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Effect of Denervation of the Pylorus and Transection of the Duodenum on Acetaminophen Absorption in Rats; Possible Mechanism for Early Delayed Gastric Emptying after Pylorus Preserving Pancreatoduodenectomy

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Tanaka, A., Ueno, T., Oka, M. and Suzuki, T. *Effect of Denervation of the Pylorus and Transection of the Duodenum on Acetaminophen Absorption in Rats; Possible Mechanism for Early Delayed Gastric Emptying after Pylorus Preserving Pancreatoduodenectomy*. Tohoku J. Exp. Med., 2000, **192** (4), 239-247 —— Early delayed gastric emptying has been reported as a frequent complication following pylorus preserving pancreatoduodenectomy (PPPD). We investigated the effect of division of the pyloric branch of the vagus nerve and/or transection of the duodenum on gastric emptying using the acetaminophen method in rats to speculate the unknown etiology of early delayed gastric emptying after PPPD. Twenty-four male Wistar rats were divided into the following four groups; Group S, sham operation as controls; Group N, disturbance of neuro-vascular supply to the pylorus; Group D, temporary interference of the duodenal continuity; and Group N+D, with both procedures in Group N and Group D. Gastric emptying was measured using the acetaminophen method at 1, 2, and 4 weeks after operations in each group. No significant difference was observed in Group S at any intervals after the operation. Gastric emptying was prolonged significantly in Group N, Group D and Group N+D compared to Group S until 2 weeks following surgery. Significant delayed gastric emptying was sustained in Group N+D at 4 weeks, although gastric emptying in Group N and Group D was improved by 4 weeks. The results in rodent models suggest that both dissection of the pyloric branch of the vagus and transection of the duodenum might be causative factors of postoperative delayed gastric emptying following PPPD. —— PPPD; early delayed gastric emptying; pyloric branch of the vagus nerve; transection of the duodenum; rat

Early delayed gastric emptying has been reported as a frequent complication

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after pylorus preserving pancreatoduodenectomy (PPPD), with incidence ranging from 27 to 57%. The etiology of early delayed gastric emptying after PPPD remains unclear. It has been reported to be caused by division of the vagus nerve, ischemia of the gastroduodenal segments (Braasch et al. 1986), alternation of the endocrinologic milieu (Kim et al. 1987; Fraser et al. 1993), intraabdominal complication such as a leakage or an abscess (Hunt and McLean 1989; Hocking et al. 1990; Traverso 1991; Miedema et al. 1992), postoperative pancreatitis (Lin and Lin 1999), and torsion of the reconstructed alimentary tract (Ueno et al. 1995).

Neurovascular injury and resection of gastrointestinal tract cause a transient disorder of motility of gastrointestinal tract. In particular, delayed gastric emptying seem to be associated with denervation of vagus nerve and transection of small intestine which is distal to pylorus. But it is unknown how these factors, i.e., denervation of vagus nerve and transection of gastrointestinal tract, develop delayed gastric emptying, how these factors interact with each other, and how the delayed gastric emptying changes after operation. Thus, it is necessary of systematic experiment using a rational method.

The purpose of this experiment is to investigate the relationship between gastric emptying and the division of the pyloric branch of the vagus nerve and/or the transection of the duodenum, using acetaminophen method, and to speculate whether these two factors are substantially causative factors of early delayed gastric emptying after PPPD.

Materials and Methods

Materials

We used *p*-acetamidophenol purchased from Wako Pure Chemical Industries, Ltd. (Tokyo).

Animals

Specific pathogen-free male Wistar rats, weighing 200 to 250 g, were used. All animals were housed under controlled temperature and humidity conditions, with a 12-hour alternating light/dark cycle, and free access to food and water. The experimental protocols used in this study were approved by the Ethics Committee for Animal Experimentation at the Yamaguchi University School of Medicine, and carried out according to the Guidelines for Animal Experimentation at Yamaguchi University School of Medicine as well as Law No.105 and Notification No.6 of the Japanese Government.

Preliminary studys

Five male Wistar rat were ligated of the pyloric ring. Before 1 week of this experiments, rats were fastened for 24 hours but were given water ad libitum, under anesthesia with diethyl ether, the jugular vein was cannulated with a polyethylene tube for sampling blood. Conscious rats were administered saline 1

ml included acetaminophen 50 mg/kg through a gastric tube and fixed in a cage. At the time of 10, 20, and 40 minutes after administration, 0.6 ml blood samples were withdrawn through this tube. The serum acetaminophen concentrations were measured by fluorescence polarization immunoassay method (Koizumi et al. 1988).

Operative procedure

Twenty-four male Wistar rats were divided randomly into the following 4 groups. 1, Group S; sham operation as controls. 2, Group N; disturbance of neurovascular supply to the pylorus. 3, Group D; temporary interference of the duodenal continuity. 4, Group N+D; performed both procedures in Group N and Group D.

Rats were anesthetized by inhalation of diethyl ether. The procedures were performed through a 5-cm upper midline laparotomy incision using clean, but not sterilized instrument.

In Group S, the pyloric ring and the duodenum were palpated only. In Group N, the surrounding structure of the pyloric ring was skeletonized by cutting and ligation with a range of 1-cm on both proximal and distal sides to denervation of the pyloric branch of the vagus nerve. As a result, the area was free not only from nerves but also from vessels. In Group D, the duodenum was transected at the point of about 4-cm distal to the pyloric ring and anastomosis was made end-to-end and performed with everted single layer sutures using running method of 6–0 monofilament polypropylene.

Acetaminophen method

Respectively, one, two, four weeks after surgery, rats were fastened for 24 hours but were given water ad libitum. One day before this experiments, under anesthesia with diethyl ether, the jugular vein was cannulated with a polyethylene tube for sampling blood. Conscious rats were administered saline 1 ml included acetaminophen 50 mg/kg through a gastric tube and fixed in a cage. At the time of 0, 10, 20, 30, 40 and 60 minutes after administration, 0.6 ml blood samples were withdrawn through this tube. The serum acetaminophen concentrations were measured by fluorescence polarization immunoassay method (Koizumi et al. 1988).

Rats were sacrificed 4 weeks after the operation and their stomach, duodenum and anastomosis were examined. If there was over 75% of stenosis of anastomosis or ulceration in the stomach and the duodenum, the data of that rat was excluded from this study.

Rats were assigned randomly to one of the four groups by blind selection from a box of a card bearing the group number. When a rat was excluded for any reason, the same operation was performed immediately on a replacement rat. All results are expressed as the mean $\pm$ S.E.. Statistical analyses were performed using

three-way analysis of variance (ANOVA). Fisher's protected least-significance difference test was used for multiple comparisons. Significance was accepted at the 5% level.

Results

Preliminary study

Mean serum acetaminophen concentrations at 10, 20, and 40 minutes after administration were 3.4 ± 1.7, 6.8 ± 2.6 and 5.5 ± 2.7 μg/ml, respectively.

Sham operation

Table 1 showed the serum acetaminophen concentrations of sham operation at each intervals after the operation. In the data, the peak of serum acetaminophen concentration was obtained at 10 minutes after the administration in the control group. No significant difference was observed in Group S at any intervals after the operation.

One week postoperation

Figure 1 represents the serial changes of the mean serum acetaminophen concentrations in the different 4 groups at 1 week after operation. Similarly to Group S, in the other three groups, the serum acetaminophen concentrations peaked at 10 minutes and reduced serially. The mean serum concentrations at 10 minutes were as follows: Group S=54.8 ± 4.5, Group N=34.5 ± 6.6, Group D=43.5 ± 4.7, Group N+D=43.3 ± 4.1. The value of Group N, D and N+D were significantly lower than Group S ($p<0.01$), and the value of group N were significantly lower than Group D and N+D ($p<0.05$). There were no other significant differences in values among the four groups at 1 week postoperatively.

Two weeks postoperation

Figure 2 represents the serial changes in the mean serum acetaminophen

Table 1. *Serum acetaminophen concentrations after sham operation*

	Minutes					
	0	10	20	30	40	60
1W	0	54.8 ± 4.5	36.9 ± 1.6	32.4 ± 1.4	26.4 ± 1.6	16.1 ± 1.5
2W	0	52.4 ± 3.4	45.6 ± 1.9	39.2 ± 1.5	32.6 ± 1.5	19.8 ± 1.4
4W	0	51.1 ± 4.8	43.0 ± 2.8	34.3 ± 2.6	27.6 ± 2.5	16.0 ± 1.7

(μg/ml)

The serum acetaminophen concentrations of sham operation at each intervals after the operation. The peak of serum acetaminophen concentration was obtained at 10 minutes after the administration in the control group. No significant difference was observed in Group S at any intervals after the operation.

W, week.

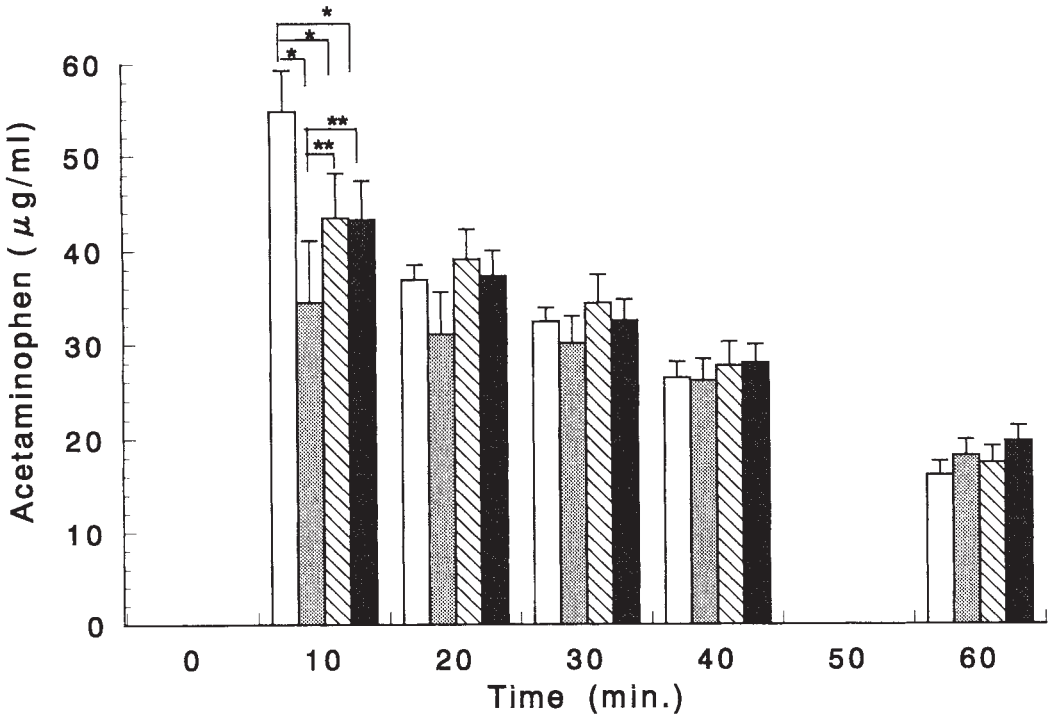

Fig. 1. The serial change of serum acetaminophen concentration (mean±S.E.) of the 4 groups at 1weeks after operation. The serum acetaminophen concentrations of all groups peaked at 10 minutes and reduced serially. The value of Groups N, D and N+D were significantly lower than Group S ($p<0.01$),and the value of Group N were significantly lower than group D and N+D ($p<0.05$). $^{*}p<0.01$, $^{**}p<0.05$.
□, S; ▨, N; ▧, D; ■, N+D.

concentrations in the different 4 groups at 2 weeks after operation. Similarly to Group S, in the Group N, the serum acetaminophen concentrations peaked at 10 minutes and reduced serially. But in Group D and Group N+D, there were no overt peak points and serum acetaminophen concentration continued lower value compared with Group S. The mean serum concentrations at 10 minutes were as follows: Group S=52.4±3.4, Group N=43.6±5.1, Group D=29.0±4.9, and Group N+D=35.7±3.2. These values of Group D and N+D were significantly lower than Group S ($p<0.01$) and the value of Group N were significantly lower than Group S ($p<0.05$). There were also significant differences between Groups N and D at 10 minutes ($p<0.01$) and between Group S and N, N+D at 20 minutes ($p<0.05$), and between Group S and D at 20 minutes ($p<0.01$).

Four weeks postoperation

Figure 3 represents the serial change of the mean serum acetaminophen concentrations in the different 4 groups. Similar to Group S, in the other three groups, serum acetaminophen concentration peaked at 10 minutes and reduced serially. The mean serum concentrations at 10 minutes were as follows: Group S=51.1±4.8, Group N=46.5±3.7, Group D=44.8±5.1, and Group N+D=37.9±5.5. There were significant differences between Groups S and N+D at 10 minutes

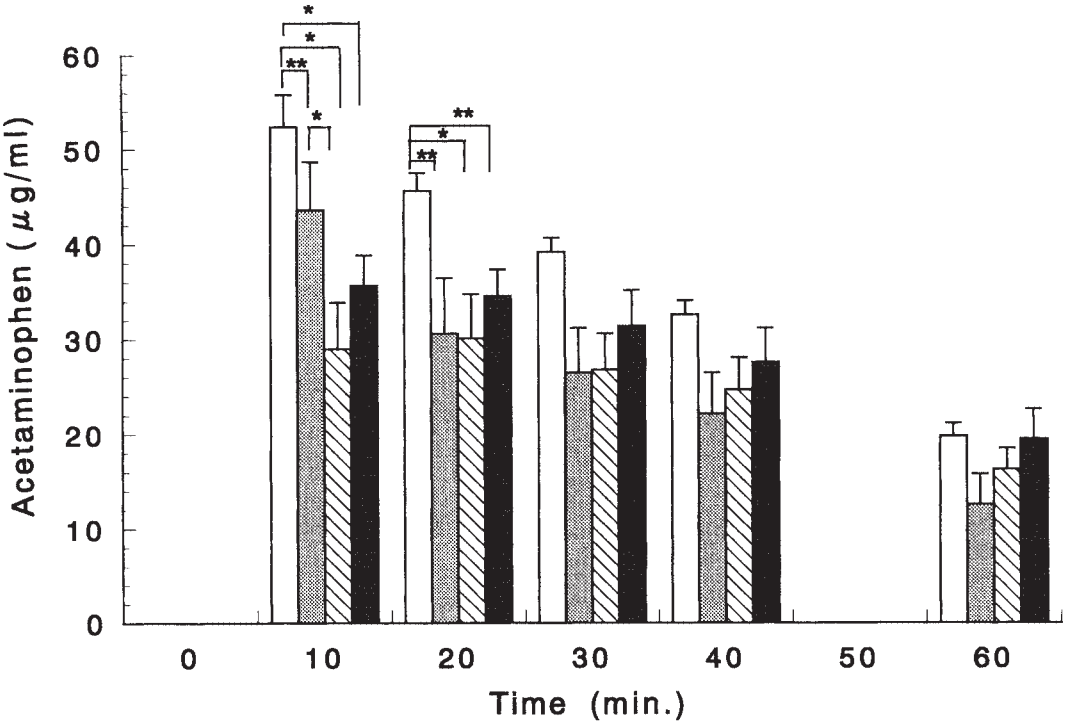

Fig. 2. The serial change of serum acetaminophen concentration (mean±S.E.) of the 4 groups at 2 weeks after operation. The serum acetaminophen concentrations of the Group N peaked at 10 minutes and reduced serially. But in Group D and Group N+D, there were no overt peak points and serum acetaminophen concentration continued lower value compared with Group S. $^{*}p<0.01$, $^{**}p<0.05$.
□, S; ▨, N; ▧, D; ■, N+D.

($p<0.05$) and between Group S and Group N+D at 20 minutes ($p<0.01$).

Discussion

There are several methods available to evaluate gastric emptying in rats (Borella and Lippmann 1980; Droppleman and Dippmann 1980; Scarpignato et al. 1980; Houghton et al. 1992; Hatanaka et al. 1994). Although a scintigraphic method is the gold standard, it requires specialized expensive equipment and is not able to apply to conscious rats. Acetaminophen is considered to be absorbed mainly and rapidly from the small intestine and to have negligible absorption from the stomach and used as a predictor for liquid gastric emptying in humans (Heading et al. 1973). However, it was reported that small amount of acetaminophen was absorbed from the stomach in rats (Bagnall et al. 1979). We performed preliminary studys in which acetaminophen administered to rats treated by complete ligation of the pyloric ring. These results indicate that acetaminophen is absorbed from the rats' stomach, however, the volume of acetaminophen absorbed from the stomach is too small to affect on our experiment.

It has been reported that there are significant correlations between the half time of gastric emptying as measured by scintiscanning and both the maximum serum acetaminophen concentration and the time taken to reach peak (Heading et

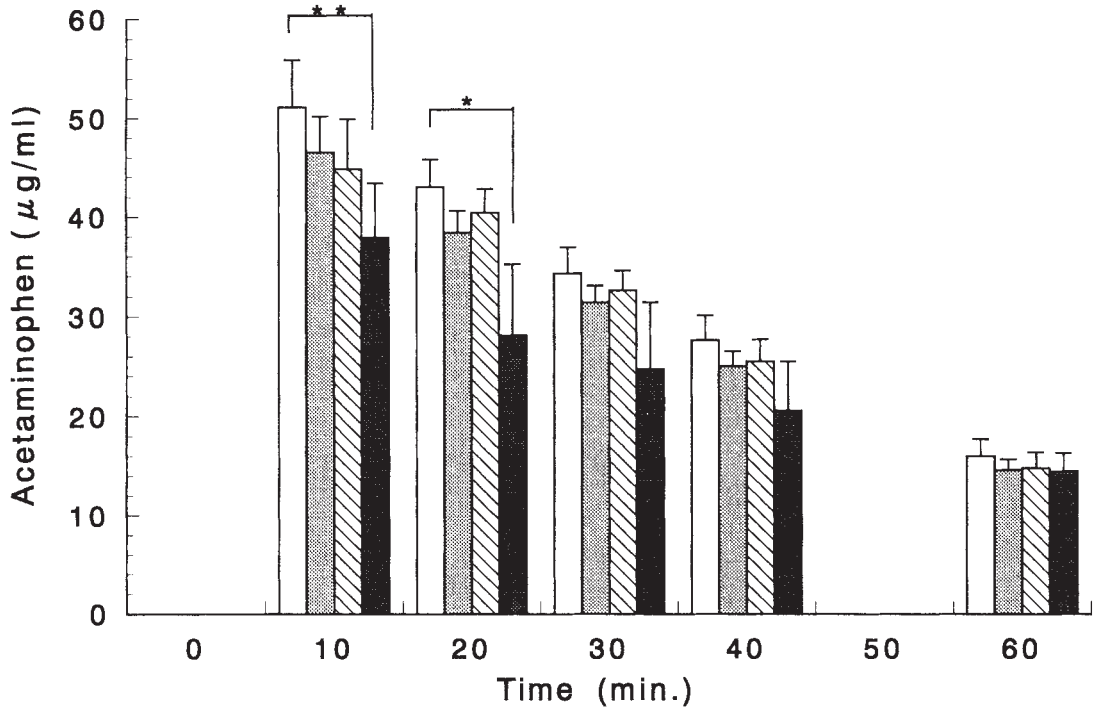

Fig. 3. The serial change of serum acetaminophen concentration (mean±S.E.) of the 4 groups at 4 weeks after operation. The serum acetaminophen concentration of all froups peaked at 10 minutes and reduced serially. There were significant differences between Groups S and N+D at 10 minutes ($p<0.05$) and between Group S and Group N+D at 20 minutes ($p<0.01$). $^{*}p<0.01$, $^{**}p<0.05$.
□, S; ▨, N; ▧, D; ■, N+D.

al. 1973; Harasawa et al. 1979; Koizumi et al. 1988). Harasawa and Miwa (1984) reported that 19.5±5.7% of the liquid meal remained in the stomach at 15 minutes after injection, and 6.2±3.4% and 4.3±4.3% at 30 and 60 minutes, respectively, in rats. In our experiment, the peak of serum acetaminophen concentration was obtained at 10 minutes after administration in the control group. As a result, we thought that the value at 10 minutes was of prime importance time and that the gastric emptying could be evaluated to compare the serum concentration at this time after administration with control group.

The etiology of early delayed gastric emptying after PPPD remains unclear. A munber of authors have reported suggestive causes by the clinical studies and some factors have been speculated. But most of these factors are too complicated to apply to the animal experiments. We therefore attempted to make simple animal models that consist of division of neural and vascular supply for the pylorus and/or the transection of the alimentary tract, in order to clarify the pathogenesis of early delay gastric emptying after PPPD.

We reported that division of the pyloric branch of the vagus impaired gastric emptying in human (Ueno et al. 1995). Aonuma (1994) also reported that gastric emptying was impaired in dogs with division of the pyloric branch for 4 weeks after the operation. In the present study, delayed gastric emptying was observed

in group which was performed pyloric skeletonization in rats, however, the prolongation was transient and improved by 4 weeks.

With regard to the intestinal continuity, Hermon-Taylor and Code (1971) reported that a duodenal pacemaker was present in the proximal 5 to 6 mm of the duodenum in dogs and an annular myotomy of the duodenum about 1 cm proximal (orad) to the biliary ampulla caused a reduction in the frequency of the pacesetter potential distal (caudad) to the conduction block. Akwari et al. (1975) documented that duodenal transection altered the pattern of small intestinal pacesetter potentials, reducing their frequency and periodicity distal to the site of transection. But they did not determine whether it was associated alteration in gastric emptying. Our results showed that the impairment of gastric emptying was observed in the group treated duodenal transection until 2 weeks after operation, though it was also transient. The effect of pyloric denervation on gastric emptying were stronger than that of the transection of duodenum at 1 week after operation, however at 2 weeks after operation, the effect of duodenal transection on gastric emptying were stronger. At 2 weeks, there were not clear peak points of serum acetaminophen concentrations of Group D and N+D. The result suggested that not only delayed gastric emptying but also slow of intestinal transit may be developed. We speculated that it was a result from adhesion around the anastomosis mainly. At 4 weeks, most severe delayed gastric emptying was recognized in the group operated with both procedures, which was prolonged significantly compared to the other two treated groups. We think that this cause was an interaction of denervation of pylorus and transection of duodenum. When we performed on the denervation, we added to ligate the vessels supplied to proximal duodenum. The proximal duodenum is the area that is thought of as the pacemaker of duodenum. Our procedure might damage the pacemaker. We speculated that since the damaged pacemaker interacts with transection of intramural nerve pathway, delayed gastric emptying of this group were severe.

Our results suggest that division of neurovascular supply to the pylorus and/or transection of the duodenum may lead to delayed gastric emptying following PPPD. Conservation of the pyloric branch of the vagus nerve, the right gastric artery and the supraduodenal artery may be recommended to avoid delayed gastric emptying after PPPD, especially for benign disease.

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