Effects of Trimoprostil on Healing and Recurrence of Acetic Acid-induced Gastric Ulcer in Rats

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Abstract—Chronic gastric ulcers were produced by injection of 20% acetic acid (0.05 ml) into the submucosal layer of the rat stomach in order to determine the effects of the prostanoid trimoprostil on the healing and recurrence of ulcers. Local injection of acetic acid solution produced large demarcated ulcers in all animals on day 5, which rapidly decreased to reach low levels on days 40–80 and then became exacerbated on day 100. The exacerbation of the ulcer is probably recurrence. Trimoprostil was administered ad libitum in drinking water containing 0.1, 0.3 and 1.0 μg/ml (average dose 12.4, 37 and 124 μg/kg/day) for a period of 14 days (day 1–15) to assess its effect on healing and for a period of 40 days (day 60–100) to assess its ability to prevent recurrence. The higher two doses of trimoprostil accelerated the spontaneous healing of the ulcers. Furthermore, trimoprostil, at both doses, prevented the observed recurrence of this type of ulcer. Trimoprostil dose-dependently (30–300 μg/kg, p.o.) inhibited gastric secretion in pylorus-ligated rats. Cimetidine at the antisecretory dose (1 mg/ml, 132 mg/kg/day) failed to affect the healing process of gastric ulcers, but tended to prevent the recurrence of gastric ulcers. Our present study suggests that trimoprostil is a promising antiulcer drug for the treatment of chronic gastric ulcer.

Acetic acid-induced gastric ulcer in rats has been suggested to be a chronic type of ulcer, characterized by healing and subsequent recurrence (1). Recently, the recurrence after healing of acetic acid-induced gastric ulcer was confirmed by sequential observations of the mucosa with an endoscope (2). Thus, this experimental ulcer seems to represent a relevant model of peptic ulcers in man.

Trimoprostil, a prostaglandin E_2 derivative, has been reported to be an orally active inhibitor of gastric hypersecretion in experimental animals (3–5) and man (6). Furthermore, this prostanoid protects the gastric mucosa of rats from lesions induced by various necrotizing agents, and it exhibits an antiulcer effect on various acute gastric and duodenal ulcers in rats (5). In view of the effects on acute lesions, it was of interest to determine the effect of trimoprostil on the healing and recurrence of chronic (acetic acid-induced) gastric ulcers in rats. Indeed, trimoprostil promoted the healing and reduced the recurrence of these ulcers.

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Material and Methods

Acetic acid-induced ulcer induction: Male Sprague-Dawley rats weighing initially 200 to 250 g were used in this study. Gastric ulcers were induced in rats under ether anesthesia according to the method of Takagi and Okabe (1, 7). Briefly, 20% acetic acid (0.05 ml) was injected into the submucosal layer at the junction of the body and antrum of the glandular stomach on the anterior wall. After injection, the abdomen was sutured, and the rats were maintained normally on rat chow and water ad libitum. Animals were sacrificed at 5, 10, 15, 20, 40, 60, 80 or 100 days after the operation to assess the extent of healing of the ulcers. Each stomach was removed, filled with 10 ml of 1% formalin, and immersed in 1% formalin for a few minutes. Subsequently, the stomach was opened along the greater curvature, examined

Table 1. Average daily intake of distilled water, trimoprostil and cimetidine (ml) during the study in rats

Exp. No.	Days after operation	Treatment	Concentration in drinking water μg/ml/day	No. of animals	Average drinking volume ml/day/rat	Average dose μg/kg/day for trimoprostil mg/kg/day for cimetidine
1	1– 14	Vehicle (distilled water)	—	15	37.0±0.5	–
	1– 14	Trimoprostil	0.1	10	36.0±0.6	12.4±0.3
	1– 14	Trimoprostil	0.3	10	36.6±0.6	37.2±1.0
	1– 14	Trimoprostil	1.0	10	37.2±0.5	124.0±3.3
	1– 14	Cimetidine	1000	9	39.5±0.9	132.0±4.8
2	60–100	Vehicle (distilled water)	—	20	39.9±0.2	
	60–100	Trimoprostil	0.3	20	42.1±1.1	23.4±0.2
	60–100	Trimoprostil	1.0	20	40.4±0.3	77.7±0.7
	60–100	Cimetidine	1000	20	38.2±0.4	74.3±0.6

Trimoprostil dissolved in absolute ethanol (10 mg/ml) and diluted with distilled water. Cimetidine (Tagamet® injection) was diluted with distilled water. Data represent the mean±S.E.

grossly for ulcers, and the length and width of ulcers were measured. The products of length times width of the ulcers (the ulcerated area) is referred to as the ulcer index. The ulcerated area was measured without knowing the treatment given. The incidence (percent of animals) with one or more ulcers was recorded.

The influence of trimoprostil and cimetidine on the healing process and the recurrence of acetic acid ulcers was studied according to the method of Takeuchi et al. (8). Trimoprostil was prepared as a stock solution in absolute ethanol at a concentration of 10 mg/ml. Aqueous solutions (in distilled water) were prepared fresh each day at trimoprostil concentrations of 0.1, 0.3 and 1.0 μg/ml in distilled water. Cimetidine (Fujisawa Co., Tagamet® injection) was prepared fresh daily at a concentration of 1.0 mg/ml. The animals were treated with trimoprostil or cimetidine (administered in their drinking water) for 14 days (from day 1) or for 40 days from day 60 after induction of the ulcers. The animals were sacrificed on day 15 or 100, respectively, after ulcer induction. The animals were weighed every other day, and the volume of water consumption was recorded daily. The average daily intake of distilled water of rats treated with trimoprostil or cimetidine is summarized in Table 1. The average daily intake in the above three groups was nearly the same throughout the study.

Gastric secretion: Male Sprague-Dawley 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. Five hours 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 0.01 N NaOH to pH 7.0 using the Beckman pH meter. Trimoprostil dissolved in 40% PEG400 solution and cimetidine (Tagamet® injection) diluted with distilled water were given either p.o. or intraduodenally (i.d.), immediately after pylorus ligation. A 40% PEG400 solution or distilled water alone was given to the control rats.

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

Results

Healing process of acetic acid-induced gastric ulcer: The healing process of acetic acid-induced ulcer is delineated in Fig. 1. Large demarcated ulcers were clearly visible in all animals on the fifth day after operation. Adhesion of the ulcer base with the liver, resulting in confined perforation, was extensive and persisted throughout the 100-day experimental period. The ulcers decreased in size and depth for the first 20 to 40 days after the induction. Thereafter, some of the

animals showed complete healing of their ulcers, but others had small ulcers at 60, 80 and 100 days. At 80 days, 9 of 15 (60%) animals showed a complete healing. However, on the 100th day, 75% of the animals had discrete ulcers. The values of the ulcerated area at 100 days showed a tendency to increase when compared to the corresponding values at 80 days, although the difference between these values were not statistically significant (P=0.07).

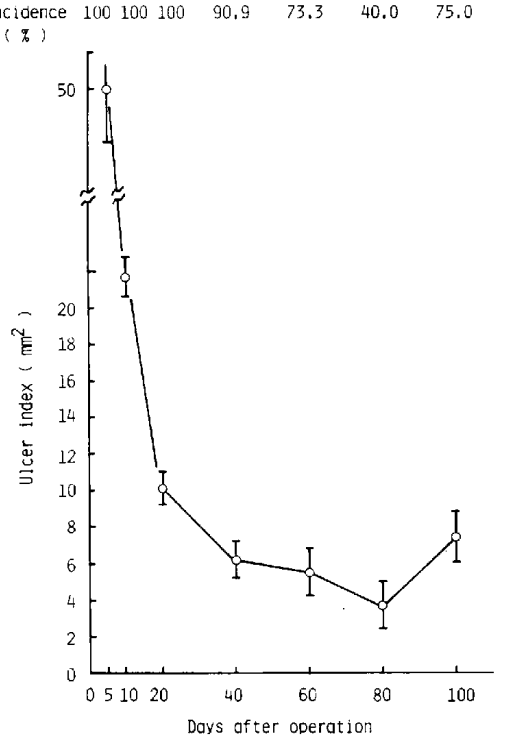

Fig. 1. Healing and recurrence of gastric ulcers produced by submucosal injection of 20% acetic acid (0.05 ml) into the anterior wall of the rat stomach.

Effects of trimoprostil and cimetidine on the healing of acetic acid-induced gastric ulcer: Trimoprostil administered ad libitum at concentrations of 0.3 and 1.0 μg/ml for 14 days from one day after ulcer induction significantly accelerated the healing of gastric ulcers (Table 2). The effects were almost the same at both doses. The lowest concentration tested (0.1 μg/ml) did not alter the healing process. Cimetidine (1.0 mg/ml, given 14 days) did not exert any significant effect on the healing process of gastric ulcers.

Effects of trimoprostil and cimetidine on the recurrence of acetic acid-induced ulcer: After maximal healing was attained (on the 80th day after operation), the recurrence of ulcers was observed on the 100th day after ulcer induction (Fig. 1). The administration of trimoprostil (0.3 and 1.0 μg/ml) for 40 days (from 60 days after the initial ulcer induction with acetic acid) significantly decreased the ulcer index (2.7±0.9 and 2.8±1.1, respectively) as compared to the control group (7.4±1.4), and it tended to reduce the incidence of recurrence of ulcers (Table 3). Cimetidine administered at a concentration of 1.0 mg/ml over the same 40 day period as trimoprostil tended to decrease the ulcer index; however, the incidence of recurrent ulcers was not different from that observed in the control rats.

Effects of trimoprostil and cimetidine on

Table 2. Effects of trimoprostil and cimetidine on the healing process of acetic acid-induced gastric ulcer in rats

Treatment	Concentration in drinking water (μg/ml)	No. of animals	Ulcer index (mm^2) mean±S.E.	% change	P value
Vehicle (distilled water)		15	10.9±1.0		
Trimoprostil	0.1	10	9.7±1.2	11.0	n.s.
	0.3	10	7.5±0.7	31.2	P<0.02
	1.0	10	6.7±1.6	38.5	P<0.05
Cimetidine	1000	9	11.0±0.8	−0.9	n.s.

Drugs were administered from one day after operation in drinking water ad libitum for a period of 14 days.

Table 3. Effects of trimoprostil and cimetidine on the recurrence of acetic acid-induced gastric ulcer in rats

Treatment	Concentration in drinking water (μg/ml)	No. of animals	Ulcer index (mm^2) mean±S.E.	Inhibition (%)	No. of animals with ulcer	Ulcer incidence (%)
Vehicle (distilled water)	—	20	7.4±1.4		15	75.0
Trimoprostil	0.3	20	2.7±0.9*	63.5	9	45.0
	1.0	20	2.8±1.1*	62.2	10	50.0
Cimetidine	1000	20	4.0±1.1	45.9	12	60.0

*: a significant difference from the control value, $P<0.05$. Drugs were given in drinking water ad libitum for a period of 40 days from 60 days after ulcer induction.

gastric acid secretion: Trimoprostil dose-dependently (0.03 to 0.3 mg/kg, p.o.) inhibited the volume of gastric juice and acid output in pylorus-ligated rats (Table 4). Trimoprostil given i.d., however, had no significant effects on gastric secretion at the doses up to 0.3 mg/kg. Cimetidine given i.d. dose-dependently (10 to 100 mg/kg) inhibited gastric secretion, whereas the agent in a dose of 100 mg/kg, p.o., did not affect gastric secretion.

Discussion

An acetic acid-induced gastric ulcer is a chronic type of ulcer which persists for a long period, and it is characterized as healing and subsequent recurrence (1, 2, 7). In these aspects, the acetic acid ulcer in rats closely resembles gastric ulcers in man. With the present ulcer model (induction by 0.05 ml of 20% acetic acid), the incidence and area of ulceration were decreased with time until 60 to 80 days after ulcer induction and then enlarged at 100 days. This pattern was quite similar to those of Okabe and Pfeiffer (7) and Fukawa et al. (2). Consequently, we might conclude that ulcers observed at 100 days are probably recurrence, although histological examination was not performed.

The prostanoid trimoprostil significantly accelerated the healing process and reduced recurrence rate of acetic acid-induced ulcers. Similar effects on the healing of chronic gastric ulcers in rats by the prostaglandin E_2 derivative, 15(S)-15-methyl-PGE_2 methyl ester, was described by Ishibashi et al. (9). Robert et al. (10) reported that several prostaglandins possess both acid-inhibitory and cytoprotective properties. The antiulcer effect of prostaglandins was once thought to be mainly due to their antisecretory action. Indeed, orally administered trimoprostil inhibits gastric hypersecretion in experimental animals (3–5) and man (6) at doses greater than cytoprotective ones. We also confirmed that orally administered trimoprostil dose-dependently (0.03–0.3 mg/kg) inhibited gastric secretion in pylorus-ligated rats. In contrast to p.o. administration, the i.d. administration of trimoprostil had no effect on gastric secretion, indicating that this agent needs to be in direct contact with gastric cells in order for it to exert its antisecretory effect. The present findings with trimoprostil might be explained as being the result of its antisecretory effect. Cimetidine, however, did not affect the healing of the acetic acid-induced ulcers, at its gastric acid inhibitory dose, in general agreement with other investigators (11, 12). Moreover, it was noted that antacid and anticholinergic agents did not improve the healing of gastric ulcer (1).

These findings suggest that the effect of trimoprostil may be more related to its cytoprotective properties. PGE_2 and 16,16 dimethyl PGE_2 (13, 14) are known to stimulate the synthesis and release of gastric mucus. In the natural healing process, Fukawa et al. (15) described an increase in the acid mucopolysaccharide layer covering the regenerated epithelium. Thus, trimoprostil may exert its healing promoting effect through increasing the defensive factors of the gastric mucosa, including the possible pre-

Table 4. Effects of trimoprostil and cimetidine on gastric acid secretion in pylorus-ligated rats

Treatment	Dose mg/kg	Route	No. of animals	Acid secretion			
				Acid output		Secretory volume	
				mean±S.E. (μEq/5 hr)	% change	mean±S.E. (ml/5 hr)	% change
Vehicle (40% PEG 400)	—	p.o.	12	1223.2± 65.6		11.1±0.5	
Trimoprostil	0.03	p.o.	8	961.5± 91.1*	−21.4	9.4±0.6*	−15.3
	0.1	p.o.	8	843.9± 35.0***	−31.0	9.0±0.4**	−18.9
	0.3	p.o.	12	541.8± 19.1***	−55.7	7.5±0.3***	−32.4
Vehicle (distilled water)	—	p.o.	8	1049.0±105.8		10.0±0.6	
Cimetidine	100	p.o.	8	1062.5± 83.0	+1.3	10.1±0.6	+1.0
Vehicle (40% PEG 400)	—	i.d.	8	904.0± 86.9		9.0±0.7	
Trimoprostil	0.3	i.d.	8	770.4± 66.5	−14.8	8.2±0.4	−8.9
Vehicle (distilled water)	—	i.d.	8	1178.6± 99.7		10.3±0.7	
Cimetidine	10	i.d.	8	903.7± 78.6*	−23.3	9.0±0.7	−12.6
	30	i.d.	8	630.5± 68.5***	−46.5	7.2±0.7**	−30.1
	100	i.d.	8	376.1± 45.0***	−68.1	5.8±0.4***	−43.7

Trimoprostil dissolved in 40% PEG 400 solution and cimetidine (commercial medicine, Tagamet® injection) diluted with distilled water were given orally (p.o.) or intraduodenally (i.d.), immediately after pylorus ligation, and 5 hr later, animals were sacrificed. Significant difference from each vehicle control value: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

vention of a decrease in acid mucopolysaccharides. This contention is supported by data which show that trimoprostil but not cimetidine, prevented the stress-induced decrease in defensive factors of the gastric mucosa (5). Furthermore, the healing of acetic acid-induced ulcers appears to be accelerated by drugs such as sucralfate (8), carbenoxolone (16), sofalcone (17) and teprenone (18) which strengthen the defensive factor. Taken together, there appears to be ample evidence that trimoprostil may exert its healing action on chronic gastric ulcers through a defense mechanism.

The clinical recurrence of ulcers in patients poses a serious problem. In this study, trimoprostil appeared to prevent recurrence of acetic acid ulcer in rats. The role of acid secretion as a mechanism in ulcer recurrence is unclear. Okabe and Pfeiffer (7) reported that acid secretion was enhanced on day 70 and thereafter in chronic fistula rats with acetic acid-induced ulcers. Cimetidine, however, tended to decrease the ulcer index, but the effect was statistically not significant. These results suggest that, at least in experimental models, gastric acid secretion appears to play a minor role in the pathogenesis of gastric ulcer recurrence. Accordingly, trimoprostil may act on chronic ulcers through other mechanisms including cytoprotection, increased acid mucopolysaccharide (5) and inhibition of gastric secretory function. The present findings indicate that mucosal defensive mechanisms rather than inhibition of gastric acid secretion may play a major role in the natural healing and prevention of recurrence of chronic gastric ulcers.

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