EFFECTS OF THE ANTIULCER DRUG
GERANYLGERANYLACETONE ON ASPIRIN-INDUCED
GASTRIC ULCERS IN RATS

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Abstract—Antiulcer effects of geranylgeranylacetone (GGA) on aspirin-
induced gastric ulcers in rats were studied, comparing them with those of
gefarnate. The oral administration of GGA prevented the development of
gastric ulcer induced by a single or repeated oral administration (5
consecutive days) of aspirin. The effects of GGA were more potent and
more definite than those of gefarnate. The intraduodenal administration
of GGA, but not the intragastrical administration, also inhibited the ulceration
induced by aspirin in pylorus-ligated rats, while the intraduodenal adminis-
tration of gefarnate did not. GGA prevented the reduction of the H+
concentration and the increment of Na+ concentration in the gastric juice
induced by aspirin. In addition, the decrease of hexosamine content in the
gastric mucosa induced by aspirin was restored to a normal level by GGA,
but not by gefarnate. From these results, it was concluded that the protective
actions of GGA on aspirin-induced gastric ulcers might be due to its pro-
tection from the weakening of gastric mucosal resistances.

We previously reported that geranylgeranylacetone (GGA), an acyclic poly-
isoprenoid, had a potent antiulcer effect on cold-restraint stress, aspirin, indomethacin,
prednisolone and reserpine-induced gastric ulcers and accelerated the healing of acetic
acid and thermocautery ulcers in rats; but GGA had no effect on the ulceration and
gastric secretion in pylorus-ligated rats (1). Recently, we have reported that the reduction
in the biosynthesis and secretion of gastric mucus is related to the ulcer formation in the
rats subjected to cold-restraint stress (4°C, for 2 hr), and also we suggested that GGA
inhibited the ulceration, probably through preventing the weakening of the so-called
"mucous barrier" induced by cold-restraint stress (2, 3). On the other hand, Takagi and
Yano (4) reported that gefarnate, an acyclic isoprenoic antiulcer drug, induced an increase
in hexosamine content of rat pyloric tissue treated with cortisone acetate, and the
decreases in hexosamine contents of the gastric juice and pyloric tissue in the 5-days
fasted rats were prevented by the treatment of gefarnate which did not affect the gastric
secretion.

In this paper, we studied the effect of
GGA in comparison to that of gefarnate on aspirin-induced ulceration which has been said to inhibit the biosynthesis of gastric mucus (5, 6) and to produce the back-diffusion of $H^+$ to the mucosa (7).

**MATERIALS AND METHODS**

Male Sprague-Dawley strain rats, weighing 220 to 250 g, were used. The animals were housed in a temp. controlled room maintained at $22\pm2^\circ C$ with the humidity at $55\pm5\%$. In all the experiments described below, except for the subacute ulcer study, the animals were deprived of food but allowed free access to water for 24 hr before each procedure.

Drugs used — Geranylgeranylacetone (GGA) was synthesized in the Chemical Synthetic Laboratories of Eisai Co. Ltd. Gefarnate was purified from the purchased material (Gefanil; Sumitomo). These drugs were emulsified in 5% gum arabic and 0.6% Tween 80. Control animals were given only the vehicle.

**Acute aspirin-ulcer:** Aspirin suspended in 5% gum arabic solution was given orally at 200 mg/kg to rats. Five hr later, the animals were sacrificed under ether anesthesia and the stomachs were removed. The stomach was inflated by injecting with 10 ml of saline through the esophageal junction and immersed in 5% neutral formalin solution for 10 min to fix the outerlayer of the gastric wall. Subsequently, the stomach was incised along the greater curvature and the length of each lesion in the glandular portion was measured under a dissecting microscope ($\times 10$). The sum of the length (mm) of all lesions for each rat was used as an ulcer index. The stomachs obtained here were examined for hexosamine content. The test drugs were given orally using a gastric tube 30 min before the administration of aspirin.

**Subacute aspirin-ulcer:** Subacute aspirin-ulcer was produced by the method of Murakami et al. (8). Briefly, after fasting for 24 hr, 100 mg/kg of aspirin suspended in 5% gum arabic solution was given orally to the rats (at AM 9:00). One hr later, the animals were permitted to take food only for 1 hr (AM 10:00–11:00), and the animals were deprived of food again. With the same schedule, aspirin was administered once a day for 5 consecutive days. On the 6th day, the animals were sacrificed under ether anesthesia and the stomachs were removed. The animals were intravenously given 1.0 ml of 1.0% pontamine sky blue (pH 7.4) 10 min before sacrifice in order to clearly visualize the erosive parts of the stomach. The stomach was examined for the lesions by the same method described above. The test drugs were administered orally once a day for 5 consecutive days immediately after the administration of aspirin.

**Aspirin-ulcer in pylorus-ligated rats:** According to the method described by Okabe et al. (9), the pylorus of each animal was ligated under ether anesthesia. After the ligation, 100 mg/kg of aspirin suspended in 5% gum arabic solution was given through a stomach tube in a volume of 0.2 ml/100 g of body weight, and then the test drugs were given intraduodenally or intragastrically. Seven hr later, the animals were sacrificed under ether anesthesia, the stomachs were removed, and then the gastric contents were collected. Subsequently, the stomach was incised along the greater curvature and examined for the presence of lesions in the glandular portion. The gastric contents collected were centrifuged and analyzed for volume and acidity; the acidity was determined by titration of the gastric juice to pH 4.2 with 0.02N NaOH. The concentrations of Na$^+$ and K$^+$ ions in the gastric juice were measured with a flame photometer (Hitachi, model 205DT).

**Determination of hexosamine in gastric tissues:** The removed stomach was cut off
at the forestomach. The gastric tissue was divided into the corpus and antrum, and then they were weighed. Both tissues were lyophilized and weighed. Hexosamine content in the tissue was measured by the method of Neuhaus and Letzring (10). The dried gastric tissue was hydrolyzed with 2 ml of 4N HCl at 100°C for 9 hr. After cooling, the hydrolysate was neutralized with 4N NaOH. The solution was then brought to 25 ml with distilled water and filtered. One ml of this filtrate was heated in a boiling water bath for 20 min with 1.0 ml of acetylacetone reagent (1 ml of acetylacetone in 50 ml of 0.75 M Na₂CO₃ and 0.25 M NaHCO₃). After cooling, 2 ml of isoamylalcohol was added to the reaction mixture, and this mixture was shaken and centrifuged at 3,000 rpm for 10 min. One ml of the organic phase was put into a tube and mixed with 0.5 ml of Earlich reagent (0.8 g of p-dimethylaminobenzaldehyde in a mixture of each 30 ml of conc. HCl and isoamylalcohol). Fifteen min later, an absorbance was measured at a wave length of 530 nm. Glucosamine was used as the standard. Hexosamine content in the tissue was expressed as µg/dried gastric tissue or µg/mg of dried tissue weight.

RESULTS

Effect of GGA and gefarnate on acute aspirin-ulcer: The oral administration of aspirin induced linear lesions which extended from the fundic area to the pyloric area in both sides of the anterior and posterior gastric walls. Especially, long linear lesions were found in the gastric mucosa of the greater curvature. In the animals treated with GGA at 200 mg/kg, the number of long linear lesions was definitely less than that of the control animals. The ulcer index of GGA-treated animals was significantly smaller when compared with that of the control animals (Table 1). Gefarnate did not inhibit the ulcer formation.

The changes of hexosamine contents in the gastric mucosa are shown in Table 2. In normal animals which were not treated with aspirin, the mean values of hexosamine content were 2317±52 µg in the corpus and 441±26 µg in the antrum. By the treatment with aspirin, hexosamine contents in both tissues of the corpus and antrum were markedly reduced, and also the hexosamine concentrations in both tissues decreased from a normal value of 15.2±0.7 µg/mg dried tissue weight to 10.8±0.4 µg/mg dried tissue weight (P<0.001) in the corpus, and from a normal value of 15.5±0.6 µg/mg dried tissue weight to 12.8±0.8 µg/mg dried tissue weight (P<0.01) in the antrum. Both reduction of the mucosal hexosamine content and concentration in the corpus provoked by aspirin were significantly restored to the normal level by treatment with GGA at 200 mg/kg. In the antrum, GGA also prevented the reduction of hexosamine

| Treatment | Dose mg/kg | Number of animals | Ulcer Index Mean±S.E. (mm) | Inhibition (%) |
|-----------|-----------|-------------------|---------------------------|---------------|
| Control   | —         | 7                 | 14.1±2.6                  | —             |
| GGA       | 100       | 8                 | 10.1±3.0                  | 28.4          |
|           | 200       | 7                 | 4.0±1.7**                 | 71.6          |
| Gefarnate | 100       | 8                 | 13.7±4.4                  | 2.8           |
|           | 200       | 8                 | 9.7±3.4                   | 31.2          |

Aspirin was given orally at 200 mg/kg. The animals were sacrificed 5 hr after the administration of aspirin. Drugs were administered orally 30 min before the aspirin-treatment. **P<0.01 when compared with the control.
Table 2. Effects of GGA and gefarnate on reduction of hexosamine content induced by aspirin

| Treatment   | Dose mg/kg | Number of animals | Hexosamine (μg) |   |   |
|-------------|------------|-------------------|-----------------|---|---|
|             |            |                   | Corpus          | Antrum |   |   |
| Normal      | ---        | 8                 | 2317±52         | 441±26  |   |   |
| Control     | ---        | 7                 | 1648±65**       | 351±17*  |   |   |
| (Aspirin)   |            |                   |                 |         |   |   |
| GGA         | 100        | 8                 | 1786±104        | 384±25  |   |   |
| 200         | 7          |                   | 1901±82*        | 395±14  |   |   |
| Gefarnate   | 100        | 8                 | 1763±126        | 353±15  |   |   |
| 200         | 8          |                   | 1606±144        | 371±18  |   |   |

All values represent the mean±S.E. *P<0.05 and **P<0.01 when compared with normal. *P<0.05 when compared with the control.

Table 3. Effects of GGA and gefarnate on subacute aspirin-ulcer in rats

| Treatment   | Dose mg/kg/day | Number of animals | Ulcer Index Mean±S.E. (mm) | Inhibition (%) |
|-------------|----------------|-------------------|---------------------------|----------------|
| Control     | ---            | 8                 | 26.2±3.1                  | ---            |
| GGA         | 25             | 7                 | 17.3±4.2                  | 34.0           |
|             | 50             | 8                 | 10.7±1.7***               | 59.2           |
|             | 100            | 7                 | 8.9±4.0**                 | 66.0           |
| Gefarnate   | 50             | 7                 | 16.5±4.1                 | 37.0           |
|             | 100            | 7                 | 14.0±3.5*                 | 46.6           |

Drugs were administered orally to rats immediately after oral administration of aspirin (200 mg/kg) for 5 consecutive days. *P<0.05, **P<0.01 and ***P<0.001 when compared with the control.

content, but not significantly. On the other hand, gefarnate did not show any effects on the reduction of hexosamine content in both tissues of the corpus and the antrum.

Effect of GGA and gefarnate on subacutely induced aspirin-ulcer: The oral administration of aspirin for 5 consecutive days produced linear ulcers in the gastric mucosa. In the histochemical study, this ulcer was more severe and deeper than that of the acutely induced aspirin-ulcer. As summarized in Table 3, GGA at oral doses of 25, 50 and 100 mg/kg/day reduced the ulcer formation dose-dependently. Gefarnate also reduced the ulcer formation at 100 mg/kg/day, but this effect was less than that of GGA.

Effect of GGA and gefarnate on gastric secretion in pylorus-ligated rats with aspirin-treatment: Results of the analysis of gastric contents obtained here are summarized in Table 5. In the pylorus-ligated rats without aspirin, the volume of gastric juice was 3.4±0.2 ml and the concentrations of H⁺, Na⁺ and K⁺ ions were 99.9±4.9, 31.5±3.5...
Table 4. Effects of GGA and gefarnate on aspirin-ulcer in pylorus-ligated rats

| Treatment | Dose mg/kg | Number of animals | Route | Ulcer Index | Inhibition (%) |
|-----------|------------|-------------------|-------|-------------|----------------|
| Control   | —          | 14                | i.d.  | 51.9±7.0    | —              |
| GGA       | 100        | 15                | i.d.  | 20.2±5.5**  | 61.1           |
|           | 200        | 15                | i.d.  | 21.9±3.7*** | 57.8           |
| Control   | —          | 8                 | i.d.  | 58.4±11.1   | —              |
| Gefarnate | 200        | 8                 | i.d.  | 45.3±8.1    | 22.4           |
| Control   | —          | 8                 | i.g.  | 38.7±10.9   | —              |
| GGA       | 200        | 8                 | i.g.  | 38.8±9.7    | -5.7           |

Aspirin at 100 mg/kg was given intragastrically (i.g.) immediately after pylorus-ligation. Drugs were intraduodenally (i.d.) or i.g. immediately after pylorus-ligation. **P<0.01 and ***P<0.001 when compared with the control.

Table 5. Effects of GGA and gefarnate on gastric secretion in pylorus-ligated rats with aspirin

| Group | Treatment          | Dose mg/kg | Number of animals | Volume (ml) | H⁺ mEq/L  | Na⁺ mEq/L | K⁺ mEq/L |
|-------|--------------------|------------|-------------------|-------------|------------|------------|----------|
| 1     | Normal (ligation alone) | —          | 8                 | 3.4±0.2     | 99.9±4.9   | 31.5±3.5   | 8.5±0.9  |
| 2     | Control (aspirin)   | —          | 14                | 4.9±0.3     | 39.7±3.4   | 94.1±2.4   | 6.9±0.5  |
| 3     | GGA                | 100        | 15                | 3.9±0.2     | 46.8±4.9   | 83.6±5.8   | 7.5±0.5  |
| 4     | GGA                | 200        | 15                | 4.3±0.2     | 48.8±2.8   | 85.6±3.1   | 7.8±0.7  |
| 5     | Control             | —          | 8                 | 5.1±0.3     | 46.9±3.6   | 92.5±2.8   | 6.0±0.3  |
| 6     | Gefarnate          | 200        | 8                 | 4.1±0.2     | 47.4±3.2   | 96.5±6.2   | 8.5±0.9  |

All values represent the mean±S.E. The animals were sacrificed 7 hr after pylorus-ligation. Aspirin at 100 mg/kg was given intragastrically immediately after pylorus-ligation.

and 8.5±0.9 mEq/L, respectively. In the pylorus-ligated rats with aspirin, the volume of gastric juice and Na⁺ ion concentration were significantly higher, but H⁺ ion concentration was lower than those of rats without aspirin. Both the reduction of H⁺ ion concentration and the increment of Na⁺ ion concentration provoked by aspirin were prevented by the pretreatment with GGA (200 mg/kg), while the volume of gastric juice was not affected. Gefarnate given intraduodenally at 200 mg/kg prevented neither the reduction of H⁺ ion concentration nor the increment of Na⁺ ion concentration provoked by aspirin.

DISCUSSION

In this experiment, it was found that GGA inhibited the ulcer formation induced by aspirin and that the effects of GGA were more potent and more definite than those of gefarnate.

As we have previously reported, GGA prevents the ulcerations induced by several
ulcerogenic agents or stress, whereas GGA does not inhibit the ulceration (Shay's ulcer) and the gastric secretion in the pylorus-ligated rats (1). We think the mechanism for the antiulcer action of GGA may be due to increasing the defensive force of the gastric mucosa because GGA prevents the reduction of hexosamine content in the gastric mucosa of rats due to cold-restraint stress (3).

It has been proposed that aspirin induces gastric ulcers by disrupting the gastric mucosal barrier resulting in the back-diffusion of acid (11). As events in the weakening of the so-called "mucosal defensive mechanism" by aspirin, reducing the biosynthesis of mucus or glycoproteins have been reported: Menguy and Masters (5) observed that the administration of aspirin to rats caused a decrease in the gastric mucosal content of mucus measured by periodic acid Schiff staining of sections of gastric mucosa and by direct measurement of the gastric mucosal content of hexosamine and fucose, and Rainsford (6) observed that aspirin caused a marked inhibition of the 
\[ {^{35}}S\]O$_4$ incorporation into gastric glycoproteins. Azuumi et al. (12) also have reported that aspirin induces the reduction of biosynthesis of gastric macromolecular glycoproteins in the gastric mucosa prior to the development of ulcers. GGA prevented the ulceration induced by a single or repeated treatment with aspirin at doses ranging from 50 to 200 mg/kg. On the other hand, gefarnate was only effective on subacute aspirin-ulcer. Takagi and Yano (4) demonstrated that gefarnate administered for 3 or 5 days consecutively increased the hexosamine content of rat gastric tissue under several experimental conditions. Takagi and Okabe (13) reported that in the curative test, gefarnate which was almost ineffective on the preventive test was effective on the immersion stress ulcer in rats. These results and our findings suggest that the effect of gefarnate on the gastric mucosa might be clear when administered consecutively for several days.

The reduction of hexosamine content in the gastric mucosa provoked by a single administration of aspirin was restored to the normal level by pretreatment with GGA. Hexosamine is one of the mucus components considered to play an important role in the maintenance of gastric mucosal integrity (14). Recently, we have reported that GGA or its hydroxylated derivative which was metabolized in rats stimulates the formation of $[^{14}\text{C}]$-mannolipids from GDP-$[^{14}\text{C}]$-mannose in rat liver microsomes using a model enzyme preparation and have also suggested the possibility that GGA is involved in glycoprotein synthesis following glycolipid formation through glycosyltransferase in the gastric mucosa (15). Hence, our results suggest that the mechanism(s) for the preventive effect of GGA may relate to the action of aspirin on the biosynthesis of gastric glycoproteins. Further investigation is required on the relationship between ulceration and biosynthesis of gastric glycoproteins.

Aspirin-ulcer in the pylorus-ligated rats was significantly inhibited by an intraduodenal administration of GGA, but not by an intragastrical administration. These results indicate that GGA at least shows the antulcer effect without an antacid or buffer action. In addition, both the reduction of H$^+$ ion concentration and the increment of Na$^+$ ion concentration in the gastric juice provoked by aspirin were prevented by the pretreatment with GGA, while the volume of gastric juice was not affected. Terano et al. (16) reported that GGA as well as prostaglandin E$_2$ and cimetidine prevented a decrease of the gastric mucosal potential difference by aspirin or taurocholate. Therefore, the results obtained here suggest that the inhibitory effect on the back-diffusion of acid to the gastric mucosa may be partially involved in
the protective action of GGA on the ulceration by aspirin.

These findings strongly suggest that GGA prevents the weakening of the gastric mucosal defensive mechanism induced by aspirin, probably through reinforcing the gastric mucosal resistance, i.e., restoring the biosynthesis of gastric glycoproteins and maintaining the gastric mucosal potential difference.

Aspirin causes damage to the human gastric mucosa, and heavy use of this drug may be an important cause of gastric ulcers in humans (17-19). The present study shows that an experimental form of such ulcers can be effectively prevented by GGA, suggesting that the use of this drug could have therapeutic applications in the prevention and/or treatment of aspirin-induced ulcers in humans.

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