INFLUENCE OF L-GLUTAMINE ON ASPIRIN-INDUCED GASTRIC LESIONS AND ABSORPTION AS WELL AS ANTIPYRETIC, ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF ASPIRIN IN RATS AND MICE

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Abstract—L-glutamine markedly suppressed the development of the gastric lesions induced by aspirin in pylorus-ligated rats. In non-ligated normal rats, aspirin was absorbed rapidly after administration and was maintained in the blood in high salicylate concentration thereafter. When aspirin was administered in combination with L-glutamine, the absorption of aspirin was at nearly the same level as when aspirin had been given alone. In pylorus-ligated rats, administration of aspirin was followed by slow increment in blood salicylate concentration. Blood salicylate level in these rats was higher when aspirin was administered in combination with L-glutamine than when it had been given alone. Combined administration of aspirin and L-glutamine produced little influence on the antipyretic, analgesic and anti-inflammatory effects of aspirin in non-ligated normal rats.

It is a well-known fact that aspirin, which is commonly used as an antipyretic, analgesic and anti-inflammatory drug, induces gastric lesions (1, 2, 3). Okabe and his colleagues previously reported that L-glutamine was remarkably effective in suppressing the development of the gastric lesions induced by aspirin (4), suggesting that L-glutamine may prevent a back diffusion of gastric acid, as reported by Davenport (5).

In the present study, an attempt was made to confirm the effect of L-glutamine on aspirin-induced gastric lesions as well as the influence of combined aspirin and L-glutamine on absorption and pharmacological actions of aspirin.

MATERIALS AND METHODS

Effect of L-glutamine on aspirin induced gastric lesions

Gastric lesions were produced in male Wistar strain rats (180-220 g) according to the method by Okabe et al. (4). Rats were kept in individual cages and were deprived of food but water was allowed ad libitum for 24 hr, after which they were anesthetized with ether and the pylorus of each was ligated (6). The animals were administered either aspirin (100 mg/kg) alone or aspirin plus L-glutamine (1,000 mg/kg) suspended in
1% carboxymethyl-cellulose (CMC) solution. They were sacrificed with an overdose of ether 40, 100, 160, 240 and 420 min after dosing aspirin with or without L-glutamine. Ten min before sacrifice, the animals were injected through the tail vein with 1 ml of a 5% solution of Pontamine sky blue 6 BX (pH 7.0) (7). After sacrificing the animals, the stomach of each was removed, treated with 1% formalin solution and observed under a dissecting microscope (10 X) with a square grid for examination of the surface of the gastric mucosa. The length of each lesion was measured and the sum of the length of the lesions for each animal was referred to as an ulcer index (mm).

Effect of L-glutamine on absorption of aspirin

Male Wistar strain rats weighing 280 to 320 g were divided into groups of 12. Non-ligated normal and pylorus-ligated rats were fixed in the supine position and a cannula was inserted into the carotid artery under ether anesthesia. After recovery from anesthesia, the rats were orally given 1% CMC solution or L-glutamine (1,000 mg/kg) suspended in 1% CMC solution and 10 min later, aspirin (100 mg/kg) was administered. Before and after administration of aspirin, approx. 50 μl of blood was collected from each rat using a glass capillary and centrifuged for 5 min at 8,000 r.p.m. to separate the plasma. Salicylate concentration in 20 μl of plasma was determined in accordance with the method of Lever and Powell (8).

Effect of L-glutamine on antipyretic action of aspirin

To male Wistar rats weighing 90 to 100 g, yeast (Brewers Yeast, Nutritional Biochem. Co.) suspended in physiological saline at the concentration of 250 mg/ml was injected s.c. in the back in an amount of 2.5 ml/rat according to the method of Gleen et al. (9) and the animals were deprived of food. After 17 hr, aspirin (200 mg/kg) and L-glutamine were suspended in 1% CMC solution and given orally separately or in combination to the rats whose rectal temperature had increased to 38°C or over. Rectal temperature was measured 60, 120, 240 and 360 min after medication.

Effect of L-glutamine on analgesic action of aspirin

Acetic acid writhing test: Aspirin (300 mg/kg) or aspirin plus L-glutamine (1,000 mg/kg) was given orally to male dd strain mice weighing 20±1 g. One hr later, 0.2 ml of 0.7% acetic acid was given i.p. in accordance with the method of Koster et al. (10). After 10 min, the frequency of writhing reactions induced by acetic acid was determined for 10 min.

Aconitine writhing test: Male dd strain mice (20±1 g) were fasted for 24 hr and given aspirin (300 mg/kg), L-glutamine (1,000 mg/kg) or aspirin plus L-glutamine. After 50 min, 3 μg of aconitine was administered i.p. by the method of Bhalla et al. (11). The animals were observed for 10 min after aconitine administration. The reaction was evaluated on the basis of "all-or-none criteria", that is, one or more writhing(s) during the observation period was referred to as a positive reaction.

Effect of L-glutamine on anti-inflammatory action of aspirin

The animals used were male Donryu strain rats weighing 90 to 100 g. They were given aspirin (200 or 400 mg/kg), L-glutamine (1,000 mg/kg) or aspirin plus L-glutamine
by the oral route and one hr later a phlogistic agent (1% carrageenin solution) was injected s.c. into the hind paw in accordance with the method of Yamasaki et al. (12). Swelling was measured 1, 3 and 5 hr after injection. The level of significance was calculated by using Student's t-test.

RESULTS

Effect of L-glutamine on aspirin-induced gastric lesions

As shown in Fig. 1, slight lesions were detected on the gastric mucosa 40 min after administration of 100 mg/kg of aspirin and these grew progressively severe with the elapse of time. L-glutamine demonstrated a significant inhibition on the development of aspirin lesions 40, 100 and 160 min, 4 and 7 hr after administration (p<0.001).

Effect of L-glutamine on absorption of aspirin

As shown in Fig. 2, in non-ligated normal rats, plasma salicylate level at 5 and 10 min after administration was significantly lower in the group given aspirin plus L-glutamine than in the group given aspirin alone (p<0.05); the level reached the peak at 40 min in both groups and then slowly decreased until the 7th hr, the level being maintained higher in rats dosed with aspirin plus L-glutamine than in those dosed with aspirin alone. As shown in Fig. 3, in pylorus-ligated rats, the results were different from those in non-ligated normal rats. Plasma salicylate level increased slowly for 7 hr after the dosing with aspirin, while the level was significantly higher in the aspirin plus L-glutamine group than in the aspirin alone group 20 min after administration and thereafter, reaching the peak at 6 hr. However there was no significant difference in the level between two groups at the 7th hr (p>0.05).
As shown in Fig. 4, L-glutamine (1,000 mg/kg) suspended in 1% CMC solution demonstrated no apparent antipyretic effect, while aspirin (200 mg/kg) showed a significant antipyretic action (p<0.01) 2 hr after administration and the action persisted until the 6th hr. L-glutamine administered in combination with aspirin did not exert any influence on the antipyretic action of aspirin.

**Effect of L-glutamine on antipyretic action of aspirin**

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Effect of L-glutamine on analgesic action of aspirin

Acetic acid writhing test: The results are shown in Table 1. Oral administration of aspirin (300 mg/kg) significantly suppressed writhing reactions caused by acetic acid (52.7%, p<0.01). L-glutamine plus aspirin slightly reduced analgesic action of aspirin but it also suppressed the writhing reactions with a statistical significance (38.1%, p<0.01)

**TABLE 1. Influence of aspirin and aspirin plus L-glutamine on writhing response caused by acetic acid in mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Writhing response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% CMC×2)</td>
<td>37</td>
<td>21±2</td>
</tr>
<tr>
<td>Aspirin (300 mg/kg)</td>
<td>39</td>
<td>10±1*</td>
</tr>
<tr>
<td>Aspirin (300 mg/kg) + L-glutamine (1000 mg/kg)</td>
<td>38</td>
<td>13±2*</td>
</tr>
</tbody>
</table>

Drugs were given orally. *Significantly different from control p<0.01.

**TABLE 2. Influence of aspirin and aspirin plus L-glutamine on writhing response caused by aconitine in mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Suppression ratio of writhing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% CMC×2)</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>L-glutamine (1000 mg kg)</td>
<td>29</td>
<td>38</td>
</tr>
<tr>
<td>Aspirin (300 mg kg)</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>Aspirin (300 mg kg) + L-glutamine (1000 mg kg)</td>
<td>29</td>
<td>48</td>
</tr>
</tbody>
</table>

Drugs were given orally.
as compared with the control. The frequency of writhing reactions did not differ significantly between the aspirin alone group and aspirin plus L-glutamine group (p > 0.05).

**Aconitine writhing test:** As shown in Table 2, it was found that L-glutamine (1,000 mg/kg) inhibited to some extent writhing reactions caused by aconitine. Aspirin (300 mg/kg) showed a significant suppression of the writhing reactions (50%, p < 0.001). The suppression ratio was unvaried by L-glutamine administered in combination with aspirin (48%, p < 0.01).

**Effect of L-glutamine on anti-inflammatory action of aspirin**

The results are shown in Table 3. L-glutamine (1,000 mg/kg) did not exert any influence on the development of carrageenin edema 5 hr after administration of carrageenin, while aspirin at 200 and 400 mg/kg showed a significant prevention of the edema (p < 0.01) as compared with the control. The anti-edematous effect of aspirin was unaffected by L-glutamine combined with aspirin.

**TABLE 3. Influence of aspirin, L-glutamine and aspirin plus L-glutamine on rat paw edema caused by carrageenin**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Swelling ratio (%) (Mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% CMC×2)</td>
<td>20</td>
<td>123.9±7.0</td>
</tr>
<tr>
<td>L-glutamine (1000 mg/kg)</td>
<td>10</td>
<td>117.2±4.7</td>
</tr>
<tr>
<td>Aspirin (200 mg/kg)</td>
<td>20</td>
<td>88.5±4.2*</td>
</tr>
<tr>
<td>Aspirin (200 mg/kg) + L-glutamine (1000 mg/kg)</td>
<td>20</td>
<td>86.3±8.5*</td>
</tr>
<tr>
<td>Aspirin (400 mg/kg)</td>
<td>20</td>
<td>65.6±5.0*</td>
</tr>
<tr>
<td>Aspirin (400 mg/kg) + L-glutamine (1000 mg/kg)</td>
<td>20</td>
<td>53.5±6.8*</td>
</tr>
</tbody>
</table>

Aspirin and L-glutamine were given orally. *Significantly different from control p < 0.01.

**DISCUSSION**

In the present experiment, it was confirmed that L-glutamine exerts a marked inhibition on gastric lesions induced by aspirin. Okabe et al. (4) consider that L-glutamine may prevent the back diffusion of gastric acid induced by aspirin. There is another suggestion concerning the mechanism of action of L-glutamine in which L-glutamine may inhibit absorption of aspirin into the gastric mucosa. Gottschalk and Menguy (13) and Brodie and Chase (14) have reported that aspirin once absorbed into the body after parenteral administration damages the gastric mucosa.

Blood salicylate level after administration of aspirin combined with L-glutamine was determined to clarify whether or not L-glutamine inhibits absorption of aspirin. It was found that both in non-ligated normal and pylorus-ligated rats, absorption of aspirin was unaffected by the combined administration with L-glutamine and that blood salicylate
level increased significantly in pylorus-ligated rats 40 min after administration of aspirin plus L-glutamine. In other words, the absorption of aspirin was accelerated with the coexistence of L-glutamine. From the above results, it is assumed that the effect of L-glutamine on aspirin-induced gastric lesions is not due to inhibition of aspirin absorption.

In pylorus-ligated rats, development of aspirin-induced gastric lesions appears to correlate with the time-course change of the blood salicylate level. However, blood salicylate level cannot be expected to play an important role in the mechanism by which L-glutamine prevents gastric lesions, as the level is maintained high even after the combined administration of L-glutamine with aspirin. Pfeiffer and Lewandowski (15) also denied correlation between aspirin-induced gastric lesions and blood salicylate level.

Blood salicylate level at various times after administration of aspirin in non-ligated normal rats is quite different from that in pylorus-ligated rats. In non-ligated normal rats, aspirin is rapidly absorbed by the small intestine, while it is slowly absorbed by the stomach in pylorus-ligated rats so that blood salicylate level increases with elapse of time. Okabe et al. have found that L-glutamine markedly inhibits gastric lesions caused by aspirin in non-ligated normal rats (16). Whether or not the high blood salicylate level at 5 to 40 min after administration of aspirin in non-ligated normal rats, as observed in the present experiment, plays a role in the etiology of aspirin-induced lesions remains to be elucidated.

A high blood salicylate level determined after the combined administration of aspirin and L-glutamine suggests that aspirin fully exerts its pharmacological actions even in the presence of L-glutamine. In fact, the present experiment verified that L-glutamine has little influence on anti-pyretic, analgesic and anti-inflammatory actions of aspirin.

Since L-glutamine proved to have a strong inhibitory effect on aspirin-induced gastric lesions and pharmacological actions of aspirin were not abolished by addition of this agent, a combined administration appears to be a favorable choice in the clinical application of aspirin.

REFERENCES


5) Davenport, H.W.: Gastroenterology, 46, 245 (1964)


