THE MECHANISM OF AGGRAVATION OF
INDOMETHACIN-INDUCED GASTRIC ULCERS BY
ADRENALECTOMY IN THE RAT

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Abstract—Bilateral adrenalectomy markedly aggravated gastric ulcers in rats induced
by 5 or 20 mg/kg of indomethacin. The degree of aggravation was much the same
in experiments done 1 and 14 days after operation. Pretreatment with prednisolone
10 mg/kg or cortisol acetate 10 mg/kg given subcutaneously significantly suppressed
the aggravated ulceration in response to 20 mg/kg of indomethacin in these adrena-
lectomized rats. Desoxycorticosterone acetate 10 mg/kg, however, had no effect on the
aggravation of indomethacin-induced ulcers. Epinephrine 0.1 or 1 mg/kg given
subcutaneously markedly suppressed the indomethacin-induced ulcers in adrena-
lectomized rats. Removal of the adrenal medulla alone did not appreciably influence
the development of indomethacin-induced ulcers. These results indicate that the
adrenal cortex, particularly the area containing glucocorticoids, plays an important
role in suppression of the noxious effect of indomethacin on the rat gastric mucosa.

In attempting to determine the pathogenesis of gastric ulcers induced by indomethacin
treatment, investigators have studied the role of the adrenal glands in development of indo-
methacin-induced ulcers by adrenalectomy in rats. Djahanguiri et al. (1) found that ad-
renalectomy produced no change in indomethacin-induced ulcers. Bhargava et al. (2),
however, showed that indomethacin-induced ulcers were apparently suppressed by removal
of the adrenal glands. In contrast to their findings, we found in preliminary studies that
adrenalectomy markedly aggravated the indomethacin-induced gastric ulcers in rats. Thus,
we attempted to clarify the mechanism by which adrenalectomy aggravates indomethacin-
induced ulcers.

MATERIALS AND METHODS

Male albino rats of Donryu strain, weighing 180-200 g, were used.

Indomethacin-induced ulceration: Prior to the experiments, the animals were deprived
of food but allowed free access to water for 24 hr. Indomethacin (Merck-Banyu) in doses
of 5 or 20 mg/kg, suspended in a trace of Tween 80 and 1% sodium carboxymethylcellulose
(CMC) solution, was given s.c. in a volume of 5 ml/kg. The animals were sacrificed by a
blow on the head 7 hr after the administration. The stomach of each was then removed,
12 ml of 1% formalin solution was injected and the organs were then immersed in formalin
solution for 10 min to fix the inner and outer layers of the gastric wall. After fixing, the
stomach was incised along the greater curvature and the length (mm) of the mucosal ulcers that appeared on the glandular portion was measured under a dissecting microscope with a square grid (10×). The sum of the length of ulcers per rat was taken as the ulcer index. The person measuring the area of ulceration was unaware of which treatment the animals had been given.

**Adrenalectomy:** Bilateral adrenalectomy was carried out under ether anesthesia by a translumbar route. The adrenalectomized rats were divided into 2 groups; 1. Immediately after operation, the rats were deprived of food but allowed free access to 1% NaCl solution for 24 hr and then used for experiments. 2. The surgically treated rats were maintained on 1% NaCl solution and the usual rat laboratory chow thereafter. Thirteen days after the operation, the rats were deprived of food but allowed free access to 1% NaCl solution for 24 hr and then used for experiments. Sham operated controls were included in the above two groups.

**Adrenal medullectomy:** Removal of the adrenal medulla alone was carried out by cutting a part of the adrenal cortex and squeezing out the medulla. The complete removal of the medulla was ascertained by histological examination after termination of the experiment. Post-operatively, the animals were deprived of food but allowed free access to 1% NaCl solution for 24 hr, and then used for experiments. Sham operation, including cutting of part of the adrenal cortex, was done in the controls. Twenty-four hr later, indomethacin 20 mg/kg was given s.c. to both sham operated and the adrenal medullectomized rats.

**Drugs:** The effects of corticosteroids or epinephrine on indomethacin-induced ulcer were studied 24 hr after adrenalectomy, during which time the animals were deprived of food but allowed free access to 1% NaCl solution. Prednisolone (Iwaki) or desoxycorticosterone acetate (DOCA) (Wako Pure Chemical) was suspended in a trace of Tween 80 and physiological saline and given s.c. in a dose of 10 mg/kg. Cortisone acetate (Merck-Banyu) or epinephrine hydrochloride (Sanko) was diluted with saline and given s.c. in doses of 1 or 10 mg/kg or 0.01, 0.1 or 1.0 mg/kg respectively. Each drug was given 10 min prior to the indomethacin treatment (20 mg/kg) in a volume of 5 ml/kg. At the end of the experiments, it was confirmed that a small amount of cortisone or prednisolone was found to have remained in the subcutaneous area while DOCA was almost completely absorbed from the area. In the control group, the animals were given saline alone.

Student’s *t*-test was employed to determine statistical significance of all tests.

**RESULTS**

As shown in Table 1, indomethacin dose-dependently produced mucosal ulcers in the glandular stomach in sham operated rats. When indomethacin at 5 or 20 mg/kg was given to the adrenalectomized rats, the development of gastric ulcers was significantly aggravated as compared to that in sham operated rats, both one and 14 days after operation (Fig. 1). As determined by the ulcer index, the ulcerations induced by 20 mg/kg of indomethacin were aggravated 8 and 5 times by adrenalectomy at 1 and 14 days, respectively. Gross observation of the ulcers indicated that both the number and the width of ulcers increased
Moreover, in these rats, the readily visible ulcers were often found in the antral portion where indomethacin-induced ulcers seldom occurred in the sham operated rats. Adrenalectomy itself produced no appreciable changes in the gastric mucosa of rats, even 14 days after operation. In contrast to adrenalectomy, adrenal medullectomy did not have an aggravating effect on indomethacin-induced ulcers in rats, as shown in Table 2.

The effects of corticosteroids and epinephrine on indomethacin-induced ulcers are shown in Table 3. The data indicate that 10 mg/kg of prednisolone or cortisone acetate significantly prevented the aggravation by adrenalectomy of indomethacin-induced ulcers. In contrast to the effect of glucocorticoids, DOCA, one of the mineralcorticoids, had no effect on indomethacin-induced ulcers at the dose of 10 mg/kg given to adrenalectomized rats. Epinephrine at 0.1 or 1 mg/kg markedly inhibited the development of indomethacin-induced ulcers in a dose-dependent manner in adrenalectomized rats.

| Group number | Days after operation | Rats        | No. of rats | Dose of indomethacin (mg/kg) | Ulcer index (mm) mean±S.E. | Ulcer incidence (%) |
|--------------|----------------------|-------------|-------------|-----------------------------|-----------------------------|---------------------|
| 1            | 1                    | Sham operated | 15          | 5                           | 0.3±0.3                     | 13.3                |
| 2            | 1                    | Adrenalectomized | 15          | 20                          | 8.5±2.1                     | 86.7                |
| 3            | 14                   | Sham operated  | 15          | 5                           | 0.2±0.1                     | 13.3                |
| 4            | 14                   | Adrenalectomized | 15          | 20                          | 8.1±2.5                     | 93.3                |
| 5            | 14                   | Sham operated  | 15          | 5                           | 64.8±6.0                    | 100                 |
| 6            | 14                   | Adrenalectomized | 15          | 20                          | 8.1±2.5                     | 93.3                |
| 7            | 14                   | 15           |              |                             |                             |                     |
| 8            | 14                   | Adrenalectomized | 15          | 20                          | 40.7±9.2                    | 100                 |

P values: 1:2 < 0.05, 1:4 < 0.05, 2:5 < 0.05, 4:5 < 0.05, 6:7 < 0.05, 6:9 < 0.05, 7:10 < 0.05, 9:10 < 0.05

Fig. 1. Gross appearances of indomethacin-induced gastric ulcers in rats (sham operated, left; adrenalectomized, right).
DISCUSSION

In the present study we found that indomethacin-induced ulcerations in rats were significantly aggravated by adrenalectomy. As the findings of Djahanguiri et al. (1) and Bhargava et al. (2) were quite the opposite, the different experimental conditions employed have to be considered. They used rats of unspecified strain, 120-150 g in body weight, both sexes and sacrificed the animals 4-8 hr after indomethacin treatment. They measured the ulcers by the all or none method; even the shedding of epithelium was counted as a sign of ulceration. Abdel-Galil and Marshall (3) reported that adrenalectomy aggravated the development of gastric ulcers induced by phenylbutazone and postulated that the aggravation was due to the hypersensitivity on adrenalectomized rats to histamine released by phenylbutazone. Since the histamine releasing effect of indomethacin is unknown, it is difficult to apply this hypothesis in the case of aggravation of indomethacin-induced ulcers. In our preliminary study, aspirin-induced ulcers in rats were seen to be significantly aggravated by adrenalectomy. In addition, there are reported data on restraint stress ulcers in which aggravation occurred with adrenalectomy (4, 5). Therefore, it appears that the aggravating effect of adrenalectomy is a general phenomenon common to a variety of experimentally-induced ulcers. As reviewed by Cooke (6), adrenalectomy is known to decrease gastric secretion in experimental animals, including rats. Thus, changes in secretion of gastric juice probably do not contribute to the aggravation of indomethacin-induced ulcers.
The present study provides evidence that the aggravation of indomethacin-induced ulcers by adrenalectomy was mainly due to the deficiency of glucocorticoids contained in the adrenal cortex. This evidence was supported by the following facts; 1) the aggravation was markedly suppressed by the administration of glucocorticoids but not by mineralcorticoids, 2) no aggravation was observed when the adrenal medulla alone was removed. The mechanism of inhibition by glucocorticoids is unknown. As reviewed by Willems, (7), studies on the influence of adrenal cortical hormones on gastric cell renewal have yielded contradictory results. Whether or not glucocorticoids, at the doses used in the present study, accelerate the gastric cell renewal in adrenalectomized rats remains to be clarified. It is of interest that stress ulcers, produced by forced muscular exercise, were reported to be prevented by prednisolone and not affected by DOCA (8).

Epinephrine was found to suppress the development of indomethacin-induced ulcers to a greater extent than glucocorticoids in adrenalectomized rats. Since adrenal medullectomy itself had no appreciable effect on indomethacin-induced ulcers, it is unlikely that the inhibition by epinephrine is due to compensation for deficient endogenous catecholamines. The mechanism involved in the inhibitory effect of epinephrine remains unknown.

It has been postulated that the ulcerogenic activity of indomethacin or other non-steroidal anti-inflammatory agents on the gastrointestinal tract might be due to inhibitory effects on prostaglandins biosynthesis (9). Chaundhury and Jacobson (10) proposed that prostaglandins have a so-called “cytoprotective” effect on the gastric mucosa by increasing intracellular levels of cyclic AMP. In addition, Foster and Perkins (11) reported that the activating effect of prostaglandins on the adenylate cyclase system in human astrocytoma cells required glucocorticoids. They concluded that glucocorticoids induced the synthesis of a protein which modified the sensitivity of adenylate cyclase to prostaglandin E1. All these data taken together suggest that both glucocorticoids and prostaglandins are essential for maintenance of normal integrity of the gastric mucosa.

We conclude that aggravation by adrenalectomy of indomethacin-induced ulcers in rats is the result of a deficiency in glucocorticoids.

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