EFFECTS OF 15(S)-15-METHYL-PGE₂ METHYL ESTER ON
HEALING OF CHRONIC GASTRIC AND DUODENAL
ULCERS IN RATS

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Prostaglandins are reportedly inhibitors of gastric secretion in animals (1–3) and man
(4), and these compounds have an anti-ulcer effect on various types of acutely induced
experimental ulcers, i.e., stress ulcers (5), histamine-induced ulcers (6), Shay or steroid-
induced ulcers and duodenal ulcers in rats or cats (7, 8). Thus, it was of interest to determine
whether one of the prostaglandin derivatives, 15(s)-15-methyl-PGE₂ methyl ester (15-Me-
PGE₂) accelerates healing of chronic types of gastric or duodenal ulcers induced in rats.
Antisecretory effect of this compound at various doses was also studied in pylorus-ligated
rats.

In male Donryu strain rats (220–240 g) chronic gastric ulcers were induced with rats
under ether anesthesia, according to the method of Takagi et al. (9). Briefly, 20% acetic
acid solution (0.03 ml) was injected into the gastric wall at the junction of the body and
antrum of the anterior wall. The animals were then maintained on rat chow and water ad
libitum. Duodenal ulcers were induced in other rats according to the method of Okabe
et al. (10). The abdomen was incised with the rats under ether anesthesia and a metal mold
(6 mm in diameter) was put in place tightly on the serosal surface of the duodenal wall, about
5 mm distal to the pylorus. Glacial acetic acid solution (0.15 ml) was poured into the mold
and allowed to remain for 25 sec. After removal of the acetic acid solution, the abdomen
was closed and the animals were fed in the usual manner. These animals with either gastric
or duodenal ulcers were sacrificed 14 or 12 days after ulceration, respectively. The stomach
or duodenum of each was removed, inflated by injecting 12 ml of 1% formalin solution,
immersed in 1% formalin solution for 10 min, and then cut along the greater curvature.
The ulcerated area (mm²) was measured as the ulcer index under the dissecting microscope
(×10) with aid of a square grid. 15-Me-PGE₂ at various doses was given by gastric intubation
twice a day from one day after ulceration for 12 or 10 consecutive days to rats with either
gastric or duodenal ulcers. As the control, saline solution with a trace of ethanol was given.
15-Me-PGE₂ or saline was given in a volume of 0.5 ml/100 g of body weight per injection.
The healing index was calculated as follows.

Healing index (%) = \( \frac{\text{Control (ulcer index)} - \text{Drug (ulcer index)}}{\text{Control (ulcer index)}} \times 100 \)
The person measuring the ulcers was not aware of which animals had been treated. Male Donryu rats (180–200 g) were deprived of food for 18 hr and had free access to water. Under ether anesthesia the abdomen of each rat was incised and the pylorus ligated. Four, seven or twelve hr after ligation the animals were sacrificed and the gastric contents were collected. After centrifugation, the volume of samples was measured and acidity was titrated with 0.1 N NaOH to pH 7.0 using the autoburette (Radiometer); acid output was expressed as μEq/hr. The pepsin activity was determined by Anson's method (11); pepsin output was expressed as mg/hr. 15-Me-PGE₂ at various doses was given intraduodenally immediately after pylorus ligation in a volume of 0.5 ml/100 g of body weight. Saline solution with a trace of ethanol was given to the control animals.

Student's t-test was employed to determine the statistical significance of the data obtained in this study.

15-Me-PGE₂ was first solubilized into 95% ethanol (0.1 ml for each milligram), then saline was added to a final volume prior to the injection. A repeated intragastrical administration of 15-Me-PGE₂ at the dose of 1 μg/kg/day for 12 days accelerated the healing of gastric ulcers in rats (healing index, 19.0%), but the effect was not statistically significant (Table 1). When the dose was increased to 4 μg/kg/day, the rate of healing of the gastric ulcers was significantly increased (healing index, 32.3%). However, 15-Me-PGE₂ had no effect on the gastric ulcers at doses of 20 μg/kg/day. Intragastrical 15-Me-PGE₂ at 4 or 20 μg/kg/day for 10 days had no effects on the healing of duodenal ulcers in rats.

In pylorus-ligated rats, 15-Me-PGE₂ dose-dependently inhibited gastric secretion (volume, acid and pepsin outputs) during the period of 3 and 7 hr (Table 2). The inhibition, however, did not persist for 12 hr.

Robert et al. (2) reported that several prostaglandins inhibit a variety of acutely induced gastric or duodenal ulcers in rats. The antiulcer effect of prostaglandins was thus ascribed chiefly to their antisecretory action. The present study also confirmed the antisecretory effect of 15-Me-PGE₂ in pylorus-ligated rats. It is unlikely, however, that this compound

| Ulcers      | Treatment | Dose (μg/kg/day) | No. of rats | Ulcer index (mm²) mean ± s.e. | Healing index (%) |
|-------------|-----------|------------------|-------------|-------------------------------|-------------------|
| Gastric     | Control   | 1                | 30          | 15.8 ± 1.7                    | 19.0              |
|             | 15-Me-PGE₂| 4                | 30          | 12.8 ± 1.6                    | 32.3              |
|             |           | 20               | 30          | 15.1 ± 1.2                    | 4.4               |
| Duodenal    | Control   | 4                | 16          | 24.8 ± 3.2                    | 2.0               |
|             | 15-Me-PGE₂| 20               | 16          | 24.3 ± 2.4                    | 8.9               |

15-Me-PGE₂ was given intragastrically by gastric intubation at the volume of 0.5 ml/100 g of body weight twice a day beginning from one day after ulceration for 12 (for gastric ulcer) or 10 (for duodenal ulcer) consecutive days. *P < 0.05.
| Time of experiment (hr) | Treatment   | Dose (μg/kg) | No. of rats | Volume (ml/rat) | % change | Gastric contents Titratable acid output (μEq/hr) | % change | Pepsin output (mg/hr) | % change |
|------------------------|-------------|--------------|-------------|----------------|----------|-----------------------------------------------|----------|-----------------------|----------|
| 3                      | Control     | 10           | 6.5±0.4     | 241.1±15.7     |          |                                               |          | 38.4±4.2              |          |
| 15-Me-PGE₂             | 2           | 10           | 5.4±0.3*    | 220.8±13.1     | 16.9     |                                               |          | 28.6±2.8              | 8.4      |
|                        | 10          | 10           | 4.2±0.3*    | 171.8±16.8*    | 35.4     |                                               |          | 27.2±2.3*             | 29.2     |
| 7                      | Control     | 10           | 12.2±0.4    | 174.3±13.3     |          |                                               |          | 23.8±1.6              |          |
| 15-Me-PGE₂             | 2           | 10           | 10.7±0.9    | 154.2±14.6     | 12.3     |                                               |          | 29.2±2.1              | 8.2      |
|                        | 10          | 10           | 9.3±0.6*    | 122.5±8.7*     | 23.8     |                                               |          | 27.5±1.4              | 13.5     |
| 12                     | Control     | 10           | 13.4±0.5    | 86.2±6.5       |          |                                               |          | 23.9±0.8              |          |
| 15-Me-PGE₂             | 2           | 10           | 13.3±0.5    | 98.7±4.0       | 0.7      |                                               |          | 24.4±1.0              | −2.1     |
|                        | 10          | 10           | 13.0±0.6    | 87.7±5.0       | 3.0      |                                               |          | 23.2±1.1              | 2.9      |

15-Me-PGE₂ was injected intraduodenally immediately after pylorus ligation at the volume of 0.5 ml/100 g of body weight. All values represent mean±s.e. *P<0.05.
enhanced the healing of gastric ulcers due to its antisecretory action. Gastric secretion was only slightly suppressed at the dose of 2 \( \mu g/kg \) even during the first 3 hr after injection. If an antisecretory effect is primarily involved in the mechanism of action of 15-Me-PGE2, an increase in the dose which inhibited the gastric secretion should have resulted in a higher healing index. In contrast, the curative effect of 15-Me-PGE2 was reduced with the increment of dose of the agent. In our preliminary study, 100 \( \mu g/kg/day \) of 15-Me-PGE2 resulted in an apparent delay of healing. These findings suggest that the effect of this compound may be related to mechanisms other than gastric inhibition. Regardless of the mechanism, the efficacy of 15-Me-PGE2 on acetic acid ulcers in the rat stomach was found to be almost equal or better than that of other anti-ulcer agents such as pirenzepine, ulcerlmin (12) or cimetidine (13). In previous work, we demonstrated that the healing of duodenal ulcers induced by acetic acid solution was accelerated by aluminum hydroxide (14), atropine sulfate or cimetidine (13), suggesting the participation of gastric juice in this healing. Therefore, it was anticipated that 15-Me-PGE2 would also enhance the healing of duodenal ulcers as it possesses antisecretory properties. We found that 15-Me-PGE2 had no effect whatever on the healing of duodenal ulcers in rats. A possible explanation is the weak inhibition of gastric secretion at the dose used in contrast to the strong inhibition induced by atropine sulfate or cimetidine. The increment of dose may yield an acceleration of healing. Since 15-Me-PGE2 had no effect on duodenal ulcers at the dose which exerted a favorable activity on gastric ulcers, the mechanism of healing of gastric and duodenal ulcers is apparently different. We conclude that 15-Me-PGE2 should be a beneficial drug for the treatment of gastric ulcers in humans.

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**EFFECT OF 6,9-THIA PROSTAGLANDIN I₂ (A STABLE PGI₂ ANALOGUE) ON PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) IN RATS**

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Biological activities of prostaglandin I₂ (PGI₂, prostacyclin) as related to platelet aggregation, the cardiovascular system, gastric function and inflammation have been well documented. We have also reported the potentiation by PGI₂ of increased vascular permeability induced by histamine in rats treated with carrageenin (1).

PGI₂ delays the onset of anaphylaxis in sensitized guinea pigs exposed to specific antigen (2) and inhibits the release of slow reacting substance of anaphylaxis (SRS-A) (3). PGI₂ is too chemically unstable (4) for clinically application.

6,9-Thia prostaglandin I₂ is a newly synthesized PGI₂ analogue which is stable in saline. It has a comparable potency to PGI₂ in inhibiting platelet aggregation (5) and the activity of the analogue does not diminish when kept in saline for several hours. The activity of PGI₂ is virtually abolished under such circumstances.

We report herein the effect of stable 6,9-thia PGI₂ in comparison with PGI₂ on the homologous passive cutaneous anaphylaxis (PCA) in rats.

Male Sprague-Dawley rats weighing 150–200 g were used. Reagin-like antibody against egg albumin was prepared according to the methods of Mota (6). The biological properties