

Relapse of Acetic Acid-Induced Gastric Ulcer and Gastric Mucosal Prostaglandin I₂ Level in Rats

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The healing process of acetic acid-induced gastric ulcer in rats was observed with an endoscope for 365 d after ulcer induction. The ulcers were induced by acetic acid solutions of various concentrations (2.5 (I), 5.0 (II), 10 (III) and 20 % (IV); 0.05 ml). On day 3, a positive correlation was observed between the ulcer index (UI) and the concentration of acetic acid solution. On day 365, cumulative healing rates in groups I, II, III and IV amounted to 100, 100, 58.3 and 51.7 %, respectively. The cumulative relapse rates in groups I, II, III and IV were 0, 13.6, 66.7 and 58.6 %, respectively. Significant correlations were observed between initial UI values and cumulative healing or cumulative relapse rate.

On day 365, rats were divided into two groups, a healed group and non-healed group, and the gastric mucosal prostaglandin I₂ (PGI₂) level was measured by bioassay. The PGI₂ level around ulcers in ulcer-induced rats was higher than in normal rats, and it was higher in non-healed rats than in healed rats. Moreover, the PGI₂ level was higher in those groups which showed a higher cumulative relapse rates.

The above results indicated that the initial ulcer size and the PGI₂ level around the ulcer might correlate to ulcer healing or exacerbation.

Keywords acetic acid-induced gastric ulcer; PGI₂; rat; healing; relapse; chronic gastric ulcer; gastric mucosa

Introduction

In 1969, Takagi *et al.* reported that the healing process of acetic acid-induced gastric ulcers in rats closely resembled that of human peptic ulcers from the standpoints of macroscopical and histological observation and chances of reulceration.¹⁾ Okabe and Pfeiffer²⁾ reported adhesion of the ulcer base with adjacent organs, delayed gastric emptying and an increase in gastric volume and acid output after ulcer induction. However, the factors responsible for the exacerbation of ulcers remained unidentified.

In the present study, gastric ulcers were induced by acetic acid solutions of various concentrations, and the healing and exacerbation processes were observed with an endoscope for 365 d. Furthermore, the prostaglandin I₂ (PGI₂) level of the gastric mucosa was measured by bioassay, to investigate the correlation between ulcer healing or exacerbation and the PGI₂ level.

Experimental

Animals Animals used were Sprague-Dawley strain (Slc: SD) male rats weighing from 220 to 240 g (7 weeks). Experimental groups and the number of animals used are shown in Table I.

Gastric Ulceration Acetic acid-induced ulcers were prepared according to the method of Takagi *et al.*¹⁾ as follows. Under pentobarbital anesthesia, a midline epigastric laparotomy was made. After exteriorizing the stomach, acetic acid solution (0.05 ml) was injected into the submucosal layer at the region between the fundus and the pylorus on the anterior wall. The concentrations of acetic acid solution used were 2.5, 5.0, 10 and 20%.

Instruments The endoscope (SES-2217S), cold light source (CLE-F) and endoscope camera (SC16-3R) used were purchased from Olympus Optical Co., Ltd. (Tokyo, Japan).

Observation of Ulcers and Determination of Ulcer Size The observation of ulcers and the determination of ulcer size were performed according to the method of Fukawa *et al.*³⁾ as follows. The animals were deprived of food but allowed free access to drinking water for 18 h prior to observation. Under ether anesthesia, the sheathed endoscope was inserted into the stomach from the mouth, about 3 to 4 ml/100 g body weight of air was pumped into the stomach through the sheath with an injection tube and the inside of the stomach observed. Dried sohmen sticks (Japanese vermicelli) cut to a length of exactly 5.0 mm were inserted into the stomach through the sheath of the endoscope after pulling out only the endoscope and the size of the acetic acid ulcer was measured by comparing the length and the width of the ulcer to the 5.0 mm sohmen stick. All of these procedures were carried out without restraining the rats. The observations were performed at 3, 10, 20, 35, 50, 70, 91, 112, 133, 154, 175, 203, 252, 301

and 365 d after ulcer induction.

Evaluation of Ulcers Ulcer Index (UI Value): The UI value was calculated as the product of the measured length and the measured width of the ulcer [UI value = length (mm) × width (mm)] and used to evaluate the ulcer size. The limit of measurement was as low as 0.5 mm.

Healing: When the white exudation and engorgement at the base of an ulcer disappeared, an ulcer was defined as healed. The healing was represented as the cumulative healing rate as follows:

cumulative healing rate =

$$(\text{No. of healed rats}/\text{No. of rats used}) \times 100 (\%)$$

Relapse: When ulceration was observed again after the healed state (reulceration) or when the UI value of an ulcer became 4 times or more larger than the previous reading (ulcer exacerbation), an ulcer was defined as relapsed. The relapse was represented as the cumulative relapse rate as follows:

cumulative relapse rate =

$$(\text{No. of relapsed rats}/\text{No. of rats used}) \times 100 (\%)$$

Generation and Bioassay of Mucosal PGI₂ Generation and bioassay of mucosal PGI₂ were performed as follows according to the methods of Whittle⁴⁾ and Gryglewsky *et al.*,⁵⁾ respectively. On the 365 th d, most of the animals were sacrificed and the stomachs were removed. A gastric mucosal sample around the ulcer (about 100 mg wet weight) was taken from the muscle layer in ice-cold saline. In the posterior wall, the gastric mucosa opposite the ulcer-induced site was taken in the same way. Blood and debris were washed away by shaking for 5 s with 1 ml of ice-cold 50 mM Tris buffer (pH 8.4) followed by centrifugation at 9000 × g for 10 s. After the supernatant had been removed, another 1 ml of 50 mM Tris buffer was added to the residual tissue. This sample was then shaken for 60 s at room temperature using a vortex mixer at a steady speed and centrifuged at 9000 × g for 15 s.

The concentration of PGI₂ in the supernatant was determined by assaying its anti-aggregatory properties. Rabbit blood was withdrawn from the carotid artery into a 3.8% solution of sodium citrate (9:1, v/v). Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 380 × g for 10 min at 20°C. PRP was aggregated at 37°C in a

TABLE I. Construction of the Experimental Groups and Ulceration Rates

Group	Acetic acid concentration (%)	No. of rats used	No. of ulcerated rats	Ulceration rate (%)
I	2.5	11	5	45.5
II	5.0	23	22	95.7
III	10.0	24	24	100
IV	20.0	29	29	100

Born aggregometer with sub-threshold proaggregatory concentrations of adenosine diphosphate (2 to 4 μ M). The anti-aggregatory potency of standard PGI₂ was measured by addition of PGI₂ to PRP at concentrations of 50 to 300 nM 1 min before adenosine diphosphate (ADP) was added. The percentage inhibition of the ADP-induced aggregation was plotted against the effective concentration of PGI₂ to obtain a standard curve. To measure the amount of PGI₂ generated by the mucosa, 30 μ l of the supernatant from incubation samples was added to PRP 1 min before the addition of ADP. The generation of a PGI₂-like activity in the tested sample was calculated by comparing the anti-aggregatory potency with that of synthetic PGI₂. Results were expressed in nanograms of generated PGI₂/g of tissue weight.

Data Analysis The values were represented as mean \pm S.E. Statistical analysis was performed using Student's *t* test, and values of *p* < 0.05 were regarded as significant.

Results

On the 3rd d after acetic acid injection, ulcers were observed in 5 rats out of 11 rats in group I (45.5%) and 22 rats out of 23 rats in group II (95.7%) (Table I). In the other groups, ulcers were observed in all rats used. Hereafter, rats with confirmed ulcers on the 3rd d were used in the present study.

UI Value The time courses of UI values are shown in Fig. 1. The initial UI values in groups I, II, III and IV were 5.3, 8.1, 28.0 and 55.6, respectively (Fig. 1). A positive correlation between mean initial UI value and concentration was observed (Fig. 2). The UI values decreased and plateaued on and after day 35. In group I, the UI value became zero on day 20.

Cumulative Healing Rate The first healed case in each

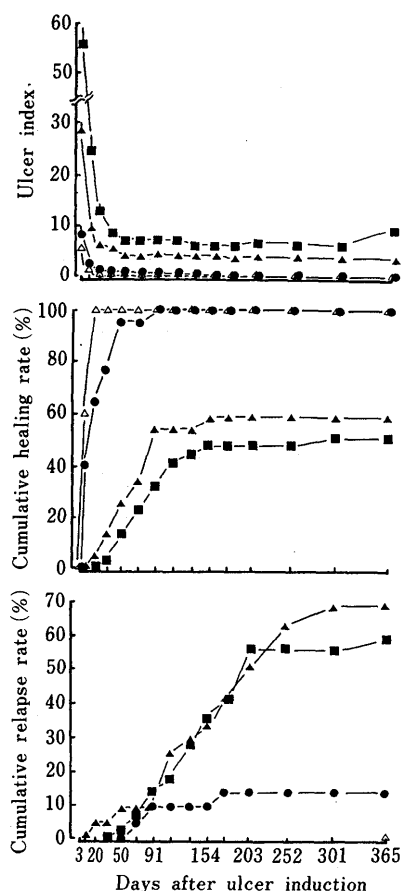


Fig. 1. Time Course of Ulcer Index and Cumulative Healing and Relapse Rates

Bars represent mean \pm S.E.M. — Δ —, group I; — \bullet —, group II; — \blacktriangle —, group III; — \blacksquare —, group IV.

group appeared after a time directly proportional to the concentration of acetic acid solution. On and after day 154, the cumulative healing rate reached a plateau; and on day 365 cumulative healing rates in groups I, II, III and IV were 100, 100, 58.3 and 51.7%, respectively (Fig. 1). Cumulative healing rate and initial UI value showed a significant negative correlation (Fig. 3).

Cumulative Relapse Rate In group I, the cumulative relapse rate was zero. The first relapsed case in each group appeared between day 20 and day 112, and the cumulative relapse rates in groups II, III and IV on day 365 were 13.6, 66.7 and 58.6%, respectively (Fig. 1). The cumulative relapse rate and initial UI value showed a significant positive correlation (Fig. 3).

Gastric Mucosal PGI₂ Level (Table II) In groups I and II, all rats were healed.

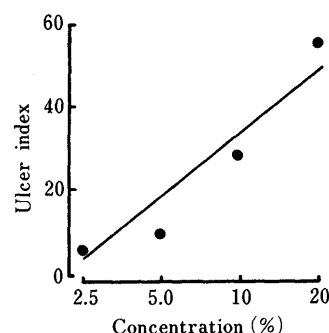


Fig. 2. Correlation between Ulcer Index and Concentration of Acetic Acid Solution

A positive correlation was observed between ulcer index and concentration of acetic acid solution. $y = 56.7 \log x - 23.9$, $r = 0.95$ ($p < 0.05$).

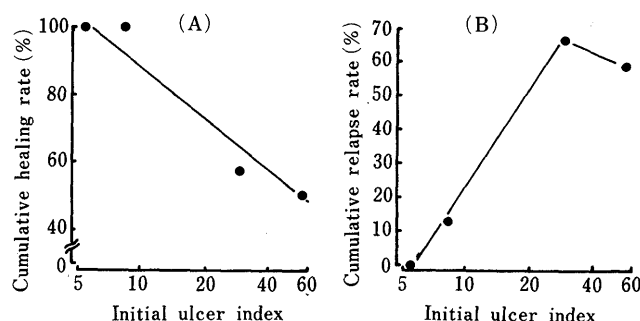


Fig. 3. Correlation between Ulcer Index and Cumulative Healing (A) or Relapse Rate (B)

A negative correlation was observed between cumulative healing rate and ulcer index. A positive correlation was observed between cumulative relapse rate and ulcer index. (A) $y = -53.9 \log x + 143$, $r = 0.975$ ($p < 0.05$). (B) $y = 93.7 \log x - 69.4$, $r = 0.999$ ($p < 0.05$).

TABLE II. Gastric Mucosal PGI₂ Level

Group	No. of rats	PGI ₂ level (ng/g, mean \pm S.E.)	
		Anterior wall	Posterior wall
Normal	12	313 \pm 23	323 \pm 23
I Healed	5	332 \pm 57	290 \pm 20
II Healed	19	432 \pm 43	383 \pm 43
III Healed	7	484 \pm 72	434 \pm 43
Non-healed	12	548 \pm 71 ^a	357 \pm 54
IV Healed	7	451 \pm 68	263 \pm 35
Non-healed	14	490 \pm 65 ^a	334 \pm 44

a) Significantly different from the normal group ($p < 0.05$).

Anterior Wall: In normal rats, the PGI_2 level was $313 \pm 23 \text{ ng/g}$. In group I, the PGI_2 level was not significantly different from that in normal rats. However, the PGI_2 levels in healed rats in groups II, III and IV were higher than in normal rats. In non-healed rats, the PGI_2 levels in groups III and IV showed a significantly higher level than in normal rats.

Posterior Wall: In the normal rats, the PGI_2 level was $323 \pm 23 \text{ ng/g}$. The PGI_2 level was not significantly different from that in normal rats in any group.

Discussion

In the present study, gastric ulcers were induced by acetic acid solutions of various concentrations, and the healing and exacerbation processes were observed sequentially with the aid of an endoscope for 365 d. Moreover, on day 365 the PGI_2 level of the gastric mucosa was measured in the healed group and the non-healed group.

Takagi *et al.*¹⁾ reported that the ulcer injury produced by a 1% acetic acid solution was limited to the epithelial layer and that at 10 and 30% the ulcerated regions involved the full thickness of the gastric wall. The present study showed that with 2.5 and 5.0% acetic acid solutions some rats had no epithelial lesion and others had only inflammatory hyperemia. The ulceration rates with 2.5 and 5.0% acetic acid solutions were 45.5 and 95.7%, respectively, on day 3. In the 10 and 20% groups, the ulceration rate was 100%. Thus, it was found that acetic acid solution at a concentration of 10% or more was necessary for inducing gastric ulcers in all rats.

It has been reported that the size of gastric ulcers is closely related to their healing and recurrence.⁶⁾ We observed a positive correlation between the mean initial UI value and the concentration of acetic acid solution. As initial UI value became larger, the cumulative healing rate became lower and the cumulative relapse rate higher. However, the cumulative relapse rate peaked at 66.7%.

One of the prominent features of acetic acid ulcer is the adhesion of the ulcer base to the neighboring organs such as the liver, pancreas or omental fat. Okabe and Pfeiffer reported that delayed gastric emptying and an increase in gastric volume and acid output were observed after ulcer induction.²⁾ However, the factors responsible for the exacerbation of acetic acid ulcer remained unidentified. Kobayashi *et al.* reported that the gastric mucosal PGE_2 level at the ulcer margin was significantly higher in tractable ulcer than in intractable ulcer.⁷⁾

PGs have been demonstrated by many investigators to have protective effects on gastric mucosa exposed to noxious substances.⁸⁾ Mølster *et al.*⁹⁾ reported that PGs are

released in the tissues near acute ulcers induced by acetic acid solutions in rats. Skarstein¹⁰⁾ reported an increased change of mucosal blood flow around the ulcer and that more prostaglandins, which may cause vasodilation, were synthesized around the ulcer than in other parts of the gastric mucosa. PGI_2 , which is reported to be a potent vasodilator¹¹⁾ and inhibitor of platelet aggregation⁵⁾ and acid secretion in rats,¹²⁾ was found in various tissues, particularly in the vascular wall, in large quantities.¹³⁾

In this study, the PGI_2 level in non-healed rats was found to be significantly higher than that in normal rats. The raised level of PGI_2 at the ulcer edge may simply reflect an increase in vascularity. The large blood supply from increased vascularity seems to reflect active reepithelization, which requires an abundant supply of glucose and oxygen. In healed rats, the PGI_2 level decreased. The present authors reported previously that vascularity around the ulcer, which increased in the active epithelization period, decreased with the healing of acetic acid-induced gastric ulcers.¹⁴⁾ Therefore, the lowered level of PGI_2 level in the healed group may reflect the decrease in vascularity.

On the other hand, PGI_2 level was higher in groups which showed higher cumulative relapse rates. Therefore, a correlation might exist between the PGI_2 level and the ulcer exacerbation.

In conclusion, it is suggested that initial ulcer size and PGI_2 level around the ulcer might correlate to ulcer healing or exacerbation.

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