Aggravation by the Capsaicin-Treatment of Gastric Antral Ulcer Induced by the Combination of 2-Deoxy-D-Glucose, Aspirin and Ammonia in Rats

Masayuki Uchida, Shingo Yano and Kazuo Watanabe

Department of Drug Evaluation and Toxicological Sciences, Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Chiba 260, Japan

Received June 20, 1991 Accepted August 7, 1991

ABSTRACT—The effect of capsaicin-sensitive nerve degeneration (capsaicin-treatment) was investigated on the antral ulcer induction by the combined administration of 2-deoxy-D-glucose (2-DG; 200 mg/kg, i.v.), aspirin (200 mg/kg, p.o.) and 1% ammonia solution (10 ml/kg, p.o.) in male Sprague-Dawley rats. On the 2nd day after ulcer induction, erosive lesions were seen in the corpus, and ulcers penetrating into the muscularis mucosae were also seen in the antrum. In the capsaicin-treated rats, the antral ulcer was significantly aggravated as compared with that in capsaicin non-treated rats, although no difference was noted in the formation of corpus lesions between capsaicin-treated rats and non-treated rats. Acid output was investigated in pylorus ligated rats. 2-DG significantly increased the acid output. A significant increase in acid output was also observed in capsaicin-treated rats, and this increase tended to be augmented by the additional treatment with 2-DG. Atropine sulfate inhibited the significant increase in acid output of the capsaicin-treated rats. In all rats, both capsaicin-treated and pylorus-ligated, 2-DG induced antral ulcers that penetrated into the muscularis mucosae. From the above results, it was suggested that capsaicin-sensitive nerve degeneration modifies the gastroprotective ability in the antral mucosa to a greater extent than in the fundic mucosa, and this aggravation may be caused by activation of the vagus nerve, although the role of acid is not completely excluded.

It has been recently reported that the capsaicin-sensitive afferent nerves play an important role in the protection of gastric mucosa (1–4). Capsaicin-sensitive nerve degeneration by capsaicin pretreatment was reported to aggravate the gastric lesions (5). The present authors also reported previously that acute administration of capsaicin inhibited the lesion formation by absolute ethanol, but the protective effect of capsaicin was not seen in capsaicin-sensitive nerve degenerated rats (6).

In various kinds of experimental ulcer models, gastric lesions or ulcers were mostly located in the corpus area in the stomach. Therefore, there are many reports on the aggravation of gastric corpus lesions by the capsaicin-sensitive nerve degeneration (capsaicin-treatment) (5, 7), while there are few reports on antral lesions (5). We have so far reported that antral ulcers and corpus erosive lesions are induced by the combined administration of 2-deoxy-D-glucose (2-DG; 200 mg/kg, i.v.), aspirin (200 mg/kg, p.o.) and 1% ammonia solution (10 ml/kg, p.o.) (8, 9). Murakami et al. (10) 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. These results suggested the possible involvement of a urea-urease-ammonia mechanism in relation to the etiological role of Helicobacter pylori (11).

In the present study, we examined the effects of capsaicin-sensitive nerve degeneration on the antral ulcer induction to clarify the sensitivity of the antral mucosa to ulcerogenic agents. In addition, basal and 2-DG-stimulated gastric acid secretion was investigated in pylorus-ligated rats.

MATERIALS AND METHODS

Animals
Male Sprague-Dawley rats weighing 220-250 g were used. The animals were deprived of food but allowed free access to tap water for 48 hr before the experiment.

Gastric ulceration
Gastric ulceration was induced according to our method reported previously (8, 9). A solution of 2-DG (Nacalai Tesque) dissolved in saline was administered intravenously at a dose of 200 mg/kg (0.1 ml/100 g). After 30 min, aspirin (Sigma) suspended in 1% gum arabic solution was orally administered; and 1 hr later, 1% ammonia solution (Wako Pure Chemical) was administered orally in a volume of 1.0 ml/100 g. Gastric ulceration was compared between capsaicin-treated rats and non-treated ones.

Observation of gastric ulcer
Two days after the ulcer induction, rats were killed with an overdose of ether. The stomach was excised, and the pylorus and the cardia were ligated. Saline (8 ml) 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 then the gastric lesions were grossly observed. Gastric erosive lesions in the fundus were expressed as the sum of the length of lesions (corpus lesion index, mm) and antral ulcer, as the product of the measured length and width (antral ulcer index, mm²).

Gastric acid secretion
Under light ether anesthesia, epigastric laparotomy was performed. After exposing the stomach, the pylorus was ligated, and the abdominal incision was sutured. Then, a solution of 2-DG dissolved in saline (200 mg/kg) or vehicle was administered intravenously in a volume of 0.1 ml/100 g. Gastric juice was collected for 4 hr after the 2-DG or vehicle treatment. The gastric juice was centrifuged at 3000 rpm for 15 min, and the volume, pH and total acidity were measured. Values of pH were measured with a pH meter, and the total acidity was determined by titrating the gastric juice with 0.05 N NaOH to the end point of pH 7.0 using an automatic titrator (Toa Electronics Co., AUT-1, ABT-1 and TIT-1, Japan). Gastric acid secretion was investigated in capsaicin-treated rats and non-treated rats. The effect of atropine sulfate (1 mg/kg) (Tanabe), subcutaneously injected 30 min before ligation, was also investigated in capsaicin-treated rats. In this study, gastric lesions were observed according to the method mentioned above.

Degeneration of the capsaicin-sensitive afferent nerves
To degenerate the capsaicin-sensitive afferent nerves, capsaicin pretreatment was performed by the method by Yonei et al. (12). Capsaicin was dissolved in vehicle consisting of 10% ethanol, 10% Tween 80 and 80% saline (vol./vol./vol.). Rats received a total dose of 125 mg/kg capsaicin, s.c. over 2 days, with 25 mg/kg in the morning and 50 mg/kg in the afternoon on the first day and 50 mg/kg once on the second day. The rats were used 10 days after the pretreatment with capsaicin. In order to check the effectiveness of the denervation treatment, a drop of a 0.01% solution of capsaicin in saline was instilled into
either eye of the rats, and their protective
wiping movements were counted. The capsaicin-
treated animals that showed any wiping move-
ment were excluded from the study.

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

Statistics
Data were expressed as mean ± S.E. Statisti-
cal analyses of the data were performed by
means of Dunnett’s multiple comparison test
and Student’s t-test.

RESULTS
Gastric ulceration

By the combined administration of 2-DG,
aspirin and ammonia solution, gastric lesions
were induced in the corpus and antrum (Fig. 1);
and the antral lesion apparently penetrated
into the muscularis mucosae (Fig. 2). The cor-
pus lesion index and the antral ulcer index are
shown in Table 1.

In the vehicle treated rats, the corpus lesion
index and antral ulcer index were 65.6 ± 7.9
(mm) and 14.3 ± 4.5 (mm2), respectively, and
the antral ulcer incidence was 100% (Table 1).
By the degeneration of the capsaicin-sensitive
nerves, significant aggravation was observed
on the antral ulcer index, but the corpus
lesion index was not different between
capsaicin-treated rats and non-treated rats
(Table 1).

Gastric acid secretion
In the vehicle treated rats, the volume, pH
and acid output were 3.9 ± 0.5 (ml/4 hr), 1.65

Fig. 1. Gross appearance of a huge antral ulcer induced by the combination of 2-deoxy-D-glucose, aspirin
and ammonia solution in capsaicin-treated rats on day 2. Most regions of the antrum were covered with
necrotic debris.
± 0.06 and 258.3 ± 37.9 (μEq/4 hr), respectively (Table 2). By the administration of 2-DG, a significant increase in acid output was observed along with a significant decrease in pH value (Table 2). In capsaicin-treated rats, significant differences were observed in the acid output and pH value, and no significant difference was observed between 2-DG-treated rats and non-treated rats, although an increase in acid output was observed by the 2-DG treatment (Table 2). The increase in acid output and the decrease in pH value in capsaicin-treated rats were attenuated by the pretreatment with atropine sulfate (Table 2).

In vehicle-treated rats, no antral lesions were observed. By the treatment with 2-DG, antral lesions were induced with a 37.5% incidence, and the antral ulcer index was 4.9 ±

### Table 1. Effect of capsaicin-sensitive nerve degeneration on the gastric lesions induced by the combined administration of 2-deoxy-D-glucose, aspirin and NH₄OH in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Corpus lesion index (mm)</th>
<th>Antral ulcer index (mm²)</th>
<th>Antral ulcer incidence¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated</td>
<td>11</td>
<td>65.6 ± 7.9</td>
<td>14.3 ± 4.5</td>
<td>11/11</td>
</tr>
<tr>
<td>Capsaicin-treated</td>
<td>11</td>
<td>53.5 ± 5.5</td>
<td>102.8 ± 14.7**</td>
<td>11/11</td>
</tr>
</tbody>
</table>

¹: No. of ulcerated rats/No. of used rats. Each value represents the mean ± S.E. **: Significant difference from the non-treated group (P < 0.01).

Fig. 2. Histological observation of the antral ulcer induced by the combination of 2-deoxy-D-glucose, aspirin and ammonia solution in capsaicin-treated rats on day 2. (HE staining, ×8).
2.6 (mm²) (Table 3). In capsaicin-treated rats, antral lesions were slightly induced (incidence and index: 12.5% and 0.6 ± 0.6 mm²), and 2-DG significantly aggravated the antral ulcers (incidence and index: 100% and 29.7 ± 6.0 mm²) (P < 0.01), which penetrated into the muscularis mucosae (Figs. 3 and 4). By the pretreatment with atropine sulfate, antral lesions were completely inhibited (incidence and index: 0% and 0 ± 0 mm²) (Table 3).

In the corpus area, gastric lesions were scarcely observed.

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>No. of rats</th>
<th>Volume (ml/4 hr)</th>
<th>Gastric acid secretion pH</th>
<th>Acid output (µEq/4 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Vehicle</td>
<td>8</td>
<td>3.9 ± 0.5</td>
<td>1.65 ± 0.06</td>
<td>258 ± 38</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2-DG</td>
<td>8</td>
<td>5.9 ± 1.0</td>
<td>1.24 ± 0.04*</td>
<td>672 ± 77**</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Vehicle</td>
<td>8</td>
<td>5.6 ± 0.8</td>
<td>1.24 ± 0.04**</td>
<td>568 ± 103*</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>2-DG</td>
<td>8</td>
<td>6.6 ± 0.9</td>
<td>1.21 ± 0.04**</td>
<td>845 ± 99**</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Atropine sulfate</td>
<td>8</td>
<td>0.9 ± 0.2**+</td>
<td>3.10 ± 0.36**+</td>
<td>43 ± 11**+</td>
</tr>
</tbody>
</table>

DISCUSSION

Capsaicin has been widely used as a pharmacological tool to assess the involvement of sensory neurons in organ functions (13, 14). There are reports about the aggravation of gastric lesions (5, 7) by the degeneration of the primary afferent neurons, and gastric lesions were almost always induced in the fundic area. Esplugues and Whittle also reported a significant aggravation of 50% ethanol-induced gastric lesions in the capsaicin-treated rats.
while the present authors reported that no significant difference between capsaicin-treated rats and non-treated rats was observed in the induction of gastric lesions by absolute ethanol (6). This finding was in accord with the report by Takeuchi et al. (15) that capsaicin-treatment did not significantly affect the formation of HCl-ethanol-induced gastric lesions, although the experimental models used in the study were different from the ones in that study. Moreover, in the present study, no significant difference was observed in the fundic erosive lesions, and this result agreed with the above reports.

In the ethanol-induced gastric lesion model, gastric lesions were induced prominently in

Fig. 3. Macroscopical observation of the antral ulcer induced by 2-deoxy-D-glucose in a capsaicin-treated and pylorus-ligated rat. Top panel: Antral ulcer was observed clearly even from the serosal side. Bottom panel: Hemorrhagic lesions were observed in the antral region.
the corpus and scarcely in the antrum. However, in capsaicin-sensitive nerve degenerated rats or in morphine pretreated rats, hyperemia or erosive lesions were often observed in the antral region. Esplugues and Whittle (5) also reported that morphine and capsaicin-treatment increased the antral damage induced by 25–100% ethanol. Therefore, it was postulated that the capsaicin-sensitive nerves play an important gastroprotective role in the antral mucosa as well as in the fundic mucosa. In the present antral ulcer model, significant ulcer-aggravation was observed between capsaicin-treated rats and non-treated rats. This finding was supported by the report by Esplugues and Whittle (5), although we used different experimental conditions than they did. From the above findings, it was suggested that the capsaicin-sensitive afferent nerves play a more important role in the antral mucosa than in the fundic mucosa.

Morphine has been known to inhibit the release of substance P (16), and capsaicin has been known to deplete the substance P or calcitonine gene-related peptide (17). Recently, Ainsworth et al. (18) reported that morphine inhibits secretion of bicarbonate from the human duodenal mucosa. Therefore, capsaicin-treatment may inhibit the bicarbonate secretion from the antral mucosa, which was known to secrete bicarbonate and mucus, though the inhibition of bicarbonate and mucus secretion by the capsaicin-treatment was not reported in the gastric mucosa. On the other hand, morphine was reported to stimulate the bicarbonate secretion in the duodenum of rats (19). Therefore, the effect of capsaicin treatment on the bicarbonate secretion in the gastric mucosa must be evaluated.

Immunohistochemical study has shown that capsaicin-sensitive afferent neurons innervating the rat stomach contain substance P (20) and calcitonin gene-related peptide (21). Therefore, studying the effect of capsaicin-
sensitive nerve degeneration on the fundic mucosal level and the antral mucosal level of substance P or calcitonin gene-related peptide may provide a means for clarifying the different susceptibility to capsaicin-sensitive nerve degeneration in the fundic mucosa and the antral mucosa.

On the gastric acid secretion in capsaicin-sensitive degenerated rats, Raybould and Tache (22) reported that capsaicin-sensitive vagal afferent fibers mediate the vagal portion of the secretory response to gastric distention. This finding suggests that the afferent signal through the vagus plays an important role in controlling the acid secretion. In this study, a significant increase in acid secretion was observed in capsaicin-treated rats. Therefore, the inhibition of the afferent signal through the vagus might increase the acid output. The increase in gastric acid secretion was inhibited by the treatment with atropine sulfate in capsaicin-treated rats. These facts suggest that the vagal efferent pathway may be involved in the activation of gastric acid secretion in capsaicin-treated rats, although direct evidence is lacking at present.

2-DG has been reported to increase the gastric secretion by activating the chemoreceptor in the lateral hypothalamic area which initiates and sustains a vagally mediated acid response. Evangelista et al. (23) reported that capsaicin-sensitive fibers are involved in the afferent branches of the reflex response activated by 2-DG to stimulate gastric acid secretion. This shows that the capsaicin-treatment depresses the increase in gastric acid secretion by 2-DG. On the contrary, in this study, 2-DG increased acid output rather than decreasing it, although the increase was not significant. In addition, Raybould and Tache (22) reported that on the basal acid output, no significant difference was observed between capsaicin-treated rats and non-treated rats. The reason for these discrepancies remains unknown, but it might be due to the different experimental conditions; we used the pylorus ligated method in the conscious state, and they used the Ghosh and Schild method in the urethane anesthetized state.

In the present study, the antral ulcer was severely induced with a 100% incidence in the 2-DG and capsaicin-treated rats, but only in the capsaicin-treated rats, antral lesions were scarcely observed, although the acid output was not significantly different between them. These facts may show that gastric acid is not a major factor for inducing antral ulcer in pylorus ligated rats, although the role of acid is not completely excluded. Therefore, it seemed that the vagal activation by 2-DG itself played an important role in the antral ulcer induction. Maeda-Hagiwara and Watanabe (24) reported that 2-DG or insulin produced antral ulcers but peripheral gastric secretagogues, bethanechol or histamine, did not produce antral ulcers at doses sufficient to stimulate gastric secretion in the indomethacin-treated rats. This finding supports the above speculation. However, further experiments will be needed to clarify the phenomena following the activation of the vagus.

In conclusion, in capsaicin-sensitive nerve degenerated rats, significant aggravation was observed in the antral ulcer induction but not in the formation of corpus lesions by the combined administration of 2-DG, aspirin and ammonia, and this may be caused by the activation of the vagus, although the role of acid was not completely excluded.

Acknowledgments
This study was supported in part by Grant-in Aids for Scientific Research from the Ministry of Education, Science and Culture of Japan and by the Suzuken Memorial Foundation, Japan.

REFERENCES
1 Holzer, P. and Sametz, W.: Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive neurons. Gastroenterology 91, 975–981 (1986)
3 Holzer, P., Pabst, M.A. and Lippe, I.T.: Intraga...
gastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa. Gastroenterology 96, 1425–1433 (1989)


