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# Effects of Cimetidine on Stress Ulcer and Gastric Acid Secretion in the Rat[1,2]

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PARÉ, W. P., G. B. GLAVIN AND G. P. VINCENT. *Effects of cimetidine on stress ulcer and gastric acid secretion in the rat.* PHARMAC. BIOCHEM. BEHAV. 8(6) 711–715, 1978. – Cimetidine at 25, 50, and 100 mg/kg significantly inhibited gastric acid secretion in rats with chronic gastric cannulas. Rats receiving either 50 or 100 mg/kg of cimetidine secreted significantly less gastric acid 3 hr after injection. Cimetidine failed to reduce the number or size of gastric lesions in rats exposed to the activity-stress procedure, but cimetidine at 100 mg/kg significantly reduced the number and size of gastric lesions in rats subjected to a supine restraint procedure.

Cimetidine Stomach ulcers Stress Stress ulcers Gastric acid secretion

FOR MANY years, antacids and anticholinergic drugs have been the mainstays of ulcer therapy. However, the recent development of histamine $H_2$-receptor antagonists indicates that these agents may now become the drugs of choice. Use of the first $H_2$ antagonists, burimamide and metiamide, was complicated by difficulty in administration and toxic side effects. These problems have been circumvented by the development of a third antagonist – cimetidine. In clinical trials, cimetidine has proved to be a potent gastric acid inhibitor [6]. It inhibits basal secretion [12,25], post-prandial secretion [12, 14, 23], histamine and penta-gastrin-stimulated secretion [7], and caffeine-induced secretion [8]. Cimetidine in doses of 0.8 or 1.6 g/day for six weeks healed ulcers in gastric ulcer patients [22].

There is little information regarding the effect of cimetidine on stress ulcer. On the other hand, many reports have indicated that metiamide reduces gastric acid secretion in animals [1, 5, 10], and also reduces the incidence of stress ulcer in the rat [1, 3, 4, 5, 10, 12, 13]. Considering cimetidine's impressive clinical trials record, one would suspect that cimetidine would also reduce the incidence of stress ulcer in the rat. For this reason, the first study in this report evaluated the effects of cimetidine on gastric acid secretion in the chronic gastric fistula rat, and the second and third studies observed the extent to which cimetidine reduced ulcer incidence in rats subjected to different ulcerogenic procedures.

## EXPERIMENT 1

This study was conducted to demonstrate in the rat the gastric acid inhibitory effect of cimetidine. The results from this study would have obvious implications in subsequent studies where the anti-ulcer properties of cimetidine were investigated. The effects of cimetidine on gastric acid secretion were studied in the chronic gastric fistula rat.

### METHOD

*Animals and Apparatus*

Ten male Sprague-Dawley rats weighing 320–426 g at the beginning of the study were used. The cage for collecting gastric samples consisted of rectangular plastic boxes 20 cm long, 15 cm deep, and 13 cm high. The floor was composed of stainless steel rods spaced 1.25 cm apart and running the length of the box. The top of the cage was also plastic and served as the entry lid to the cage.

*Procedure*

Chronic gastric cannulas were surgically implanted in all 10 rats. The cannula and surgical procedure are described in detail in earlier reports [18,21].

After a 14-day postoperative period, gastric collections were started. Following a 19-hr deprivation period, the plugging screw of the cannula was removed. The stomach was flushed and drained through the cannula with 5–10 ml of warm saline until the effluent was clear. A Silastic tube was then screwed into the cannula and the rat was placed in one of the collection cages with the Silastic tube positioned between the two middle floor rods. Gastric juice was not collected for the first 30 min in order to allow any residual saline to drain from the stomach. Following this 30-min period, a plastic collection vial was attached to the distal

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end of the Silastic tubing and gastric samples were collected in the vial.

During the first phase of the study, three consecutive 1-hr baseline collections were obtained from all rats. Baseline samples were collected on three days from all rats with at least three days intervening between any two collection days.

The second phase of the study was the drug treatment phase. Five treatment conditions were observed. These were: cimetidine at 25, 50 and 100 mg/kg, saline placebo, and a no-injection control treatment. A rat was subjected to the five treatment conditions in random order, but a particular treatment condition was administered on two consecutive collection days in order to evaluate habituation effects. In this fashion, each rat was subjected to the five treatment conditions twice. During the drug treatment phase 1-hr gastric samples were collected for four consecutive hours. Injections, when appropriate, were administered IP after the first hour gastric sample had been collected.

In the third phase of the study post-drug baseline gastric collections were obtained in the same fashion as in Phase 1.

The gastric juice for each hourly sample was measured for volume and total acidity. The concentration of the hydrogen ions was determined electrometrically with a pH meter by titrating 1 ml samples with 0.01 N NaOH to a pH of 7.0. Total acidity was obtained by multiplying the volume of the sample of the hydrogen ion concentration.

The cimetidine (Smith, Kline and French Laboratories, Philadelphia, PA) was prepared in quantities of 30 cc for each dose level of 25, 50, and 100 mg/kg. Doses were prepared for rats averaging 250 g. The 25 mg/kg solution consisted of 375.0 mg of cimetidine, 1.7 ml of 1.00 N HCl, 3.1 ml of 0.1 N NaOH, 10.0 ml of $H_2O$, adjusted to a pH of 6 with NaOH and increased to 30.0 cc with 0.9% NaCl. The 50 mg/kg solution consisted of 750.0 mg of cimetidine in 3.4 ml of 1.00 N HCl with 6.2 ml of 0.1 N NaOH plus 10.0 ml of $H_2O$, adjusted to a pH of 6, with 0.1 N NaOH and filled to a 30.0 ml solution with 0.9% NaCl. The 100 mg/kg solution consisted of 1.5 g of cimetidine in 6.8 ml of 1.00 N HCl with 12.4 ml of 0.1 N NaOH plus 5 ml of $H_2O$, adjusted to a pH of 6 with 0.1 N NaOH and filled to solution (30 cc) with 0.9% NaCl. Stock solutions were prepared daily. Injections were administered IP in volumes of 0.5 cc. Placebo injection consisted of 0.5 cc injections of 0.9% saline.

## RESULTS

Gastric secretion and total acid output are illustrated in Fig. 1. There were no significant differences between the three days on which collections were made for the predrug baseline and the postdrug baseline phases, Predrug: $F(2,27) = 0.76$, $p>0.05$; Postdrug: $F(2,27) = 1.31$, $p>0.05$. During the drug phase, the differences between the gastric samples collected on the two days for each drug treatment were not significant, therefore these data were pooled. Cimetidine had no effect on the volume of gastric juice secreted, $F(4,45) = 1.40$, $p>0.05$, but a significant treatment effect was obtained for total acid output, $F(4,45) = 11.83$, $p<0.01$. The three cimetidine treatment conditions significantly inhibited gastric acid secretion when compared to the placebo and no-injection control conditions, Tukey (a) test, $p<0.05$. Inhibition was most pronounced for the

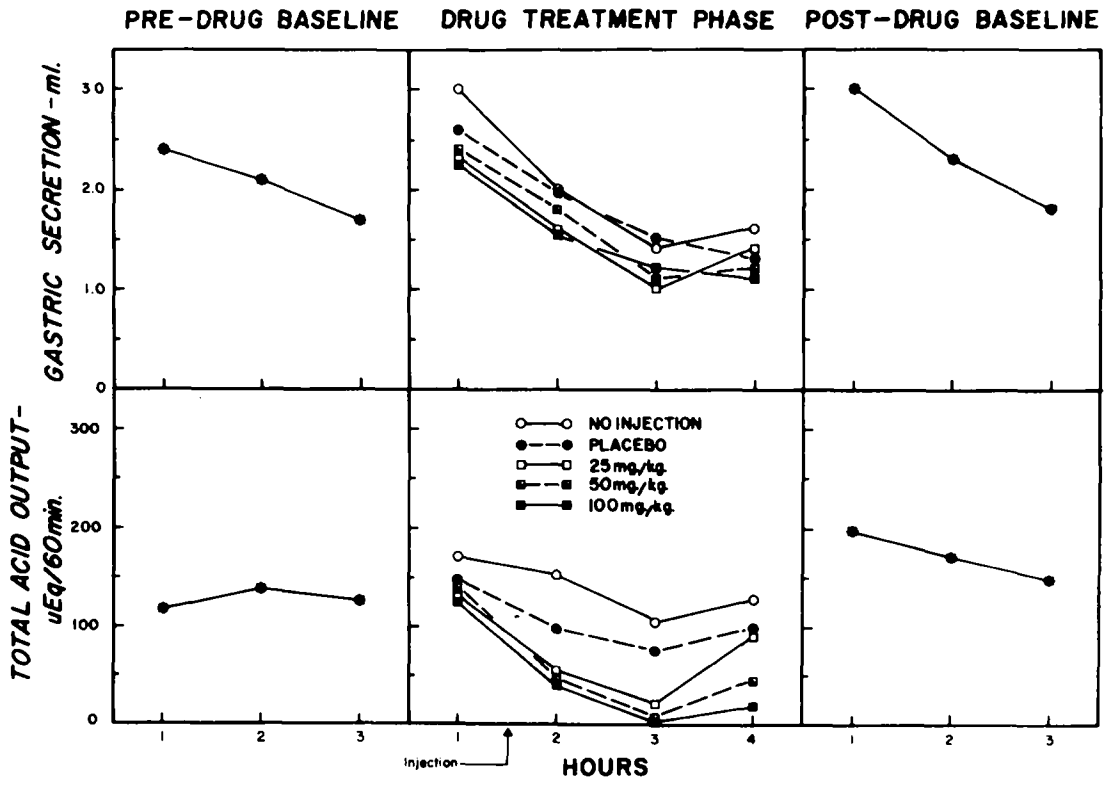


FIG. 1. Mean gastric secretion and mean total acid output for the predrug baseline phase, the drug treatment phase, and the postdrug baseline phase.

TABLE 1

SUMMARY OF STOMACH CONDITIONS FOR ACTIVITY-STRESS ANIMALS

| Treatment | N | Rats With Ulcers | Mean No. Ulcers Per Rat | Mean Length (mm) | Mortalities |
|---|---|---|---|---|---|
| 25 mg/kg | 10 | 7 | 8.0 | 28.7 | 3 |
| 50 mg/kg | 10 | 5 | 14.0 | 37.5 | 1 |
| 100 mg/kg | 10 | 9 | 14.3 | 36.2 | 2 |
| Placebo | 10 | 9 | 18.0 | 45.5 | 2 |
| No injection | 10 | 7 | 20.5 | 52.4 | 2 |

second hourly collection following cimetidine injection. The first 1-hr collection, which was made just before cimetidine was injected, showed no significant differences between treatment conditions. The last hourly collection revealed a significant depression in acid secretion for the doses of cimetidine at 50 and 100 mg/kg, Tukey (a) test.

## EXPERIMENT 2

The first study demonstrated that cimetidine can significantly depress gastric acid secretion. The purpose of the second study was to observe if cimetidine reduced the incidence of stress ulcers in rats. The stress ulcer procedure used in this study was the activity-stress ulcer technique [19] which produces extensive stomach lesions in the rat. With this procedure rats are housed in running-wheel activity cages and fed only 1 hr each day. Rats on this feeding schedule demonstrate excessive daily running activity and within 4–12 days die and reveal extensive lesions in the glandular stomach.

### METHOD

*Animals and Apparatus*

Fifty male Sprague-Dawley rats (220–257 g) were used in this study. The apparatus consisted of standard running-wheel activity cages (Wahmann Manufacturing Co., Baltimore, MD). Each activity wheel was equipped with an adjoining cage measuring 25 x 15 x 13 cm. Wheel revolutions were recorded on digital counters. Room temperature was controlled at 22–23°C, and daylight conditions were artificially maintained between 6 a.m. and 6 p.m.

*Procedure*

Rats were individually housed in activity cages for a 3-day habituation period during which food (granular Purina Rat Chow) and water were available. Following the habituation period, animals were divided equally into five weight-equivalent groups and each group was randomly assigned to one of five treatment conditions. These conditions were: cimetidine at either 25.0, 50.0, or 100.0 mg/kg; a saline-injected placebo condition; and a no-injection control condition. On Day 4, food was withdrawn from all rats at 9:30 a.m. On Day 5, and all subsequent days, rats were fed for 1 hr daily between 9:30 and 10:30 a.m. Rats which were in injection groups received their appropriate treatment three times daily, at 10:30 a.m., 4:30 p.m., and 12 midnight. All injections were presented IP in 0.5 cc volumes. Food consumption, body weight and activity in terms of wheel revolutions were recorded daily for all rats. When an animal was moribund, it was killed with ether. The stomach was removed immediately and cut open along the greater curvature. It was rinsed with water, spread and pinned on a flat surface and covered with 10% Formalin to fix the stomach in a flat attitude. The stomach was examined with a binocular microscope. One eyepiece of the scope was fitted with a reticle permitting lesions to be quantified in terms of millimeters of abnormal tissue. The number, location (i.e., glandular stomach or forestomach) and size of lesions were recorded. After five days of 1-hr feeding, the study was terminated. At this time, surviving animals were killed with ether and stomachs were inspected for lesions. The dependent variables subjected to statistical analysis included daily body weight, food consumption and running activity as well as number of stomach lesions, size of lesions and mortalities per treatment group.

### RESULTS

During the habituation period, there were no significant differences between treatment groups for either daily running activity, $F(4,45) = 1.90$, $p>0.05$, food consumption, $F(4,45) = 1.25$, $p>0.05$, or body weight, $F(4,45) = 0.07$, $p>0.05$. During the 1-hr feeding period, treatment groups did not differ with respect to activity, $F(4,45) = 1.35$, $p>0.05$, food consumption, $F(4,45) = 0.48$, $p>0.05$, and body weight, $F(4,45) = 0.55$, $p>0.05$. The number of mortalities and the number and size of stomach lesions are shown in Table 1. There were no significant differences with respect to the number of rats which died, $\chi^2(4) = 1.25$, $p>0.05$ or the number of rats with ulcers, $\chi^2(4) = 7.61$, $p>0.05$ which could be attributed to the five treatment conditions. Cimetidine did not reduce the number of ulcers per rat, $F(4,45) = 1.05$, $p>0.05$, nor did it significantly reduce the size of the lesions, $F(4,45) = 0.55$, $p>0.05$.

These results essentially replicated the results obtained from a similar study conducted earlier in this laboratory. In

TABLE 2

SUMMARY OF STOMACH CONDITIONS FOR SUPINE RESTRAINT ANIMALS

| Treatment | N | Rats With Ulcers | Mean No. Ulcers Per Rat | Mean Length (mm) |
|---|---|---|---|---|
| 25 mg/kg | 9 | 7 | 4.4 | 7.3 |
| 50 mg/kg | 9 | 7 | 1.9 | 4.8 |
| 100 mg/kg | 9 | 4 | 1.3 | 2.7 |
| Placebo | 9 | 6 | 9.2 | 9.4 |
| No injection | 9 | 8 | 9.6 | 17.8 |

the earlier study, rats had been injected only twice each day, i.e., at 10:30 a.m. and 4:30 p.m., and had failed to reduce the incidence of ulcers. Since cimetidine is rapidly absorbed [12,23], the present study was conducted in which rats were injected three times, instead of only twice daily. But the negative results of the earlier study and the present experiment were not expected. The clinical literature had led us to believe that cimetidine would reduce ulcer incidence in rats exposed to the activity-streee procedure. From these results, one may conclude that cimetidine is not an effective anti-ulcer drug, or that, more specifically, it is not effective in preventing stress ulcer as investigated in this experiment. The clinical literature would argue against the former conclusion; however, some doubt still existed regarding the effect of cimetidine on stress-ulcer formation. For this reason, the anti-ulcer action of cimetidine was again studied using a different ulcerogenic procedure.

## EXPERIMENT 3

The procedure used in this experiment was a modification of the restraint technique [26].

### METHOD

*Animals*

Forty-five male Sprague-Dawley rats (223–339 g) were used. These rats were bred in our laboratory.

*Procedure*

All animals were placed in individual cages five days prior to being restrained and were allowed continuous access to food and water. Rats were randomly assigned to one of five treatment conditions which were: cimetidine at either 25, 50, or 100 mg/kg, a saline placebo, and a no-injection control. There were nine rats in each group. All rats were deprived of food 24 hr before being restrained. One hr prior to restraint, rats were injected with their appropriate drug treatment. All injections were presented IP in 0.5 cc volumes. The restraint procedure is described in detail elsewhere [26]. Briefly, it consisted of restraining the animals in a supine position. This was accomplished by taping the animal's limbs to a board while the animal lay on its back. Rats were placed in a ventilated refrigerator with temperature maintained at 4°–7°C. After 3 hr of restraint, all rats were sacrificed and stomachs were inspected as described in Experiment 2.

### RESULTS

Table 2 summarizes the results obtained. There were no differences between treatment groups with respect to ulcer incidence, $\chi^2(4) = 4.97$, $p<0.29$. However, there was a significant treatment effect with respect to the number, $F(4,40) = 2.73$, $p<0.05$ and the size, $F(4,40) = 2.83$, $p<0.05$, of lesions. Rats injected with cimetidine at 100 mg/kg had fewer and smaller lesions as compared to the two control groups, Tukey (a) test.

## DISCUSSION

Experiment 1 demonstrated that cimetidine inhibited gastric acid secretion in rats with chronic gastric cannulas. However, in Experiment 2 cimetidine failed to significantly reduce the number of stomach lesions in rats subjected to the activity-stress procedure. In the last experiment, rats were exposed to a different ulcerogenic technique. In this study, in which rats were subjected to supine restraint, cimetidine at 100 mg/kg resulted in fewer and smaller lesions as compared to control conditions.

This report, and many other studies have observed that cimetidine does inhibit gastric secretion [12, 14, 23, 25], but there is some question whether the acid inhibitory function of cimetidine can be used to explain the results of the present investigation. A recent report by Okabe, Takeuchi, Urashidani and Takagi [17] revealed that acidity could be reduced dramatically by intraduodenal cimetidine at 100 mg/kg without reducing the incidence of stomach lesions. Dai, Ogle and Lo [10] reported the inhibition of cold restraint ulcers by metiamide, indicating that the effect was not due to gastric secretion but to stress-related gastric motility. Houser, Cash and Van Hart [13] observed a reduction in lesion incidence in activity-stress rats injected with metiamide, but since none of their animals were ulcer-free and none survived beyond 11 days, they suggested that, "other factors may be involved in the formation of these lesions (e.g., reduced mucus secretion, vascular changes, etc.) or that metiamide in the dosages tested was not sufficient to block totally the hypersecretion that may occur in this animal model" (p. 40). A subsequent

study [20] was to show that activity-stress rats secreted less gastric acid as compared to controls.

Other investigators have reported that gastric secretion plays a secondary role in stress-ulcer formation [2, 9, 15, 16]. Okabe *et al.* [17] concluded that cimetidine prevented experimental duodenal ulcers in the rat by suppressing gastric secretion, but that its suppression of experimental gastric ulcer was mediated by an alternative mechanism, probably by the improvement of impaired gastric circulation which occurs under stress. The conclusion that gastric secretion is related to duodenal ulcers but not gastric ulcer is consistent with clinical observations [11,27].

The results of Experiment 2 are not consistent with the data reported by Houser, Cash and Van Hart [13]. As mentioned earlier, these investigators observed that metiamide reduced ulcer incidence in rats subjected to the activity-stress procedure. Besides the obvious fact that metiamide was used in the Houser *et al.* study and cimetidine was used in this investigation, other differences may account for the apparent discrepancies. The Houser *et al.* animals were younger (i.e., 151–200 g vs. 220–277 g in the present report). Younger animals are more susceptible to the activity-stress procedure [19]. Room temperature was maintained at 67°F (as compared to 72°F in the present report) in order to initiate greater running activity. Their aimals also received only two days of habituation and the experimental period was extended until all animals had died at eleven days. Therefore, the age of the animals, the environmental conditions, and the length of the observation period suggest that their method was more severe than the procedure used in the present report. Consequently, the probability of observing a drug-induced lesion suppression effect was greater in the Houser *et al.* study as compared to the present study, wherein rats were submaximally stressed. After four days of 1-hr feeding, 80% of the saline control rats in the Houser *et al.* study were dead as compared with a 20% mortality for saline controls in this report. These data support the conclusion that the rats in the Houser *et al.* study were more severely stressed and this difference may account for the different outcomes.

Cimetidine prevented supine-restraint ulcers, but it failed to reduce the incidence of activity-stress ulcers. This report, as well as others, indicates that gastric acidity cannot be considered the sufficient cause of stress ulcer. The differential effects observed with cimetidine on suppression of supine-restraint ulcers and activity-stress ulcers may also reflect the different developmental variables inherent in these two procedures

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