Change in Formation of Gastric Lesions by Aspirin during Aging in Rats

Masayuki UCHIDA,*,*** Noriyuki MISAKI, Osamu KAWANO,*, Shingo YANO, and Kazuo WATANABE**

Research Laboratories, Grelan Pharmaceutical Co., Ltd.,* 12-3, Sakurashimmachi, Setagaya-ku, Tokyo, 154, Japan and Department of Drug Evaluation and Toxicological Sciences, Faculty of Pharmaceutical Sciences, Chiba University,** 1-33, Yayoi-cho, Chiba, 260, Japan

(Received February 15, 1990)

Formation of gastric lesions induced by orally administered aspirin (100 mg/kg) was examined in 4 to 86 week-old Sprague-Dawley male rats. Gastric mucosal prostaglandin E_2 (PGE_2) level and gastric secretion in basal state were also examined in these rats. Gastric mucosal PGI_2 level was measured by bioassay and gastric secretion was collected by the pyloric ligation method for 4 h. Gastric lesions reached the maximum value in 7 week-old, and lowest in 60 week-old. Acid output also reached the maximum value in 7 week-old. As for PGI_2 level, it showed the maximum value in 20 week-old, and moderately decreased thereafter. In 86 week-old, PGI_2 level was further lowered to about 35% of 20 week-old rats. A linear positive correlation was noted between formation of aspirin-induced gastric lesion and acid secretion. From these results, it was concluded that formation of gastric lesions by aspirin was closely related to acidity of the gastric secretion. It was also suggested that in aged rats, aspirin-induced gastric lesions may at least partly be associated with the reduced PGI_2 level.

Keywords — aging; rat; gastric acid secretion; prostaglandin E_2; aspirin; gastric lesion

Introduction

Maitra et al.\(^1\) reported that aging decreases capacity of the gastric mucosa to secrete acid in rats under both basal and pentagastrin-stimulated conditions and this decrease could be attributed in part to mucosal atrophy. Maeda-Hagiwara et al.\(^2\) also reported that the gastric acid secretory sensitivity to thyroid-releasing hormone decreased with aging.\(^2\) These findings are also consistent with the reports that in humans both basal and secretagogue-induced gastric acid output decreases with advancing age and that atrophic gastritis is rather common among elderly.\(^3\)–\(^5\)

In 1967, Davenport et al.\(^6\) reported that aspirin damages the gastric mucosal barrier by a back diffusion of strongly acidic gastric juice which induces mucosal erosive lesions and bleeding. It has been known that achlorhydric patients are less susceptible to aspirin-induced gastric damage than normal persons\(^7\) and the aspirin-induced gastric damage markedly decreased when gastric acid was buffered to a level of pH 6–7.\(^8\) On the other hand, prostaglandins (PGs) were reported to have an intimate implication in protection of the gastric mucosa.\(^9\)–\(^11\) Endogenous gastric PGI_2 synthesis has been known to be inhibited by the treatment with non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, naproxen and indomethacin.\(^12\)

In the present study, gastric lesions induced by orally administered aspirin were examined in 4 to 86 week-old rats in association with gastric mucosal PGI_2 level and gastric acid secretion to elucidate the susceptibility to gastric lesion formation with aging.

Materials and Methods

Animals — Sprague-Dawley male rats (7 to 86 week-old) were used after 24 h fasting. They were housed in individual cages with wide mesh bottoms to prevent coprophagy.

Aspirin-Induced Gastric Lesion — Aspirin (100 mg/kg) was suspended in 1% gum arabic solution and administered orally at a volume of 5 ml/kg. Four hours after the aspirin administration, the stomach was excised and opened along the greater curvature. The length of each gastric erosive lesion in the glandular portion was measured, and the sum of the length was ex-
pressed as lesion index (LI).

**Gastric Acid Secretion** — Under light ether anesthesia, epigastric laparotomy was performed. After exposing the stomach, the pylorus was ligated, and the abdominal incision was sutured. Gastric juice was collected for 4 h after the pylorus ligation. The gastric juice was centrifuged at 3000 × g for 5 min, and the volume, pH and total acidity were measured. Values of pH were measured with a pH meter (Hitachi-Horiba pH meter/M-7) and total acidity by a titration method using phenolphthalein. Acid output was calculated by the following formula: acid output (μeq/4 h) = total acidity (μeq/ml) × volume (ml/4 h)

**Bioassay of Mucosal PGI2 Level** — Bioassay of mucosal PGI2 was performed according to the method reported previously. In brief, the gastric mucosal layer (about 100 mg wet weight) was separated from the muscle layer in ice cold saline. This specimen was then shaken for 60 s at room temperature (22 °C) using a steady speed of a vortex stirrer and centrifuged at 9000 × g for 15 s. The concentration of PGI2 in the supernatant was determined by assaying its anti-aggregatory properties. Results were expressed in ng of the generated PGI2/g wet weight (ww) of tissue.

**Data Analysis** — Data were represented as mean ± S.E. Statistical analysis was performed using the Dunnett’s multiple comparison test and statistical significance was evaluated at p < 0.05. A regression analysis was calculated to determine a correlation coefficient between two different variables.

**Results**

**LI Values**

Aspirin developed the linear erosive lesions in the glandular portion of the stomach. The LI value was 36.6 ± 2.8 mm in 4 week-old (Fig. 1). It markedly increased in 7 week-old and decreased thereafter; the value was lowest in 60 week-old.

**Gastric Secretion**

The volume gradually increased, and then reached the maximum value of 7.0 ± 0.9 ml in 20 week-old (Table I). Thereafter, it was definitely reduced in 40 to 86 week-old. On the other hand, pH value showed the minimum value in 7 week-old, and increased stepwise with aging (Table I). In 86 week-old, it amounted to the highest value of 3.30 ± 0.46, significantly differ-

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**Fig. 1.** Formation of Gastric Lesions by Aspirin in Aging Rats

Each value represents mean ± S.E. (n = 5—10). a) Significant difference from 4 week-old rats (p < 0.05). b) Significant difference from 7 week-old rats (p < 0.01).

**Table I.** Change of Gastric Acid Secretion in Pylorus-Ligated Rats for 4 h

<table>
<thead>
<tr>
<th>Age (week-old)</th>
<th>No. of rats</th>
<th>Gastric acid secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Volume (ml)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>6.5 ± 1.9</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>7.0 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
<td>3.4 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>3.3 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>86</td>
<td>5</td>
<td>3.1 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E. a, b) Significant difference from 4 week-old rats (p < 0.05, 0.01). c, d) Significant difference from 7 week-old rats (p < 0.05, 0.01). e) Significant difference from 40 week-old rats (p < 0.01).
ent from that in 40 week-old rats. As for acid output, it showed the maximum value of 701 ± 138 μeq/4 h in 7 week-old, and thereafter decreased markedly (Table I).

**Correlation between Formation of Aspirin-Induced Gastric Lesions and Acid Secretion**

A linear correlation was observed between aspirin-induced gastric lesion and acid secretion; its correlation coefficient was $r = 0.903$ ($p < 0.05$) (Fig. 2).

**PGI$_2$ Level**

PGI$_2$ level showed the maximum value of 473 ± 22 ng/g ww in 20 week-old (Fig. 3). It was moderately decreased in 40 to 60 week-old. In 86 week-old, PGI$_2$ level was further lowered to a value of 173 ± 26 ng/g ww, significantly different from that in 40 week-old rats.

**Discussion**

Pare et al.\textsuperscript{14} reported that elder rats were not susceptible to stress-ulcer and gastric acid was not significantly related to degree of ulceration. Olson et al.\textsuperscript{15} also reported the circadian variation in the susceptibility to gastric lesions induced by aspirin. On the other hand, the age-related difference on aspirin-induced gastric lesion has been studied little as yet. In the present study, formation of aspirin-induced gastric lesions, mucosal PGI$_2$ level and gastric acid secretion during aging were investigated in 4 to 86 week-old rats and it became obvious that acid secretion decreases with aging, and formation of aspirin-induced gastric lesions well correlated to the acid secretory activity. In addition, it was indicated that the susceptibility of the stomach to aspirin may be different in age, and in aged rats, the decrease of defensive ability may be associated with formation of gastric lesions by aspirin.

In 1967, Davenport et al.\textsuperscript{6} reported that aspirin induces gastric lesions by the back diffusion of gastric acid. In the strongly acidic environment of gastric juice, aspirin is mostly non-ionized and freely diffuses into the mucosal cell. As the pH value of the intracellular environment is much higher, aspirin is dissociated and trapped inside the cell, and as a result, it induces gastric damage. In the present study, a statistically significant positive correlation was observed between aspirin-induced gastric lesion and acid secretion. This relationship is in good agreement with the above-mentioned observations.

There are several reports concerning the age-related difference on gastric secretion.\textsuperscript{14,16} Maitra et al.\textsuperscript{19} also reported that aging decreases capacity of the gastric mucosa to secrete acid in 4 to 21 month-old rats. These finding are consistent with the results obtained in the present study. The reason why aging decreases acid secretion was explained by gastric mucosal atrophy.\textsuperscript{13} The present finding that the pH
value at 86 week-old was highest among 7—86 week-old groups would be attributed to the same reason.

In 86 week-old, PGI₂ level showed the minimum value and aspirin-induced gastric erosive lesions were comparable to those in 40 week-old rats, while the pH value in 86 week-old showed the highest value of 3.30 ± 0.46. Gastric mucosal PG plays an important role in maintaining the gastric integrity. Exogenously administered PG has been reported to be protective against NSAID-induced gastric lesions. Whittle et al. reported that the inhibition of PGI₂ synthesis by NSAIDs parallels the extent to which these drugs induce gastric lesion. Then, in aged rats the weakened gastric integrity resulting from the decrease of PGI₂ level may be associated with formation of gastric lesions by aspirin, although the involvement of other types of PG could not be disregarded.

In conclusion, gastric acid secretion decreased with aging, and formation of gastric lesions by aspirin well correlated to the acid secretory activity. In the aged animal, the decrease of defensive ability along with the reduced mucosal PGI₂ level may be at least partly associated with formation of gastric lesions induced by aspirin.

References

6) H. W. Davenport: Salicylate damage to the gastric mucosal barrier, Gastroenterology, 276, 1307—1312 (1967).