AGGRAVATING EFFECT OF SEROTONIN ON GASTRIC ULCERATION INDUCED BY THERMOCAUTERY UNDER THE HEALING PROCESS IN MICE

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Abstract—The influence of various ulcerogenic treatments on healing gastric ulcers induced by thermocautery was studied in mice. A low dose of serotonin (5HT) which did not produce ulceration, was found to aggravate gastric ulcers at 15th or 30th day after thermocauterization, but other ulcerogenic treatments including histamine, norepinephrine, vasopressin, acetic acid ingestion and cold-restraint stress did not affect this induced gastric ulcer. Bleeding and ulceration, however, occurred in the gastric glandular portion in addition to thermocauterization ulcer by the treatment of acetic acid ingestion or cold-restraint stress. Histological sections of gastric ulcers (15th and 30th day) 24 hr after 5HT injection, showed severe necrosis of the regenerated mucosal layer. Microvessel structure in the gastric mucosa as revealed by the Indian-ink infusion, showed a local obstruction of blood flow on the edge of ulcers 1 or 3 hr after 5HT injection. Although acetic acid ingestion increased transmucosal fluxes of Na⁺ and K⁺, 5HT had no effect on the ion flux in normal mice. Thus the healed ulcer area was resistant to various ulcerogenic stimulants, except for 5HT, and the vasoactive factor of 5HT may be involved in the aggravating process of gastric ulcers induced by thermocautery.

The recurrence of peptic ulcer is a most important problem from the point of pharmacotherapeutics and pathology. The experimental approaches, however, on this subject leave much to be desired. Some workers have induced re-ulceration in the experimental animals which had previously been given ulcerogenic treatment, and found that gastric ulcers during the healing process were resistant to the various ulcerogenic factors (1–3). It has been reported that gastric mucosal blood flow may play a major role in the ulceration and healing process of gastric ulcers. Milne and Cohn (4) reported that gastric lesions enhanced by serotonin (5HT) seemed likely to occur as a result of ischemic necrosis by vasoconstriction and they suggested the involvement of this amine in the pathogenesis of ulceration.

We previously reported a simple method for induction of gastric or duodenal ulceration by thermocautery in mice (5). In the present study, we examined the influence of 5HT and other various ulcerogenic treatments on this form of gastric ulcer, during the healing process in an attempt to clarify the mechanism of aggravation or recurrence of gastric ulcers. We also examined the possibility that the vasoactive factor of 5HT might be involved in this process.

MATERIALS AND METHODS

Gastric ulcer induced by thermocautery: Male ddY mice, weighing 25–30 g, were
anesthetized with pentobarbital Na. Laparotomy was performed through a midline incision and the stomach was withdrawn from the abdomen. The head of the thermocautery, which was 5 mm in diameter and heated to 72°C, was then applied to the junctional portion of the gastric antrum and body in the anterior wall for 5 sec with slight pressure as described previously (5). The abdomen was closed and the animals fed the usual laboratory chow diet. After 24 hr deprivation of solid food only, the animals were sacrificed at various intervals (1st to 30th day) 30 min after the injection of 1% Pontamine Sky Blue (10 ml/kg i.v.). The stomach was inflated with 2 ml of saline solution and immersed into 5% formalin solution for 10 min to fix lightly the outer surface. The stomach was then incised along the greater curvature. The ulcerated area (long diameter X short diameter, mm²) was measured under the dissection microscope.

**Histological procedure:** Tissue taken from area with gastric lesion was stained with Hematoxylin-Eosin (H.E.). Microvessel structure of the gastric mucosal layer was examined after injection of Indian-ink into the aorta dorsalis.

**Effects of various ulcerogenic factors:** Test drugs dissolved in saline solution were administered s.c. except for vasopressin (i.p.) and acetic acid (p.o.) to the animals with gastric ulcer. Serotonin, histamine and acetic acid were administered of the 14th or 29th day after ulceration and 24 hr later the animals were sacrificed. In some experiments, 5HT and histamine were given once a day (10:00 a.m.) for 3 days before the 15th or 30th day after ulceration. Animals were sacrificed 24 hr after the last administration. To study the microvessel structure of the gastric mucosa, animals were sacrificed 1 or 3 hr after the drug administration. Norepinephrine and vasopressin were administered twice a day (10:00 a.m. and 6:00 p.m.) for 3 days (27th, 28th and 29th day after ulceration). Animals were sacrificed 18 hr after the last administration. To load stress, the mouse was put into the cylindric holder and immersed into water at 17°C for 5 hr after 24 hr fasting, according to the method described by Takagi et al. (6), with modification for application to mice.

**Ion flux into the gastric lumen in the mouse:** Male ddY mice (25-30 g) were fasted for 24 hr and then anesthetized with 2 g/kg i.p. of urethane. A tracheal cannula was inserted, laparotomy was performed through a midline incision, and the stomach was withdrawn from the abdomen. A dual polyethylene cannula was introduced into the gastric lumen through a forestomach incision after ligation of the pylorus and esophagus. The stomach was washed with 5% glucose in distilled water (6-9) prior to intragastric instillation of 5% glucose solution (0.5 ml). The intragastric glucose solution was washed out with the glucose solution (6 ml), and 0.5 ml of glucose solution was again instilled into the stomach. This procedure was performed 3 times at 30 min intervals to stabilize the initial ion flux before 5HT (10 mg/kg s.c.) or 5% acetic acid in 5% glucose solution (0.5 ml/mouse p.o.) was administered. Three hours later, intragastric solution was washed out with isotonic glucose solution. The contents of sodium and potassium ions in the perfusate were determined using a flame photometer (COLEMAN-MODEL 51).

**Drugs:** Drugs used were serotonin creatinine-sulfate (Merck), histamine dihydrochloride (Nakarai), 1-noradrenaline bitartrate (Wako) and vasopressin (Pitressin®-Park Davis).
RESULTS

Comparison of aggravating effects of ulcerogenic treatments on the ulcer during the healing process: By thermocautery of the serosal surface of the mouse stomach, a clearly defined gastric ulcer approximately 25 mm² in size was produced. The ulcer healed rapidly and was nearly completely repaired within 30 days (Fig. 1). Microscopical observation of H.E. stained specimens of the 1st day ulcer indicated severe necrosis of the mucosal layer, however, the muscularis mucosa was intact. On the 4th day after ulceration, muscularis mucosa was completely lost and on the 7th day fibroblasts in the submucosa began to proliferate. On the 15th day, fibroblasts were markedly proliferated and the

![Fig. 1. Healing process of gastric ulcers by thermocautery in mice. Gastric ulcers were produced by thermocautery of the junctional portion of the gastric antrum and body in anesthetized mice. Pontamine Sky Blue (1%) was given in a dose of 10 ml/kg into the tail vein, 30 min before removal of the stomach. Ulcer score was calculated as the product of long and short diameter of ulcer area (mm²). Each points represent the mean and standard error of mean of 10-15 mice.](image)

![Fig. 2. Anatomical subdivision of the mouse stomach used for analyzing the regional distribution of ulcers. Deepness of color indicates the occurrence percent of gastric lesions; ■■■, more than 75%, ■■, more than 50% and less than 75%, ■□□, more than 25% and less than 50%, □, less than 25%. Each group included 15 mice.](image)
mucosal layer was partially regenerated. The ulcerated area was covered with regenerated mucosa and was almost completely repaired 30 days after the initial ulceration.

Figure 2 shows the schema of the mouse stomach used to analyze the regional distribution of the lesion induced by various ulcerogenic stimulants. Five percent acetic acid (10 ml/kg p.o.) or cold-restraint stress for 5 hr at 17°C did not affect the pre-existing gastric ulcer (30th day after thermocauterization) while the ulcer index in 5HT (5 mg/kg s.c.)-treated mice (12.2±2.6 mm²) was significantly larger than that in control group (3.6±0.8 mm²), p<0.05. In the case of the former, however, bleeding and stress ulcer were recognized in the gastric glandular portion other than thermocauterization area. Incidence percent in the regional distribution of gastric lesion as shown in Fig. 2 indicates that 5HT aggravated the gastric ulceration in the healing process while gastric ulcer portion was resistant to other ulcerogenic stimulants, acetic acid and stress. Figure 3 shows a typical thermocauterization ulcer treated with 5HT (5 mg/kg s.c.) 30 days after operation.

Effects of vasoactive agents: Figure 4 summarizes the influence of various vasoactive agents on gastric ulcers 30 days after ulceration. The administration of histamine (10 mg/kg s.c.) once daily ×3, did not aggravate the healing process of gastric ulcers. Neither norepinephrine (0.5 and 2.5 mg/kg s.c.) nor vasopressin (2 and 10 U/kg i.p.) twice daily ×3, had any effect on the healing gastric ulcer. High doses of histamine (80 mg/kg s.c.) slightly aggravated the severity of gastric ulcers on the 30th day.

Serotonin was administered s.c. to the mouse on the 14th day after ulceration, and 24 hr later the ulcer score was significantly larger than that of control group. This aggravating effect of serotonin on gastric ulcer during the healing process was dose dependent from 2.5 to 20 mg/kg s.c. and reached a plateau at 10 mg/kg s.c. as shown in Fig. 5. However, the so-called 5HT induced ulcer was evident in the gastric glandular portion after the administration of 20 mg/kg s.c. in 4 of 10 mice. With a dose of 5 mg/kg of serotonin, only the
thermocauterization induced ulcer was evident (Fig. 2).

Serotonin was found to have the same aggravating effect, 15 and 30 days after thermocauterization, on the healing process of gastric ulcers, and the ulcer index was not significantly different between repeated (3 consecutive days) and a single administration of 5HT, as shown

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**FIG. 4.** Influence of various vasoactive agents on gastric ulcers 30 days after thermocauterization in mice. All drugs dissolved in saline were given to animals. Serotonin (5HT) was administered 24 hr before removal of the stomach. Histamine was administered once daily × 3. Norepinephrine (NE) and vasopressin were given twice daily × 3. The stomach was removed 24 hr after the last administration. Each group included 10–15 mice. *The mean ulcer index is statistically different from control, p<0.05, **, p<0.01.

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**FIG. 5.** Dose-response relationship of aggravating effect of 5HT on gastric ulcers 15 days after thermocauterization. Serotonin (5HT) dissolved in saline was administered 24 hr before removal of the stomach. Each group included 10–15 mice.

*A significant difference from control, p<0.05, **, p<0.01.

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**TABLE 1.** Aggravating effect of 5HT on gastric ulcers induced by thermocautery in mice

| Treatments       | 30th Day Ulcer Index (mm²) | 15th Day Ulcer Index (mm²) |
|------------------|-----------------------------|-----------------------------|
| Control          | 3.6±0.9 n=14                | 12.0±1.9 n=19               |
| 5HT 5 mg/kg s.c. | 12.2±2.6* n=10              | 22.3±2.2* n=15              |
| 5 mg/kg s.c. × 3 | 16.9±3.3* n=10              | 22.3±2.2* n=15              |

Serotonin (5HT) was given once daily for one or three days and the stomach was removed 24 hr after last administration. The values are mean±S.E. *The mean value is statistically different from corresponding control, p<0.05.
Microscopical observation: Histological sections of the 15th day gastric ulcer, 24 hr after 5HT administration, show a severe necrosis of the regenerated mucosal layer (Fig. 6). When the microvessel structure of gastric mucosa was examined by the Indian-ink injection method, the local obstruction of blood flow was observed 1 or 3 hr after 5HT injection, on the edge of ulcer 14 (Fig. 7) and ulcer 29 (Fig. 8) days after ulceration.

Na⁺ and K⁺ ion fluxes: Effects of 5HT and orally administered 5% acetic acid on Na⁺ and K⁺ fluxes into the gastric lumen of normal mice are shown in Fig. 9. Fluxes of Na⁺ and K⁺ across the gastric mucosa were fairly constant for 5 hr except for the first 30 min on urethane anesthetized mouse. Na⁺ and K⁺ fluxes into the gastric lumen during 3 hr from

![Fig. 6](image_url)

**Fig. 6.** Photomicrographs of sections from gastric ulcers 15 days after thermocauterization. H.E.-stained sections from typical gastric ulcer of non-treated (A) and 10 mg/kg s.c. of 5HT-treated (B) mouse 15 days after thermocauterization (×40). Severe necrosis in the regenerated mucosa is evident in tissue from the 5HT-treated animal.

![Fig. 7](image_url)

**Fig. 7.** Photomicrographs of sections from gastric ulcers 14 days after thermocauterization. Microvessel structure in the mucosa infused with Indian-ink (×40), A: normal mucosa, B: surrounding area of non-treated thermocauterization ulcer, C: 1 hr after 5HT (20 mg/kg s.c.) in thermocauterization ulcer, D: 3 hr after 5HT (20 mg/kg s.c.) in thermocauterization ulcer.
immediately after the administration of 10 mg/kg s.c. of 5HT, were not different from control, but intragastric instillation of 5% acetic acid (0.5 ml) increased remarkably Na⁺ and K⁺ fluxes at 2 and 4 times, respectively (Fig. 9).

FIG. 8. Photomicrographs of sections from gastric ulcers 29 days after thermocauterization. Microvessel structure in the mucosa infused with Indian-ink (×40). A: normal mucosa, B: surrounding area of non-treated thermocauterization ulcer, C: 1 hr after 5HT (5 mg/kg s.c.) in thermocauterization ulcer, D: 3 hr after 5HT (5 mg/kg s.c.) in thermocauterization ulcer.

FIG. 9. Effects of 5HT and acetic acid on Na⁺ and K⁺ fluxes in the normal stomach of mice. The esophagus and phylorus were ligated under urethane anesthesia after a 24 hr fast. Acetic acid was given through a dual cannula introduced into the forestomach. Each column represents the mean of 8 mice. The mean values are statistically different from the corresponding control, *, p<0.01.

DISCUSSION

The most striking observation in this study was the appearance, in mice given a low dose of serotonin (5HT), of aggravation in healing process of thermocautery-induced gastric ulcers. Other vasoactive agents, histamine, norepinephrine and vasopressin, except for a high dose of histamine (80 mg/kg s.c.), and ulcerogenic treatments (acetic acid ingestion and cold-restraint stress) did not influence gastric ulcers in mice. In the sham operated
mice, a low dose of 5HT did not induce ulcers. These findings indicate that the aggravation of gastric ulcers during the healing process in mice was apparently different from so-called 5HT ulcer in rats (7–10) and that 5HT produced new injury in regenerated mucosal layer of the mouse stomach.

Ulcers induced in rats by clamping were apparently aggravated by cortisone (11). Some investigators (12) using cautery ulcer in rats, confirmed the high incidence of re-ulceration of a completely healed ulcer area, with cortisone treatment. On the other hand, it was reported that cortisone aggravated the healing process of chronic gastric ulcers (2, 11, 13–15), but exerted no aggravating effects on completely healed gastric ulcers (2). Hale and Grossman (1) also reported that animals with a completely healed ulcer showed a strong resistance to ulceration by histamine.

Milne and Cohn (4) reported that serotonin played an active role in blood coagulation in addition to its role as a vasoconstrictor, and serotonin-related lesions seemed to be the result of ischemic necrosis induced by platelet aggregation and thrombus formation. In the present study, microvessel damage in the mucosal layer of the edge of gastric ulcers was observed 1 to 3 hr after 5HT injection in the specimens perfused with Indian-ink (Figs. 7 and 8), while the local obstruction of blood flow was not observed after norepinephrine or vasopressin injection, under this same experimental condition. When gastric blood flow was measured by the aminopyrine clearance method, it was found to be decreased by the injection of 5HT in a dose which was capable of producing gastric erosions (7). This seems to reflect the constriction of the collecting venula of the gastric mucosa observed by some workers (16, 17) in rats given 5HT.

It has been reported that gastric movement was abnormally enhanced by the administration of 5HT and noradrenaline, and then hemorrhage occurred in the area where the abnormally enhanced movement was observed (18, 19). Thus, gastric movement appeared to be one of the factors indispensable for the production of a gastric lesion. Further studies are required to clarify whether 5HT increases the gastric motility in this experimental condition of mice.

Peptic ulcers are probably the result of an imbalance between the aggressive and the defensive mechanisms in the gastrointestinal tract, but 5HT does decrease gastric acid and pepsin output (7, 20, 21). Therefore, it is unlikely that acid secretion plays a role as a trigger for aggravation of gastric lesions by 5HT.

Serotonin-induced gastric ulcerations in the rat resulted in the release of lysosomal enzymes, and pretreatment with PGE1 reduced the number of ulcers and stabilized the lysosomal membranes (22). Serotonin might inhibit active ion transport thereby leading to an intracellular accumulation of sodium, anions and water. The resultant osmotic swelling of epithelial cells could produce severe damage, and alter permeability and disruption of lysosomes. The normal gastric mucosa has a barrier which resists the tendency of H+ to diffuse from the lumen into the mucosa and of Na+ to diffuse in the other direction (23, 24). Although acetic acid producing lesions on the mucosal layer increased transmucosal fluxes of Na+ and K+ in normal mice, 5HT had no effects.
Acute and chronic peptic ulcers can be produced in laboratory animals by the administration of histamine, under a variety of experimental conditions (25). The vascular factor has been accepted as a primary cause of gastric lesion by histamine (26–29). Initial ischemia, hemorrhage, stasis, infarction, and finally necrosis are the stages of acute ulceration of gastric mucosa after histamine, according to Watt (30). We found that even a high dose of histamine (80 mg/kg s.c.) had only a slight effect on thermocautery-induced ulcers and a histamine-induced ulcer was not produced at the other portion of pre-existing gastric ulcers.

Among the vasoactive drugs having ulcerogenic effects on gastric mucosa, histamine is one of the representative vasodilators. Some vasoconstrictors can also induce gastric ulcers in animals (31, 32). Nedzel (31) produced gastric lesions in animals by the administration of pitressin and pointed out "spasm theory" as the most logical one to explain the ulcers produced by this method. It has been reported that vasopressin (about 0.5 U/kg i.v.) and noradrenaline (0.75 mg/kg s.c.) enhanced the Shay ulcer, especially in the gastric antrum, by the spasm of blood vessels (33). In rats subjected to cold and restraint stress, Brodie and Hooke (34) have shown that vasodilators reduced, while vasopressin increased, the incidence of gastric hemorrhage. However, in the present study using mice, neither a very high dose of vasopressin nor noradrenaline affected gastric ulcers 30 days after thermocauterization. This discrepancy may be, in part, attributed to the differences of experimental methods, species, and the healing process of gastric ulcers.

Vagus stimulation mediated by the central nervous system (35), and vasoactive agents 5HT and histamine released from mast cell by stress (32), may produce the peptic ulcer, by the disturbance of circulation in the gastrointestinal tract. Although stress which may play an important role of re-ulceration in humans and acetic acid which produces lesions in the gastric mucosa, were applied during the healing process of gastric ulcers (30th day), the ulcers were not aggravated. These findings coincide with the report that healed mucosa had some resistance to stimulation (1).

When the regional distribution of gastric lesion by the various agents above mentioned was analyzed in mice, with or without gastric ulcers by thermocauterization, the ulcer area was, to some degree, resistant to all the ulcerogenic stimulants, except for 5HT. It has been stated in clinical reports (36, 37) that recurrence of ulcers was seen in the area nearest the original ulcer in the early stages of healing and occurred in the portion distant from the original one in the late stages of healing. It is also reported that re-ulceration occurred in the surrounding area of the original ulcer with concentricity (3). Experimental research on this subject indicated that in the cinchophen ulcer, re-ulceration occurred in the area distant from the former ulcer and the new ulcer was deeper and more severe as compared to the former one (38).

Our study suggests that since numerous microvessels were newly generated in the healing process of ulcers (39), the vasoactive effect of 5HT may be involved in the aggravating process of gastric ulcers induced by thermocauterization in mice.

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