PATHOGENESIS OF GASTRIC LESIONS INDUCED
BY ASPIRIN IN THE PYLORUS-LIGATED RAT

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Abstract—A standard method for the production of gastric lesions by aspirin in rats
was elaborated, and the mechanisms of the deleterious effects of aspirin were inter-
preted. The method consisted of pylorus ligation of the rat immediately before as-
pirin dosing, resulting in severe and consistent gastric lesions in the glandular por-
tion of the rat stomach 7 hr later. Sodium bicarbonate and L-glutamine showed a
strong inhibitory effect on the development of aspirin-induced lesions, at almost the
same dose level. Aspirin itself reduced the gastric acidity in pylorus-ligated rats.
Sodium bicarbonate with or without aspirin markedly lowered the gastric acidity,
whereas L-glutamine with or without aspirin restored the reduced acidity by aspirin
or increased the acidity more than the normal level. These findings suggest that L-
glutamine may inhibit the back diffusion of HCl into the gastric mucosa caused by
aspirin. Amylopectine sulfate and sulfated glyptide, in a dose sufficient to suppress
the peptic activity of gastric contents, slightly inhibited the aspirin-induced lesions.
Atropine sulfate, which strongly reduced gastric juice volume but not acidity, did not
exert a marked influence on aspirin-induced lesions.

It is well known that aspirin is capable of damaging the gastric mucosa in humans and
experimental animals, and that this can be detected by a variety of techniques (1, 2). In
animals, however, the incidence and severity of gastric lesions induced by aspirin was re-
portedly rather weak at the time of autopsy. This fact renders the quantitative measure-
ment of each lesion in the glandular stomach quite difficult, and thus observations of the
damage have been performed grossly, frequently employing “all or none” criteria.

In a recent review, Cooke (2) concluded that the basis of aspirin damage to the gastric
mucosa is the presence of acid in the lumen of the stomach. Thus, an attempt was made
to aggravate the aspirin-induced lesions in the rat, by means of ligation of the pylorus im-
mediately prior to aspirin dosing, allowing for an easier quantitation of the lesions and in-
crease in sensitivity of test procedure. By this method hypersecreted gastric juice is accu-
cumulated in the stomach and at the same time the emptying of aspirin into the duodenum
is completely prevented. Quite recently, Johnson (3) reported that salicylic acid signifi-
cantly increased the pepsin output in dogs as a result of accelerated back diffusion of HCl.
Several pharmacologic agents, including antipeptic agents, were thus examined for their
effects on “standardized” gastric lesions, as well as on gastric secretion in order to test the
concept of aspirin-induced damage.
MATERIALS AND METHODS

Male Donryu strain rats, weighing 195-215 g, were deprived of food but allowed free access to water for 24 hr prior to experiments.

Induction of aspirin-induced lesions: After fasting, the animals were divided into 4 groups: group 1 was subjected to gastric intubation of aspirin (100 mg/kg) suspended in 1% carboxymethylcellulose (CMC) solution; Group 2 was subjected to aspirin at the same dose and route immediately after laparotomy; Group 3 was subjected to aspirin immediately after pylorus ligation and Group 4 was subjected to 1% CMC solution given immediately after the pylorus ligation. The dosage of aspirin was based upon previous data published by Brodie and Chase (4) and Pfeiffer and Lewandowski (5). Ligation of the pylorus was done according to Shay's original method (6), and ether anesthesia was used. Seven hr later the animals were sacrificed by an overdose of ether. Ten min before death, the animals (while under ether anesthesia) were injected with 1 ml of a 5% solution of pontamine sky blue 6 BX dissolved in saline (adjusted the pH to 7.2 with 0.5 N HCl) i.v., in the femoral vein, according to the method of Brodie, Tate and Hooke (7). After sacrificing the animals, the stomach of each was removed, then slightly inflated by injecting 1% formalin solution through the esophageal junction, and immersed in 1% formalin solution for 10 min to fix the inner and outer layers of the gastric wall (8). Subsequently, the stomachs were incised along the greater curvature and examined for the presence of gastric hemorrhagic areas and mucosal defects without hemorrhages. The length of each lesion, dark blue areas, or clearly demarcated mucosal defects against a pale blue background, were measured under the dissecting microscope (10 X) with a square grid. The sum of the lengths of all lesions for each animals was used as an ulcer index. In order to evaluate the effects of pharmacologic agents upon the aspirin-induced lesions, the next group of rats were given the following agents by gastric intubation immediately after pylorus ligation; sodium bicarbonate and L-glutamine, both suspended in 1% CMC solution; amyllopectine sulfate (Nihonkayaku), and sulfated glyptide (Kyowa) were dissolved in saline and given by gastric intubation; atropine sulfate (Merck) was dissolved in saline and injected subcutaneously. Corresponding control solutions were given orally or subcutaneously. Ten min after dosing by each agent, aspirin 100 mg/kg was given orally. Seven hr after aspirin treatment, the animals were sacrificed and the stomachs were treated as described above in order to evaluate the lesions.

Gastric secretion: In order to determine the effect of aspirin alone, aspirin plus specific other agents or other agents themselves on gastric secretion, the pylorus ligation preparation was also employed by applying the same time schedule (7hr ligation after 24 hr fasting), the only difference being that the intravenous injection of PSB 6 BX solution was not used. As the control, a corresponding volume of 1% CMC solution was given orally. The combined treatments of aspirin and the various agents described above were given in order to evaluate whether or not aspirin would interfere with the effects of these drugs in the stomach, due to some physical or chemical interaction among the agents. Atropine
sulfate was given to rats without dosing with aspirin as the agent was injected s.c. so as to exclude any interaction. Seven hr after pylorus ligation, the animals were sacrificed by an overdose of ether, and the gastric contents were collected. After centrifugation, samples were analyzed for volume and titrated with 0.1 N NaOH to pH 7.4 on the Hitachi electromatic pH meter for titratable acidity, which is expressed as mEq/L. The pepsin concentration was determined by Anson's hemoglobin method (9), and was expressed as mg pepsin per ml. The level of significance was calculated by using Student's t-test (10).

RESULTS

Influence of pylorus ligation on gastric lesions induced by aspirin

Aspirin 100 mg/kg, given to intact rats, evoked inconsistent and rather vaguely demarcated damage to the glandular portion of both the gastric fundus and antrum; 3 out of 20 rats had negligible or no lesions (Fig. 1). In laparotomized rats, however, aspirin produced a significantly lesser degree of lesion formation in comparison with those of the normal rats (61.9% reduction) on aspirin. The lesion index was not significantly different from that observed in pylorus ligation alone (no aspirin) in which 9 out of 15 rats subjected to 1%
CMC solution had minute lesions in the stomach. In contrast, aspirin-induced lesions were strongly aggravated by ligation of the pylorus in all animals as compared with those of the laparotomized rats (82.4% aggravation) or even with those of the normal rats (53.7% aggravation); these values being highly significant (P<0.001). In these cases, the gastric lesions consisted of severe hemorrhagic erosions of various sizes and mucosal defect without hemorrhages, both of which were clearly defined from the surrounding intact mucosa by a difference in the color of the mucosal surface (Fig. 2). The forestomach of the rats, dosed either with aspirin or 1% CMC solution, was seldom, if ever, influenced by this procedure.

**Effects of several agents on aspirin-induced lesions in pylorus-ligated rats**

**Antacid:** As expected, sodium bicarbonate had a strong inhibitory effect on aspirin-induced lesions at dose levels of 750 and 250 mg/kg, and at 750 mg/kg, only a few erosions

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![Fig. 2. Gastric lesions induced by aspirin at 100 mg/kg (P.O.) in a pylorus ligated rat (7 hr ligation after 24 hr fasting).](image)

![Fig. 3. Effects of several pharmacologic agents on aspirin-induced gastric lesions in pylorus-ligated rats. Ten to seventeen rats were used for each experiment.](image)
were observed on the surface of the gastric mucosa (94.7% inhibition) (Fig. 3). In addition, even a low dose of the agent, such as 83.3 mg/kg, remarkably inhibited the lesion formation (67.0% inhibition).

**Amino acid:** L-glutamine elicited a significant inhibition of aspirin-induced lesions at 750 mg/kg (86.6% inhibition), which was comparable to that of sodium bicarbonate. Even at 83.3 mg/kg, L-glutamine had a considerable effect on aspirin-induced lesions (38.3% inhibition).

**Antipeptic agents:** Amylopectine sulfate at 600 and 200 mg/kg, showed a lesser degree of inhibitory effects on aspirin-induced lesions, but the inhibition was statistically significant at 66.7 mg/kg (32.1% inhibition). Likewise, sulfated glyptide, at 200 mg/kg, had a weak effect though significant (P<0.05) on aspirin-induced lesions.

**Anticholinergic agents:** Atropine sulfate at 10 mg/kg did not significantly inhibit (28.8% inhibition) the aspirin-induced lesions. In contrast to the normal appearance of aspirin-induced lesions, most of the lesions observed after atropine sulfate administration consisted of mucosal defects without hemmorrhages.

**Table 1. Effects of several agents on gastric secretion in pylorus-ligated rats (7 hr ligation after 24 hr fasting).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose &amp; route</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Volume (ml)</th>
<th>Gastric contents (mEq/l)</th>
<th>Pepsin (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% CMC)*</td>
<td>P.O. 17</td>
<td>183</td>
<td>12.2±0.5</td>
<td>118.6±4.6</td>
<td>27.4±0.6</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>P.O. 17</td>
<td>188</td>
<td>12.4±0.3</td>
<td>67.9±4.8**</td>
<td>28.0±0.5</td>
<td></td>
</tr>
<tr>
<td>Control (Aspirin 1% CMC): Sodium bicarbonate</td>
<td>P.O. 10</td>
<td>183</td>
<td>12.5±0.5</td>
<td>65.1±5.7</td>
<td>31.8±1.0</td>
<td></td>
</tr>
<tr>
<td>Aspirin + Sodium bicarbonate</td>
<td>P.O. 10</td>
<td>188</td>
<td>12.6±0.4</td>
<td>22.6±3.4**</td>
<td>18.1±1.0***</td>
<td></td>
</tr>
<tr>
<td>83.3 P.O. 10</td>
<td>180</td>
<td>12.2±0.5</td>
<td>82.5±6.5</td>
<td>27.2±0.8**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin 1-glutamine</td>
<td>P.O. 10</td>
<td>185</td>
<td>12.0±0.4</td>
<td>128.6±2.7**</td>
<td>26.8±0.7**</td>
<td></td>
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<tr>
<td>83.3 P.O. 10</td>
<td>183</td>
<td>12.4±0.5</td>
<td>92.2±5.6**</td>
<td>23.5±0.5**</td>
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<td></td>
</tr>
<tr>
<td>Control (1% CMC)</td>
<td>P.O. 8</td>
<td>179</td>
<td>13.3±0.6</td>
<td>118.5±3.2</td>
<td>27.4±0.6</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>P.O. 8</td>
<td>179</td>
<td>13.1±0.5</td>
<td>47.6±3.1***</td>
<td>18.5±1.5***</td>
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</tr>
<tr>
<td>250 P.O. 8</td>
<td>185</td>
<td>13.5±0.7</td>
<td>93.4±1.8***</td>
<td>21.0±1.4**</td>
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<td></td>
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<tr>
<td>83.3 P.O. 8</td>
<td>181</td>
<td>13.8±0.4</td>
<td>103.6±1.9***</td>
<td>27.5±0.9</td>
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<tr>
<td>1-glutamine</td>
<td>P.O. 8</td>
<td>179</td>
<td>13.5±0.3</td>
<td>143.8±2.2**</td>
<td>25.3±0.2*</td>
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<tr>
<td>83.3 P.O. 8</td>
<td>186</td>
<td>13.3±0.4</td>
<td>120.3±2.8</td>
<td>23.3±0.6***</td>
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<tr>
<td>Control (Aspirin + saline)</td>
<td>P.O. 16</td>
<td>183</td>
<td>12.3±0.5</td>
<td>87.5±6.1</td>
<td>30.1±1.4</td>
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<tr>
<td>Aspirin Amylopectine sulfate</td>
<td>P.O. 10</td>
<td>184</td>
<td>12.3±0.6</td>
<td>56.2±3.2***</td>
<td>15.0±0.8***</td>
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<tr>
<td>66.7 P.O. 10</td>
<td>179</td>
<td>10.3±0.4**</td>
<td>51.3±3.8***</td>
<td>14.9±2.0***</td>
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<td></td>
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<tr>
<td>Aspirin Sulfated glyptide</td>
<td>P.O. 12</td>
<td>190</td>
<td>10.8±0.7</td>
<td>79.7±3.7</td>
<td>8.7±1.8***</td>
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<tr>
<td>Control (saline) b</td>
<td>S.C. 10</td>
<td>182</td>
<td>11.1±0.3</td>
<td>129.6±4.8</td>
<td>30.0±0.5</td>
<td></td>
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<tr>
<td>Atropine sulfate</td>
<td>S.C. 10</td>
<td>187</td>
<td>2.4±0.5***</td>
<td>116.0±7.3</td>
<td>31.1±1.9</td>
<td></td>
</tr>
</tbody>
</table>

All figures represent mean ± SE. Significantly different from control (*P<0.05, **P<0.01, ***P<0.001).

a The volume given was 0.5 ml 100 g of body weight.

b The volume given was 0.2 ml 100 g of body weight.
Effects of aspirin and several agents on gastric secretion in pylorus-ligated rats

Aspirin alone: Aspirin at 100 mg/kg caused severe bleeding, consequently the gastric contents were found to be colored brown at the time of autopsy. Aspirin had no effect on the gastric volume and pepsin activity, but significantly reduced the acidity (42.7% reduction) in comparison with the control (Table I).

Aspirin plus antacid: Sodium bicarbonate at 750 mg/kg plus aspirin at 100 mg/kg considerably reduced the acidity and pepsin activity (P<0.001) as compared with control values, although the volume did not change. The gastric juice was quite clear and did not reveal any notable bleeding. Although the volume and pepsin values were slightly but significantly decreased with 250 mg/kg, the acidity showed little change. Sodium bicarbonate at 83.3 mg/kg did not change the volume but slightly increased the acidity. The pepsin activity was slightly but significantly reduced as compared with the control.

Aspirin plus amino acid: L-glutamine at 750 mg/kg given simultaneously with aspirin (100 mg/kg) prevented alteration in the acidity attributable to aspirin administration; however, the pepsin activity was reduced. Even at 83.3 mg/kg, a tendency to recovery was evident.

Antacid and amino acid: Sodium bicarbonate significantly (P<0.001) reduced the acidity dose dependently and reduced the pepsin activity at 750 mg/kg (P<0.001) and 250 mg/kg (P<0.01). The acidity of gastric juice of rats given L-glutamine at 750 mg/kg was found to be significantly higher than the control value but almost equal to the control at 83.3 mg/kg. Pepsin activity was slightly but significantly reduced at 750 (P<0.05) and 83.3 mg/kg (P<0.001) of L-glutamine.

Aspirin plus antipeptic agents: Amylopectine sulfate or sulfated glyptide given with aspirin significantly reduced pepsin activity (P<0.001) at the dose levels of 600, 200 and 66.7 mg/kg or 200 mg/kg, respectively. Amylopectine sulfate at 600 and 200 mg/kg also reduced the acidity significantly. At 200 mg/kg, the agent reduced the volume in comparison with the control treatment. At 66.7 mg/kg, the agent slightly but significantly (P<0.05) reduced the acidity. In contrast, sulfated glyptide slightly reduced the volume and acidity.

Anticholinergic agent: It was found that atropine sulfate at 10 mg/kg markedly reduced the volume (78.4% reduction), but that the acidity and pepsin activities were unchanged.

DISCUSSION

Aspirin-induced gastric lesions were strongly exacerbated in rats subjected to pylorus ligation in contrast to the normal or laparotomized animals. Several investigators have also demonstrated severe damage to the gastric mucosa of experimental animals by the addition of various amounts of HCl into the stomach of denervated dogs (11, 12) or rats (13), and pylorus ligation prior to aspirin dosing (5). Aspirin lesions were found to be significantly less severe after laparotomy in comparison with those produced in the normal rats. Moreover, the ulcer index in the former was not different from that observed after pylorus ligation in contrast to the normal or laparotomized animals.
Aspirin-induced gastric lesions in rats

Ligation alone (no aspirin). Menguy (14) and Brodie, Marshall and Moreno (15) have reported that stress situations decreased the gastric acidity in rats, therefore, laparotomy, which is a traumatic stress, may decrease the gastric acidity (basal secretion), leading to the suppressed formation of aspirin-induced lesions. On the other hand, pylorus ligation, which is empirically known to elicit a strong stimulation of gastric secretion during the early stage, appears to overcome the influence of laparotomy followed by the exacerbated development of aspirin lesions as reported in the present study.

In any case, the mechanisms of the acceleration of aspirin-induced gastric lesions by pylorus ligation are subject to the following explanations. One consideration is that accumulated, acidic gastric contents elicited the enhancement of the so-called back diffusion of HCl through the broken barrier in response to aspirin administration (16), resulting in severe damage to the gastric mucosa. In fact, the titratable acidity was found to be significantly lowered by aspirin in comparison with the control. Sodium bicarbonate, which reduced the acidity to a greater extent by neutralization, strongly prevented the aspirin-induced gastric lesions, dose dependently. This is consistent with the data by Brodie and Chase (4), Pfeiffer and Lewandowski (5), and Anderson (17), who reported marked inhibition of aspirin-induced gastric lesions by antacids in rats and guinea pigs. The administration of aspirin plus sodium bicarbonate resulted in a lower acidity than that of sodium bicarbonate alone, probably due to the fact that aspirin caused slight back diffusion of acid into the gastric mucosa in addition to the neutralization by the antacid. The decrease of pepsin activity is presumably caused by the lowered acidity of gastric juice.

In contrast to the findings of Brodie and Chase (4) and Anderson (17), atropine sulfate did not show any notable inhibitory effect on aspirin-induced lesions. However, the character of the lesions was considerably different from typical lesions, i.e., instead of severe hemorrhagic erosions superficial mucosal defects without hemorrhage were observed. Hollander (18) has reported that atropine sulfate reduced the volume of gastric juice but maintained the acidity at normal level in dogs. The present authors also confirmed the data presented by Hollander as to the effect of atropine sulfate. The lesser effect of atropine sulfate on the aspirin-induced lesions may be caused by quick absorption of aspirin from the stomach with a small amount of gastric juice but high acidity, leading to mucosal ulceration. Since the effect of aspirin plus atropine sulfate on gastric acidity was not determined herein, participation of back diffusion of acid is as yet not clear. These facts suggest that the enhanced formation of aspirin-induced lesions after pylorus ligation appear to be somewhat independent of the amount of gastric juice accumulated in the stomach, but rather dependent on the degree of acidity.

It is of great interest that an amino acid, L-glutamine, which has showed an accelerating activity on the healing process of stress-induced gastric ulcer in rats (19), strongly inhibited aspirin-induced lesions. The effect was found to be comparable to that induced by sodium bicarbonate at the same dose level. Simultaneous administration of aspirin plus L-glutamine in pylorus-ligated rats resulted in higher acidic gastric contents than that of control (aspirin +1% CMC). It is possible that L-glutamine prevented back diffusion...
of HCl into the gastric mucosa, thus inhibition of the development of aspirin-induced lesions took place. It should be noted that L-glutamine alone resulted in an evident higher acidity than that of control, suggesting that either prevention of back diffusion of acid occurring under a pylorus ligated condition or that stimulation of secretion was due to the amino acid. With preliminary in vitro experiments, there was convincing evidence that the titration of dilute HCl with L-glutamine or L-glutamine plus aspirin with dilute NaOH indicated a negligible increase of the acidity after incubation at 37°C for 7 hr. The participation of the proteolytic activity of pepsin was considered in addition to the acid factor in this lesion model, a factor that has seldom been taken into consideration with regard to the mechanisms of aspirin-induced lesions. Quite recently, Johnson has found that pepsin output was directly related to the back diffusion of hydrogen ions, and that this pepsin output may be increased in abnormally permeable mucosa, therefore, may be of etiologic significance in ulcer diseases. The present authors did not find that there was any appreciable stimulation of pepsin secretion with aspirin, as suggested by Johnson (20) with salicylic acid. Actually, amylpectine sulfate and sulfated glyptide significantly suppressed the pepsin activity, but the degree of lesion inhibition was considerably weak in comparison with the antacid. The doses of these agents were thought to be adequate enough to exert an antipeptic effect, as there was remarkable inhibition of Shay ulceration in rats, in which model, pepsin as well as acid presumably contributes to the pathogenesis, as reported at a relatively lower dose level (less than 50 mg/kg) by Bianchi and Cooke (21), and Prino et al (22). Accordingly, the role of pepsin as an aggressive factor in production of aspirin-induced gastric lesions is apparently minor. The decreased pepsin activity by L-glutamine and L-glutamine plus aspirin, therefore, appears to be insignificant in relation to the effect of L-glutamine.

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