EFFECTS OF CL-1700 AND ITS CONSTITUENTS ON ACUTE OR CHRONIC GASTRIC LESIONS AND GASTRIC SECRETION IN RATS

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Abstract—Effects of CL-1700 (N-acetyl-L-carnosine aluminum) and its constituents, L-carnosine, N-acetyl-L-carnosine and Al(OH)₃ on acute or chronic gastric lesions in intact rats and on gastric secretion in pylorus-ligated rats were studied. CL-1700 at 600 or 1,000 mg/kg p.o. markedly inhibited Shay ulcers or indomethacin-induced erosions, but its constituents at the doses contained in CL-1700 did not. Also, CL-1700 at 300 mg/kg i.p. significantly inhibited water-immersion stress-induced erosions, but its constituents did not. CL-1700 at 600 mg/kg i.p. and Al(OH)₃ at 143 mg/kg i.p. significantly inhibited Shay ulcers. CL-1700 at 1,000 mg/kg p.o. almost completely inhibited aspirin- or histamine-induced erosions, but both L-carnosine at 639 mg/kg p.o. and N-acetyl-L-carnosine at 817 mg/kg p.o. also markedly inhibited the formation of erosions. CL-1700 at 1,000 mg/kg p.o. increased the volume and raised the pH value, and the agent at 600 mg/kg i.p. reduced the acid output. CL-1700 at 1,000 mg/kg/day p.o. given twice daily for 3 weeks diminished the size of acetic acid ulcers and significantly increased the number of rats with healed ulcers.

In this communication, we report that CL-1700 has a much stronger effect on several acute gastric lesions than its constituents and accelerates the healing of gastric ulcers.

MATERIALS AND METHODS

Male Donryu rats, weighing 200–250 g, were used.

Induction of acute lesions

Shay ulcers: Rats were deprived of food but allowed free access to water for 48 hr prior to experiments. Under ether anesthesia, the abdomen was incised and the pylorus ligated. The animals were killed 15 hr later by an overdose of ether, and the stomach of each rat removed. The stomach was incised along the greater curvature and examined for
gastric ulcers developed in the forestomach. Test compounds dissolved in distilled water or distilled water alone as a control were given either p.o. (by gastric intubation) or i.p. (intraperitoneally) immediately after pylorus ligation.

Stress-induced gastric erosions: Rats not fasted prior to experiments were placed in a stress cage and immersed to the level of the xiphoid process in a water bath (23°C) for 7 hr. The animals were then immediately killed by a blow on the head. The stomach of each rat was removed, inflated by injecting 13 ml of 2% formalin, and immersed in 2% formalin for 10 min. This formalin treatment was performed in all the following experiments. The stomach was then incised along the greater curvature and examined for erosions developed in the glandular portion. Test compounds or distilled water alone as a control were given i.p. to the rats 10 min before the water immersion.

Aspirin-induced gastric erosions: Rats were deprived of food but allowed free access to water for 24 hr after which the pylorus was ligated. Aspirin (100 mg/kg, Maruishi) suspended in 1% CMC solution was given p.o. to the rats 5 min after pylorus ligation in a volume of 0.5 ml/100 g of body weight. The animals were killed 7 hr after aspirin dosing. The stomach of each rat was removed and examined for erosions developed in the glandular portion. Test compounds or distilled water alone as a control were given p.o. to the rats immediately after pylorus ligation.

Indomethacin-induced gastric erosions: Rats were deprived of food but allowed free access to water for 24 hr, and then indomethacin (25 mg/kg, Sigma) suspended in 1% CMC solution was given s.c. to the rats. The animals were killed 7 hr later, and the stomach of each rat was removed and examined for erosions developed in the glandular portion. Test compounds or distilled water alone as a control were given p.o. to the rats 10 min before the indomethacin treatment.

Histamine-induced gastric erosions: Rats were deprived of food but allowed free access to water for 24 hr, and then histamine 2 HCl (100 mg/kg, Nakarai) was given i.p. to the rats. The rats were killed 4 hr later, and the stomach of each rat was removed and examined for erosions developed in the glandular portion. Test compounds or distilled water alone as a control were given p.o. to the rats 10 min before the histamine treatment.

Gastric secretory studies: Rats were deprived of food but allowed free access to water for 18 hr. Under ether anesthesia, the abdomen of each rat was incised and the pylorus ligated. The animals were killed 7 hr after the pylorus ligation, and the gastric contents collected and analyzed for volume, pH, acidity, and pepsin activity. The acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (Autoburette, Radiometer). The pepsin activity was determined by Anson's method using bovine albumin as a substrate (2). Titratable acid and pepsin output were expressed as μEq/hr and mg tyrosine/hr, respectively. Test compounds or distilled water alone as a control were given either p.o., i.p., or i.d. to the rats immediately after pylorus ligation.

Induction of chronic ulcers: The abdomen of rats was incised under ether anesthesia, and a 20% acetic acid solution (0.015 ml) was injected into the subserosal layer of the glandular stomach. Postoperatively, the animals were maintained on rat chow and water ad libitum. CL-1700 or distilled water alone as a control was given p.o. from one day after the operation for 21 consecutive days to rats with gastric ulcers. As the reference drugs, aluminum sucrose sulfate (Chugai) dissolved in distilled water,
gefarnate (Teikoku Kagaku) suspended in 1% CMC solution, and cimetidine (Dott Bonapace) dissolved in 0.3 N HCl and then neutralized with 0.3 N NaOH solution were used. As a control, either distilled water alone or a 1% CMC solution was given to the rat. The animals were killed the next day after the final administration of drugs and the stomachs removed and examined for ulcers.

Ulcer or erosion index

Each area (mm²) of damaged mucosa in Shay ulcers was measured under the dissecting microscope (×10) with a square grid, summed, and arbitrarily classified into 5 degrees by an ulcer index as follows:

Ulcerated area (mm²): 1–6, 7–12, 13–18, 19–24, >25 or perforation

Ulcer index: 1 2 3 4 5

The area (mm²) of acetic acid-induced ulcers was also measured under the dissecting microscope and used as an ulcer index. The length (mm) of each of the erosions induced by water-immersion stress, aspirin, indomethacin, or histamine was measured under the dissecting microscope, summed, and used as an erosion index.

Dose

The dose of CL-1700 used in this study is the one which was effective on several gastric lesions in rats as shown in previous experiments (1). The doses of L-carnosine, N-acetyl-L-carnosine, and Al(OH)₃ were selected according to the ratio of each of their molecular weights to the molecular weight of CL-1700 according to the composition of CL-1700.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M.W.</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-1700</td>
<td>328.2</td>
<td>1000 600 300</td>
</tr>
<tr>
<td>L-carnosine</td>
<td>226.2</td>
<td>689 414 207</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine</td>
<td>268.2</td>
<td>817 490 245</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>78.0</td>
<td>238 143 72</td>
</tr>
</tbody>
</table>

Each compound alone or the combination of some of the constituents was given in a volume of 0.5 ml/100 g of body weight.

Analysis of data

The Student’s t-test and the z² test were employed to determine the statistical significance of the data obtained in this study at the level of P<0.05.

RESULTS

Acute gastric lesions

Shay ulcers: CL-1700 at 600 mg/kg p.o. significantly inhibited the development of Shay ulcers by 77.4% (Table 1). Neither L-carnosine at 414 mg/kg, N-acetyl-L-carnosine at 490 mg/kg, or Al(OH)₃ at 143 mg/kg nor the combination of N-acetyl-L-carnosine at 490 mg/kg and Al(OH)₃ at 143 mg/kg had any appreciable effects on Shay ulcers when they were given p.o. CL-1700 at 600 mg/kg i.p. also showed a potent inhibitory effect on Shay ulcers, the inhibition being 74.3%. Neither L-carnosine at 414 mg/kg i.p. nor N-acetyl-L-carnosine at 490 mg/kg i.p. had any effects on the ulcers. However, Al(OH)₃ at 143 mg/kg i.p. and the combination of N-acetyl-L-carnosine at 490 mg/kg and Al(OH)₃ at 143 mg/kg i.p. significantly inhibited Shay ulcers, the inhibitions being 57.1% and 51.4%, respectively.

Stress-induced gastric erosions: CL-1700 at 300 mg/kg i.p. significantly inhibited the development of gastric erosions induced by water-immersion stress by 51.1% (Table 2). Neither L-carnosine at 207 mg/kg, N-acetyl-L-carnosine at 245 mg/kg, or Al(OH)₃ at 72 mg/kg nor the combination of N-acetyl-L-carnosine at 245 mg/kg and Al(OH)₃ at 72 mg/kg had a significant effect on the erosions when they were given i.p.

Aspirin-induced gastric erosions: CL-1700
Table 1. Effects of CL-1700, L-carnosine, N-acetyl-L-carnosine, and aluminum hydroxide on Shav ulcers in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose &amp; Route (mg/kg)</th>
<th>No. of rats</th>
<th>Ulcer index mean±S.E.</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>p.o.</td>
<td>15</td>
<td>3.1±0.5</td>
<td></td>
</tr>
<tr>
<td>CL-1700</td>
<td>600 p.o.</td>
<td>15</td>
<td>0.7±0.4*</td>
<td>77.4</td>
</tr>
<tr>
<td>L-carnosine</td>
<td>414 p.o.</td>
<td>15</td>
<td>3.2±0.5</td>
<td>-3.2</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine</td>
<td>490 p.o.</td>
<td>15</td>
<td>3.4±0.5</td>
<td>-9.7</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>143 p.o.</td>
<td>15</td>
<td>3.1±0.5</td>
<td>0</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine+</td>
<td>490 p.o.</td>
<td>15</td>
<td>3.3±0.5</td>
<td>-6.5</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>143 i.p.</td>
<td>15</td>
<td>3.5±0.4</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>600 i.p.</td>
<td>15</td>
<td>0.9±0.5*</td>
<td>74.3</td>
</tr>
<tr>
<td>CL-1700</td>
<td>414 i.p.</td>
<td>15</td>
<td>3.7±0.4</td>
<td>-5.7</td>
</tr>
<tr>
<td>L-carnosine</td>
<td>490 i.p.</td>
<td>15</td>
<td>3.3±0.5</td>
<td>5.7</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine</td>
<td>143 i.p.</td>
<td>15</td>
<td>1.5±0.4*</td>
<td>57.1</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>490 i.p.</td>
<td>15</td>
<td>1.7±0.5*</td>
<td>51.4</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine+</td>
<td>143 i.p.</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance as compared to the controls, *P<0.05.

Table 2. Effects of CL-1700, L-carnosine, N-acetyl-L-carnosine, and aluminum hydroxide on water-immersion stress-induced gastric erosions in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose &amp; Route (mg/kg)</th>
<th>No. of rats</th>
<th>Erosion index (mm) mean±S.E.</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>i.p.</td>
<td>15</td>
<td>22.3±2.6</td>
<td></td>
</tr>
<tr>
<td>CL-1700</td>
<td>300 i.p.</td>
<td>15</td>
<td>10.9±3.6*</td>
<td>51.1</td>
</tr>
<tr>
<td>L-carnosine</td>
<td>207 i.p.</td>
<td>15</td>
<td>23.3±1.7</td>
<td>-4.5</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine</td>
<td>245 i.p.</td>
<td>15</td>
<td>20.4±2.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>72 i.p.</td>
<td>15</td>
<td>16.8±2.8</td>
<td>24.7</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine+</td>
<td>245 i.p.</td>
<td>15</td>
<td>17.9±2.9</td>
<td>23.8</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>72 i.p.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance as compared to the control, *P<0.05.

at 1,000 mg/kg p.o. almost completely inhibited the gastric erosions induced by aspirin, the inhibition being 99.5% (Table 3). L-carnosine at 639 mg/kg p.o. and N-acetyl-L-carnosine at 817 mg/kg p.o. also potently inhibited the erosions with inhibitions of 92.3% and 91.4%, respectively. While Al(OH)₃ at 238 mg/kg p.o. had a weak effect on the development of aspirin-induced gastric erosions, the combination of N-acetyl-L-carnosine at 817 mg/kg and Al(OH)₃ at 238 mg/kg p.o. showed a marked inhibitory effect (86.1%) on the formation of erosions.

Indomethacin-induced gastric erosions: CL-1700 at 1,000 mg/kg p.o. almost completely inhibited the development of gastric erosions induced by indomethacin (Table 3). Neither L-carnosine at 689 mg/kg, N-acetyl-L-carnosine at 817 mg/kg, or Al(OH)₃ at 238 mg/kg nor the combination
Table 3. Effects of CL-1700, L-carnosine, N-acetyl-L-carnosine, and aluminum hydroxide on aspirin- and indomethacin-induced gastric erosions in rats

<table>
<thead>
<tr>
<th>Erosions induced by</th>
<th>Treatment</th>
<th>Dose &amp; Route (mg/kg)</th>
<th>No. of rats</th>
<th>Erosion index (mm) mean±S.E.</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Control</td>
<td>p.o.</td>
<td>15</td>
<td>20.8±3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CL-1700</td>
<td>1000 p.o.</td>
<td>15</td>
<td>0.1±0.1*</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td>L-carnosine</td>
<td>689 p.o.</td>
<td>15</td>
<td>1.6±0.5*</td>
<td>92.3</td>
</tr>
<tr>
<td></td>
<td>N-acetyl-L-carnosine</td>
<td>817 p.o.</td>
<td>15</td>
<td>1.8±0.5*</td>
<td>91.4</td>
</tr>
<tr>
<td></td>
<td>Al(OH)₃</td>
<td>238 p.o.</td>
<td>15</td>
<td>14.9±2.8</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td>N-acetyl-L-carnosine+</td>
<td>817 p.o.</td>
<td>15</td>
<td>2.9±1.4*</td>
<td>86.1</td>
</tr>
<tr>
<td></td>
<td>Al(OH)₃</td>
<td>238 p.o.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Control</td>
<td>p.o.</td>
<td>15</td>
<td>23.1±2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CL-1700</td>
<td>1000 p.o.</td>
<td>15</td>
<td>0.7±0.6*</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>L-carnosine</td>
<td>689 p.o.</td>
<td>15</td>
<td>23.6±3.3</td>
<td>-2.2</td>
</tr>
<tr>
<td></td>
<td>N-acetyl-L-carnosine</td>
<td>817 p.o.</td>
<td>15</td>
<td>25.5±2.6</td>
<td>-10.4</td>
</tr>
<tr>
<td></td>
<td>Al(OH)₃</td>
<td>238 p.o.</td>
<td>15</td>
<td>24.9±3.3</td>
<td>-7.8</td>
</tr>
<tr>
<td></td>
<td>N-acetyl-L-carnosine+</td>
<td>817 p.o.</td>
<td>15</td>
<td>25.5±2.8</td>
<td>-10.4</td>
</tr>
<tr>
<td></td>
<td>Al(OH)₃</td>
<td>238 p.o.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance as compared to the controls, *P<0.05.

Table 4. Effects of CL-1700, L-carnosine, N-acetyl-L-carnosine, and aluminum hydroxide on histamine-induced gastric erosions in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose &amp; Route (mg/kg)</th>
<th>No. of rats</th>
<th>Erosion index (mm) mean±S.E.</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>p.o.</td>
<td>15</td>
<td>13.7±2.1</td>
<td></td>
</tr>
<tr>
<td>CL-1700</td>
<td>1000 p.o.</td>
<td>15</td>
<td>2.1±0.6*</td>
<td>84.7</td>
</tr>
<tr>
<td>L-carnosine</td>
<td>689 p.o.</td>
<td>15</td>
<td>5.3±0.8*</td>
<td>61.3</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine</td>
<td>817 p.o.</td>
<td>15</td>
<td>3.3±1.0*</td>
<td>75.9</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>238 p.o.</td>
<td>15</td>
<td>13.1±2.5</td>
<td>4.4</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine+</td>
<td>817 p.o.</td>
<td>15</td>
<td>7.1±1.5*</td>
<td>48.2</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>238 p.o.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance as compared to the control, *P<0.05.

of N-acetyl-L-carnosine at 817 mg/kg and Al(OH)₃ at 238 mg/kg had any appreciable effects on the erosions when they were given p.o.

Histamine-induced gastric erosions: CL-1700 at 1,000 mg/kg p.o. potently inhibited the development of gastric erosions induced by histamine by 84.7% (Table 4). Both L-carnosine at 689 mg/kg p.o. and N-acetyl-L-carnosine at 817 mg/kg p.o. also had potent inhibitory effects on the formation of erosions with inhibitions of 61.3% and 75.9%, respectively. Al(OH)₃ at 238 mg/kg p.o. had no effect on the erosions. The combination of N-acetyl-L-carnosine at 817 mg/kg and Al(OH)₃ at 238 mg/kg p.o.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose &amp; Route</th>
<th>No. of rats</th>
<th>Volume</th>
<th>pH</th>
<th>Acid output</th>
<th>Pepsin output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/kg)</td>
<td></td>
<td>ml/rat</td>
<td></td>
<td>mg/hr</td>
<td>mg tyrosine/hr</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>15</td>
<td>7.0±0.6</td>
<td>1.4</td>
<td></td>
<td>18.9±1.6</td>
</tr>
<tr>
<td>Control</td>
<td>1000</td>
<td>15</td>
<td>11.8±0.8*</td>
<td>3.3</td>
<td></td>
<td>19.6±1.8</td>
</tr>
<tr>
<td>CL-1700</td>
<td>689</td>
<td>15</td>
<td>10.1±0.6*</td>
<td>1.5</td>
<td></td>
<td>22.7±1.5</td>
</tr>
<tr>
<td>L-carnosine</td>
<td>817</td>
<td>15</td>
<td>9.8±0.4*</td>
<td>1.4</td>
<td></td>
<td>24.1±1.0*</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine</td>
<td>238</td>
<td>15</td>
<td>9.1±0.8</td>
<td>1.4</td>
<td></td>
<td>20.0±1.6</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>817+238</td>
<td>15</td>
<td>7.5±0.6</td>
<td>1.4</td>
<td></td>
<td>21.9±1.3</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine + Al(OH)₃</td>
<td>i.p.</td>
<td>10</td>
<td>7.4±0.9</td>
<td>1.2</td>
<td>87.6±12.9</td>
<td>19.9±2.4</td>
</tr>
<tr>
<td>CL-1700</td>
<td>600</td>
<td>10</td>
<td>5.4±0.7</td>
<td>1.4</td>
<td>51.7±9.3*</td>
<td>14.1±2.1</td>
</tr>
<tr>
<td>L-carnosine</td>
<td>414</td>
<td>10</td>
<td>8.0±1.0</td>
<td>1.2</td>
<td>97.0±15.9</td>
<td>21.3±3.1</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine</td>
<td>490</td>
<td>10</td>
<td>7.2±0.7</td>
<td>1.2</td>
<td>86.1±9.7</td>
<td>18.9±1.4</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>490+143</td>
<td>10</td>
<td>7.7±0.9</td>
<td>1.2</td>
<td>88.1±12.6</td>
<td>18.5±1.8</td>
</tr>
<tr>
<td>N-acetyl-L-carnosine + Al(OH)₃</td>
<td>i.d.</td>
<td>10</td>
<td>5.2±0.8</td>
<td>1.4</td>
<td>52.8±11.1</td>
<td>31.0±1.9</td>
</tr>
<tr>
<td>Control</td>
<td>1000</td>
<td>10</td>
<td>8.1±0.8</td>
<td>1.2</td>
<td>99.1±12.8</td>
<td>21.8±1.9</td>
</tr>
<tr>
<td>CL-1700</td>
<td></td>
<td></td>
<td>6.7±0.5</td>
<td>1.2</td>
<td>79.3±6.8</td>
<td>19.6±1.6</td>
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</table>

All values represent the mean±S.E. Statistical significance as compared to the controls, *P<0.05.
significantly inhibited (48.2%) the development of the erosions.

**Gastric secretion**

The intragastric administration of CL-1700 at 1,000 mg/kg significantly increased the volume of gastric juice and raised the pH value in pylorus-ligated rats (Table 5). The agent had no effect on pepsin output. Both L-carnosine at 689 mg/kg p.o. and N-acetyl-L-carnosine at 817 mg/kg p.o. significantly increased the volume of gastric juice, but they had no effect on the pH value. However, N-acetyl-L-carnosine at 238 mg/kg p.o. significantly increased the pepsin output as compared to the control. Neither Al(OH)₃ at 238 mg/kg p.o. nor the combination of N-acetyl-L-carnosine at 817 mg/kg and Al(OH)₃ at 238 mg/kg p.o. had any significant effects on the gastric secretion (volume, pH and pepsin output). CL-1700 at 600 mg/kg i.p. tended to reduce the volume and pepsin output, but significantly reduced the acid output. Neither L-carnosine at 414 mg/kg i.p., N-acetyl-L-carnosine at 490 mg/kg i.p. nor Al(OH)₃ at 143 mg/kg i.p. had any effects on gastric secretion. The combination of N-acetyl-L-carnosine at 490 mg/kg and Al(OH)₃ at 143 mg/kg i.p. tended to decrease the volume and acid output, but significantly reduced the pepsin output. CL-1700 at 1,000 mg/kg i.d. had no effects on gastric secretion (volume, pH, acid and pepsin output).

**Chronic gastric ulcers**

CL-1700 at 300 mg/kg, twice daily, had no effects on the healing of acetic acid-induced gastric ulcers with regard to both the index and incidence (Table 6). However, the agent at 1,000 mg/kg, twice daily, tended to accelerate the healing of gastric ulcers and significantly increased the number of animals with healed ulcers as compared to that in the control group. Aluminum sucrose sulfate at 100 mg/kg, twice daily, tended to accelerate the healing of ulcers but 300 mg/kg of this agent, twice daily, significantly accelerated the healing of ulcers with respect to the index. Gefarnate at 100 mg/kg, twice daily, showed a tendency to delay the healing of the ulcers with respect to both the index and incidence. The administration of 300 mg/kg of the agent, twice daily, had a weak effect on the ulcer index, but decreased the number of animals

<table>
<thead>
<tr>
<th>Table 6. Effects of CL-1700, aluminum sucrose sulfate, gefarnate, and cimetidine on healing of acetic acid-induced gastric ulcers in rats</th>
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<tbody>
<tr>
<td><strong>Treatment</strong></td>
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<tr>
<td>Control</td>
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<tr>
<td>CL-1700</td>
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<td>Control</td>
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<td>Aluminum sucrose sulfate</td>
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<td>Gefarnate</td>
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<td>Control</td>
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<tr>
<td>Cimetidine</td>
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</tbody>
</table>

Statistical significance as compared to the controls, *P<0.05 (t-test), **P<0.05 (χ²-test).
with healed ulcers in contrast to the control. While cimetidine at 10 mg/kg, twice daily, had a weak effect on both ulcer index and incidence, and 100 mg/kg of this agent, twice daily, tended to accelerate the healing, especially the index.

**DISCUSSION**

The present studies confirm our previous findings (1) that CL-1700, given either p.o. or i.p., markedly inhibits the development of various types of acute gastric lesions in rats. The mechanism by which CL-1700 exerts its anti-lesion effects remains unknown. As provided in the present study, the administration of CL-1700 at 1,000 mg/kg p.o. or at 600 mg/kg i.p. increased the volume of gastric secretion and raised its pH or reduced acid output in pylorus-ligated rats. We also found in an additional study that CL-1700 at 600 mg/kg given p.o. and at 300 mg/kg given i.p. raised the pH value and significantly reduced the acid output, respectively. Shay ulcers or gastric lesions induced by water-immersion stress, aspirin, indomethacin, or histamine are inhibited by potent antisecretory agents such as anticholinergic agents or histamine H₂-receptor antagonists (3–5). Thus, the anti-lesion activity of CL-1700 might be partly caused by its antisecretory properties. Since CL-1700 given i.p. showed its effects on Shay ulcers and stress-induced gastric lesions, it is likely that the agent exerts its anti-lesion effect systemically.

As new findings, we found that the constituents of CL-1700, i.e., L-carnosine, N-acetyl-L-carnosine, and Al(OH)₃ or the combination of N-acetyl-L-carnosine and Al(OH)₃ at the doses contained in the effective doses of CL-1700 had little or no effects on Shay ulcers, indomethacin-induced erosions (p.o.), or water-immersion stress-induced erosions (i.p.). This fact indicates that the entire molecule of CL-1700 is essential for exertion of its full anti-lesion effect on the above lesions.

Some constituents, however, had marked inhibitory effects on Shay ulcers and aspirin- or histamine-induced erosions. Concerning Shay ulcers, the i.p. administration of Al(OH)₃ and the combination of N-acetyl-L-carnosine and Al(OH)₃ significantly inhibited the ulcers; the degrees of inhibition in these two groups were almost the same. It should be noted that Al(OH)₃ i.p. at the dose which inhibited Shay ulcers had no effect on gastric secretion in pylorus-ligated rats. Therefore, the reason that Al(OH)₃ inhibits Shay ulcers is unknown. Concerning the aspirin-induced erosions, L-carnosine, N-acetyl-L-carnosine, and the combination of N-acetyl-L-carnosine and Al(OH)₃ potently inhibited the erosions as effectively as CL-1700. This finding suggests that the active part of CL-1700 might be the L-carnosine molecule itself. Since the anti-lesion dose of L-carnosine on aspirin-induced erosions did not appreciably affect the pH and pepsin output, the inhibitory effect of L-carnosine on aspirin-induced erosions might be unrelated to the gastric secretion. It is likely that L-carnosine might strengthen the so-called gastric mucosal barrier by some means. With respect to the histamine-induced gastric erosions, both L-carnosine and N-acetyl-L-carnosine had a potent inhibition on the erosions even though the degree of inhibition was slightly weaker than that of CL-1700. N-acetyl-L-carnosine might be the main active part of CL-1700, at least with respect to the inhibition of histamine-induced erosions. However, we cannot explain the reason why the combination of N-acetyl-L-carnosine and Al(OH)₃ showed a weaker inhibition on histamine-induced erosions in contrast to N-acetyl-L-carnosine alone. On the whole, it was apparent that CL-1700 appears to be much more effective in preventing acute gastric lesions than its constituents. L-carnosine is originally reported to be
related to the repair mechanism of vital tissue (6, 7). Recently, it was found that CL-1700 had also accelerated wound healing in rat skin (unpublished). Thus, it was expected that CL-1700 might enhance the healing of a chronic type of gastric ulcer. When CL-1700 was given twice daily for 3 weeks to rats with acetic acid ulcers, the agent diminished the size of the ulcerated area and increased the number of completely healed ulcers as compared to the control group. The dose of CL-1700 which accelerated the healing of the ulcers is the dose which raised the pH in the gastric juice in pylorus-ligated rats. In contrast, the i.d. administration of CL-1700 at 1,000 mg/kg had no effect on gastric secretion in pylorus-ligated rats. Therefore, the mechanism of action of CL-1700 in accelerating acetic acid ulcers is likely to occur partially by gastric inhibition and partially by some other unknown action. Whether or not CL-1700 speeds up the regeneration of epithelial cells themselves is the subject of ongoing studies. In the previous report, we have determined the effects of CL-1700 on existing ulcers were slightly weaker than those of aluminum sucrose sulfate but stronger than those of cimetidine and gefarnate. This finding was almost the same as that obtained in acute gastric lesions.

We conclude that CL-1700 is a promising drug for the treatment of acute and chronic types of peptic ulcers in humans.

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