THE EFFECTS OF DRUGS ON THE PRODUCTION AND RECOVERY PROCESSES OF THE STRESS ULCER

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Various procedures have been reported on the biossay of antiulcer drugs. Pylorus ligation of the rat (1–3), restraint of the rat (4, 5), water immersion of the restrained rat (7) and the application of ulcerogenic agents on various animals (8–11) were used for producing experimental ulcers. In these methods the drug had been administered to the animal before the ulcer production and the preventive effect on the ulcer producing processes was tested. Although some drugs such as glutamine or methylmethionine sulfonium chloride were recently introduced to promote the repair of the ulcer which had existed already, suitable methods are lacking for their pharmacological test, and so it was hoped to develop a new method for pharmacological assay of curative activity of the drugs on existing ulcers. In the present paper curative effects of some drugs on the stress ulcer of the rat are reported.

METHODS AND MATERIALS

The method reported by Takagi et al. (6) was modified for the purpose of producing

![Fig. 1. Stress cage.](image-url)
deeper ulceration in a number of rats. Male rats of Donryu strain weighing 240 to 260 g were fed as usual for several days and each animal was immobilized in each compartment of the stress cage. The cage was made of aluminum board and galvanized wire net to hold the animal body as illustrated in Fig. 1. The details of the size are shown in Fig. 2. The cages were then immersed vertically in a water bath kept on 23°C for 20 hours to the height of the xiphoid of the animals. After this procedure the animals were freed from the cage and bred as usual. No superficial injury was observed on the stressed animals. More than thirty animals could be applied at a time by using those stress cages. Because fasting before the stress decreased the incidence of the ulcer, the rats fed normally were used for the experiments. They were successively killed at proper intervals after the stress, the isolated stomachs were inflated with about 10 ml of water and placed in 0.5% formalin solution for 5 minutes according to Brodie et al. (12). After washing the stomach was cut open along the greater curvature and extended on the green pad. The length of a ulcer which is approximately proportionate to the area of the ulcer was totaled on each animal and it was indicated as the ulcer index (UI).

For the examination of the preventive effect of drugs, they were given orally or subcutaneously 30 minutes before the stress and the animals were killed immediately after the stress. The stomachs were then examined. For the curative test the animals received a drug once or twice a day orally or subcutaneously during 10 consecutive days after the stress and were sacrificed to remove and examine the stomachs on the
The control animals received the same procedure and the same solution without the drug.

Curative ratio (CR) is expressed as follows.

$$\text{CR} = \frac{\text{UI Control} - \text{UI Test}}{\text{UI Control}} \times 100(\%)$$

Sodium bicarbonate, synthetic aluminum silicate and precipitated calcium carbonate were suspended in 0.5% CMC solution and given orally twice a day. Carrageenin as an antipepsinic drug was dissolved in water and given orally once a day, and atropine methylbromide subcutaneously once a day. Eighteen amino acids and methylmethionine sulfonium chloride were dissolved in 0.5% CMC solution respectively and given orally twice a day. In the combination of glutamine and atropine methylbromide, the former was administered orally twice a day and the latter subcutaneously once a day respectively for 10 days. Gefarnate (geranyl farnesyl acetate) was suspended in water with a trace of Tween 80 and given orally once a day or subcutaneously twice a day. Bilateral subdiaphragmatic vagotomy was made before or after the stress and at the same time sham operation was done as the corresponding controls.

RESULTS

The rats lost about 20 g of body weight during the stress but approached gradually to the level of the control animals. Although a few animals died during or immediately after the stress, no animals died after that period. As the period of stress was increased from 2 to 24 hours, the index and severity of the ulcer were increased as shown in Fig. 3. Just after the stress extensive superficial erosions with various lengths and congestion of the surrounding portion were observed in the fundus of the stomach. However, the

![Graph](image-url)

**Fig. 3.** Time course of the incidence of and recovery from gastric ulcers in the rat. Vertical bar indicates the standard error.
necrotic portion was eliminated 2 or 3 days after the stress probably by the ingested foods. Fig. 4 indicates typical ulcers on the 2nd and 11th day after the stress and it was shown that the number and shape of the ulcer became smaller as time passed (Fig. 3). The complete recovery of the ulcer took 3 to 4 weeks after the stress.

At the histological examination almost all of the lesions were erosion of mucosa and they extended down to muscularis mucosae and submucosae in some cases but not to the muscle layer (Fig. 5).

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**Fig. 4.** Rat stomachs showing the ulcers at the 2nd day (left) and 11th day (right) after the stress.
Note the ulcers which appear in the glandular area and not in the forestomach. Blood coagulums can be seen at the base of ulcers (left).

**Fig. 5.** Microphotograph of the ulcer.
(a) shows the ulcer at the 2nd day and (b) is one at the 23rd day after the stress.
Antacids and antipepsinic drugs: The curative ratios of sodium bicarbonate and synthetic aluminum silicate were 16.1% and 19.8% in doses of 2 g/kg twice a day, and that of precipitated calcium carbonate was 25.1% which was significant (P=0.05) as indicated in Table 1. Although this dose seemed enough to neutralize free acid in gastric juice, it had little effect on the curative test. On the curative test carrageenin (300 mg/kg by oral administration) showed only weak effect, though the dose seemed to be enough to inhibit the pepsin of gastric juice.

**TABLE 1. Effect of antacids and an antipepsinic drug in the curative test.**

<table>
<thead>
<tr>
<th>Treatment Drug</th>
<th>Dose g/kg</th>
<th>No. of animals</th>
<th>Ulcer index m±s.e.</th>
<th>Curative ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>11</td>
<td>16.7±1.3</td>
<td>16.1</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>2</td>
<td>10</td>
<td>14.0±1.7</td>
<td>16.1</td>
</tr>
<tr>
<td>Synthetic aluminum silicate</td>
<td>2</td>
<td>9</td>
<td>13.4±1.5</td>
<td>19.8</td>
</tr>
<tr>
<td>Precipitated calcium carbonate</td>
<td>2</td>
<td>11</td>
<td>12.4±1.5</td>
<td>25.1</td>
</tr>
<tr>
<td>Antipepsinic drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>15</td>
<td>12.4±1.6</td>
<td>25.2</td>
</tr>
<tr>
<td>Carrageenin</td>
<td>0.3</td>
<td>9</td>
<td>9.5±2.0</td>
<td>25.2</td>
</tr>
</tbody>
</table>

Fig. 6. Preventive and curative effects of atropine methylbromide at various doses and bilateral vagotomy. Ten to fourteen animals were used for each treatment.
Anticholinergic drugs and vagotomy: In the preventive test atropine methylbromide was strikingly effective and the maximal effect of 94% was obtained in a dose of 5 mg/kg given subcutaneously, but complete inhibition of the ulcer production was not realized even when a high dose such as 40 mg/kg was administered. However, the drug was not so effective in the curative test as in the preventive test. The maximal effect of 40.1% was obtained at 10 mg/kg, and the increase of doses to 20 and 40 mg/kg lowered the curative ratio to 23.2 and -0.2%.

On the vagotomized animals the preventive ratio was 100% but the curative ratio was 25.1%, when the laparotomized animals were used as the control (Fig. 6).

Amino acids and methylmethionine sulfonium chloride: Glutamine was the most effective on existing ulcer among eighteen amino acids in a dose of 2 g/kg and its curative ratio was 61.8% as shown in Fig. 7. The curative ratios of tyrosine, ornithine, methionine and ornithine aspartate were 49.6, 39, 36 and 29% respectively. Lysine, proline and threonine were ineffective. The curative ratio of methylmethionine sulfonium chloride was 25.7% (Table 2).

Combined use of glutamine and atropine methylbromide: Glutamine 2 g/kg orally and atropine methylbromide 10 mg/kg subcutaneously were administered at the same time. The curative ratio was 59.1%, which was almost the same as that of glutamine alone. Gluta-
mine 1 g/kg and atropine methylbromide 40 mg/kg, each of which was not effective after the single administration, showed definite effectiveness of 60.3% (Table 3).

**Gefarnate:** The curative ratios of gefarnate were 18.6 and 43.7% at the doses of 100 and 200 mg/kg respectively by oral administration. By subcutaneous administration they were 32.8 and 55.7% at the same doses as the oral route. Therefore the effect of subcutaneous administration was slightly higher than oral one. In the preventive test gefarnate was given orally in doses of 200 and 500 mg/kg before the stress, but no effect was observed (Table 4).

**DISCUSSION**

Evaluation of curative effect of drugs on existing ulcers of experimental animals had not been reported, because it seemed to be difficult to produce the ulcer which would take a long time for the complete healing. In the restrained rat (12) the ulcer produced recovered within 3 days and so the method seemed to be useless for the curative test. Shay rat (3) could not be adopted because the ligation at pylorus portion must be freed again after the ulcer producing process. The thermocouteric method (14, 15) was able to evoke the deep injuries on the gastric wall, and it took about fifty-five days for their complete recovery. In addition they showed striking similarity to the human ulcer in its shape, recovery process and aggravation with cortisone, but no report using this ulcer for the evaluation of antiulcer agents, as far as we knew, had been found. Among the

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**TABLE 3. Effect of the combination of atropine methylbromide and glutamine in the curative test.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose/kg</th>
<th>No. of animals</th>
<th>Curative ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine methylbromide</td>
<td>10 mg</td>
<td>10</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>40 mg</td>
<td>10</td>
<td>-0.7</td>
</tr>
<tr>
<td>Glutamine</td>
<td>1 g</td>
<td>10</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>2 g</td>
<td>11</td>
<td>61.8</td>
</tr>
<tr>
<td>Atropine methylbromide + Glutamine</td>
<td>10 mg + 2 g</td>
<td>8</td>
<td>59.1</td>
</tr>
<tr>
<td></td>
<td>40 mg + 1 g</td>
<td>11</td>
<td>60.3</td>
</tr>
</tbody>
</table>

**TABLE 4. Effect of gefarnate in the preventive and the curative test.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug</th>
<th>Dose mg/kg</th>
<th>No. of animals</th>
<th>Ulcer index m±s.e.</th>
<th>Preventive &amp; curative ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preventive test</td>
<td>Control</td>
<td>200</td>
<td>7</td>
<td>39.3±5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gefarnate (p.o.)</td>
<td>500</td>
<td>7</td>
<td>40.6±1.3</td>
<td>-3.3</td>
</tr>
<tr>
<td>Curative test</td>
<td>Control</td>
<td>100</td>
<td>9</td>
<td>18.3±1.9</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>Gefarnate (p.o.)</td>
<td>200</td>
<td>10</td>
<td>14.9±1.6</td>
<td>43.7</td>
</tr>
<tr>
<td></td>
<td>Gefarnate (s.c.)</td>
<td>200</td>
<td>9</td>
<td>8.1±1.3</td>
<td>55.7</td>
</tr>
</tbody>
</table>
FIG. 8. Influence of repetition of the stress on the ulcer.
(a) is the result of daily repetition of 8 hours stress for 6 days, and
(b) is the result of weekly repetition of 17 hours stress for 7 weeks. In
(a) the ordinate shows the average value of the ulcer unit which is
graded for 1 to 5 by its shape. Vertical bar is the standard error.
experimental ulcers induced by the various ulcerogenic drugs, histamine ulcer of the guinea pig is the most excellent in the similarity to human ulcer in its depth and shape, which was used by Adami (9) for the screening of gefarnate. However, the drug was given before and during the ulcer producing process and so this method was not exactly a curative test. We also tried this method but it was a little difficult to estimate the effect of the drug, because the ulcer was not produced consistently in the control group.

It was tried after all to produce the definite ulcer at the glandular portion of the rat stomach without any surgical or chemical intervention in our laboratory. If mild conditions of the stress factors such as duration and temperature of the bathing were used, the incidence of the ulcer decreased and it was restored rapidly. When the stress was stronger, the mortality increased but no increase of the severity and incidence of ulcer was found. It also failed to induce the deeper ulcer by daily repetition of the stress of 8 hours at 23°C for several days (Fig. 8) or weekly repetition of the stress of 17 hours at 23°C (Fig. 8). Moreover in the latter case the animals adapted themselves to the stress after 6 weeks just as reported by Guth (16). After stress of 17 hours at 25°C was applied daily for 7 days, the severity of the ulcer increased and concurrently duodenal ulcer was produced in some cases. Since the mortality was very high, it couldn’t be utilized for the screening.

Treatment with drugs less than ten days was not favorable, because the action of drugs seemed to take much time to exert the full effect on ulcer.

Antacids, anticholinergic drug and bilateral vagotomy which were effective in the preventive test were all proved to have little effect in the curative test. Carrageenin was well known as an antipepsinic and antiulcerogenic agent in the histamine ulcer, ulcer induced by pylorus ligation or cortisone ulcer (3). However, carrageenin was ineffective in the curative test.

In the eighteen amino acids, glutamine was the most effective in the curative test. Shive et al. (17) already reported its effect on the ulcer and they suggested that glutamine would have some relation to the synthesis of the hexosamine in the mucosa, but in this study hexosamine in a dose of 250 mg/kg orally did not accelerate the repair of the deficit mucosa. Glutamic acid was ineffective (11.2%) at the same dose as glutamine (2 g/kg twice a day p.o.). In the preventive test glutamine administered orally, 2 g/kg once a day for 8 days prior to the stress exerted no effect. Although histidine and tryptophan had been recognized as effective for Mann-Williamson ulceration in dogs by Weiss and Aron (20), these amino acids showed only a little effect in the present study. Methylmethionine sulphonium chloride (vitamin U) which had been reported as a curative drug of ulcer by Cheney (18) had little effect on preventive and a little effect on curative test. Although gefarnate was ineffective on the preventive test, its curative effect was excellent by either of parenteral or oral administration. Adami et al. (9) reported the definite effect of gefarnate on preventive and curative tests on several types of experimental ulcers at dosages varying between 1.25 and 50 mg/kg and concluded that the action of gefarnate was due to the acceleration of the regeneration process of the mucosa.
Preventive drugs such as atropine methylbromide and antacids either inhibited contraction of gastric wall or lowered the acidity of gastric contents. On the other hand, curative drugs such as glutamine (19) and gefarnate (9) did not inhibit gastric contraction and secretion. It can be supposed from these results that the repairing process of experimental ulcers is a separate and independent one from ulcer producing factors like gastric secretion and contraction, and accordingly antiulcer drugs must be divided into two classes as protective and curative drugs.

SUMMARY

A method for estimating the curative effects of drugs on the gastric ulcer of the rat was devised. The method was able to produce the comparatively deep ulcer at the glandular portion of the rat stomach under rather physiological condition. The effect of several drugs was examined by this method. On the preventive test an anticholinergic drug, atropine methylbromide, showed the most excellent effect but it had only weak effect on existing ulcer. However, on the curative test glutamine and gefarnate which were almost ineffective on the preventive test were more effective than antacids and the anticholinergic drug. Those drugs seemed to accelerate the repairing of the deficit mucosa without inhibition of the gastric secretion and motility.

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