CRITICAL STUDIES OF THE PYLORUS LIGATED RAT (SHAY RAT)

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The pylorus ligated rat (Shay rat) was described by Shay *et al.* (1) for the production of acute ulcers in the forestomach, and it has been also employed as a means of studying gastric secretion. This technique is now used extensively as a screening method of gastric secretory depressants and antipeptic ulcer agents (2-4) because of the following advantages: 1) the simplicity of surgical procedure, 2) the high rate and rapid occurrence of ulceration, and 3) the possibility of measurements of gastric secretory rate and severity of ulceration in the same animals; which make up for the shortcoming that ulcers are present only in the forestomach.

The pathogenesis of ulceration in the pylorus ligated rat has been discussed by many investigators, but the definite conclusion is not yet offered. Several factors have been considered as causes of ulceration; such as the digestive effect of gastric juice, the interference of blood circulation or the decrease of mucosal resistance. Sun and Chen (5) concluded that the rumenal ulcers in the pylorus ligated rat might be merely a result of the volume and the concentration of gastric hydrochloric acid and pepsin upon the mucosa. But other factors can not be ruled out completely.

There remains some obscurities also in the gastric secretory mechanism of the pylorus ligated rat. It is often said that the pylorus ligated rat is inadequate for the estimation of stimulants of gastric secretion, for they can hardly increase in gastric secretion in response to stimuli owing to the hypersecretion. The mechanism of the hypersecretion in the pylorus ligated rat is now still controversial.

The present study is an attempt to clarify the effects of various factors on gastric secretory rate and ulceration and to discuss the mechanisms of ulceration and gastric hypersecretion in the pylorus ligated rat.

METHODS

Male *Donryu* and Sprague-Dawley rats weighing between 100 and 200 g (except where otherwise noted) were used for this study. They were maintained at 23°C and 60% humidity throughout the experiment. They were fasted for the fixed time in each experiment but were allowed free access to water, and weighed just prior to the surgical

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operation. Under light ether anesthesia, the pylorus was ligated through a midline abdominal incision with care not to damage any blood vessels. After wound closure, the animals were caged individually without food and water during this period. At the end of experiments, the animals were sacrificed by exsanguination and the stomachs were removed. Gastric contents were drained from the stomach and centrifuged at 3,000 cps for 5 minutes, and the volume of supernatant solution was recorded. If the debris of gastric content was over 0.6 ml, the animal was excluded from the study because of possible contamination of the gastric contents by food residue or feces. Free and total acidities were estimated by titration with 0.1 N NaOH using Töpfer and phenolphthalein as indicators. Acid output was expressed in mEq by multiplying the volume in ml by the acid concentration in mEq/l.

The pylorus ligated rat with acute fistula was prepared by the same surgical procedure to the pylorus ligated rat except the fixation of acute fistula made of polyethylene tube at the forestomach. Immediately after the operation, the rats were placed in individual cages and the gastric juice was collected into the graduated centrifuge tube.

RESULTS

1. Studies on rumenal ulceration

1) Effect of various factors on ulceration

In order to study the various factors influencing the severity of ulcers, the experiment was designed using the orthogonal array of L_8 . As shown in Table 1, 4 factors consisting of 2 levels respectively, and 3 interactions between 2 factors were investigated. The experiment was repeated 4 times in the same design. The result was analysed according to the cumulative method devised by Taguchi (6). As the analytical value, the severity of ulceration was adopted, which was classified into the following 3 groups: (---) no ulcer, (+) unperforated ulcer and (++) perforated ulcer. The results are shown in Tables 2 and 3, which show that the strain of rat and the interaction between the time of starvation and the time after ligation are significant at $p=0.01$, and the time of starvation and the time after ligation are significant at $p=0.05$. The body weight of the rat did not influence on the severity of ulceration. Namely, *Donryu* rats were more susceptible to ulceration than Sprague-Dawley rats, and the increase of the time of starvation to 2 days or the increase of the time after ligation to 19 hours augmented the severity of ulceration. Judg-

TABLE 1. Design of experiment on pylorus ligated rats using L_8 orthogonal arrays.

Factor	Level	
A : Strain	A ₁ : Donryu ♂	A ₂ : Sprague-Dawley ♂
B : Body weight	B ₁ : 110-140 g	B ₂ : 160-190 g
C : Time of starvation	C ₁ : 48 hr	C ₂ : 24 hr
D : Time after ligation	D ₁ : 19 hr	D ₂ : 19 hr
B × C		
B × D		
C × D		

TABLE 2. Analysis of variance of experiment on pylorus ligated rats using L_8 orthogonal array.

Factor	Degree of freedom	Sum of square	Mean square	F
A	2	13.35	6.68	10.19**
B	2	2.02	1.02	1.82
C	2	4.70	2.35	4.20*
D	2	6.51	3.25	5.81*
B $\times$ D	2	0.68	0.34	—
B $\times$ D	2	0.51	0.25	—
C $\times$ D	2	8.04	4.02	7.18**
e	48	28.18	0.59	
T	62			
(e)	52	29.37	0.56	

* $p=0.05$ ** $p=0.01$ TABLE 3. The estimation of effects of factors showing significant difference at $p=0.01$ or 0.05 .

Factor	Severity of ulcer			Total
	—	+	++	
A ₁	3 (18.8%)	1 (6.2%)	12 (75.0%)	16 (100%)
A ₂	5 (31.3%)	9 (56.2%)	2 (12.5%)	16 (100%)
C ₁	2 (12.5%)	5 (31.3%)	9 (56.2%)	16 (100%)
C ₂	6 (37.4%)	5 (31.3%)	5 (31.3%)	16 (100%)
D ₁	7 (43.7%)	3 (18.8%)	6 (37.5%)	16 (100%)
D ₂	1 (6.3%)	7 (43.7%)	8 (50.0%)	16 (100%)
(CD) ₁	1 (6.3%)	6 (37.5%)	9 (56.2%)	16 (100%)
(CD) ₂	7 (43.7%)	4 (25.0%)	5 (31.3%)	16 (100%)
C ₁ D ₁	1 (12.5%)	2 (25.0%)	5 (62.5%)	8 (100%)
C ₁ D ₂	1 (12.5%)	3 (47.5%)	4 (50.0%)	8 (100%)
C ₂ D ₁	6 (75.0%)	1 (12.5%)	1 (12.5%)	8 (100%)
C ₂ D ₂	0 (0%)	4 (50.0%)	4 (50.0%)	8 (100%)

— : no ulcer + : unperforated ulcer ++ : perforated ulcer

ing from this result, the most appropriate condition as a screening method of anti-peptic ulcer agents is A₂, C₂ and D₂, which can produce unperforated ulcers at the highest rate. If a condition of Sprague-Dawley rats, 160–190 g of body weight, 24 hour starvation and 19 hour ligation are used, anticipated rates of ulcer production calculated by the omega method devised by Taguchi (6) are as follows: (—) 13.8%, (+) 70.2% and (++) 16.0%.

The difference of ulceration between strains may be not due to the difference of gastric secretory activity. Because the volumes of gastric juice in 6 hour pylorus ligated rats were 11.5 ± 1.2 ml in *Donryu* rats and 11.2 ± 0.5 ml in Sprague-Dawley rats and there was no significant difference between them.

2) Effect of glucose and protein hydrolysate on ulceration

Since the ulcerogenic effect of starvation was considered to be due to the decrease

TABLE 4. Effect of administration of glucose and protein hydrolysate on ulceration in the pylorus ligated rats.

Treatment	Decrease of body weight (%)	Severity of ulcer	Gastric secretion			
			Volume (ml)	pH	Free acid (mEq)	Total acid (mEq/l)
Saline 20 ml/kg $\times$ 5 (i.p.)	19.6	##	—	—	—	—
	17.2	+	25.4	1.6	27	53
	25.1	++	19.7	1.1	45	88
	22.1	##	—	—	—	—
	21.7	+	19.8	1.3	34	66
Mean	21.1		21.6	1.3	35	69
20% Glucose and 5% protein hydrolysate 10 ml/kg $\times$ 5 (i.p.)	19.1	+	19.9	1.5	30	56
	16.9	+	21.1	1.2	41	71
	19.8	—	15.9	1.2	43	72
	19.8	+	19.9	1.0	67	110
	20.1	+	24.2	1.3	40	63
Mean	19.1		20.2	1.4	44	74

: Perforated ulcer ++ : Severe ulceration + : Slight ulceration — : No ulcer

of mucosal resistance caused by undernourishment, the effect of administration of glucose and protein hydrolysate on ulceration was examined. The rats were treated with the intraperitoneal injections of glucose (2 g/kg) and protein hydrolysate (10 ml/kg of 5% "Polytamin", Daigo-Eiyo-Kagagu Co., Ltd.) twice a day during 2 days of starvation and immediately after the ligation (5 times in total). The result is shown in Table 4.

Fig. 1. The influence of body weight on the volume of gastric secretion in the 19 hour pylorus ligated rats. The volume of gastric secretion is expressed by ml per animal in (A) and by ml per 100 g body weight in (B).

Though the decrease of body weight and the volume of gastric secretion of treated animals were not significantly different from those of controls, the severity of ulceration of treated animals was clearly low compared with controls.

II. Studies on gastric secretion

1) Influence of body weight on gastric secretion

The relationship between the volume of gastric secretion and the body weight of rats fasted for 48 hours was investigated in the 19 hour pylorus ligated rats. As shown in Fig. 1, a linear relationship between the volume of gastric secretion and the body weight within the 100 to 170 g was observed. Above those weights, the volume did not

Fig. 2. The influence of time after pylorus ligation on gastric secretion.
 (A) : Influence on volume of gastric secretion
 (B) : Influence on free and total acidities of gastric secretion
 Each point is a mean with standard error of 6 animals.

increase with increasing body weight and the variance of it grew large.

2) Influence of the time after pylorus ligation

The influence of the time after pylorus ligation on the volume and acidity of gastric juice was studied in rats fasted overnight. As shown in Fig. 2 (A, B), a linear relationship between the volume or acidity and the time after pylorus ligation was observed within 6 hours, but above 6 hours the volume got nearly plateau and the free and total acidities slightly declined.

FIG. 3. The inhibitory effect of duodenal souring on gastric secretion in the 5 hour pylorus ligated rats. Each group consists of 5 animals.

FIG. 4. Effects of acidification, alkalization or local anesthesia of stomach on gastric secretion in the 4 hour pylorus ligated rats. Each group consists of 6 animals.

3) *Effects of gastric or duodenal acidification, gastric alkalization and local anesthesia of gastric mucosa*

These experiments were carried out in order to study the contribution of humoral stimulating mechanism of gastric secretion in the pylorus ligated rat. The effects of gastric acidification or alkalization and local anesthesia of gastric mucosa were investigated in the 4 hour pylorus ligated rats, and 1 ml of 0.1 N HCl, 0.1 N NaOH or 1% dibucaine was injected intragastrically after the pylorus ligation. The values of volume and acidity of gastric secretion were corrected for the values of injected solutions. For the purpose of studying the effect of duodenal acidification, 1 ml of 0.1 N HCl was injected immediately after the pylorus ligation in the 5 hour pylorus ligated rats. The results are summarized in Figs. 3 and 4. The injection of acid into the duodenum inhibited the gastric secretion, while the injections of acid, alkali or the local anesthetic into the stomach did not affect the gastric secretion.

4) *Gastric secretion in the pylorus ligated rats with acute fistula*

The relationship between the volume of gastric secretion and the time after pyloric ligation is shown in Fig. 5. The volume of gastric secretion increased in proportion to the time after ligation up to 10 hours. The total volume and the pattern of gastric secretion are apparently different from those of the pylorus ligated rats without acute fistula (refer to Fig. 2).

FIG. 5. The influence of time after pylorus ligation on gastric secretion in the pylorus ligated rats with acute fistula. Each point is a mean with standard error of 6 animals.

DISCUSSION

The etiology of ulceration in the pylorus ligated rats has been eagerly discussed, but the definitive conclusion is not yet given. The digestive action of gastric juice accumulated into the stomach is considered to be the primary cause of mucosal damages in the forestomach (5). In our present experiment using L_8 orthogonal array, the time of starvation had a great influence on the severity of ulceration, which suggests that the undernutrition by starvation decreases the mucosal defense mechanism and accelerates the ulcer production. The intraperitoneal administration of glucose and protein hydrolysate during starvation markedly prevented the ulcer production in the forestomach without any influence on body weight and gastric secretion. Shay *et al.* (7) reported that the administration of high concentration of protein hydrolyzed products with normal foods prior to the starvation for 4 weeks decreased the ulcer production. These experiments show that the decrease of mucosal defense mechanism can not be neglected as the cause of ulceration in the pylorus ligated rats besides the accumulation of gastric juice in the stomach and

obstruction of blood supply to the forestomach.

When the technique of the pylorus ligated rats is used as a screening method of anti-ulcer agents, unperforated ulcers should be produced at the highest rate because rats with perforated ulcer died before the end of experiments and precise information can not be obtained. In our experiment, a condition of Sprague-Dawley rats, 24 hour starvation and 19 hour pylorus ligation is considered to be most appropriate for this purpose.

The experimental data concerning gastric secretion in the pylorus ligated rats are conflicting, which may be due to the difference of analytical method of data. The volume of gastric juice in the pylorus ligated rat is often recorded as ml per 100 g rat body weight on the basis of the direct relationship between gastric secretion and body weight reported by Madden *et al.* (8). In contrast, Brown *et al.* (3) described that the body weight had not any significant effect on the volume of secretion and therefore there was no need to adjust for weight in expressing the volume of secretion. Our present result shows that there is a linear relationship between body weight and volume of secretion up to about 170 g body weight after overnight starvation, and above that weight the volume was nearly constant in spite of the increase of body weight and the variance grew large. Therefore, as far as *Donryu* strain is concerned the rats weighing below 170 g after starvation should be used because of the small variance of values.

A linear relationship between the volume of secretion and the time after pylorus ligation within 6 hours and after that the volume of secretion was nearly plateau, which is slightly different from the result described by Sun *et al.* (5). The total and free acidities also got the maximum response at the 8th hour after ligation. The time after pylorus ligation must be set within 8 hours for the study of gastric secretion.

It has been usually difficult to demonstrate a substantial increase of a gastric acid output in good response to a gastric secretory stimulant, because the control value is high by itself and moreover the mechanism of the hypersecretion is not clear. Several opinions on the mechanism of the hypersecretion have been presented. Gati *et al.* (9) reported that the duodenal souring reduced the gastric secretory rate in the pylorus ligated rat, but Brodie (10) failed to ascertain this phenomenon. In our present experiment, the intra-duodenal injection of acid decreased gastric secretion but the effect of gastric acidification, alkalization or mucosal anesthesia of the stomach on gastric secretion was not demonstrated. It shows that the humoral mechanism such as gastrin release has little effect on the hypersecretion in the pylorus ligated rat. Brodie (10) presumed that the mechanism of this hypersecretion was due to the stimulation of the pressure receptors in the antral mucosa by ligation. The present experiment shows that the volume and the pattern of gastric secretion of the pylorus ligated rat with acute fistula is different from those of the originally described pylorus ligated rats. This result is somewhat inconsistent with the conclusion of Brodie (10) and somewhat consistent with the suggestion of Levine (11) saying the possibility of the stimulation of gastric secretion by saliva. It may be reasonable to consider that the mechanism of hypersecretion in the pylorus ligated rat is very complicated and further investigations have to be carried out.

SUMMARY

1. Various factors influencing the rumenal ulceration in the pylorus ligated rat were investigated using the experimental design of L_8 orthogonal array, and the most appropriate condition for the screening of antiulcer agents was presented.

2. The decrease of mucosal defense mechanism may be a cause of ulceration besides the peptic activity of gastric juice.

3. The relationship between the body weight of the rat and the volume of gastric secretion is linear between 100 to 170 g after starvation.

4. The volume and acidity of gastric juice increased in proportion to the gain of body weight up to 6 hours after pylorus ligation.

5. The duodenal souring suppressed the gastric secretory rate, but the humoral mechanism of gastric phase has little effect on it.

6. The pylorus ligated rat showed the hypersecretion and the mechanism of it may be complicated.

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