Effects of Prolonged Treatment with Compound 48/80 on the Gastric Mucosa and Mast Cells in the Rat

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Abstract—Effects of prolonged administration of compound 48/80 (48/80) on the gastric mucosa, serotonin and histamine levels in serum, and mast cells of rats were studied. Daily administration of 48/80 (0.75 mg/kg, i.p.) for 2 or 4 days produced widespread gastric lesions. Further administration of the agent for up to 12 days did not aggravate the lesions which had developed in the early period of administration of the drug. There were only a few visible lesions and numerous healed ones. Almost the same phenomenon was observed with the daily administration of serotonin plus histamine (10 mg/kg each, i.p.) for 2 to 12 days. While 48/80 given for 2 or 4 days increased serotonin and histamine levels in serum, it induced no appreciable increase of these amines after 8 or 12 days of treatment. Serotonin and histamine levels in peritoneal mast cells significantly decreased after the treatment with 48/80, over a 4 day period. The decrease in gastric lesions after prolonged treatment with 48/80 is due to both the depletion of serotonin and histamine from mast cells and an increased resistance of the gastric mucosa with healed lesions.

We reported that a daily intraperitoneal administration of compound 48/80 (48/80) for 4 days to rats consistently produced widespread gastric lesions (1, 2). The mechanism by which 48/80 induces these lesions appears to involve primarily the release of serotonin and partially the release of histamine from extragastric sources. While the gastric lesions were observed throughout the oxyntic glandular area, the damage remained within the mucosal layer. In the present work, we attempted to determine whether or not further administration of 48/80, or serotonin plus histamine, would produce even more severe lesions which would penetrate the muscularis mucosa. We herein discussed the relationship between the lesion formation and levels of these amines in both serum and intraperitoneal mast cells.

Materials and Methods
Male Donryu rats (200–230 g) were fed a normal rat chow and given tap water ad libitum during the entire period. Experiments were carried out using 8 rats in each group.

Lesion induction: 48/80 (Sigma) was given intraperitoneally at 0.75 mg/kg once daily for either 2, 4, 8 or 12 days, in a volume of 0.5 ml/100 g body weight. Serotonin (serotonin creatinine sulfate, Wako) plus histamine 2HCl (Nakarai) were given intraperitoneally in a dose of 10 mg/kg each for 2, 4, 8 or 12 days. On the next day after the final administration of these agents, the rats were killed, the stomachs removed, inflated with 10 ml of 2% formalin, and put into 2% formalin for 10 min. The stomachs were then opened along the greater curvature and examined for lesions in the oxyntic glandular stomach under a dissecting microscope (×10). The area of damaged mucosa (mm²) was measured, and the sum of each damage per stomach was used as the lesion index. The person (SO) measuring the lesions had no knowledge of which treatment an animal had received. Tissue samples were fixed in 10% formalin for microscopical study.
Serotonin and histamine levels in serum and mast cells: The effects of 48/80 on serotonin and histamine levels both in serum and mast cells were studied as follows: 48/80 was given intraperitoneally at 0.75 mg/kg once daily for 2, 4, 8, and 12 days. Serotonin and histamine levels in serum were determined 0.5 hr after the final administration of 48/80. Under ether anesthesia, blood was collected from the abdominal aorta and centrifuged at 4°C for 20 min at 1,600 g. Serum samples were deproteinized by adding perchloric acid at a final concentration of 3% and then centrifuged at 4°C for 10 min at 10,000 g. Serotonin and histamine levels in the mast cells were determined 24 hr after the final administration of 48/80. Mast cells were obtained by injecting extracting fluid (15 ml/rat) into the peritoneal cavity after decapitation of the ether anesthetized rats. The extracting fluid was prepared according to the method of Sullivan et al. (3) and was of the following composition: 150 mM NaCl, 3.7 mM KCl, 1.0 mM CaCl₂, 3 mM Na₂HPO₄, 3.5 mM KH₂PO₄, 5.6 mM glucose, 10 units/ml heparin, 0.1% bovine serum albumin (BSA), and 0.1% gelatin (pH 6.8). After 90 sec gentle massage of the abdomen, the fluid was collected through an incision in the abdominal wall, into icecold polycarbonate tubes. Fluid so obtained was centrifuged at 500 g for 5 min at room temperature. The supernatant was discarded and the precipitate with mast cells was resuspended in 1 ml of a fresh extracting fluid. Three mM perchloric acid (0.1 ml) was added to this suspension which was then centrifuged at 10,000 g for 10 min. Serotonin and histamine levels in the supernatant as well as levels in serum were determined using a fluorometer HPLC-autoanalyzer system (Hitachi) (4), and they were expressed as nmole/animal and nmole/ml serum. Determination of histamine levels was done using the o-phthalaldehyde method of Shore et al. (5). All experiments were performed in duplicate.

Analysis of data: Statistical evaluation of the results was performed using Student’s t-test. P values <0.05 being considered as statistically significant.

Results

Effects of 48/80 and serotonin plus histamine on the gastric mucosa: The daily administration of 48/80 for 2 or 4 days produced widespread lesions throughout the oxyntic glandular area at the incidence of 90% and 100%, respectively (Fig. 1). The lesion index in the 4 day’s treatment group was higher than that in the 2 day’s treatment group, although it was not statistically significant. Further administration of 48/80 for up to 8 or 12 days also produced gastric lesions, but the lesion indices were significantly smaller than those observed after 4 day’s treatment. The incidence of the lesions was 80% in the group treated for 8 days and 50% in the group treated for 12 days. Most of the stomachs showed evidence of healing of the previously developed lesions, by both gross and microscopical observations (Fig. 2).

The combined administration of serotonin plus histamine for 2 or 4 days also produced widespread lesions which were similar to the 48/80-induced lesions in both location and appearance. As seen in cases of 48/80, the gastric lesions produced by 8 or 12 day’s treatment with these amines were smaller than the lesions observed after 4 day’s treatment (Fig. 1). Healed lesions were also observed in most of the animals.

Effects of 48/80 on serum serotonin and histamine levels: The administration of 48/80
for 2 and 4 days (once daily) increased the serum serotonin and histamine levels 30 min after the final administration (Fig. 3). However, there was no appreciable increase of these amines when 48/80 was given for 8 or 12 days.

**Effects of 48/80 on serotonin and histamine levels in mast cells:** The administration of 48/80 for 2 days (once daily) had little or no influence on the serotonin and histamine levels in mast cells (Fig. 4). However, the repeated administration of the agent for 4, 8 or 12 days resulted in a significant decrease of both serotonin and histamine levels in mast cells, as compared to the level in the control group. The decrease in serotonin levels after 4, 8 and 12 day’s treatment was 66.3%, 65.2% and 68.5%, respectively. The decrease in histamine levels after 4, 8 and 12 day’s treatment was 80.2%, 89.1% and 91.1%, respectively.

**Discussion**

The present study confirmed our previous findings that a daily administration of 48/80 for 2 to 4 days induced gastric lesions in rats. However, we found that further administration of these agents for up to 12 days failed to aggravate the lesions, rather the lesion formation was reduced. As shown in this study, the increase of administration times of 48/80 reduced both serotonin plus histamine contents in mast cells, resulting in a reduction of these amines in the serum. The extent of reduction of histamine in the mast cells was more evident than that of serotonin. Our earlier study showed that antiserootonin agents, such as methysergide and cyproheptadine, all but completely inhibited the development of 48/80-induced gastric lesions (1, 2). We also found that histamine \(H_1\)- and \(H_2\)-receptor blocking agents, such as tripelennamine or cimetidine, showed only a tendency to inhibit the 48/80-induced lesions (1, 2). Moreover, serotonin alone at 10 mg/kg produced apparent gastric lesions, while histamine alone at the same dose did not induce any lesions (2). Accordingly, reduction in serotonin levels in the serum...
appears to be a more critical factor in the reduced development of 48/80-induced lesions than that of histamine levels. The reduced lesion formation in response to 48/80 given for more than 4 days may be due to the depletion of these amines, particularly serotonin in the mast cells. Similar to the 48/80-induced lesions, a daily administration of serotonin plus histamine for 2 to 4 days induced gastric lesions, whereas the prolongation of administration times for up to 12 days resulted in a reduced lesion formation. Most of the stomachs of rats on 48/80 or serotonin plus histamine for 8 or 12 days had healed lesions, i.e., regenerated epithelial cells covered the surface of the damaged area. Thus, it is apparent that the healing process exceeded the development of new lesions by these agents. It is likely that the regenerated epithelial cells or newly formed vascular system in the regenerated mucosal layer has a specific property which resists the irritative effect of 48/80 or serotonin plus histamine. These results suggest that the reduced development of 48/80-induced lesions after repeated treatment is not totally due to a depletion of these amines in mast cells.

One of the present authors (SO) reported that weekly repetition of water-immersion stress to rats reduced the development of stress-induced gastric lesions (6). Other investigators (7, 8) also reported that repeated administration of aspirin resulted in an increased resistance of gastric mucosa against the agent. Determination of mucosal blood flow or generation of endogenous prostaglandins in the gastric mucosa after prolonged treatment with 48/80 or serotonin plus histamine may clarify the mechanism of increased resistance. We conclude that prolonged administration of 48/80 as well as serotonin plus histamine did not induce penetrating lesions in the gastric mucosa of rats. The decrease in gastric lesions may be due to both the depletion of serotonin and histamine from mast cells, and an increased resistance of the gastric mucosa with reduced lesions.

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