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# Preventive effect of ebselen on acute gastric mucosal lesion development in rats treated with compound 48/80

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**Abstract**

The preventive effect of ebselen, a seleno-organic compound, which is known to possess glutathione peroxidase-like activity and antioxidative and anti-inflammatory properties, on the development of acute gastric mucosal lesions was examined in rats with a single injection of compound 48/80 (0.75 mg/kg, i.p.), a mast cell degranulator. Pre-administration of ebselen (p.o.) at a dose of 50 or 100 mg/kg, but not 10 mg/kg, prevented gastric mucosal lesion development at 3 h, but not gastric mucosal lesion formation at 0.5 h, after compound 48/80 injection, although any dose of pre-administered ebselen did not affect decreased gastric mucosal blood flow and increased serum serotonin and histamine concentrations found at 0.5 and 3 h after compound 48/80 injection. A decrease in Se-glutathione peroxidase activity and increases in the activities of myeloperoxidase, an index of tissue neutrophil infiltration, and xanthine oxidase and the concentration of thiobarbituric acid reactive substances, an index of lipid peroxidation, were found in gastric mucosal tissues at 0.5 h after compound 48/80 injection and these changes were further enhanced at 3 h. Pre-administration of ebselen (p.o.) at a dose of 50 or 100 mg/kg, but not 10 mg/kg, attenuated all these changes found at 3 h after compound 48/80 injection. These preventive effects of ebselen occurred in a dose-dependent manner. The present results indicate that pre-administered ebselen prevents the development of compound 48/80-induced acute gastric mucosal lesions in rats, and suggest that this preventive effect of ebselen could be due to its glutathione peroxidase-like activity and antioxidative and anti-inflammatory properties. © 2001 Published by Elsevier Science B.V.

*Keywords:* Ebselen; Gastric mucosal lesion, acute rat; Compound 48/80; Neutrophil infiltration; Xanthine oxidase; Glutathione peroxidase; Lipid peroxidation

## 1. Introduction

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)one) is a seleno-organic compound. This seleno-organic compound is known to possess glutathione (GSH) peroxidase-like activity and antioxidative and anti-inflammatory properties (Wendel et al., 1984; Müller et al., 1984, 1985; Issekutz and Lopes, 1992; Noguchi et al., 1992; Goa and Issekutz, 1993a,b, 1994). Ebselen has been reported to protect against various types of experimentally induced gastric mucosal lesions such as aspirin-, diclofenac-, HCl-, acidified ethanol-, ethanol-, water immersion restraint stress-, burn stress-, and ischemia–reperfusion-induced gastric mucosal lesions (Ueda et al., 1989, 1990; Kurebayashi et al., 1989; Leyck and Parnham, 1990; Tabuchi and Kurebayashi, 1993; Tabuchi et al., 1995; Unlucerci et al., 1999). Ebselen has also been reported to inhibit gastric acid secretion in pylorus-ligated rats (Tabuchi and Kurebayashi, 1993) and in parietal cells or gastric vesicles by interference with sulfhydryl groups of the gastric proton pump, $H^+$, $K^+$–ATPase (Beil et al., 1990; Tabuchi et al., 1994), although there is a report showing that ebselen has no effect on gastric acid secretion in pylorus-ligated rats (Ueda et al., 1990).

Compound 48/80 is known to cause degranulation of connective tissue mast cells with release of serotonin and histamine from the cells (Enerback and Lundin, 1974; Befus et al., 1982; Irman-Florjanc and Erjavec, 1983). Recently, it has been reported that ebselen inhibits compound 48/80-induced histamine release in rat peritoneal

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mast cells (Tchoumken-Nzouessa and Rebel, 1998). Gastric mucosal lesions in rats injected with compound 48/80 resemble variolifomred gastritis occurring in humans (Feldberg and Talenski, 1953; Takeuchi et al., 1986; Yasuhiro et al., 1997). We have shown that, in the gastric mucosa of rats with a single compound 48/80 injection, lesion development occurs with decreases in Se-glutathione (Se-GSH) peroxidase activity and vitamin E and hexosamine contents and increases in neutrophil infiltration, xanthine oxidase activity, and lipid peroxide content and that, in the compound 48/80-injected rats, gastric mucosal blood flow decreases with gastric mucosal lesion formation and the decreased blood flow recovers with the lesion development (Ohta et al., 1997). We have also shown that, in rats with a single injection of compound 48/80, neutrophils infiltrating into the gastric mucosal tissue participate in gastric mucosal lesion formation, while the xanthine–xanthine oxidase system in the gastric mucosal tissue takes part in the lesion development rather than the lesion formation (Ohta et al., 1999a). Furthermore, we have shown that, in rats with a single compound 48/80 injection, acutely released endogenous serotonin causes gastric mucosal lesion formation with a decrease in gastric mucosal blood flow, while released endogenous histamine mainly contributes to the lesion development and that gastric acid plays little role in the pathogenesis of the compound 48/80-induced gastric mucosal lesion (Ohta et al., 1997, 1999b). These findings may allow us to assume that ebselen prevents the development of acute gastric mucosal lesions in rats with a single injection of compound 48/80 through its GSH peroxidase-like activity and antioxidative and anti-inflammatory properties.

In the present study, therefore, we attempted to confirm the preventive effect of ebselen on the development of acute gastric mucosal lesions in rats with a single injection of compound 48/80. Namely, we examined the effect of pre-oral administration of ebselen on gastric mucosal lesion development and changes in the activities of gastric mucosal Se-GSH peroxidase, myeloperoxidase, an index of tissue neutrophil infiltration (Krawisz et al., 1984), and xanthine oxidase and the content of gastric mucosal thiobarbituric acid reactive substances, an index of lipid peroxidation, with the lesion development in compound 48/80-injected rats. We further examined the effect of pre-orally administered ebselen on changes in gastric mucosal blood flow and serum serotonin and histamine concentrations with gastric mucosal lesion development in the compound 48/80-injected rats.

## 2. Materials and methods

### 2.1. Materials

Ebselen was kindly provided by Daichi Pharmaceutical (Tokyo, Japan). Compound 48/80, methyl serotonin, 3,3′,5,5′-tetramethylbenzidine, xanthine, and yeast glutathione reductase were purchased from Sigma (St. Louis, MO, USA); 2,2′-azobis(2-amindinopropane) dihydrochloride (AAPH), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), NADPH, *o*-phthalaldehyde, 2-thiobarbituric acid, and other chemicals from Wako (Osaka, Japan).

### 2.2. Animals

Male Wistar rats aged 6 weeks were obtained from Nippon SLC (Hamamatsu, Japan). The animals were housed in cages in a ventilated animal room with controlled temperature (23 ± 2°C) and relative humidity (55 ± 15%) and with 12 h of light (7:00 to 19:00). They were maintained on standard laboratory chow (Oriental MF, Oriental Yeast, Tokyo, Japan) and tap water ad libitum for 1 week. All animals received humane care in compliance with the guideline of the Animal Care and Use Committee of Fujita Health University.

### 2.3. Gastric mucosal lesion induction by compound 48 / 80

Compound 48/80 (0.75 mg/kg), 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). The control rats received an i.p. injection of an equal volume 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 and 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): 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 ebselen

Ebselen was suspended in 0.5% carboxymethylcellulose sodium solution at a constant dosing volume of 5 ml/kg. Ebselen (10, 50 or 100 mg/kg) was orally administered to fasted rats with a stomach tube at 0.5 h before compound 48/80 injection. Ebselen-untreated rats received an equal volume of 0.5% carboxymethylcellulose sodium solution at the same time point.

### 2.5. Determinations of gastric mucosal Se-GSH peroxidase, myeloperoxidase, xanthine oxidase, and thiobarbituric acid reactive substances

Gastric mucosal Se-GSH peroxidase and myeloperoxdiase were assayed by the methods of Hochstein and Utley (1968) and Hashimoto (1974), respectively. For assays of both enzymes, gastric mucosal tissues were homogenized in nine volumes of ice-cold 0.05 M Tris–HCl buffer (pH 7.4). After sonication on ice for 20 s using a Handy Sonic model UR-20P (Tomy Seiko, 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-GSH 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 of this activity is defined as the amount of enzyme oxidizing 1 μmol NADPH per min. Myeloperoxidase activity was assessed by measuring the $H_2O_2$-dependent oxidation of tetramethylbenzidine at 37°C. One unit of this enzyme is defined as the amount of enzyme causing a change in absorbance of 1.0/min at 655 nm. Gastric mucosal xanthine oxidase was assayed by the method of Suzuki et al. (1983). 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 (10,000 × *g*, 20 min), and the resultant supernatant was dialyzed against 100 volumes 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 of this enzyme is defined as the amount of enzyme forming 1 μmol uric acid/min. Gastric mucosal thiobarbituric acid reactive substances were 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 volumes of ice-cold 20 mM EDTA. The amount of thiobarbituric acid reactive substances is expressed as that of malondialdehyde equivalents.

### 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, Japan), as described in our previous reports (Ohta et al., 1997, 1999a,b). 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, Japan), 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.

### 2.8. Determinations of lipid peroxidation and xanthine oxidase activity in gastric mucosal tissues treated with and without ebselen

AAPH-induced lipid peroxidation and xanthine oxidase activity were measured in gastric mucosal tissues treated with and without ebselen as follows: gastric mucosal tissues collected from three different rats sacrificed at 3 h after compound 48/80 were homogenized in 9 volumes of ice-cold 0.15 M KCl or 0.25 M sucrose. The homogenate prepared with 0.15 M KCl was centrifuged at 4°C (10,000 × *g*, 20 min), and the resultant supernatant was used for the determination of AAPH-induced lipid peroxidation. The homogenate prepared with 0.25 M sucrose 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 volumes of 0.25 M sucrose at 4°C for 24 h. The dialyzed supernatant was used for the assay of xanthine oxidase. AAPH-induced lipid peroxidation was conduced in the reaction mixture consisting of 0.1 M NaCl–10 mM potassium phosphate buffer (pH 7.4) (0.4 ml), 0.5 M AAPH (0.1 ml), gastric mucosal tissue sample (0.4 ml) and either ebselen dissolved in absolute ethanol (final concentration: 0.5%) (0.1 ml) or absolute ethanol (final concentration: 0.5%) (0.1 ml) at 37°C for 60 min under aerobic conditions with agitation. The formation of lipid peroxides in the reaction was measured by the thiobarbituric acid method of Beuge and

Aust (1978). The concentration of lipid peroxides formed is expressed as that of malondialdehyde equivalents. The MDA concentration was quantified by use of the molecular extinction coefficient $\varepsilon = 1.56 \times 10^5$ $M^{-1}$ $cm^{-1}$ (Beuge and Aust, 1978). Xanthine oxidase activity in gastric mucosal tissue samples in the presence of either ebselen dissolved in absolute ethanol (final concentration: 0.5%) or absolute ethanol (final concentration: 0.5%) was measured by the same method as described above and was assessed by measuring the increase in absorbance at 292 nm following the formation of uric acid at 30°C. One unit of this enzyme is defined as the amount of enzyme forming 1 μmol uric acid/min.

### 2.9. 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.

## 3. Results

### 3.1. Effect of ebselen on gastric mucosal lesions induced by compound 48/80

As shown in Table 1, gastric mucosal lesions appeared 0.5 h after a single i.p. injection of compound 48/80 (0.75 mg/kg) and this gastric mucosal lesion formation was not prevented by pre-administration of ebselen (p.o.) at a dose of 10, 50 or 100 mg/kg, although the lesion formation tended to be attenuated by ebselen pre-administered at a dose of 100 mg/kg. Gastric mucosal lesions developed 3 h after the compound 48/80 injection and this gastric mucosal lesion development was significantly prevented by pre-administration of ebselen at a dose of 50 or 100 mg/kg, but not 10 mg/kg (Table 1). In addition, this preventive effect of pre-administered ebselen occurred in a dose-dependent manner.

### 3.2. Effect of ebselen on changes in serum serotonin and histamine concentrations and gastric mucosal blood flow following compound 48/80 injection

Serum serotonin and histamine concentrations in rats with a single compound 48/80 injection alone were 3.4- and 21.5-fold, respectively, higher than those in control rats without the injection at 0.5 h after the injection, while gastric mucosal blood flow in the compound 48/80-injected group was 25% of that in the control group (Fig. 1). Pre-administration of ebselen at a dose of 10, 50 or 100 mg/kg did not affect the increases in serum serotonin and histamine concentrations and the decrease in gastric mucosal blood at 0.5 after the compound 48/80 injection (Fig. 1). As shown in Fig. 2, serum serotonin and histamine concentrations in the compound 48/80-injected group were 2.4- and 6.1-fold higher than those in the control group at 3 h after the injection, while gastric mucosal blood flow in the compound 48/80-injected group was 75% of that in the control group. These changes in serum serotonin and histamine concentrations and gastric mucosal blood flow were not attenuated by ebselen pre-administered at a dose of 10, 50 or 100 mg/kg (Fig. 2).

### 3.3. Effect of ebselen on gastric mucosal thiobarbituric acid reactive substances concentration and Se-GSH peroxidase, myeloperoxidase, and xanthine oxidase activities at 0.5 h after compound 48/80 injection

As shown in Fig. 3A, rats with a single compound 48/80 injection alone had a significantly higher thiobarbi-

Table 1
Effect of ebselen pre-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 | 0 | 50 | 40 | 10 | 0 | 0 | 0 | – |
| + Ebselen (10 mg/kg) | 0 | 0 | 50 | 50 | 0 | 0 | 0 | 0 | NS |
| + Ebselen (50 mg/kg) | 0 | 0 | 60 | 40 | 0 | 0 | 0 | 0 | NS |
| + Ebselen (100 mg/kg) | 0 | 10 | 60 | 30 | 0 | 0 | 0 | 0 | NS |
| *3 h* | | | | | | | | | |
| Compound 48/80 | 0 | 0 | 0 | 0 | 0 | 20 | 50 | 30 | – |
| + Ebselen (10 mg/kg) | 0 | 0 | 0 | 0 | 0 | 50 | 50 | 0 | NS |
| + Ebselen (50 mg/kg) | 0 | 0 | 0 | 20 | 50 | 30 | 0 | 0 | 0.05 |
| + Ebselen (100 mg/kg) | 0 | 0 | 10 | 60 | 30 | 0 | 0 | 0 | 0.05 |

Rats received oral administration of ebselen (10, 50 or 100 mg/kg) at 0.5 h before intraperitoneal injection of compound 48/80 (0.75 mg/kg) and were sacrificed 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.

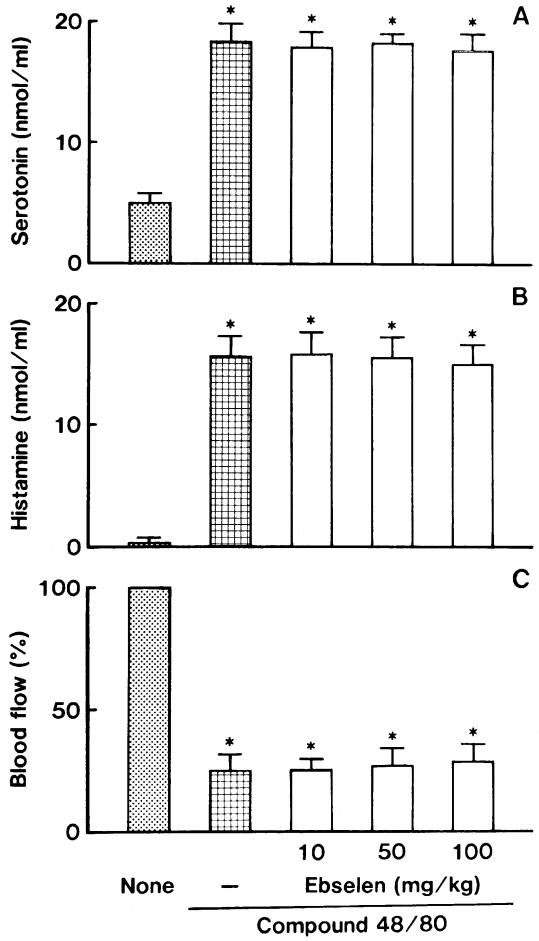


Fig. 1. Effect of ebselen on serum serotonin (A) and histamine (B) concentrations and gastric mucosal blood flow (C) at 0.5 h after compound 48/80 injection. Rats received p.o. administration of ebselen (10, 50 or 100 mg/kg) at 0.5 h before i.p. injection of compound 48/80 (0.75 mg/kg). Control rats untreated with both ebselen and compound 48/80 received each vehicle in the same manner at the same time point. All animals were sacrificed 0.5 h after the compound 48/80 injection. Serum serotonin and histamine and gastric mucosal blood flow were determined as described in Section 2. Each value is a mean ± S.D. ($n = 10$ for each group). * $P < 0.05$ (vs. control rats).

turic acid reactive substances concentration than control rats without compound 48/80 injection at 0.5 h after the compound 48/80 injection. This increase in gastric mucosal thiobarbituric acid reactive substances concentration was not attenuated by pre-administration of ebselen at a dose of 10, 50 or 100 mg/kg (Fig. 3A). The compound 48/80-injected group had significantly lower gastric mucosal Se-GSH peroxidase activity than the control group at 0.5 h after compound 48/80 injection (Fig. 3B). This decrease in gastric mucosal Se-GSH peroxidase activity by compound 48/80 injection was not attenuated by pre-administration of ebselen at a dose of 10, 50 or 100 mg/kg (Fig. 3B). Gastric mucosal myeoperoxidase and xanthine oxidase activities in the compound 48/80-injected group were significantly higher than those in the control group (Fig. 3C and D). These increases in gastric mucosal myeloperoxidase and xanthine oxidase activities were not attenuated by ebselen pre-administered at a dose of 10, 50 or 100 mg/kg (Fig. 3C and D).

### *3.4. Effect of ebselen on gastric mucosal thiobarbituric acid reactive substances concentration and Se-GSH peroxidase, myeloperoxidase, and xanthine oxidase activities at 3 h after compound 48/80 injection*

As shown in Fig. 4A, gastric mucosal thiobarbituric acid reactive substances concentration in rats with a single compound 48/80 injection alone was 1.9-fold higher than that in control rats without compound 48/80 injection at 3 h after the compound 48/80 injection. This increase in gastric mucosal thiobarbituric acid reactive substances concentration by compound 48/80 injection was significantly

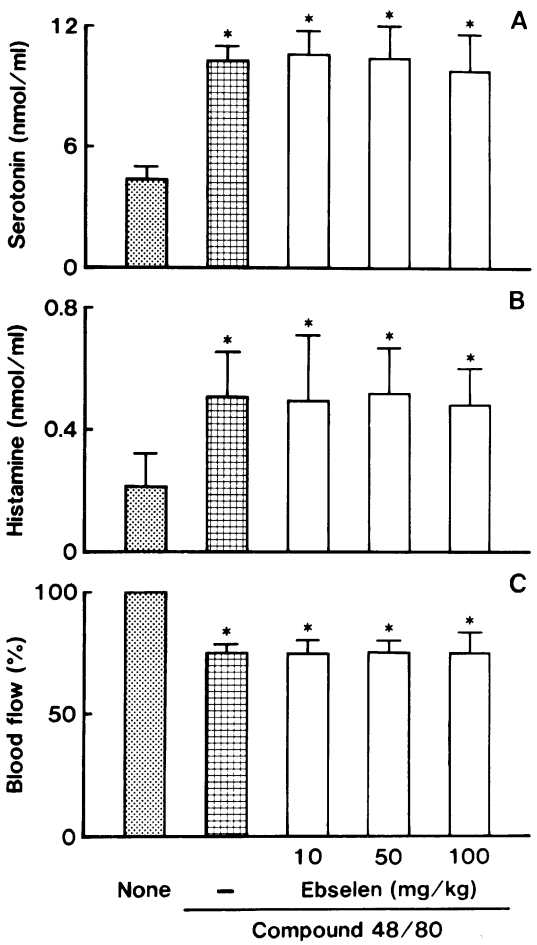


Fig. 2. Effect of ebselen on serum serotonin (A) and histamine (B) concentrations and gastric mucosal blood flow (C) at 3 h after compound 48/80 injection. Rats received p.o. administration of ebselen (10, 50 or 100 mg/kg) at 0.5 h before i.p. injection of compound 48/80 (0.75 mg/kg). Control rats untreated with both ebselen and compound 48/80 received each vehicle in the same manner at the same time point. All animals were sacrificed 3 h after the compound 48/80 injection. Serum serotonin and histamine and gastric mucosal blood flow were determined as described in Section 2. Each value is a mean ± S.D. ($n = 10$ for each group). * $P < 0.05$ (vs. control rats).

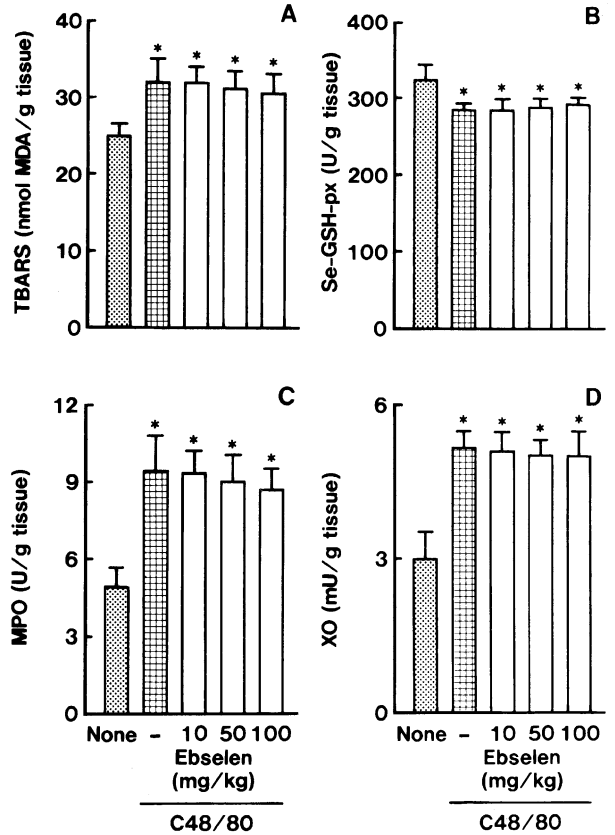


Fig. 3. Effect of ebselen on gastric mucosal thiobarbituric acid reactive substances (TBARS) concentration (A) and Se-GSH peroxidase (Se-GSH-px) (B), myeloperoxidase (MPO) (C), and xanthine oxidase (XO) (D) activities at 0.5 h after compound 48/80 (C48/80) injection. Rats received p.o. administration of ebselen (10, 50 or 100 mg/kg) at 0.5 h before i.p. injection of C48/80 (0.75 mg/kg) and were sacrificed 0.5 h after the C48/80 injection. Control rats untreated with both ebselen and C48/80 received each vehicle in the same manner at the same time point. All animals were sacrificed 0.5 h after the C48/80 injection. Gastric mucosal TBARS, Se-GSH-px, MPO, and XO were assayed as described in Section 2. MDA represents malondialdehyde. Each value is a mean ± S.D. ($n = 10$ for each group). $^{*}P < 0.05$ (vs. control rats).

attenuated by ebselen pre-administered at a dose of 50 or 100 mg/kg, but not 10 mg/kg; gastric mucosal thiobarbituric acid reactive substances concentrations in the compound 48/80-injected groups pre-administered with 50 and 100 mg/kg of ebselen were 1.5- and 1.3-fold, respectively, higher than that in the control group (Fig. 4A). The compound 48/80-injected group had 28.6% of gastric mucosal Se-GSH peroxidase activity in the control group (Fig. 4B). This decrease in gastric mucosal Se-GSH peroxidase activity by compound 48/80 injection was significantly attenuated by pre-administration of ebselen at a dose of 50 or 100 mg/kg, but not 10 mg/kg; gastric mucosal Se-GSH peroxidase activities in the compound 48/80-injected groups pre-administered with 50 and 100 mg/kg of ebselen were 55.5 and 76.2%, respectively, of that in the control group (Fig. 4B). Gastric mucosal myeloperoxidase activity in the compound 48.80-injected group was 2.7-fold higher than that in the control group (Fig. 4C). This increase in gastric mucosal myeloperoxidase activity by compound 48/80 injection was significantly attenuated by ebselen pre-administered at a dose of 50 or 100 mg/kg, but not 10 mg/kg; gastric mucosal myeloperoxidase activities in the compound 48/80-injected groups pre-administered with 50 and 100 mg/kg of ebselen were 2.0- and 1.4-fold, respectively, higher than that in the control group (Fig. 4C). As shown in Fig. 4D, the compound 48/80-injected group had 3.6-fold higher gastric mucosal xanthine oxidase activity than the control group. This increase in gastric mucosal xanthine oxidase activity by compound 48/80 injection was significantly attenuated by ebselen pre-administered at a dose of 50 or 100 mg/kg, but not 10 mg/kg; gastric mucosal xanthine oxidase activities in the compound 48/80-injected groups pre-administered with 50 and 100 mg/kg of ebselen were 3.0- and 2.0-fold, respectively, higher than that in the control group (Fig. 4D).

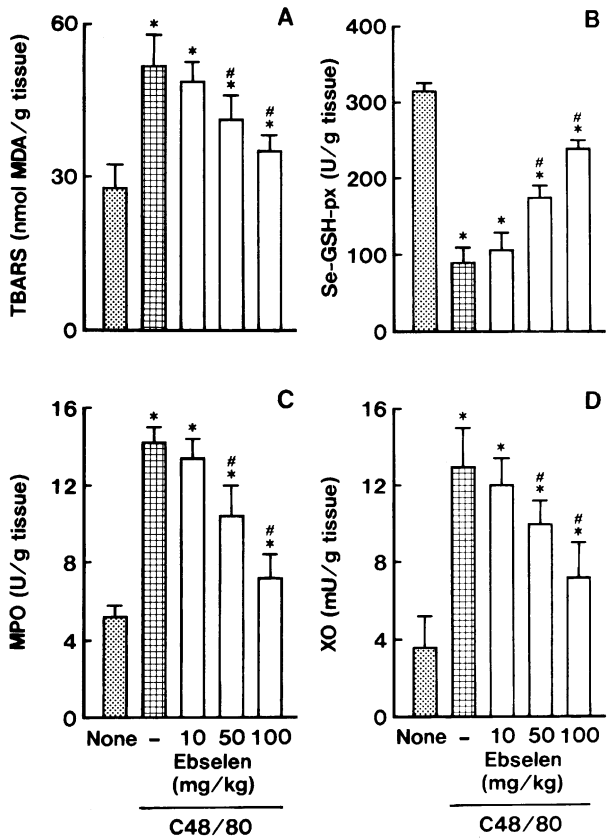


Fig. 4. Effect of ebselen on gastric mucosal thiobarbituric acid reactive substances (TBARS) concentration (A) and Se-GSH peroxidase (Se-GSH-px) (B), myeloperoxidase (MPO) (C), and xanthine oxidase (XO) (D) activities at 3 h after compound 48/80 (C48/80) injection. Rats received p.o. administration of ebselen (10, 50 or 100 mg/kg) at 0.5 h before i.p. injection of C48/80 (0.75 mg/kg). Control rats untreated with both ebselen and C48/80 received each vehicle in the same manner at the same time point. All animals were sacrificed 3 h after the C48/80 injection. Gastric mucosal TBARS, Se-GSH-px, MPO, and XO were assayed as described in Section 2. MDA represents malondialdehyde. Each value is a mean ± S.D. ($n = 10$ for each group). $^{*}P < 0.05$ (vs. control rats); $^{\#}P < 0.05$ (vs. rats injected with C48/80 alone).

Table 2
Effect of ebselen on AAPH-induced lipid peroxidation and xanthine oxidase activity in gastric mucosal tissue preparations from compound 48/80-injected rats

| Ebselen (μg/ml) | AAPH-induced lipid peroxidation (nmol MDA/g tissue per hour) | Xanthine oxidase activity (mU/g tissue) |
|---|---|---|
| 0 | 7.92 ± 0.33 | 1.29 ± 0.12 |
| 10 | 6.35 ± 0.23[a] | 1.26 ± 0.17 |
| 50 | 4.75 ± 0.41[a] | 1.22 ± 0.14 |
| 100 | 3.95 ± 0.24[a] | 1.20 ± 0.14 |

AAPH-induced lipid peroxidation was conducted in gastric mucosal tissue preparations from compound 48/80-injected rats in presence or absence of ebselen at 37°C for 1 hour under aerobic conditions. The amount of lipid peroxides formed in the reaction was determined by the method of Beuge and Aust (1978). Xanthine oxidase activity in gastric mucosal tissue preparations from compound 48/80-injected rats in the presence or absence of ebselen was measured at 30°C by checking the formation of uric acid at 292 nm according to the method of Suzuki et al. (1983). Each value is expressed as the mean ± S.D. for three different animals.

[a] $P < 0.05$ (vs. the absence of ebselen).

### 3.5. Effect of ebselen on in vitro lipid peroxidation and xanthine oxidase activity in gastric mucosal tissues

When the effect of ebselen on AAPH-induced lipid peroxidation in gastric mucosal tissue preparations from three different compound 48/80-injected rats was examined at its concentrations of 10, 50, and 100 μg/ml, the seleno-organic compound inhibited the lipid peroxidation in a dose-dependent manner (Table 2) When the effect of ebselen on xanthine oxidase activity in gastric mucosal tissue preparations from three different compound 48/80-injected rats was examined at its concentrations of 10, 50, and 100 μg/ml, the seleno-organic compound had little effect at any concentration used (Table 2).

## 4. Discussion

The model of acute gastric mucosal lesions in rats with a single compound 48/80 treatment 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 the present study, it has been shown clearly that pre-orally administered ebselen prevents the development of acute gastric mucosal lesion in rats with a single injection of compound 48/80 (0.75 mg/kg), a mast cell degranulator, in dose-dependent manner. However, pre-administration of ebselen at doses used in the present study, i.e., 10, 50, and 100 mg/kg, was found to have no preventive effect on the formation of acute gastric mucosal lesions in the compound 48/80-injected rats.

It has been shown that, in rats with a single compound 48/80 injection, endogenous serotonin released from connective tissue mast cells plays a major role in gastric mucosal lesion formation, while endogenous histamine released from connective tissue mast cells contributes to the lesion development (Ohta et al., 1999b). Tchoumken-Nzouessa and Rebel (1998) have reported that ebselen inhibits compound 48/80-induced histamine release from isolated rat peritoneal mast cells, although this inhibitory effect of ebselen is counteracted by GSH. In the present study, pre-administration of ebselen to compound 48/80-injected rats at doses of 10, 50, and 100 mg/kg had no effect on the increases in serum serotonin and histamine concentrations found at early and progressed stages of gastric mucosal lesions. These results suggest that pre-administered ebselen prevents gastric mucosal lesion development in rats with a single compound 48/80 injection without affecting release of not only histamine but also serotonin from the connective mast cells.

It has been shown that ischemia–reperfusion occurs in the gastric mucosal tissue of rats with a single compound 48/80 injection (Ohta et al., 1997, 1999a,b). It has also been shown that the reduction of gastric mucosal blood flow in compound 48/80-injected rats is due to endogenous serotonin released from connective tissue mast cells (Ohta et al., 1997, 1999b). Pretreated ebselen is known to prevent gastric mucosal injury induced by ischemia–reperfusion without affecting a change in gastric mucosal blood flow in rats (Ueda et al., 1990). In the present study, any dose of pre-administered ebselen did not affect the change in gastric mucosal blood flow with the formation and development of compound 48/80-induced gastric mucosal lesions. These results indicate that pre-administered ebselen prevents gastric mucosal lesion development in rats with a single compound 48/80 injection without affecting the change in gastric mucosal blood flow. It has been reported that when ebselen at a dose of 10, 30 or 100 mg/kg is orally administered to normal mice, serum ebselen concentration increases dose-dependently 0.5 h after the administration and the increased serum ebselen concentration is reduced gradually thereafter (Tabuchi et al., 1995). Accordingly, it can be thought that ebselen administered orally to rats at doses of 10, 50, and 100 mg/kg at 0.5 h before compound 48/80 injection has an increase in its concentration in the serum just before the injection. However, a marked decrease in gastric mucosal blood flow occurred 0.5 h after compound 48/80 injection at which time gastric mucosal lesions appeared and the decreased gastric mucosal blood flow was partially recovered 3 h after the injection at which time progressed gastric mucosal lesions occurred, as described above. Therefore, it may be assumed that pre-administered ebselen cannot achieve an amount enough to exert its pharmacological actions in the gastric mucosal tissue of compound 48/80-injected rats at an early stage of gastric mucosal lesions, resulting in no preventive effect on gastric mucosal lesion formation. It may be also assumed that pre-administered ebselen can achieve an amount enough to exert its phar-

macological actions in the gastric mucosal tissue of compound 48/80-injected rats at a progressed stage of gastric mucosal lesions, leading to prevention of gastric mucosal lesion development. However, further investigation is required to confirm these assumptions.

It has been shown that, in the gastric mucosal tissue of rats with a single compound 48/80 injection, Se-GSH peroxidase activity decreases with an increase in lipid peroxidation at an early stage of gastric mucosa lesions and further decreases with further increase in lipid peroxidation at a progressed stage of the lesion (Ohta et al., 1997). Ebselen possesses GSH peroxidase-like and anti-lipid peroxidative activities (Wendel et al., 1984; Müller et al., 1984, 1985; Noguchi et al., 1992). In the present study, ebselen pre-administered to compound 48/80-injected rats attenuated the decreased gastric mucosal Se-GSH peroxidase activity and the increased concentration of gastric mucosal thiobarbituric acid reactive substances, an index of lipid peroxidation, found at a progressed stage, but not an early stage, of gastric mucosal lesions in a dose-dependent manner. In addition, ebselen at a concentration of 10 to 100 μg/ml inhibited in vitro lipid peroxidation induced by APPH, a water-soluble radical initiator, in gastric mucosal tissue preparations from compound 48/80-injected rats in a dose-dependent manner. It has been shown that, in rats with a single compound 48/80 injection, neutrophils infiltrating into the gastric mucosal tissue contributes to gastric mucosal lesion formation and development, while the xanthine–xanthine oxidase system in the gastric mucosal tissue mainly contributes to the lesion development (Ohta et al., 1999a). Ebselen inhibits the adhesion and transendothelial migration of polymorphonuclear leukocytes, i.e., neutrophils, both in vitro (Issekutz and Lopes, 1992) and in vivo (Goa and Issekutz, 1993a,b, 1994). In the present study, ebselen pre-administered to compound 48/80-injected rats attenuated the increases in the activities of gastric mucosal myeloperoxidase, an index of tissue neutrophil infiltration (Krawisz et al., 1984), and xanthine oxidase found at a progressed stage, but not an early stage, of gastric mucosal lesions in a dose-dependent manner. These results suggest that pre-administered ebselen could exert a preventive effect on gastric mucosal lesion development in rats with a single compound 48/80 injection through its GSH peroxidase-like activity and antioxidative and anti-inflammatory properties. The mechanism by which pre-administered ebselen attenuates increased gastric mucosal xanthine oxidase activity in compound 48/80-injected rats has not been clarified in the present study. However, ebselen at a concentration of 10 to 100 μg/ml had no effect on in vitro xanthine oxidase activity in gastric mucosal tissue preparations from compound 48/80-injected rats. Our previous report (Ohta et al., 1999a) has shown that, in rats with a single compound 48/80 injection, treatment with anti-neutrophil antiserum or NPC 14686 (L-homophenylalanine), an inhibitor of neutrophil recruitment, attenuates increased gastric mucosal xanthine oxidase activity at a progressed stage, but not an early stage, of compound 48/80-induced gastric mucosal lesions. Accordingly, these findings may allow us to think that, in rats with a single compound 48/80 injection, pre-administered ebselen attenuates a neutrophil-mediated increase in xanthine oxidase activity in the gastric mucosal tissue by inhibiting neutrophil infiltration into the gastric mucosal tissue, although the mechanism by which infiltrating neutrophils cause an increase in gastric mucosal xanthine oxidase activity is unclear at present.

It has been shown that ebselen at a dose of 30 to 300 mg/kg inhibits gastric acid secretion in pylorus-ligated rats (Tabuchi and Kurebayashi, 1993), although there is a report showing no effect of the seleno-organic compound at a dose of 200 mg/kg on gastric acid secretion in pylorus-ligated rats (Ueda et al., 1990). It has also been shown that ebselen inhibits gastric acid secretion by interfering with sulfhydryl groups of the gastric proton pump, $H^+,K^+$–ATPase, in vitro (Beil et al., 1990; Tabuchi et al., 1994). However, our previous report (Ohta et al., 1999b) has shown that pretreatment with cimetidine or famotidine, a histamine $H_2$ receptor antagonist, at doses enough to inhibit gastric acid secretion has no effect on gastric mucosal lesion development in rats with a single compound 48/80 injection. Accordingly, it seems unlikely that pre-administered ebselen exerts a preventive effect on gastric mucosal lesion development in rats with a single compound 48/80 injection by inhibiting gastric acid secretion.

In conclusion, the results of the present study indicate that pre-administered ebselen exerts a preventive effect on the development of acute gastric mucosal lesions in rats with a single compound 48/80 injection, and suggest that this preventive effect of ebselen could be due to its GSH peroxidase-like activity and antioxidative and anti-inflammatory properties.

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