Note

A Change in Gastric Mucosal Ascorbic Acid Status with the Formation, Progression, and Recovery of Compound 48/80-induced Acute Gastric Mucosal Lesions in Rats

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Summary We examined whether gastric mucosal ascorbic acid status changes with the formation, progression, and recovery of acute gastric mucosal lesions in rats treated with compound 48/80, a mast cell degranulator. Fasted Wistar rats received a single intraperitoneal injection of compound 48/80 (0.75 mg/kg). Apparent gastric mucosal lesions occurred 0.5 h after compound 48/80 treatment, progressed gastric mucosal lesions were observed at 3 h, and a partial recovery of the progressed lesions was found at 6 h. The gastric mucosal concentrations of total and reduced ascorbic acids in compound 48/80-treated rats decreased to approximately 60% of the levels of untreated rats at 3 h after the treatment but the decreased concentrations of total and reduced ascorbic acids were almost completely returned to the levels of untreated rats at 6 h. The gastric mucosal concentration of oxidized ascorbic acid in compound 48/80-treated rats showed little change. The serum concentrations of total and reduced ascorbic acids in compound 48/80-treated rats increased at 0.5 h after the treatment and further increased at 3 h but the increased concentrations of total and reduced ascorbic acids were almost completely returned to the levels of untreated rats at 6 h. The serum concentration of oxidized ascorbic acid in compound 48/80-treated rats increased transiently at 0.5 h after the treatment. The hepatic concentrations of total, reduced, and oxidized ascorbic acids in compound 48/80-treated rats increased 3 h after the treatment, but these increases were not observed at 6 h. These results indicate that gastric mucosal ascorbic acid status is disrupted with the progression of acute gastric mucosal lesions in rats treated with compound 48/80.

Key Words ascorbic acid status (gastric mucosa, liver, and serum), compound 48/80, gastric mucosal lesion (rat)

Ascorbic acid (vitamin C) exerts an antioxidant action not only by itself but also by interacting with reduced glutathione (GSH) or vitamin E (1, 2). It is known that ascorbic acid scavenges reactive oxygen species (ROS) generated in activated neutrophils and in the hyoxanthine-xanthine oxidase (XO) system and hypochlorous acid derived from myeloperoxidase present in neutrophils in vitro (3–5).

Ekman et al. (6, 7) have reported that a single intraarterial pretreatment with ascorbic acid reduces gastric bleeding after hemorrhagic shock and retransfusion in rats and that the same ascorbic acid pretreatment prevents gastric mucosal energy depletion after hemorrhagic shock and gastric mucosal vessel injury after retransfusion in rats. Alptekin et al. (8) have shown in rats exposed to water immersion restraint stress for 2.5 h that pre-administration of ascorbic acid in drinking water for 15 d reduces microscopically observed gastric mucosal injury, although neither the ascorbic acid pre-administration nor 2.5 h of water immersion restraint stress causes any change in ascorbic acid, GSH, and lipid peroxide levels in the gastric mucosal tissue. Thus, administered ascorbic acid protects against acute gastric mucosal lesions induced by hemorrhagic shock and retransfusion or stress. However, it is still unknown whether gastric mucosal ascorbic acid status changes with the development of experimentally induced acute gastric mucosal lesions.

Compound 48/80 is known to cause degranulation of connective tissue mast cells, but not mucosal mast cells, with release of serotonin and histamine from the cells (9, 10). We have shown in rats with a single compound 48/80 treatment that gastric mucosal lesions develop with decreases in Se-glutathione peroxidase activity and vitamin E content and increases in neutrophil infiltration, XO activity, and lipid peroxide level in the gastric mucosal tissue and that gastric mucosal blood flow is reduced with gastric mucosal lesion forma-
tion, while the decreased blood flow is recovered with the lesion progression and recovery (11). We have also shown in rats treated once with compound 48/80 that neutrophils infiltrating into the gastric mucosal tissue participate in gastric mucosal lesion formation and progression, while the xanthine-XO system in the gastric mucosal tissue takes part mainly in the lesion progression (12). These findings may allow us to assume that gastric mucosal ascorbic acid status may change during the development of compound 48/80-induced acute gastric mucosal lesions in rats.

In the present study, therefore, we examined the changes in the gastric mucosal levels of total, reduced, and oxidized ascorbic acids with the formation, progression, and recovery of acute gastric mucosal lesions in rats treated once with compound 48/80. Ascorbic acid can be synthesized in the liver of rats (13). Hepatocytes have the capacity to release ascorbic acid in its reduced form (14). It is known that a single administration of compound 48/80 to rats affects the intrahemat microvascular regulatory mechanisms by releasing mast cell constituents, such as serotonin and histamine, following mast cell degranulation induced by the administered compound (15). Accordingly, the changes in the serum and hepatic levels of total, reduced, and oxidized ascorbic acids with the formation, progression, and recovery of compound 48/80-induced acute gastric mucosal lesions were examined in order to clarify whether the change in systemic ascorbic acid status is related with the changes in gastric mucosal ascorbic acid status in the compound 48/80-treated rats.

Results

Materials and Methods

Materials. Compound 48/80 was purchased from Sigma Chemicals (St. Louis, MO, USA); L-ascorbic acid (reduced from), α,α-dipiridyld, dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), N-ethylmaleimide (NEM), trichloroacetic acid (TCA), and other chemicals from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). All chemicals were used without further purification.

Animals. Male Wistar rats aged six weeks were purchased 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%) with 12 h of light (7:00 to 19:00). The animals were maintained with free access to rat 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 guidelines of the Animal Care and Use Committee of Fujita Health University.

Induction and observation of gastric mucosal lesions. Rats (7 wk old) fasted for 24 h received a single intraperitoneal (i.p) injection of compound 48/80 (0.75 mg/kg body weight), dissolved in distilled water, as described previously (11, 12). 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, 3 or 6 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 (11, 12):

- 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.

Sample collection and ascorbic acid determination in gastric mucosa, liver, and serum. Rats with and without compound 48/80 treatment were sacrificed under ether anesthesia at just before or 0.5, 3 or 6 h after the treatment at which time blood was collected from the inferior vena cava. Serum was obtained from the collected blood by centrifugation. Immediately after sacrifice, livers were perfused with ice-cold 0.15 M NaCl through the portal vain to remove residual blood in the tissue as much as possible. Then, livers and stomachs were isolated. Gastric mucosal tissues were collected from the isolated stomachs. The collected gastric mucosal tissues, livers, and serum were stored at −80°C until use. Total, reduced, and oxidized ascorbic acids in gastric mucosal and hepatic tissues and serum were determined by the method of Zannoi et al. (16) and Okamura (17) as follows: gastric mucosal or hepatic tissue was homogenized in 9 volumes of ice-cold 0.15 M KCl containing 1 mM EDTA to prepare 10% homogenate. For the determination of total ascorbic acid, 0.3 mL of 10% gastric mucosal homogenate, 10% liver homogenate or serum was incubated with 0.1 mL of 10 mM DTT at 37°C for 30 min to convert all ascorbic acid in an oxidized form in the homogenate or serum to its reduced form and then the excess DTT was removed with 0.1 mL of 0.5% NEM. An aliquot of the supernatant obtained after deproteinization with 0.5 mL of ice-cold 10% TCA was used for the assay of the resultant reduced ascorbic acid plus the original reduced ascorbic acid. For the determination of reduced ascorbic acid, 0.3 mL of 10% gastric mucosal homogenate, 10% liver homogenate or serum was mixed with 0.2 mL of a solution of 10 mM DTT-0.5% NEM. An aliquot of the supernatant obtained after deproteinization with 0.5 mL of ice-cold 10% TCA was used for the assay of the reduced form of ascorbic acid. Reduced ascorbic acid in each sample was measured by the α,α-dipiridyld method. The concentration of reduced ascorbic acid was determined using the standard curve of authentic L-ascorbic acid in a reduced form. The concentration of oxidized ascorbic acid in gastric mucosa, liver or serum was estimated from the difference between the concentrations of total ascorbic acid and reduced ascorbic acid determined.

Statistical analysis. Results obtained for total, reduced, and oxidized ascorbic acids in the gastric mucosal and hepatic tissues and serum are expressed as
Table 1. Time course of gastric mucosal lesions in compound 48/80-treated rats.

<table>
<thead>
<tr>
<th>Time after compound 48/80</th>
<th>0.5 h</th>
<th>3 h</th>
<th>6 h</th>
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<td>Lesion index (%)</td>
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<td>I</td>
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<td>II</td>
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<td>VI</td>
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Rats received a single i.p. injection of compound 48/80 (0.75 mg/kg) and the severity of gastric mucosal lesions was estimated at 0.5, 3, and 6 h after the compound 48/80 injection as described in Materials and Methods. The number of rats used in each group is 10.

Fig. 1. Time courses of the gastric mucosal concentrations of total, reduced, and oxidized ascorbic acids in compound 48/80-treated rats. Total, reduced, and oxidized ascorbic acids in the gastric mucosal tissue of rats treated with (striped column) and without (open column) compound 48/80 were determined just before and 0.5, 3, and 6 h after compound 48/80 treatment as described in Materials and Methods. Each value is a mean±SD (n=10). * Significantly different from control rats without compound 48/80 treatment at p<0.05.

Fig. 2. Time courses of the serum concentrations of total, reduced, and oxidized ascorbic acids in compound 48/80-treated rats. Total, reduced, and oxidized ascorbic acids in the sera of rats treated with (striped column) and without (open column) compound 48/80 were determined just before and 0.5, 3, and 6 h after compound 48/80 treatment as described in Materials and Methods. Each value is a mean±SD (n=10). * Significantly different from control rats without compound 48/80 treatment at p<0.05.

Means±SD. The results were analyzed by Student’s t-test. Values of significance were set at p<0.05.

Results

As shown in Table 1, compound 48/80-treated rats showed apparent gastric mucosal lesions at 0.5 h after the treatment, progressed gastric mucosal lesions at 3 h, and a recovery of the progressed gastric mucosal lesions at 6 h when the severity of gastric mucosal lesions was estimated using the lesion gradation. Untreated control rats showed no gastric mucosal lesion when the gastric mucosal concentrations of total, reduced, and oxidized ascorbic acids in compound 48/80-treated and untreated rats were determined 0.5, 3, and 6 h after the treatment, the results shown in Fig. 1 were obtained. There were no significant differences in the gastric mucosal concentrations of total and reduced ascorbic acids between the compound 48/80-treated and untreated control rats at 0.5 h after the treatment, although the gastric mucosal concentration of oxidized ascorbic acid in the compound 48/80-treated group tended to be higher than that in the control group. The gastric mucosal tissue of the compound 48/80-treated
group contained significantly less total and reduced ascorbic acids than that of the control group at 3 h after the treatment, although there was no difference in the gastric mucosal concentration of oxidized ascorbic acid between the two groups: the gastric mucosal concentrations of total and reduced ascorbic acids in the compound 48/80-treated group were 58.2 and 58.5%, respectively, of those in the control group. There were no significant differences in the gastric mucosal concentrations of total, reduced, and oxidized ascorbic acids between the compound 48/80-treated and control groups at 6 h after the treatment.

When the serum concentrations of total, reduced, and oxidized ascorbic acids in the compound 48/80-treated and untreated control groups were determined 0.5, 3, and 6 h after the treatment, the results shown in Fig. 2 were obtained. The serum of the compound 48/80-treated group contained significantly more total, reduced, and oxidized ascorbic acids than that of the control group at 0.5 h after the treatment. Further increases in the serum concentrations of total and reduced ascorbic acids in the compound 48/80-treated group occurred 3 h after the treatment. The increased serum concentrations of total and reduced ascorbic acids in the compound 48/80-treated group decreased 6 h after the treatment and the decreased concentrations of total and reduced ascorbic acids in the compound 48/80-treated group were not significantly different from those concentrations in the control group. However, there was no significant difference in the serum concentration of oxidized ascorbic acid between the compound 48/80-treated and control groups at 3 and 6 h after the treatment.

When the hepatic concentrations of total, reduced, and oxidized ascorbic acids in the compound 48/80-treated and untreated control groups were determined just before and 0.5, 3, and 6 h after compound 48/80 treatment as described in Materials and Methods. Each value is a mean±SD (n=10). * Significantly different from control rats without compound 48/80 treatment at p<0.05.

Discussion

In the present study, the gastric mucosal concentrations of total and reduced ascorbic acids decreased significantly with the progression of acute gastric mucosal lesions in rats with a single compound 48/80 treatment and the decreased concentrations of total and reduced ascorbic acids were almost completely returned to the levels of untreated rats at a recovery stage of the lesions, although the gastric mucosal concentration of oxidized ascorbic acid showed no significant change with the formation, progression, and recovery of the lesions. These results indicate that gastric mucosal ascorbic acid status is disrupted with the progression of compound 48/80-induced acute gastric mucosal lesions in rats.

We have shown in rats treated once with compound 48/80 that gastric mucosal Se-glutathione peroxidase activity and vitamin E level decrease with the progression of gastric mucosal lesions, while gastric mucosal neutrophil infiltration, XO activity, and lipid peroxide level increase with the formation and progression of the lesions and all these changes found at a progressed stage of the lesions are attenuated at a recovery stage of the lesions (11). Se-glutathione peroxidase decomposes both hydrogen peroxide and lipid hydroperoxides (18). Vitamin E functions as a chain-breaking antioxidant for lipid peroxidation in cell membranes and also as a scavenger of ROS (19). The xanthine-XO system and activated NADPH oxidoreductase in neutrophils generate ROS such as superoxide radical and hydrogen peroxide (20, 21). It is known that lipid peroxidation occurs via ROS generated not only by the xanthine-XO system but also by activated NADPH oxidoreductase in neutrophils (22, 23). It is also known that myeloperoxidase present in neutrophils mediates lipid peroxidation in the pres-
ence of hydrogen peroxide and halide ions (24). Reduced ascorbic acid not only scavenges ROS and hypochlorous acid but also supports the chain-breaking antioxidant action of vitamin E by reduction of vitamin E radical to vitamin E at the lipid/aqueous interface (1–5). These findings suggest that the loss of reduced ascorbic acid with lesion progression found in the gastric mucosa of rats treated once with compound 48/80 could be associated with the antioxidant action of the vitamin to scavenge ROS and hypochlorous acid generated by infiltrated neutrophils and ROS generated by the xanthine-XO system and to recycle vitamin E in the gastric mucosal tissue.

It is known that oxidized ascorbic acid, i.e., dehydroascorbic acid, is converted to its reduced form via GSH itself in a non-enzymatic manner or via the enzyme-dependent ascorbic acid recycling system in which 3α-hydroxysteroid dehydrogenase functions as an NADPH-dependent dehydroascorbate reductase and thiolttransferase (glutaredoxin), protein disulide isomerase, and thioredoxin reductase function as a GSH-dependent dehydroascorbate reductase (25–28). It has been reported that oxidized ascorbic acid is enzymatically converted to its reduced form in the stomach and small intestine tissue preparations of guinea pigs (29). It has also been reported that an enzyme to reduce oxidized ascorbic acid in the presence of GSH, i.e., GSH-dependent dehydroascorbate reductase, exists in the jejunum, ileum, and colon tissues of rats (30). Therefore, a possibility cannot be ruled out that a decrease in the activity of an enzyme to reduce oxidized ascorbic acid contributes to the loss of reduced ascorbic acid in the gastric mucosal tissue of rats treated once with compound 48/80.

Ascorbic acid can be synthesized in the liver of rats (13). Hepatocytes releases ascorbic acid in its reduced form (14). It is known that a single administration of compound 48/80 to rats affects the intrahepatic microvascular regulatory mechanisms (15). Therefore, we further examined whether a change in systemic ascorbic acid status contributes to the disruption of gastric mucosal ascorbic acid status with gastric mucosal lesion progression in rats treated once with compound 48/80. The serum concentrations of total and reduced ascorbic acids in compound 48/80-treated rats increased significantly with the formation of gastric mucosal lesions and further increased with the progression of the lesions but the increased concentrations of total and reduced ascorbic acids were almost completely returned to the levels of untreated rats at a recovery stage of the lesions. A significant increase in the serum concentration of oxidized ascorbic acid in compound 48/80-treated rats occurred with the formation of gastric mucosal lesions but no significant increase in the serum concentration of oxidized ascorbic acid was observed during the progression and recovery of the lesions. Compound 48/80-treated rats showed significant increases in the hepatic concentrations of total, reduced, and oxidized ascorbic acids during the progression of gastric mucosal lesions. These results indicate that hepatic ascorbic acid synthesis is stimulated in compound 48/80-treated rats possibly in response to oxidative stress. These results also suggest that the increase in the hepatic concentration of reduced ascorbic acid during the progression of gastric mucosal lesions in compound 48/80-treated rats is related to the increase in the serum concentration of reduced ascorbic acid found during the lesion progression. Thus, systemic ascorbic acid status was found to change with the formation and progression of acute gastric mucosal lesions in compound 48/80-treated rats. The changes in the serum and hepatic concentrations of total and reduced ascorbic acids found in compound 48/80-treated rats were clearly different from the above-described changes in the gastric mucosal concentrations of total and reduced ascorbic acids. At present, we cannot explain the reason why, in compound 48/80-treated rats, the concentrations of total, reduced, and oxidized ascorbic acids in the serum increase despite no changes in their concentrations in the liver tissue at an early stage of gastric mucosal lesions, i.e., at 0.5 h after the treatment. However, there seems to be a possibility that, in compound 48/80-treated rats, the amount of reduced ascorbic acid released into the blood flow from the liver is equal to the amount of reduced ascorbic acid synthesized in the liver at 0.5 h after the treatment even if hepatic ascorbic acid synthesis is stimulated at that time point, so that an increase in the concentration of reduced ascorbic acid occurs in the serum, but not in the liver. In addition, it seems likely that, in compound 48/80-treated rats, reduced ascorbic acid increasing in the serum through stimulation of ascorbic acid synthesis in the liver at a progressed stage of gastric mucosal lesions contributes to the return of the decreased gastric mucosal concentration of reduced ascorbic acid to the level of untreated control rats at a recovery stage of the lesions.

In the present study, the gastric mucosal concentration of oxidized ascorbic acid did not change at a progressed stage of acute gastric mucosal lesions in compound 48/80-treated rats despite a marked decrease in the gastric mucosal concentration of reduced ascorbic acid. It is known that oxidized ascorbic acid is rapidly degraded to 2,3-diketogulonic acid in animal tissues if not converted back to reduced ascorbic acid by enzymatic and/or non-enzymatic recycling, since oxidized ascorbic acid is unstable at physiological pH (31). From this finding, it is assumed that oxidized ascorbic acid formed upon the oxidation of reduced ascorbic acid is rapidly degraded to 2,3-diketogulonic acid without its conversion back to reduced ascorbic acid in the gastric mucosal tissue of rats treated once with compound 48/80, resulting in no increase in the gastric mucosal concentration of oxidized ascorbic acid.

In conclusion, the results of the present study indicate that gastric mucosal ascorbic acid status is disrupted with the progression of compound 48/80-induced gastric mucosal lesions in rats. However, further investigation is needed to clarify the exact mechanisms by which a single compound 48/80 treatment
causes changes in ascorbic acid concentrations in the serum and gastric mucosal and hepatic tissues of rats.

REFERENCES


