 200 mg/kg did not affect the recovery of gastric mucosal blood flow found at 3 h after the treatment. In addition, no change in gastric mucosal blood flow occurred in normal rats given teprenone at the same doses. These results indicate that administered teprenone prevents acute gastric mucosal lesion progression in rats treated once with compound 48/80 without altering the change in gastric mucosal blood flow.

It has been reported that orally administered teprenone exerts a preventive effect on the development of stress-induced acute gastric mucosal lesions in rats 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 (Nishida et al., 1998). Teprenone is known to inhibit the adhesion of neutrophils activated by *H. pylori* water extract to endothelial cells and the expression of CD11b/CD18a on the activated neutrophils (Yoshikawa et al., 1999). The drug is also known to protect against gastric mucosal injury in rats with water immersion restraint stress by inhibiting increases in the concentrations of adenosine, which is metabolized to hyoxanthine and xanthine via xanthine oxidase, uric acid, a metabolite of the xanthine oxidase-catalyzed oxidation of xanthine, and lipid peroxide, and a decrease in hypoxanthine concentration in the gastric mucosal tissue (Itoh et al., 1991). Xanthine oxidase generates reactive oxygen species such as superoxide radical and hydrogen peroxide during the oxidation of hyoxanthine or xanthine (Fridovich, 1970; Porras et al., 1981). It has been shown in rats with a single compound 48/80 treatment that neutrophil infiltration, increased xanthine oxidase activity, decreased Se-glutathione peroxidase activity, and enhanced lipid peroxidation in the gastric mucosal tissue contribute to gastric mucosal lesion progression (Ohta et al., 1997, 1999a). Our recent report has suggested that the protective effect of orally administered teprenone against compound 48/80-induced acute gastric mucosal lesions in rats is associated with its inhibitory action on neutrophil infiltration and enhanced lipid peroxidation in the gastric mucosal tissue (Ohta et al., 2003a). In the present study, teprenone post-administered to compound 48/80-treated rats attenuated the further increases in the activities of gastric mucosal myeloperoxidase, an index of tissue neutrophil infiltration (Krawisz et al., 1984), and xanthine oxidase found at 3 h after the treatment in a dose-dependent manner. In addition, teprenone administered to normal rats in the same manner reduced the gastric mucosal myeloproxidase activity in a dose-dependent manner, as shown previously (Nishida et al., 1998). However, none of the doses of teprenone affected gastric mucosal xanthine oxidase activity in normal rats. It is known that teprenone does not inhibit gastric mucosal myeloperoxidase activity in vitro (Nishida et al., 1998, 1999). It has been suggested that an increase in gastric mucosal xanthine oxidase activity in compound 48/80-treated rats is related to neutrophil infiltration into the gastric mucosal tissue during the progression of acute gastric mucosal lesions (Ohta et al., 1999a). We have observed that teprenone at a concentration of 10–100 μg/ml has no inhibitory effect on xanthine oxidase activity in gastric mucosal tissue preparations from rats treated with compound 48/80 (unpublished data). Lipid peroxidation is known to occur via reactive oxygen species generated not only by the xanthine–xanthine oxidase system but also by activated NADPH oxidoreductase in neutrophils (Fukuzawa et al., 1995; Zimmerman et al., 1997). It is also known that myeloperoxidase mediates lipid peroxidation in the presence of hydrogen peroxide with halide ions (Stelmaszynska et al.,

1992). In the present study, teprenone post-administered to compound 48/80-treated rats attenuated the further increase in the gastric mucosal content of thiobarbituric acid reactive substances, an index of lipid peroxidation, and the further decrease in the gastric mucosal activity of Se-glutathione oxidase, an enzyme to metabolize hydrogen peroxide and lipid hydroperoxides in the presence of GSH, found at 3 h after the treatment in a dose-dependent manner. We have shown that teprenone at a concentration of 10–100 μg/ml does not inhibit in vitro lipid peroxidation induced by a water-soluble radical initiator, 2,2′-azobis(2-amidinopropane), in gastric mucosal tissue preparations from compound 48/80-treated rats (Kobayashi et al., 2001). It has also been shown that teprenone at various concentrations up to 100 μg/ml has no activity to scavenge superoxide radical and hydroxyl radical (Nishida et al., 1998). In addition, teprenone administered to normal rats at a dose of 20, 100 or 200 mg/kg had no effect on thiobarbituric acid reactive substances content and Se-glutathione peroxidase activity in the gastric mucosal tissue. Se-glutathione peroxidase is known to be inactivated by superoxide radical, hydroxyl radical and hypochlorous acid, which is generated in the presence of hydrogen peroxide and chloride ion via myeloperoxidase, in vitro (Aruoma and Halliwell, 1987; Pigeolet et al., 1990; Tabatabaie and Floyd, 1994). Therefore, these findings suggest that orally administered teprenone could exert a preventive effect on acute gastric mucosal lesion progression in rats with a single compound 48/80 treatment through its action to inhibit 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 (Kanuitz, 1999). It is known that mucin interacts with reactive oxygen species in vitro (Grisham et al., 1987). It is also known that gastric mucus plays an important role in protecting the gastric mucosa of rats against ischemia–reperfusion stress (Seno et al., 1995). We have suggested that the protective effect of orally administered teprenone against compound 48/80-induced acute gastric mucosal lesions is associated with its stimulatory action on gastric mucus synthesis and secretion in addition to its inhibitory action on neutrophil infiltration and enhanced lipid peroxidation in the gastric mucosal tissue (Ohta et al., 2003a). In the present study, an apparent decrease in the gastric mucosal content of hexosamine, a maker of gastric mucus, in rats treated once with compound 48/80 was found 3 h, but not 0.5 h, after the treatment, as shown in our previous report (Ohta et al., 1997). Teprenone post-administered to compound 48/80-treated rats attenuated the decreases in gastric mucosal hexosamine found at 3 h after the treatment in a dose-dependent manner. In addition, teprenone administered to normal rats at a dose of 20, 100 or 200 mg/kg caused a dose-dependent increase in gastric mucosal hexosamine content, as reported previously (Nishida et al., 1998). Teprenone is known to protect cultured rat gastric mucosal cells against reactive oxygen species by increasing the production of mucus (Hiraishi et al., 1993). However, the drug has no activity to scavenge reactive oxygen species and to inhibit lipid peroxidation in vitro, as described above. Accordingly, it is conceivable that orally administered teprenone prevents acute gastric mucosal lesion progression in rats treated once with compound 48/80 by protecting the gastric mucosal barrier and tissue against the attack of reactive oxygen species derived from infiltrated neutrophils and the xanthine–xanthine oxidase system and/or lipid peroxidation mediated by the reactive oxygen species through its stimulatory action on gastric mucus synthesis. Vitamin E is well known to function as a chain-breaking antioxidant for lipid peroxidation and as a scavenger of reactive oxygen species (Liebler, 1993). In the present study, an apparent decrease in gastric mucosal vitamin E content in rats treated once with compound 48/80 was found 3 h, but not 0.5 h, after the treatment, as reported previously (Ohta et al., 1997). Teprenone post-administered to compound 48/80-treated rats attenuated the decrease in gastric mucosal vitamin E content found at 3 h after the treatment in a dose-dependent manner. However, none of the doses of teprenone administered to normal rats in the same manner affected the gastric mucosal vitamin E content. These results suggest that orally administered teprenone contributes to the maintenance of gastric mucosal vitamin E in rats treated once with compound 48/80, which could be related to its preventing effect on acute gastric mucosal lesion progression.

It has been reported that gastric mucus depletion associated with decreases in gastric mucosal nitric oxide synthase (NOS) activity, neuronal NOS expression and cyclic GMP content contributes, at least in part, to the development of ischemia–reperfusion-induced gastric mucosal injury in rats (Kim and Kim, 2001). It has been shown that teprenone exerts stimulatory actions on gastric mucus synthesis and secretion through a nitric oxide (NO)-dependent pathway (Tatsuta et al., 1995; Nishida and Ohta, 1998). It has been indicated that teprenone exerts an inhibitory action on neutrophil infiltration and decreases in mucus synthesis and secretion in the gastric mucosa of rats with water immersion restraint stress by maintaining constitutive NOS activity in the gastric mucosal tissue (Nishida et al., 1999). In addition, our recent report has suggested 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 (Ohta et al., 2003a). Therefore, it seems likely that teprenone exerts a preventing effect on the progression of compound 48/80-induced acute gastric mucosal lesions by eliciting NO-mediated gastric mucus synthesis and secretion and NO-mediated inhibition of neutrophil infiltration into the gastric mucosal tissue.

Mikami et al. (1997) have shown that endogenous prostaglandins in the gastric mucosa of rats may play, 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. (1986a,b) 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 (Terano et al., 1986b), while it has been suggested that the protective effect of teprenone against ethanol-induced gastric mucosal injury is not mediated by endogenous prostaglandins (Bilski et al., 1988). We have shown 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 had no effect on gastric mucosal lesion formation and progression in the compound 48/80-treated rats (Ohta et al., 2003b). Therefore, it seems unlikely that teprenone exerts a preventive effect on the progression of compound 48/80-induced acute gastric mucosal lesions through endogenous prostaglandins.

In conclusion, the results of the present study indicate that orally administered teprenone exerts a preventive effect on the progression of compound 48/80-induced acute gastric mucosal lesions in rats possibly through suppression of gastric mucus depletion, neutrophil infiltration and oxidative stress in the gastric mucosal tissue.

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