Effect of Oral Vitamin E Administration on Acute Gastric Mucosal Lesion Progression in Rats Treated with Compound 48/80, a Mast Cell Degranulator

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The effect of oral vitamin E administration on acute gastric mucosal lesion progression was examined in rats treated once with compound 48/80 (C48/80) (0.75 mg/kg, i.p.) in comparison with that of subcutaneously administrated superoxide dismutase (SOD) plus catalase (CAT). Vitamin E (50, 100 or 250 mg/kg) administrated at 0.5 h after C48/80 treatment reduced progressive gastric mucosal lesions at 3 h after the treatment dose-dependently, like SOD plus CAT administrated at the same time point. The gastric mucosa of C48/80-treated rats had decreased Se-glutathione peroxidase activity and vitamin E, ascorbic acid, and hexosamine contents and increased myeloperoxidase and xanthine oxidase activities and thiobarbituric acid reactive substances content at 3 h after the treatment. Administered vitamin E attenuated all these changes found at 3 h after C48/80 treatment dose-dependently, like administrated SOD plus CAT. C48/80-treated rats administrated with vitamin E (100 or 250 mg/kg) had higher gastric mucosal vitamin E content than C48/80-untreated rats. Neither administrated vitamin E nor SOD plus CAT had any effect on the increases in serum serotonin and histamine concentrations and the decrease in gastric mucosal blood flow found at 3 h after C48/80 treatment. In the gastric mucosa of C48/80-untreated rats administrated with vitamin E, thiobarbituric acid reactive substances content decreased with an increase in vitamin E content. These results indicate that orally administrated vitamin E prevents acute gastric mucosal lesion progression in C48/80-treated rats possibly by suppressing oxidative stress, neutrophil infiltration, and mucus depletion in the gastric mucosa like administrated SOD plus CAT.

Key words compound 48/80; gastric mucosal lesion (rat); vitamin E; lipid peroxidation; neutrophil infiltration; oxidative stress

Compound 48/80 (C48/80) is known to cause degranulation of connective tissue mast cells, but not mucosal mast cells, with release of serotonin and histamine from the cells.1,2) We have shown in rats with a single C48/80 treatment that the development of gastric mucosal lesions occurs with decreases in Se-glutathione peroxidase (Se-GSHpx) activity and vitamin E and hexosamine levels and increases in neutrophil infiltration, xanthine oxidase (XO) activity, and lipid peroxide level in the gastric mucosal tissue and that gastric mucosal blood flow changes like ischemia-reperfusion during gastric mucosal lesion development.3) We have also shown in rats treated once with C48/80 that neutrophils infiltrating into the gastric mucosal tissue participate in gastric mucosal lesion formation and progression, while the xanthine–XO system in the gastric mucosal tissue takes part mainly in the lesion progression.4) Furthermore, it has been shown in rats treated once with C48/80 that acutely released endogenous serotonin contributes to gastric mucosal lesion formation, while released endogenous histamine mainly contributes to the lesion progression, although gastric acid plays no important role in the pathogenesis of the C48/80-induced gastric mucosal lesion.5) Ascorbic acid (vitamin C) is a water-soluble antioxidant and it scavenges reactive oxygen species (ROS), such as superoxide radical (O2·−), hydroxyl radical (OH·), hydrogen peroxide (H2O2), singlet oxygen, and hypochlorous acid, by itself and also supports the chain-breaking antioxidant action of vitamin E by reducing vitamin E radical to vitamin E at the liquid/aqueous interface.6—11) In addition, ascorbic acid prevents neutrophil adherence to endothelium by scavenging ROS derived from activated neutrophils.12) Our recent reports have shown that ascorbic acid content decreases with a concomitant decrease in vitamin E content during lesion progression in the gastric mucosa of rats treated once with C48/80 and that gastric mucosal ascorbic acid plays a critical role in the progression of C48/80-induced gastric mucosal lesions, which is closely associated with its antioxidant and anti-inflammatory actions in the gastric mucosa.13,14)

Vitamin E is a lipid-soluble antioxidant and it functions as a chain-breaking antioxidant for lipid peroxidation in cell membranes and also as a scavenger of ROS such O2·−, OH·, and singlet oxygen.15) Vitamin E exerts an anti-inflammatory action by inhibiting the production O2·− in activated neutrophils, adhesion of neutrophils to endothelial cells, and transendothelial migration of neutrophils.16—20)

Yoshikawa et al.21) reported that both a decrease in gastric mucosal vitamin E level and an increase in gastric mucosal lipid peroxidation occurred during the development of ischemia-reperfusion-induced gastric mucosal injury in rats and that the severity of ischemia-reperfusion-induced gastric mucosal injury was enhanced in vitamin E-deficient rats. Naito et al.22) have shown in nitric oxide-depleted rats that vitamin E plays an important role in protecting against ischemia-reperfusion-induced gastric mucosal injury, and have suggested that this gastroprotective effect of vitamin E is due to not only its antioxidant action but also its inhibitory action on neutrophil infiltration into the gastric mucosa. Al-Dho- hyan and Al-Tuwaijri23) reported that a single oral pre-admin-

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oration of α-tocopherol acetate to rats prevented ischemia-reperfusion-induced gastric mucosal injury. It is also known that 2-(α-d-glucopyranosyl)ethyl-2,5,7,9-tetramethylchroman-6-ol, a water-soluble vitamin E derivative, protects against gastric mucosal damage induced by ischemia-reperfusion in rats by suppressing lipid peroxidation and inflammation in the gastric mucosal tissue. However, it is unknown whether orally administered vitamin E exerts a preventive effect on the progression of C48/80-induced acute gastric mucosal lesions in rats by exerting its antioxidant and anti-inflammatory actions.

The purpose of the present study was to clarify whether orally administered vitamin E prevents acute gastric mucosal lesion progression in rats treated with C48/80. In the present study, we examined the effect of orally administered vitamin E on gastric mucosal lesion progression and changes in the gastric mucosal activities of Se-GSHpx, myeloperoxidase (MPO), an index of tissue neutrophil infiltration, and XO and the gastric mucosal contents of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation, vitamin E, ascorbic acid, and hexosamine, an index of gastric mucus, with the lesion progression in rats treated once with C48/80. The effect of orally administered vitamin E on changes in serum serotonin and histamine concentrations and gastric mucosal blood flow with gastric mucosal lesion progression were also examined in the C48/80-treated rats. In addition, the preventive effect of vitamin E administration on the progression of C48/80-induced acute gastric mucosal lesions was compared with that of the administration of antioxidant enzymes, i.e., superoxide dismutase (SOD), an enzyme to scavenge O2 to H2O2 and O2, plus catalase (CAT), an enzyme to decompose H2O2 to H2O and O2, on the gastric mucosal lesion progression.

MATERIALS AND METHODS

Materials C48/80, methyl serotonin, 3,3',5,5'-tetramethylbenzidine, RRR-α-Toc., xanthine, CAT (purified from bovine liver), Cu,Zn-SOD (purified from bovine erythrocyte), yeast glutathione reductase were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); l-ascorbic acid (reduced form), α,α′-dipirydyl, ethylenediaminetetraacetic acid (EDTA), glucosamine, reduced glutathione (GSH), NADPH, o-phthalaldehyde, 2-thiobarbituric acid, and other chemicals from Wako Pure Chemicals Ind., Co. (Osaka, Japan).

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

Gastric Mucosal Lesion Induction by Compound 48/80 C48/80 (0.75 mg/kg), dissolved in distilled water, was intraperitoneally injected to 7-week-old rats fasted for 24 h at a dosing volume of 3 ml/kg, as described previously. The control rats received an i.p. injection of an equal volume of distilled water. All animals were maintained with free access to water and without food during the experiment. The animals were sacrificed under ether anesthesia 0.5 or 3 h after C48/80 injection. The stomachs were removed, inflated with 10 ml of 0.9% NaCl, and put into 10% formalin for 10 min. The stomachs were then opened along the greater curvature and examined for lesions in the glandular part under a dissecting microscope (×10). The severity of gastric mucosal lesions was estimated using the index of the following eight grades of lesions as described in our previous reports: grade 0, no lesion (normal); grade I, edema only; grade II, damaged area of 1—10 mm2; grade III, damaged area of 11—20 mm2; grade IV, damaged area of 21—30 mm2; grade V, damaged area of 31—40 mm2; grade VI, damaged area of 41—50 mm2; grade VII, damaged area of >51 mm2.

Administration of Vitamin E or SOD Plus CAT Vitamin E (RRR-α-Toc.) (50, 100 or 250 mg/kg), dissolved in 5% Tween 80, was orally administered to rats treated with and without C48/80 at a constant dosing volume of 5 ml/kg BW with a stomach tube. A mixture of SOD (50000 U/kg, 11.4 mg protein/kg) and CAT (90000 U/kg, 3.6 mg protein/kg), dissolved in 0.9% NaCl, was injected subcutaneously into rats treated with and without C48/80 at a volume of 1 ml/kg. Vitamin E or SOD plus CAT were administered 0.5 h after C48/80 treatment. Half of C48/80-treated and untreated rats with neither vitamin E nor SOD plus CAT administration received an equal volume of 5% Tween 80 at the same time point. Half of C48/80-treated and untreated rats with neither vitamin E nor SOD plus CAT administration received an equal volume of 0.9% NaCl at the same time point. There were no differences in the condition of gastric mucosal tissue and the levels of all parameters determined between C48/80-treated rats receiving 5% Tween 80 and 0.9% NaCl or between C48/80-untreated rats receiving 5% Tween 80 and 0.9% NaCl.

Determinations of Gastric Mucosal Enzymes and Components Gastric mucosal Se-GSHpx and MPO were assayed by the methods of Hochstein and Utley and Suzuki et al., respectively. For assays of both enzymes, gastric mucosal tissues were homogenized in 9 volumes of ice-cold 0.05 M Tris–HCl buffer (pH 7.4). After sonication on ice for 20 s using a Handy Sonic model UR-20P (Tony Seiko Co., Tokyo, Japan), the homogenate was centrifuged at 4°C (10000 × g, 20 min), and the resultant supernatant was dialyzed against 100 volumes of the same buffer at 4°C for 24 h. Se-GSHpx activity was determined at 37°C by recording the decrease in absorbance at 340 nm following the oxidation of NADPH in the presence of H2O2, GSH, and yeast glutathione reductase. One unit (U) of this activity is defined as the amount of enzyme oxidizing 1 μmol NADPH per min. 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. For this enzyme assay, gastric mucosal tissues were homogenized in 9 volumes of ice-cold 0.25 M sucrose. The homogenate was sonicated as described above. The sonicated homogenate was centrifuged at 4°C (10000 × g, 20 min), and the resultant supernatant was dialyzed against 100 volumes of the same...
solution at 4 °C for 24 h. XO activity was assessed by measuring the increase in absorbance at 292 nm following the formation of uric acid at 30 °C. One unit (U) of this enzyme is defined as the amount of enzyme forming 1 μmol uric acid per min. For the determinations of gastric mucosal TBARS and ascorbic acid, gastric mucosal tissues were homogenized in 9 volumes of ice-cold 0.15 M KCl–1 mM EDTA. Gastric mucosal thiobarbituric acid reactive substances were spectrophotometrically determined by the thiobarbituric acid method of Ohkawa et al. except that 1.0 mM EDTA was added to the reaction medium. The amount of TBRAS is expressed as that of malondialdehyde (MDA) equivalents. Gas- 

tric tissue was performed after evaluation of the lesion index. Samples of the corpus were excised and transformed to 10% fresh formalin and later processed by routine techniques before embedding in paraffin. Sections (2 μm thick) were mounted on glass slides and stained with hematoxylin and eosin. Coded slides were examined by an experienced pathologist blinded to the treatment using a light microscope.

**Histological Examination**  Histological study of the gastric tissue was performed after evaluation of the lesion index. Samples of the corpus were excised and transformed to 10% fresh formalin and later processed by routine techniques before embedding in paraffin. Sections (2 μm thick) were mounted on glass slides and stained with hematoxylin and eosin. Coded slides were examined by an experienced pathologist blinded to the treatment using a light microscope.

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

**RESULTS**

**Effect of Vitamin E or SOD Plus CAT Administration on Gastric Mucosal Lesion Development**  As shown in Table 1, gastric mucosal lesions appeared 0.5 h after treatment with C48/80 (0.75 mg/kg) and progressed at 3 h. Oral administration of vitamin E at a dose of 100 or 250 mg/kg, but not 50 mg/kg, which was conducted 0.5 h after C48/80 treatment, significantly prevented progressive gastric mucosal lesions at 3 h after the treatment and this preventive effect occurred in a dose-dependent manner (Table 1). In addition, the C48/80-treated group administered with vitamin E

<table>
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<tr>
<th>Time after C48/80 treatment and groups</th>
<th>Lesion index (%)</th>
<th>p-value (vs. C48/80-treated rats, 0.5 h)</th>
<th>p-value (vs. C48/80-treated rats, 3 h)</th>
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<tr>
<td></td>
<td>0</td>
<td>I</td>
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<tr>
<td>0.5 h C48/80</td>
<td>0</td>
<td>10</td>
<td>50</td>
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<tr>
<td>3 h C48/80</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>+ Vitamin E (50 mg/kg)</td>
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<td>+ SOD + CAT</td>
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The number of rats used in each group is 10. NS indicates not significant.
(250 mg/kg) showed the severity of gastric mucosal lesions almost equal to that found just before vitamin E administration, i.e., at 0.5 h after C48/80 treatment (Table 1). The severity of gastric mucosa lesions in the C48/80-treated group administered with SOD plus CAT was significantly weaker than that in the C48/80-treated group and was similar to that in the C48/80-treated group administered with vitamin E (250 mg/kg) (Table 1). No gastric mucosal lesion was found in C48/80-untreated rats administered with and without either vitamin E (50, 100 or 250 mg/kg) or SOD plus CAT in the same manner (data not shown).

Effect of Vitamin E Administration on Histological Changes in Gastric Tissues Hemorrhage and superficial exfoliation (Fig. 1C) and inflammatory cells including neutrophils (Fig. 1D) were observed in the gastric tissue of the C48/80-treated group in comparison with the gastric tissue of the C48/80-untreated group (control) (Figs. 1A, B). The gastric tissue of the C48/80-treated group administered with vitamin E (250 mg/kg) showed much lighter histological changes than that of the C48/80-treated group (Figs. 1E, F).

Effect of Vitamin E or SOD Plus CAT Administration on Serum Serotonin and Histamine Concentrations and Gastric Mucosal Blood Flow Rats treated with C8/80 alone had 4.4- and 64.2-fold higher serum serotonin and histamine concentrations, respectively, than C48/80-untreated rats (control) at 0.5 h after the treatment and the C48/80-treated group had 2.3- and 6.1-fold higher serum serotonin and histamine concentrations, respectively, than the control group at 3 h (Figs. 2A, B). Oral administration of vitamin E (50, 100 or 250 mg/kg) or SOD plus CAT at 0.5 h after C48/80 treatment did not affect the increases in serum serotonin and histamine concentrations found at 3 h after the treatment (Figs. 2A, B). The level of gastric mucosal blood flow in the C48/80-treated group was 26 and 78% of that in the control group at 0.5 and 3 h, respectively, at 3 h after the treatment (Fig. 2C). The administration of vitamin E (50, 100 or 250 mg/kg) or SOD plus CAT did not affect the change in gastric mucosal blood flow found at 3 h after C48/80 treatment (Fig. 2C). There were no changes in serum serotonin and histamine concentrations and gastric mucosal blood flow in C48/80-untreated rats administered with and without either vitamin E (50, 100 or 250 mg/kg) or SOD plus CAT (Fig. 2).

Effect of Vitamin E or SOD Plus CAT Administration on Gastric Mucosal Vitamin E Content As shown in Fig. 2, there was no significant difference in gastric vitamin E content between the C48/80-treated and control groups at 0.5 h after the treatment but the C48/80-treated group had significantly lower gastric vitamin E content than the control group at 3 h. Vitamin E (50, 100 or 250 mg/kg) administered at 0.5 h after C48/80 treatment significantly attenuated the decrease in gastric mucosal vitamin E content found at 3 h after the treatment in a dose-dependent manner (Fig. 3). Administration of vitamin E (50, 100 or 250 mg/kg) to C48/80-untreated rats significantly increased the gastric mucosal vitamin E content and the increasing effect occurred dose-dependently (Fig. 3). In addition, the C48/80-treated group administered with vitamin E (50 mg/kg) had almost as much gastric mucosal vitamin E content as the control group (Fig. 3). The C48/80-treated group administered with vitamin E (100 or 250 mg/kg) had significantly higher gastric mucosal vitamin E content than the control group and also higher gastric mucosal vitamin E content than the C48/80-untreated groups administered with the same doses of vitamin E (Fig. 3). The C48/80-treated group administered with SOD plus CAT had almost as much gastric mucosal vitamin E content as the control group and the C48/80-untreated group administered with SOD plus CAT alone showed no change in gastric mucosal vitamin E content (Fig. 3).

Effect of Vitamin E or SOD Plus CAT Administration on Gastric Mucosal TBARS Content and MPO and XO Activities As shown in Fig. 4A, the C48/80-treated group...
had 1.3- and 1.8-fold higher gastric mucosal TBARS content than that in the control group at 0.5 and 3 h after the treatment, respectively. Oral administration of vitamin E (50, 100 or 250 mg/kg) at 0.5 h after C48/80 treatment significantly attenuated the increase in gastric mucosal TBARS content found at 3 h after the treatment and the attenuating effect occurred in a dose-dependent manner (Fig. 4A). In addition, the gastric mucosal TBARS content in the C48/80-treated group administered with vitamin E (250 mg/kg) was near that in the control group (Fig. 4A). Administration of SOD plus CAT at 0.5 h after C48/80 treatment reduced the increase in the gastric mucosal TBARS content found at 3 h after the treatment significantly and the reduced gastric mucosal TBARS content was almost equal to that of the C48/80-treated group administered with vitamin E (250 mg/kg) (Fig. 4A). As shown in Fig. 4B, the C48/80-treated group had 1.7- and 3.4-fold higher gastric mucosal MPO activity than that in the control group at 0.5 and 3 h after the treatment, respectively. Administration of vitamin E at a dose of 100 or 250 mg/kg, but not 50 mg/kg, at 0.5 h after C48/80 treatment significantly attenuated the increases in gastric mucosal MPO activity found at 3 h after the treatment, although the attenuating effect was a little larger at a dose of 250 mg/kg than at a dose of 100 mg/kg (Fig. 4B). Administration of SOD plus CAT at 0.5 h after C48/80 treatment reduced the increase in the gastric mucosal MPO activity found at 3 h after the treatment significantly and the reduced gastric mucosal MPO activity was almost equal to that of the C48/80-treated group administered with vitamin E (250 mg/kg) (Fig. 4B). As shown in Fig. 4C, the C48/80-treated group had 1.7- and 5.7-fold higher gastric mucosal XO activity than that in the control group at 0.5 and 3 h after the treatment, respectively. Administration of vitamin E at a dose of 100 or 200 mg/kg, but not 50 mg/kg, at 0.5 h after C48/80 treatment significantly attenuated the increase in gastric mucosal XO activity found at 3 h after the treatment, although the attenuating effect was a little larger at a dose of 250 mg/kg than at a dose of 100 mg/kg (Fig. 4C). Administration of SOD plus CAT at 0.5 h after C48/80 treatment reduced the increase in gastric mucosal XO activity found at 3 h after the treatment significantly and the reduced gastric mucosal XO activity was almost equal to that of the C48/80-treated group administered with vitamin E (250 mg/kg) (Fig. 4C). When vitamin E (50, 100 or 250 mg/kg) was administered to C48/80-untreated rats, no dose of vitamin E had any effect on the gastric mucosal MPO and XO activities (Figs. 3A, B). However, administration of vitamin E to C48/80-untreated rats at a dose of 100 or 250 mg/kg significantly reduced the gastric mucosal TBARS content, although the reducing effect was larger at a dose of 250 mg/kg than at 100 mg/kg (Fig. 3C). The C48/80-untreated group administered with SOD plus CAT showed no changes in gastric mucosal TBARS content and MPO and XO activities (Fig. 3).

**Effect of Vitamin E or SOD Plus CAT Administration on Gastric Mucosal Se-GSHpx Activity and Ascorbic Acid and Hexosamine Contents** As shown in Fig. 5A, the
C48/80-treated group had 73.5 and 40.4% of gastric mucosal Se-GSHpx activity in the control group at 0.5 and 3 h after the treatment, respectively. Oral administration of vitamin E at a dose of 100 or 250 mg/kg, but not 50 mg/kg, at 0.5 h after C48/80 treatment significantly attenuated the decrease in gastric mucosal Se-GSHpx activity found at 3 h after the treatment and the attenuating effect occurred dose-dependently (Figs. 4B, C). Administration of SOD plus CAT at 0.5 h after C48/80 treatment attenuated the decreases in gastric mucosal ascorbic acid and hexosamine contents found at 3 h after the treatment significantly and the restored gastric mucosal ascorbic acid and hexosamine contents were almost equal to those of the C48/80-treated group administered with vitamin E (250 mg/kg) (Figs. 5B, C). Administration of vitamin E (50, 100 or 250 mg/kg) or SOD plus CAT to C48/80-untreated rats did not affect the gastric mucosal Se-GSHpx activity and ascorbic acid and hexosamine contents (Fig. 5).

DISCUSSION

The model of acute gastric mucosal lesions in rats treated with C48/80, a mast cell degranulator, has been thought to be important for clarifying the roles of ischemia-reperfusion, oxidative stress, and inflammation in the pathogenesis of gastritis in humans.3-5,14) Recently, it has been indicated that Helicobacter pylori might be involved in gastric mucosal inflammation and the reduction of gastric mucosal blood flow...
The present study has clearly shown that orally administered vitamin E at a dose of 100 or 250 mg/kg, but not 50 mg/kg, prevents acute gastric mucosal lesion progression in rats with a single C48/80 treatment significantly in comparison with the administration of SOD plus CAT, antioxidant enzymes. Histological observation of the gastric tissue of C48/80-treated rats administered with vitamin E (250 mg/kg) supported the preventive effect of orally administered vitamin E on the progression of C48/80-induced acute gastric mucosal lesions. To the best of our knowledge, this study is the first report showing that orally administered vitamin E prevents the progression of acute gastric mucosal lesions in experimental animal models.

It has been shown that vitamin E inhibits C48/80-induced histamine release from connective tissue mast cells in vitro. In the present study, vitamin E administered orally to rats at 0.5 h after a single treatment with C48/80 (0.75 mg/kg, i.p.) had no effect on the changes in serum serotonin and histamine concentrations found at 3 h after the treatment, as found in C48/80-treated rats administered with SOD plus CAT. Takeuchi et al. have shown in rats treated once with C48/80 (0.75 mg/kg, i.p.) that serotonin and histamine are released from the extravascular connective tissue mast cells and that the release of serotonin and histamine reaches a maximum within 0.5 h after the treatment. Therefore, it seems unlikely that orally administered vitamin E prevents the progression of acute gastric mucosal lesions in C48/80-treated rats by affecting the C48/80-mediated release of histamine and serotonin from the connective tissue mast cells. It has been shown in rats treated once with C48/80 (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. Thus, gastric mucosal blood flow shows an ischemia-reperfusion-like change in C48/80-treated rats. In the present study, administration of vitamin E (50, 100 or 250 mg/kg) to C48/80-treated rats at 0.5 h after the treatment had no effect on the recovery of reduced gastric mucosal blood flow at 3 h as found in C48/80-treated rats administered with SOD plus CAT. These results indicate that orally administered vitamin E prevents acute gastric mucosal lesion progression in rats treated once with C48/80 without affecting the change in gastric mucosal blood flow.

Yoshikawa et al. have shown that gastric mucosal vitamin E concentration decreases with an increase in gastric mucosal TBARS concentration during the development of ischemia-reperfusion-induced gastric mucosal injury in rats and that the severity of ischemia-reperfusion-induced gastric mucosal injury is enhanced in vitamin E-deficient rats. The authors have indicated that when the gastric mucosa of rats is subjected to ischemia-reperfusion, vitamin E in the gastric mucosal tissue is consumed in the process of lipid peroxidation induced by ROS generated mainly in reperfusion to prevent the development of gastric mucosal injury. In the present study, C48/80-treated rats showed a decrease in gastric mucosal vitamin E content with an enhanced increase in gastric mucosal TBARS content at a progressed stage of gastric mucosal lesions, i.e., at 3 h after the treatment, as shown in our previous report. These results suggest that vitamin E in the gastric mucosa of rats with a single C48/80 treatment is consumed in the enhancing process of gastric mucosal lipid peroxidation to prevent the progression of gastric mucosal lesions during which the gastric mucosal blood flow shows a reperfusion-like change as described above. Vitamin E (50, 100 or 250 mg/kg) administered to C48/80-treated rats at 0.5 h after the treatment attenuated both the decrease in gastric mucosal vitamin E content and the increase in gastric mucosal TBARS content found at 3 h after the treatment in a dose-dependent manner. In addition, vitamin E (50, 100 or 250 mg/kg) administered to C48/80-untreated rats increased the gastric mucosal vitamin E content with a decrease in the gastric mucosal TBARS content dose-dependently. However, vitamin E administered to C48/80-treated rats at a dose of 50 mg/kg had no significant preventive effect on gastric mucosal lesion progression as described above although the administered vitamin recovered the decreased gastric mucosal vitamin E content near to the level of C48/80-untreated rats and reduced the increased gastric mucosal TBARS content significantly. These results suggest that orally administered vitamin E could exert a preventive effect on acute gastric mucosal lesion progression in C48/80-treated rats by raising gastric vitamin E content over the level of the C48/80-untreated rats. In fact, C48/80-treated rats administered with vitamin E at a dose of 100 or 250 mg/kg had higher gastric mucosal vitamin E content than not only C48/80-untreated rats but also C48/80-untreated rats administered with the same doses of vitamin E at 3 h after the treatment. We cannot explain this difference in increased gastric mucosal vitamin E content after oral vitamin E administration between C48/80-treated and untreated rats at present, because the mechanism for the uptake of vitamin E in gastric mucosal tissues has not been clarified yet. However, the mechanisms for the uptake of vitamin E in peripheral tissues have been thought as follows; the uptake of vitamin E occurs during catabolism of triacylglycerol-rich lipoproteins by the activity of lipoprotein lipase via the low-density lipoprotein receptor or non-receptor-mediated uptake. Therefore, the aforementioned phenomenon that C48/80-treated rats administered with vitamin E at a dose of 100 or 250 mg/kg had higher gastric mucosal vitamin E content than C48/80-untreated rats administered with the same doses of vitamin E may be explained as follows: the regulation of vitamin E transport into the gastric mucosal tissue of C48/80-treated rats is impaired at 0.5 h after the treatment, so that vitamin E administered to the C48/80-treated rats at 0.5 h after the treatment is transported into the gastric mucosal tissue under the condition where the vitamin E uptake regulation is impaired, resulting in an excess transport of vitamin E into the gastric mucosal tissue of the C48/80-treated rats at 3 h after the treatment. Administration of SOD plus CAT to C48/80-treated rats prevented gastric mucosal lesion progression with decreases in both the increase in gastric mucosal TBARS content and the decrease in gastric mucosal vitamin E content found at 3 h after the treatment significantly, although the restored gastric mucosal vitamin E content was almost equal to the level of C48/80-untreated rats. Thus, there was a clear difference in the preventive effect on gastric mucosal lesion progression in C48/80-treated rats through attenuation of increased lipid peroxidation and decreased vitamin E content in the gastric mucosa between administered vitamin E and SOD plus CAT.

Vitamin E is known to exert an anti-inflammatory action...
by inhibiting the production of $\text{O}_2^·$ in activated neutrophils, adhesion of neutrophils to endothelial cells, and trans-endothelial migration of neutrophils.16—20 Naito et al.21 have shown that vitamin E protects against ischemia-reperfusion-induced gastric mucosal injury under nitric oxide depletion in rats by inhibiting not only lipid peroxidation but also neutrophil infiltration in the gastric mucosal tissue. We have reported that neutrophil infiltration into gastric mucosal tissues contributes to not only the formation but also the progression of C48/80-induced acute gastric mucosal lesions in rats.43 In the present study, vitamin E (100 or 250 mg/kg) administered to C48/80-treated rats at 0.5 h after the treatment attenuated an enhancement of the increased gastric mucosal activity of MPO, an index of neutrophil infiltration, at 3 h significantly. No doses of vitamin E administered to C48/80-untreated rats had any effect on gastric mucosal MPO activity. In addition, we have observed that vitamin E at concentrations of 5 to 100 $\mu$g/ml cannot inhibit MPO activity in gastric mucosal tissue preparations from rats treated with C48/80 in vitro (unpublished data). These results suggest that orally administered vitamin E prevents acute gastric mucosal lesion progression in rats treated with C48/80 by attenuating an enhancement of increased neutrophil infiltration into gastric mucosal tissues. It is known that activated neutrophils mediate lipid peroxidation via ROS generated by NADPH oxidoreductase.42 It is also known that MPO mediates lipid peroxidation in the presence of $\text{H}_2\text{O}_2$ with halide ions.43 Therefore, a part of the aforementioned reduction of gastric mucosal TBARS content in C48/80-treated rats by vitamin E administration may be due to the inhibitory effect of the administered vitamin on neutrophil infiltration into gastric mucosal tissues. We have reported that an increase in the gastric mucosal activity of XO, an enzyme to generate $\text{O}_2^·$ and $\text{H}_2\text{O}_2$, in the presence of hypoxanthine or xanthine, is mainly associated with acute gastric mucosal lesion progression in rats treated with C48/80, and have suggested that this increase in gastric mucosal XO activity is closely related to neutrophil infiltration into the gastric mucosal tissue.34 In the present study, vitamin E (100 or 250 mg/kg) administered to C48/80-treated rats attenuated an enhancement of the increased gastric mucosal XO activity found at 3 h after the treatment significantly. No doses of vitamin E administered to C48/80-untreated rats had any effect on gastric mucosal XO activity. In addition, we have observed that vitamin E at concentrations of 5 to 100 $\mu$g/ml cannot inhibit XO activity in gastric mucosal tissue preparations from rats treated with C48/80 in vitro (unpublished data). These results suggest that orally administered vitamin E prevents acute gastric mucosal lesion progression in C48/80-treated rats by attenuating an enhancement of increased gastric mucosal XO activity through its inhibitory action on neutrophil adherence to endothelium.15—20 Administration of SOD plus CAT to C48/80-treated rats attenuated the decrease in gastric mucosal Se-GSHpx activity found at 3 h after the treatment, although the administration of SOD plus CAT to C48/80-untreated rats did not affect the gastric mucosal Se-GSHpx activity. Therefore, these findings suggest that orally administered vitamin E exerts a preventive effect on acute gastric mucosal lesion progression in rats treated with C48/80 by maintaining gastric mucosal Se-GSHpx activity through its action to scavenge $\text{O}_2^·$ and OH‘ and to inhibit neutrophil-derived $\text{O}_2^·$ production and neutrophil infiltration in the gastric mucosal tissue like administered SOD plus CAT.

Our recent reports have shown that gastric ascorbic acid content decreases with acute gastric mucosal lesion progression in rats treated with C48/80 and that gastric mucosal ascorbic acid plays a critical role in the progression of C48/80-induced acute gastric mucosal lesions.11,14 In the present study, post-administered vitamin E (100 or 250 mg/kg) attenuated the decrease in gastric mucosal ascorbic acid content found at 3 h after C48/80 treatment significantly, although the same doses of vitamin E administered to C48/80-untreated rats did not affect the gastric mucosal ascorbic acid content. SOD plus CAT administered to C48/80-treated rats reduced the decrease in gastric mucosal ascorbic acid content found at 3 h after C48/80 treatment significantly, although the same doses of vitamin E administered to C48/80-untreated rats did not affect the gastric mucosal ascorbic acid content. SOD plus CAT administered to C48/80-treated rats reduced the decrease in gastric mucosal ascorbic acid content found at 3 h after C48/80 treatment significantly, although the same doses of vitamin E administered to C48/80-untreated rats did not affect the gastric mucosal ascorbic acid content. Vitamin E scavenges $\text{O}_2^·$ and OH‘ in vitro and also inhibits the production of $\text{O}_2^·$ in activated neutrophils in addition to its inhibitory action on neutrophil adherence to endothelium. Vitamin E scavenges $\text{O}_2^·$ and OH‘ in vitro and also inhibits the production of $\text{O}_2^·$ in activated neutrophils in addition to its inhibitory action on neutrophil adherence to endothelium.
Gastric mucus plays a critical role in the primary defense of the gastric mucosa and provides a protective barrier in the gastric epithelium. It is known to interact with ROS, especially OH\(^-\), in vitro. Gastric mucus plays an important role in protecting the gastric mucosa of rats against ischemia-reperfusion stress. In the present study, rats treated with C48/80 showed an apparent decrease in the gastric mucosal content of hexosamine, a maker of gastric mucus, at 3 h after the treatment, as reported previously. Vitamin E (100 or 250 mg/kg) administered to C48/80-treated rats attenuated the decrease in gastric mucosal hexosamine content at 3 h after the treatment significantly. SOD plus CAT administered to C48/80-treated rats did not affect the gastric mucosal hexosamine content, although SOD plus CAT administered to C48/80-un-treated rats possibly through its antioxidant and anti-inflammatory actions like administered SOD plus CAT, which may contribute to its preventive effect on the progression of C48/80-induced acute gastric mucosal lesions.

In conclusion, the results of the present study indicate that orally administered vitamin E exerts a preventive effect on acute gastric mucosal lesion progression in C48/80-treated rats possibly by suppressing oxidative stress, neutrophil infiltration, and mucus depletion in the gastric mucosal tissue and provides a protective barrier in the gastric epithelium. These results indicate that orally administered vitamin E prevents the degradation of gastric mucus in C48/80-treated rats possibly through its antioxidant and anti-inflammatory actions like administered SOD plus CAT, which may contribute to its preventive effect on the progression of C48/80-induced acute gastric mucosal lesions.

REFERENCES