A Reliable Method for the Production of Antral Gastric Ulcer by a Combination of 2-Deoxy-D-Glucose, Aspirin and Ammonia in Rats

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ABSTRACT—In order to establish a reliable method for the production of gastric antral ulcer in rats, combined treatments with three factors: a vagal stimulant, a mucosal barrier breaker and a necrotizing agent were investigated. By the combined administration of 2-deoxy-D-glucose (2-DG; 200 mg/kg, i.v.), aspirin (100–400 mg/kg, p.o.) and hydrochloric acid (0.15 and 0.35 N, 0.5–1.5 ml/100 g, p.o.) or ammonia solution (0.5–1.0%, 0.5–1.5 ml/100 g, p.o.), gastric lesions were prominently induced in sites of both the corpus and antrum on day 2. The largest antral ulcer was induced by the combination of 2-DG (200 mg/kg), aspirin (200 mg/kg) and ammonia solution (1%, 10 ml/kg); and the mean antral ulcer index (mm²) was 43.1 ± 4.4 and the incidence was 100%. The antral ulcer was found to penetrate the muscularis mucosae and still observed on day 21 and day 28 after ulcer induction in a few cases. From these findings, it was indicated that this antral ulcer would be a useful model for studying the etiology and therapy of gastric ulcer disease.

It is well-known that most of the gastric ulcers in humans are located in the antral area along the lesser curvature (1). However, experimental gastric lesions or ulcers were mostly located in the corpus, and there are few experimental models for producing antral ulcer in small animals. Therefore, there is a need to develop an experimental antral ulcer model to study the etiology and therapy of antral gastric ulcer disease.

In 1981, Satoh et al. (2) produced antral ulcers by the oral administration of indomethacin followed by a 1 hr refeeding period in 24 hr-fasted rats. Maeda-Hagiwara and Watanabe (3) also reported that antral ulcers were induced by combining indomethacin with certain centrally acting drugs such as ergot alkaloids or 2-deoxy-D-glucose (2-DG) in rats. In these methods, a rather large dose of indomethacin was used. The ulcers were induced in the intestine as well, and the animal mortality was high. Kolbasa et al. (4) reported the indomethacin-induced gastric antral ulcer in hamsters, but the dose was also high. In 1985, Hirose et al. (5) reported the rat antral ulcer induced by the combination of acid, indomethacin and ischemia formed by the surgical operation (ligation of blood vessels). However, this method requires a surgical procedure, although the dosage of indomethacin was low.

Thus, in the present study, we tried to produce antral ulcers in rats by the combined administration of aspirin with 2-DG and hydrochloric acid, avoiding the need for surgery or the use of indomethacin which induces in-
testinal ulcers.

Recently, Murakami et al. (6) reported that ammonia can induce gastric lesions and ulcers in rats. Because we have also been interested in the gastric toxicity of ammonia in relation to the etiological role of Helicobacter pylori (7), we also investigated the effect of ammonia instead of hydrochloric acid.

MATERIALS AND METHODS

Animal

Male Sprague-Dawley strain rats weighing 230–250 g were used after 48-hr fasting, but allowed free access to drinking water. They were housed in cages with wide mesh bottoms to prevent coprophagy.

Gastric ulceration

2-DG dissolved in saline was administered intravenously at a dose of 200 mg/kg. After 30 min, aspirin suspended in 1% gum arabic solution was orally administered; and 1 hr later, hydrochloric acid or ammonia solution in various concentrations was administered orally. Experimental conditions were examined for the reliable production of gastric antral ulcers with respect to factors such as dose of aspirin, concentration and volume of hydrochloric acid or ammonia solution. The rats were returned to the home cage 2 hr after the final treatment and allowed free access to chow pellets and drinking water.

Observation of gastric lesions

Two days after the final treatment, the rats were killed with an overdose of ether. The stomach was excised, and the pylorus and the cardia were ligated. Eight milliliters of saline was instilled into the stomach, and the outside of the stomach was fixed with 5% formalin. Ten minutes after the formalin fixation, the stomach was rinsed with saline and cut along the greater curvature and gastric lesions were grossly observed. Gastric erosive lesions in the corpus were expressed as the sum of the length of lesions (corpus lesion index, mm) and antral ulcers, as the product of the measured length and width (antral ulcer index, mm²).

Healing process of the antral ulcer

The antral ulcer was induced by the combined administration of 2-DG (200 mg/kg), aspirin (200 mg/kg) and 1% ammonia solution (10 ml/kg). The observation of the ulcer was performed at appropriate intervals until day 28 after the ulcer induction. The gastric lesions were observed according to the above-described method.

Histological observation

Immediately after the observation of gastric lesions, the specimens were fixed in the formalin solution. Tissue sections were prepared and stained with hematoxylin and eosin and examined under a microscope.

Materials

2-DG (Nacalai Tesque), aspirin (Sigma), hydrochloric acid and ammonia solution (Wako Pure Chemical) were used in this study.

RESULTS

By the combined administration of 2-DG, aspirin and hydrochloric acid or ammonia solution, gastric lesions were induced in the corpus and antrum (Fig. 1), and the antral lesion penetrated the muscularis mucosae (Fig. 2). The corpus lesion index and the antral ulcer index are shown in Tables 1–3.

The combinations of 2-DG and hydrochloric acid without aspirin (Table 1) and 2-DG and aspirin without hydrochloric acid did not induce antral ulcer. The combination of aspirin and hydrochloric acid did not induce antral ulcer. With respect to the dosage of aspirin, the largest antral ulcer was induced at 200 mg/kg, and its incidence was 60%.

Regarding hydrochloric acid concentration and volume, the largest antral ulcer was induced at the concentration of 0.15 N and the volume of 15 ml/kg (Table 2), and its incidence was about 50%. By increasing the con-
Fig. 1. Antral ulcer induced by the combined administration of 2-deoxy-D-glucose (200 mg/kg), aspirin (200 mg/kg) and 1.0% ammonia solution (10 ml/kg) on day 2 in a rat.

Fig. 2. Histological section of antral ulcer induced by the combined administration of 2-deoxy-D-glucose (200 mg/kg), aspirin (200 mg/kg) and 1.0% ammonia solution (10 ml/kg) on day 2 in a rat (×25).
centration of hydrochloric acid, the antral ulcer size became smaller, but by increasing the volume of hydrochloric acid, it became larger. The antral ulcer incidence did not greatly change among the groups.

By using ammonia solution instead of hydrochloric acid, the antral ulcer size became larger and the incidence became higher (Table 3). The largest ulcer was induced at the volume of 10 ml/kg (1% concentration), and its incidence was 100%. The combination of 2-DG and ammonia solution induced antral ulcers to a small degree; the antral ulcer index was 2.5 ± 1.3 and the ulcer incidence was 3/5.

Corpus lesions did not differ in each group except in the aspirin non-treated group, where

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### Table 1. Effects of aspirin dosage on antral ulcer induction in rats receiving 200 mg/kg of 2-deoxy-D-glucose and 0.15 N hydrochloric acid (10 ml/kg)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>0.6 ± 0.6</td>
<td>0 ± 0</td>
<td>0/5</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>54.4 ± 3.2</td>
<td>2.3 ± 1.4</td>
<td>3/5</td>
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<tr>
<td>200</td>
<td>5</td>
<td>40.6 ± 7.2</td>
<td>4.6 ± 2.4</td>
<td>3/5</td>
</tr>
<tr>
<td>400</td>
<td>5</td>
<td>44.2 ± 5.3</td>
<td>2.6 ± 1.3</td>
<td>4/5</td>
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</tbody>
</table>

[1]Values represent the mean ± S.E. of used rats (mm).
[2]Values represent the mean ± S.E. of used rats (mm²).
[3]Number of ulcerated rats/number of used rats.

### Table 2. Effect of hydrochloric acid on antral ulcer induction in rats receiving 200 mg/kg of 2-deoxy-D-glucose and 200 mg/kg of aspirin

<table>
<thead>
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<tbody>
<tr>
<td>0.15</td>
<td>5</td>
<td>5</td>
<td>40.6 ± 7.2</td>
<td>4.6 ± 2.4</td>
<td>3/5</td>
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<tr>
<td>0.15</td>
<td>10</td>
<td>5</td>
<td>59.2 ± 4.4</td>
<td>2.4 ± 2.2</td>
<td>2/5</td>
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<tr>
<td>0.15</td>
<td>15</td>
<td>15</td>
<td>43.3 ± 2.9</td>
<td>7.9 ± 3.9</td>
<td>7/15</td>
</tr>
<tr>
<td>0.35</td>
<td>15</td>
<td>5</td>
<td>52.2 ± 5.8</td>
<td>6.4 ± 4.5</td>
<td>3/5</td>
</tr>
</tbody>
</table>

[1]Values represent the mean ± S.E. of used rats (mm).
[2]Values represent the mean ± S.E. of used rats (mm²).
[3]Number of ulcerated rats/number of used rats.

### Table 3. Effect of ammonia solution of antral ulcer induction in rats receiving 200 mg/kg of 2-deoxy-D-glucose and 200 mg/kg of aspirin

<table>
<thead>
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<tbody>
<tr>
<td>0.5</td>
<td>15</td>
<td>5</td>
<td>66.6 ± 11.6</td>
<td>0.8 ± 0.8</td>
<td>1/5</td>
</tr>
<tr>
<td>0.7</td>
<td>15</td>
<td>5</td>
<td>34.0 ± 6.3</td>
<td>7.6 ± 4.2</td>
<td>4/5</td>
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<tr>
<td>1.0</td>
<td>5</td>
<td>5</td>
<td>54.4 ± 3.9</td>
<td>14.8 ± 6.1</td>
<td>3/5</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>10</td>
<td>51.9 ± 3.6</td>
<td>43.1 ± 4.4</td>
<td>10/10</td>
</tr>
<tr>
<td>1.0</td>
<td>15</td>
<td>5</td>
<td>64.2 ± 10.2</td>
<td>19.6 ± 8.3</td>
<td>4/5</td>
</tr>
</tbody>
</table>

[1]Values represent the mean ± S.E. of used rats (mm).
[2]Values represent the mean ± S.E. of used rats (mm²).
[3]Number of ulcerated rats/number of used rats.
only slight erosive lesions were observed, and the mean corpus lesion index was 0.6 ± 0.6 (Table 1).

Healing process of the antral ulcer: The initial ulcer index and corpus lesion index were 43.1 ± 4.4 and 51.9 ± 3.6, respectively (Table 4). On day 7, the healing ratio was 5/10, and the ulcer index markedly decreased to about 1/20 of the initial ulcer index (on day 2). However, even on day 21 and day 28, the antral ulcer was still observed in a few cases. No gastric erosive lesions were observed on and after day 7.

Table 4. Healing process of the antral ulcer induced by the combination of 2-deoxy-D-glucose (200 mg/kg), aspirin (200 mg/kg) and 1% ammonia solution (10 ml/kg) in rats

<table>
<thead>
<tr>
<th>Days after ulcer induction</th>
<th>Antral ulcer index&lt;sup&gt;1)&lt;/sup&gt;</th>
<th>Healing ratio&lt;sup&gt;2)&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>7</td>
<td>2.0 ± 0.8</td>
<td>8/10</td>
</tr>
<tr>
<td>14</td>
<td>1.9 ± 0.8</td>
<td>7/10</td>
</tr>
<tr>
<td>21</td>
<td>0.6 ± 0.4</td>
<td>9/10</td>
</tr>
<tr>
<td>28</td>
<td>0.3 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1)</sup>Values represent the mean ± S.E. of used rats (mm<sup>2</sup>). <sup>2)</sup>Number of healed rats/number of used rats. <sup>3)</sup>The data are cited from Table 3.

DISCUSSION

Most human gastric ulcers are located in the antrum along the lesser curvature or marginal area between the corpus and the antrum as reported by Oi et al. (1). However, there are few experimental models of gastric ulcers in this position (2–5). Therefore in the present study, we tried to produce an antral ulcer by the combination of 2-DG, aspirin and hydrochloric acid or ammonia solution, avoiding surgical operation; aspirin was substituted for indomethacin because it does not cause intestinal lesions. By the combined administration of 2-DG (200 mg/kg), aspirin (200 mg/kg) and ammonia solution (1%, 10 ml/kg), a large antral ulcer was induced, which penetrated to the muscularis mucosae, and its incidence was 100%. Moreover, the ulcer was still observed on day 28 after ulcer induction.

Nonsteroidal anti-inflammatory drugs such as indomethacin and aspirin have been reported to induce gastric lesions in experimental animals and humans (8, 9). Indomethacin has been known to induce intestinal ulcer as well as gastric lesions, while aspirin did not induce intestinal lesions and antral lesions when it was administered orally. The present authors also reported previously that most of the lesions are present in the corpus when aspirin was administered orally and that this would result from the difference in hexosamine content between the corpus and antrum (10).

In the parenteral route, gastric antral lesions were reported to be produced by a combination of intravenous infusion of aspirin and gastric perfusion with hydrochloric acid in rats (11) and by intravenous infusion of aspirin alone or with histamine in cats (12, 13). In the present study, in combination with 2-DG and hydrochloric acid, aspirin induced both the antral ulcer and the corpus lesions in rats, although the administration route of aspirin was per os. The combination of 2-DG and hydrochloric acid, however, did not induce antral ulcer. Therefore, prostaglandin (PG) deficiency by aspirin might be needed in the induction of antral ulcer.

PGs have been known to play an important role in maintaining gastrointestinal mucosal integrity. Koblaska et al. (4) also reported that mucosal PG depletion seems to play an important role in inducing antral ulcer in hamsters. On the other hand, Ligumsky et al. (14) reported that aspirin-induced gastric lesions were observed only by the per os route, although parenteral aspirin inhibited PG synthesis by about 90%, suggesting that aspirin has a local irritant effect unrelated to its effects on gastric PG synthesis. Therefore, in the present model, studying the parenteral route of aspirin administration would clarify whether gastric mucosal PG deficiency and/or a local irritant effect by aspirin may play an important role in inducing antral ulcer.
In 1979, Urushidani et al. (15) reported that the adrenal cortex, particularly the area containing glucocorticoids, plays an important role in suppressing the noxious effects of indomethacin on the rat gastric corpus mucosa and that aspirin-induced ulcers in rats were significantly aggravated by adrenalectomy. Moreover, in the adrenalectomized rats, readily visible ulcers were often found in the antral portion where indomethacin-induced ulcers seldom occurred in the sham operated rats. 2-DG has been reported to increase gastric secretion by activating the chemoreceptor in the lateral hypothalamic area that initiates and sustains a vagally mediated response. Thus, the activation of the vagus (parasympathetic nerves) by 2-DG may be concerned with the induction of antral ulcer, if the activation of the vagus by 2-DG predominates over the sympathetic nerve activity. Satoh et al. (2), however, reported that indomethacin-induced antral ulcers were protected by adrenalectomy. To clarify these points, further examinations are needed.

Instillation of alkaline solution has been known to be toxic to the mucosa of gastrointestinal tracts. In 1980, Vancula et al. (16) reported the esophageal ulceration by alkaline solution. Murakami et al. (6) reported ammonia toxicity in ammonia-induced gastric lesions and ulcers. Recently, ammonia production by Helicobacter pylori has attracted the interest of many researchers in the field of gastroenterology. We have also studied the ammonia production in the stomach of experimental animals (7, 17, 18) by examining the effect of ammonia on antral ulcer formation. By using ammonia instead of hydrochloric acid, the antral ulcer index became larger and its incidence became higher. It has been known that alkaline compounds cause liquefactive necrosis which in turn causes ongoing invasion into deeper layers of tissue, while acids cause coagulation necrosis that sets up a tissue barrier to prevent ongoing destruction (16). Against acid, gastric mucosa has a protective ability to secrete alkali (bicarbonate) and mucus, and the mucus layer plays an important role in the gastroprotection (19). However, an excessive amount of ammonia is permeable to the cell membrane and may be cytotoxic towards the gastric mucosa.

Of the few studies on antral ulcer induction, most did not examine the healing process of antral ulcer, except in the report by Satoh et al. (2), who reported that antral lesions did not heal for at least 7 days. In the present study, even on day 28, the antral ulcer was still observed, though the healing ratio was 9/10. Therefore, it would be worth modifying the experimental conditions for the production of a more chronic antral ulcer model.

In conclusion, a large antral ulcer was induced by the combination of 2-DG (200 mg/kg), aspirin (200 mg/kg) and 1% ammonia solution (10 ml/kg), and its incidence was 100%. Thus, this antral ulcer would be a useful model for studying the etiology and therapy of gastric ulcer diseases.

Acknowledgments

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