EFFECTS OF VARIOUS DRUGS AND VAGOTOMY ON INDOMETHACIN-INDUCED GASTRIC ULCERS IN THE RAT

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Various drugs inhibit gastric ulcerations induced by indomethacin administration to rats (1-4). Our recent studies also showed that several amino acids, e.g., tryptophan, methionine etc., prevented indomethacin-induced gastric ulcers in this same species (5). In the present study we examined the effects of various drugs and surgical treatment on gastric ulcers induced by indomethacin in an attempt to elucidate the mechanism of ulcerogenic activity of this compound.

Male Donryu rats, 180-210 g, were fasted for 24 hr but allowed free access to water. Indomethacin (Merck-Banyu) at 20 mg/kg, suspended in a trace of tween 80 and 1% carboxymethylcellulose (CMC) solution, was given s.c. in a volume of 0.5 ml per 100 g of body weight. The animals were sacrificed 7 hr after the indomethacin administration by a blow on the head. The stomach of each was removed, 12 ml of 1% formalin solution was injected and the preparation was immersed in 1% formalin solution for 10 min. The stomach was then incised along the greater curvature and the length (mm) of each mucosal ulcer developed in the glandular portion was measured under a dissecting microscope (10×). The sum of the length of each mucosal ulcer per rat was used as the ulcer index. The person measuring
the ulcers was unaware of which pretreatment the animals had been given. The mixture of aluminum hydroxide plus magnesium oxide (2:1) (Shionogi), amylopectine sulfate (Nihon-Kayaku), atropine sulfate (Sanko) was suspended in 1% CMC solution and given p.o. in a volume of 0.5 ml per 100 g of body weight 10 min before the indomethacin treatment. Chlorpromazine hydrochloride (Yoshitomi), hexamethonium bromide (Yamanouchi), Phenylephrine hydrochloride (Kowa), isoproterenol hydrochloride (Kaken Kagaku), phentolamine mesylate (CIBA) or propranolol hydrochloride (Sumitomo Kagaku) was dissolved in saline and given s.c. in a volume of 0.2 ml per 100 g of body weight 10 min before indomethacin. Control animals were given either 1% CMC solution or saline alone. Reserpine (Daichi Seiyaku) was given s.c. once a day for consecutive days in a volume of 0.2 ml per 100 g of body weight. Indomethacin was given s.c. 24 hr after the final administration of reserpine. After a 24 hr fast, subdiaphragmatic bilateral vagotomy was carried out with the rats under ether anesthesia. Indomethacin 20 mg/kg was given s.c. 10 min after vagotomy. Sham operated animals served as the control. The vagotomized rats were sacrificed 7 hr after indomethacin treatment. Student’s t-test was employed to determine the statistical significance of the data.

Indomethacin 20 mg/kg induced multiple gastric mucosal ulcers in every animal in the

| Table 1. Effects of various agents on indomethacin-induced gastric ulcers in rats |
|------------------|---------|---------------|--------|--------|-----|------|
| Treatment        | Dose (mg/kg) | Route | No. of rats | Ulcer index (mm) mean ± s.e. | Inhibition (%) | P    |
| Control (1% CMC) | p.o.     | 30 | 15.3 ± 2.3  | 66.0  | <0.05 |
| Aluminum hydroxide | 250  | p.o. | 10 | 5.2 ± 2.1 | 98.0 | <0.001 |
| + Magnesium oxide | 750 | p.o. | 10 | 0.3 ± 0.3 | 98.0 | <0.001 |
| Amylopectine      | 200 | p.o. | 10 | 13.5 ± 6.1 | 11.8 | N.S. |
| Atropine          | 0.1 | p.o. | 10 | 9.5 ± 5.2 | 37.8 | N.S. |
|                   | 1   | p.o. | 10 | 0.1 ± 0.1 | 99.3 | <0.001 |
| Control (saline)  | s.c. | 30 | 18.1 ± 2.9  |       |       |      |
| Chlorpromazine    | 1   | s.c. | 10 | 8.2 ± 2.7 | 54.7 | N.S. |
|                   | 3   | s.c. | 10 | 2.9 ± 1.6 | 84.0 | <0.01 |
| Hexamethonium     | 30  | s.c. | 10 | 17.7 ± 4.4 | 2.2  | N.S. |
| Phenylephrine     | 0.1 | s.c. | 10 | 6.8 ± 2.1 | 62.4 | <0.05 |
|                   | 3   | s.c. | 10 | 2.7 ± 0.9 | 85.1 | <0.01 |
| Isoproterenol     | 0.1 | s.c. | 10 | 8.0 ± 3.5 | 55.8 | N.S. |
|                   | 3   | s.c. | 10 | 0.3 ± 0.3 | 98.3 | <0.01 |
| Phentolamine      | 30  | s.c. | 10 | 17.9 ± 4.5 | 1.1  | N.S. |
| Propranolol       | 20  | s.c. | 10 | 16.5 ± 4.6 | 8.8  | N.S. |
|                   | 50  | s.c. | 10 | 16.0 ± 4.6 | 11.6 | N.S. |
| Reserpine         | × 3 days | s.c. | 10 | 11.9 ± 2.7 | 34.3 | N.S. |

Each drug, dissolved in saline or suspended in 1% CMC solution, was given p.o. or s.c. 10 min before indomethacin (20 mg/kg, s.c.) treatment. Animals were sacrificed 7 hr after indomethacin treatment. N.S. non-significant at the level of P=0.05.
control groups. As shown in Table 1, the mixture of aluminum hydroxide plus magnesium oxide or atropine dose-dependently inhibited the ulcerations. Amylopectine at 200 mg/kg did not inhibit the indomethacin-induced ulcers. Chlorpromazine, phenylephrine or isoproterenol markedly inhibited indomethacin-induced ulcers, in a dose-dependent manner. Hexamethonium 30 mg/kg, phenolamine 30 mg/kg, or propranolol 50 mg/kg failed to show any significant inhibition of indomethacin-induced ulcers. With administration of reserpine for 3 days there was a tendency toward inhibition of the indomethacin-induced ulcers but the response was not statistically significant. As shown in Table 2, bilateral vagotomy markedly inhibited the development of these induced ulcers.

These results indicate that gastric ulcers induced by indomethacin are quite sensitive to various types of drugs and surgical treatment. We found that an antacid potentially inhibited the ulcerations in response to indomethacin. In addition, we confirmed the results reported by Lee et al. (1) that an anticholinergic agent and vagotomy markedly inhibited the indomethacin ulcers, whereas an antipeptic agent did not. It should be noted that administration of several drugs, i.e., chlorpromazine, phenylephrine or isoproterenol resulted in a significant inhibition of indomethacin-induced ulceration. Regarding the effect of an adrenergic beta stimulating agent, our data are consistent with those of Fielding et al. (6) who found a marked inhibition of indomethacin-induced ulcers when salbutamol was given to rats. These adrenergic alpha and beta stimulating agents suppress gastric secretion in rats (7, 8). Our previous study (9) showed that cimetidine, a potent inhibitor of gastric secretion, markedly protected the animals from indomethacin-induced ulcers. All these data taken together suggest that the presence of gastric acid in the stomach is a sine qua non for the production of ulcers by indomethacin. Hexamethonium, phentolamine, propranolol and reserpine also suppress gastric secretion in rats (7, 10, 11), but these drugs failed to inhibit the ulceration induced by indomethacin. As described previously, the effect of several amino acids on indomethacin ulcers was not in parallel with their neutralizing or buffering actions on gastric acid. Thus, other factors are probably involved in the pathogenesis. It may be that indomethacin induces gastric ulcers by inhibiting the biosynthesis of prostaglandins in the stomach (3). Ascik (12) found that catecholamines stimulated prostaglandin synthetase activity in the rat stomach. Therefore, it is possible that the adrenergic nerve stimulating agents such as phenylephrine and isoproterenol may stimulate prostaglandin synthesis and protect the gastric mucosa from ulceration. As to the sympa-

### Table 2. Influence of vagotomy on indomethacin-induced ulcers in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Ulcer index (mm) mean±s.e.</th>
<th>Inhibition</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>20</td>
<td>2.4±0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vagotomy</td>
<td>20</td>
<td>0.4±0.2</td>
<td>83.3</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Indomethacin 20 mg/kg was given s.c. 10 min after operation. Animals were sacrificed 7 hr after indomethacin treatment.
thetic nervous system, Djahanguiri et al. (13) emphasized the role of endogenous catechol-
amines in the etiology of indomethacin-induced ulceration. Our findings herein confirm ob-
servations in our recent report (14) that surgical sympathectomy had no preventive effect
on indomethacin-induced ulcer. Segawa et al. (15) reported that the turnover rate of
norepinephrine in the rat stomach was not influenced by the ulcerogenic dose of
indomethacin. All these observations suggest that the sympathetic nervous system probably
plays a minor role in the pathogenesis of indomethacin-induced ulceration. The reduction
of mucous secretion by indomethacin as reported by Menguy and Desbaillets (16) also has
to be considered.

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