ELSEVIER

European Journal of Pharmacology 354 (1998) 51–58

ejp

Protective and preventive effects of teprenone on gastric mucosal lesions in rats

Keiji Nishida *, Yoshiji Ohta, Isao Ishiguro

Department of Biochemistry, School of Medicine, Fujita Health University, Toyoake, Aichi 470-1192, Japan

Accepted 3 June 1998

Abstract

We have reported that neutrophil infiltration into gastric mucosa is closely related to gastric mucosal lesion development in rats with water immersion restraint stress. In this study, we examined the effect of teprenone, which is known to prevent gastric mucosal injury through stimulation of gastric mucus synthesis and secretion, on neutrophil infiltration into the gastric mucosa of rats with water immersion restraint stress. Pre- and post-administration of teprenone (200 mg/kg, p.o.) significantly attenuated the neutrophil infiltration into the gastric mucosa and the lesion development found at 6 h of water immersion restraint stress with preservation of gastric mucosal hexosamine and adherent mucus levels. These results indicate that teprenone exerts protective and preventive actions against water immersion restraint stress-induced gastric mucosal lesions in rats and suggest that these actions could be related to the preservation of gastric mucus synthesis and secretion and inhibition of neutrophil infiltration into the gastric mucosal tissue.

Keywords: Gastric mucosal lesion; Lipid peroxidation; Myeloperoxidase; Neutrophil infiltration; Teprenone; Water immersion restraint stress

1. Introduction

Teprenone (6,10,14,18-tetramethyl-5,9,13,17-nonadecatetraen-2-one, geranylgeranylacetone) is an anti-ulcer drug developed in Japan (Murakami et al., 1981) and clinically used for the treatment of gastric ulcer and gastritis. It is known that teprenone enhances mucus synthesis and secretion in gastric cells (Terano et al., 1986). Furthermore, it has been demonstrated that teprenone protects gastric mucosal tissues against injury in various experimental gastric ulcer models through preservation of gastric mucus synthesis or secretion (Murakami et al., 1981, 1982; Ito et al., 1986; Kunisaki and Sugiyama, 1992; Seno et al., 1995; Tanoue et al., 1996).

It has been suggested that lipid peroxidation plays an important role in the pathogenesis of gastric mucosal lesions induced by water immersion restraint stress (Yoshikawa et al., 1986; Itoh et al., 1991). We have recently reported that in rats subjected to water immersion restraint stress, the development of gastric mucosal lesions is closely related to the enhanced formation of lipid peroxide and enhanced oxidation of non-protein sulfhydryl (non-protein SH) which depend on an increased generation of oxygen free radicals, such as superoxide anion (O_2^-) via the xanthine–xanthine oxidase system and neutrophils (Nishida et al., 1997). Itoh et al. (1991) have reported that when administered to rats before the onset of water immersion restraint stress, teprenone protects gastric mucosal tissue against the stress-induced injury with attenuations of increased adenine nucleotide degradation and lipid peroxidation in gastric mucosal tissue and have suggested that teprenone could exert this protective effect through inhibition of the xanthine–xanthine oxidase system in gastric mucosal tissues. It has also been reported that teprenone exerts a protective effect against cold-restraint stress-induced gastric mucosal injury with suppression of increased gastric mucosal lipid peroxide formation in rats (Kunisaki and Sugiyama, 1992). Therefore, it can be thought that teprenone prevents gastric mucosal injury both through the maintenance of gastric mucus synthesis or secretion of gastric mucus and inhibition of gastric mucosal lipid peroxide formation. As mentioned, a potential source of reactive oxygen metabolites in tissues is the neutrophils which contain NADPH oxidase that reduces molecular oxygen to O_2^-, resulting in its conversion to H_2O_2 (McCord and

* Corresponding author. Tel.: +81-562-93-2451; Fax: +81-562-93-1193; E-mail: knishida@fujita-hu.ac.jp

0014-2999/98/$19.00

Fridovich, 1979). These findings may allow us to assume that suppression of neutrophil infiltration into the gastric mucosa of rats with water immersion restraint stress leads to the prevention of lipid peroxide formation in the gastric mucosal tissue. In fact, our previous report indicates that in rats pre-treated with anti-polymorphonuclear anti-serum, lipid peroxide formation in the gastric mucosa of rats with water immersion restraint stress is significantly attenuated with the prevention of neutrophil infiltration into the gastric mucosal tissue. Therefore, there is a possibility that the preventive effect of teprenone on gastric mucosal lipid peroxide formation in rats with water immersion restraint stress is in part due to its inhibitory effects on neutrophil infiltration into the gastric mucosal tissue (Nishida et al., 1997). However, it is unknown whether or not teprenone has an inhibitory effect on neutrophil infiltration into the gastric mucosa of rats with water immersion restraint stress.

In addition, it has been demonstrated that gastric mucin interacts with oxygen free radicals in vitro (Grisham et al., 1987). Seno et al. (1995) have reported that an increase in gastric mucin, which is caused by teprenone-stimulated gastric mucus synthesis and secretion, results to the protection of gastric mucosal tissue against ischemia/reperfusion-induced injury in rats. It has been known that ischemia/reperfusion-induced gastric mucosal lesions are caused by oxygen free radicals derived from xanthine–xanthine oxidase system and/or neutrophils (Yoshikawa et al., 1989; Andrew et al., 1992). Therefore, there is also a possibility that teprenone-mediated increase in gastric mucin contributes to the suppression of increases in gastric mucosal lipid peroxide formation and non-protein SH oxidation in rats with water immersion restraint stress, which leads to the prevention of gastric mucosal lesion development.

In this study, therefore, we examined the effect of teprenone administration on the infiltration of neutrophils into the gastric mucosal tissues during the development of gastric mucosal lesions in rats with water immersion restraint stress. This neutrophil infiltration into the gastric mucosal tissue was assessed based on myeloperoxidase activity, an index of neutrophil infiltration (Krawisz et al., 1984), in the tissue. In addition, we examined the effect of teprenone administration on changes in the concentrations of lipid peroxide, non-protein SH, hexosamine (a marker of gastric mucin) and the adherent mucus in gastric mucosal tissues during the development of gastric mucosal lesions in the water immersion restraint-stressed rats was examined.

2. Materials and methods

2.1. Chemicals

3,3′,5,5′-tetramethylbenzidine (Sigma, St. Louis, MO, USA); dioctyl sodium sulfosuccinate (docusate sodium) (DSS) (Tokyo Kasei Kogyo, Tokyo, Japan); ethylenediaminetetraacetic acid (EDTA), *N*,*N*-dimethylformamide, thiobarbituric acid, 5,5′-dithiobis (2-nitrobenzoic acid), alcian blue 8gx, glucosamine and other chemicals (Wako, Osaka, Japan); and teprenone (vitamin E-free) (Eisai, Tokyo, Japan) were used.

2.2. Induction of gastric mucosal lesions

Seven-week-old male Wistar rats weighing 200–210 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used. These animals were starved for 24 h prior to the experiments, but were allowed free access to water. Rats were restrained in a wire cage and immersed up to the depth of the xiphoid process in a 23°C water bath for 3 and 6 h to produce water immersion restraint stress-induced gastric mucosal lesions as described by Takagi and Okabe (1968). Rats were killed under ether anesthesia after application of 0, 3 and 6 h of water immersion restraint, blood was collected from the inferior vena cava and the stomachs were removed. The collected blood was separated into serum. The removed stomachs were cut open along with the greater curvature and the gastric mucosa was removed on ice using a small pair of scissors. For the observation of gastric lesion, the stomachs of the rats with and without water immersion restraint, after ligation of the esophagus at 5 mm proximal to the gastroesophageal junction and the duodenum at 10 mm distal to the pylorus, were infused with approximately 10 ml of saline and secured by ligatures at both the esophagus and the duodenum. The stomachs were removed and were fixed with 10% formaldehyde for 10 min and cut open along the greater curvature. The gastric mucosa was carefully examined for lesions recognized as linear breaks (erosions) at the mucosal surface of the glandular part under a stereoscopic microscope (×10). The extent of the lesion (lesion index) is expressed as the sum of the length of these breaks per stomach. All animals received human care in compliance with the guideline of the Animal Care and Use Committee of Fujita Health University.

2.3. Teprenone treatment

Teprenone (50, 100 and 200 mg/kg) suspended in 5% arabic gum, was orally administered at 0.5 h before the beginning of water immersion restraint stress. Another group of rats orally received teprenone (200 mg/kg) suspended in 5% arabic gum 3 h after the onset of the stress. Five percent arabic gum, used as vehicle, was given to control rats in the same manner. The dosage of teprenone was decided according to previous reports (Ito et al., 1986; Bilski et al., 1988; Morimoto et al., 1991; Terano et al., 1991; Itoh et al., 1991; Namiki et al., 1994).

2.4. Biochemical determinations

Immediately after gastric mucosal collection, the mucosa was disrupted in 9 vols of ice-cold 50 mM Tris–HCl buffer (pH 7.5) using a microhomogenizer and the prepared homogenate was sonicated on ice for 60 s using a Handy Sonic model UR-20P (Tomy Seiko, Tokyo). The sonicated homogenate was centrifuged at $10\,000 \times g$ for 20 min at 4°C and the resultant supernatant was used for the assay sample. Gastric mucosal myeloperoxidase was assayed by the method of Suzuki et al. (1983) measuring the H_2O_2-dependent oxidation of tetraethylbenzidine at 37°C. One unit (U) of this activity is defined as the amount of enzyme causing a change in the absorbance of 1.0 per min at 655 nm. To investigate the direct effect of teprenone on myeloperoxidase activity in gastric mucosal tissues, 10 to 100 μg/ml of teprenone dissolved in 0.01% Tween 80 were added to the supernatant of gastric mucosal homogenates obtained from rats with water immersion restraint stress for 6 h. Myeloperoxidase activities in the supernatants containing various concentrations of teprenone were assayed using the above-mentioned method.

Immediately after the collection of gastric mucosal tissues, the mucosal tissue was disrupted in 9 vols of 0.15 M KCl containing 1 mM EDTA using a microhomogenizer. Lipid peroxide in the homogenate was determined according to the method of Ohkawa et al. (1979) using the thiobarbituric acid reaction. The content of lipid peroxide is expressed as the amount of malondialdehyde. Non-protein SH in the homogenate was measured by the 5,5′-dithiobis (2-nitrobenzoic acid) method of Sedlak and Lindsay (1968).

Gastric mucosal mucin extracted with Triton X-100 was hydrolyzed with hydrochloric acid. Hexosamine obtained from the hydrolyzed mucin was assayed by the method of Neuhaus and Letzring (1957) using glucosamine as a standard. The determined gastric mucosal hexosamine was used as an indicator for gastric mucosal mucus synthesis. Briefly, the prepared sample and standard were incubated with acetylacetone at 100°C for 15 min. The mixtures were further mixed with Ehrlich's reagent and were allowed to stand at room temperature for 40 min. The optical density of the resultant red pigment was read at 550 nm. The concentration of gastric mucosal hexosamine was estimated from the optical density of the sample in comparison with that of the standard.

The removed stomach was cut open along the greater curvature and was rinsed with 10 ml of ice-cold 0.25 M sucrose. 50 mm^2 (approximately 8 mm in diameter) of the glandular portion of the stomach was excised with a scalpel and its wet weight was obtained. Gastric mucosal adherent mucus was assayed by the method of Kitagawa et al. (1986) using alcian blue staining. The determined gastric mucosal adherent mucus was used as an indicator for gastric mucus secretion. Briefly, 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 the dye complex with mucus was extracted with 2 ml of 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 was expressed as that of alcian blue that adhered to the gastric mucosal surface (μg/g tissue).

2.5. Statistical analysis

Results obtained were expressed as means ± S.D. Results were analyzed by computerized statistical packages (Super Analysis of Variance, Statview II) if necessary. Each mean value was compared by One-way ANOVA and Fisher's protected least significant difference for multiple comparisons as the post-hoc test. The level of significance was taken as $P < 0.05$.

3. Results

When rats were subjected to water immersion restraint stress over a 6-h period, gastric mucosal lesions developed as shown in Fig. 1. The magnitude and development of gastric mucosal lesions in the present study were identical with those shown in previous reports (Hirota et al., 1989, 1990; Hamajima et al., 1994). In contrast, there were no lesions in rats without the stress (data not shown). Oral administration of teprenone (50, 100 and 200 mg/kg) at 0.5 h prior to water immersion restraint stress induction prevented the lesion development at 3 and 6 h of the stress in a dose-dependent manner, with IC_{50} values of 62 and 141 mg/kg, respectively (Fig. 1).

As shown in Fig. 2, myeloperoxidase activity, an indicator of neutrophil infiltration (Krawisz et al., 1984), in the gastric mucosa of rats subjected to water immersion restraint stress for 3 and 6 h, significantly increased; rats with 3 and 6 h of the stress had 2.2- or 3.2-fold higher gastric mucosal myeloperoxidase activity, respectively, than control rats without the stress. This water immersion restraint stress-induced increase in gastric mucosal myeloperoxidase activity was significantly prevented by pre-treatment with teprenone at doses of 50, 100 and 200 mg/kg (Fig. 2). Furthermore, when teprenone was administered at the same doses to control rats without water immersion restraint stress, the gastric mucosal myeloperoxidase activity was slightly, but significantly decreased in a dose-dependent manner (Fig. 2).

Lipid peroxide concentrations in the gastric mucosa of rats with 3 and 6 h of water immersion restraint stress

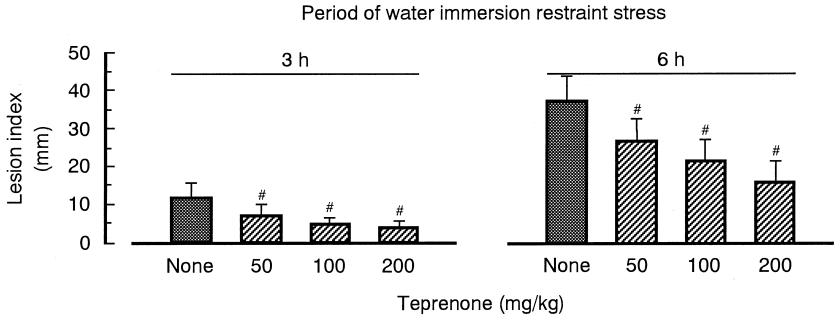

Fig. 1. Effect of pre-treatment with teprenone on the development of gastric mucosal lesions in rats with water immersion restraint stress over a 6-h period. Rats received oral administration of teprenone (50, 100 and 200 mg/kg) at 0.5 h prior to water immersion restraint stress induction. Rats without teprenone pre-treatment received oral administration of an equal volume of 5% arabic gum used as vehicle. These rats were subjected to water immersion restraint stress for 3 and 6 h. Each value represents the mean ± S.D. for 7–10 animals. $\#P < 0.05$ compared with water immersion restraint-stressed rats without teprenone pre-treatment.

were 1.3- and 1.6-fold higher than those of control rats without the stress, respectively (Fig. 3A). Non-protein SH concentrations in the gastric mucosa of rats subjected to water immersion restraint stress for 3 and 6 h were 55 and 50% of those of control rats without the stress, respectively (Fig. 3B). Pre-treatment with teprenone at doses of 50, 100 and 200 mg/kg almost dose-dependently attenuated the increase in gastric mucosal lipid peroxide concentration and the decrease in gastric mucosal non-protein SH concentration in rats subjected to water immersion restraint stress for 3 and 6 h (Fig. 3). However, treatment with teprenone at the same doses had no effect on gastric mucosal lipid peroxide and non-protein SH concentrations in control rats without the stress (Fig. 3).

Gastric mucosal hexosamine concentration, which was used as an index for gastric mucus synthesis, in rats subjected to 3 and 6 h of water immersion restraint stress was significantly decreased; gastric mucosal hexosamine concentrations in the rats subjected to the stress for 3 and 6 h were 82 and 72% of those in control rats without the stress, respectively (Fig. 4A). Pre-treatment with 100 and 200 mg/kg of teprenone significantly prevented the decrease in gastric mucosal hexosamine concentration in rats subjected to water immersion restraint stress for 3 and 6 h, and also significantly increased gastric mucosal hexosamine concentration in control rats without the stress (Fig. 4A). When the change in gastric mucosal adherent mucus concentration, which was used as an indicator for gastric mucus secretion, was examined in rats subjected to water immersion restraint stress for 3 and 6 h, the adherent mucus concentrations in the rats with 3 and 6 h of the stress were 47 and 41% of those in control rats without the stress, respectively (Fig. 4B). The decrease in gastric adherent mucus concentration at 3 or 6 h of water immersion restraint stress was significantly prevented by pre-treatment with 100 and 200 mg/kg of teprenone. Furthermore, pre-treatment with teprenone at the same doses increased gastric adherent mucus concentration in control rats without water immersion restraint stress in a dose-dependent manner (Fig. 4A). In addition, regarding the preservative actions of teprenone on the gastric mucus synthesis and secretion in rats with water immersion re-

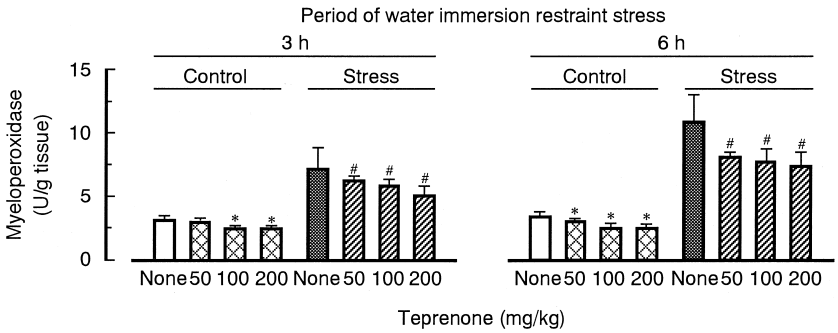

Fig. 2. Effect of pre-treatment with teprenone on the change in gastric mucosal myeloperoxidase activity in rats with water immersion restraint stress over a 6-h period. Rats received oral administration of teprenone (50, 100 and 200 mg/kg) at 0.5 h prior to water immersion restraint stress induction. Rats without teprenone pre-treatment received oral administration of an equal volume of 5% arabic gum used as vehicle. These rats were subjected to water immersion restraint stress for 3 and 6 h. Each value represents the mean ± S.D. for 7–10 animals. $\#P < 0.05$ compared with water immersion restraint-stressed rats without teprenone pre-treatment. $*P < 0.05$ compared with control rats without teprenone pre-treatment and water immersion restraint stress.

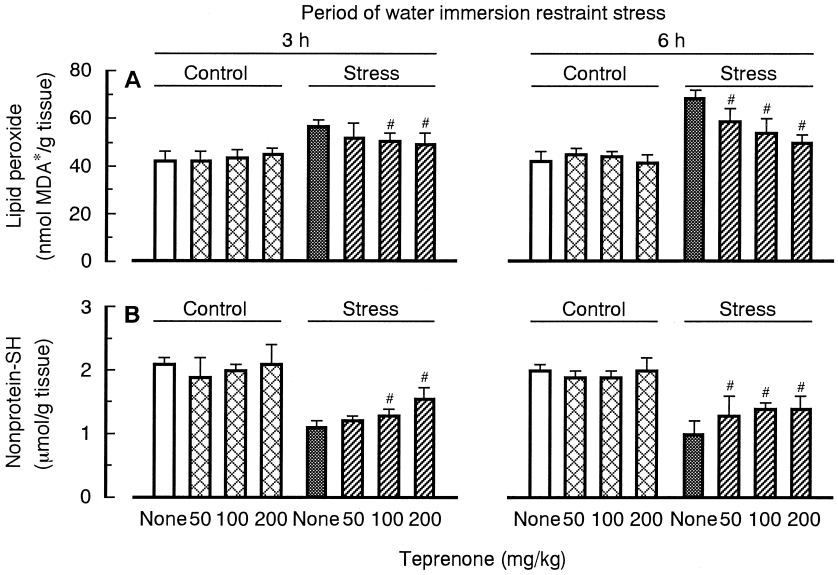

Fig. 3. Effect of pre-treatment with teprenone on the changes in gastric mucosal lipid peroxide (A) and non-protein SH (B) concentrations in rats with water immersion restraint stress over a 6-h period. Rats received oral administration of teprenone (50, 100 and 200 mg/kg) at 0.5 h prior to water immersion restraint stress induction. Rats without teprenone pre-treatment received oral administration of an equal volume of 5% arabic gum used as vehicle. These rats were subjected to water immersion restraint stress for 3 and 6 h. Each value represents the mean ± S.D. for 7–10 animals. #$P < 0.05$ compared with water immersion restraint-stressed rats without teprenone pre-treatment. ∗Malondialdehyde.

straint stress, the action of teprenone on gastric mucus synthesis was slightly weaker than the action on gastric mucus secretion.

When 200 mg/kg of teprenone was orally administered to water immersion restraint-stressed rats at 3 h after the onset of the stress, this post-teprenone administration prevented not only gastric mucosal lesion development in rats subjected to 6 h of the stress, but also increased gastric mucosal myeloperoxidase activity and lipid peroxide concentration and decreased gastric mucosal non-protein SH,

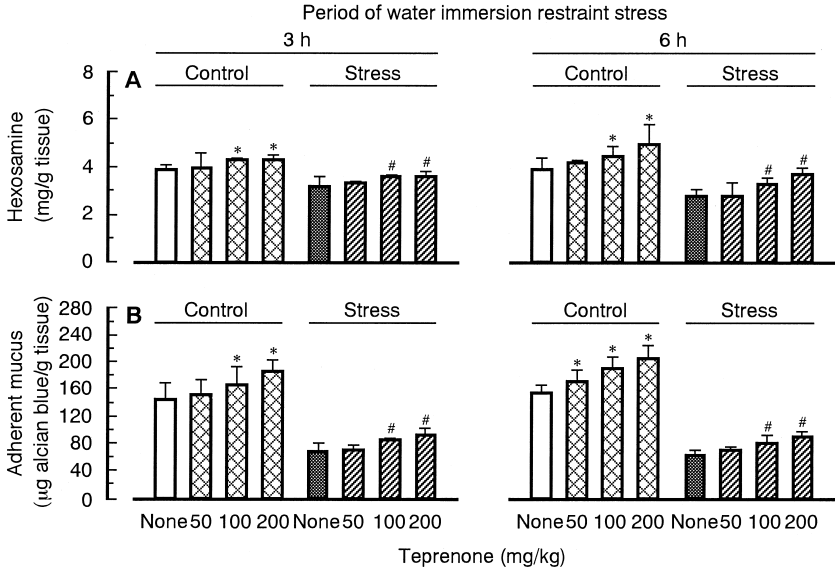

Fig. 4. Effect of pre-treatment with teprenone on the changes in gastric mucosal hexosamine (A) and adherent mucus (B) concentrations in rats with water immersion restraint stress over a 6-h period. Rats received oral administration of teprenone (50, 100 and 200 mg/kg) at 0.5 h prior to water immersion restraint stress induction. Rats without teprenone pre-treatment received oral administration of an equal volume of 5% arabic gum used as vehicle. These rats were subjected to water immersion restraint stress for 3 and 6 h. Each value represents the mean ± S.D. for 7–10 animals. #$P < 0.05$ compared with water immersion restraint-stressed rats without teprenone pre-treatment. ∗ $P < 0.05$ compared with control rats without teprenone pre-treatment and water immersion restraint stress.

Table 1
Effect of post-administration of teprenone on the progression of gastric mucosal lesions and changes in gastric mucosal myeloperoxidase activity and lipid peroxide, non-protein SH, hexosamine and adherent mucus concentrations in rats subjected to water immersion restraint stress over a 6-h period

	Control	Water immersion restraint		
		3 h	6 h	
			Arabic gum	Teprenone
Lesion index (mm)	0	11.8 ± 3.6	38.4 ± 9.1	22.7 ± 5.5[b]
Myeloperoxidase (U/g tissue)	3.3 ± 0.5	7.2 ± 1.7[a]	10.9 ± 2.8[a]	7.9 ± 1.2[a,b]
Lipid peroxide (nmol malondialdehyde/g tissue)	42.3 ± 4.1	55.4 ± 2.9[a]	68.1 ± 3.5[a]	54.7 ± 4.1[a,b]
Non-protein SH (μmol/g tissue)	2.0 ± 0.1	1.1 ± 0.1[a]	1.0 ± 0.1[a]	1.4 ± 0.1[a,b]
Hexosamine (mg/g tissue)	3.9 ± 0.4	3.1 ± 0.2[a]	2.8 ± 0.1[a]	3.4 ± 0.1[a,b]
Adherent mucus (μg alcian blue/g tissue)	155.1 ± 14.0	71.2 ± 18.7[a]	61.9 ± 1.5[a]	80.0 ± 5.0[a,b]

Rats were restrained in a wire cage and immersed up to the depth of the xiphoid process in a 23°C water bath to produce water immersion restraint stress-induced gastric mucosal lesions. Rats received p.o. administration of teprenone (200 mg/kg) at 3 h after the onset of water immersion restraint stress. Rats without teprenone pre-treatment received p.o administration of an equal volume of 5% arabic gum used as vehicle. Rats were killed 3 and 6 h after the onset of water immersion restraint stress. Each value represents the mean ± S.D. ($n = 7–10$). [a]$P < 0.05$ compared with control rats without water immersion restraint. [b]$P < 0.05$ compared with vehicle-treated rats.

hexosamine and adherent mucus concentrations in the rats subjected to 6 h of the stress (Table 1).

4. Discussion

The results obtained in this study have clearly shown the protective and preventive effects of teprenone on the development of gastric mucosal lesions in rats with water immersion restraint stress, both through the inhibition of neutrophil infiltration into the gastric mucosal tissue and preservation of mucus synthesis and secretion in the gastric mucosal tissue. As described previously (Nishida et al., 1997), gastric mucosal myeloperoxidase activity, as an indicator of neutrophil infiltration (Krawisz et al., 1984), increased with the development of gastric mucosal lesions in rats with water immersion restraint stress. Furthermore, our previous study (Nishida et al., 1997) showed that neutrophil accumulation in the damaged gastric mucosa of rats with water immersion restraint stress occurred in parallel with an increase in lipid peroxide concentration and a decrease in non-protein SH concentration in the tissue. Therefore, we have postulated that lipid peroxide formation and sulfhydryl oxidation in the gastric mucosa of rats with water immersion restraint stress could occur via neutrophils-derived oxygen free radicals such as O_2^- and H_2O_2, resulting in the development of gastric mucosal lesions.

Pre-administration of teprenone attenuated neutrophil infiltration into the gastric mucosa of rats with water immersion restraint stress with the prevention of an increase in lipid peroxide concentration and a decrease in non-protein SH concentration in the tissue. Because in in vitro study, teprenone in various concentrations up to 100 μg/ml did not affect myeloperoxidase activity in the gastric mucosa obtained from rats subjected to 6 h of water immersion restraint stress (data not shown), it was assumed that the decrease in gastric mucosal myeloperoxidase activity in rats with teprenone treatment was due to the inhibition of neutrophil infiltration, and not the direct inhibition of myeloperoxidase activity by the drug. Because we have observed that teprenone in various concentrations up to 100 μg/ml has no O_2^-- and ·HO-scavenging activities when the O_2^-- and ·HO-scavenging activities were checked by the cytochrome *c* method (Forman and Fridovich, 1973) and the deoxyribose method (Halliwell et al., 1987), respectively, teprenone itself may not act as a free radical scavenger against these oxygen radicals. From these findings, it is suggested that teprenone could attenuate lipid peroxide formation and sulfhydryl oxidation in the gastric mucosa of rats with water immersion restraint stress through the inhibition of neutrophil accumulation in the tissue, but not its free radical scavenging effect. Kurose and Granger (1994) have suggested the following mechanisms of neutrophil infiltration into tissues: first, production of neutrophil chemoattractants, such as leukotriene B_4 (lipoxygenase products), C5a and xanthine oxidase-derived O_2^- promote the neutrophil chemotaxis; second, expressions of neutrophil–endothelial cell adherent molecules such as P-selectin, CD11b/CD18, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) mediate neutrophil adherence and its emigration into the tissues. Recently, Yoshida et al. have observed that teprenone in concentrations of 10^{-5}–10^{-4} M significantly inhibit *Heliobacter pylori* extract-induced expressions of CD11b/CD18 on neutrophils and ICAM-1 and VCAM-1 on endothelial cells (personal communication with Dr. N. Yoshida in Kyoto Prefectural University of Medicine, Japan). Therefore, there is a possibility that teprenone attenuates neutrophil infiltration into the gastric mucosa of rats with water immersion restraint stress through the inhibition of the expression of neutrophil–endothelial cell adherent molecules. In the present study, it was found that teprenone pre-treatment slightly, but significantly, decreased gastric mucosal myeloperoxidase activity in control rats without water immersion re-

straint stress. Although this phenomenon cannot be explained precisely at present, teprenone may affect the physiological neutrophil recruitment and expulsion in the normal gastric mucosa.

Teprenone pre-treatment preserved both gastric mucus synthesis and secretion in rats with water immersion restraint stress, although the ability of teprenone to preserve gastric mucus synthesis was slightly lower than of preserving gastric mucus secretion. Accordingly, these preservative effects of teprenone on gastric mucus synthesis and secretion, as well as the preventive effect of this drug on neutrophil infiltration into gastric mucosal tissues could participate in the amelioration of gastric mucosal lesions induced by water immersion restraint stress. However, it seems likely that administered teprenone shows stronger prevention against gastric mucosal lesion development than against a decrease in gastric mucus level or neutrophil infiltration into gastric mucosal tissues in rats with water immersion restraint stress. Therefore, one can think that both the preservative effect of teprenone on gastric mucus level and the preventive effect of this drug on neutrophil infiltration into gastric mucosal tissues synergistically contribute to the attenuation of gastric mucosal lesion development in rats with water immersion restraint stress. As mentioned, it has been known that gastric mucin interacts with oxygen free radicals in vitro (Grisham et al., 1987) and that teprenone exerts a protective effect against ischemia/reperfusion-induced gastric mucosal lesion by stimulating gastric mucus synthesis and secretion (Seno et al., 1995). From these findings, it can be thought that the preservative effects of teprenone on the gastric mucus synthesis and secretion in rats with water immersion restraint stress may be related to the inhibition by the drug of increases in lipid peroxide formation and sulfhydryl oxidation in the gastric mucosal tissue.

On the other hand, anti-gastric ulcer drugs, such as teprenone, are usually administered to patients with gastritis or gastric ulcer after the patients are diagnosed as having these diseases. Furthermore, neutrophil infiltration has been observed in the gastric mucosa of patients with severe gastritis and chronic peptic ulcers (Cutin et al., 1987). Hence, we examined the effect of post-administration of teprenone on the progression of gastric mucosal injury and the enhanced infiltration of neutrophils into gastric mucosal tissues in rats with water immersion restraint stress. Administration of teprenone (200 mg/kg) to water immersion restraint-stressed rats 3 h after the beginning of the stress was found to significantly attenuate the progression of gastric mucosal lesions and the enhanced neutrophil infiltration into gastric mucosal tissues with preservations of gastric mucus synthesis and secretion 6 h after the beginning of the stress. These results indicate the possibility that teprenone exerts a therapeutic effect on stress-induced gastric mucosal lesions with inhibition of neutrophil infiltration into gastric mucosal tissues and preservation of gastric mucus synthesis and secretion in the clinical use of this drug. In addition, increased lipid peroxide concentration and decreased non-protein SH concentration found in the gastric mucosa of rats subjected to 6 h of water immersion restraint stress were attenuated by this post-administration of teprenone, as well as the pre-administration of this drug. Therefore, one can think that the prevention of neutrophil infiltration into gastric mucosal tissues and preservation of gastric mucus synthesis and secretion in water immersion restraint-stressed rats with post-teprenone administration result in the suppression of the increase in lipid peroxide concentration and the decrease in non-protein SH concentration in the gastric mucosal tissue.

It has been demonstrated that in rats that had oral administration of teprenone (125 mg/kg), the peak of teprenone concentration in the serum was observed 8–10 h after the drug administration, while the peak of teprenone concentration in the gastric mucosal tissue was found 2–4 h after the drug administration (Nishizawa et al., 1983). When 150 mg (approximately 2 mg/kg) of teprenone was orally administered to normal healthy subjects ($n = 12$), the peak of teprenone concentration in the serum was found 5 h after the administration, although the peak in the gastric mucosal tissue was not determined (personal communication with Eisai, Tokyo, Japan). Furthermore, in patients with severe peptic ulcer who have been orally administered with 50 mg of teprenone thrice daily for 1–13 days prior to surgery, the level of gastric mucosal teprenone in the ulcer region is 11-fold higher than that in the surrounding normal region (Nakazawa et al., 1983). These findings indicate that in rats with oral teprenone administration, a peak of an increase in teprenone concentration in the gastric mucosal tissue is observed before an increase in serum teprenone concentration reaches a peak and suggest that orally administered teprenone is easily transported to gastric mucosal tissues, resulting in an effective increase in its concentration in the tissue of rats and possibly of human subjects. In addition, it can be thought that orally administered teprenone reaches the lesion area in the stomach relatively early after the drug administration and shows protective and preventive effects on the gastric mucosal lesions.

5. Conclusion

The results obtained in this study indicate that teprenone has protective and preventive effects on gastric mucosal injury induced by water immersion restraint stress in rats and suggest that these effects could be related to the preservation of gastric mucus synthesis and secretion and inhibition of neutrophil infiltration into the gastric mucosal tissue. In addition, it can be expected that teprenone exerts a therapeutic action in patients with gastric mucosal injury with inflammation through the above-mentioned two mechanisms.

References

Andrew, F.J., Malcontenti, C., O'Brien, P.E., 1992. Sequence of gastric mucosal injury following ischemia and reperfusion. Role of reactive oxygen metabolites. Dig. Dis. Sci. 37, 1356–1361.

Bilski, J., Murty, V.L., Nadziejko, C., Sarosiek, J., Aono, M., Moriga, M., Slomiany, A., Slomiany, B.L., 1988. Protection against alcohol-induced gastric mucosal injury by geranylgeranylacetone: effect of indomethacin. Digestion 41, 22–33.

Cutin, J., Haase, H., Moura, M.A., 1987. Evaluation of electrical potential difference across gastric mucosa in patients with chronic gastritis according to site, histology, and degree of inflammation. Dig. Dis. Sci. 32, 239–243.

Forman, H.J., Fridovich, I., 1973. Superoxide dismutase: a comparison of rate constants. Arch. Biochem. Biophys. 158, 396–400.

Grisham, M.B., Von, R.C., Smith, B.F., Lamont, J.T., Granger, D.N., 1987. Interaction between oxygen radicals and gastric mucin. Am. J. Physiol. 253, G93–G96.

Halliwell, B., Gutteridge, J.M., Aruoma, O.I., 1987. The deoxyribose method: a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem. 165, 215–219.

Hamajima, E., Sugiyama, S., Hoshino, H., Goto, H., Tsukamoto, Y., Ozawa, T., 1994. Effects of FK506, an immunosuppressive agent, on genesis of water-immersion stress-induced gastric lesions in rats. Dig. Dis. Sci. 39, 713–720.

Hirota, M., Inoue, M., Ando, Y., Hirayama, K., Morino, Y., Sakamoto, K., Mori, K., Akagi, M., 1989. Inhibition of stress-induced gastric injury in the rat by glutathione. Gastroenterology 97, 853–859.

Hirota, M., Inoue, M., Ando, Y., Morino, Y., 1990. Inhibition of stress-induced gastric mucosal injury by a long acting superoxide dismutase that circulates bound to albumin. Arch. Biochem. Biophys. 280, 269–273.

Ito, M., Tanaka, T., Suzuki, Y., 1986. Increasing action of teprenone, a new antiulcer agent, on high-molecular-weight glycoprotein in gastric mucus during the healing process of acetic acid-induced ulcer in rats. Jpn. J. Pharmacol. 41, 117–125.

Itoh, Y., Suzuki, K., Matsubara, M., Takeyama, N., Kitazawa, Y., Tanaka, T., 1991. Changes in gastric mucosal content of adenosine, xanthine and hypoxanthine induced by restrained water immersion stress: antiulcer effects of tetraprenylacetone. Digestion 48, 25–33.

Kitagawa, H., Takeda, F., Kohei, H., 1986. A simple method for estimation of gastric mucus and effects of antiulcerogenic agents on the decrease in mucus during water-immersion stress in rats. Arzneim. Forsch. Drug Res. 36, 1240–1244.

Krawisz, J.E., Sharon, P., Stenson, W.F., 1984. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology 87, 1344–1350.

Kunisaki, C., Sugiyama, M., 1992. Effect of teprenone on acute gastric mucosal lesions induced by cold-restraint stress. Digestion 53, 45–53.

Kurose, I., Granger, D.N., 1994. Evidence implicating xanthine oxidase and neutrophils in reperfusion-induced microvascular dysfunction, Vol. 723, Ann. N.Y. Acad. Sci., 158–179.

McCord, J.M., Fridovich, J., 1979. The biology and pathology of oxygen radicals. Ann. Intern. Med. 89, 122–129.

Morimoto, Y., Shimohara, K., Oshima, S., Sukamoto, T., 1991. Effects of the new antiulcer agent KB-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, as compared to those of teprenone and cimetidine. Jpn. J. Pharmacol. 57, 495–505.

Murakami, M., Oketani, K., Fujisaki, H., Wakabayashi, T., Ohgo, T., 1981. Antiulcer effect of geranylgeranylacetone, a new acyclic polyisoprenoid on experimentally induced gastric and duodenal ulcers in rats. Arzneim. Forsch. Drug Res. 31, 799–804.

Murakami, M., Oketani, K., Fujisaki, H., Wakabayashi, T., 1982. Effect of geranylgeranylacetone (GGA) on gastric lesions induced by topical aspirin plus HCl. Jpn. J. Pharmacol. 32, 921–924.

Nakazawa, S., Tsuboi, Y., Tsukamoto, Y., Yoshino, J., Okada, M., 1983. Serum and stomach tissue levels of geranylgeranylacetone in patients. Int. J. Clin. Pharmacol. Ther. Toxicol. 21, 267–270.

Namiki, T., Egawa, M., Inoue, S., Tominaga, S., Takamura, Y., 1994. Gastric damage and body weight loss in LHA-lesioned rats: effects of cytoprotective geranylgeranylacetone. Am. J. Physiol. 266, R503–R509.

Neuhaus, O.W., Letzring, M., 1957. Determination of hexosamine in conjunction with electrophoresis. Anal. Chem. 29, 1230–1233.

Nishida, K., Ohta, Y., Kobayashi, T., Ishiguro, I., 1997. Involvement of the xanthine–xanthine oxidase system and neutrophils in the development of acute gastric mucosal lesions in rats with water immersion restraint stress. Digestion 58, 340–351.

Nishizawa, Y., Tanaka, K., Kinoshita, K., 1983. Absorption, distribution, metabolism, and excretion of tetraprenylacetone in rats and guinea pigs. Prog. Med. 3, 1029–1035.

Ohkawa, H., Onishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Sedlak, J., Lindsay, H.R., 1968. Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192–205.

Seno, K., Joh, T., Yokoyama, Y., Itoh, M., 1995. Role of mucus in gastric mucosal injury induced by local ischemia/reperfusion. J. Lab. Clin. Med. 126, 287–293.

Suzuki, K., Ota, H., Sasagawa, S., Sakatani, T., Fujikura, T., 1983. Assay method for myeloperoxidase in human polymorphonuclear leukocytes. Anal. Biochem. 132, 345–353.

Takagi, K., Okabe, S., 1968. The effects of drugs on the stress ulcer in rats. Jpn. J. Pharmacol. 18, 9–18.

Tanoue, K., Tarnawski, A.S., Kishihara, F., Ohta, M., Hashizume, M., Sugimachi, K., Sarfeh, I.J., 1996. Effect of teprenone on portal hypertensive gastric mucosa. Digestion 57, 35–40.

Terano, A., Hiraishi, H., Ota, S., Sugimoto, T., 1986. Geranylgeranylacetone, a novel antiulcer drug, stimulates mucus synthesis and secretion in rat gastric cultured cells. Digestion 33, 206–210.

Terano, A., Shiga, J., Mutoh, H., Hiraishi, H., Shiina, S., Kurita, M., Ota, S., Itoh, Y., Sugimoto, T., 1991. Tetraprenylacetone promotes healing process of ethanol-induced gastric damage in the rat. Jpn. J. Pharmacol. 55, 115–120.

Yoshikawa, T., Miyagawa, H., Yoshida, N., Sugino, S., Kondo, M., 1986. Increase in lipid peroxidation in rat gastric mucosal lesions induced by water-immersion restraint stress. J. Clin. Biochem. Nutr. 1, 271–277.

Yoshikawa, T., Ueda, S., Naito, Y., Takahashi, S., Oyamada, H., Morita, Y., Yoneta, T., Kondo, M., 1989. Role of oxygen-derived free radicals in gastric mucosal injury induced by ischemia or ischemia–reperfusion in rats. Free Radic. Res. Commun. 7, 285–291.