INTRODUCTION

Compound 48/80 (C48/80) is a condensation product of N-methyl-p-methoxy phenylethylamine and formalin (1). It has been demonstrated that, in rats treated once with C48/80, connective tissue mast cells such as peritoneal mast cells, but not mucosal mast cells, are degranulated, resulting in release of histamine and serotonin from the connective tissue mast cells (2-4).

We have reported the formation, progression, and recovery of gastric mucosal lesions in rats with a single treatment with C48/80 (5). That is, the C48/80-induced lesions appeared with decreases in Se-glutathione peroxidase (Se-GSH-px) activity and non-protein SH (NPSH) content and increases in neutrophil infiltration and xanthine oxidase (XO) activity and lipid peroxide (LPO) content in the gastric mucosa at 0.5h after C48/80 treatment. The lesions progressed with decreases in vitamin E (VE) and mucus contents in addition to further decrease in Se-GSH-px activity and further increases in neutrophil infiltration, XO activity, and LPO content in the gastric mucosa at 3 h. The lesions recovered fairly well with marked attenuations of the decreased Se-GSH-px activity and VE and mucus contents and the increased neutrophil infiltration, XO activity, and LPO contents in the gastric mucosa at 12 h. We have also reported that gastric mucosal blood flow changes like ischemia-reperfusion in rats treated once with C48/80 and that the gastric mucosal blood flow decreases with gastric mucosal lesion formation and the decreased blood flow recovers rapidly with the lesion progression (5). Furthermore, we have reported the following findings in rats with a single C48/80 treatment. Neutrophils infiltrating into the gastric mucosal tissue participates in the formation of gastric mucosal lesions, while the xanthine-XO system in the gastric mucosal tissue participates in the progression rather than the formation of gastric mucosal lesions (6). Acutely released endogenous serotonin mainly contributes to the formation of gastric mucosal lesions by decreasing gastric mucosal blood flow, while released endogenous histamine mainly contributes to the progression of the lesions by recovering the decreased blood flow (7). Gastric mucosal ascorbic acid (AA) content decreases with the progression of gastric mucosal lesions (8) and AA plays a critical role in the progression of C48/80-induced gastric mucosal lesions (9). In addition, our previous report has shown that when rats are treated once with compound 48/80, gastric mucosal lesions are aggravated again with an increase in gastric mucosal lipid peroxide content and decreases in Se-GSH-px and catalase activities at 24h after treatment (10).

We examined the recurrence of gastric mucosal lesions in rats after a single treatment with compound 48/80 (C48/80), a mast cell degranulator. During the period of 0.5 h to 24 h after treatment with C48/80 (0.75 mg/kg, i.p.), an apparent recurrence of gastric mucosal lesions was found 18 and 24 h after the lesion formation, progression, and recovery occurred during the period of 12 h. Gastric mucosal blood flow showed the maximum reduction at 0.5, 16, and 22 h after treatment followed by the maximum recovery of the decrease at 12, 20, and 24 h, respectively. Gastric mucosal myeloperoxide and xanthine oxidase activities and lipid peroxide content showed the maximum increase at 3, 18, and 24 h after treatment. Gastric mucosal superoxide dismutase activity unchanged after treatment and gastric mucosal catalase activity decreased only at 24 h. Gastric mucosal Se-glutathione peroxidase activity and vitamin E, ascorbic acid, and hexosamine contents showed their maximum decrease at 3, 18, and 24 h after treatment. Gastric mucosal non-protein SH content showed the maximum decrease at 0.5, 16, and 22 h after treatment. Serum histamine and serotonin concentrations increased rapidly after treatment but the increases in serum histamine and serotonin concentrations diminished completely until 12 and 14 h, respectively. These results indicate that lesions recur repeatedly accompanied with an ischemia-reperfusion-like change in blood flow, inflammation, and disruption of antioxidant defense systems in the gastric mucosa of rats in no relation to released histamine and serotonin after a single C48/80 treatment.

Key words: compound 48/80, mast cell degranulator, gastric mucosal lesions, blood flow, inflammation, antioxidant defense system

INTRODUCTION

Compound 48/80 (C48/80) is a condensation product of N-methyl-p-methoxy phenylethylamine and formalin (1). It has been demonstrated that, in rats treated once with C48/80, connective tissue mast cells such as peritoneal mast cells, but not mucosal mast cells, are degranulated, resulting in release of histamine and serotonin from the connective tissue mast cells (2-4).

We have reported the formation, progression, and recovery of gastric mucosal lesions in rats with a single treatment with C48/80 (5). That is, the C48/80-injected lesions appeared with decreases in Se-glutathione peroxidase (Se-GSH-px) activity and non-protein SH (NPSH) content and increases in neutrophil infiltration and xanthine oxidase (XO) activity and lipid peroxide (LPO) content in the gastric mucosa at 0.5 h after C48/80 treatment. The lesions progressed with decreases in vitamin E (VE) and mucus contents in addition to further decrease in Se-GSH-px activity and further increases in neutrophil infiltration, XO activity, and lipid peroxide content in the gastric mucosa at 3 h. The lesions recovered fairly well with marked attenuations of the decreased Se-GSH-px activity and VE and mucus contents and the increased neutrophil infiltration, XO activity, and LPO contents in the gastric mucosa at 12 h. We have also reported that gastric mucosal blood flow changes like ischemia-reperfusion in rats treated once with C48/80 and that
The purpose of the present study was to clarify how gastric mucosal lesions recur in rats with a single treatment of C48/80. Namely, we examined the recurrence of gastric mucosal lesions in rats treated once with C48/80 during the period of 0.5 h to 24 h after treatment. We also examined changes in the activities of myeloperoxidase (MPO), an index of tissue neutrophil infiltration (11), XO, superoxide dismutase (SOD), catalase, and Se-GSH-px, the contents of NPSH, VE, AA, hexosamine, an index of gastric mucus (12), and lipid peroxide, and blood flow in the gastric mucosa of C48/80-treated rats during the period of 0.5 h to 24 h after treatment.

MATERIALS AND METHODS

Reagents

C48/80, 3,3',5,5'-tetramethylbenzidine, xanthine, bovine erythrocyte SOD, and yeast glutathione reductase were purchased from Sigma Chemical Co. (St. Louis, MO); milk XO from Roche-Diagnostic Co. (Tokyo, Japan); and α-tocopherol, L-AA, 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman’s reagent), ethylenediaminetetraacetic acid (EDTA), glucosamine, reduced glutathione (GSH), NADPH, 2-thiobarbituric acid, tocol, RRR-α-tocopherol, and other chemicals from Wako Pure Chemicals Ind., Co. (Osaka, Japan).

Animals

Male Wistar rats aged six weeks were obtained from Nippon 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±15%) 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 of the Animal Care and Use Committee of Fujita Health University. This animal experiment was approved by the Institutional Animal Care and Use Committee.

Induction and observation of gastric mucosal lesions

C48/80 (0.75 mg/kg), dissolved in distilled water, was intraperitoneally injected to 7-week-old rats fasted for 24 h, as described previously (5-10). The control rats received an equal volume of distilled water in the same manner. All animals were maintained with free access to water and without food under unaesthetized conditions during the experiment. The animals were anesthetized with ether at 0.5, 1.5, 3, 6, 12, 14, 16, 18, 20, 22 or 24 h after treatment at which time blood was collected from the inferior vena cava. Serum was obtained from the collected blood by centrifugation. Immediately after sacrifice, stomachs were isolated and gastric mucosal tissues were collected from the isolated stomachs. The collected gastric mucosal tissues and serum were stored at -80°C until use. For the assays of NPSH, AA, and LPO, gastric mucosal tissue was homogenized in 9 volumes of ice-cold 0.15 M KCl containing 1 mM EDTA on ice using a microhomogenizer M-100 (Tokai Irika Co., Tokyo, Japan). Gastric mucosal NPSH was determined by the method of Sedlak and Lindsay (13) using Ellman’s reagent and GSH as a standard. AA in gastric mucosal tissues and serum were determined by the dipiridyl method of Zannoni et al. (14) using L-AA as a standard. Gastric mucosal LPO was determined by the method of Okhawa et al. (15) using the thiobarbituric acid reaction except that 1.0 mM EDTA was added to the reaction mixture. The amount of gastric mucosal LPO was expressed as that of malondialdehyde (MDA) equivalents. Gastric mucosal VE was determined by the high-performance liquid chromatographic method of Abe et al. (16) using fluoresceence detection. RRR-α-tocopherol was used as an authentic standard and tocol was used as an internal standard. VE in gastric mucosal tissues was extracted with n-hexane. The amount of VE in gastric mucosal tissues was expressed as that of α-tocopherol (α-Toc). Gastric hexosamine was determined by the method of Oktani et al. (17) using acetylatedene and Ehrlich’s reagent. The amount of gastric mucosal hexosamine is expressed as that of glucosamine.

For the assays of SOD, catalase, Se-GSH-px, MPO, and XO, gastric mucosal samples were prepared as follows: gastric mucosal tissue was homogenized in 9 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5) using a microhomogenizer M-100. The homogenate was sonicated two times on ice for 30 s using a Handy Sonic model UR-20P (Tomy Seiko Co., Tokyo, Japan). The sonicated homogenate was centrifuged at 10000 x g for 20 min at 4°C and the resultant supernatant was dialyzed against 100 volumes of the same buffer at 4°C for 24 h. Gastric mucosal SOD, catalase, and Se-GSH-px were assayed by the methods of Oyanagi (18), Bergmeyer (19), and Hochstein and Utley (20), respectively. SOD activity was determined at 37°C by the XO-NH2OH method using purified bovine erythrocyte SOD (5000 units/mg solid) as a standard. This activity is expressed as the amount of the erythrocyte SOD showing activity equivalent to the determined activity. Catalase activity was measured at 37°C by recording hydrogen peroxide (H2O2) decomposition at 240 nm. One unit (U) of this activity is defined as the amount of enzyme decomposing 1 µmol H2O2 as a substrate per min. Sed-GSH-px activity was determined at 37°C by recording the decrease in absorbance at 340 nm following the oxidation of NADPH in the presence of GSH and yeast glutathione reductase. One unit (U) of this activity is defined as the amount of enzyme oxidizing 1 µmol NADPH per min. Gastric mucosal XO activity was assayed by the method of Suzuki et al. (21) using xanthine as a substrate. XO activity was assessed by the formation of uric acid at 30°C. One unit (U) of this activity is defined as the amount of enzyme forming 1 µmol uric acid per min. Gastric mucosal MPO was assayed by the method of Hashimoto (22). MPO activity was assessed by measuring the H2O2-dependent oxidation of tetramethylbenzidine at 37°C. One unit (U) of this enzyme was defined as the amount of enzyme causing a change in absorbance of 1.0 per min at 655 nm.

Serum samples were deproteinized by adding perchloric acid at a final concentration of 3% and then centrifuged at 4°C for 10 min (10000 x g). Serum serotonin was measured by the method of Shibata et al. (23) 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 Lorenz et al. (24) and Shore et al. (25). Histamine was reacted with o-phthalaldehyde and the intensity of the resulting fluorescence was measured using a spectrofluorometer (the excitation wavelength, 360 nm; the emission wavelength, 450 nm).

Measurement of gastric mucosal blood flow

Gastric mucosal blood flow was measured using a laser Doppler flowmeter, Laser Flow BRL-100 (Bio Research Center Co., Nagoya), as described in our previous reports (5, 7, 9). Rats used for this measurement were anesthetized with pentobarbital sodium 10 min before the onset of the measurement and the abdomen was opened on an operation mat. The mat was heated at 37°C during the operation and blood flow measurement. The laser probe was attached to the serosal side of the corpus mucosa by aid of a cyanoacrylate-typed instantaneous adhesive, Aron Alpha (Toha Gosei Co., Tokyo), and the blood flow changes were monitored on a recorder for at least 5 min after the onset of the measurement. Gastric mucosal blood flow in C48/80-treated rats was expressed as a relative percentage toward the mean value of gastric mucosal blood flow determined in control rats without C48/80 treatment. The values of gastric mucosal blood flow measured in C48/80-untreated rats were constant within at least 5% in standard deviation.

Statistical analysis

Results of biochemical determinations in the gastric mucosal tissue and serum were expressed as the mean±SD. 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 protected least significance difference (PLSD) for multiple comparisons as the post hoc test. Statistical analyses of the severity of mucosal lesions were carried out using the Kruskal-Wallis test. Values of significance were set at P<0.05 for both tests.

RESULTS

Change in the severity of gastric mucosal lesions after C48/80 treatment

When gastric mucosal lesions were checked in rats during the period of 0.5 h to 24 h after a single treatment with C48/80 (0.75 mg/kg), the results shown in Figs. 1 and 2 were obtained. Gastric mucosal lesions appeared 0.5 h after C48/80 treatment, became the worst at 3 h, and recovered fairly well at 12 h. However, gastric mucosal lesions became worse two times later than 12 h after C48/80 treatment and the lesions showed the maximum recurrence at 18 and 24 h. The gross features of typical gastric mucosal lesions found at 3, 18, and 24 h after C48/80 treatment were similar as shown in Fig. 3.

Changes in serum histamine and serotonin concentrations after C48/80 treatment

Serum histamine concentration in C48/80-treated rats was significantly higher than that in untreated rats between 0.5 h and 6 h after treatment although the increase was reduced time-dependently and there was no significant difference in that concentration between the two groups thereafter (Fig. 4A). Serum serotonin concentration in C48/80-treated rats was significantly higher than that in untreated rats between 0.5 h and 12 h after treatment although the increase was reduced time-dependently and there was no significant difference in that concentration between the two groups thereafter (Fig. 4B).

![Fig. 1. Change in the severity of gastric mucosal lesions during the period of 0.5 h to 12 h after a single C48/80 treatment. C48/80 (0.75 mg/kg) was intraperitoneally injected to rats fasted for 24 h. The severity of gastric mucosal lesions was estimated as described in the section of Materials and Methods. The number of rats used at each time point after C48/80 treatment is 10. *Significantly different from the previous value, P<0.05.](image-url)
Change in gastric mucosal blood flow after C48/80 treatment

When gastric mucosal blood flow was determined in rats with a single C48/80 treatment during the period of 0.5 h to 24 h after treatment, the results shown in Fig. 5 were obtained. An apparent change in reduction in gastric mucosal blood flow occurred three times after C48/80 treatment; the maximum reduction was found at 0.5, 16, and 22 h. The maximum recovery of the reduced gastric mucosal blood flow was observed at 12, 20, and 24 h after C48/80 treatment. Thus, an ischemia-reperfusion-like change in gastric mucosal blood flow occurred repeatedly in rats treated once with C48/80.

Changes in gastric mucosal LPO content and XOD and MPO activities after C48/80 treatment

When LPO content and XOD and MPO activities in the gastric mucosal tissue of rats treated once with C48/80 were determined during the period of 0.5 h to 24 h after treatment, they were changed as shown in Fig. 6. Apparent changes in
increases in gastric mucosal LPO content and XOD and MPO activities occurred three times after C48/80 treatment; the maximum increases in gastric mucosal LPO content and XOD and MPO activities were found at 3, 18, and 24 h.

Changes in gastric mucosal SOD, catalase, and Se-GSH-px activities after C48/80 treatment

When SOD, catalase, and Se-GSH-px activities in the gastric mucosal tissue of rats with a single compound 48/80 treatment were determined during the period of 0.5 h to 24 h after treatment, the results shown in Fig. 7 were obtained. No significant change in gastric mucosal SOD activity occurred after C48/80 treatment (Fig. 7A) and a significant reduction of gastric mucosal catalase activity was found at 24 h (Fig. 7B). In contrast, an apparent change in increase in gastric mucosal Se-GSH-px activity occurred three times after C48/80 treatment and the maximum activity was found at 3, 18, and 24 h (Fig. 7C).

Changes in gastric mucosal NPSH, VE, and AA contents after C48/80 treatment

When NPSH, VE, and AA contents in the gastric mucosal tissue of rats with a single C48/80 treatment were determined during the period of 0.5 h to 24 h after treatment, they were changed as shown in Fig. 8. Apparent changes in decreases in gastric mucosal VE and AA contents occurred three times after C48/80 treatment; the maximum decreases in both contents were
Fig. 6. Changes in gastric mucosal LPO content (A) and XOD (B) and MPO (C) activities during the period of 0.5 h to 24 h after a single C48/80 treatment. C48/80 (0.75 mg/kg) was intraperitoneally injected to rats fasted for 24 h. Gastric mucosal MPO, XOD, and MPO were measured as described in the section of Materials and Methods. Open bar, control rats without C48/80 treatment; closed bar, C48/80-treated rats. Data represent the mean±SD (n=5 for untreated control rats; n=8 for C48/80-treated rats). *Significantly different from the corresponding control, P<0.05. +Significantly different from the corresponding previous value, P<0.05.

Fig. 7. Changes in gastric mucosal SOD (A), catalase (B), and Se-GSH-px (C) activities during the period of 0.5 h to 24 h after a single C48/80 treatment. C48/80 (0.75 mg/kg) was intraperitoneally injected to rats fasted for 24 h. Gastric mucosal SOD, catalase, and Se-GSH-px were measured as described in the section of Materials and Methods. Open bar, control rats without C48/80 treatment; closed bar, C48/80-treated rats. Data represent the mean±SD (n=5 for untreated control rats; n=8 for C48/80-treated rats). *Significantly different from the corresponding control, P<0.05. +Significantly different from the corresponding previous value, P<0.05.
found at 3, 18, and 24 h (Fig. 8A and B). An apparent change in decrease in gastric mucosal NPSH content occurred three times after C48/80 treatment; the maximum decrease in that content was found at 0.5, 16, and 22 h (Fig. 8C).

Change in gastric mucosal hexosamine content after C48/80 treatment

An apparent change in decrease in gastric mucosal hexosamine content occurred three times during the period of 0.5 h to 24 h after C48/80 treatment as shown in Fig. 9. The maximum decrease in gastric mucosal hexosamine content was found at 3, 18, and 24 h.

DISCUSSION

The present study has clearly shown that, in rats with a single treatment with C48/80 (0.75 mg/kg), gastric mucosal lesions recur repeatedly during the period of 12 h to 24 h after treatment. The maximum recurrence of gastric mucosal lesions was found 18 and 24 h after C48/80 treatment. To the best of our knowledge, this is the first report as to the repeated recurrence of acute gastric mucosal lesions in experimental animals, although we have reported that, in rats treated once with C48/80, gastric mucosal lesions are aggravated again at 24 h after treatment (10). It has been demonstrated that histamine and serotonin

Fig. 8. Changes in gastric mucosal VE (A), AA (B), and NPSH (C) contents during the period of 0.5 h to 24 h after a single C48/80 treatment. C48/80 (0.75 mg/kg) was intraperitoneally injected to rats fasted for 24 h. Gastric mucosal VE, AA, and NPSH were measured and was estimated based on gastric mucosal blood flow obtained from the control group as described in the section of Materials and Methods. Open circle, control rats without C48/80 treatment; closed circle, C48/80-treated rats. Data represent the mean±SD (n=5 for untreated control rats; n=8 for C48/80-treated rats). *Significantly different from the corresponding control, P<0.05. †Significantly different from the corresponding previous value, P<0.05.

Fig. 9. Change in gastric mucosal hexosamine content during the period of 0.5 h to 24 h after a single C48/80 treatment. C48/80 (0.75 mg/kg) was intraperitoneally injected to rats fasted for 24 h. Gastric mucosal hexosamine was measured as described in the section of Materials and Methods. Open circle, control rats without C48/80 treatment; closed circle, C48/80-treated rats. Data represent the mean±SD (n=5 for untreated control rats; n=8 for C48/80-treated rats). *Significantly different from the corresponding control, P<0.05. †Significantly different from the corresponding previous value, P<0.05.
released from degranulated connective tissue mast cells contribute to the development of gastric mucosal lesions in rats treated once with C48/80 (5, 7). However, no increases in serum histamine and serotonin concentrations were found later than 12 and 14 h after C48/80 treatment, respectively. Furthermore, we have observed that when ketotifen, an inhibitor of connective tissue mast cells (25), is orally administered to rats at 12 h after C48/80 treatment, the severity of gastric mucosal lesions found at 18 h after treatment is not reduced at all (unpublished data). These findings indicate that the recurrence of gastric mucosal lesions induced by a single C48/80 treatment does not occur via mediators such as histamine and serotonin released from connective tissue mast cells degranulated by the treatment.

We have reported that an ischemia-reperfusion change in gastric mucosal blood flow due to serotonin and histamine released from degranulated connective tissue mast cells is involved in the development of gastric mucosal lesions in rats with a single C48/80 treatment (5, 7). In the present study, an ischemia-reperfusion-like change in gastric mucosal blood flow occurred two times during the period of 12 h to 24 h after C48/80 treatment and the maximum reduction of the blood flow was found at 16 and 22 h. The change in gastric mucosal blood flow was well consistent with the recurrence of gastric mucosal lesions found at 18 and 24 h after C48/80 treatment. Accordingly, such a change in gastric mucosal blood flow could contribute to the recurrence of gastric mucosal lesions in rats treated once with C48/80, although the mechanism by which gastric mucosal blood flow shows an ischemia-reperfusion-like change later than 12 h after C48/80 treatment is not unclear at present.

It has been shown that the neutrophil infiltration into gastric mucosal tissues and changes in gastric mucosal reactive oxygen species metabolism are closely related to the development of gastric mucosal lesions in rats with a single C48/80 treatment (5, 6). It has also been shown in rats treated once with C48/80 that the aggravation of gastric mucosal lesions found at 24 h after treatment is accompanied with increases in gastric mucosal LPO content and XOD activity and decreases in gastric mucosal Se-GSH-px and catalase activities (8). In the present study, a change in increase in gastric mucosal LPO content occurred two times during the period of 12 h to 24 h after C48/80 treatment and the maximum increase in that content was found at 18 and 24 h. It has been shown that the change in LPO content with lesion development in the gastric mucosa of C48/80-treated rats is closely associated with neutrophil infiltration and an increase in XOD activity in the tissue (5, 6). Changes in the activities of gastric mucosal MPO, an index of tissue neutrophil infiltration (11) and XOD occurred two times during the period of 12 h to 24 h after C48/80 treatment. The maximum increases in both activities were found at 18 and 24 h after C48/80 treatment. Thus, the changes in increases in gastric mucosal LPO content and MPO and XOD activities found later than 12 h after C48/80 treatment were well consistent with the recurrence of gastric mucosal lesions. In addition, we have observed that when NPC 14686, an inhibitor of neutrophil recruitment (26), allopurinol, an inhibitor of XOD (27) or VE is intraperitoneally injected to rats at 12 h after C48/80 treatment, the severity of gastric mucosal lesions found at 18 h after treatment is reduced significantly (unpublished data). These findings suggest that increases in gastric mucosal LPO content and XOD activity and infiltration of neutrophils into gastric mucosal tissues could contribute to the recurrence of gastric mucosal lesions in rats treated once with C48/80.

When the activities of antioxidant enzymes such as SOD, catalase, and Se-GSH-px, in the gastric mucosal tissue of rats treated once with C48/80 were determined during the period of 0.5 h to 24 h after treatment, gastric mucosal SOD activity showed no significant change at any time point and gastric mucosal catalase activity showed a significant decrease only at 24 h. However, gastric mucosal Se-GSH-px activity showed an apparent change in decrease two times during the period of 12 h to 24 h after C48/80 treatment. The maximum decrease in gastric mucosal Se-GSH-px activity was found 18 and 24 h after C8/80 treatment. Thus, the changes in decreases in gastric mucosal catalase and gastric mucosal Se-GSH-px activities found later than 12 h after C48/80 treatment were well consistent with the recurrence of gastric mucosal lesions. These findings suggest that disruption of gastric mucosal antioxidant system associated with catalase and Se-GSH-px could contribute to the recurrence of gastric mucosal lesions in rats treated once with C48/80.

It has been reported that gastric mucosal VE and AA contents decrease with the development of gastric mucosal lesions in rats with a single C48/80 treatment, while gastric mucosal NPSH content decreases following the formation of the lesions and the decrease is recovered following the progression of the lesions (5, 8, 9). In the present study, changes in decreases in gastric mucosal VE and AA contents occurred two times during the period of 12 h to 24 h after C48/80 treatment and their decreases reached the maximum at 18 and 24 h. Thus, the changes in decreases in gastric mucosal VE and AA contents found later than 12 h after C48/80 treatment were well consistent with the recurrence of gastric mucosal lesions. In contrast, a change in decrease in gastric mucosal NPSH occurred two times during the period of 12 h to 24 h after C48/80 treatment but the maximum decrease in that content was found at 16 and 22 h. Thus, gastric mucosal NPSH content in C48/80-treated rats began to decrease before the recurrence of gastric mucosal lesions reached the maximum and the decrease recovered when the recurrence of the lesions reached the maximum. In addition, the change in gastric mucosal NPSH content found later than 12 h after C48/80 treatment was well consistent with the above-described change in gastric mucosal blood flow. We have assumed that a rapid change in gastric mucosal blood flow with the formation and progression of gastric mucosal lesions in C48/80-treated rats affects the consumption and uptake of GSH, the major SH-compound of NPSH, in the gastric mucosal tissue, resulting in the blood flow-associated change in NPSH content in the tissue (5). Therefore, the change in blood flow during the recurrence of lesions in the gastric mucosa of C48/80-treated rats may affect the consumption and uptake of GSH in the gastric mucosal tissue, resulting in the blood flow-associated change in NPSH content in the tissue. These findings suggest that disruption of gastric mucosal antioxidant system associated with antioxidants such as VE, AA, and NPSH could contribute to the recurrence of gastric mucosal lesions in rats treated once with C48/80.

Gastric mucus plays a critical role in the primary defense of the gastric mucosa and provides a protective barrier in the gastric epithelium (29). Gastric mucin is known to interact with reactive oxygen species in vitro (30). We have reported that gastric mucosal mucus content is reduced with the development of gastric mucosal lesions in rats with a single C48/80 treatment (5). In the present study, a change in decrease in the content of gastric mucosal hexosamine, an index of gastric mucus (12), occurred two times during the period of 12 h to 24 h after C48/80 treatment and the maximum decrease in that content was found at 18 and 24 h. Thus, the barrier in the gastric mucosa C-48/80-treated rats was found to break down consistently with the recurrence of gastric mucosal lesions. These findings suggest that breakdown of the gastric mucosal barrier could contribute to the recurrence of gastric mucosal lesions in rats treated once with WIRS.

Liu et al. (31) have shown in rats with a 10-min inhalation with C48/80 that construction of the airway occurs two times, i.e., at 0.5 h and between 6 h to 8 h after inhalation, and have concluded that mediators such as histamine, serotonin, and tumor necrosis factor-α released from connective tissue mast
cells degranulated by C48/80 is responsible for the early phase of airway response which subsequently triggers the late phase of airway response via mediators such as leukotriene C₄, a metabolite of the lipoxigenase pathway, derived from mucosal tissue mast cells. We have observed that when C48/80-treated rats are orally administered with AA-861, an inhibitor of 5-lipoxigenase (32), at 12 h after treatment, the severity of gastric mucosal lesions found at 18 h after treatment is reduced significantly (unpublished data). Accordingly, there seems to be a possibility that the recurrence of gastric mucosal lesions in rats treated once with C48/80 is caused by mediators derived from mucosal tissue mast cells, which appears late after activation of connective tissue mast cells by the compound.

Yildirim et al. (33) have suggested a physiological regulatory role of adrenal gland in the maintenance of oxidant/antioxidant balance in gastric mucosal tissues in rats. Our recent report has shown that a single C48/80 treatment causes oxidative damage in the adrenal gland of rats through mast cell degranulation (34). Therefore, there may be a possibility that adrenal gland plays an important role in the recurrence of gastric mucosal lesions in rats treated once with C48/80.

In conclusion, the results obtained from the present study indicate that lesions recur repeatedly accompanied with an ischemia-reperfusion-like change in blood flow, inflammation, and disruption of antioxidant defense systems in the gastric mucosa of rats in no relation to released histamine and serotonin after a single C48/80 treatment. However, further investigation is needed to clarify the mechanism by which gastric mucosal lesions recur repeatedly in rats treated once with C48/80.

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