Significance of Local Circulatory Disturbance in the Experimental Production of Gastric Ulcer

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SEKINE, T. and SHIRATORI, T. Significance of Local Circulatory Disturbance in the Experimental Production of Gastric Ulcer. Tohoku J. exp. Med., 1974, 114 (2), 121-130 — Experimental gastric ulcers were produced by ligating gastric vessels accompanied with or without histamine administration. A total of 62 adult mongrel dogs were divided into five groups, and intramuscular injection of histamine-beeswax mixture was given. In each group, macroscopic findings of the gastric mucosa, microscopic findings of ulcer, incidence of production of ulcers and acid secretory response to histamine were observed. Duration of observations ranged from 1 to 4 weeks. The results were as follows: (1) Gastric ulcers were closely similar to those seen in human except little regeneration of mucosa or proliferation of connective tissue at the base and margin of the ulcer. (2) The incidence of gastric ulcers was extremely high in groups underwent both histamine administration and gastric vessel ligation as compared with those where only histamine injection was given. Gastric acid secretion in response to histamine also showed a fall in free acidity and a marked decrease in volume. These results suggest that decrease in mucosal resistance induced by ligation of gastric vessels may play an important role as an etiologic factor rather than a factor of gastric acid secretion in the pathogenesis of gastric ulcers.

Since Cruveilhier (1835-1842) described the chronic gastric ulcer as an independent disease, numerous reports have appeared on the etiology of gastric ulcer. On the other hand, Hollander (1954) and Shay and Sun (1963) pointed out that the unbalanced mutual relationship between aggressive and defensive factors is the cause of gastric ulcer and they demonstrated that these factors, forming complicated causality with each other, contribute to the production of gastric ulcer. Of the theories based on these factors, those which have been most influential are the theories on acid-pepsin activity and local circulatory disturbance. Among these factors participating in the production of ulcers, acid-pepsin factor has received considerable attention as an important factor. However, there have been few systematic studies concerning the etiologic factor of local circulatory disturbance. In the present study, to elucidate the etiology of gastric ulcer, the relationship between experimental production of gastric ulcer and local circulatory disturb-
ance was investigated by ligating gastric vessels accompanied with or without histamine injection.

**MATERIALS AND METHODS**

A total of 62 healthy adult mongrel dogs of both sexes, weighing 12–20 kg were used in this study. They were housed in individual cages and daily fed a routine diet consisting of horse-meat and milk. Dogs were divided into five groups and the following preparations were made to produce ulcers.

- **Group 1**: Histamine injection only.
- **Group 2**: Ligation of the main gastric arteries. The right and left gastric and gastroepiploic arteries were ligated.
- **Group 3**: Ligation of the main gastric arteries followed by a daily histamine injection. The right and left gastric and gastroepiploic arteries were ligated, and then a daily histamine injection was given.
- **Group 4**: Ligation of the main gastric arteries and veins. The right and left gastric and gastroepiploic arteries and veins were ligated.
- **Group 5**: Ligation of the main gastric arteries and veins followed by a daily histamine injection. The right and left gastric and gastroepiploic arteries and veins were ligated, and a daily histamine injection was given.

Prior to the experiment, dogs were fasted for at least 24 hr. Under pentobarbital sodium anesthesia, the abdomen was opened through a mid-line epigastric incision, and the right and left gastric and gastroepiploic arteries were ligated at the proximal sites of the branches. Care was taken not to ligate the short gastric arteries. Veins were ligated at the same sites as done in arteries. According to the method of Code and Varco (1940), the histamine-beeswax was prepared in batches mixing histamine dihydrochloride, beeswax and liquid paraffin containing 100 mg histamine dihydrochloride per cubic centimeter. As a routine, 0.5 ml of the histamine-beeswax mixture was injected into the muscles of the back. The injection was continued once a day for 1 to 4 weeks starting on the first postoperative day until the date of sacrifice.

Dogs were sacrificed 1 to 4 weeks after each preparation and the stomach was examined macroscopically as well as microscopically. The stomach was removed, and an incision was made along the greater curvature and the gastric mucosa was examined macroscopically. Then, the stomach was fixed in 10% formalin solution and was examined microscopically with hematoxylin-eosin and hematoxylin-eosin, alcianblue, and PAS-Masson quintuple stain. On the other hand, for collection of gastric juice, 12 dogs were used, 6 of which received no ligation of gastric vessels (control dogs) and in the other 6 dogs the main gastric arteries and veins were ligated in the same manner as in Group 4. In these dogs a cannula was inserted through the anterior wall into the corpus of the fundic gland area. Gastric acid secretion was observed for 1 to 4 weeks after each preparation.

Samples of gastric juice were collected at 15-min intervals for 24 hr after the intramuscular injection of the histamine-beeswax mixture. Volume was measured and free acidity determined by titration with Töpfer's reagent. Acid output was expressed as milliequivalents per 15 min. On completion of the experiment, all dogs were examined to ascertain that ligation of gastric vessels had been effected.

**RESULTS**

*Macroscopic findings of the gastric mucosa*

In the Groups 2 and 4 to which ligation of gastric vessels was made without histamine injection, the border between the esophageal and gastric mucosa was clearly discerned with the naked eye. Moreover, the gastric mucosa was divided
Experimental Gastric Ulcer

Fig. 1. Erosion and gastric ulcer (arrows) developed at the border between the fundic and pyloric gland areas after 4 week injections of histamine-beeswax mixture.

Fig. 2. A perforated gastric ulcer following ligation of gastric vessels in addition to histamine injection. Ulcer is localized at the pyloric antrum and along the lesser curvature.

into the reddish fundic gland area and the pale pyloric gland area, and the pyloric ring was connected with the duodenal mucosa. In the Groups 1, 3 and 5 to which histamine injection was given, the fundic gland area showed a pronounced hyper-
emia and hypertrophy in the greater part of cases, regardless of ligation of gastric vessels. Furthermore, there were seen a great many erosions at the sites close to the border between the fundic and pyloric gland areas (Fig. 1). These erosions were often accompanied by petechia, in the form of the so-called hemorrhagic erosion. However, these findings were not significantly different from the group to which only histamine injection was given, and the group to which ligation of the main gastric arteries was done with histamine injection.

Macroscopic findings of ulcers

Ulcers were round or oval in the majority of cases, and sometimes irregular-shaped. Round ulcers were often found along the border line between the fundic and pyloric gland areas, but there was no linear ulcer. These ulcers were in a great variety of depth. They were also of all sizes, some as small as the tip of a needle and others measuring 2 to 3 cm in diameter. However, there was no ulcer accompanied by remarkable cicatricial tissue which is often observed in the chronic ulcer of humans. In the Group 5 to which histamine injection as well as ligation of the main gastric arteries and veins was given, perforation of ulcer was observed in 7 of 10 cases (Fig. 2).

Localization of ulcers

Almost all the ulcers were localized at the region of the antrum along the
Table 1. Incidence of production of gastric and duodenal ulcer

<table>
<thead>
<tr>
<th>Stomach*</th>
<th>Number of dogs</th>
<th>Gastric ulcer</th>
<th>Duodenal ulcer</th>
<th>Coexisting ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of dogs</td>
<td>%</td>
<td>Number of dogs</td>
<td>%</td>
</tr>
<tr>
<td>Group 1</td>
<td>13</td>
<td>2</td>
<td>15.4</td>
<td>3(2)</td>
</tr>
<tr>
<td>Group 2</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 3</td>
<td>14</td>
<td>7(2)</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Group 4</td>
<td>13</td>
<td>4</td>
<td>30.8</td>
<td>4</td>
</tr>
<tr>
<td>Group 5</td>
<td>10</td>
<td>10(7)</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* Preparations for each group are described in text.

The figures in parentheses indicate the perforated ulcer.

lesser curvature adjacent to the border between the fundic and pyloric gland areas. Ulcers were found slightly more in number on the posterior than on the anterior wall. In the Group 5 to which histamine injection as well as ligation of main gastric vessels was given, all the cases developed gastric ulcer at the antrum. On the other hand, duodenal ulcers were developed within 3 to 5 cm from the pyloric ring (Fig. 3).

Incidence of ulcers

The incidence of production of gastric and duodenal ulcer was determined on the basis of ulcers judged by macroscopic findings (Table 1). As to gastric ulcer, there were 2 (15.4%) out of 13 cases in Group 1, no case in Group 2, 7 (50%) out of 14 cases in Group 3, 4 (30.8%) out of 13 cases in Group 4, and 10 (100%) out of 10 cases in Group 5, of which 7 (70%) were perforated ulcers. Incidence of duodenal ulcers was as follows: There were 3 (23.1%) out of 13 cases in Group 1, no case in Group 2, 1 (7.1%) out of 14 cases in Group 3, 4 (30.8%) out of 13 cases in Group 4, and no case in Group 5. In Group 5, however, duodenal mucosa frequently showed pronounced hyperemia, edema and erosion. Besides, the findings of duodenitis were observed evidently.

Microscopic findings of the gastric wall

Microscopic findings of ulcers. There was no characteristic finding referable to each group. The ulcers were mostly acute and often had perforated, accompanied with slight granulomatous inflammation at the base of the ulcer. Often the muscular layer showed degeneration or necrobiosis. However, cell infiltration in the submucosa was extremely slight. And, there was almost no regeneration of mucosa or proliferation of the connective tissue at the base and margin of the ulcer.

Microscopic findings of the gastric wall other than the part of ulcers. There was no significant difference referable to each group. In Group 1, some cases showed necrobiosis and dissociation of the glandular cells. Decrease in stainability and swelling of the parietal cells were frequently observed. On the contrary, the
Fig. 4. Effect of ligation of gastric vessels upon gastric acid secretion; acid secretory response to the intramuscular injection of histamine-beeswax mixture in dogs with gastric fistula at 4 weeks after ligation of the gastric arteries and veins.

(a) Free acidity (mEq/l) and volume (ml).
(b) Acid output (mEq/15 min). It would be noted that the volume of gastric juice showed a marked decrease.
muscular layer showed little change. In Group 3, pronounced necrosis and necrobiosis of the epithelium were observed as well as acute ulcer and erosion. In Group 5, as compared with other groups, there were higher incidences of perforated ulcers, in addition to necrosis and excoriation of the epithelium.

**Effect of ligation of gastric vessels upon gastric acid secretion**

Acid secretory response to the intramuscular injection of histamine-beeswax mixture was observed in dogs without ligation of gastric vessels (Group 1) and in those with ligation of the gastric arteries and veins (Group 4), at 1 to 4 weeks intervals (Fig. 4a and b). In Group 4, gastric acid secretion showed a gradual rise in free acidity and an increase in volume of gastric juice after histamine injection, and both reached the maximum at 90 min. After injection, a temporal reflux of bile was seen, often mingled with blood clots. Expressed in terms of acid output, the peak acid output (PAO: mEq/15 min) was 9.1 mEq/15 min in the case of control dogs and 3.7 mEq/15 min in Group 4, on the average. Acid secretory response reduced gradually 3 hr after injection, but kept a definitely high value for 24 hr. Thus, acid secretory response to histamine injection following ligation of gastric vessels showed, as compared with the control dogs, a fall in free acidity, and a considerably marked decrease in volume of gastric acid secretion. Although the influence of the surgical insult could not be ruled out completely 1 or 2 weeks after preparation, the acid secretory response showed almost the same tendency as 4 weeks after operation.

**DISCUSSION**

The experimental production of gastric and duodenal ulcer by the use of histamine was initiated by Büchner and Malloy (1927). Hence, Code and Varco (1940) succeeded in producing gastric and duodenal ulcers in animals closely similar to those occurring in human by the use of the histamine-beeswax mixture which consisted of finely crystallized histamine suspended in beeswax and mineral oil. It is necessary for the production of ulcer that the doses of histamine should be sufficient to give a maximal stimulus to the parietal cells. In the present experiment, according to the method of Code and Varco (1940) and Hay et al. (1942), the authors injected intramuscularly 50 mg of histamine dihydrochloride, equivalent in amount of 30 mg of histamine base, in terms of the histamine-beeswax mixture. This histamine-beeswax preparation provides for a slow release of the drug and a sustained stimulation of gastric acid secretion throughout the 24 hr period. When daily injections of the mixture were given to animals, some signs of alterations of the gastrointestinal tract were often developed. On the 3rd or 4th day of the consecutive injection, anorexia and loss of weight were not uncommon, and the occasional passage of a tarry stool was observed in dogs which developed ulcer.

Concerning the localization of ulcers, it has been reported by Yo (1955), Motojima (1957), and Fushiya (1959) that the development of gastric ulcers was limited to the region of the antrum adjacent to the border between the fundic
and pyloric gland areas, as frequently observed clinically in the chronic gastric ulcer in human (Oi et al. 1959, 1969). In the present experiment, as reported by these investigators, gastric ulcers were mostly found on the posterior wall alongside the lesser curvature. In such a case it is noteworthy that even when local circulatory disturbance of the stomach was employed by ligating gastric vessels, gastric ulcers were developed nearly at the same site as in the case where histamine injection alone was given. The incidence of gastric ulcers produced by histamine-beeswax injection was 43% as given by Yo (1955), and 47% by Fushiya (1959). However, in the present experiment, the incidence of gastric ulcers was somewhat lower, being 15.4%, in the group to which only histamine injection was given.

Extensive studies have been made on the correlation between local circulatory disturbance and ulcer production. Layne and Bergh (1943) reported that ligation of two or three of the large arteries supplying the stomach produced no significant alteration of gastric acid secretion, but ligation of four large arteries of the stomach elicited necrosis in the dog. Imanari (1957) attempted to produce gastric ulcers experimentally by means of circulatory disturbance. He reported that when the entire blood flow to the stomach was blocked, the stomach fell into a state of necrosis, but with ligation of arteries only no ulcer production resulted. On the other hand, Le Veen et al. (1970) reported that in an attempt to study the relative roles of histamine-induced acid secretion and steroid-induced reduced mucosal resistance, gastric acid secretion was still necessary for ulceration. In the present experiment, ligation of the four main gastric arteries except short gastric arteries resulted in marked ischemia of the antrum, while complete necrosis failed to occur. However, though ligation of the main gastric arteries did not produce gastric ulcers, ligation of the main gastric arteries and veins produced gastric ulcers in 30.8% of the dogs. This fact leads us to presume that ligation of gastric vessels causes local circulatory disturbance of the gastric wall, producing various pathologic changes from temporary anoxia to necrosis and other changes to the tissue, eliciting erosion, hemorrhage, cell infiltration, hyperemia, and resulting in so-called locus minoris resistantiae. Therefore, it would be conceivable that such a locus minoris resistantiae provides sufficient preparation for the production of gastric ulcer. With respect to the combined effects of histamine injection and ligation of gastric vessels, Baronofsky (1948) reported that ligation of three of the large gastric arteries of the stomach, and additional small ones coming from the gastro-epiploic arteries, failed to protect the histamine-provoked ulcer. It was demonstrated in the present experiment that when histamine injection was given in addition to ligation of gastric vessels, ulcers were produced at an extremely high incidence as compared with the cases where only histamine injection was given.

On the other hand, it seems highly probable that gastric acid secretory function is affected by ligation of gastric vessels. Le Veen (1948) reported that ligation of the major gastric vessels could effectively reduce the amount of hydrochloric acid secreted by the stomach, and this reduction in acid secretion was sufficient to protect dogs from development of ulcers induced by histamine injection.
Recently Herbst and Barnett (1966), however, reported that temporary total gastric arrest was accomplished by the clamps on the left gastroepiploic, left gastric, and short gastric arteries, after ligation of the right gastric and gastroepiploic vessels, but even such gastric circulatory arrest failed to reduce the incidence of ulceration occurring within an interposed segment of ileum between stomach and duodenum. In the present experiment, gastric acid secretion in response to the sustained administration of histamine was investigated when the main gastric arteries and veins were ligated at the same time. Peak acid output was markedly reduced less than one half as compared with the control dogs. Acid secretory response to histamine injection showed a fall in free acidity, and especially a considerably marked decrease in volume of gastric acid secretion. Thus, it was one of the striking features that the production of gastric ulcers was observed with a higher incidence, despite the fact that acid secretory response to histamine was reduced after ligation of gastric vessels. Therefore, these findings would suggest that decrease in mucosal resistance induced by ligation of gastric vessels of the stomach may play an important role as an etiologic factor rather than a factor of gastric acid secretion per se in the production of gastric ulcers.

References


