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Preventive Effect of Pirenzepine on Atrophic Erosive Gastritis in Rats

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ABSTRACT

The preventive effect of pirenzepine on atrophic erosive gastritis induced experimentally after long exposure of the gastric mucosa in rats to sodium taurocholate (TCA) described. Gastritis was produced in male Wistar rats by giving drinking water ad libitum containing 5 mM TCA for 11 months (Group T). Preventive effect of pirenzepine was designed by giving powder meal containing 100 mg/kg of pirenzepine with TCA-containing drinking water for 11 months (Group P). Rats were also raised with the standard powder meal which is the same as in other two groups but without the agent for 11 months (Group N). The parameters employed in the present study for atrophic erosive gastritis are mucosal surface injury (erosion), reduction of parietal cells per unit area, inflammatory cell infiltration, decreased mucosal thickness and proliferation of collagenous fiber. Significant decrease in the length of total erosions and in the number of ulcers were present in rats with Group P when compared to those in Group T. The mucosal thickness and the number of parietal cells in Group P were not different from those in Group N. Fibrotic proliferation and cyst formation were significantly decreased in Group P when compared to those in Group T. These data indicate that pirenzepine has a preventive effect on the development of atrophic erosive gastritis by TCA in rats.

Key words: *Experimental gastritis, Pirenzepine, Prevention*

Pirenzepine is known to be an M1 receptor antagonist on parietal cells, having a potent antisecretory action and antiulcer effect in man and animals[8-10,15,17,23]. Lately, the agent has been employed in the treatment of gastritis in man and its therapeutic effect on gastritis has reported at the endoscopic level[19], but this should be confirmed in animal models of gastritis. For this purpose, an animal model of experimental gastritis must be first produced. It has been suggested that duodenal contents, particularly bile acids, can cause gastritis, for it has been reported that bile reflux-gastritis can develop in the remaining stomach after gastric surgery[1,7,16]. The authors have also reported that taurocholic acid can induce atrophic erosive gastritis in rats[11-14]. The aim of this study as the first step was to determine whether or not pirenzepine has a preventive effect on atrophic erosive gastritis induced by taurocholic acid in rats.

MATERIALS AND METHODS

1. Experimental animals

Male Wistar rats, weighing about 100 g at the beginning of the experiment, were used for this study. Rats were raised in an air conditioned room during the experimental period. Drinking water and feed were provided to them every morning.

2. Induction of atrophic erosive gastritis

The method of inducing atrophic erosive gastritis in rats has been described elsewhere[12] and will only be presented briefly here. The rats were fed a standard pellet meal (Japan Clea Co., Tokyo, Japan) daily for 11 months, 100 g of which consisted mainly of 24.0 g of protein, 7.0 g of water, 5.1 g of fat, 6.2 g of inorganic substances, 3.2 g of fiber and 54.5 g of soluble substances without nitrogen, and water containing 5 mmol/liter sodium salt of taurocholic acid (TCA; Difco, Detroit, MI, USA). Atrophic erosive gastritis was thus induced experimentally.

3. Administration of pirenzepine and experimental groups

One group of rats was fed a standard powder meal which was adjusted to contain 100 mg/kg of a meal (Japan Clea Co. Tokyo) and drinking water containing TCA for 11 months (Group P). A second group of rats was fed the same standard powder meal and drinking water containing TCA for 11 months (Group T). A third group of rats was fed a standard powder meal and drinking water for 11 months (normal, Group N). The standard powder meal used in the three groups was oderless, all having the same quality, hardness, and components. Drinking water was added to TCA solution (adjust-

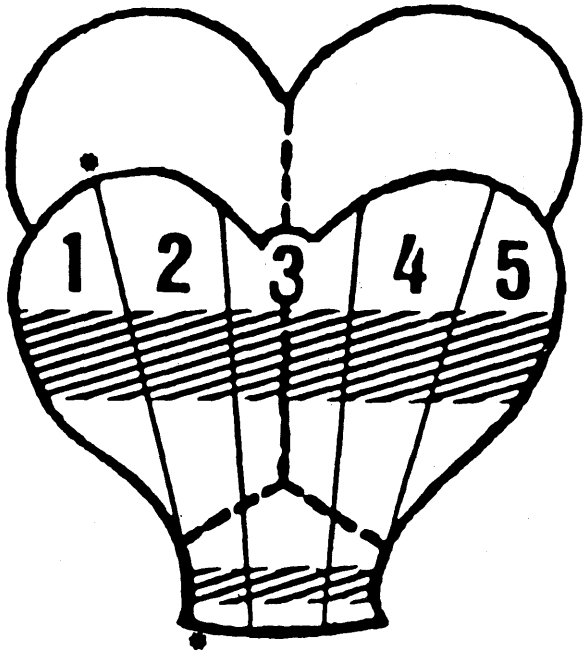

Fig. 1. The tissue samples were taken from each part of the stomach and rolled in a Swiss manner from asteric to asteric. The gastric mucosa was divided into 5 parts and each part of the mucosa was cut along the longitudinal line from the cardia to the pylorus. After that it was rolled in the manner of Swiss roll for examining the gastric mucosa from the cardia to the pylorus. This examination was performed with a microscope. The number of parietal cells and the thickness of the mucosa were counted or measured in the hatched area of the mucosa because their number and their length were constant there.

Table 1

BW. (g)

Month \ Group	P	T	N
1	246	258	264
2	299	307	314
3	323	328	337
4	341	343	347
5	345	353	359
6	353	361	365
7	366	370	379
8	375	384	385
9	380	383	398
10	386	392	400
11	392	399	410

Table 2

Meal. (g)

Month \ Group	P	T	N
1	108	122	126
2	132	138	137
3	129	130	135
4	127	131	130
5	125	133	129
6	128	133	134
7	131	133	138
8	129	129	133
9	130	129	129
10	130	133	133
11	119	121	124
12	122	129	128

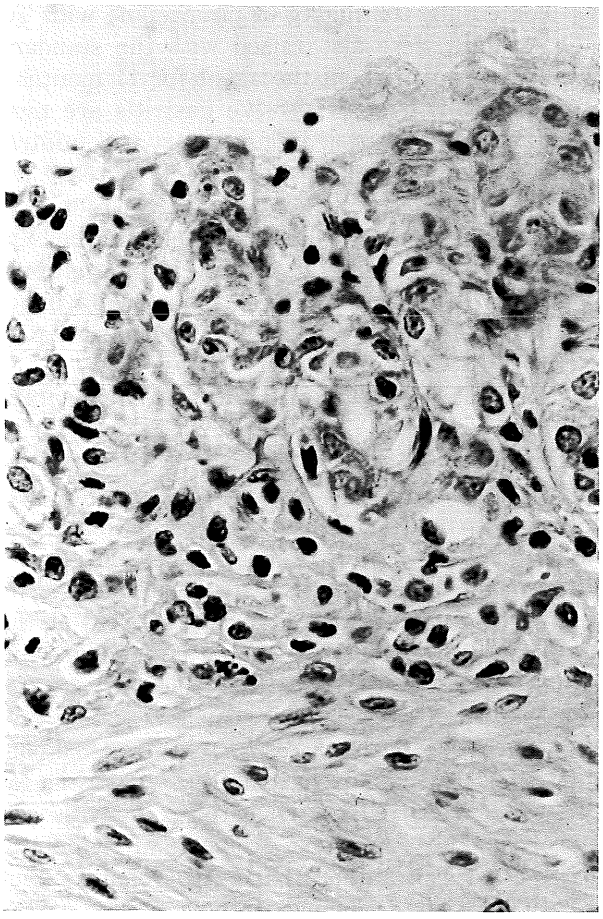

Fig. 2. The antral mucosa of a rat treated with TCA. There were marked infiltration of inflammatory cells and fibrotic proliferation instead of loss of pyloric glands. Gastric ulcer is present. (HE stain, ×200).

ed to 5 mmol/liter) in Groups P and T. Tap water given to rats in Group N.

The dose used here was decided based on the dose in the case of humans and the effective dose in the case of rats with inhibition of acid secretion and with acute gastric ulcer. The maximum effective dose of the agent was administered. The total amount of pirenzepine ingested by rats was calculated from the consumed amount of powder meal. The amount was expressed as mg/kg/week taking into consideration to increase in body weight for one month.

4. Preparation for morphology

The rats fasted for 24 hr prior to the experiment

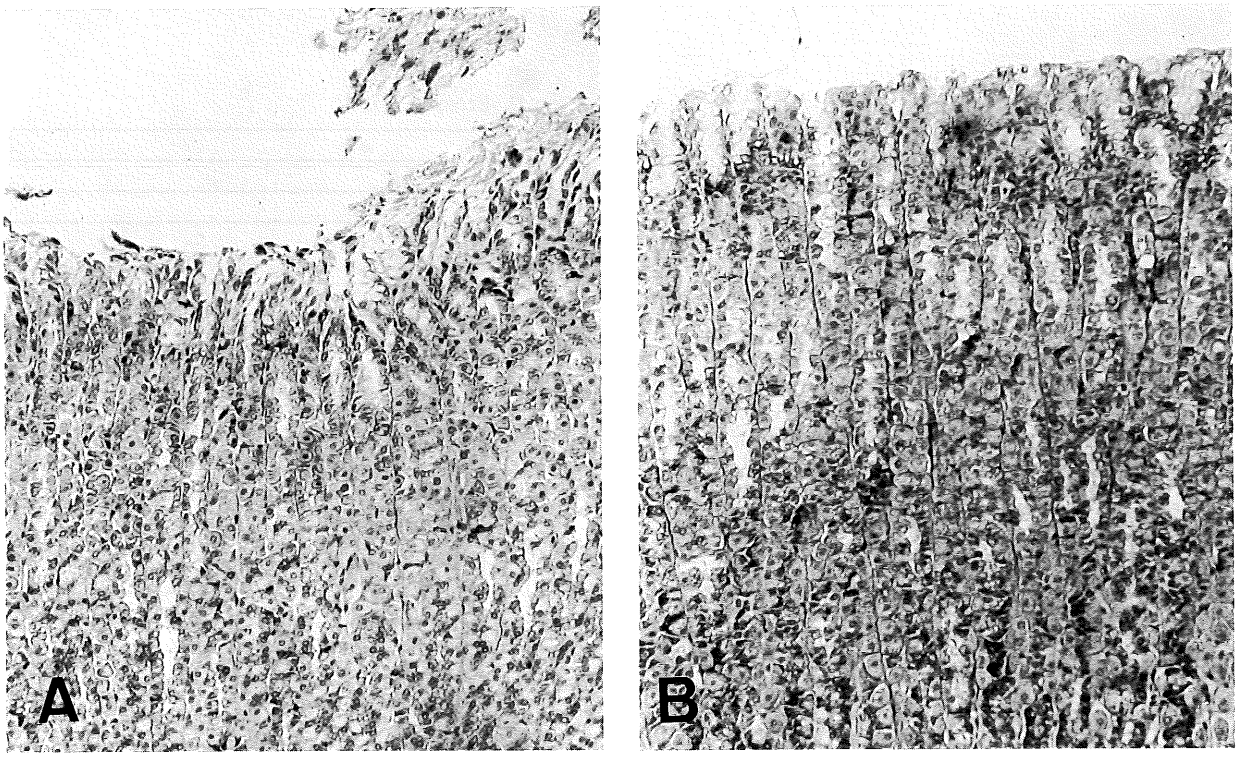

Fig. 3. Gastric erosion is present on the surface of the oxyntic mucosa of a rat in T group (A), while gastric erosion is not present on the mucosal surface of the oxyntic mucosa of a rat in P group (B). Thus, pirenzepine prevents development of erosion after long exposure of the mucosa to TCA.

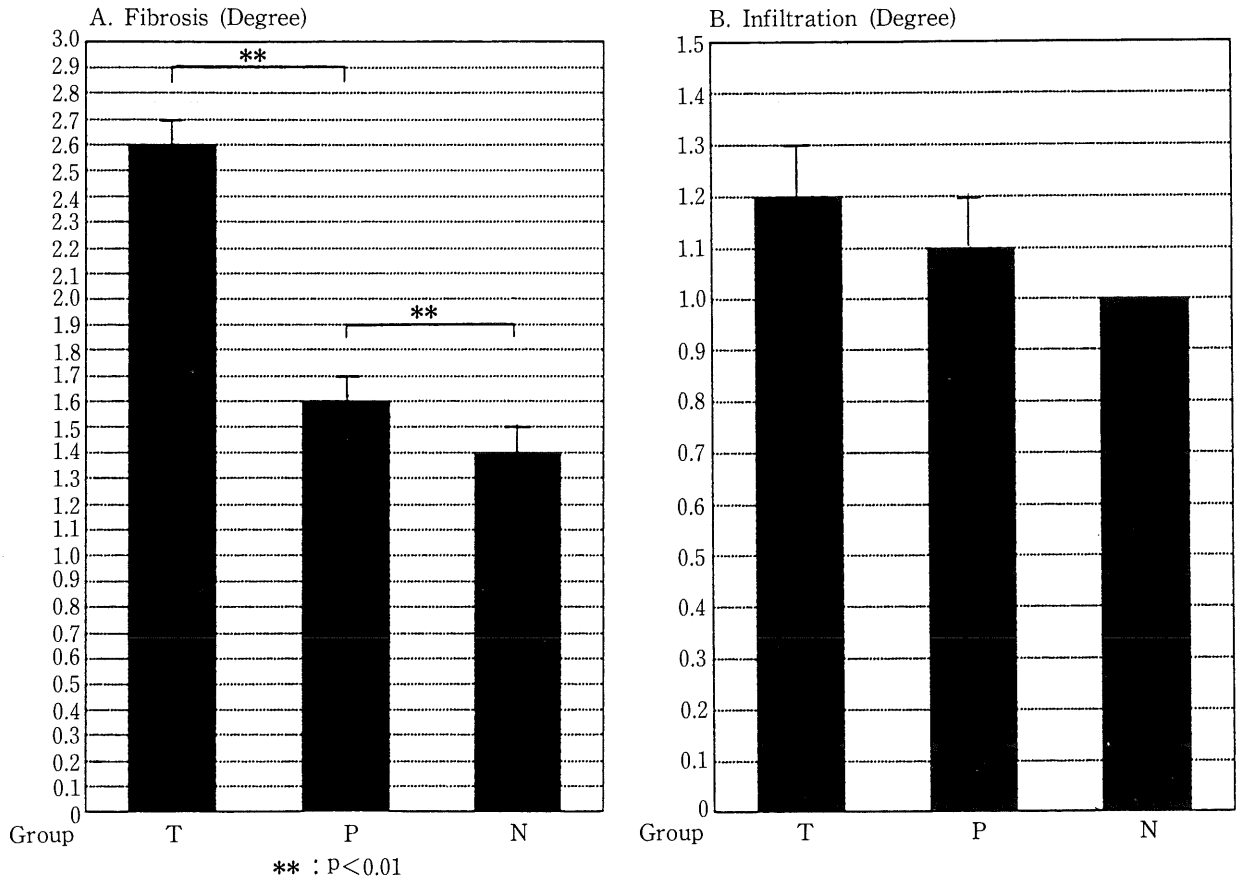

Fig. 4. Proliferation of interstitial fibrosis (A) and inflammatory cell infiltration (B). Fibrotic proliferation is significantly marked in T group while this is not so prominent in Groups P and N although there is a significant difference between P and N group. Celluar infiltration composed of mainly lymphocytes, plasmocytes, neutrophiles, is increased in all three groups by TCA. However the infiltration is most marked in T group. Wilcoxon rank-sum test. n=5/group.

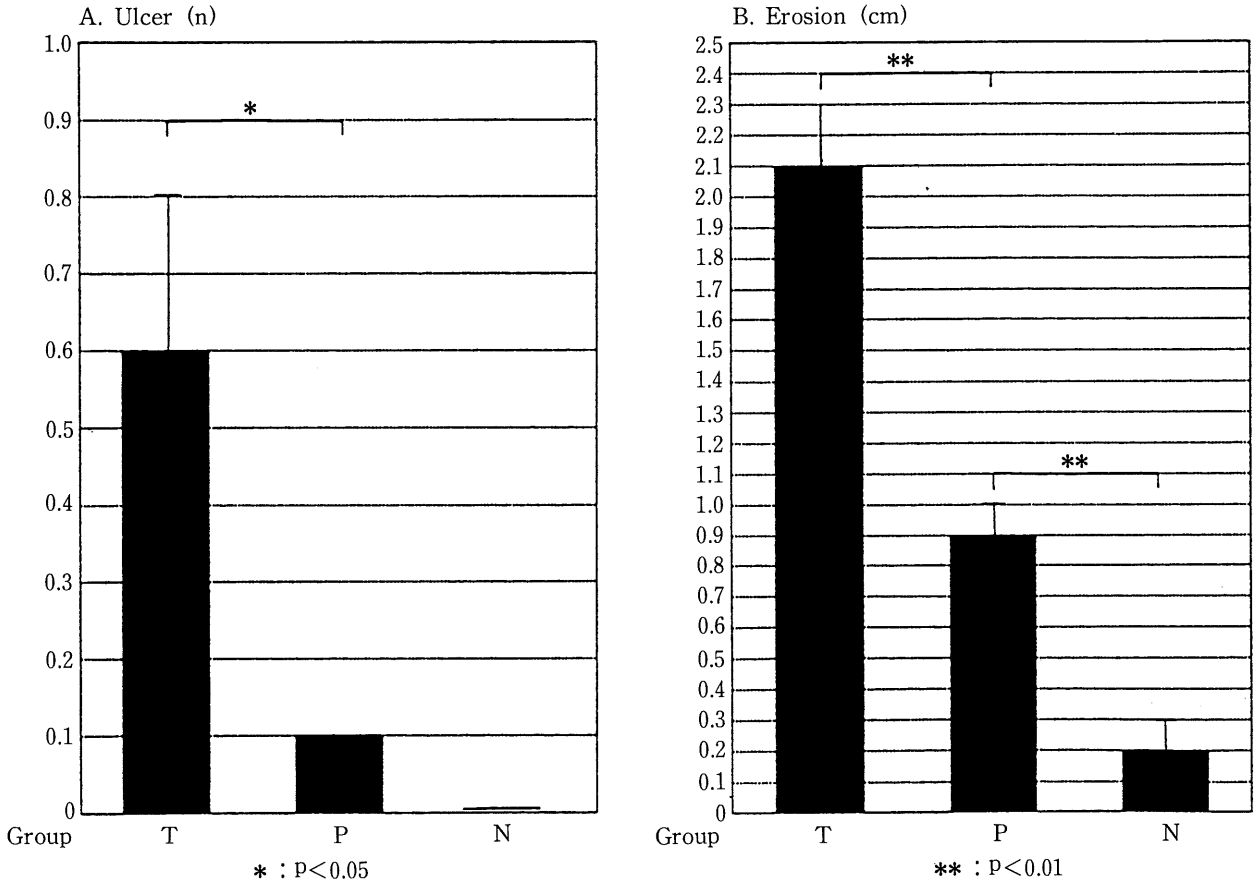

Fig. 5. The number of gastric ulcers (A) and the total length of erosion per each stomach (B). The number of gastric ulcers is significantly increased in T group when compared to that in P group, while there is not any ulcer in N group. Student's t test. n=5/group.

were sacrificed by decapitation and the abdomen was opened. The stomach was then excised, opened along the greater curvature, laid flat by fingers without any artificial damage, and examined carefully for any evidence of gross macroscopic damage. Each flattened stomach was divided into five parts with each excised and rolled like a Swiss roll for observation from the cardiac to pyloric region (each from asteric to asteric, Fig. 1). The Swiss-rolled tissue specimens were fixed in Bouin's solution after 12 hr at 4°C and the paraffin-wax tissues of the embedded blocks were prepared by the usual method.

The tissue sections were stained with hematoxylin and eosin for histopathological evaluation. Azan stained sections were used for interstitial fibrosis.

5. Atrophic erosive gastritis in the present study

The parameters employed in the present study for experimental atrophic erosive gastritis were follows: (1) mucosal surface injury (erosion), (2) reduction of parietal cells per unit area, (3) inflammatory cell infiltration, (4) shortened mucosal thickness, and (5) proliferation of collagenous fiber.

6. Evaluation of erosion, number of parietal cells, mucosal thickness inflammatory cell infiltration, and proliferation of collagenous fiber.

The histopathological evaluation was made by two examiners who were not provided any information on the experimental conditions. Using an ocular eye grid provided with an objective lens, the length of erosion was measured in the entire length of each tissue section from the asteric to the asteric as shown in Fig. 1 under a visual field of 100-fold magnification. The erosion (mm) was expressed as the total sum of five parts per stomach. The number of gastric ulcers was counted in the stomach mucosa and it was expressed per stomach. The number of parietal cells was counted per unit area (0.25 cm^2) under a visual field of 400-fold magnification and mucosal thickness was measured under a visual filed of 100-fold magnification both using the same ocular eye grid as in the case of measuring erosion. The number of parietal cells and mucosal thickness were evaluated in all the vertical sections of the shaded area of five parts as shown in Fig. 1. These were averaged to obtain a single value for each group. The extent of fibrosis was evaluated in grades (±)=1, (+)=2, and (++)=3, from the largeness of stained area and the degree of the cellular infiltration was evaluat-

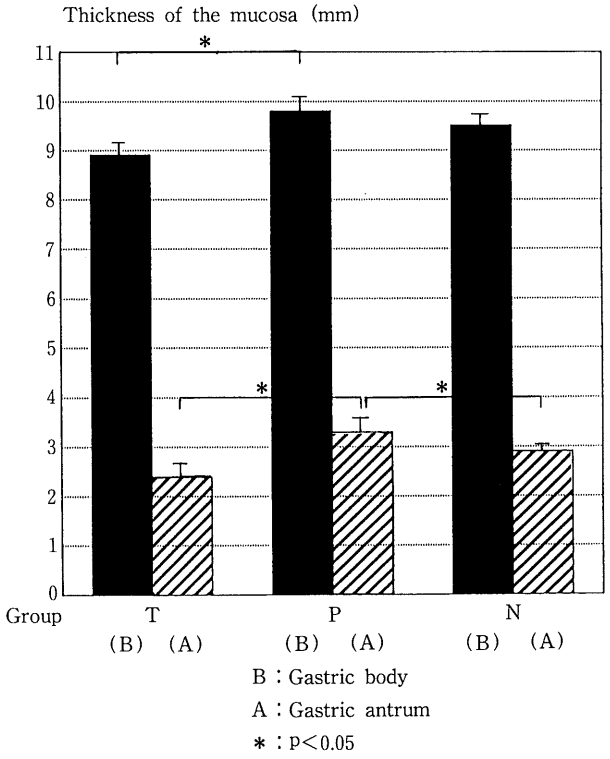

Fig. 6. The thickness of the body (oxyntic, B) and antral mucosa (A). A significant shrinkage of the mucosa, which means atrophic mucosa, is present in T group, while such mucosal shrinkage is not in either P or N groups. Student's t test. n=5/group.

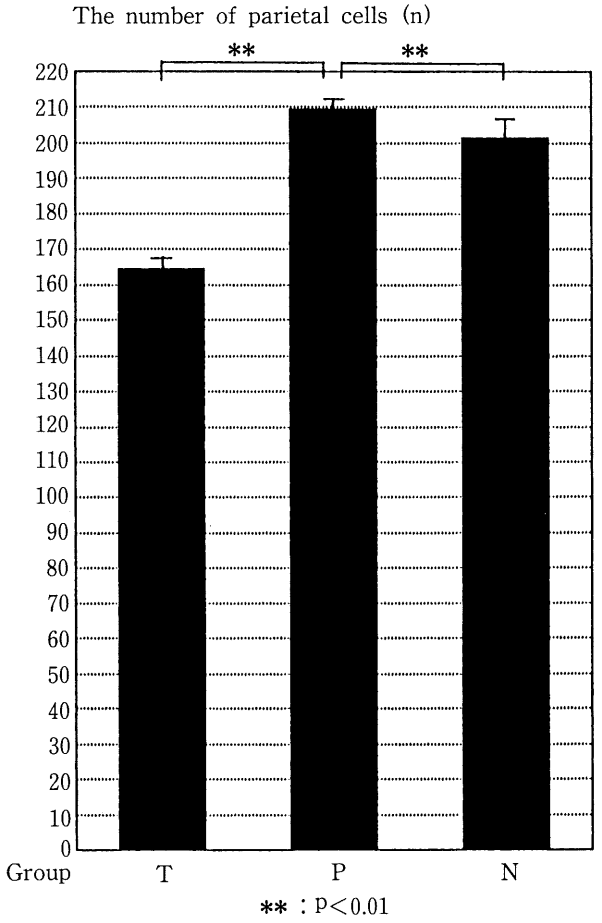

Fig. 7. The number of parietal cells per unit area. A significant reduction in the number of paretal cells is noticed in T group when compared to those in both P and N groups. The reduction of parietal cells indicates atrophic mucosa. Student's t test. n=5/group.

ed in 1= the number of cells less than 20, 2= 21 to 30, and 3= more than 30 under a visual field of 100-fold magnification, and the number of microcysts was counted and expressed in 1= 1 cyst, 2= 2—5 cysts, and 3= more than 3 cysts under 8 visual fields of 100-fold magnification. Gastric mucosal pH was checked by pH test-paper.

7. Statistical evaluation

The length of erosion, number of parietal cells and mucosal thickness are presented as mean ± SEM. The each difference between the P and T group, P and N group, and T and N group was evaluated statistically by student's t test for the length of erosion, number of parietal cells and mucosal thickness. Wilcoxon rank-sum test was used for the extent of fibrosis, cellular infiltration. A p value of less than 0.05 was considered to represent statistical significance.

RESULTS

The body weight in rats of each group is shown in Table 1, indicating that the rats were fully developed without sickness and any spontaneous death by taking standard powder meal and TCA solution and/or tap water for 11 months (Table 2). The amount per week of pirenzepine taken by a rat was mean 30 mg/kg in Group P. The amount was not significantly varied from one week to another for a month.

No gastric lesions were observed macroscopically on the mucosa of rats belonging to the three groups, while there were marked changes in the gastric mucosa at a microscopic level in Group T, such as erosion, gastric ulcers, irregular mucosal structure, cystic formation in glandular tubules, acute and chronic infiltration of inflammatory cells, reduction of parietal cells as well as pyloric glands, and proliferation of collagenous fibers, which indicated atrophic erosive gastritis (Fig. 2), while the gastric mucosal structure in Group P was nearly similar to that in rats in Group N (Fig. 3A and B).

In Group T (1.2 ± 0.1), cell infiltration was thus observed in the same degree as in Group P (1.1 ± 0.1), while the degree of collagenous fiber proliferation was statistically significantly smaller in Groups P (1.6 ± 0.1) and N (1.4 ± 0.1) than that in Group T (2.6 ± 0.1) (Fig. 4A and B). The number of ulcers was significantly fewer in Group P (0.1) than that in Group T (0.6 ± 0.2) (Fig. 5A). The length of the erosion was significantly shorter in Groups P (0.9 ± 0.1 cm) and N (0.2 ± 0.1 cm)

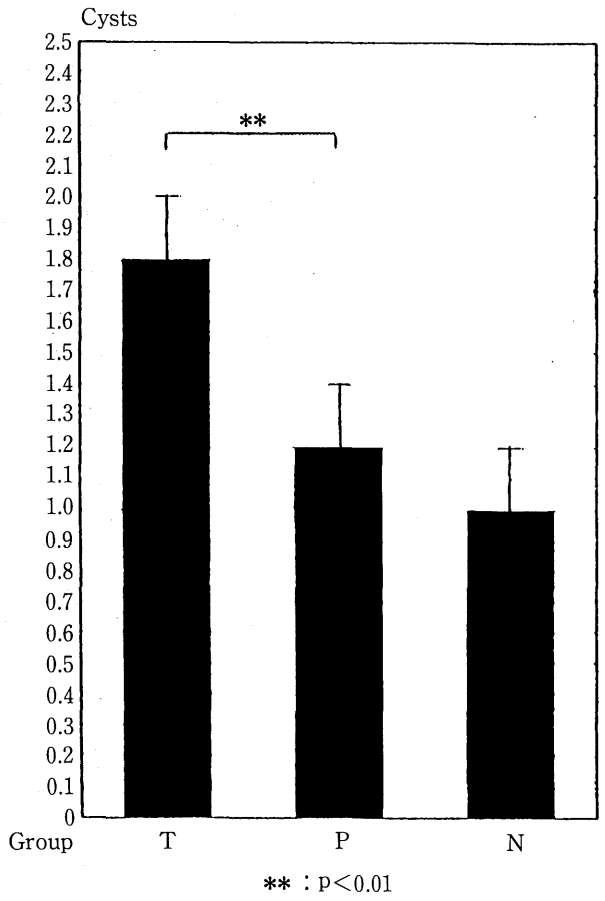

Fig. 8. The number of microcysts. There is a significant increase in the number of microcysts in the stomach mucosa of T and P groups. The difference in the number is statistically significant between T and P, and between T and N groups.

compared to Group T (2.1 ± 0.2 cm) (Fig. 5B). There was no ulcer in Group N. Both parietal cells and chief cells were reduced and cellular spaces between the glandular thickness being shortened in Group T.

The mucosal thickness in the gastric oxyntic mucosa (9.8 ± 0.2 mm) and in the antrum (3.3 ± 0.3 mm) of Group P was the same as those in Group N (9.5 ± 0.2 mm in the oxyntic mucosa and 2.9 ± 0.1 mm in the antral mucosa) but these were significantly thicker when compared to those (8.9 ± 0.3 mm in the oxyntic mucosa and 2.4 ± 0.2 mm in the antral mucosa) in Group T (Fig. 6). The number of parietal cells per unit area in Group P (209.5 ± 2.5 cells) was similar to that in Group N (201.6 ± 3.0 cells), while a significant decrease in the number of parietal cells per unit area was noted in Group T (164.4 ± 4.8 cells) when compared to that in Groups P and N (Fig. 7). Cystic formation of various sizes was noted in rats of Group P (1.2 ± 0.2) and Group N (1.0 ± 0.2) and the number was significantly smaller than that (1.8 ± 0.2) in Group T (Fig. 8). The mucosal pH checked by examining the mucosal surface carefully was weak acidic (pH around 4) in Group P, while it was acidic (pH 2 to 3) in Groups T and N.

DISCUSSION

Pirenzepine, a selective muscarinic receptor antagonist, markedly inhibits gastric acid secretion both in animals and man[8-10,15,17,23]. In particular, pirenzepine is highly effective against hypersecretion induced by penta-and tetragastrin[4,16,17]. Similarly, the agent has shown its potency to heal both gastric and duodenal ulcers in many therapeutic trials and is widely used in treating peptic ulcers in the world[2,5,18,21].

Lately, pirenzepine has also shown a therapeutic effect on gastritis associated with hyperemia, erosion, and mucosal edema on the gastric mucosa diagnosed at the endoscopic level[19]. The authors therefore conducted a preliminary study in an attempt to determine whether or not pirenzepine can prevent production of experimental atrophic erosive gastritis after long exposure of the gastric mucosa to TCA in rats. Pirenzepine inhibited development of erosion, reduction of parietal cells, and shrinkage of mucosal thickness which were all induced by TCA. However, fibrotic proliferation and cellular infiltration were not inhibited by pirenzepine more than we expected. The results obtained in the present study indicate that pirenzepine has a preventive effect on such atrophic erosive gastritis.

As possible mechanism by which pirenzepine has such a preventive effect, there are two actions of pirenzepine. One is due to acid inhibition of pirenzepine. Bile acids including TCA break the gastric mucosal barrier and increase back-diffusion of hydrogen ion into the gastric mucosa in animals[3,22]. These have been shown to occur only in strong acidic solution in the stomach. Gastric mucosal pH was weak acidic in rats of Group P and thus TCA may not cause damage to the gastric mucosa under such condition. The resulting back-diffusion of hydrogen ion can be inhibited in this situation. Thus, the gastric mucosa of P group was protected from the damaging effect of TCA. This is probably one of the reasons why gastric mucosal erosion did not develop in P group. Bile acids including TCA have been said to be cytotoxic to gastric cells, particularly parietal cells, in acidic environment in the stomach[6]. However, the cytotoxity of TCA did not act on parietal cells in P group because the gastric environment was mildly acidic as mentioned before. Therefore, the number of parietal cells of which reduction was one of parameters for atrophic erosive gastritis was kept to the normal number. The other mechanism is due to cytoprotective action of pirenzepine[24], this is that gastric mucosa can be protected by mucosal blood flow and mucous secretion increased by pirenzepine. TCA can reduce the amount of endogenous prostaglandin E_2 in the gastric mucosa

after long exposure of TCA[20]. This is one of the main causes that TCA can induce atrophic erosive gastritis. It is well known that prostaglandins have cytoprotective actions on the gastric mucosa. It is usually believed that gastric mucosa is protected by defensive mechanisms. The main one of the mechanism is prostaglandins. Prostaglandin is reduced by the presence of TCA. Because of prostaglandins' reduction the gastric mucosa is damaged. Once the gastric mucosa is damaged, atrophic erosive gastritis develops. Pirenzepine might inhibit the reduction of the amount of prostaglandin E_2 through an unknown mechanism. This is the third possible mechanism by which pirenzepine prevents the development of atrophic erosive gastritis.

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