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Preventive Effect of Proglumide on Erosive Gastritis in the Rat

Shinya KISHIMOTO, Hirofumi KOBAYASHI, Norio TAKABA, Reiko KONEMORI, Kazuma OKAMOTO, Satoru SHIMIZU, Toshio KUNITA, Goro KAJIYAMA and Akima MIYOSHI

The 1st Department Internal Medicine, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan

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ABSTRACT

The purpose of this study is to determine whether or not proglumide has a preventive effect on the erosive gastritis induced by sodium salt of taurocholic acid (TCA) in the rat. Its effect on the formation of gastric erosions, serum gastrin levels and secretion of acid and pepsin were also studied.

The rats were given standard feed containing 0.25% proglumide and water containing 5mM TCA (experimental E group). The control rats were given standard feed and water containing 5mM TCA (TCA group). All rats were killed at the end of the 3 months. The tissue specimens of the resected gastric mucosa were stained with hematoxylin eosin for histopathology and with azan for evaluation of fibrous ploriferation.

From microscopic observation of the stained specimens, the following results were obtained. TCA-group showed long mucosal surface injury (erosion), inflammatory cell infiltration, a reduction in the number of parietal cells, a decrease of mucosal thickness, and proliferation of collagenous fiber. In contrast, in the E group, these morphological and morphoquantitative changes were significantly small. The length of erosion and inflammatory cell infiltration were significantly reduced in the E group when compared with the TCA group. Furthermore, mucosal thickness was almost normal and fibrous proliferation was significantly scarce in the E group.

Proglumide had an insignificant effect on pH on the mucosa, volume and pH of gastric juice, serum gastrin levels and tetragastrin-induced secretion of acid and pepsin.

It is, thus, evident that proglumide has a preventive effect on the induction of erosive gastritis caused by TCA in the rats. Since it is difficult to explain its mechanism for the prevention of gastritis from only the already known facts that it has protective action on gastric mucosa and an inhibitory effect on secretion of acid and pepsin, unknown mechanisms are suspected to be involved.

Proglumide inhibits not only acid secretion but also gastrin release from gastrin G cells in man and animals. Furthermore, it can promote the healing of peptic ulcers in man[1,11,14,15]. However, it is not clear whether or not proglumide has an effect on acute erosive gastritis or acute gastric mucosal lesions. Other than proglumide, there is no other profitable data on agents in experimental and clinical traials on the treatment of gastritis at the present time, although prevention of development of gastritis or the medical treatment of gastritis have been discussed for a long time. This may be a result of limited understanding about gastritis and the difficulty in developing an experimental mode of gastritis for the examination of the preventive

or curative efficacy of an agent of gastritis in the animal.

The authors have succeeded in developing erosive gastritis in the rat by the oral administration of taurocholic acid (sodium salt: TCA) over an extended period[5,6]. In the present study, we tried to examine the preventive action of proglumide on erosive gastritis in the rat after administrating TCA. The precise purpose of this study is to determine whether or not proglumide has a preventive effect on erosive gastritis in the gastric mucosa produced by TCA.

EXPERIMENTAL PROCEDURE

1. Development of experimental erosive gastritis in the rat

Erosive gastritis was developed by the oral administration of 5mM TCA (Difco) to male wistar rats weighing 200 g. for 3 months. Intake of TCA was about 0.2 to 0.5 mg/kg/day although rats could take it freely from a water bottle.

2. The administration of proglumide and the control.

(1) Experimental group (E group)

Experimental group was fed standard meal (Japan Clea, CE-2) containing 0.25% (N = 8). Intake of the drug was around 1.070 mg/head/week after due consideration of increasing body weight of a rat.

(2) Control groups

1) TCA group (N = 8)

TCA alone was administered to rats for 3 months. The amounts of TCA given were the same as those in the experimental groups.

2) Normal control group (N group, N = 8)

The rats in this group received standard feed without any drugs and TCA (non treatment).

3) Proglumide alone group (PG group, N = 8)

The intention of the group was to examine the action of proglumide on the gastric mucosa in rats without TCA. The dose of proglumide given was 0.25% in standard feed.

Proglumide was administered to rats of E group at the same time. All rats were fed in an air-conditioned room for 3 months.

3. Preparation for samples

The rats were killed by venesection from the heart under ether anesthesia, having been treated for 3 months. Having opened the abdomen, the stomach was taken out of the abdomen. Gastric juice was collected to check pH and volume. And then the stomach was opened from the greater curvature and examined carefully. Gastric mucosal pH was measured and mucosal lesions were checked macroscopically. After that the gastric mucosa was divided into 5 parts as shown in fig. 1 and each part of the mucosa was cut along the longitudinal line from the cardia to the pylorus. After that it was rolled in the manner of a Swiss roll, and these rolls were fixed in Bouin's solution for 6 hr at 4°C. This procedure was performed to examine the gastric mucosa from the cardia to the pyloric end under a microscope. The following markers of the mucosa were evaluated in the fundubody and the antropyloric mucosa separately.

4. Histopathology

Four micron-wax tissue sections were prepared for histopathology. Hematoxylin/eosin stain was done for evaluation of histopathology and azan stain was made for evaluation of fibrotic proliferation.

5. Procedure to evaluate the effect of drugs on erosive gastritis

For the purpose of evaluation of the preventive effect of drugs on erosive gastritis, five rats were selected from 8 rats in each group at random. The following 6 markers were utilized for the evaluation; (1) total length of mucosal surface lesion (erosion), (2) the thickness of the mucosa, (3) the number of parietal cells, (4) the grade of inflammatory cell infiltration, (5) the grade of proliferation of collagenous fiber.

The total length of erosion was measured by an ocular eyegrid equipped in an objective glass at 100 times magnitude under a microscope, and was expressed as mm.

The thickness of the gastric mucosa was measured by the same method as the length of erosion and was expressed as mm.

The number of parietal cells was counted in unit area (0.25 cm^2) by using the ocular eyegrid under a microsopic visual field at 400 times magnitude. In order to obtain constant data, both were measured or counted in the shaded area of the mucosa as shown in Fig. 1.

The grade of inflammatory cell infiltration and fibrotic proliferation was estimated as (–), (±), (+), and (++) and then as a mean grade by an examiner without knowledge of the experimental conditions.

6. Serum gastrin

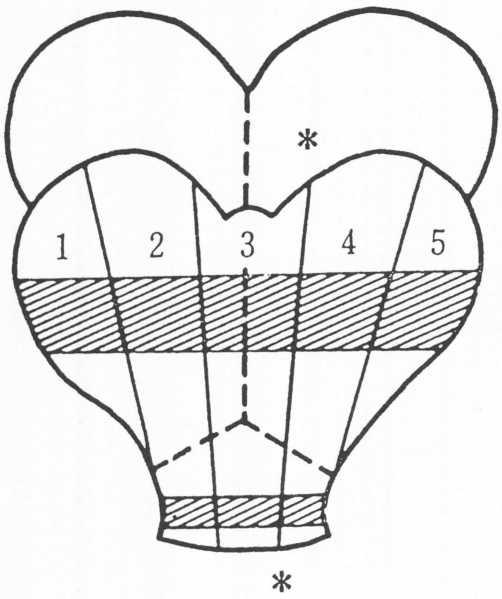

Fig. 1. The gastric mucosa was divided into 5 parts and each part of the mucosa was cut along the longitudinal line from the cardia to the pylorus. After that it was rolled in the manner of a swiss roll, and these rolls were fixed in Bouin's solution.

Basal gastrin was determined in the serum by the radioimmunoassay method.

7. Gastric acid and pepsin

Pylorus-ligated rats, fasted for 24 hr prior to the determination of acid and pepsin, were used for this. Volume, acidity, acid output, and pepsin output of gastric juice were measured for 4 hr after the ligation with a subcutaneous injection of 500 micrograms per kg in a dose of tetragastrin.

8. The criteria for erosive gastritis

Erosive gastritis is defined as the mucosal surface injury (erosion), inflammatory cell infiltration in lamina propria mucosae, reduction in the number of parietal cells, shortened mucosal thickness, and proliferation of collagenous fiber.

9. Statistical analysis

The results were given as medians ± standard error. The data were analyzed by Wilcoxon's rank sum test for paired variates. Value of $p<0.05$ were considered significant.

RESULTS

1. Erosion (Fig. 2, 3)

There were not any gastric lesions on the gas-

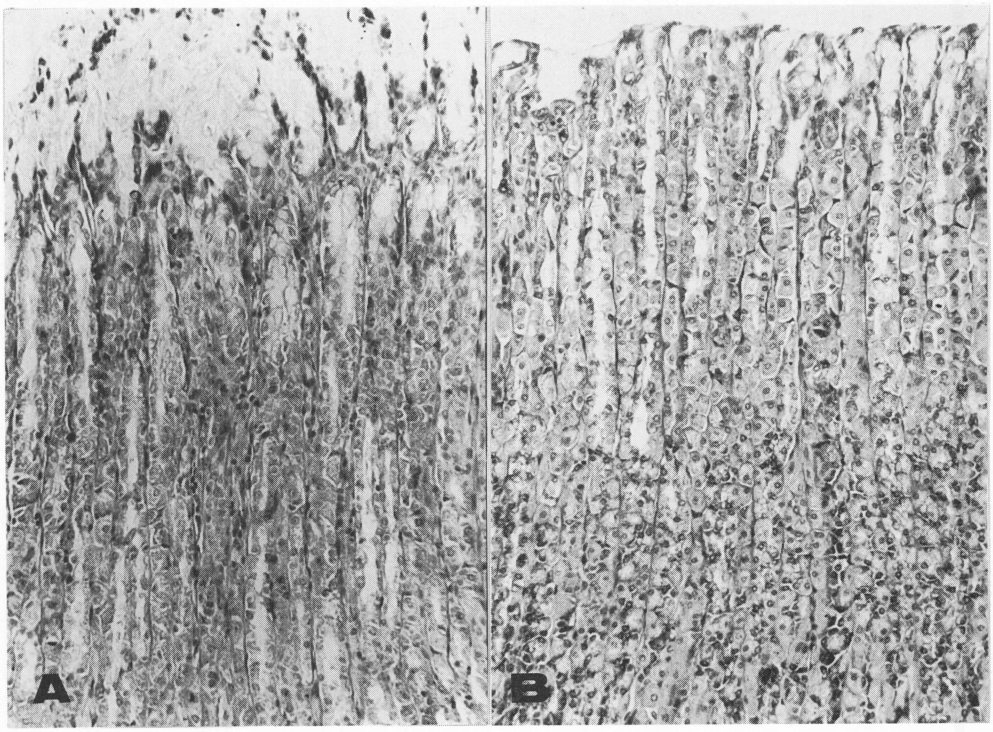

Fig. 2. Gastric erosion caused by TCA in the TCA group (A), and normal gastric mucosal surface after the administration of TCA with proglumide of the E group (B). HE stain (× 100).

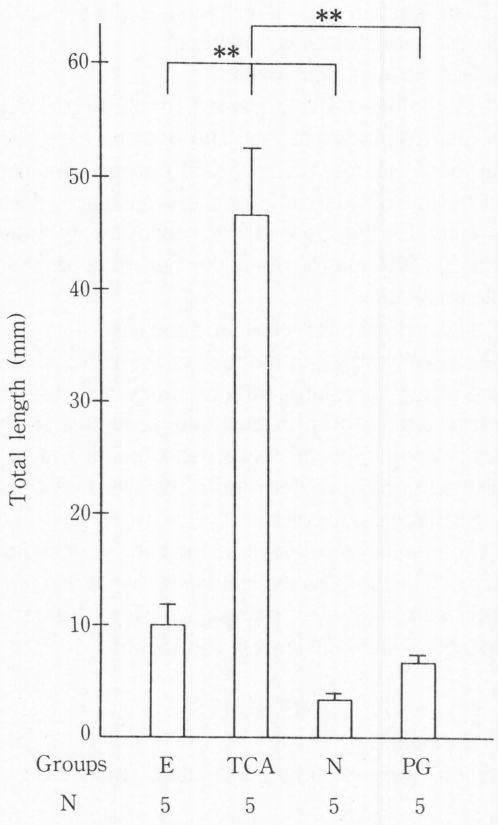

Fig. 3 Total length of erosion **: $p<0.01$

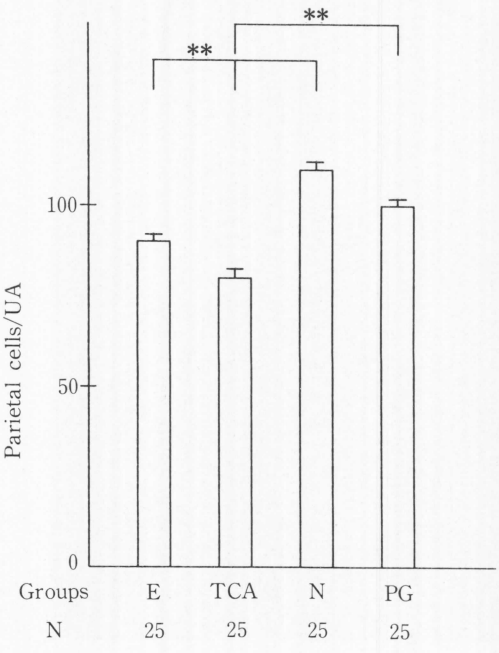

Fig. 5 The number of parietal cells per unit area
**: $p<0.01$

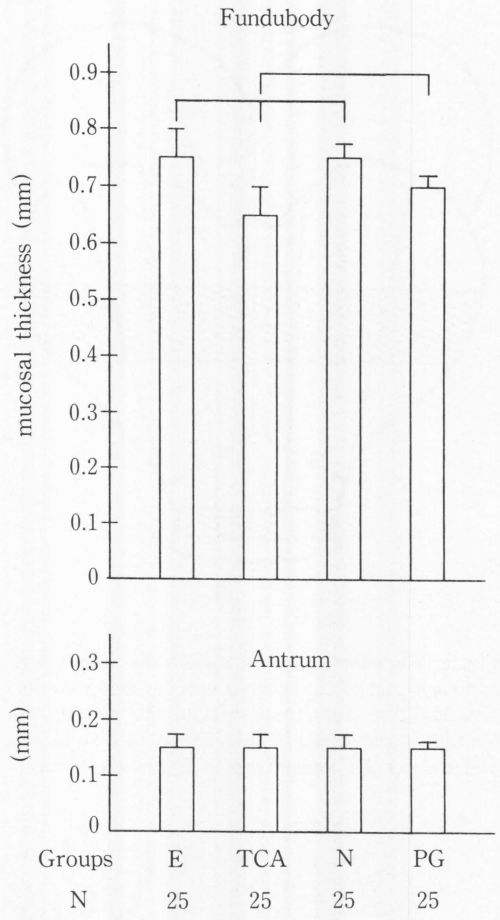

Fig. 4 Gastric mucosal thickness : $p<0.01$

tric mucosal surface. The total length of erosion was 1.00 ± 0.32 mm in the N group, 47.40 ± 5.72 mm in the TCA group, 9.90 ± 2.83 mm in the E groups and 5.8 ± 0.83 mm in the PG group. There was a statistically significant difference between E and TCA groups ($p<0.01$). There were also statistically significant differences between PG and TCA groups ($p<0.01$).

2. Mucosal thickness (Fig. 4)

The mucosal thickness was 0.77 ± 0.02 mm in the fundubody mucosa and 0.17 ± 0.01 mm in the antrum in the E group. The mucosal thickness of the TCA group was 0.65 ± 0.02 mm in the fundubody and 0.16 ± 0.01 mm in the antrum, while that of the N group was 0.77 ± 0.01 mm in the fundubody and 0.17 ± 0.01 mm, and that of PG group was 0.72 ± 0.01 mm in the fundubody and 0.18 ± 0.002 mm in the antrum. The mucosal thickness of the fundubody mucosa was significantly longer in the E groups

them TCA group ($p<0.01$), and was similar to that of N group.

3. The number of parietal cells (Fig. 5)

The number of parietal cells per unit area (0.25 cm^2) was 96.0 ± 0.1 cells in the E group, 87.0 ± 1.4 cells in the TCA group, 117.0 ± 1.1 cells in the N group, and 105.0 ± 0.9 cells in the PG group. There was a statistically significant difference between E and TCA groups ($p<0.01$), between N and TCA groups ($p<0.01$), and between PG and TCA groups ($p<0.01$).

4. Proliferation of collagenous fiber (Table 1, Fig. 6)

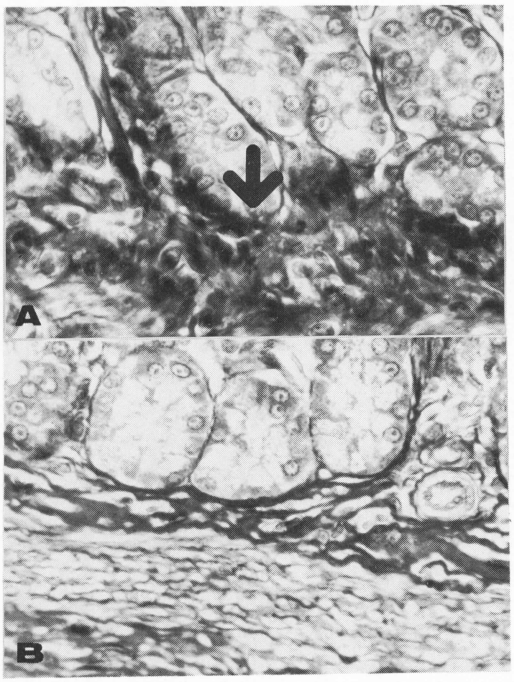

Fig. 6. Proliferation of collagenous fiber in the gastric mucosa in the TCA group (A) and prevention of it by proglumide in the E group (B). Azan stain (× 100).

Table 1. The grade of proliferation of collagenous fiber

Grade \ Group	E	TCA	N	PG
—	25	19		25
+	0	6	0	0
++	0	0	0	0
N	25	25	25	25

**: $p<0.01$

Collagenous fiber colored blue with azan stain was found in the base of the gastric mucosa in TCA groups. The fibrosis grew up towards the mucosa from the base. There was no fibrotic changes in the mucosa in the E, N, and PG groups

5. Inflammatory cell infiltration (Table 2, Fig. 7)

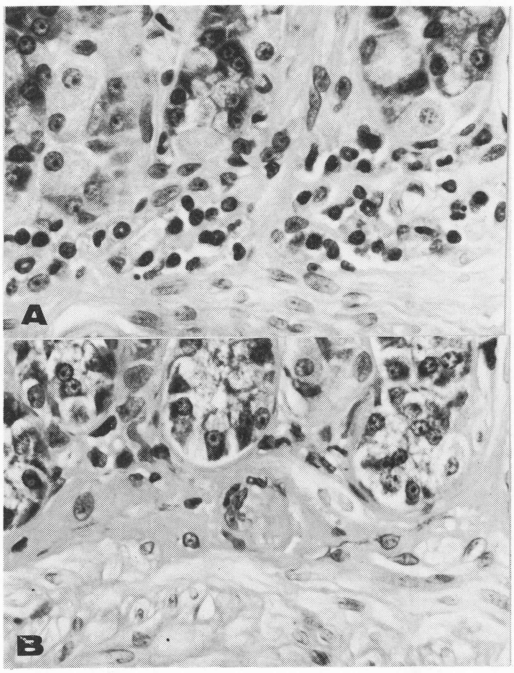

Fig. 7. Inflammatory cell infiltration in the TCA group (A) and no inflammatory cell infiltration in the E group (B) in the lamina propria mucosae of the gastric mucosa. HE stain (× 100).

Table 2. Inflammatory cell infiltration

Grade \ Group	E	TCA	N	PG
—	21	19	25	17
+	4	6	0	8
++	0	0	0	0
N	25	25	25	25

*: $p<0.05$
**: $p<0.01$

Inflammatory cell infiltration was significantly less in the gastric mucosa of the E group than in the TCA group ($p<0.05$). There was no inflammatory cell infiltration in the gastric mucosa of the N and very slight infiltration in

Table 4. Gastric mucosal pH, gastric juice volume, acidity, acid output, and pepsin output.

Group	E	TCA	N	PG
Mucosal pH				
Fundubody	3.76 ± 0.17	3.81 ± 0.20	3.79 ± 0.15	3.99 ± 0.22
Antrum	4.87 ± 0.32	4.82 ± 0.42	4.07 ± 0.45	4.28 ± 0.20
Volume (ml)	9.44 ± 0.96	10.13 ± 1.10	10.27 ± 1.32	9.13 ± 0.76
Acidity (μEq/ml)	106 ± 5	103 ± 9	112 ± 4	99 ± 8
Acid Output (μEq/hr)	254 ± 33	272 ± 52	292 ± 44	230 ± 34
Pepsin Output (mgTyr/min/hr)	1.202 ± 0.109	1.302 ± 0.168	1.142 ± 0.123	1.043 ± 0.098

the PG group.

6. Gastric pH, gastric juice pH (Table 3)

Gastric mucosal pH was 4.5 to 4.0 in the fundubody and 3.8 to 5.0 in the antrum in all groups. Gastric juice pH was less than 3.0 in all groups.

7. Serum gastrin (Fig. 8)

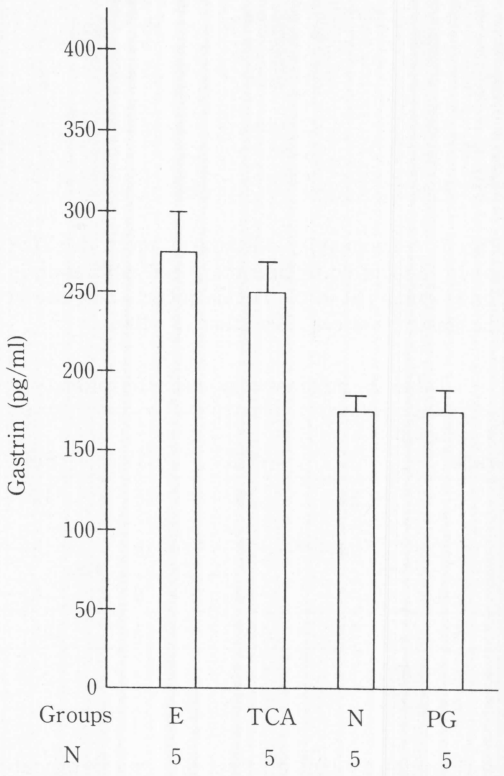

Fig. 8 Basal levels of serum gastrin

The basal levels of gastrin in the serum were 275 ± 32 pg/ml in the E group, 256 ± 26 pg/ml in the TCA group, 181 ± 13 pg/ml in the N group, and 177 ± 19 pg/ml in the PG group.

8. Gastric juice analysis

Gastric volume was 9.44 ± 0.96 ml the E group, 10.13 ± 1.10 ml in the TCA group, 10.27 ± 1.32 ml in the N group, 9.13 ± 0.76 ml in the PG group. Acidity was 106 ± 5 μEq/ml in the E group, 103 ± 9 μEq/ml in the TCA group, 112 ± 4 μEq/ml in the N group, and 99 ± 8 μEq/ml in the PG group. Acid output was 254 ± 33 μEq/hr in the E group, 272 ± 52 μEq/hr in the TCA group, 292 ± 44 μEq/hr in the N group, and 230 ± 34 μEq/hr in the PG group. Pepsin output was 1.202 ± 0.109 mgTyr/min /hr in the E group, 1.302 ± 0.168 mgTyr/min/hr in the TCA group, 1.142 ± 0.123 mgTyr/min/hr in the N group, and 1.043 ± 0.098 mgTyr/min/hr in the PG group.

DISCUSSION

The authors have demonstrated that the mucosal changes developed after the 3 month-administration of TCA are erosive gastritis based on the histopathological findings in the previous studies[7,10]. In the study erosive gastritis was also present in the gastric mucosa treated with TCA for 3 months. The histoquantative data in which total length of erosion and inflammatory cell infiltration are significantly less in the E group than in the TCA group indicates that proglumide prevents the development of erosive gastritis caused by TCA. Furthermore, the study showed that proglumide can also prevent the development of atrophic change in the gastric mucosa, since the reduction of parietal cells, the shortening of mucosal thickness and

the proliferation of fibers which are markers of atrophic gastritis, are all very slight in the E group and are significantly lower than in the TCA group.

Proglumide can inhibit basal and stimulated acid secretion and gastrin release[2,4,12,18]. Therefore the agent may be a gastrin receptor antagonist[4,18]. Prevention of the development of erosion after TCA in the E group may be due to the inhibition of acid and gastrin by the agent. Acid-pepsin secretion and gastrin levels of the E group, however, are not different from those of the TCA group. This means that acid-pepsin and gastrin might be not involved in the mechanism of the preventive effect on proglumide on erosive gastritis. Proglumide has a protective action on the gastric mucosa and can increase gastric mucosal blood flow[13,16,17]. The actions may protect the gastric mucosa from TCA, a mucosal barrier breaker[3], which is largely involved in the preventive mechanism of proglumide.

The number of parietal cells and the mucosal thickness in the E group were similar to those in the N or PG group. This may also be due to the protective actions of the agent. The mucosal thickness was almost within normal range in the E group. This may be due to the fact that the normal number of parietal cells was kept.

Inflammatory cell infiltration and proliferation of collagenous fiber were less marked in the E group than in the TCA group. This might be due to inhibition of hydrogen ion back diffusion and its resulting damaged blood vessels and repeated mucosal damage.

CONCLUSION

Proglumide had a preventive effect on the development of erosive gastritis caused by the 3 month-administration of TCA in the rat. However, some complicated factors which are not explained by acid-pepsin and gastrin alone may be involved in the preventive mechanism of proglumide.

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